
# Maximisation of extraction of phytochemicals from *Salacia chinensis* L. and encapsulation of its enriched extract

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BSc in Genetics (VNU); MSc in Biochemistry and Molecular Biology (BFU)

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Food Science

August 2019

## Statement of Originality

*I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.*

**Thanh Van Ngo**

Date:

**Thesis by publication**

*I hereby certify that this thesis is in the form of a series of papers. I have included as part of the thesis a written declaration from each co-author, endorsed in writing by the Faculty Assistant Dean (Research Training), attesting to my contribution to any jointly authored papers.*

**Thanh Van Ngo**

Date:

**Acknowledgments**

First of all, I would like to express my sincere gratitude to my supervisors Dr Quan Van Vuong, Prof Christopher Scarlett and Prof Michael Bowyer for their continuous guidance and support, for their patience, motivation and immense knowledge.

I would like to acknowledge The University of Newcastle and Vietnam International Education Development (VIED) for awarding me the PhD scholarship.

I would also like to thank my colleagues in Vietnam National University of Forestry, particularly A/Prof Thang Van Bui and A/Prof Nam Quang Vu for their help and support before and during my PhD. Many thanks to my fellows PhD students at Ourimbah campus, especially Mrs Xuan Tran, Mrs Anh Le, Mr Chuyen Hoang, Mrs Thuy Pham, Mrs Hang Vu, Mrs Rebecca McCullum, Mr Saifullah Emon for their help and friendship.

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Last but not least, I would like to thank my wife Hien Nguyen, my son Anh Tuan Ngo and my daughter Ha Khanh Ngo for encouraging and supporting me spiritually during the last 4 years of my PhD.

***This thesis is dedicated to my wife, my son and my daughter with all of my love.***

## List of publications included as part of the thesis

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**Major research and review papers either published or under review in peer-reviewed journals**

1. Thanh Van Ngo, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2019). The *Salacia*: Phytochemicals and Health Benefits (submitted to *Journal of Herbal Medicines*).

2. Thanh Van Ngo, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2017). Phytochemical and antioxidant properties from different parts of *Salacia chinensis* L. *Journal of Biologically Active Products from Nature*, 7(5), 401-410. http://dx.doi.org/10.1080/22311866.2017.1383186

3. Thanh Van Ngo, Christopher James Scarlett, Michael Christian Bowyer, Phuong Duc Ngo, and Quan Van Vuong (2017). Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. *Journal of Food Quality*, 2017, 1 – 8. DOI: https://doi.org/10.1155/2017/9305047

4. Thanh Van Ngo, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2019). Ultrasonic assisted extraction as an advanced technique for the extraction of bioactive

compounds from *Salacia chinensis* root: A comparison with decoction and continuously shaking extraction (submitted to *Journal of Plant Biochemistry and Biotechnology*).

5. Thanh Van Ngo, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2019). Isolation and maximisation of extraction of mangiferin from the root of *Salacia chinensis* L. *Separations*, 6(44), 1-10.

6. Thanh Van Ngo, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2019). Maximising the conditions for encapsulation of enriched crude extract of *Salacia chinensis* root (submitted to *Journal of Microencapsulation*).

## Statement of contribution of others

26 August 2019

**To Whom It May Concern**

This is to confirm that Thanh Van Ngo has contributed to the series of major research papers, which are submitted as a part of his PhD thesis as shown in each paper below.

| | | |
|---|---|---|
| ..... | ..... | ..... |
| Date: ..... | Date: ..... | Date: ..... |
| Prof Michael C. Bowyer | Prof Christopher J. Scarlett | Dr Quan V. Vuong |
| ..... | ..... | |
| Date: ..... | Date: ..... | |
| Phuong Duc Ngo | Rebecca McCullum | |

Endorsed by

Date:

Prof Frances Martin, Assistant Dean (Research Training)

**Publications included in the thesis**

1. **Thanh Van Ngo**, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2019). The *Salacia*: Phytochemicals and Health Benefits (submitted to *Journal of Herbal Medicines*).

| Contributor | Statement of contribution |
|---|---|
| Thanh Van Ngo | Reviewed the literature (100%)<br>Wrote the paper (80%) |
| Michael Christian Bowyer | Critically reviewed the manuscript (20%) |
| Christopher J. Scarlett | Critically reviewed the manuscript (20%) |
| Quan V. Vuong | Wrote the paper (20%)<br>Critically review the manuscript (60%) |

2. **Thanh Van Ngo**, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2017). Phytochemical and antioxidant properties from different parts of *Salacia chinensis* L. *Journal of Biologically Active Products from Nature*, 7(5), 401-410. http://dx.doi.org/10.1080/22311866.2017.1383186

| Contributor | Statement of contribution |
|---|---|
| Thanh Van Ngo | Designed experiments (60%)<br>Performed experiments (100%)<br>Analysed data (100%)<br>Wrote the manuscript (80%) |
| Christopher J. Scarlett | Designed experiments (5%)<br>Critically reviewed the manuscript (20%) |
| Michael Christian Bowyer | Designed experiments (5%)<br>Critically reviewed the manuscript (20%) |
| Quan V. Vuong | Designed experiment (30%)<br>Wrote the manuscript (20%)<br>Critically review the manuscript (60%) |

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| Contributor | Statement of contribution |
|---|---|
| Thanh Van Ngo | Collected plant materials (50%)<br>Designed experiments (60%)<br>Performed experiments (100%)<br>Analysed data (100%)<br>Wrote the manuscript (80%) |
| Christopher J. Scarlett | Designed experiment (5%)<br>Critically reviewed the manuscript (20%) |
| Michael C. Bowyer | Designed experiment (5%)<br>Critically reviewed the manuscript (20%) |
| Phuong Duc Ngo | Collected plant materials (50%) |
| Quan V. Vuong | Designed experiment (30%)<br>Wrote the manuscript (20%)<br>Critically review the manuscript (60%) |

4. **Thanh Van Ngo**, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2019). Ultrasonic assisted extraction as an advanced technique for the extraction of bioactive compounds from *Salacia chinensis* root: A comparison with decoction and continuously shaking extraction (submitted to *Journal of Plant Biochemistry and Biotechnology*).

| Contributor | Statement of contribution |
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| Thanh Van Ngo | Designed experiments (70%)<br>Performed experiments (100%)<br>Analysed data (100%)<br>Wrote the manuscript (80%) |
| Christopher J. Scarlett | Design experiments (5%)<br>Critically reviewed the manuscript (20%) |
| Michael Christian Bowyer | Design experiments (5%)<br>Critically reviewed the manuscript (20%) |
| Quan V. Vuong | Designed experiment (20%)<br>Wrote the manuscript (20%)<br>Critically review the manuscript (60%) |

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| Michael Christian Bowyer | Design experiments (5%)<br>Critically reviewed the manuscript (20%) |
| Quan V. Vuong | Designed experiment (20%)<br>Wrote the manuscript (20%)<br>Critically review the manuscript (60%) |

6. **Thanh Van Ngo**, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2019). Optimising conditions for encapsulation of *Salacia chinensis* root extract enriched with phenolic compounds (submitted to *Journal of Microencapsulation*)

| Contributor | Statement of contribution |
|---|---|
| Thanh Van Ngo | Designed experiments (70%)<br>Performed experiments (100%)<br>Analysed data (100%)<br>Wrote the manuscript (80%) |
| Christopher J. Scarlett | Design experiments (5%)<br>Critically reviewed the manuscript (10%) |
| Michael Christian Bowyer | Design experiments (5%)<br>Critically reviewed the manuscript (10%) |
| Rebecca McCullum | Critically reviewed the manuscript (30%) |
| Quan Van Vuong | Designed experiment (20%)<br>Wrote the manuscript (20%)<br>Critically review the manuscript (50%) |

## Conference oral presentations/proceedings

1. Thanh Van Ngo (2017). Phytochemical and antioxidant properties from different parts of *Salacia chinensis* L. *Proceedings of the 5th International Conference and Exhibition on Pharmacognosy, Phytochemistry & Natural Products*, 24-25 July, 2017, Melbourne, Australia.

# Table of Contents

**Abstract**

*Salacia chinensis* L., a common species in genus *Salacia*, is distributed in many Asian countries, such as China, Vietnam, India and Sri Lanka. For thousands of years, *S. chinensis* has been used for the prevention and treatment of various illnesses including arthritis, inflammation, diabetes, obesity and liver disorders. Studies have shown that *S. chinensis* has relatively high content of bioactive compounds, such as total phenolics and total flavonoids as well as strong antioxidant properties. Numerous individual compounds, isolated and identified from *S. chinensis* such as mangiferin, catechins, salacinol and kotalanol, have been linked with therapeutic potential against various diseases, such as diabetes and certain common types of cancers. However, the conditions for maximum recovery of bioactive compounds from this plant material have not been thoroughly assessed. In addition, encapsulation to stabilise the extract and make it more available and convenient for further applications has not been conducted previously. Therefore, overall aim of this study was to optimise the conditions for maximum extraction of bioactive compounds from *S. chinensis* and further encapsulation of its enriched extract to improve the stability. To achieve this overall aim, four specific objectives were addressed as follows: (1) to compare bioactive compounds in different parts of *S. chinensis* to determine the most suitable part for further extraction of bioactive compounds; (2) to study the impact of different solvents on extraction efficiency of bioactive compounds from *S. chinensis* to identify the most suitable solvent for further extraction; (3) to optimise conditions for maximum extraction of total bioactive compounds and mangiferin, the major bioactive compound from *S. chinensis*; and (4) to investigate the optimal encapsulation conditions for improving stability of *S. chinensis* enriched extract.

The results are presented in six research articles through four chapters (chapter 3 to chapter 6). Firstly, we found that the root of *S. chinensis* had higher levels of phenolics, flavonoids,

proanthocyanidins and saponins as well as antioxidant capacity as compared to those of its stem and leaf (Chapter 3). Therefore, the root of *S. chinensis* was used in subsequent experiments for further extraction and isolation. The impact of different solvents on extraction efficiency of bioactive compounds from *S. chinensis* root was tested and the results (Chapter 4) revealed that solvents significantly affected extraction efficiency of bioactive compounds from *S. chinensis* root. Absolute organic solvents and water were found to be ineffective, but 50% ethanol and 50% acetone were effective for extraction of bioactive compounds and antioxidant capacity from *S. chinensis* root. Mixtures of ethanol or acetone with water were then applied for opimisation of ultrasound assisted extraction (UAE) conditions. Extraction efficiency of UAE was compared with continuous shaking extraction and decoction, which is known as the traditional method for preparation of extracts from *S. chinensis*. The optimal UAE conditions for maximum extraction of phenolic compounds, flavonoids, proanthocyanidins, and saponins with high antioxidant activities were an ethanol concentration of 50%, extraction time of 60 min, temperature of 50 ºC and ultrasonic power of 250 W; whereas, optimal UAE conditions for maximum extraction of mangiferin were 40% acetone, temperature of 50 ºC, 60 min extraction time, and 250W. In comparison with the two most common conventional extraction methods: decoction and continuously shaking extraction, UAE had comparable extraction yields of total bioactive compounds to continuously shaking extraction, but had significantly higher extraction yields than the decoction method. In addition, UAE could extract 3% and 57% more mangiferin in comparison with continuously shaking extraction and decoction, respectively (Chapter 5). UAE was then employed to prepare the enriched extract for further encapsulation. Encapsulating conditions such as maltodextrin to extract ratio, inlet spraying temperature, and feed rate were found to significantly affect recovery yields of powdered extract and its quality. The optimal conditions for encapsulation

were maltodextrin to extract ratio of 20/100 (g/mL), inlet temperature of 130ºC and feed rate of 30% (9 mL/min). In comparison with freeze drying, encapsulation using spray drying produced the powder with comparable contents of total phenolics and mangiferin as well as antioxidant properties (Chapter 6). As it is more cost effective than freeze drying, these conditions are recommended for preparation of *S. chinensis* extract enriched with bioactive compounds for further applications.

## List of figures

## List of Abbreviations

| | |
|---|---|
| ABTS | 22′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid |
| ANOVA | Analysis of variance |
| CE | Catechin equivalent |
| CUPRAC | Cupric ion reducing antioxidant capacity |
| CSE | Continuously shaking extraction |
| DPPH | 11-diphenyl-2-picrylhydrazyl |
| FRAP | Ferric reducing antioxidant power |
| GAE | Gallic acid equivalent |
| HPLC | High performance/pressure liquid chromatography |
| MS | Mass spectrometry |
| RSM | Response surface methodology |
| TE | Trolox equivalents |
| TFC | Total flavonoid content |
| TPC | Total phenolic content |
| TPrC | Total proanthocyanidin content |
| TPTZ | Tripyridyltriazine |
| TSC | Total saponin content |
| UAE | Ultrasound assisted extraction |

# *CHAPTER 1.*

# INTRODUCTION AND LITERATURE REVIEW

## 1.1. Introduction

The genus *Salacia* belongs to the family of *Celastraceae* and comprises between 100 and 200 individual plant species, which are typically characterised as woody shrubs with dichotomous branching or small trees (Paarakh, Patil, & Thanga, 2008; Ramakrishna, Shashank, G.K., Kiran, & Ravishankar, 2016). *Salacia* are shade–loving plants, growing vigorously in the lower forest canopy by either climbing on other trees or as a stand-alone plant up to 2 meters in height (Ramakrishna et al., 2016). *Salacia* leaves are simple, usually opposite, petioled, coriaceous structures being glabrous underneath and shiny on the upper face. Leaf shapes vary from ovate-oblong, acuminate, elliptic-oblong, base acute to apex abruptly acuminate. *Salacia* flowers are small and bisexual in nature, occurring as clusters of 2 to 8 units per leaf axil. The flower colours are typically greenish white to greenish yellow, with entire calyx lobes and anthers dehiscing transversely. *Salacia* fruits occur as pinkish- orange globes when ripe. Each fruit contains 1 to 4 seeds, which have an almond-like shape. The colour of root bark is golden or yellow depending on species (Paarakh et al., 2008; Ramakrishna et al., 2016) (Figure 1).


**Figure 1.** *Salacia chinensis*: stem and leaf (A), root (B) and root cross-cut (C) (TVN, 2015)

*S. chinensis* is a common species in genus *Salacia*. The species is distributed in many Asian countries, such as China, Vietnam, Malaysia, Indonesia, India and Sri Lanka. For thousands of years, *S. chinensis* has been used for the treatment of various illnesses such as arthritis, leucorrhoea, inflammation, fever, skin diseases, menstrual disorders and spermatorrhoea. It has been also linked with prevention of diabetes, obesity and liver disorders (Jaykumar J. Chavan, Ghadage, Bhoite, & Umdale, 2015; Ramakrishna et al., 2016). For example, in Vietnam decoction of *S. chinensis* root has been traditionally used for treatment of rheumatism, back-pain and debility. The decoction is prepared by washing the root, followed by slicing into small pieces which are then air-dried. Decoction is then performed using the fresh dried preparation as required and can be used independently or in combination with other herbs to strengthen the efficacy (Vo, 1997). In Laos, decoction of the stem of *S. chinensis* is used for treatment of back pain (Delang, 2007). *S. chinensis* stems are also utilised in Thailand for anti-diabetic and laxative treatment (Muraoka, 2011; Ramakrishna, Shashank, Shinomol, Kiran, & Ravishankar, 2015). In both India and Sri Lanka, *Salacia* is powdered and packed in a filter bag for use as herbal tea for daily consumption and it is believed to improve the health of diabetics (Jayawardena, de Alwis, Hettigoda, & Fernando, 2005). More recently, in Japan, Korea, United States and India, powdered extracts from *S. chinensis*, *S. reticulata*, and *S. oblonga* have been marketed as food supplements to prevent and manage obesity and diabetes (Li, Huang, & Yamahara, 2008; Singh & Duggal, 2010).

The popularity of *S. chinensis* in traditional medicine has led to significant research being undertaken to isolate and identify key bioactive constituents from different parts of this plant material. Root, root bark, stem, leaf and fruit pulp of *S. chinensis* have been used for the extraction of numerous phytochemicals in different categories, such as sesquiterpenoids, triterpenoids, xanthonoids, flavan-3-ols, and thiosugar sulfonium sulfate compounds (J. J. Chavan, Ghadage,

Kshirsagar, & Kudale, 2015; Kishi, Morikawa, Matsuda, & Yoshikawa, 2003; Morikawa, Kishi, Pongpiriyadacha, Matsuda, & Yoshikawa, 2003; Muraoka, 2011; Muraoka et al., 2010; Muraoka et al., 2008; Sellamuthu, Arulselvan, Muniappan, & Kandasamy, 2012; Tewari, Narayan Ayengar, & Rangaswami, 1974; Tran, Nguyen, Vu, & Tran, 2009, 2010; Yoshikawa et al., 2008). However, a procedure for maximising the extraction of total bioactive compounds with high antioxidant activities has not been reported.

Mangiferin, a "super antioxidant", anti-viral, anti-cancer, anti-diabetic, anti-aging, immunomodulatory, hepatoprotective and analgesic compound, has been reported as a major bioactive component of *S. chinensis* (Imran et al., 2017). To date, over 450 articles have been published on its occurrence, chemical nature, synthesis and medicinal properties over the last 50 years (Asif et al., 2016; Saha, Sadhukhan, & Sil, 2016). Nevertheless, the conditions for extraction and isolation of this phytochemical have not been optimised.

Generally, phytochemicals are susceptible to adverse environmental factors, including physical, chemical and biological conditions such as temperature, humidity and oxidation (Munin & Edwards-Levy, 2011; Papoutsis et al., 2018). Therefore, for commercial uses, they must be encapsulated to minimise their degradation. The conditions for encapsulation should also be optimised to improve the stability of the bioactive compounds for further utilisation and applications.

## 1.2. Literature review

The *Salacia*: Phytochemicals and Health Benefits (Review paper, Under Review)

## 1.3. Research hypotheses and aims

### *1.3.1. Hypotheses*

This study hypothesizes that *S. chinensis* contains bioactive compounds which can be extracted effectively by suitable solvents and proper extraction techniques. Encapsulation conditions can be optimised to improve the stability of the bioactive compounds extracted from *S. chinensis*.

### *1.3.2. Overall aim*

This study aimed to optimise the conditions for extraction of bioactive compounds from *S. chinensis* as well as encapsulation of its enriched extract for further applications.

### *1.3.3. Specific aims*

Aim 1: To compare bioactive compounds in different parts of *S. chinensis* to determine the most suitable part for further extraction of bioactive compounds.

Aim 2: To study the impact of different solvents on extraction efficiency of bioactive compounds from *S. chinensis* to identify the most suitable solvent for further extraction.

Aim 3: To optimise conditions for maximum extraction of total bioactive compounds and mangiferin, the major bioactive compound, from *S. chinensis.*

Aim 4: To investigate the optimal encapsulation conditions for improving stability of *S. chinensis* enriched extract.

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Manuscript Draft

Manuscript Number: HERMED-D-19-00750R1

Title: The Salacia: Phytochemicals and Health Benefits

Article Type: Review article

Keywords: Anti-cancer; Anti-diabetes; Health benefit; Phenolic; Salacia; Triterpenoid.

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Order of Authors: Thanh Van Ngo, MSc; Christopher J Scarlett, PhD; Michael C Bowyer, PhD; Quan V Vuong, PhD

Abstract: Background: The genus Salacia (Celastraceae family), with over 100 species, grows in a number of climatic regions of the World and has been used as a traditional medicine for the treatment of a range of ailments including arthritis, leucorrhoea, inflammation, diabetes, obesity and liver disorders. Due to its popularity in ethnopharmacy, significant research time has been devoted to the identification and mode of action of bioactive constituents of Salacia species.
Methods: Electronic databases including NCBI, Scopus, Science Direct, PubMed, Scifinder and Google Scholar were searched using the related specific keywords to make sure that most related information was collected. The data were then screened for writing different sections based on quality and eligibility of the publications to assure the quality of the data.
Results: From over 200 publications related to Salacia, this review identified 68 publications, which provided direct information for this review. Salacia root, stem and leaf have been used as herbal medicine. To date, 168 individual bioactive compounds have been successfully catalogued, including 123 terpenes and terpenoids, 18 phenolics and 27 compounds belonging to other chemical families. The activity of these compounds has been linked to action against a range of human ailments including several types of cancer, diabetes, obesity and malaria.
Conclusions: Published evidence has shown that Salacia is a abundant source of terpenes and terpenoids as well as phenolics, which can be potential therapeutic agents. Further studies are needed to isolate phytochemicals from Salacia and test their individual or synergistic effects on various health benefits.

**Cover Letter with handwritten signature**

**FACULTY OF SCIENCE AND INFORMATION TECHNOLOGY**

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Thursday, 11th April 2019

Prof B. Pendry,

*Editor-in-Chief of Journal of Herbal Medicine*

Dear Prof B. Pendry,

The genus *Salacia* (*Celastraceae* family), with over 100 species, grows in a number of climatic regions of the World and has been used widely as a traditional medicine for the treatment of a range of ailments including arthritis, leucorrhoea, inflammation, fever, skin diseases, menstrual disorder and spermatorrhoea. It has been also linked with prevention of diabetes, obesity and liver disorders. Due to its popularity in ethnopharmacy, significant research time has been devoted to the identification and mode of action of bioactive constituents of *Salacia* species.

*Journal of Herbal Medicine* is a peer-reviewed journal which aims to serve its readers as an authoritative resource on the profession and practice of herbal medicine. We therefore wish to submit a review article entitled **"The *Salacia*: Phytochemicals and Health Benefits"** for publication in your journal. A brief of the content of our review as follows:

**Introduction**

The first part of this review describes biological characteristics of genus *Salacia* which distributes widely in Asian countries, such as China, Vietnam, India. This section also gives brief information about phytochemical constituents and medicinal properties of *Salacia*.

**Ethnopharmacology**

This section describes medicinal properties of different species of *Salacia* in different cultures in the world. The ways of preparation and use are also included in this section.

**Bioactive compounds as potential therapeutic agents**

This section is a main part of the review and catalogues bioactive constituents from different species of *Salacia* up-to-date into three groups: terpenes and terpenoids, phenolics and other compounds. In this section,

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gaps between researches related to extraction of bioactive components from *Salacia* materials are also suggested for future studies.

**Potential health benefits**

This is another main part of this review. This section discusses the link between *Salacia* extracts and their bioactive compounds with various health benefits which include anti-cancer, anti-diabetes, anti-obesity and anti-malaria properties. This section also suggests gaps between studies related to medicinal properties of *Salacia* extracts and their bioactive compounds for future studies.

**Trend for future studies**

This section focuses on potential future trends and opportunities for future research.

**Conclusions**

This last section concludes the important points from above sections.

We hope that our article entitled **"The *Salacia*: Phytochemicals and health benefits"** will be of suitable interest to be considered for publication as a review in your journal. Any comments on the content of our review will be appreciated.

With Best Regards,

On behalf of the article's other authors Prof. Michael Bowyer, Prof. Christopher Scarlett and Dr. Quan Vuong.

Thanh V. Ngo,

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# The *Salacia*: Phytochemicals and health benefits

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*Detailed Response to Editors

26 August 2019

Dr. Barbara Pendry

Chief Editor

*Journal of Herbal Medicine*

Manuscript ID: HERMED-D-19-00750

Dear Dr. Barbara Pendry,

On behalf of my co-authors, I would like to resubmit our revised manuscript entitled "***The Salacia: Phytochemicals and Health Benefits***", which requires revision for consideration to be published on *Journal of Herbal Medicine*. We have made a revision based on editors' comments. We thank the editors for their supportive comments and we have addressed each query of the editors as following:

**Editors comments:**

*A review is generally intended to consider the strengths and weaknesses of published research and as such is required to have a detailed robust methodology to indicate how your data has been accessed and selected e.g. inclusion/exclusion criteria for data filtering. a robust methodology eliminates author bias. PRISMA guidelines are useful for constructing review papers. Thus on this basis we would request that you consider the editors comments and revise your manuscript accordingly to continue the review process.*

**Response from authors:**

We have added a new section describing how the data were collected and analysed for this review article. We also significantly revised the abstract, which includes the methods of collecting and analysing the data for this review.

The new methodology section now reads: "The data for the review were collected from peer-reviewed research papers and review articles published by the reputed publishers, such as Springer, Elsevier, Taylor & Francis, Wiley. Online databases, including NCBI, Scopus, Science Direct, PubMed, Scifinder and Google Scholar were searched using the following keywords: *Salacia; Salacia chinensis; Salacia cochinchinensis; Salacia oblonga*; traditional medicine; diabetes; obesity; bioactive compounds; phytochemicals; terpene; terpenoids; triterpenoids; pristimerin; phenolic; mangiferin; salacinol; kotalanol; health benefit; anti-cancer; α-glucosidase inhibitory; aldose reductase inhibitory; pancreatic lipase inhibitory. From over 200 relevant publications, data were screened for this review based on the relevance, quality and eligibility of the research."

We thank editors for their supportive comments and are confident that we have now addressed the above points concerning the manuscript. We are pleased that the editorial board continues to share our enthusiasm for these data and accept to publish our work in the *Journal of Herbal Medicine*.

With best regards,

Thanh Van Ngo

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# The *Salacia*: Phytochemicals and Health Benefits


## Abstract

*Background*: The genus *Salacia* (*Celastraceae* family), with over 100 species, grows in a number of climatic regions of the World and has been used as a traditional medicine for the treatment of a range of ailments including arthritis, leucorrhoea, inflammation, diabetes, obesity and liver disorders. Due to its popularity in ethnopharmacy, significant research time has been devoted to the identification and mode of action of bioactive constituents of *Salacia* species.

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*Conclusions*: Published evidence has shown that *Salacia* is a abundant source of terpenes and terpenoids as well as phenolics, which can be potential therapeutic agents. Further studies are needed to isolate phytochemicals from *Salacia* and test their individual or synergistic effects on various health benefits.


**Keywords:** Anti-cancer; Anti-diabetes; Phenolic; *Salacia*; Triterpenoid.

## 1. Introduction

The genus *Salacia* belongs to the family of *Celastraceae* and comprises between 100 and 200 individual plant species typically characterised as woody shrubs with dichotomous branching or small trees [1, 2]. *Salacia* are typically shade–loving plants, growing vigorously in the lower forest canopy by either climbing on other trees or as a stand-alone plant up to 2 metres in height [2]. *Salacia* leaves are simple, usually opposite, petioled, coriaceous structures being glabrous underneath and shiny on the upper face. Leaf shapes vary from ovate-oblong, acuminate, elliptic-oblong, base acute to apex abruptly acuminate. *Salacia* flowers are small and bisexual in nature, occurring as clusters of 2 to 8 units per leaf axil. The flower colours are typically greenish white to greenish yellow, with entire calyx lobes and anthers dehiscing transversely. *Salacia* fruits occur as pinkish- orange globes when ripe. Each fruit contains 1 to 4 seeds, which have an almond-like shape. The colour of root bark is golden or yellow depending on species [1, 2] (Figure 1).

***Please insert Figure 1 here***

*Salacia* occurs in different parts of the world. For example, *S. chinensis* (synonym *S. prinoides*), *S. cochinchinensis*, *S. oblonga* and *S. reticulata* are native to China, Vietnam, Malaysia, Indonesia, India, Sri Lanka and other Asian countries [3-5]. By contrast, *S. macrosperma* and *S. wayanadica* have been found in India [6], while *S. petenensis, S. cordata, S. crassifolia, S. elliptica, S. grandifolia, S. impressifolia, S. alwynii* and *S. arbore* occur in Central and South America, and *S. lehmbachii* and *S. madagascariens* in Africa [2].

*Salacia* has been used as herbal medicine for the treatment of various ailments including arthritis, leucorrhoea, inflammation, fever, skin diseases, menstrual disorders, spermatorrhoea, diabetes and obesity [2, 7], revealing that it might contain functional bioactive compounds, which act as therapeutic agents. Numerous studies have attempted to identify bioactive compounds in *Salacia*. Over 120 terpenes and terpenoids, 18 phenolic

compounds and 27 other bioactive compounds have been identified in *Salacia* species. Extracts and several bioactive compounds derived from *Salacia* have been tested *in vitro* and/or *in vivo* to assess their potential health benefits for ailments such as anti-cancer, cardiovascular diseases, and diabetes. This review comprehensively outlines the traditional use of *Salacia* as herbal medicine, describes bioactive compounds which have been identified in *Salacia*, discusses the link between *Salacia* extracts and their bioactive compounds with various health benefits, and finally proposes a trend for future studies.

## 2. Methodology

The data for the review were collected from peer-reviewed research papers and review articles published by the reputed publishers, such as Springer, Elsevier, Taylor & Francis, Wiley. Online databases, including NCBI, Scopus, Science Direct, PubMed, Scifinder and Google Scholar were searched using the following keywords: *Salacia; Salacia chinensis; Salacia cochinchinensis; Salacia oblonga*; traditional medicine; diabetes; obesity; bioactive compounds; phytochemicals; terpene; terpenoids; triterpenoids; pristimerin; phenolic; mangiferin; salacinol; kotalanol; health benefit; anti-cancer; α-glucosidase inhibitory; aldose reductase inhibitory; pancreatic lipase inhibitory. From over 200 relevant publications, data were screened for this review based on the relevance, quality and eligibility of the research.

## 3. Ethnopharmacology

*Salacia* has been traditionally used for the treatment of various illnesses for thousands of years (Table 1). *S. chinensis* has been used for treatment of arthritis, leucorrhoea, inflammation, fever, skin diseases, menstrual disorders, and spermatorrhoea. It has been also linked with prevention of diabetes, obesity and liver disorders [2, 7]. Traditionally, people use simple techniques including infusion and decoction to prepare solutions for oral administration to treat these ailments. Infusion refers to preparation of a solution by adding soft plant materials (e.g. leaves and flowers) to cold or hot water to form a tea or broth. In a

decoction, the solution is prepared by boiling harder plant materials (e.g. roots and stems) in water for a certain time period to extract bioactives.

The roots and stems of *Salacia* have been found to be more effective in disease treatment, thus decoction is generally the preferred method for extract preparation. However, the plant parts and species utilised for treatment preparations varies significantly between cultures. In Vietnam, use of the root of *S. chinensis* dominates as the preferred treatment for rheumatism, back-pain and debility. The decoction is prepared by washing the root, followed by slicing into small pieces which are then air-dried. Decoction is then performed using the fresh dried preparation as required and can be used independently or in combination with other herbs to strengthen the efficacy [8]. By contrast, in Laos, decoction of the stem of the same *Salacia* species (*S. chinensis*) is preferred for the treatment of back pain [9]. *S. chinensis* stems are also utilised in Thailand as an anti-diabetic and laxative treatment [10, 11].

In Sri Lanka, *S. reticulata* root is used to treat skin ailments including itching and swelling and a range of other ailments including asthma, thirst, amenorrhea and dysmenorrhea [4]. In Ayurvedic medicinal system, stems and roots of *S. reticulata* and *S. oblonga* are used for treatment of rheumatism, gonorrhea, skin diseases and diabetes [11]. In India, the leaf of *S. macrosperma* is similarly used to treat eczema. A range of other *Salacia* species including the root of *S. reticulata, S. macrosperma, S. grandiflora* (synonym *S. longifolia*) *S. macrophylla* (synonym *S. flavescens*) and *S. ovalis* are also prescribed to Indian and Sri Lankan people by traditional practitioners as a glycemic control [6, 12]. Interestingly, it is believed that drinking water by mugs made from *Salacia* wood is a good way to treat diabetes mellitus [12, 13]. However, studies conducted on female rats found daily oral administration of *Salacia reticulata* Wight extracts resulted in an increased incidence of post-implantation losses and low birth weight. Therefore, this herbal medicine is not recommended for women during pregnancy [12].

Powdered *Salacia* packed in a filter bag is used as herbal tea for daily consumption in both India and Sri Lanka. Known traditionally as Kothala Himbutu, consumption is believed to improve the health of diabetics [14]. More recently, in Japan, Korea, United States and India, powdered extracts from *S. chinensis*, *S. reticulata*, and *S. oblonga* have been marketed as food supplements to prevent and manage obesity and diabetes [15, 16]. No scientific evidence supporting these claims has yet been published however.

***Please insert Table 1 here***

## 4. Bioactive compounds as potential therapeutic agents

The popularity of *Salacia* in traditional medicine has led to significant research being undertaken to identify key bioactive constituents. To date some 168 individual bioactive compounds have been identified across various *Salacia* species (see Appendix 1, 2 and 3 and Figure 2). These compounds are typically classified into three groups: terpenes and terpenoids, phenolics and other compounds.

***Please insert Figure 2 here***

### *4.1. Terpenes and terpenoids*

Since the first terpenoids were successfully isolated from *S. prinoides* syn *chinensis* in 1950s [17], more than 120 terpenes and terpenoids have been characterised from different species of *Salacia* (Appendix 1). Of these, triterpenoids are the most common terpene sub-group, comprising approximately 86% (106 out of 123) of the known *Salacia* terpenes and terpenoids. Other known *Salacia* terpene structures include 5 monoterpenes, 3 monoterpenoids, 2 sesquiterpenes, 4 sesquiterpenoids and 3 diterpenoids and 3 compounds of unknown structure.

Depending on carbon skeleton framework, triterpenoids are divided into various subgroups, including acyclic, monocyclic, bicyclic, tricyclic, tetracyclic, pentacyclic and miscellaneous

structures [18]. All of the 106 triterpenoids isolated from *Salacia* species are pentacyclic structures, including 47 friedelanes, 11 oleananes, 4 ursanes, 20 lupanes, 2 hopanes, 3 tirucalls, 16 quinonemethides and 3 compounds of unknown structure. Pentacyclic triterpenoids have received significant attention from researchers due to their remarkable pharmacological activities, which include anti-cancer, anti-inflammatory, antioxidant, anti-viral, anti-microbial, anti-diabetic and hepato- and cardio- protective effects [18, 19]. It is promising research data that signifies the potential use of *Salacia* bioactives as the basis for the development of new pharmaceutical drug leads.

Pristimerin, a quinonemethide triterpenoid (Figure 2), has been reported to inhibit tumour cell proliferation and induce apoptosis in both breast and prostate cancer cells lines *in vitro* [20]. In genus *Salacia*, currently two species (*S. cochinchinensis* and *S. impressifolia*) have been formally identified as containing pristimerin [21, 22]; being isolated from the root of *S. cochinchinensis* and trunk and twings of *S. impressifolia* and tested for its cytotoxic effect on human breast cancer cell line. Purified pristimerin was found to inhibit the cancer cell proliferation in both a dose- and time- dependent manner and was found to downregulate the mTOR/p70S6K/4EBP1 pathway (related to the invasion and angiogenesis of the tumour), inhibit metastasis and promote caspase-dependent apoptosis in breast cancer cells [21].

The pharmacological properties of terpenes and terpenoids derived from only 12 of the 100-200 *Salacia* species have to date been investigated in any detail (Appendix 1), with new terpene and terpenoid derivatives undoubtedly waiting to be identified as the phytochemical knowledge base of *Salacia* expands into the future. In addition, most of studies conducted on *Salacia* occur under the fixed conditions, which could only extract selected compounds from *Salacia* for identification. Therefore, many compounds have not been identified from *Salacia.* Many extraction factors, such as temperature, length of extraction, extraction time (one time or more), sample-to-solvent ratio, pH of solvent, particle size of sample, and extraction

methods (conventional or advanced techniques) can significantly influence extraction efficiency of phytochemicals [23]. Consequently, there is a need for further studies to establish the optimal extraction conditions for the isolation of biologically important terpenes and terpenoids from *Salacia*.

### *4.2. Phenolic compounds*

Phenolic compounds are well-known for their ability to protect plants from external impact including pathogen infection, insect attack and damage by UV radiation [24, 25]. Studies have also demonstrated that phenolic compounds have strong antioxidant properties, hence these natural phytochemicals have been linked with the prevention and treatment of ailments such as diabetes, obesity, cardiovascular disease and cancer [24].

To date, 18 individual phenolic compounds have been identified from *Salacia* species; (predominantly from *S. chinensis* and *S. reticulata*). These phenolics are divided into various subgroups, such as xanthonoid (mangiferin), flavan-3-ols (catechins), flavones and lignans (Appendix 2).

The most important phenolic compound to be identified from *Salacia* to date is mangiferin. Mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-β-D glucoside – Figure 2) is known as a "super antioxidant" due to its strong antioxidant character [26]. Different cultural groups throughout India, China, and Southeast Asia have traditionally utilised mangiferin-rich plants for the prevention and treatment of various illnesses including cardiovascular diseases, diabetes, infection and cancer [27].

In Ayurvedic medicine, *S. chinensis* has been shown to contain high levels of mangiferin and have been associated with hypo-lipidaemic, anti-diabetic, hepato-protective and antioxidant properties [28]. Mangiferin action in relation to diabetes has been linked to the alleviation of

symptoms associated with retinal and neural damage via aldose reductase inhibitory activities [29, 30]. Mangiferin has been reported to possess anti-inflammatory, anti-proliferative, anti-apoptotic, anti-oxidative, anti-genotoxic, anti-viral and anti-cancer properties [28]. Mangiferin has also been identified in the root bark of *S. reticulata* and the root of *S. chinensis* [31-34].

Other phenolic bioactives isolated from *Salacia* include catechins, a well-known antioxidant family present in leaf tea (*Camellia sinensis*) [23]. A series of studies conducted by Japanese researchers successfully isolated 9 catechins from *S. chinensis* (stem), *S. reticulata* (root and leaf) and *S. oblonga* (root) (Appendix 2). Catechins have been linked to a range of health benefits, such as prevention of cancers and cardiovascular diseases, as well as improvement of immune response [23].

To date, the phenolic profile of only four *Salacia* species - *S. chinensis*, *S. reticulata, S. oblonga* and *S. amplifolia* has been established, offering further opportunities in natural products research for structural identification of new compounds and the assessment of their biological properties. Even within the species already studied, opportunity exists to optimise extraction methods in terms of solvent formulation and method of extraction (conventional extraction methods verses new technologies such as ultrasonic- and microwave-assisted extraction) [32, 34-37]. Previous studies have indicated that choice of solvent, extraction method and extraction conditions significantly impact the extraction efficiency of phenolic compounds [38, 39]. Further study is therefore required to establish the most suitable solvent and extraction conditions for isolation and identification of phenolic compounds from *Salacia* species.

### *4.3. Other compounds*

To date, 27 compounds which belong to other chemical families have also been identified in *Salacia* species (Appendix 3). Thiosugar sulfonium sulfate compounds, including salacinol, kotalanol, neosalacinol, neokotalanol, salaprinol and ponkoranol (Figure 2) which possess α-glucosidase inhibitory properties, have been identified in *S. chinensis, S. reticulata* and *S.oblonga.* α-Glucosidase inhibitors slow down the process of digestion and absorption of carbohydrates by competitively through inhibition of glucosidase activity, the enzyme present in the intestinal chorionic epithelium of mammals that is responsible for carbohydrate degradation. Some of these compounds, such as acarbose and voglibose, have been used clinically for treatment of diabetes mellitus [40]. Olibanumol J, isolated from the root of *S. hainanensis* by Huang, Guo, Cheng, Sun and Gao [41], and de-O-sulfated kotalanol isolated from *S. reticulata* stem by Ozaki, Oe and Kitamura [42] were also found to inhibit α-glucosidase. Of note, only 7 species have been screened for these compounds. Further studies on other species are needed to identify bioactive compounds in *Salacia* and to test their biological activities.

## 5. Potential health benefits

To date, 165 bioactive compounds have been isolated and characterised from *Salacia* species, including 123 terpenes and terpenoids and 16 phenolics. Of these, 61 compounds, including 43 terpenes and 10 phenolics have been reported as possessing biological activity (Table 2).

***Please insert Table 2 here***

### *5.1. Anti-cancer*

Thirteen compounds to date, isolated from various *Salacia* species have been found to possess activity *in vitro* against a range of cancer cell types including colon, liver, gastric, lung, breast, mouth, prostate, pancreastic cancers [5, 43-45]. Structurally, all are triterpenoids and include 6 friedelanes, 1 lupane, 1 hopane and 5 quinonemethides. Individual compound activities vary from moderate to strong. According to Somwong, Suttisri and Buakeaw [44],

26-hydroxyfriedelane-1,3-dione and 21α-hydroxy-D:A-friedo-olean-3-one were found to be moderately cytotoxic to colon, liver and gastric cancers while friedelane-1,3-dione was strongly active against colon cancer cell lines, displaying high level specificity.

Three compounds isolated from *S. chinensis* stem - 7α,21α-dihydroxyfriedelane-3-one, 28-hydroxy-3-oxo-30-lupanoic acid and 3,4-seco-friedelane-3-oic acid, exhibited broad specturm anti-cancer activity against liver, lung, mouth and breast cancers. By contrast, friedelane-1,3-dione, isolated from *S. verrucosa* stem and 29-nor-21α-H-hopane-3,22-dione, isolated from *S. chinensis* stem, were found to be specifically cytotoxic against colon and lung cancer cell lines, respectively.

### *5.2. Anti-diabetes*

α-glucosidase as discussed previously, is secreted from intestinal chorionic epithelium during digestion and is responsible for carbohydrate degradation in mammals. In blocking α-glucosidase activity, α-glucosidase inhibitors, slow down the rate of breakdown and absorption of carbohydrates, leading to reduce blood sugar levels [40].

*Salacia* is traditionally recognised as an anti-diabetes treatment in ethnopharmacy, resulting in numerous scientific studies being undertaken to validate these claims. To date, some 35 phytochemicals reported as possessing α-glucosidase inhibitory activities have to date been isolated from *Salacia* species. These include 23 terpenoids and 4 phenolics, with many showing strong activity.

Extracts from different parts of *Salacia* plants exhibit different levels of α-glucosidase inhibitory activity. Five compounds from *S. hainanensis* root were found to inhibit α-glucosidase activity, with activities 5 - 17 times higher than that of Acarbose – the positive experimental control [41]. Mangiferin activity was found to be comparable with Glibenclamide, a commercial anti-diabetic drug, in regulating blood glucose, glycosylated

hemoglobin, insulin and hemoglobin levels and the activity of selected liver enzymes in diabetic rats [34].

The highest α-glucosidase inhibitory activity is seen in *S. chinensis* stem and *S. reticulata* root, followed by *S. oblonga* root and *S. chinensis* root. *S. chinensis* leaf and fruit samples did not exhibit α-glucosidase inhibition activity [46]. Growing location and time of harvest were also found to affect α-glucosidase inhibitory activity in *Salacia* samples harvested from different locations in Thailand [10].

*Salacia* derived compounds have also been found to possess aldose reductase inhibitory activity, which helps to prevent the damage to the eye and nervous system in patients with diabetes [47]. Eleven compounds, including 2 diterpenoids, 8 triterpenoids (3 oleananes, 2 friedelanes, 2 quinonemethides and 1 norfriedelane) and 1 phenolic compound (mangiferin) displayed the activity, with mangiferin being the most active [32]. These compounds are potentially genesis compounds for the development of new therapeutic agents to combat the chronic effects of diabetes.

### *5.3. Other diseases*

Compounds derived from *Salacia* have also been linked to activity against a range of other diseases including obesity and malaria, with the level of observed biological activity being linked to different parts of the plant. Leaf extracts of *Salacia reticulata* have displayed potent pancreatic lipase inhibitory activity in comparison with stem extracts with epigallocatechin thought to be the source compound responsible for the reported activity [36]. Pancreatic lipase, the lipolytic enzyme synthesized and secreted by the pancreas, plays a key role in the efficient digestion of triglycerides, being responsible for the hydrolysis of 50 – 70% of total dietary fats. Inhibition of this enzyme therefore represents an opportunity to control caloric uptake in the human body [48].

*Salacia* derived compounds isolated from *S. kraussi* root have been found to be active against the malaria pathogen, with 17-(methoxycarbonyl)-28-nor-isoiguesterin and isoiguesterol, being effective in preventing the growth of the two strains of *Plasmodium falciparum*. Activities of these two compounds were found to be stronger than that of chloroquine, the current standard malaria medication [43].

## 6. Trend for future studies

In general, *Salacia* is a rich source of phytochemicals that explains why this genus has been widely used as a traditional medicine. To date, only 11 *Salacia* species have been screened and their phytochemical profile established. In most of these cases, optimisation of extraction conditions (choice of solvent and extraction method) has yet to be completed. Only limited studies have so far been undertaken to assess potential health benefits including antioxidant and anti-microbial activities. Therefore, the full potential of *Salacia* as a source of bioactive compounds remains under utilised. Future studies on *Salacia* are needed to further screen for phytochemicals and test for their antioxidant, anti-microbial and therapeutic properties for maximising the use of *Salacia* in the food, cosmetic or pharmaceutical industries.

As many factors can affect the extraction and activity of phytochemicals in *Salacia,* a standardised protocol/s for comprehensively extracting and screening phytochemicals, testing their properties, and assessing their therapeutic potential is needed. A trend for future studies is proposed in Figure 3. *Salacia* needs to be identified and authenticated by a professional botanist to assure the correct species is acquired and examined. The plant then needs to be rapidly transferred to the laboratory for pre-treatment. An optimal drying treatment then needs to be applied to ensure that a high yield of phytochemicals is retained, as drying has been demonstrated to significantly affect phytochemical retention [49]. Further treatment, such as grinding to reduce particle size and homogenisation of the sample is also necessary.

***Please insert Figure 3 here***

Extraction is an essential step in the liberation of phytochemicals from the plant tissue matrix. Solvent character, temperature, sample to solvent ratio, pH, particle size, extraction techniques (conventional or advanced methods) and extraction time all significantly affect the extraction efficiency of phytochemicals [50, 51]. The targeting of individual compounds within a phytochemical profile may require the use of different techniques, and then identified using Mass Spectrometry (MS) and Nuclear Magnetic Resonance Spectroscopy (NMR).

Crude extracts, enriched fractions and individual compounds can be further tested for their antioxidant activity *in vitro* or *in vivo*. These can be further tested for anti-microbial properties against various types of microorganisms to propose their potential use in the food industry. Phytochemicals with potent antioxidant and anti-microbial properties can be potentially fortified in food products to extend their shelf-life because these bioactive compounds can prevent the fat oxidation and microbial growth, which directly affects the quality of foods [52]. These extracts, fractions and compounds can be further tested for their potential health benefits to indicate the potential use as therapeutic agents.

## 7. Conclusions

*Salacia* has been traditionally used as herbal medicines in different regions around the world, and previous studies have revealed that this genus is a rich source of phytochemicals, which play important roles in primary health care. 168 individual compounds, including 123 terpenes and terpenoids, 18 phenolics and 27 other compounds have been identified from 11 *Salacia* species to date, revealing that many potentially beneficial and functional compounds are yet to be identified. In addition, limited studies have been conducted to test the antioxidant and anti-microbial properties as well as examine the potential health benefits of

*Salacia*-derived bioactive compounds. Therefore, the potential use of *Salacia* has not been fully utilised. Future studies on *Salacia* are necessary to explore bioactive compounds present in other *Salacia* species and to investigate their potential use as anti-microbial agents, functional food ingredients, and therapeutic agents.

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**Declaration of interest statement**

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Appendix 1. Terpenes isolated and identified in different species of *Salacia*

| Species | Part used | Phytochemicals | Solvent used/ identification method | References |
|---|---|---|---|---|
| *S. chinensis* | Stem | **Eudesmane sesquiterpene:** Salasol A, Salasol B, Celahin C. **Oleanane triterpenoid:** 3β,22β-dihydroxyolean-12-en-29-oic acid; 22α-hydroxy-3-oxoolean-12-en-29-oic acid; β-amyrenone; β-amyrin; Maytenfolic acid; **Friedelane triterpenoid:** Maytenoic acid; Salaspermic acid; Orthosphenic acid; Salasone A, Salasone B, Salasone C; Salasone D; Salasone E; 15α-hydroxyfriedelane-3-one; Regeol A; friedelane-3-one-29-ol; 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one); 7α,21α-dihydroxyfriedelane-3-one; 7α,29-dihydroxyfriedelane-3-one; 21α,30-dihydroxyfriedelane-3-one; 29-hydroxyfriedelane-3-one (29-hydroxy-D:A-friedo-olean-3-one); Wilforic acid C. | Methanol 80%, ethylacetate, n-hexane/HPLC and NMR | [32, 45, 53, 54] |

| | | | | |
|---|---|---|---|---|
| | | **Ursane triterpene:** Tripterygic acid A; Demethylregelin. **Lupane triterpenoid:** 28-hydroxy-3-oxo-30-lupanoic acid; 3-oxo-lupane-30-al; Betulin (lup-20-(29)-ene-3β,28-diol); **Hopane triterpenoid:** 29-nor-21α-H-hopane-3,22-dione; 21α-H-hop-22(29)-ene-3β ,30-diol; **Quinonemethide triterpenoid:** Maytenin (Maitenin or tingenone); Tingenin B (22β-hydroxytingenone). **Norfriedelane triterpene:** Triptocalline A; Salaquinone A; Salaquinone B | | |
| | Root bark | **Friedelane triterpenoid:** Friedel-1-en-3-one; Friedelane-1,3-dione; 1,3-dioxofriedelane-24-al; 7α-hydroxy-friedelane-1,3-dione | | [55] |
| | Leaf | **Friedelane triterpenoid:** Friedelin (friedelane-3-one); 3β – hydroxyfriedelane; 3,4-seco-friedelane-3-oic acid. **Norfriedelane triterpenoid:** Taraxer-14-ene-3β-ol | n-hexane/NMR | [45] |
| *S. oblonga* | Root | **Diterpenoid:** 19-hydroxyferruginol; Lambertic acid **Oleanane triterpenoid:** 3β,22α-dihydroxyolean-12-en-29-oic | Methanol 80%/NMR, FT- | [35] |

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|---|---|---|---|---|
| | | acid; Maytenfolic acid;<br>**Friedelane triterpenoid:** Kotalagenin 16-acetate; 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione) | IR | |
| | Aerial | **Diterpenoid:** Phytol.<br>**Ursane triterpenoid:** Ursa-9(11), 12-dien-3-ol. | Methanol/GC-MS | [56] |
| *S. reticulata* var. *β-diandra* | Root bark | **Quinonemethide triterpenoid:** Salaciquinone; isoiguesterin. | Hexane, benzene/NMR | [57] |
| | Outer root bark | **Friedelane triterpenoid:** Salacenonal; Epi-kokoondiol (21α,26-dihydroxy-D:A-friedo-oleanan-3-one); Kokoondiol (21β,26-dihydroxy-D:A-friedo-oleanan-3-one); Kokoononol (26-hydroxy-D:A-friedo-oleanane-3,21-dione); Kokzeylanol (6β,26-dihydroxy-D:A-friedo-aleanan-3-one); Kokoonol (26-hydroxy-D:A-friedo-oleanane-3-one); Kokzeylanonol (6β,26-dihydroxy-D:A-friedo-oleanane-3,21-dione);<br>**Quinonemethide triterpenoid:** Pristimerin; 30-hydroxy-pristimerin; Isoiguesterol (isoiguesterinol); Maytenin (Maitenin | Hexane, benzene/NMR | [58, 59] |

| | | | | |
|---|---|---|---|---|
| | | or tingenone); Tingenin B (22β-hydroxytingenone); Celastrol; Salaciquinone; Isoiguesterin; Netzahualcoyene. | | |
| | Outer stem bark | Quinonemethide triterpenoid: Iguesterin; pristimerin; epi-kokoondil | Benzene/NMR | [60] |
| *S. verrucosa* | Stem | **Friedelane triterpenoid:** 21α-hydroxyfriedelane-1,3-dione; 30-hydroxyfriedelane-1,3-dione; Friedelane-1,3-dione; 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); Friedelin (friedelane-3-one); 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one); Kokoonol | n-hexane, chloroform, methanol/NMR | [44] |
| *S. amplifolia* | Root | **Oleanane triterpenoid:** Wilforlide A; Wilforlide B; β-amyrin; Gult-5-en-3β-ol; **Friedelane triterpenoid:** Salacenonal; 15α-hydroxyfriedelan-3-one; 3β-hydroxy-D:A-friedoolean-3-en-2-one-29-oic acid; 7-tetraene-24-nor-friedelane-29-oic acid methylester; Regeol A. **Ursane triterpenoid:** α-amyrin. **Quinonemethide triterpenoid:** Isoiguesterin; Netzahualcoyene; | Ethanol 80%/ IR, MS, NMR | [37] |

| | | | | |
|---|---|---|---|---|
| | | Pristimerin. | | |
| *S. beddomei* | Stem bark | **Friedelane triterpenoid:** Friedelin (friedelane-3-one); 15α-hydroxyfriedelane-3-one; 15α-hydroxyfriedelane-1,3-dione **Lupane triterpenoid:** Salacianone (lup-20(29)-en-3,21-dione); Salacianol (21β-hydroxy lup-20(29)-en-3-one); Lup-20(29)-en-3-one; Quinonemethide triterpenoid: Pristimerin; | n-hexane/IR, NMR, MS | [61] |
| *S. cochinchinensis* | Root | Quinonemethide triterpenoid: Pristimerin; | Methanol/HPLC | [21] |
| *S. impressifolia* | Trunk and twings | **Friedelane triterpenoid:** Friedelin, β-amyrin; Lupane triterpenoid: Lupeol; 2-oxo-20(29)-lupen-3β-ol; Salacinin B; 2β,3β-dihydroxylup-20(29)-ene; Regeol A; **Oleanane triterpenoid:** β-amyrin **Ursane triterpenoid:** α-amyrin; **Quinonemethide triterpenoid:** Pristimerin; 30-hydroxypristimerin; Isoiguesterin; Tingenone; 22- | Ethanol/NMR, MS | [22] |

| | | | | |
|---|---|---|---|---|
| | | hydroxytingenone; Netzahualcoyene. | | |
| *S. kraussii* | Root | **Quinonemethide triterpenoid:** Pristimerin; Isoiguesterol (isoiguesterinol); 17-(methoxycarbonyl)-28-nor-isoiguesterin; 28-hydroxyisoiguesterin; 28-nor-isoiguesterin-17-carbaldehyde; Celastrol; | | [43] |
| *S. campestris* | Root bark | **Quinonemethide triterpenoid:** Pristimerin; Salacin; Maytenin (Maitenin or tingenone); 20α-hydroxymaytenin; Netzahualcoyene | Chloroform/NMR | [62] |
| *S. senegalensis* | Leaf | **Monoterpene:** α-terpinene; α-phellandrene; α-pinene; cymene; β-pinene. **Monoterpenoid:** Linalool; Carvacrol; 1,8-Cineole. **Sesquiterpene:** Germacrene D; α-caryophyllene. **Sesquiterpenoid:** Caryophyllene oxide. | Chloroform/GC | [63] |
| **Total (123):** Monoterpene (**5**); Monoterpenoid (**3**); Sesquiterpene (**2**); Sesquiterpenoid (**4**); Diterpenoid (**3**); Triterpenoid (**106**; include: 11 oleananes, 47 friedelanes, 4 ursanes, 20 lupanes, 2 hopanes, 3 tirucalls, 16 quinonemethides and 3 compounds of unknown structure. | | | | |

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Appendix 2. Phenolic compounds isolated and identified in different species of *Salacia*

| Species | Part used | Phytochemicals | Solvent used/ identification method | References |
|---|---|---|---|---|
| *S. chinensis* | Stem | **Xanthonoid:** Mangeferin.<br>**Flavan-3-ol:** (-)-Epigallocatechin; (-)-epicatechin; (+)-catechin.<br>**Flavone:** Vitexin; Isovitexin.<br>**Lignan:** (+)-lyoniresinol; (+)-isolariciresinol; (+)-8-methoxyisolariciresinol. | Methanol/ NMR, MS | [32] |
| | Root | **Xanthonoid:** Mangeferin. | Methanol/HPLC, FT-IR | [31, 34] |
| *S. oblonga* | Root | **Flavan-3-ol:** (-)-4'-*O*-methylepigallocatechin. | Methanol 80%/NMR | [35] |
| *S. reticulata* | Root bark | **Xanthonoid:** Mangeferin. | Methanol/MS, NMR | [33] |
| | Root | **Flavan-3-ol:** Epigallocatechin; epicatechin; Epiafzelechin; (-)- | | [33] |

| | | | | |
|---|---|---|---|---|
| | | epiafzelechin-(4β→8)-(-)-4'-*O*-methylepigallocatechin; (-)-epicatechin-(4β→8)-(-)-4'-*O*-methylepigallocatechin. | | |
| | Leaf | **Flavan-3-ol:** Epigallocatechin; epicatechin; Epiafzelechin; Epigallocatechin-4-(2hydroxyethyl)thio ether; Epicatechin-4-(2hydroxyethyl)thio ether; Epiafzelechin-4-(2hydroxyethyl)thio ether. | Ethanol 60%/NMR | [36] |
| *S. amplifolia* | Root | **Simple phenolic:** Dibutyl phthalate; Coniferaldehyde; Sinapic aldehyde. | Ethanol 80%/IR, MS, NMR | [37] |
| **Total: 18**: Xanthonoid (**1**); Flavan-3-ol (Flavonoid; **9**); Flavone (**2**); lignan (**3**) and simple phenolic (**3**). | | | | |

Appendix 3. Other compounds isolated and identified in different species of *Salacia*

| Species | Part used | Phytochemicals | Solvent used/ identification method | References |
|---|---|---|---|---|
| *S. chinensis* | Root | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neosalacinol; Neokotalanol; Salaprinol; Ponkoranol. | Water, methanol/LC-MS | [10, 46, 64] |
| | Stem | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neosalacinol; Neokotalanol; Salaprinol; Ponkoranol. **Steroid precursor:** Squalene. | Water, methanol/LC-MS | [10, 32, 46, 64] |
| | Leaf | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neokotalanol | Water, methanol/LC-MS | [10, 46] |
| | Fruit | **Thiosugar sulfonium sulfate compound**: Salacinol; Kotalanol; Neokotalanol | Water, methanol/LC-MS | [10, 46] |
| *S. oblonga* | Root | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neosalacinol; Neokotalanol. **Alkanes and alkenes:** cyclotrisiloxane; Hexamethyl. | Water, methanol, ethanol/LC-MS, NMR | [10, 35, 46, 56, 65] |

| | | | | |
|---|---|---|---|---|
| | | **Aliphatic ester:** Silicic acid diethyl bis(trimethylsilyl) ester. **Benzene ring:** Benzo [h] quinoline, 2,4-dimethyl; Benzene 1,1', 1", 1"'-oxydimethylidyne)tetrakis-. **Phenols:** Pyridine-3-carboxamide.oxime.N-(2-trifluromethylphenyl); 5-methyl-2-phenylindolizine. **Saturated fatty acid:** n-Hexadecanoic acid; n-Octadecanoic acid; **Ester:** Hexadecanoic acid, 3-hydroxy methyl ester; 1,2 Benzene dicarboxylic acid mono(2-ethylhexyl)ester. **Others:** N-methoxy-N-methylacetamide; 2-Ethylacridine. | | |
| | Aerial | **Steroid:** γ-sitosterol **Aliphatic ester:** Carbonic acid, 2,2,2-trichloroethyl-undec-10-enyl ester; trichloroacetic acid, undec-10-enyl ester. **Ketone:** 2-p-nitrophenyl-Oxadiazol-1,3,4 one-5; Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl. **Aliphatic amine:** 2,3-Dimethylamphetamine. **Aliphatic alcohol:** Phytol. | Methanol, ethanol/GC-MS | [56, 65] |

| | | | | |
|---|---|---|---|---|
| | | **Benzene ring:** Benzeneethanol α,α,β-triphenyl. **Phenols:** Pyridine-3-carboxamide.oxime.N-(2-trifluromethylphenyl); 5-methyl-2-phenylindolizine **Saturated fatty acid:** n-Hexadecanoic acid; n-Octadecanoic acid; **Ester:** Hexadecanoic acid, 3-hydroxy methyl ester; 1,2 Benzene dicarboxylic acid mono(2-ethylhexyl)ester. | | |
| *S. reticulata* | Root | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neosalacinol; Neokotalanol | Water, methanol/LC-MS | [10, 46] |
| | Stem | A polyhydroxylated cyclic 13-membered sulfoxide compound | Water/NMR,MS | [42] |
| *S. hainanensis* | root | Olibanumol J | Methanol/HPLC | [41] |
| *S. amplifolia* | Root | **Polyol:** Dulcitol | Ethanol 80%/IR, MS, NMR | [37] |
| *S. beddomei* | Stem bark | **Steroid:** Sitosterol; | Hexane/NMR | [61] |
| *S. impressifolia* | Trunk and twings | **Steroid:** β-sitosterol | Ethanol/NMR, MS | [22] |

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Table 1. Traditional use of *Salacia* for various ailments

| Species | Way of preparation | Health benefits | References |
|---|---|---|---|
| *S. chinensis* (root) | Decoction | Rheumatism, back-pain and debility | [8] |
| *S. chinensis* (stem) | Decoction | Back-pain | [9] |
| *S. reticulata* (root) | Decoction | Itching and swelling, asthma, thirst, amenorrhea and dysmenorrhea | [4] |
| *S. macrosperma, S. grandiflora, S. macrophylla* and *S. ovalis* (root) | Decoction | For women after parturition | [6] |
| *S. macrosperma* (leaf) | ND (No data) | Eczema | [6] |
| *S. reticulata* and *S. oblonga* (root and stem) | ND | Rheumatism, gonorrhea, skin diseases and diabetes | [6, 11] |
| *S. chinensis* | ND | Anti- diabetes and laxative | [10, 11] |
| *Salacia sp.* | Herbal tea (infusion) | Diabetes | [14] |
| *S. chinensis, S. reticulata and S. oblonga* | Powder extracts | Obesity and diabetes | [15, 16] |

| | | | |
|---|---|---|---|
| *Salacia sp* (wood from root or stem) | Making mugs for drinking water | Diabetes | [12, 13] |
| *S. chinensis* (root) | ND | Diabetes, amenorrhoea, dysmenorrhoea and genito-urinary and venereal diseases | [6] |
| *S. oblonga* (root bark) | ND | Rheumatism, gonorrhoea, swellings and skin diseases | [6] |
| *S. oblonga* (whole plant) | ND | Mild antiseptic | [6] |
| *S. reticulata* (root bark) | ND | Gonorrhoea, skin diseases and inflammation and hypoglycaemic activity | [6] |
| *S. reticulata* (whole plant) | ND | Mild antiseptic | [6] |

Table 2. Individual compounds isolated from *Salacia* species showed activities against diseases

| No | Disease | Tested compounds | References |
|---|---|---|---|
| 1 | Liver cancer | **Friedelane triterpenoid:** 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one); 7α,21α-dihydroxyfriedelane-3-one; 3,4-seco-friedelane-3-oic acid; **Lupane triterpenoid:** 28-hydroxy-3-oxo-30-lupanoic acid; | [44, 45] |
| 2 | Colon cancer | **Friedelane triterpenoid:** Friedelane-1,3-dione; 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one). **Quinonemethide triterpenoid:** Pristimerin; Isoiguesterol (isoiguesterinol); 17-(methoxycarbonyl)-28-nor-isoiguesterin; 28-hydroxyisoiguesterin; Celastrol | [43, 44] |
| 3 | Gastric cancer | **Friedelane triterpenoid: 26**-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one) | [44] |
| 4 | Lung cancer | **Friedelane triterpenoid:** 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 7α,21α-dihydroxyfriedelane-3-one; 3,4-seco-friedelane-3-oic acid. | [44, 45] |

| | | | |
|---|---|---|---|
| | | **Lupane triterpenoid:** 28-hydroxy-3-oxo-30-lupanoic acid.<br>**Hopane triterpenoid:** 29-nor-21α-H-hopane-3,22-dione | |
| 5 | Breast cancer | **Friedelane triterpenoid:** 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 7α,21α-dihydroxyfriedelane-3-one; 3,4-seco-friedelane-3-oic acid;<br>**Lupane triterpenoid:** 28-hydroxy-3-oxo-30-lupanoic acid.<br>**Quinonemethide triterpenoid:** Pristimerin | [44, 45] |
| 6 | Mouth cancer | **Friedelane triterpenoid:** 7α,21α-dihydroxyfriedelane-3-one; 3,4-seco-friedelane-3-oic acid.<br>**Lupane triterpenoid:** 28-hydroxy-3-oxo-30-lupanoic acid. | [45] |
| 7 | Pancreatic cancer | Quinonemethide triterpenoid: Pristimerin | [5] |
| 8 | Diabetes | **Xanthonoid:** Mangiferin | [34] |
| 9 | α-glucosidase inhibitory (Diabetes) | **Oleanane triterpenoid:** β-amyrin; 3β,30-dihydroxy-olean-12-ene; oleanic acid.<br>**Friedelane triterpenoid:** Salasone C; 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one); 29-hydroxyfriedelane-3-one (29-hydroxy-D:A-friedo-olean-3-one); 30-hydroxy-friedelan-3-one; 7α,21α-dihydroxy-D:A-friedo-oleanane-3-one; 30-hydroxy-D:A-friedo-olean-1-en-3-one; D:A-friedo-oleanane-7α,30- | [33, 41, 66-68] |

| | | | |
|---|---|---|---|
| | | dihydroxy-3-one;<br>**Lupane triterpenoid:** Salacianone (lup-20(29)-en-3,21-dione); 2β,3β-dihydroxylup-20(29)-ene; 2α,3β,22α-trihydroxy-lup-20(29)-ene; 3β-hydroxy-2-carbonyl-lupan-29-oic acid; 2,3-seco-lup-20(29)-en-2,3-dioic-2-methylate; 3β,30-dihydroxy-lup-20(29)-en-2-one; 3α,28-dihydroxy-lup-20(29)-en-2-one; 3α-hydroxy-lup-20(29)-en-2-one; 2,3-seco-lup-20(29)-en-2,3-dioic acid; 2-oxo-20(29)-lupen-3β-ol; Lupeol;<br>**Tirucall triterpenoid:** 24S,25-dihydroxy-tirucall-7-en-3-one; 24,25,26-trihydroxytirucall-7-en-3-one.<br>**Flavan-3-ol:** Epicatechin; Epigallocatechin; (-)-4'-*O*-methylepigallocatechin | |
| 10 | Aldose reductase inhibitory (Diabetes) | **Diterpenoid:** 19-hydroxyferruginol; Lambertic acid.<br>**Oleanane triterpenoid:** 3β,22α-dihydroxyolean-12-en-29-oic acid; 3β,22β-dihydroxyolean-12-en-29-oic acid; Maytenfolic acid.<br>**Friedelane triterpenoid:** Kotalagenin 16-acetate; Regeol A.<br>**Quinonemethide triterpenoid:** Maytenin (Maitenin, tingenone); Tingenin B (22β-hydroxytingenone);<br>**Norfriedelane triterpenoid:** Triptocalline A. | [32, 35] |

| | | | |
|---|---|---|---|
| 11 | Pancreatic lipase inhibitory (Obesity) | **Flavan-3-ol:** Epigallocatechin | [36] |
| 12 | Malaria | **Quinonemethide triterpenoid:** Pristimerin; Isoiguesterol (isoiguesterinol); 17-(methoxycarbonyl)-28-nor-isoiguesterin; 28-hydroxyisoiguesterin; 28-nor-isoiguesterin-17-carbaldehyde; Celastrol | [43] |


Figure 1. *Salacia chinensis*: stem and leaf (A), root (B) and root cross-cut (C)

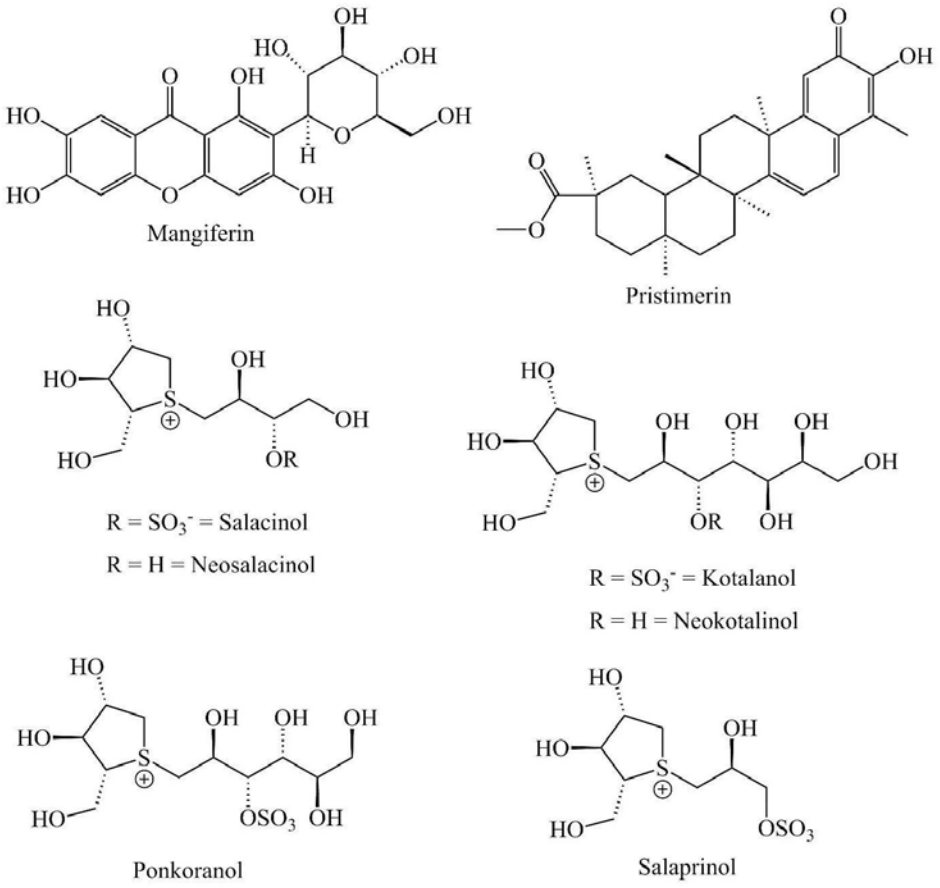



Figure 2. Bioactive compounds isolated from *Salacia* species

**Table**

Table 1. Traditional use of *Salacia* for various ailments

| Species | Way of preparation | Health benefits | References |
|---|---|---|---|
| *S. chinensis* (root) | Decoction | Rheumatism, back-pain and debility | (Vo, 1997) |
| *S. chinensis* (stem) | Decoction | Back-pain | (Delang, 2007) |
| *S. reticulata* (root) | Decoction | Itching and swelling, asthma, thirst, amenorrhea and dysmenorrhea | (Arunakumara and Subasinghe, 2010) |
| *S. macrosperma, S. grandiflora, S. macrophylla* and *S. ovalis* (root) | Decoction | For women after parturition | (Khare, 2007) |
| *S. macrosperma* (leaf) | ND (No data) | Eczema | (Khare, 2007) |
| *S. reticulata* and *S. oblonga* (root and stem) | ND | Rheumatism, gonorrhea, skin diseases and diabetes | (Khare, 2007; Ramakrishna et al., 2015) |
| *S. chinensis* | ND | Anti- diabetes and laxative | (Muraoka, 2011; Ramakrishna et al., 2015) |
| *Salacia sp.* | Herbal tea (infusion) | Diabetes | (Jayawardena et al., 2005) |

| | | | |
|---|---|---|---|
| *S. chinensis, S. reticulata and S. oblonga* | Powder extracts | Obesity and diabetes | (Li et al., 2008; Singh and Duggal, 2010) |
| *Salacia sp* (wood from root or stem) | Making mugs for drinking water | Diabetes | (Pinto, 2009) |
| *S. chinensis* (root) | ND | Diabetes, amenorrhoea, dysmenorrhoea and genito-urinary and venereal diseases | (Khare, 2007) |
| *S. oblonga* (root bark) | ND | Rheumatism, gonorrhoea, swellings and skin diseases | (Khare, 2007) |
| *S. oblonga* (whole plant) | ND | Mild antiseptic | (Khare, 2007) |
| *S. reticulata* (root bark) | ND | Gonorrhoea, skin diseases and inflammation and hypoglycaemic activity | (Khare, 2007) |
| *S. reticulata* (whole plant) | ND | Mild antiseptic | (Khare, 2007) |

**Table**

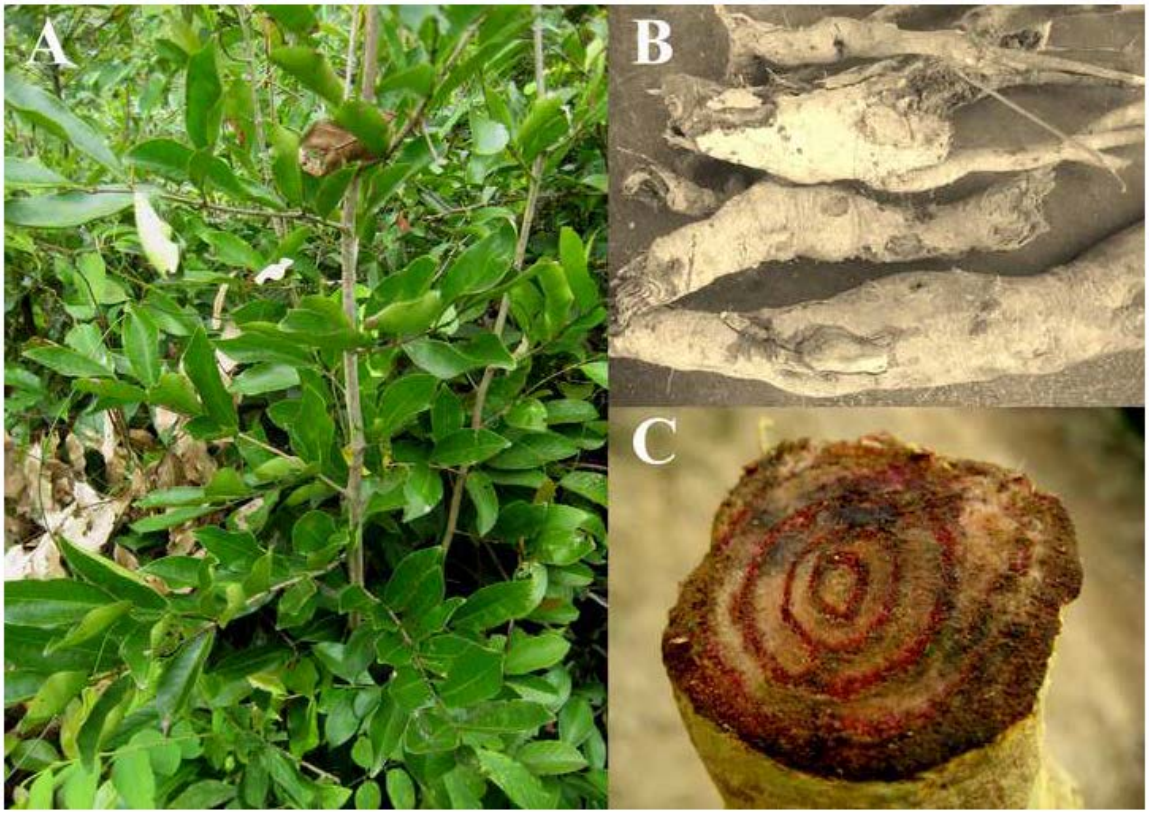
Table 2. Individual compounds isolated from *Salacia* species showed activities against diseases

| No | Disease | Tested compounds | references |
|---|---|---|---|
| 1 | Liver cancer | **Friedelane triterpenoid**: 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one); 7α,21α-dihydroxyfriedelane-3-one; 3,4-seco-friedelane-3-oic acid;<br>**Lupane triterpenoid**: 28-hydroxy-3-oxo-30-lupanoic acid; | (Somwong et al., 2011; Tran et al., 2010) |
| 2 | Colon cancer | **Friedelane triterpenoid**: Friedelane-1,3-dione; 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one).<br>**Quinonemethide triterpenoid**: Pristimerin; Isoiguesterol (isoiguesterinol); 17-(methoxycarbonyl)-28-nor-isoiguesterin; 28-hydroxyisoiguesterin; Celastrol | (Figueiredo et al., 1998; Somwong et al., 2011) |
| 3 | Gastric cancer | **Friedelane triterpenoid: 26**-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one) | (Somwong et al., 2011) |
| 4 | Lung cancer | **Friedelane triterpenoid**: 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 7α,21α-dihydroxyfriedelane-3-one; 3,4-seco-friedelane-3-oic acid.<br>**Lupane triterpenoid**: 28-hydroxy-3-oxo-30-lupanoic acid.<br>**Hopane triterpenoid**: 29-nor-21α-H-hopane-3,22-dione | (Somwong et al., 2011; Tran et al., 2010) |
| 5 | Breast cancer | **Friedelane triterpenoid**: 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); 7α,21α-dihydroxyfriedelane-3-one; | (Somwong et al., 2011; Tran et al., 2010) |

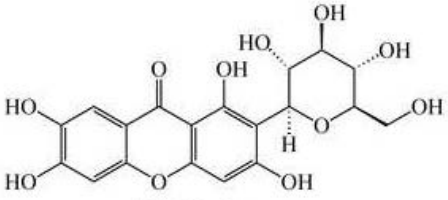
| | | | |
|---|---|---|---|
| | | 3,4-seco-friedelane-3-oic acid;<br>**Lupane triterpenoid:** 28-hydroxy-3-oxo-30-lupanoic acid.<br>**Quinonemethide triterpenoid:** Pristimerin | |
| 6 | Mouth cancer | **Friedelane triterpenoid:** 7α,21α-dihydroxyfriedelane-3-one; 3,4-seco-friedelane-3-oic acid.<br>**Lupane triterpenoid:** 28-hydroxy-3-oxo-30-lupanoic acid. | (Tran et al., 2010) |
| 7 | Pancreatic cancer | Quinonemethide triterpenoid: Pristimerin | (Phuong et al., 2015) |
| 8 | Diabetes | **Xanthonoid:** Mangiferin | (Sellamuthu et al., 2009) |
| 9 | α-glucosidase inhibitory (Diabetes) | **Oleanane triterpenoid:** β-amyrin; 3β,30-dihydroxy-olean-12-ene; oleanic acid.<br>**Friedelane triterpenoid:** Salasone C; 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one); 29-hydroxyfriedelane-3-one (29-hydroxy-D:A-friedo-olean-3-one); 30-hydroxy-friedelan-3-one; 7α,21α-dihydroxy-D:A-friedo-oleanane-3-one; 30-hydroxy-D:A-friedo-olean-1-en-3-one; D:A-friedo-oleanane-7α,30-dihydroxy-3-one;<br>**Lupane triterpenoid:** Salacianone (lup-20(29)-en-3,21-dione); 2β,3β-dihydroxylup-20(29)-ene; 2α,3β,22α-trihydroxy-lup-20(29)-ene; 3β-hydroxy-2-carbonyl-lupan-29-oic acid; 2,3-seco-lup-20(29)-en-2,3-dioic-2-methylate; 3β,30-dihydroxy-lup-20(29)-en-2-one; 3α,28-dihydroxy-lup-20(29)-en-2-one; 3α-hydroxy-lup-20(29)-en-2-one; 2,3-seco-lup-20(29)-en-2,3-dioic acid; 2-oxo-20(29)-lupen-3β-ol; Lupeol; | (Gao et al., 2010; Guo et al., 2013; Huang et al., 2012; Yoshikawa et al., 2001; Yu et al., 2014) |

| | | | |
|---|---|---|---|
| | | **Tirucall triterpenoid:** 24S,25-dihydroxy-tirucall-7-en-3-one; 24,25,26-trihydroxytirucall-7-en-3-one.<br>**Flavan-3-ol:** Epicatechin; Epigallocatechin; (-)-4'-*O*-methylepigallocatechin | |
| 10 | Aldose reductase inhibitory (Diabetes) | **Diterpenoid:** 19-hydroxyferruginol; Lambertic acid.<br>**Oleanane triterpenoid:** 3β,22α-dihydroxyolean-12-en-29-oic acid; 3β,22β-dihydroxyolean-12-en-29-oic acid; Maytenfolic acid.<br>**Friedelane triterpenoid:** Kotalagenin 16-acetate; Regeol A.<br>**Quinonemethide triterpenoid:** Maytenin (Maitenin, tingenone); Tingenin B (22β-hydroxytingenone);<br>**Norfriedelane triterpenoid:** Triptocalline A. | (Matsuda et al., 1999; Morikawa et al., 2003) |
| 11 | Pancreatic lipase inhibitory (Obesity) | **Flavan-3-ol:** Epigallocatechin | (Koga et al., 2013) |
| 12 | Malaria | **Quinonemethide triterpenoid:** Pristimerin; Isoiguesterol (isoiguesterinol); 17-(methoxycarbonyl)-28-nor-isoiguesterin; 28-hydroxyisoiguesterin; 28-nor-isoiguesterin-17-carbaldehyde; Celastrol | (Figueiredo et al., 1998) |

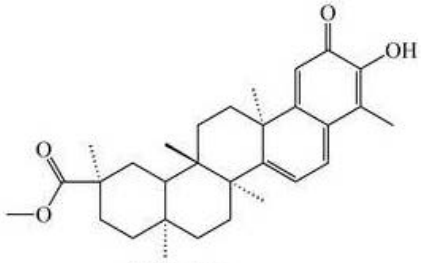
Figure



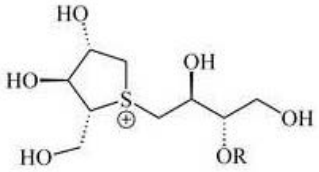
Figure



Mangiferin

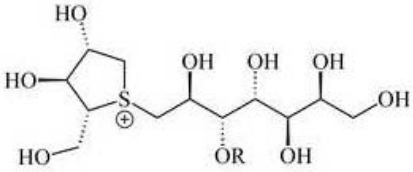


Pristimerin



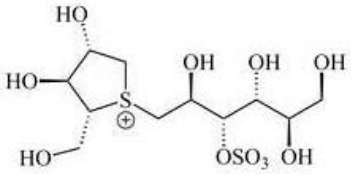
R = $SO_3^-$ = Salacinol

R = H = Neosalacinol

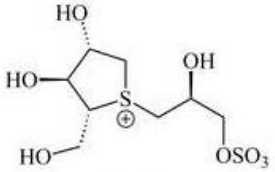


R = $SO_3^-$ = Kotalanol

R = H = Neokotalinol

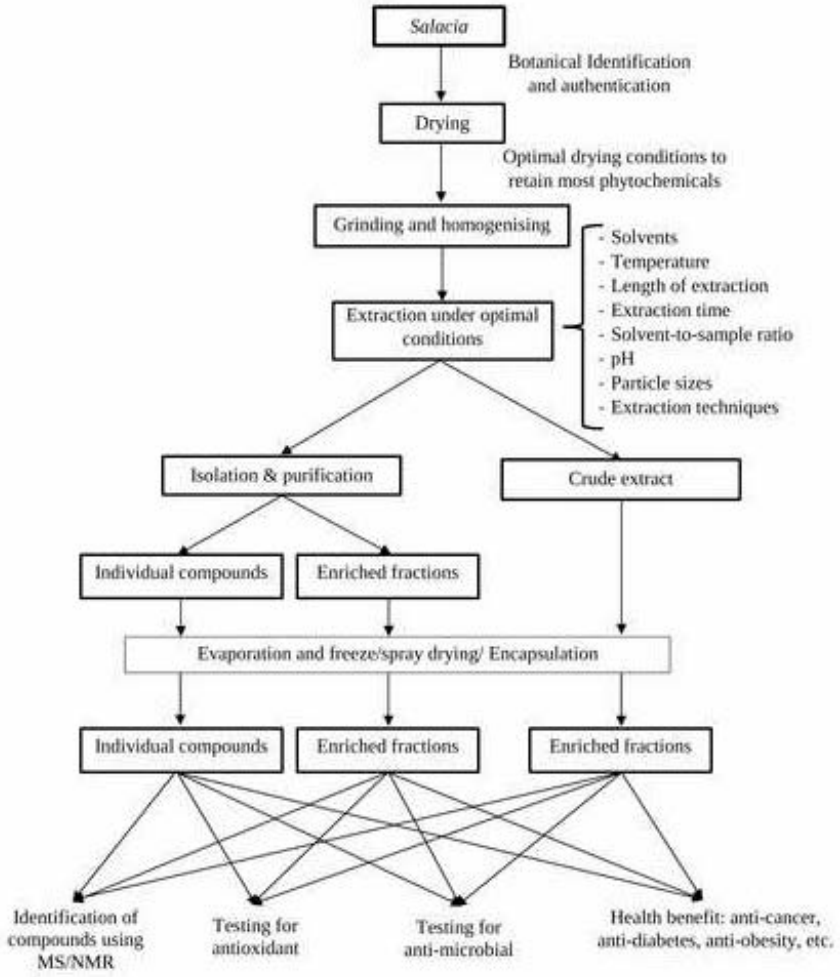


Ponkoranol



Salaprinol

Figure


Salacia
Botanical Identification and authentication
Drying
Optimal drying conditions to retain most phytochemicals
Grinding and homogenising
Extraction under optimal conditions
- Solvents
- Temperature
- Length of extraction
- Extraction time
- Solvent-to-sample ratio
- pH
- Particle sizes
- Extraction techniques
Isolation & purification
Crude extract
Individual compounds
Enriched fractions
Evaporation and freeze/spray drying/ Encapsulation
Individual compounds
Enriched fractions
Enriched fractions
Identification of compounds using MS/NMR
Testing for antioxidant
Testing for anti-microbial
Health benefit: anti-cancer, anti-diabetes, anti-obesity, etc.

**e-Supplemental material**

Appendix 1. Terpenes isolated and identified in different species of *Salacia*

| Species | Part used | Phytochemicals | Solvent used/ identification method | References |
|---|---|---|---|---|
| *S. chinensis* | Stem | **Eudesmane sesquiterpene:** Salasol A, Salasol B, Celahin C.<br>**Oleanane triterpenoid:** 3β,22β-dihydroxyolean-12-en-29-oic acid; 22α-hydroxy-3-oxoolean-12-en-29-oic acid; β-amyrenone; β-amyrin; Maytenfolic acid;<br>**Friedelane triterpenoid:** Maytenoic acid; Salaspermic acid; Orthosphenic acid; Salasone A, Salasone B, Salasone C; Salasone D; Salasone E; 15α-hydroxyfriedelane-3-one; Regeol A; friedelane-3-one-29-ol; 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one); 7α,21α-dihydroxyfriedelane-3-one; 7α,29-dihydroxyfriedelane-3-one; 21α,30-dihydroxyfriedelane-3-one; 29-hydroxyfriedelane-3-one (29-hydroxy-D:A-friedo-olean-3-one); Wilforic acid C.<br>**Ursane triterpene:** Tripterygic acid A; Demethylregelin.<br>**Lupane triterpenoid:** 28-hydroxy-3-oxo-30-lupanoic acid; 3-oxo-lupane-30-al; Betulin (lup-20-(29)-ene-3β,28-diol);<br>**Hopane triterpenoid:** 29-nor-21α-H-hopane-3,22-dione; 21α-H-hop-22(29)-ene-3β ,30-diol;<br>**Quinonemethide triterpenoid:** Maytenin (Maitenin or tingenone); Tingenin B (22β-hydroxytingenone).<br>**Norfriedelane triterpene:** Triptocalline A; Salaquinone A; Salaquinone | Methanol 80%, ethylacetate, n-hexane/HPLC and NMR | (Kishi et al., 2003; Morikawa et al., 2003; Tran et al., 2009, 2010) |

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| | | B | | |
| | Root bark | **Friedelane triterpenoid:** Friedel-1-en-3-one; Friedelane-1,3-dione; 1,3-dioxofriedelane-24-al; 7α-hydroxy-friedelane-1,3-dione | | (Tewari et al., 1974) |
| | Leaf | **Friedelane triterpenoid:** Friedelin (friedelane-3-one); 3β – hydroxyfriedelane; 3,4-seco-friedelane-3-oic acid.<br>**Norfriedelane triterpenoid:** Taraxer-14-ene-3β-ol | n-hexane/NMR | (Tran et al., 2010) |
| *S. oblonga* | Root | **Diterpenoid:** 19-hydroxyferruginol; Lambertic acid<br>**Oleanane triterpenoid:** 3β,22α-dihydroxyolean-12-en-29-oic acid; Maytenfolic acid;<br>**Friedelane triterpenoid:** Kotalagenin 16-acetate; 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione) | Methanol 80%/NMR, FT-IR | (Matsuda et al., 1999) |
| | Aerial | **Diterpenoid:** Phytol.<br>**Ursane triterpenoid:** Ursa-9(11), 12-dien-3-ol. | Methanol/GC-MS | (Musini et al., 2015) |
| *S. reticulata* var. *β-diandra* | Root bark | **Quinonemethide triterpenoid:** Salaciquinone; isoiguesterin. | Hexane, benzene/NMR | (Tezuka et al., 1994) |
| | Outer root bark | **Friedelane triterpenoid:** Salacenonal; Epi-kokoondiol (21α,26-dihydroxy-D:A-friedo-oleanan-3-one); Kokoondiol (21β,26-dihydroxy-D:A-friedo-oleanan-3-one); Kokoononol (26-hydroxy-D:A-friedo-oleanane-3,21-dione); Kokzeylanol (6β,26-dihydroxy-D:A-friedo-aleanan-3-one); Kokoonol (26-hydroxy-D:A-friedo-oleanane-3-one); Kokzeylanonol (6β,26-dihydroxy-D:A-friedo-oleanane-3,21-dione);<br>**Quinonemethide triterpenoid:** Pristimerin; 30-hydroxy-pristimerin; Isoiguesterol (isoiguesterinol); Maytenin (Maitenin or tingenone); | Hexane, benzene/NMR | (Dhanabalasingham et al., 1996; Gunatilaka et al., 1993) |

| | | | | |
|---|---|---|---|---|
| | | Tingenin B (22β-hydroxytingenone); Celastrol; Salaciquinone; Isoiguesterin; Netzahualcoyene. | | |
| | Outer stem bark | Quinonemethide triterpenoid: Iguesterin; pristimerin; epi-kokoondil | Benzene/NMR | (Kumar et al., 1985) |
| *S. verrucosa* | Stem | **Friedelane triterpenoid:** 21α-hydroxyfriedelane-1,3-dione; 30-hydroxyfriedelane-1,3-dione; Friedelane-1,3-dione; 26-hydroxyfriedelane-1,3-dione (26-hydroxy-1,3-friedelanedione); Friedelin (friedelane-3-one); 21α-hydroxyfriedelane-3-one (21α-hydroxy-D:A-friedo-olean-3-one); Kokoonol | n-hexane, chloroform, methanol/NMR | (Somwong et al., 2011) |
| *S. amplifolia* | Root | **Oleanane triterpenoid:** Wilforlide A; Wilforlide B; β-amyrin; Gult-5-en-3β-ol;<br>**Friedelane triterpenoid:** Salacenonal; 15α-hydroxyfriedelan-3-one; 3β-hydroxy-D:A-friedoolean-3-en-2-one-29-oic acid; 7-tetraene-24-nor-friedelane-29-oic acid methylester; Regeol A.<br>**Ursane triterpenoid:** α-amyrin.<br>**Quinonemethide triterpenoid:** Isoiguesterin; Netzahualcoyene; Pristimerin. | Ethanol 80%/ IR, MS, NMR | (Wang et al., 2011) |
| *S. beddomei* | Stem bark | **Friedelane triterpenoid:** Friedelin (friedelane-3-one); 15α-hydroxyfriedelane-3-one; 15α-hydroxyfriedelane-1,3-dione<br>**Lupane triterpenoid:** Salacianone (lup-20(29)-en-3,21-dione); Salacianol (21β-hydroxy lup-20(29)-en-3-one); Lup-20(29)-en-3-one; Quinonemethide triterpenoid: Pristimerin; | n-hexane/IR, NMR, MS | (Hisham et al., 1995) |
| *S.* | Root | Quinonemethide triterpenoid: Pristimerin; | Methanol/HPLC | (Lee et al., 2013) |

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| *cochinchinensis* | | | | |
| *S. impressifolia* | Trunk and twings | **Friedelane triterpenoid:** Friedelin, β-amyrin;<br>Lupane triterpenoid: Lupeol; 2-oxo-20(29)-lupen-3β-ol; Salacinin B; 2β,3β-dihydroxylup-20(29)-ene; Regeol A;<br>**Oleanane triterpenoid:** β-amyrin<br>Ursane **triterpenoid:** α-amyrin;<br>**Quinonemethide triterpenoid:** Pristimerin; 30-hydroxypristimerin; Isoiguesterin; Tingenone; 22-hydroxytingenone; Netzahualcoyene. | Ethanol/NMR, MS | (da Silva et al., 2016) |
| *S. kraussii* | Root | **Quinonemethide triterpenoid:** Pristimerin; Isoiguesterol (isoiguesterinol); 17-(methoxycarbonyl)-28-nor-isoiguesterin; 28-hydroxyisoiguesterin; 28-nor-isoiguesterin-17-carbaldehyde; Celastrol; | | (Figueiredo et al., 1998) |
| *S. campestris* | Root bark | **Quinonemethide triterpenoid:** Pristimerin; Salacin; Maytenin (Maitenin or tingenone); 20α-hydroxymaytenin; Netzahualcoyene | Chloroform/NMR | (Carvalho et al., 2005) |
| *S. senegalensis* | Leaf | **Monoterpene:** α-terpinene; α-phellandrene; α-pinene; cymene; β-pinene.<br>**Monoterpenoid:** Linalool; Carvacrol; 1,8-Cineole.<br>**Sesquiterpene:** Germacrene D; α-caryophyllene.<br>**Sesquiterpenoid:** Caryophyllene oxide. | Chloroform/GC | (Adumanya et al., 2014) |

Appendix 2. Phenolic compounds isolated and identified in different species of Salacia

| Species | Part used | Phytochemicals | Solvent used/ identification method | References |
|---|---|---|---|---|
| *S. chinensis* | Stem | **Xanthonoid:** Mangiferin.<br>**Flavan-3-ol:** (-)-Epigallocatechin; (-)-epicatechin; (+)-catechin.<br>**Flavone:** Vitexin; Isovitexin.<br>**Lignane:** (+)-lyoniresinol; (+)-isolariciresinol; (+)-8-methoxyisolariciresinol. | Methanol/ NMR, MS | (Morikawa et al., 2003) |
| | Root | **Xanthonoid:** Mangiferin. | Methanol/HPLC, FT-IR | (Chavan et al., 2015b; Sellamuthu et al., 2009) |
| *S. oblonga* | Root | **Flavan-3-ol:** (-)-4'-*O*-methylepigallocatechin. | Methanol 80%/NMR | (Matsuda et al., 1999) |
| *S. reticulata* | Root bark | **Xanthonoid:** Mangiferin. | Methanol/MS, NMR | (Yoshikawa et al., 2001) |
| | Root | **Flavan-3-ol:** Epigallocatechin; epicatechin; Epiafzelechin; (-)-epiafzelechin-(4β→8)-(-)-4'-*O*-methylepigallocatechin; (-)-epicatechin-(4β→8)-(-)-4'-*O*-methylepigallocatechin. | | (Yoshikawa et al., 2001) |
| | Leaf | **Flavan-3-ol:** Epigallocatechin; epicatechin; Epiafzelechin; Epigallocatechin-4-(2hydroxyethyl)thio ether; Epicatechin-4-(2hydroxyethyl)thio ether; Epiafzelechin-4-(2hydroxyethyl)thio ether. | Ethanol 60%/NMR | (Koga et al., 2013) |

| | | | | |
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| *S. amplifolia* | Root | **Simple phenolic:** Dibutyl phthalate; Coniferaldehyde; Sinapic aldehyde. | Ethanol 80%/IR, MS, NMR | (Wang et al., 2011) |

Appendix 3. Other compounds isolated and identified in different species of *Salacia*

| Species | Part used | Phytochemicals | Solvent used/ identification method | References |
|---|---|---|---|---|
| *S. chinensis* | Root | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neosalacinol; Neokotalanol; Salaprinol; Ponkoranol. | Water, methanol/LC-MS | (Muraoka, 2011; Muraoka et al., 2010; Yoshikawa et al., 2008) |
| | Stem | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neosalacinol; Neokotalanol; Salaprinol; Ponkoranol. **Steroid precursor:** Squalene. | Water, methanol/LC-MS | (Morikawa et al., 2003; Muraoka, 2011; Muraoka et al., 2010; Yoshikawa et al., 2008) |
| | Leaf | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neokotalanol | Water, methanol/LC-MS | (Muraoka, 2011; Muraoka et al., 2010) |
| | Fruit | **Thiosugar sulfonium sulfate compound**: Salacinol; Kotalanol; Neokotalanol | Water, methanol/LC-MS | (Muraoka, 2011; Muraoka et al., 2010) |
| *S. oblonga* | Root | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neosalacinol; Neokotalanol. **Alkanes and alkenes:** cyclotrisiloxane; Hexamethyl. **Aliphatic ester**: Silicic acid diethyl bis(trimethylsilyl) ester. **Benzene ring:** Benzo [h] quinoline, 2,4-dimethyl; Benzene 1,1', 1'', 1'''-oxydimethylidyne)tetrakis-. | Water, methanol, ethanol/LC-MS, NMR | (Matsuda et al., 1999; Muraoka, 2011; Muraoka et al., 2010; Musini et al., 2015; Musini et al., 2013) |

| | | | | |
|---|---|---|---|---|
| | | **Phenols:** Pyridine-3-carboxamide.oxime.N-(2-trifluromethylphenyl); 5-methyl-2-phenylindolizine.<br>**Saturated fatty acid:** n-Hexadecanoic acid; n-Octadecanoic acid;<br>**Ester:** Hexadecanoic acid, 3-hydroxy methyl ester; 1,2 Benzene dicarboxylic acid mono(2-ethylhexyl)ester.<br>**Others:** N-methoxy-N-methylacetamide; 2-Ethylacridine. | | |
| | Aerial | **Steroid:** γ-sitosterol<br>**Aliphatic ester:** Carbonic acid, 2,2,2-trichloroethyl-undec-10-enyl ester; trichloroacetic acid, undec-10-enyl ester.<br>**Ketone:** 2-p-nitrophenyl-Oxadiazol-1,3,4 one-5; Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl.<br>**Aliphatic amine:** 2,3-Dimethylamphetamine.<br>**Aliphatic alcohol:** Phytol.<br>**Benzene ring:** Benzeneethanol α,α,β-triphenyl.<br>**Phenols:** Pyridine-3-carboxamide.oxime.N-(2-trifluromethylphenyl); 5-methyl-2-phenylindolizine<br>**Saturated fatty acid:** n-Hexadecanoic acid; n-Octadecanoic acid;<br>**Ester:** Hexadecanoic acid, 3-hydroxy methyl ester; 1,2 Benzene dicarboxylic acid mono(2-ethylhexyl)ester. | Methanol, ethanol/GC-MS | (Musini et al., 2015; Musini et al., 2013) |
| *S. reticulata* | Root | **Thiosugar sulfonium sulfate compound:** Salacinol; Kotalanol; Neosalacinol; Neokotalanol | Water, methanol/LC-MS | (Muraoka, 2011; Muraoka et al., 2010) |
| | Stem | A polyhydroxylated cyclic 13-membered sulfoxide compound | Water/NMR,MS | (Ozaki et al., 2008) |

| | | | | |
|---|---|---|---|---|
| *S. hainanensis* | root | Olibanumol J | Methanol/HPLC | (Huang et al., 2012) |
| *S. amplifolia* | Root | **Polyol:** Dulcitol | Ethanol 80%/IR, MS, NMR | (Wang et al., 2011) |
| *S. beddomei* | Stem bark | **Steroid:** Sitosterol; | Hexane/NMR | (Hisham et al., 1995) |
| *S. impressifolia* | Trunk and twings | **Steroid:** β-sitosterol | Ethanol/NMR, MS | (da Silva et al., 2016) |

*Conflict of Interest Statement

**Declaration of interest statement**

Authors Thanh V. Ngo, Christopher J. Scarlett, Michael C. Bowyer and Quan V. Vuong declare that they have no potential conflict of interest.

# *CHAPTER 2.*

# MATERIALS, EXPERIMENTAL DESIGN AND METHODS

## 2.1. Materials

The root, stem and leaf of *S. chinensis* L. were collected from the forest in Nghe An province (Vietnam) in May 2015 and March 2016 and then authenticated by Associate Professor Vu Quang Nam, Department of Forest Plant Resources, College of Forestry Biotechnology, Vietnam National University of Forestry. The voucher specimen of this plant material can be found in the Herbarium of National Institute of Medicinal Materials, Ha Noi (SA 611/04). After collection, these materials were cut into small pieces and sun-dried for 2 days and then ground into small particles using a commercial cutter. The powders were then sieved using a steel mesh sieve (1.4 mm EFL 2000; Endecotts Ltd., London, England_ and stored at -20$^{o}$C for further analysis.

## 2.2. Experimental design

The overall experiment design is shown in Figure 2 to achieve the specific aims of the research.

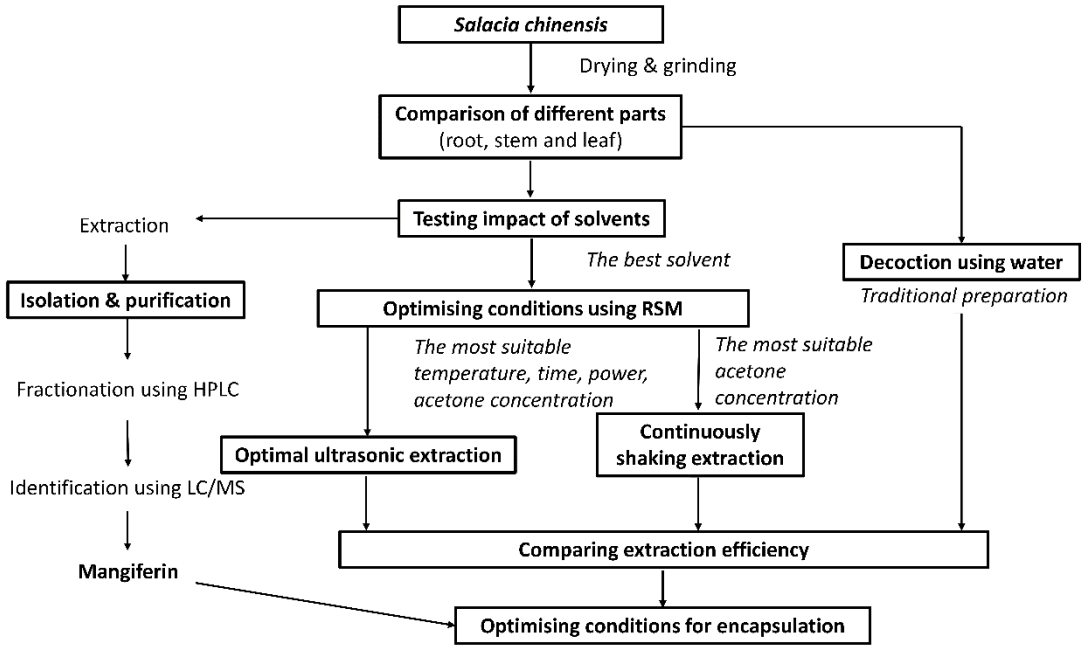


**Figure 2.** Overall experiment design of the research

## 2.3. Methods

### *2.3.1. Preparation of S. chinensis crude extract*

*S. chinensis* root, stem and leaf were extracted in acetone 50% by an ultrasonic bath (Soniclean 220 V, 50 Hz, 250 W, Soniclean Pty Ltd., Thebarton, Australia). Firstly, 100 g of sample was added into 2 L of acetone 50% (sample-to-solvent ratio 5:100 (w/v)). The mixture was then put in the ultrasonic bath with preset conditions (the mixture was vortexed thoroughly once every 3-5 min). Next, the extract was immediately cooled on ice to room temperature and then filtered using filter paper (Whatman, 11 µm pore size). This crude extract was stored in dark containers at -20ºC for further analysis.

For powdered crude extract, the extract was condensed to the volume of 150 mL using a rotary evaporator (Buchi Rotavapor B-480, Buchi Australia, Noble Park, Victoria, Australia) and then freeze-dried to yield powdered crude extract.

### *2.3.2. Determination of physical properties*

*Extractable solids*

Extractable solids were determined according to the method described previously with a minor modification (Vuong, Golding, Nguyen, & Roach, 2012). 3 mL of the extract was put in a pottery tray and then placed in an oven set at 120 °C for drying over 5 hours to remove all moisture. Extractable solids (ES) were calculated by the following formula:

$$ES\ (\%) = W\ x\ 100/3$$

(*W*: Weight of 3mL of the extraction after drying, in grams).

*Solubility*

The solubility of samples were determined by a method described by Şahin Nadeem, Torun, and Özdemir (2011). 1 g of sample was added to 100 mL of DI water at ambient temperature and the mixture was agitated with a magnetic stirrer at 600 rpm for 5 min. The solution was then centrifuged at 3000 rpm for 5 min. 20 mL of supernatant was transferred to a pre-weighed petri dish and dried in an oven at 70 ºC for 24 h until constant weight. The percent solubility was calculated by weight difference and is expressed as dry basis, considering the moisture content of each sample.

*Bulk density*

Bulk density was determined by the tapping method described by Beristain, García, and Vernon-Carter (2001). 2 g of powder was loosely weighed into 10 mL graduate cylinder. The cylinder containing the powder was tapped on a flat surface to a constant volume. The final volume was recorded and bulk density was calculated by dividing the sample weight by the volume.

*Moisture content*

The moisture content of samples were determined by method described by Şahin Nadeem et al. (2011). 0.5 g of each sample was weighed in triplicate and dried in an oven at 70 ºC for 24 h until constant weight. The moisture content was calculated as g water loss x 100/ g of powder sample.

*Water activity*

The water activity ($a_w$) was determined using a water activity meter (Decagon Devices, Inc., Pullman, WA) by the method described by Vuong et al. (2012)

### *2.3.3. Determination of chemical properties*

*Total phenolic content (TPC):*

TPC was determined according to the method described by Vuong et al. (2013). Briefly, 2.5 mL of Folin – Ciocalteu 10% (v/v) reagent was mixed with 0.5 mL of 10x diluted sample. The solution was then added to 2mL of $Na_2CO_3$ 7.5% (w/v), followed by thoroughly mixing and incubating in the dark at room temperature for 1 h. The absorbance at 765 nm was taken using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). Gallic acid was used as the standard and the results are expressed as mg of gallic acid equivalents per g of sample dry weight (mg GAE/g DW).

*Total flavonoids content (TFC):*

TFC was determined as previously described by (Vuong et al., 2013). Briefly, 2 mL of deionized water was mixed with 0.15 mL of $NaNO_2$ 5% (w/v) and 0.5 mL of 5x diluted sample. The solution was mixed thoroughly and then left at room temperature for 6 min. Subsequently, 0.15 mL of $AlCl_3$ 10% (w/v) was added and the solution was mixed well and allowed to stand for 6 min. Finally, 2 mL of NaOH 4% (w/v) and 0.7 mL of DI water were added to get the final volume of 5.5 mL. The solution was then mixed thoroughly and allowed to stand for 15 min at room temperature. The absorbance at 510 nm was taken. Catechin was used as the standard and the results are expressed as mg of catechin equivalents per gram of sample dry weight (mg CE/g DW).

*Total proanthocyanidins content (TPrC):*

TPrC was measured as previously described by (Vuong et al., 2013). Briefly, 0.5 mL of 5x diluted sample was mixed with 3 mL of vanillin 4% and followed by adding 1.5 mL of HCl 37%. The solution was mixed and allowed to stand for 15 min. The absorbance was measured at 500 nm. Catechin was used as the standard and the results are expressed as mg of catechin equivalents per gram of sample dry weight (mg CE/g DW).

*Total saponin content (TSC):*

TSC was determined as previously described by Vuong *et al.* (2013). Firstly, 0.5 mL of 5x diluted sample was mixed with 0.5 mL of vanillin 8%, followed by adding 5 mL of $H_2SO_4$ 72%. The solution was mixed thoroughly and placed on ice to cool. The mixture was then incubated in a water bath at 60ºC for 15 min. The mixture was cooled on ice for approximately 10 min and the absorbance was measured at 560 nm. Escin was used as the standard and the results are expressed as mg of escin equivalents per gram of sample dry weight (mg EE/g DW).

### *2.3.4. Determination of antioxidant properties*

To obtain a greater understanding on the antioxidant properties of *S. chinensis*, four antioxidant assays were employed including the ABTS assay; the DPPH assay; the CUPRAC assay; and the FRAP assay.

*ABTS (2,2'- azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) scavenging activity assay*

The ABTS assay described by Thaipong *et al.* (2006) was applied with some modifications. A stock solution was prepared by adding 10 mL of 7.4 mM ABTS solution to 10 mL of 2.6 mM $K_2S_2O_8$, left at room temperature in the dark for 15 h, and then stored at -20ºC until required. The working solution was freshly prepared by diluting 1 mL of stock solution with approximately 60 mL of methanol to obtain an absorbance value of 1.1 ± 0.02 at 734 nm on the day of analysis. 2.85 mL of the working solution was added to 0.15 mL of 10x diluted sample and left in the dark at room temperature for 2 h before its absorbance was read at 734 nm. Trolox was used as a standard and the results are expressed as mM trolox equivalents per gram of dry weight (mM TE/g dw).

*DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity assay*

The DPPH assay introduced by Thaipong *et al.* (2006) was applied with some modifications. A stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and stored at −20 °C until required. The working solution was prepared daily by mixing 10 mL stock solution with approximately 45 mL methanol to obtain an absorbance of 1.1±0.02 at 515 nm. 2.85 mL of working solution was added to 0.15 mL of diluted sample and then left under darkness at room temperature for 3 h before measuring the absorbance at 515 nm. Trolox was used as the standard and the results are expressed as mM of trolox equivalents per g of dry weight (mM TE/g dw).

*CUPRAC (Cupric reducing antioxidant capacity) assay*

The CUPRAC assay described by Apak *et al.* (2004) was employed with some modifications. Firstly, 1 mL of $CuCl_2$ was mixed with 1 mL of neocuproine and 1 mL of $NH_4Ac$ and 1.1 mL of 10x diluted sample. The sample was mixed well and incubated at room temperature for 1.5 h before measuring the absorbance at 450 nm. Trolox is used as the standard and the results are expressed as mM of trolox equivalents per g of sample (mM TE/g dw).

*FRAP (Ferric reducing antioxidant power) assay*

The FRAP assay as described by Thaipong *et al.* (2006) was employed with some modifications. A working FRAP solution was prepared by mixing acetate buffer 300 mM, Tripyridil-s-triazine (TPTZ) 10 mM (which was dissolved in HCl 40 mM) and $FeCl_3$ 20 mM in the ratio of 10:1:1 and mixed at 37ºC in a water bath (Ratek Instruments Pty. Ltd., Victoria, Australia) before use. 2.85 mL of the working FRAP solution was added to 0.15 mL of 10x diluted sample and incubated at room temperature in the dark for 30 min before its absorbance was read at 593 nm. Trolox was used as a standard and the results are expressed as mM trolox equivalents per gram of dry weight (mM TE/g dw).

### *2.3.5. HPLC analysis*

The crude extract of *S. chinensis* root were analysed using the Shimadzu HPLC system (M20, Shimadza Australia, Rydalmere, NSW, Australia) connected with a 250 mm x 4.6 mm Prodigy 5 µm ODS3-100A reversed phase column (Phenomenex Australia Pty. Ltd., Lane Cove, NSW, Australia) maintained at 35 ºC. The mobile phase consisted of solvent systems A and B; solvent A was deionized water: acetonitrile: orthophosphoric acid in the ratio of 96.8: 3: 0.2 (v/v/v); solvent B was 100% acetonitrile. The detector was set at 254 nm.

A linear gradient elution schedule was used as follows: 100% A from 1 to 10 min; 100% A to 90 A from 10 to 15 min; remaining at 90% A to 25 min; 90% A to 85% A from 25 to 40 min; 85% A to 10 % A from 40 min to 42 min; 10% A to 0% A from 42 to 52 min, remaining at 0% A to 57 min and then back to 100% A at 60 min with a post-run re-equilibration time of 15 min with 100% A before the next injection.

For fractionation, an EC-C18 reversed-phase column (Agilent Technologies Pty Ltd) was used. The linear gradient elution schedule was modified as follow: 100% A from 0 to 3 min; 100% B from 3 to 6 min; 100% A to 0% A from 6 to 20 min; remained at 0% A to 25 min; and 100% A from 25 to 30 min before the next injection. The injection volume was 50 µL and the flow rate was 1 mL/min. The column was maintained at 28ºC. The detector was set at 254 nm. Based on the retention time, the major peak was fractionated using an auto fraction collector and then freeze dried to powder form.

### *2.3.6. LC-MS analysis*

The fractionated compound was then identified using a Shimadzu LC/MS (LCMS 2020, Shimadzu) equipped with an electrospray ionization (ESI) interface. The mobile phase A was delivered at a flow rate of 0.2 mL/min. The injection volume was 10 µl. The mass spectrometer was operated at

negative mode with selected ion monitoring (SIM) and the parameters as follow: nebulizing gas flow 1.5 L/min, drying gas pressure 0.15 MPa, CDL temperature 250 ºC, block heater temperature 200 ºC.

### *2.3.7. Statistical analysis*

All analyses were performed at least in triplicate. Differences in means were considered statistically significant at $p<0.05$ and assessed using independent sample t-test and one way ANOVA with Duncan's post hoc multiple comparisons and Tukey Honest Significant Difference tests. The RSM experiments were designed using JMP software (version 11, 12, 13 and 14).

# *CHAPTER 3.*

# DETERMINATION OF THE SUITABLE PART OF *S. CHINENSIS* FOR EXTRACTION OF BIOACTIVE COMPOUNDS

## 3.1. Introduction

Different parts of *S. chinensis* including root, stem, leaf and fruit pulp have been studied previously. For example, Sellamuthu, Arulselvan, and Fakurazi (2014) isolated mangiferin from *S. chinensis* root and found that its anti-diabetic property is comparable to glibenclamide, the positive control drug. Tran et al. (2010) isolated three compounds from the ethyl acetate stem extract, seven compounds from the *n*-hexane stem extract and four compounds from the n-hexane leaf extract of *S. chinensis*. They further tested their anti-cancer property *in vitro* and found that three of the compounds from the stem and one from the leaf has activities against liver, lung, mouth and breast cancers. These studies revealed that composition or concentration of bioactive compounds could be varied in different parts of *S. chinensis*. However, none of the previous studies compared phytochemicals and antioxidant properties between different parts of *S. chinensis*. Therefore, the aim of this study was to analyse and compare phytochemical and antioxidant properties of the root, stem and leaf of *S. chinensis* for further extraction and isolation.

## 3.2. Results and Discussions

The results and detailed discussions of this study were published in the following paper:

Thanh Van Ngo, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2017). Phytochemical and antioxidant properties from different parts of *Salacia chinensis* L. *Journal of Biologically Active Products from Nature*, 7(5), 401-410.

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## 3.3. Conclusions

Different parts of *S. chinensis* had different levels of phytochemicals and various antioxidant properties. The *S. chinensis* root had the highest levels of phenolic compounds, flavonoids, proanthocyanidins and saponins; followed by the stem and the leaf. The root also had the higher

antioxidant capacity than the stem and the leaf. There were three major compounds in the root and the stem extracts; whereas, there were more compounds in the leaf, however in small quantities. From the results of this research, the root of *S. chinensis* was chosen as the plant material for all further steps.

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# Phytochemical and Antioxidant Properties from Different Parts of *Salacia chinensis* L.

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**Abstract:** The aim of this study was to compare phytochemical and antioxidant properties of different parts of *Salacia chinensis* L. The root of *S. chinensis* had the highest content of phenolics, flavonoids, proanthocyanidins and saponins (64.4 mg gallic acid equivalent /g dried weight (DW), 106 mg catechin equivalent (CE)/g DW, 43.5 mg CE/g DW and 799.9 mg escin equivalent /g DW, respectively), followed by the stem and leaf. Similarly, results from the four antioxidant assays (ABTS, DPPH, FRAP and CUPRAC) showed that the root of *S. chinensis* had the highest antioxidant capacity (632.2, 577.1, 443.3 and 365.0 µM trolox equivalent/g DW, respectively), followed by the stem while the leaf had the lowest antioxidant capacity. From HPLC chromatograms, three major compounds were detected from the root and stem of *S. chinensis*, while more major individual compounds in lower quantities were seen in the leaf. As the root contained high phytochemical and antioxidant properties, future studies are recommended to isolate and identify the major bioactive compounds from the root for further industrial utilization.

**Key words:** Antioxidant property; bioactive compound; *Salacia chinensis* L.



## Introduction

*Salacia chinensis* L., which belongs to the family *Celastraceae,* is distributed in numerous Asian countries, such as China, Vietnam, Malaysia, Thailand, India, and Sri Lanka [1, 2]. In traditional medicine, different parts of *S. chinensis*, including root, stem, leaf and fruit pulp, have been used for treatment of various ailments, such as arthritis, leucorrhoea, inflammation, fever, skin diseases, menstrual disorders, and spermatorrhoea [3-5]. For example, Vietnamese and Lao people use the root and stem for treatment of rheumatism, back-pain and debility [4, 5]. The root of *S. chinensis* (or the combination of this plant with other species in genus *Salacia*) is used by Indian people for prevention and treatment of diabetes and obesity [3, 6]. Therefore, it is suggested for researchers that these parts of *S. chinensis* might contain potent therapeutic agents. .

Different components of *S. chinensis* including root, stem, leaf and fruit pulp have been used in numerous researches.. For example, Ngo *et al.*[10] has investigated the impact of different solvents on recovery of total phenolics, flavonoids, proanthocyanidins and saponins as well as antioxidant properties of the root of *S. chinensis*. Sellamuthu *et al.* [1] has used the same material to isolated mangiferin, an anti-diabetic compounds which is comparable to glibenclamide, the positive control drug. In another research, from stem

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**Fig. 1.** *Salacia chinensis* L. (collected by TVN in Nghe An province (Vietnam) in March 2016)

and leaf of *S. chinensis*, Tran *et al.* [2] has isolated three compounds from ethyl acetate partition and seven compounds from n-hexane partition of the stem and four compounds from n-hexane partition of the leaf. These compounds were then investigated for anti-cancer activities. The result showed that three of compounds from the stem and one from the leaf have activities against liver, lung, mouth and breast cancers with different levels.

However, although the fact that there are so many reports of *S. chinensis* these studies have not compared the phytochemical and antioxidant properties from the root, stem and leaf of *S. chinensis*. Therefore, the aim of this study was to analyse and compare phytochemical and antioxidant properties of the root, stem and leaf of *S. chinensis* for further extraction and isolation.

## Material and methods

### *Material*

The root, stem and leaf of *S. chinensis* L. were collected from the forest in Nghe An province (Vietnam) in March 2016 and authenticated by Associate Professor Vu Quang Nam, Department of Forest Plant Resources, College of Forestry Biotechnology, Vietnam National University of Forestry. After collection, these materials were cut into small pieces and sun-dried for 2 days and then ground into small particles using a commercial cutter. The powders were then sieved using a steel mesh sieve (1.4 mm EFL 2000; Endecotts Ltd., London, England) and kept at -20°C for further analysis.

### Methods

#### *Extraction process*

The extraction method, which was optimized in our previous study [10], was employed for extraction of the *S. chinensis* L. materials in the current study. Briefly, 1 g of sample was added into 100 mL of 50 % ethanol. The mixture was then put in an ultrasonic bath (Soniclean, 220 V, 50 Hz and 250 W; Soniclean Pty Ltd., Thebarton, Australia) with pre-set conditions: temperature of 35°C, time of 30 min and power of 150 W (the mixture was vortexed thoroughly once every five minutes). Next, the extract was immediately cooled on ice to room temperature (RT) and then filtered using filter paper (Whatman, 11 µm pore size). Subsequently, the extract was stored in the dark at -18°C for further determination of extractable solids, total phenols content (TPC), total flavonoids content (TFC), total proanthocyanidins content (TPrC), total saponins content (TSC), and antioxidant capacity (DPPH, FRAP, CUPRAC and ABTS assays).

### Determination of chemical properties

#### *Total phenolic content (TPC)*

TPC was determined as previously described by Vuong *et al.* [11]. 2.5 mL of Folin - Ciocalteu reagent 10 % (v/v) was mixed with 0.5 mL of diluted sample. The solution was then added to 2 mL of $Na_2CO_3$ 7.5 % (w/v), followed by thoroughly mixing and incubating in the dark at RT for 1 h. The absorbance at 760 nm was taken using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia), a reagent blank was set at base level (zero). Gallic acid was used as the standard for a calibration curve and the results were expressed as mg of gallic acid equivalents per g of sample dry weight (mg GAE/g DW).

#### Total flavonoids content (TFC)

TFC was determined as previously described by Dailey and Vuong [12]. 2 mL of deionized water

was mixed with 0.15 mL of $NaNO_2$ 5 % (w/v) and 0.5 mL of diluted sample. The solution was mixed thoroughly and then left at room temperature for 6 min. Subsequently, 0.15 mL of $AlCl_3$ 10 % (w/v) was added and the solution was mixed well and allowed to stand for 6 min. Finally, 2 mL of NaOH 4 % (w/v) and 0.7 mL of deionized water were added to get the final volume of 5.5 mL. The solution was then mixed thoroughly and allowed to stand for 15 min at RT. The absorbance at 510 nm was taken using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia), a reagent blank was set at base level (zero). Catechin was used as the standard for a calibration curve and the results were expressed as mg of catechin equivalents per gram of sample dry weight (mg CE/g DW).

**Total proanthocyanidins content (TPrC)**

TPrC was measured as previously described by Dailey and Vuong [12]. 0.5 mL of diluted sample was mixed with 3 mL of vanillin 4 % and followed by adding 1.5 mL of HCl 37 %. The solution was mixed and allowed to stand for 15 min. The absorbance was measured using a spectrophotometer at 500 nm, a reagent blank was set at base level (zero). Catechin was used as the standard for a calibration curve and the results were expressed as mg of catechin equivalents per gram of sample dry weight (mg CE/g DW).

**Total saponin content (TSC)**

TSC of *Salacia chinensis* root, stem and leaf was determined as previously described by Vuong *et al.* [11]. 0.5 mL of diluted sample was mixed with 0.5 mL of vanillin 8 %, followed by adding 5 mL of $H_2SO_4$ 72 %. The solution was mixed thoroughly and placed on ice to cool. The mixture was then incubated in a water bath at 60°C for 15 min. The mixture was then cooled on ice for approximately 10 min and the absorbance was then measured at 560 nm, a reagent blank was set at base level (zero). Escin was used as the standard for a calibration curve and the results were expressed as mg of escin equivalents per gram of sample dry weight (mg EE/g DW).

**Determination of antioxidant properties**

To compare the antioxidant properties between the root, stem and leaf of *Salacia chinensis*, four antioxidant assays were employed including the ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) assay, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, the CUPRAC (cupric reducing antioxidant capacity) assay, and the FRAP (Ferric reducing antioxidant power) assay.

**ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) assay**

ABTS assay described by Thaipong *et al.* [13] was applied with some modifications. A stock solution was prepared by adding 10 mL of 7.4 mM ABTS solution to 10 mL of 2.6 mM $K_2S_2O_8$ and left at RT in the dark for 15 h, and then stored at -20°C until required. The working solution was freshly prepared by diluting 1 mL of stock solution with approximately 60 mL of methanol to obtain an absorbance value of 1.1 ± 0.02 at 734 nm at the day of analysis. 2.85 mL of the working solution was added to 0.15 mL of diluted sample and left in the dark at RT for 2 h before its absorbance was read at 734 nm using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). Trolox was used as a standard and the results were expressed as mg trolox equivalents per gram of dry weight (mg TE/g dw).

**DPPH (2,2-diphenyl-1-picrylhydrazyl) assay**

DPPH assay introduced by Thaipong *et al.* [13] was applied with some modifications. A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL methanol, then stored at -20°C until required. The working solution was then prepared fresh by mixing 10 mL stock solution with approximately 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 at 515 nm. 2.85 mL of working solution was added to 0.15 mL of diluted sample and then left under darkness at RT for 3 h before measuring the absorbance at 515 nm using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). Trolox was used as standard for a calibration curve and the results were expressed as mg of trolox equivalents per g of dry weight (mg TE/g dw).

**Cupric Reducing Antioxidant Capacity assay (CUPRAC)**

CUPRAC assay described by Apak *et al.* [14]

was employed with some modifications. 1 mL of $CuCl_2$ was mixed with 1 mL of neocuproine and 1 mL of $NH_4Ac$ and 1.1 mL of diluted sample. The sample was mixed well and incubated at RT for 1.5 h before measuring the absorbance at 450 nm using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). Trolox was used as standard for a calibration curve and the results were expressed as mg of trolox equivalents per g of sample (mg TE/g dw).

**Ferric Reducing Antioxidant Power assay (FRAP)**

FRAP assay described by Thaipong *et al.* [13] was employed with some modifications. A working FRAP solution was prepared by mixing 300 mM Acetate buffer, 10 mM tripyridil-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$ in the ratio of 10:1:1 and mixed at 37°C in a water bath (Ratek Instruments Pty. Ltd., Victoria, Australia) before use. 2.85 mL of the working FRAP solution was added to 0.15 mL of diluted sample and incubated at RT in the dark for 30 min before its absorbance was read at 593 nm using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). Trolox was used as a standard and the results were expressed as mg trolox equivalents per gram of dry weight (mg TE/g dw).

**Analysis of major bioactive components using High performance Liquid Chromatography (HPLC)**

The acetone extracts of *S. chinensis* root, stem and leaf were filtered using a 0.45 µm Phenex Syringe filter (Phenomenex). The bioactive components were then measured using a Shimadzu HPLC system (Shimadza Australia, Rydalmere, NSW, Australia) using UV detection at 254 nm, on a 250 mm x 4.6 mm Prodigy 5 µm ODS3 - 100A reversed-phase column (Phenomenex Austra-lia Pty. Ltd., Lane Cove, NSW, Australia), which was maintained at 35°C. The mobile phases consisted of solvent systems A and B; solvent A was deionized water : acetonitrile : orthophosphoric acid, 96.8:3:0.2 (v/v/v), solvent B was 100 % acetonitrile.

A gradient elution schedule was used as follow: 100 % A from 0 to 10 min; a linear gradient from 100 % A to 90 % A from 10 to 15 min and remained at 90 % A to 25 min; from 90 % A to 85 % A from 25 to 40 min; from 85 % A to 10 % A from 40 min to 42 min; 10 % A to 0 % A from 42 to 52 min and remained at 0 % A to 57 min and then back to 100 % A at 60 min with a post-run re-equilibration time of 15 min with 100 % A before the next injection. The injection volume was 40 µL of the extract solution onto the HPLC and the flow rate was 1 mL/min. In the HPLC graph, each peak represented one individual compound. The concentration of the compound was calculated by comparing the area of the peak with the area of gallic acid and expressed by mg gallic acid equivalents/g (mg GAE/g).

**Statistical analysis**

The one-way analysis of variance (ANOVA) and the Least Significance Difference (LSD) were conducted using the IBM SPSS statistical software version 23. Data were reported as means ± standard deviations. Differences between the mean levels of the components in the different experiments were taken to be statistically significant at $p<0.05$.

**Results and discussion**

***Total phenolic compounds***

The total phenolic compounds in the root, stem and leaf extracts of *S. chinensis* are presented in Table 1. The results showed that the levels of TPC varied in different parts of *S. chinensis*, in which the root had the highest content of phenolics (64.4 mg GAE/g DW), followed by stem and leaf extracts (41.9 and 35.9 mg GAE/g DW, respectively). These results revealed that the stem and leaf only accounted for 65 % and 55 % of the TPC of the root. These findings are supported by previous studies, which found that different parts of plant materials had different levels of phenolic compounds. For example, Martin-Puzon and Rivera [15] found that the leaves of *Glinus oppositifolius* L. had the highest content of phenolics, followed by the root and stem. Ferracane *et al.* [16] reported that the best source of phenolic compounds in burdock (*Arctium lappa*) came from the seeds but not the leaves or roots. Interestingly, the result of this study is contrary to a

**Table 1. Total phenolics, flavonoids and proanthocyanidins in *S. chinensis* root, stem and leaf extracts**

| | TPC (mg GAE/g DW) | TFC (mg CE/g DW) | TPrC (mg CE/g DW) |
|---|---|---|---|
| Root | $64.4 \pm 2.0^{a}$ | $106.5 \pm 3.0^{a}$ | $43.5 \pm 0.14^{a}$ |
| Stem | $41.9 \pm 4.6^{b}$ | $79.3 \pm 2.1^{b}$ | $41.0 \pm 1.4^{b}$ |
| Leaf | $35.9 \pm 1.4^{b}$ | $42.0 \pm 1.1^{c}$ | $27.5 \pm 1.1^{c}$ |

Data are the means ± standard deviations for at least triplicates.
Figures with different superscript letters in the same column are significantly different at $p<0.05$
TPC: Total phenolic content
GAE: Gallic acid equivalents
TFC: Total flavonoid content
CE: Catechin equivalents
TPrC: Total proanthocyanidin content
DW: Dry weight



study conducted by Keeragalaarachchi *et al.* [17] which showed that total phenolic and flavonoid content of *S. chinensis* leaf were higher than those of the stem. The difference might come from the difference of the material's ages, the places that the materials were collected. However, the result of this research is supported by the fact that in different traditional medicine systems (such as Vietnamese, Lao and Indian medicines), people always prefer using the root and stem parts than the leaf of this plant.

In comparison with other *Salacia* species, levels of TPC of *S. chinensis* root, stem and leaf extracts are much higher than those of *S. pallescens* (8.6 mg GAE/g DW) [18], but are lower than those of *S. oblonga* aerial and root extracts (297 and 275 mg GAE/g DW, respectively) [19]. In comparison with other plant materials, such as *Dalbergia saxatilis* and *Icacina tricantha*, *S. chinensis* root had more than 10 times the levels of TPC than these plant materials (4.47 and 5.00 mg GAE/g, respectively) [18], revealing that *S. chinensis* root is a rich source of phenolic compounds.

**Total flavonoids**

The total flavonoids in the root, stem and leaf of *S. chinensis* also varied considerably (Table 1). The root had the highest level of flavonoids (106.5 mg CE/g DW), followed by stem and leaf, which had significantly lower levels of flavonoids (79.3 and 42.0 mg CE/g DW, respectively). Flavonoids in the stem and leaf accounted for only 74 % and 39 % of the flavonoids found in the root.

These findings revealed that flavonoids of *S. chinensis* are mainly developed in the root, and that the root is excellent starting material for the extraction of flavonoids from *S. chinensis*. As mentioned in previous section, these findings differ to results reported in the study of Keeragalaarachchi *et al.* [17]. Another study on *Glinus oppositifolius* [15] also found that different parts of plant had different levels of flavonoids. In particular, the leaves of *G. oppositifolius* was the best source of flavonoids when compared to the roots and stems.

**Proanthocyanidins**

Proanthocyanidins are an important subclass of phenolic compounds due to their potent antioxidant properties [20]. The results in Table 1 showed that the root also had the highest content of proanthocyanidins (43.5 mg CE/g DW), followed by the stem and the leaf (41.0 and 27.5 mg CE/g DW, respectively). The content of proanthocyanidins in the root and stem of *S. chinensis* were higher than those of *Senecio anteuphorbium* (36.7 mg CE/ g DW) [21] and lemon pomace (1.86 mg CE/g DW) [22]. These findings indicated that the root of *S. chinensis* is also a rich source of proanthocyanidins.

**Saponins**

The results (Table 2) showed that the root of *S. chinensis* contained the highest content of saponins (799.9 mg EE/g DW), followed by the stem (733.4 mg EE/g DW); whereas, the saponin content in the leaf extract accounted for only approximately 50 % of the saponin content of the root (436.4 mg EE/g DW). The saponin content of *S. chinensis* root is higher than those of other species, such as *Artemisia capillaries, Codonopsis pilosula* and *Rehmannia glutinosa* (121.7, 97.4 and 90.8 mg EE/g DW, respectively) [23]. As saponins have been linked with numerous health benefits, such as improvement of the immune system, decreasing the risk of heart attack and the prevention of certain cancers [24,25], it is necessary to isolate saponins from *S. chinensis* for further assessment of their potential health benefits.

**Antioxidant properties**

Results from four antioxidant assays, including ABTS, DPPH, FRAP and CUPRAC (**Table 3**), showed that the root had the highest antioxidant properties, followed by the stem and leaf extracts. These findings can be explained by the fact that the bioactive compounds, such as phenolics and saponins are the major antioxidants in *S. chinensis*, particularly in the root. This statement is in agreement with previous studies which demonstrated that phenolic compounds are mainly responsible for antioxidant properties of *S. chinensis* [26], burdock (*Arctium lappa*) [16].

As previously reported, the root had higher levels of phenolics and saponins than the stem and leaf; consequently the root had significantly higher antioxidant properties than those of the stem and leaf. These findings further confirmed that the root of *S. chinensis* should be used as starting mate-

**Table 2. Total saponins content in *S. chinensis* root, stem and leaf extracts**

| Part of plant | TSC (mg EE/g DW) |
|---|---|
| Root | 799.9 ± 18.9[a] |
| Stem | 733.4 ± 54.7[b] |
| Leaf | 436.4 ± 18.0[c] |

Data are the means ± standard deviations for at least triplicates
Figures with different superscript letters are significantly different at $p < 0.05$
TSC: Total saponin content
EE: Escin equivalents
DW: Dry weight

**Table 3. Antioxidant properties of *S. chinensis* root, stem and leaf extracts**

| Part of plant | ABTS | DPPH | FRAP | CUPRAC |
|---|---|---|---|---|
| Root | 632.2 ± 10.7[a] | 577.1 ± 18.6[a] | 443.3 ± 08.3[a] | 365.0 ± 05.2[a] |
| Stem | 565.2 ± 10.9[b] | 442.1 ± 22.1[b] | 291.4 ± 18.4[b] | 167.0 ± 11.5[b] |
| Leaf | 476.8 ± 04.1[c] | 352.6 ± 18.0[c] | 286.6 ± 10.4[c] | 214.3 ± 14.8[c] |

Data are expressed as μM TE/g DW and are the means ± standard deviations for at least triplicates.
Figures with different superscript letters in the same column are significantly different at $p<0.05$
ABTS: ABTS radical scavenging capacity
DPPH: DPPH radical scavenging capacity
FRAP: Ferric reducing antioxidant power
CUPRAC: Cupric reducing antioxidant capacity
TE: Trolox equivalents
DW: Dry weight

rial for further extraction, isolation and testing for its potential health benefits.

**Individual compounds identified by HPLC**

The HPLC chromatogram (Fig. 2), revealed that there were three major bioactive compounds in the root and stem of *S. chinensis;* whereas, there were more individual bioactive compounds identified in the leaf of *S. chinensis*. Overall, the total content of major bioactive compounds in *S. chinensis* root (113.13 mg GAE/g DW) was significantly higher than those of the stem or the leaf (75.9 and 91.25 mg GAE/g DW, respectively; Table 4). Of note, there are two compounds present in all of the three parts of *S. chinensis*; compound numbers 1 and 3. Compound 5 was found in the root and stem but not in the leaf of *S. chinensis*, while the remaining compounds (2, 4, 6, 7 and 8) were only found in the leaf of the plant. These findings indicated that the root had less bioactive compounds but in higher quantities; whereas the leaf had more individual compounds but in lower quantities.

From Fig. 2, we can see that the peaks are well - separated, and hence these individual compounds should be easy to be isolated by using HPLC preparative system. Therefore, future studies are needed to isolate, identify and quantify these compounds for further testing their biological properties.

**Conclusion**

This study found that *S. chinensis* is a rich source of phenolic compounds and saponins. However, different parts of *S. chinensis* had different levels of phytochemicals and various antioxidant

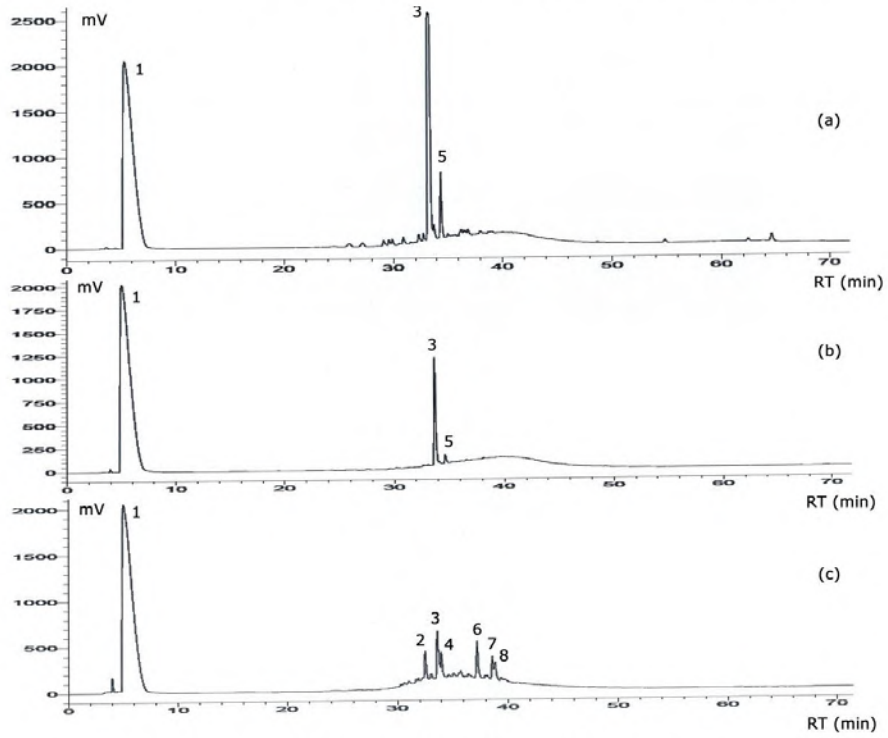


**Fig. 2.** Representative HPLC chromatogram detected at 254 nm for the root (a), stem (b) and leaf (c) extracts of *S. chinensis*

**Table 4. Individual compounds in different parts of *S. chinensis***

| Peak/ compound | Quantity (mg GAE/g DW) | | |
|---|---|---|---|
| | Root | Stem | Leaf |
| 1 | 77.41 ± 3.49[a] | 68.13 ± 1.20[b] | 67.51 ± 1.03[b] |
| 2 | - | - | 4.26 ± 0.79 |
| 3 | 30.40 ± 4..51[a] | 6.94 ± 0.66[b] | 4.69 ± 0.52[b] |
| 4 | - | - | 3.54 ± 0.32 |
| 5 | 5.32 ± 0.19[a] | 0.83 ± 0.65[b] | - |
| 6 | - | - | 5.59 ± 0.12 |
| 7 | - | - | 2.46 ± 0.05 |
| 8 | - | - | 3.20 ± 0.63 |
| Total | 113.13 | 75.9 | 91.25 |

Data are the means ± standard deviations for at least triplicates
Figures with different superscript letters in the same row are significantly different at $p<0.05$
GAE: Gallic acid equivalents

properties. The *S. chinensis* root had the highest levels of phenolic compounds, flavonoids, proanthocyanidins and saponins; it was followed by the stem and the leaf. The root also had the higher antioxidant capacity than the stem and the leaf. There were three major compounds in the root and the stem extracts; whereas, there were more compounds which were found in the leaf, however in small quantities. Future studies are recommended to extract and isolate major bioactive compounds from the root, stem and leaf of *S. chinensis* for further examination of their biological properties.

**Competing interests**

The authors declare no conflict of interests.

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# *CHAPTER 4.*

# DETERMINATION OF A SUITABLE SOLVENT FOR EXTRACTION OF BIOACTIVE COMPOUNDS FROM *S. CHINENSIS*

## 4.1. Introduction

One of the most important factors affecting the extraction efficiency of bioactive compounds from plant materials, and their subsequent health benefits, is the extraction solvent. Traditionally, *S. chinensis* has been brewed or decocted in water for use as a traditional medicine in some Asian countries, such as India, Sri Lanka and Vietnam (Jayawardena *et al.*, 2005, Vo, 1997). As plant materials contain bioactive compounds with a wide ranges of polarities, previous studies have used methanol, petroleum ether, chloroform, ethanol, acetone and water as the solvents for extracting bioactive compounds from *S. chinensis* for further analysis (Periyar *et al.*, 2014, Sikarwar and Patil, 2012, Chavan *et al.*, 2012). Although, extraction solvents have been extensively studied in other plant materials, such as macadamia skin waste (Dailey and Vuong, 2015), *S. chinensis* fruit pulp (Chavan *et al.*, 2015b) and basil leaf (Złotek *et al.*, 2016), none of the previous studies have compared the impact of different solvents on the extraction efficiency of bioactive compounds from the *S. chinensis* root. Therefore, this study aimed to determine the impact of different common solvents (water, absolute methanol, ethanol, acetone, 50% methanol, 50% ethanol and 50% acetone) on the extraction efficiency of bioactive compounds, as well as antioxidant capacity from the root of *S. chinensis,* in order to identify the most suitable solvent for further extraction and isolation of bioactive and antioxidant compounds from *S. chinensis.*

## 4.2. Results and discussions

The results and detailed discussions were published in the following Research paper:

Thanh Van Ngo, Christopher James Scarlett, Michael Christian Bowyer, Phuong Duc Ngo, and Quan Van Vuong (2017). Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. *Journal of Food Quality*, 2017, 1 – 8. https://doi.org/10.1155/2017/9305047

### 4.3. Conclusions

This study demonstrated that the extraction solvents play an important role in the extraction of important bioactive groups from *S. chinensis*. Absolute organic solvents, or water, were not effective, whereas 50% ethanol and 50% acetone were solvents of choice for yielding a high content of extractable solids, phenolic compounds as well as flavonoids.

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*Research Article*

# Impact of Different Extraction Solvents on Bioactive Compounds and Antioxidant Capacity from the Root of *Salacia chinensis* L.

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This study aimed to study the impact of selected common organic solvents on extractable solids, phytochemical composition, and antioxidant capacity of *S. chinensis*. The results showed that the tested solvents played an important role in extraction of total solid and phytochemical composition as well as antioxidant capacity of *S. chinensis*. Acetone (50% v/v) was found to be the optimal extraction solvent for extractable solids (12.2%), phenolic compounds (60 mg GAE/g DW), flavonoids (100 mg CE/g DW), proanthocyanidins (47.4 mg CE/g DW), and saponins (754 mg EE/g DW) as well as antioxidant capacity (ABTS 334 mM TE/g DW, DPPH 470 mM TE/g DW, FRAP 347 mM TE/g DW, and CUPRAC 310 mM TE/g DW). The extract prepared from 50% acetone had high levels of bioactive compounds (TPC 555 mg GAE/g CRE, flavonoids 819 mg CE/g CRE, proanthocyanidins 392 mg CE/g CRE, and saponins 1,880 mg EE/g CRE) as well as antioxidant capacity (ABTS 414 mM TE/g, DPPH 407 mM TE/g, FRAP 320 mg TE/g, and CUPRAC 623 mM TE/g), thus further confirming that 50% acetone is the solvent of choice. Therefore, 50% acetone is recommended for extraction of phenolic compounds, their secondary metabolites, saponins, and antioxidant capacity from the root of *S. chinensis* for further isolation and utilisation.

## 1. Introduction

*Salacia chinensis* L. (*S. chinensis*) belongs to the genus *Salacia* of the family Celastraceae. The *S. chinensis* tree can grow up to 3–10 m in height and 16 cm in diameter in the tropical forests of Africa, China, India, and South East Asia including Laos, Cambodia, and Vietnam. *S. chinensis* has been widely used as a traditional medicine to treat various ailments such as arthritis, leucorrhoea, inflammation, fever, as an astringent, and amenorrhea [1]. In Vietnam, the root of *S. chinensis* has been used for the treatment of rheumatism, back-pain, and debility [2]. *S. chinensis* has been linked with antimicrobial, antidiabetic, antioxidant, antimutagenic, and anticancer properties due to the material being found to contain high levels of phenolic and flavonoid compounds and possessing strong antioxidant capacity [1].

One of the most important factors affecting the extraction efficiency of bioactive compounds from plant materials and their consequent health benefits is the extraction solvent. Traditionally, *S. chinensis* has been brewed or decocted in water for use as a traditional medicine in some Asian countries, such as India, Sri Lanka, and Vietnam [2, 3]. Previous studies have used methanol, petroleum ether, chloroform, ethanol, acetone, and water as the solvents for extracting bioactive compounds from *S. chinensis* for further analysis [4–6]. Although extraction solvents have been extensively studied in other plant materials, such as macadamia skin waste [7], *S. chinensis* fruit pulp [8], and basil leaf [9], none of the previous studies have compared the impact of different common solvents on the extraction efficiency of phenolic compounds from the *S. chinensis* root. Therefore, this study aimed to determine the impact of different common solvents

(water, absolute methanol, ethanol, acetone, 50% methanol, 50% ethanol, and 50% acetone) on the extraction efficiency of bioactive compounds, as well as antioxidant capacity from the root of *S. chinensis*, in order to identify the most appropriate solvent for further extraction and isolation of bioactive compounds and antioxidant capacity from *S. chinensis*.

## 2. Materials and Methods

*2.1. Materials.* The root of *S. chinensis* L. was collected from Nghe An Province (Vietnam) in May 2015. After collection, the root was sun-dried, which is the traditional preparation method to obtain the dried sample. The dried root was then ground into small particles using a commercial blender (John Morris Scientific, Chatswood, NSW, Australia) and then sieved using a steel mesh sieve (1.4 mm EFL 2000; Endecotts Ltd., London, England). The ground root was kept at −20°C for further analysis.

*2.2. Methods for Characterisation of the Root of S. chinensis*

*2.2.1. Extraction Process.* Seven common solvents were used for the extraction of bioactive compounds from the ground root of *S. chinensis* L. including water, absolute methanol, ethanol, acetone (the polarity indexes are 10.2, 5.1, 4.3, and 5.1, resp.), 50% methanol, 50% ethanol, and 50% acetone. The sample was extracted in these solvents by firstly adding 1 g of sample into 100 mL of solvent. The mixture was then put in an ultrasonic bath (Soniclean, 220 V, 50 Hz and 250 W; Soniclean Pty Ltd., Thebarton, Australia) with preset conditions: temperature of 35°C, time of 30 min, and power of 150 W (the mixture was vortexed thoroughly once every five minutes). Next, the extract was immediately cooled on ice to room temperature and then filtered using filter paper (Whatman, 11 $\mu$m pore size). Subsequently, the extract was stored in the dark at −18°C for further determination of the extractable solids, total phenols content (TPC), total flavonoids content (TFC), total proanthocyanidins content (TPrC), total saponins content (TSC), and antioxidant capacity (DPPH, FRAP, CUPRAC, and ABTS assays).

*2.2.2. Preparation of Saponin and Phenolic Enriched Extract from S. chinensis.* Firstly, 100 g of sample was added into 2 L of 50% acetone. The mixture was then put in an ultrasonic bath (Soniclean, 220 V, 50 Hz and 250 W, Soniclean Pty Ltd., Thebarton, Australia) with preset condition: temperature of 35°C, time of 30 min, and power of 150 W (the mixture was vortexed thoroughly once every five minutes). Next, the extract was immediately cooled on ice to room temperature and then filtered using filter paper (Whatman, 11 $\mu$m pore size). Subsequently, the extract was condensed to the volume of 150 mL using a rotary evaporator (Buchi Rotavapor B-480, Buchi Australia, Noble Park, Victoria, Australia) and then freeze-dried to yield the crude extract. This crude extract was stored in dark containers at −20°C for further analysis, including TPC, TFC, TPrC, TSC, and antioxidant capacities (DPPH, FRAP, CUPRAC, and ABTS assays).

*2.2.3. Determination of Extractable Solids.* Extractable solids were determined according to a method described previously with minor modification [10]. 3 mL of the extract was put in a pottery tray and then placed in an oven set at 120°C for drying during 5 h to remove all moisture. Extractable solids (ES) were calculated by the following formula:

$$\text{ES}(\%) = \frac{W \times 100}{3} \quad (1)$$

($W$ is weight of 3 mL of the extraction after drying, in grams).

*2.2.4. Determination of Chemical Properties*

*Total Phenolic Content (TPC).* TPC of *Salacia chinensis* root was determined as previously described by [11]. 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent was mixed with 0.5 mL of diluted sample. The solution was then added to 2 mL of 7.5% (w/v) $Na_2CO_3$, followed by thorough mixing and incubating in the dark at room temperature for 1 h. The absorbance at 760 nm was taken using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia); a reagent blank was set at base level (zero). Gallic acid was used as the standard for a calibration curve and the results were expressed as mg of gallic acid equivalents per g of sample dry weight (mg GAE/g DW).

*Total Flavonoids Content (TFC).* TFC of *Salacia chinensis* root was determined as previously described by Dailey and Vuong [12]. 2 mL of deionized water was mixed with 0.15 mL of 5% (w/v) $NaNO_2$ and 0.5 mL of diluted sample. The solution was mixed thoroughly and then left at room temperature for 6 min. Subsequently, 0.15 mL of 10% (w/v) $AlCl_3$ was added and the solution was mixed well and allowed to stand for 6 min. Finally, 2 mL of 4% (w/v) NaOH and 0.7 mL of deionized water were added to get the final volume of 5.5 mL. The solution was then mixed thoroughly and allowed to stand for 15 min at room temperature. The absorbance at 510 nm was taken using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia); a reagent blank was set at base level (zero). Catechin was used as the standard for a calibration curve and the results were expressed as mg of catechin equivalents per gram of sample dry weight (mg CE/g DW).

*Total Proanthocyanidins Content (TPrC).* TPrC of *Salacia chinensis* root was measured as previously described by Dailey and Vuong [12]. 0.5 mL of diluted sample was mixed with 3 mL of 4% vanillin and followed by adding 1.5 mL HCl 37%. The solution was mixed and allowed to stand for 15 min. The absorbance was measured using a spectrophotometer at 500 nm; a reagent blank was set at base level (zero). Catechin was used as the standard for a calibration curve and the results were expressed as mg of catechin equivalents per gram of sample dry weight (mg CE/g DW).

*Total Saponin Content (TSC).* TSC of *Salacia chinensis* root was determined as previously described by Vuong et al. [11]. 0.5 mL of diluted sample was mixed with 0.5 mL of 8% vanillin, followed by adding 5 mL $H_2SO_4$ (72%). The solution was mixed thoroughly and placed on ice to cool. The mixture was then incubated in a water bath at 60°C for 15 min. The

mixture was then cooled on ice for approximately 10 min and the absorbance was then measured at 560 nm; a reagent blank was set at base level (zero). Escin was used as the standard for a calibration curve and the results were expressed as mg of escin equivalents per gram of sample dry weight (mg EE/g DW).

*2.2.5. HPLC Analysis of Bioactive Components in the Crude Extract of S. chinensis Root.* The solution made of 0.02 g of crude extract of *S. chinensis* root diluted in 2 mL of 50% acetone was filtered using a 0.45 $\mu$m Phenex Syringe filter (Phenomenex). The bioactive components were then measured using a Shimadzu HPLC system (Shimadzu Australia, Rydalmere, NSW, Australia) using UV detection at 254 nm, on a 250 mm × 4.6 mm Prodigy 5 $\mu$m ODS3–100A reversed-phase column (Phenomenex Australia Pty. Ltd., Lane Cove, NSW, Australia) which was maintained at 35°C. The mobile phases consisted of solvent systems A and B; solvent A was deionized water : acetonitrile : orthophosphoric acid, 96.8 : 3 : 0.2 (v/v/v); solvent B was 100% acetonitrile.

A gradient elution schedule was used as follows: 100% A from 0 to 10 min; a linear gradient from 100% A to 90% A from 10 to 15 min and remaining at 90% A to 25 min; from 90% A to 85% A from 25 to 40 min; from 85% A to 10% A from 40 min to 42 min; 10% A to 0% A from 42 to 52 min and remaining at 0% A to 57 min and then back to 100% A at 60 min with a postrun reequilibration time of 15 min with 100% A before the next injection. The injection volume was 50 $\mu$L of the crude extract solution onto the HPLC and the flow rate was 1 mL/min.

*2.2.6. Determination of Antioxidant Properties.* To obtain a greater understanding on the antioxidant properties of the *Salacia chinensis* root, four antioxidant assays were employed including the ABTS (2,2′- azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) assay, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, the CUPRAC (cupric reducing antioxidant capacity) assay, and the FRAP (ferric reducing antioxidant power) assay.

*ABTS Assay.* ABTS assay described by Thaipong et al. [13] was applied with some modifications. A stock solution was prepared by adding 10 mL of 7.4 mM ABTS solution to 10 mL of 2.6 mM $K_2S_2O_8$ and left at room temperature in the dark for 15 h and then stored at −20°C until required. The working solution was freshly prepared by diluting 1 mL of stock solution with approximately 60 mL of methanol to obtain an absorbance value of 1.1 ± 0.02 at 734 nm at the day of analysis. 2.85 mL of the working solution was added to 0.15 mL of diluted sample and left in the dark at room temperature for 2 h before its absorbance was read at 734 nm using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). Trolox was used as a standard and the results were expressed as mg trolox equivalents per gram of dry weight (mg TE/g dw).

*DPPH Assay.* DPPH assay introduced by Thaipong et al. [13] was applied with some modifications. A stock solution was prepared by dissolving 24 mg DPPH in 100 mL methanol and then stored at −20°C until required. The working solution was then prepared fresh by mixing 10 mL stock solution with approximately 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 at 515 nm. 2.85 mL of working solution was added to 0.15 mL of diluted sample and then left under darkness at room temperature for 3 h before measuring the absorbance at 515 nm using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). Trolox was used as standard for a calibration curve and the results were expressed as mg of trolox equivalents per g of dry weight (mg TE/g dw).

*CUPRAC Assay.* CUPRAC assay described by Apak et al. [14] was employed with some modifications. 1 mL of $CuCl_2$ was mixed with 1 mL of neocuproine and 1 mL of $NH_4Ac$ and 1.1 mL of diluted sample. The sample was mixed well and incubated at room temperature for 1.5 h before measuring the absorbance at 450 nm using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). Trolox was used as standard for a calibration curve and the results were expressed as mg of trolox equivalents per g of sample (mg TE/g dw).

*FRAP Assay.* FRAP assay described by Thaipong et al. [13] was employed with some modifications. A working FRAP solution was prepared by mixing 300 mM Acetate buffer, 10 mM tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$ in the ratio of 10 : 1 : 1 and mixed at 37°C in a water bath (Ratek Instruments Pty. Ltd., Victoria, Australia) before use. 2.85 mL of the working FRAP solution was added to 0.15 mL of diluted sample and incubated at room temperature in the dark for 30 min before its absorbance was read at 593 nm using a UV spectrophotometer (Varian Australia Pty. Ltd., Victoria, Australia). Trolox was used as a standard and the results were expressed as mg trolox equivalents per gram of dry weight (mg TE/g dw).

*2.3. Statistical Analysis.* The one-way analysis of variance (ANOVA) and the Least Significance Difference (LSD) were conducted using the IBM SPSS statistical software version 23. Data were reported as averages ± standard deviations. Differences between the mean levels of the components in the different experiments were taken to be statistically significant at $p < 0.05$. The Pearson correlation test was employed to determine the correlation coefficients among bioactive compounds and different antioxidant assays.

## 3. Results and Discussion

*3.1. Impact of Extraction Solvents on Extractable Solids.* The result of this study showed that different solvents had significant effects on the extractable solids yield of *S. chinensis* root (Figure 1). Absolute methanol had the highest extractable solids (15.6%), followed by 50% ethanol, 50% methanol, and 50% acetone (14.3%, 12.3%, and 12.2%, resp.). Water extracted half of extractable solids in comparison with absolute methanol, whereas absolute ethanol and absolute acetone only extracted ~25% of extractable solids extracted by absolute methanol. These findings indicated that recovery yields of crude powder extract prepared from *S. chinensis*

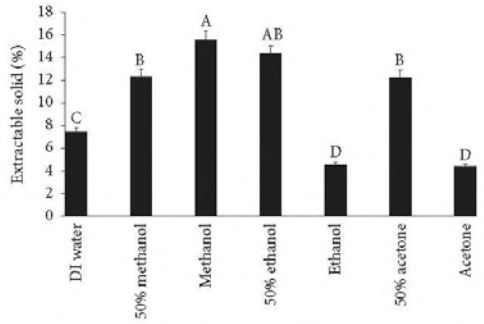


FIGURE 1: Effect of solvents on extractable solids from the root of *S. chinensis*. The values are the mean average of three replications for each solvent ± standard deviation. Columns not sharing the same superscript letter are significantly different $p < 0.05$ (DI water: deionized water).

root could be significantly affected by the extraction solvents. These findings were in agreement with previous studies on *Limnophila aromatica* [15] and *Phoenix dactylifera* L. [16], whereby the variation can be explained by the difference in solubility of different compounds in the sample. In general, these findings suggested that absolute methanol or mixture of 50% (v/v) water with methanol, ethanol, or acetone was the solvents of choice for yielding high levels of extractable solids.

*3.2. Impact of Extraction Solvents on Total Phenolic Content (TPC).* Our data showed that extraction solvents had a significant impact on the extraction yields of TPC from the root of *S. chinensis* (Figure 2). The mixture of 50% (v/v) water with methanol, ethanol, and acetone had the highest extraction yields of TPC, followed by absolute methanol and absolute ethanol, which accounted for approximately 66% and 43% of the TPC extracted by 50% acetone. Water extracted approximately 30% of TPC in comparison to 50% acetone, whereas absolute acetone only extracted about 12% of the TPC extracted by 50% acetone. These findings further confirmed that extraction solvents play an important role in the extraction of phenolic compounds from the sample, and the mixture of 50% (v/v) water with methanol, ethanol, and acetone is the best solvents for maximum extraction of TPC.

These findings were supported by previous studies, which also found that different extraction solvents significantly affected the extraction yields of TPC [6, 7, 15, 17]. However, the extraction yields of TPC were different depending on the types of solvent used. For example, Chavan et al. [6] reported that methanol was the best extraction solvent for TPC from the fresh fruit pulp of *S. chinensis*, while Dailey and Vuong [7] reported that 50% acetone with water was the best solvent for the extraction of TPC from macadamia skin. Furthermore, Do et al. [15] found that absolute ethanol and acetone were the best extraction solvents for TPC from *Limnophila aromatic*. The differences can be explained by the variation in polarities of the solvents, which selectively extract different hydrophobic or hydrophilic phenolic compounds in the sample, thus highlighting the importance of investigating and identifying the optimal extraction solvent for each sample type.

*3.3. Impact of Extraction Solvents on Total Flavonoid Content (TFC).* Our study showed that the extraction solvents had a significant effect on the extraction of flavonoids ($p < 0.05$) (Figure 2). The mixture of 50% (v/v) water with acetone and ethanol had the highest extraction yields of flavonoids (100 and 89 mg CE/g DW, resp.). This was followed by 50% methanol (85 mg CE/g DW), while absolute methanol, absolute ethanol, and water could only extract 50%, 30%, and 20% of flavonoids, respectively, in comparison with those of 50% acetone and 50% methanol. Absolute acetone extracted the lowest flavonoid levels from *S. chinensis*. Our findings were supported by previous studies on *S. chinensis* fruit pulp, *Limnophila aromatica*, and *Macadamia tetraphylla* skin waste, which reported that extraction solvents significantly affected flavonoids [6, 7, 15]. The variation can be also explained by the different polarities of compounds which were selectively more soluble in different solvents.

*3.4. Impact of Extraction Solvents on Total Proanthocyanidin Content.* The current study found that absolute acetone had the highest extraction of proanthocyanidins (61 mg CE/g DW), followed by 50% acetone (47.4 mg CE/g DW) (Figure 2). 50% methanol or ethanol only extracted 50% of proanthocyanidins in comparison to that extracted by absolute acetone. Water was found to extract the lowest content of proanthocyanidins. These findings indicated that extraction solvents play an important role in the extraction efficiency of proanthocyanidins. Water has the highest polarity index, whereas acetone has the lowest polarity index among the tested solvents; thus most proanthocyanidins from *S. chinensis* are more hydrophilic and thus acetone is the best solvent for extraction of these phenolic compounds.

*3.5. Impact of Extraction Solvents on Total Saponin Content.* The results of this study showed that the best solvent for extraction of saponins was 50% acetone (754 mg EE/g DW), followed by absolute methanol, ethanol, and 50% (v/v) of these solvents with water. The results also revealed that water and absolute acetone had the lowest content of saponins (Figure 2). Previous studies also reported that different extraction solvents significantly affected the extraction efficiency of saponins [18, 19]. In comparison with some Chinese herbal medicines reported by Chen et al. [20], the total content of saponins in *S. chinensis* is higher than those of various species of herbs, such as *Artemisia capillaries, Codonopsis pilosula, Euryale ferox*, and *Coix lacryma-jobi*.

*3.6. Impact of Extraction Solvents on Antioxidant Properties.* Four antioxidant assays were used for determining the effect of extraction solvents on the antioxidant capacity of *S. chinensis* extracts. Figure 3 shows that the extraction solvent significantly affected the antioxidant capacity of *S. chinensis*. All four antioxidant assays revealed that antioxidant capacity is in decreasing order with the corresponding solvents used:

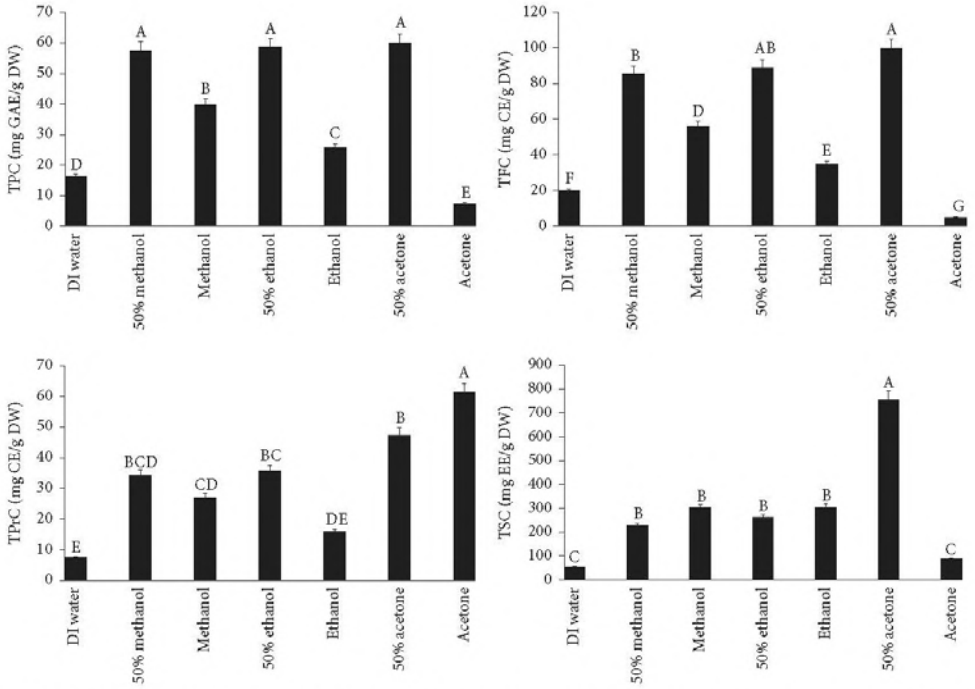


FIGURE 2: Effect of solvents on recovery of total phenolics (TPC), flavonoids (TFC), proanthocyanidins (TPrC), and saponins (TSC) from the root of *S. chinensis*. The values are the mean of three replications for each solvent ± standard deviation. Columns not sharing the same superscript letter are significantly different $p < 0.05$ (DI water: deionized water, GAE: gallic acid equivalent, CE: catechin equivalent, EE: escin equivalent, and DW: dry weight).

50% acetone > 50% ethanol > 50% ethanol > absolute methanol > absolute ethanol > water > absolute acetone. Previous studies found that extraction solvents significantly affected antioxidant capacities of Saptarangi (*S. chinensis* L.) fruit pulp [6] and macadamia skin [7]. However, the impact of individual solvents on the antioxidant capacity of different solvents was different. For example, Chavan et al. [6] reported that absolute methanol gave the highest antioxidant capacity, followed by ethanol, acetone, and water that had the lowest antioxidant capacity from Saptarangi (*S. chinensis* L.) fruit pulp, whereas Dailey and Vuong [7] revealed that the highest antioxidant capacities were seen in the combination of organic solvents (methanol, ethanol, acetonitrile, and acetone) with water in the ratio of 1 : 1 (v/v).

The differences in impact of solvents on antioxidant capacity of *S. chinensis* in the current study can be explained by the variation of bioactive groups extracted by the different solvents. Each bioactive group contributed with a different antioxidant power as these groups were found to have differing correlation with antioxidant capacity (Table 2). Table 2 shows that phenolic compounds had a strong correlation with the four antioxidant properties ($r > 0.95$), followed by flavonoids ($r > 0.67$) and saponins ($r > 0.6$). Proanthocyanidins were found to have a weak correlation with antioxidant capacity of *S. chinensis*. These findings revealed that antioxidant capacity of *S. chinensis* was mainly contributed by phenolic compounds, flavonoids, and saponins. These findings were supported by studies on fruit pulp of *Salacia chinensis*, lilly pilly, and bitter melon, which reported that phenolic compounds, flavonoids, and saponins were mainly responsible for antioxidant activity for these tested materials [6, 21, 22].

*3.7. Phytochemical and Antioxidant Properties and HPLC Analysis of the Enriched Extract.* As 50% acetone was found to be the best extraction solvent for both phenolic and saponin compounds from *S. chinensis* root, a crude extract was prepared using 50% acetone and the results are shown in Table 1. The results showed that the crude extract had high level of phenolics and saponins as well as potent antioxidant capacity. Compared to the result of previous studies, the TPC, TFC, and TPrC of enriched extract of *S. chinensis* root (555 mg GAE, 819 mg CE, and 392 mg CE per g crude extract, resp.) are much higher than those of *Davidsonia pruriens* F.

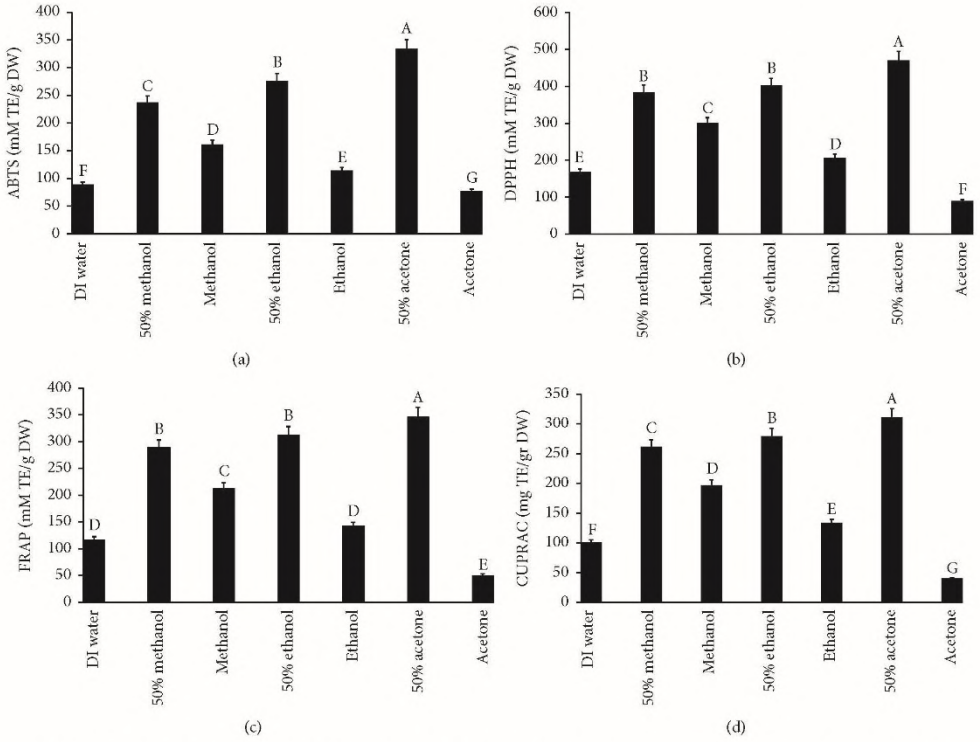


FIGURE 3: Effect of solvents on antioxidant properties from the root of *S. chinensis* using various antioxidant assays, including ABTS, DPPH, FRAP, and CUPRAC. The values are the mean of three replications for each solvent ± standard deviation. Columns not sharing the same superscript letter are significantly different $p < 0.05$ (DI water: deionized water).

TABLE 1: Physicochemical and antioxidant properties of the enriched extract prepared by optimal solvent.

| Properties | Values |
|---|---|
| Total phenolic compounds (mg GAE/g CRE) | 555.22 ± 11.22 |
| Flavonoids (mg CE/g) | 819.47 ± 27.06 |
| Proanthocyanidins (mg CE/g) | 392.09 ± 2.38 |
| Saponins (mg EE/g) | 1,880.83 ± 246.68 |
| DPPH (mM TE/g) | 407.47 ± 11.40 |
| ABTS (mM TE/g) | 414.38 ± 18.21 |
| CUPRAC (mM TE/g) | 623.82 ± 9.77 |
| FRAP (mM TE/g) | 320.24 ± 15.70 |

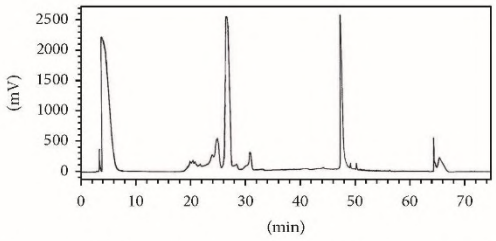


FIGURE 4: HPLC chromatogram detected at 254 nm for the crude extract of *S. chinensis* root.

Muell (45 mg GAE, 22 mg CE, and 3.2 mg CE per g crude extract, resp.) [23]. Similarly, the saponin content of the *S. chinensis* root enriched extract (1,880 mg EE/g crude extract) is many times higher than that of *Carica papaya* leaf (32 mg EE/g) [24]. Therefore, these findings further confirmed that 50% acetone is the solvent of choice for extraction of bioactive compounds from *S. chinensis*. In addition, these findings also indicated that the crude extract prepared from 50% acetone has the potential for further testing biological activities such as antimicrobial and anticancer properties.

From the HPLC analysis (Figure 4), there are several peaks in the extract, which can be further isolated for identification as well as testing their properties.

Table 2: Correlation between bioactive compounds and antioxidant properties of the root of *S. chinensis*.

| | TPC | | TFC | | TPrC | | TSC | |
|---|---|---|---|---|---|---|---|---|
| | $r$ | $p$ value | $r$ | $p$ value | $r$ | $p$ value | $r$ | $p$ value |
| ABTS | 0.957 | **** | 0.730 | **** | 0.322 | ns | 0.664 | *** |
| FRAP | 0.981 | **** | 0.727 | **** | 0.202 | ns | 0.606 | ** |
| CUPRAC | 0.989 | **** | 0.739 | **** | 0.184 | ns | 0.613 | ** |
| DPPH | 0.977 | **** | 0.672 | *** | 0.248 | ns | 0.602 | ** |

*Note:* ****: extremely significant ($p$ value < 0.0001), ***: extremely significant (0.0001 < $p$ value < 0.001), **: very significant (0.001 < $p$ value < 0.01), and ns: not significant ($p$ value ≥ 0.05).

## 4. Conclusion

This study demonstrated that the extraction solvents play an important role in the extraction of important bioactive groups from *S. chinensis*. Absolute organic solvents or water was not effective, whereas 50% ethanol and 50% acetone were solvents of choice for yielding high content of extractable solids, phenolic compounds, and flavonoids. Among these two solvents, 50% acetone was found to have the highest levels of saponins as well as high antioxidant capacity. This study also prepared phenolic and saponin enriched extracts using 50% acetone and further confirmed that 50% acetone was the solvent of choice for yielding high content of phenolics, saponins, and antioxidant properties. Therefore, 50% of acetone is recommended for extraction of phenolic compounds, their secondary metabolites, and saponins from the root of *S. chinensis* for further isolation and utilisation.

## Additional Points

*Practical Applications.* The medicinal properties of herbal plants are mostly determined by the contents of bioactive compounds, such as phenolic compounds, flavonoids, and saponins and the antioxidant capacities of the plants. *Salacia chinensis* has been used widely for prevention and treatment of various diseases, such as arthritis, diabetes, and obesity, and therefore it is a potential material for further research. In this study, we have optimised the conditions for extraction of bioactive compounds and determined the antioxidant properties in *S. chinensis* root. The result showed that optimal conditions for extraction of bioactive compounds from *S. chinensis* root can be applied for further isolation and utilisation in the food and pharmaceutical industries.

## Competing Interests

The authors declare no conflict of interests.

## Acknowledgments

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# *CHAPTER 5.*

# OPTIMISATION OF CONDITIONS FOR EXTRACTION OF BIOACTIVE COMPOUNDS FROM *S. CHINENSIS*

## 5.1. Introduction

Numerous methods have been employed to extract bioactive compounds from plant materials. These methods can be categorised into conventional extraction methods, such as decoction, continuously shaking extraction (CSE), soxhlet and reflux; and more advanced methods, such as microwave – assisted extraction (MAE), ultrasonic – assisted extraction (UAE) and supercritical fluid extraction (SFE) (Handa et al. 2008; Azmir et al. 2013; Nayak et al. 2015). The advanced extraction methods are more rapid techniques in comparison with conventional methods (Nayak et al. 2015). The root of *S. chinensis* has been extracted using infusion, decoction, soxhlet, CSE and MAE (Karunanayake and Sirimanne 1985; Jansakul et al. 2005; Tran et al. 2008, 2010; Chavan et al. 2012; Periyar et al. 2014; Chavan et al. 2015). UAE has also been applied to extract bioactive compounds from *S. chinensis* root (Ngo et al. 2017a; Ngo et al. 2017b); however, UAE conditions have to date, not been optimised for extraction of total bioactive compounds, such as phenolics, saponins and antioxidants from *S. chinensis* root.

Mangiferin is considered as a "super antioxidant" and has attracted the interest of researchers around the world. Over 450 articles have been published on its occurrence, chemical nature, synthesis and medicinal properties over the last 50 years (Asif et al., 2016; Saha et al., 2016). It exhibits anti-viral, anti-cancer, anti-diabetic, anti-aging, immunomodulatory, hepatoprotective and analgesic properties (Imran et al., 2017). Mangiferin is a major bioactive compounds of *S. chinensis* root. However, optimal conditions for extraction of mangiferin from *S. chinensis* root have not been reported.

The aims of this research were (1) to determine the optimal conditions for extraction of total bioactive compounds and their subsequent antioxidant activities, and (2) to identify the optimal conditions for extraction of mangiferin from *S. chinensis* L. root using UAE as an advanced

extraction technique. Effectiveness of UAE was then compared with two conventional extraction methods - decoction and CSE.

### 5.2. Results and discussions

Results and detailed discussions were presented in two Research Papers:

Thanh V. Ngo, Christopher J. Scarlett, Michael C. Bowyer, and Quan V. Vuong (2019). Ultrasonic assisted extraction as an advanced technique for the extraction of bioactive compounds from *Salacia chinensis* root: A comparison with decoction and continuously shaking extraction. (submitted to *Journal of Plant Biochemistry and Biotechnology*).

Thanh Van Ngo, Christopher James Scarlett, Michael Christian Bowyer, and Quan Van Vuong (2019). Isolation and maximisation of extraction of mangiferin from the root of *Salacia chinensis* L. *Separations*, 6(44), 1-10.

### 5.3. Conclusions

UAE effectively extracted phenolic compounds, flavonoids, proanthocyanidins, and saponins with high antioxidant activities from *S. chinensis* root. This technique was also effective for extraction of mangiferin from *S. chinensis* root. The optimal UAE conditions for extraction of total bioactive compounds were: 50% ethanol, 60 min, 50 ºC and 250 W. Optimal UAE conditions for extraction of mangiferin were a temperature of 50 ºC, acetone of 40%, extraction time of 60 min, and ultrasonic power of 250 W. In comparison with the two most common conventional extraction methods: decoction and CSE, UAE had comparable extraction yields of total bioactive compounds to CSE, but had significantly higher extraction yields than the decoction method. For extraction of mangiferin, UAE extracted 3% and 57% more mangiferin in comparison with CSE and decoction, respectively.

As only a short time is required, UAE is recommended for extraction of total bioactive compounds and mangiferin from *S. chinensis* root for further isolation and utilisation.

Journal of Plant Biochemistry and Biotechnology

# Ultrasonic assisted extraction as an advanced technique for the extraction of bioactive compounds from Salacia chinensis root: A comparison with decoction and continuously shaking extraction

--Manuscript Draft--

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|---|---|
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| **Abstract:** | This study aimed to determine the impact of a range of extraction parameters including ethanol concentration, extraction time, temperature, and ultrasonic power on extraction yields of bioactive compounds and antioxidant activities from Salacia chinensis L. root, and to optimise extraction conditions using Response Surface Methodology (RSM). The ultrasonic assisted extraction method (UAE) was then further compared with two conventional extraction methods, which included decoction and continuously shaking extraction (CSE). Ethanol concentration, extraction time, temperature, and power significantly affected the yield of phenolic compounds (TPC), flavonoids (TFC), proanthocyanidins (TPrC) and saponins (TSC) as well as antioxidant capacities of S. chinensis root. The RSM models revealed that the optimal conditions for maximum extraction of these bioactive compounds from S. chinensis root were 50% ethanol, 60 min., 50 oC and 250 W. Under these optimal conditions, a phytochemical enriched extract was obtained with high levels of TPC (68 mg GAE/g), TFC (91 mg CE/g), TPrC (39 mg CE/g) and TSC (200 mg EE/g). UAE had comparable extraction yields for bioactive compounds and antioxidant properties with CSE, but had significantly higher extraction yields than the decoction method. As UAE was significantly more time efficient than CSE, UAE is recommended for extraction of bioactive compounds with antioxidant properties from S. chinensis root for further isolation and utilisation. |
| **Response to Reviewers:** | 4 July 2019<br><br>Dr. Tilak Raj Sharma<br>Chief Editor<br>Journal of Plant Biochemistry and Biotechnology<br>Manuscript ID: JPBB-D-18-00290R2 |

Dear Dr. Tilak Raj Sharma,

On behalf of my co-authors, I would like to resubmit our revised manuscript entitled "Ultrasonic assisted extraction as an advanced technique for the extraction of bioactive compounds from Salacia chinensis root: A comparison with decoction and continuously shaking extraction", which requires revision for consideration to be published on Journal of Plant Biochemistry and Biotechnology. We have made a minor revision based on the Editorial comments as following:

1.iThenticate shows 39% plagiarism

We would like to thank the editor for the comment. We have used Turnitin for detecting Similarity Index and realized that the similarity mostly came from Method Section (actually it matched with our previous published paper). We already removed all sentences which matched with our previous paper.

2.Include only 3-4 most important tables in the MS. Rest can be included as supplementary information. See instructions to authors for formatting and citation of the supplementary info

We keep 4 tables in the manuscript, other 3 tables have been changed into supplementary files as suggested by the editor.

3.MS is not in the format of JPBB. Include all the table and figures at the end of Text and all caption should be given on a separate page

We have moved all tables and figures to the end of the text and all captions on a separate page.

4.Check language and spelling mistakes carefully

We have gone through the manuscript and then checked and corrected the language and spelling mistakes.

We thank the editorial board for their supportive comments and are confident that we have now addressed the above points concerning the manuscript. We are pleased that the editorial board continues to share our enthusiasm for these data and accept to publish our work in the Journal of Plant Biochemistry and Biotechnology.

With best regards,

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# Ultrasonic assisted extraction as an advanced technique for the extraction of bioactive compounds from *Salacia chinensis* root: A comparison with decoction and continuously shaking extraction

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**ABSTRACT**

This study aimed to determine the impact of a range of extraction parameters including ethanol concentration, extraction time, temperature and ultrasonic power on extraction yields of bioactive compounds and antioxidant activities from *Salacia chinensis* L. root, and to optimise extraction conditions using Response Surface Methodology (RSM). The ultrasonic assisted extraction method (UAE) was then further compared with two conventional extraction methods, which included decoction and continuously shaking extraction (CSE). Ethanol concentration, extraction time, temperature and power significantly affected the yield of phenolic compounds (TPC), flavonoids (TFC), proanthocyanidins (TPrC) and saponins (TSC) as well as antioxidant capacities of *S. chinensis* root. The RSM models revealed that the optimal conditions for maximum extraction of these bioactive compounds from *S. chinensis* root were 50% ethanol, 60 min., 50 °C and 250 W. Under these optimal conditions, a phytochemical enriched extract was obtained with high levels of TPC (68 mg GAE/g), TFC (91 mg CE/g), TPrC (39 mg CE/g) and TSC (200 mg EE/g). UAE had comparable extraction yields for bioactive compounds and antioxidant properties with CSE, but had significantly higher extraction yields than the decoction method. As UAE was significantly more time efficient than CSE, UAE is recommended for extraction of bioactive compounds with antioxidant properties from *S. chinensis* root for further isolation and utilisation.

**KEYWORDS:**

*Salacia chinensis* L.; optimisation; UAE; CSE; decoction.

**ABBREVIATION**

UAE: Ultrasonic-assisted extraction

CSE: Continuously shaking extraction

RSM: Response surface methodology

TPC: Total phenolic content

TFC: Total Flavonoid content

TPrC: Total Proanthocyanidin content

TSC: Total saponin content

ABTS: (2,2'-azino-bis(3-ethylbenzthiazoline- 6-sulphonic acid)) assay

DPPH: (2,2-diphenyl-1-picrylhydrazyl) assay

FRAP: Ferric Reducing Antioxidant Power assay

GAE: Gallic acid equivalent

CE: Catechin equivalent

EE: Escin equivalent

TE: Trolox equivalent

DW: Dry weight

**Introduction**

*Salacia chinensis* L. (synonym *S. prinoides*) belongs to the family *Celastraceae* and is widely distributed in many Asian countries, including China, Vietnam, Malaysia, Thailand, India and Sri Lanka (Rong et al. 2008; Arunakumara and Subasinghe 2010). Different parts of *S. chinensis*, including root, stem, leaf and fruit pulp have been used for the treatment of various ailments including arthritis, leucorrhoea, inflammation, fever, skin diseases, menstrual disorders and spermatorrhoea. In Vietnamese and Lao cultures, the root and stem of *S. chinensis* is used for the treatment of rheumatism, back-pain and debility (Vo 1997; Delang 2007). The root of *S. chinensis* (or the combination of this plant with other species in genus *Salacia*) is used in India for the prevention and treatment of diabetes and obesity (Chavan et al. 2015; Ramakrishna et al. 2016).

Numerous methods have been employed to extract bioactive compounds from plant materials, including conventional extraction methods, such as decoction, continuously shaking extraction (CSE), soxhlet and reflux; and newer, more advanced methods such as microwave – assisted extraction (MAE), ultrasonic – assisted extraction (UAE) and supercritical fluid extraction (SFE) (Handa et al. 2008; Azmir et al. 2013; Nayak et al. 2015). The advanced extraction methods are more rapid techniques in comparison with conventional methods (Nayak et al. 2015). The root of *S. chinensis* has been extracted using infusion, decoction, soxhlet, CSE and MAE (Karunanayake and Sirimanne 1985; Jansakul et al. 2005; Tran et al. 2008, 2010; Chavan et al. 2012; Periyar et al. 2014; Chavan et al. 2015). UAE has also been applied to extract bioactive compounds from *S. chinensis* root (Ngo et al. 2017a; Ngo et al. 2017b); however, UAE extraction conditions have to date, not been optimised. The aim of this research was to determine the impact of a range of extraction parameters including ethanol concentration, ultrasonic time, temperature and power on extraction yields of bioactive compounds and their subsequent antioxidant activities from *Salacia chinensis* L. root and to optimise these conditions using Response Surface Methodology (RSM). UAE was then compared with two conventional extraction methods - decoction and continuously shaking extraction to determine the most suitable method for extraction of bioactive compounds with antioxidant properties from *Salacia chinensis* L. root.

**Materials and methods**

***Materials***

The sample (*S. chinensis* root) was collected from the forest in Nghe An province (Vietnam) in March 2016 and authenticated by Associate Professor Vu Quang Nam, Department of Forest Plant Resources, College of Forestry Biotechnology, Vietnam National University of Forestry. After collection, the material was cut into small pieces,

sun-dried for two days then ground to a powder using a commercial cutter, sieved using a steel mesh sieve (1.4mm EFL 2000; Endecotts Ltd., London, England) and stored at -20ºC until required for analysis.

***Experimental design***

This study compared the optimal ultrasound – assisted extraction (UAE) method as an advanced extraction technique with two conventional extraction techniques including decoction and CSE methods for phytochemical and antioxidant properties. UAE extraction conditions were optimised using Response Surface Methodology (RSM). Experimental procedures in details for each extraction method are described in the following sections. In this study, aqueous ethanol was chosen as the extraction solvent because of (1) its safety and cost effectiveness in comparison with other organic solvents and (2) its comparable extraction efficiency over other solvents, such as aqueous acetone, aqueous methanol (Ngo et al. 2017a).

*Optimisation of ultrasound – assisted extraction*

To optimise the UAE conditions, optimal ranges of four parameters, including ethanol concentration, extraction power, time and temperature were determined using the single factor experiments (one factor at a time). The optimal ranges were then applied using RSM. UAE was conducted in an ultrasonic bath (Soniclean 1000HD, 220 V, 50/60 Hz and 250 W, Soniclean Pty Ltd, Thebarton, SA, Australia).

*Single factor experiments*

The extraction procedures for single factor experiments were applied as described in a previous study (Ngo et al. 2017a) with some modification. Briefly, 5 g of sample was added into 100 mL of solvent. The mixture was then placed in an ultrasonic bath with pre-set conditions for temperature, time and power (the frequency was set as default at 50/60 Hz). An external digital thermometer was employed to measure the temperature in the ultrasonic bath, and ice and hot water were used to maintain the desired temperature. Post-extraction, the solution was immediately cooled to room temperature (RT) using an ice bath, then filtered using filter paper (Whatman, 11µm pore size). The extract was then stored in the dark at 4°C for further analysis.

To test the impact of ethanol concentration on bioactives and antioxidant capacity of *S. chinensis* L. root, a range from 40 to 70 % of aqueous ethanol concentrations (40, 50, 60 and 70 %, v/v) were used. Other extraction conditions were set at temperature of 35 ºC, extraction time of 20 minutes and extraction power of 150W.

For the extraction time, the optimal concentration of ethanol (50 %) was applied to test the impact of the extraction time ranging from 5 to 80 minutes (5, 10, 15, 20, 25, 30, 35, 40, 50, 60 and 80 minutes). Other extraction conditions were set at a temperature of 35 ºC and extraction power of 150 W.

For the extraction temperature, the optimal concentration of ethanol (50 %) and time (60 min.) were applied to determine the impact of temperature ranging from 20 to 60 °C (at 10 degree intervals) and room temperature (17 °C) on bioactives and antioxidant capacity of *S. chinensis* L. root. Extraction power was set at 150 W.

*UAE optimisation using Response Surface Methodology (RSM)*

JMP software (version 13.0.0) was employed for RSM experimental design using a three level, four factorial, Box – Behnken methodology. Based on the results of preliminary single – factor experiments, four factors were applied for optimisation, with the following ranges: solvent concentration (50 - 60 %), time (30 - 60 min.), extraction temperature (30 - 50 °C) and power (150 - 250 W). The ratio of solvent to sample was 100:5 mL/g. Experimental design and the true run results are shown in Table 1.

To predict the optimal extraction conditions, a second order polynomial equation (Eq. 1) was used to assess the relationship between independent variables and the targeted responses.

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3X_4 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2 \quad (1)$$

Where $X_1$: ethanol concentration; $X_2$: time, $X_3$: temperature and $X_4$: power; $\beta_o$: intercept; $\beta_1$, $\beta_2$, $\beta_3$ and $\beta_4$: linear regression coefficients; $\beta_{12}$, $\beta_{13}$, $\beta_{13}$, $\beta_{23}$, $\beta_{14}$, $\beta_{24}$ and $\beta_{34}$: interaction regression coefficients; $\beta_{11}$, $\beta_{22}$, $\beta_{33}$ and $\beta_{44}$: quadratic regression coefficients.

*Decoction*

Decoction of the *S. chinensis* L. root was conducted in a manner similar to a previous study by Karunanayake and Sirimanne (1985). Briefly, 10 g of sample was added into 200 mL DI water (ratio 5:100 g/mL). The mixture was then put in a small container and boiled for 3 hours. Subsequently, the extract was filtered (Whatman filter paper, 11µm pore size), collected and stored in the dark at 4°C for further analysis.

*Continuously shaking extraction*

Continuously shaking extraction (CSE), previously reported by Chavan et al. (2012) was employed in this study. Briefly, 5 g of sample was added into 100 mL 50% ethanol (ratio 5:100 g/mL). The extraction was then carried out on an orbital shaker for 24 hours at room temperature. Subsequently, the extract was filtered (Whatman filter paper, 11µm pore size), collected and stored in the dark at 4°C for further analysis.

*Determination of phytochemical contents and antioxidant capacities*

Assays for determination of TPC, TFC, TPrC and TSC) and antioxidant abilities (including ABTS, DPPH and FRAP) were conducted by methods described in our previous study (Ngo et al, 2017a).

*HPLC and LC/MS analysis of major bioactive components*

Protocol for running HPLC were described in our previous study (Ngo et al, 2017a). In the HPLC graph, each peak represented one individual compound. The concentration of the compound was calculated by comparing the area of the peak with the area of gallic acid and expressed by mg gallic acid equivalents/g (mg GAE/g).

Peak 1 was isolated using a Shimadzu auto fraction collector and then freeze dried to powder form. The compound was then identified using a Shimadzu LC/MS (LCMS 2020, Shimadzu) equipped with an electrospray ionization (ESI) interface. The mobile phase was delivered at a flow rate of 0.2 mL/min. The injection volume was 1 µl. The mass spectrometer was operated at negative mode with selected ion monitoring (SIM) and the parameters as follow: nebulizing gas flow 1.5 L/min, drying gas pressure 0.15 MPa, CDL temperature 250 °C, block heater temperature 200 °C.

*Statistical analysis*

JMP version 13.0.0 (64-bit, SAS Institute Inc.) was employed for RSM design and statistical analysis in this study. 2D and 3D contour plots of variable responses, as well as the predictive optimal values and extraction conditions of four independent variables were generated by the program.

**Results and Discussion**

***Impact of individual UAE extraction factors on extraction efficiency of bioactive compounds and antioxidant capacity***

*Impact of solvent concentration*

The results showed that ethanol concentration significantly affected the extraction efficiency of bioactive compounds and antioxidant properties of *S. chinensis* root (see Appendix 1). The optimal ethanol concentration for extraction (55%) produced yields of phenolics, flavonoids, proanthocyanidins and saponins of 59 mg GAE/g, 85 mg CE/g, 33 mg CE/g and 190 mg EE/g, respectively. At this concentration (55% ethanol), levels of bioactive compounds were 33 % higher than other ethanol concentrations examined using UAE. Similarly, antioxidant properties measured by three different assays (DPPH, ABTS and FRAP assays) were also greatest at 55% ethanol concentration. These levels were 42 % higher than those extracted by other concentrations, revealing that 55% ethanol is the best solvent for further testing. The findings are in agreement with previously reported results by

Ncube and Okoh (2008) and Tiwari et al. (2011) who found that solvent concentrations significantly affected the extraction efficiency of bioactive compounds. This can be explained by the relative polarities of the two solvents. Water has relative polarity of 1, whereas ethanol has relative polarity of 0.654. Thus, water can extract effectively the polar compounds, while ethanol can extract both polar and non-polar compounds; and their right combination would give a better extraction efficiency for both polar and non-polar compounds.

*Extraction time*

Extraction time is a well-known parameter affecting the extraction efficiency of bioactive compounds (Azmir et al. 2013; Nayak et al. 2015). This study found that variation in extraction time produced different levels of bioactive compounds and antioxidant properties extracted from *S. chinensis* root (see Appendix 2). The content of phenolics, flavonoids, proanthocyanidins and saponins increased with increasing extraction time, plateauing at 50 min. (58 mg GAE/g, 85 mg CE/g, 33 mg CE/g and 178 mg EE/g, respectively). Levels of these major bioactive compounds at 50 min. were 31 %, 27%, 18% and 47 %, respectively, higher than those extracted for the shorter times. The three antioxidant assays (DPPH, ABTS and FRAP) used to assess antioxidant activity showed a similar trend, again reaching maximum value at 50 min. These results align with previously reported studies on carrot pomace and *Carica papaya* leaf, which found that extraction time significantly affected extraction efficiency of bioactive compounds (Vuong et al. 2013; Jabbar et al. 2014).

*Extraction temperature*

Temperature is another important factor that affects the yield of bioactive compounds from plant materials (Tiwari et al. 2011; Azmir et al. 2013). Principally, higher extraction temperature increases the solubility of active constituents into solvent, which leads to the increased extraction yield. However, prolonged exposure to elevated temperatures may result in thermal decomposition of sensitive constituents (Azmir et al. 2013). Therefore, the extraction temperature should be optimised to get the optimal extraction results. The increase of extraction temperature led to the rise of the yields of bioactive compounds in *S. chinensis* root. At 40 °C levels of phenolics, flavonoids, proanthocyanidins and saponins were 59 mg GAE/g, 85 mg CE/g, 35 mg CE/g and 185 mg EE/g, respectively, which were 23 %, 30 %, 26 % and 36 %, respectively, higher than those extracted at other temperatures. Results from three antioxidant assays also revealed that antioxidant capacity of *S. chinensis* root was highest at 40 °C (Appendix 3).

***Optimisation of UAE using RSM***

Based on the single factor results, the optimal range for each variable for further RSM assessment was chosen as follows: Ethanol concentration (50 – 60%), extraction time (30 – 60 min.), extraction temperature (30 – 50ºC). In addition, ultrasonic power with a range of 150 – 250 W was also used as the fourth factor for RSM.

To evaluate the reliability of RSM mathematical model in accurately predicting the optimal variances and representing the correlations between the selected parameters, fitting the models for TPC, TFC, proanthocyanidins, saponins and antioxidant properties of the *S. chinensis* root extract was carried out. The Box – Behnken design representing the analysis of variances for determination of the model fit is shown in Table 2. Overall, mathematical models for phytochemicals and antioxidant capacity are reliable and can be effectively applied for prediction because the models were significant ($p<0.05$) and $R^2$ of all models were greater than 0.75, revealing that at least 75% of predicted values would match with the actual values.

Based on the reliable predictive models, equations for estimation of extraction yields of bioactive compounds and antioxidant properties are proposed as follows:

$$Y_{TPC} = -18.265 + 1.108X_1 + 1.109X_2 + 0.5695X_3 + 0.104X_4 - 0.022X_1X_2 - 0.023X_1X_3 - 0.0007X_2X_3 - 0.0042X_1X_4 + 0.0016X_2X_4 - 0.0011X_3X_4 + 0.008X_1^2 + 0.0012X_2^2 + 0.013X_3^2 + 0.0009X_4^2 \quad \textbf{(2)}$$

$$Y_{TFC} = 25.20 + 0.552X_1 + 1.235X_2 - 0.083X_3 + 0.1465X_4 - 0.02X_1X_2 - 0.0174X_1X_3 - 0.006X_2X_3 - 0.007X_1X_4 + 0.0061X_2X_4 - 0.0025X_3X_4 + 0.0124X_1^2 - 0.0027X_2^2 + 0.0233X_3^2 + 0.0009X_4^2 \quad \textbf{(3)}$$

$$Y_{TPrC} = 38.91 - 0.707X_1 + 1.055X_2 - 0.822X_3 + 0.102X_4 - 0.021X_1X_2 + 0.008X_1X_3 - 0.001X_2X_3 - 0.0056X_1X_4 + 0.003X_2X_4 + 0.0024X_3X_4 + 0.015X_1^2 - 0.0004X_2^2 + 0.0046X_3^2 - 0.0001X_4^2 \quad \textbf{(4)}$$

$$Y_{TSC} = 313.2725 + 2.915X_1 - 1.047X_2 - 2.0715X_3 - 4.8105X_4 - 0.019X_1X_2 - 0.058X_1X_3 + 0.0105X_2X_3 + 0.0161X_1X_4 + 0.006X_2X_4 - 0.0055X_3X_4 - 0.0092X_1^2 + 0.0157X_2^2 + 0.0744X_3^2 + 0.0253X_4^2 \quad \textbf{(5)}$$

$$Y_{DPPH} = 2486.185 - 62.549X_1 + 1.548X_2 - 10.976X_3 - 5.154X_4 - 0.173X_1X_2 + 0.047X_1X_3 + 0.012X_2X_3 + 0.079X_1X_4 + 0.007X_2X_4 - 0.017X_3X_4 + 0.552X_1^2 + 0.087X_2^2 + 0.134X_3^2 + 0.01X_4^2 \quad \textbf{(6)}$$

$$Y_{FRAP} = 378.2 - 6.22X_1 + 1.35X_2 + 11.15X_3 - 3.703X_4 - 0.002X_1X_2 - 0.215X_1X_3 - 0.055X_2X_3 + 0.059X_1X_4 + 0.013X_2X_4 - 0.023X_3X_4 + 0.08X_1^2 + 0.004X_2^2 + 0.081X_3^2 + 0.007X_4^2 \quad \textbf{(7)}$$

$$Y_{ABTS} = -483.92 + 34.34X_1 + 2.641X_2 + 3.579X_3 - 4.386\,X_4 - 0.076X_1X_2 - 0.157X_1X_3 + 0.02X_2X_3 + 0.005X_1X_4 + 0.039X_2X_4 - 0.015X_3X_4 - 0.236X_1^2 - 0.019X_2^2 + 0.093X_3^2 + 0.02X_4^2 \quad \textbf{(8)}$$

According to the predictive models, the results (Fig. 1) showed that solvent concentration had a negative correlation on extraction efficiency of bioactive compounds and antioxidant properties, while extraction time, temperature and irradiative power had positive correlations. In the ranges examined, solvent concentration, extraction time, temperature and power significantly affected the extraction yield of phenolic compounds and flavonoids, as well as ferric antioxidant power. Temperature and power in the tested ranges also had a significant effect on the obtained yields of proanthocyanidins and saponins, while power did not have a significant impact.

In the tested ranges, the results (Table 3) showed that interaction between ethanol concentration and extraction time had significant influence on extraction yields of phenolics, flavonoids and proanthocyanidins, but not on saponins. Interactive effect between ethanol concentration and temperature only had significant impact on phenolics, not on other bioactive compounds. Similarly, interactive effect between time and power only had significant impact on flavonoids and proanthocyanidins. There was no interactive effect between ethanol concentration and power, as well as temperature and power on the extraction yields of bioactive compounds.

Based on the predictive models with consideration of interactive effects between the factors, optimal conditions for extraction of phenolics, flavonoids, proanthocyanidins, saponins and antioxidant capacity were predicted as follows: 50% ethanol concentration, extraction time of 60 min, extraction temperature of 50 °C and power of 250 W.

To validate the optimal conditions predicted by the models, *S. chinensis* root was extracted in triplicate using these predicted optimal conditions. The results showed that the actual values for all tested parameters (TPC: 68 mg GAE/g, TFC: 91 mg CE/g, TPrC: 39 mg CE/g, TSC: 201 mg EE/g, DPPH: 490 μM TE/g, ABTS: 520 μM TE/g and FRAP: 347 μM TE/g) were similar to the predicted values ($p>0.05$), revealing that the predictive conditions were reliable and thus these optimal conditions were used for further comparison with two other conventional extraction methods.

***Comparison of UAE as advanced technique with conventional techniques***

Results obtained from the optimal UAE conditions were further compared with two other conventional extraction techniques, including decoction and continuously shaking extraction (CSE) techniques and the results are shown in Table 4.

The results indicated that UAE and CSE had similar extraction yields of phenolics and proanthocyanidins; however, UAE had a significantly higher level of flavonoids and a lower level of saponins in comparison with CSE. UAE had approximately 23% higher extraction yield for flavonoids and about 7% lower extraction yield for saponins as compared to CSE. The possible reason for the reduced extractability of saponins using ultrasound is the degradation of saponins under high temperature. Of note, UAE produced a significantly higher extraction yield for all tested bioactive compounds in comparison with the decoction method. Overall, UAE gave approximately 3 times greater extraction yield than the decoction method. Results from three antioxidant assays also revealed that UAE and CSE had similar antioxidant capacity, and extracts prepared from these two extraction methods had significantly higher antioxidant capacity than that of the decoction method (Table 4).

Scanning results for major compounds using HPLC (Fig. 2) revealed that UAE gave higher extraction yield of the compound 1 (38 mg GAE/g DW) than that of CSE (35 mg GAE/g DW) but had similar extraction yield of the compound 2. These two extraction methods had significantly higher extraction yields of these two major compounds than those extracted by the decoction method. The decoction method only extracted 10% of these two major compounds in comparison with CSE and UAE. These findings further confirm that extraction methods significantly affect extraction yields of bioactive compounds and their antioxidant capacity from plant materials.

Compound 1 was isolated using HPLC auto fraction collector and the fraction was then freeze dried to get a yellow amorphous powder. By using LCMS, the molecular mass of the purified compound was determined as 422. From its physical property (yellow amorphous powder form) and molecular mass (422), the compound was identified as mangiferin, an important active component which has been found previously in *S. chinensis* (Karunanayake and Sirimanne 1985; Sellamuthu et al. 2014).

The higher extraction yields of bioactive compounds and antioxidant capacity in UAE can be explained by the improvement in dissolving rates of solutes which is created by the ultrasonic high shear and cavitation forces, leading to a faster and more complete extraction (Hielscher 2017). By contrast, the lowest extraction efficiency of decoction was due to the high temperature which was used in the extraction process (i.e. boiling the mixture of solvent and sample for several hours) leading to the degradation of thermolabile compounds and the evaporation of volatile substances (Azmir et al. 2013). The continuously shaking extraction, which took place at room temperature, was comparable with UAE with regards to the extraction efficiency, and this was due to a long extraction process with the application of agitation.

In general, both UAE and CSE are effective for extraction of bioactive compounds and their antioxidant capacity from *S. chinensis* root. CSE does not require expensive equipment to complete the extraction, however it is a time consuming method with 24h needed for completion. Whereas, UAE is more expensive for setting up the equipment, but it takes only 60 min to complete the extraction process. As the cost of initial setting up can be profitable for the long run, UAE is therefore recommended for extraction of bioactive compounds and antioxidant activities from *S. chinensis* root.

**Conclusion**

UAE has been shown to effectively extract phenolic compounds, their secondary metabolites: flavonoids, proanthocyanidins, and saponins with high antioxidant activities of *S. chinensis* root. Ethanol concentration, extraction time, temperature and power were found significantly affect bioactive compounds and the antioxidant activities of *S. chinensis* root. Response Surface Methodology revealed that these extraction parameters also had interactive effects on the extraction yields and antioxidant capacities of bioactive compounds. The optimal UAE conditions were: 50% ethanol, 60 min., 50 °C and 250 W. In comparison with the two most common conventional extraction methods: decoction and continuously shaking extraction, UAE had comparable extraction yields of bioactive compounds to continuously shaking extraction but had significantly higher extraction yields than the decoction method. As only a short time is required for the extraction, UAE with conditions of 50% ethanol, 60 min, 50 °C and 250W is recommended for extraction of bioactive compounds from *S. chinensis* root for further isolation and utilisation.

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**Conflict of Interest**

Author Thanh V. Ngo, Author Christopher J. Scarlett, Author Michael C. Bowyer and Author Quan V. Vuong declare that they have no conflict of interest.

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**Tables**



**Figures**

**Table 1** Box-Behnken design and observed responses

| Run | Ultrasonic conditions | | | | Experimental values (n = 3) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | EtOH conc. | Time | Temp. | Power | TPC | TFC | TPrC | TSC | Antioxidant capacity | | |
| | | | | | | | | | DPPH | ABTS | FRAP |
| **1** | 50 | 60 | 40 | 80 | 59.99 | 83.34 | 35.64 | 171.27 | 490.34 | 342.13 | 471.55 |
| **2** | 60 | 45 | 50 | 80 | 55.07 | 83.40 | 34.22 | 171.97 | 436.65 | 325.72 | 470.70 |
| **3** | 60 | 45 | 40 | 60 | 52.97 | 77.30 | 31.24 | 167.83 | 392.58 | 314.65 | 450.27 |
| **4** | 55 | 45 | 40 | 80 | 53.45 | 79.63 | 31.68 | 156.80 | 390.03 | 316.73 | 457.01 |
| **5** | 50 | 45 | 40 | 100 | 57.61 | 85.18 | 34.32 | 175.01 | 431.35 | 340.90 | 476.81 |
| **6** | 55 | 60 | 40 | 60 | 54.48 | 77.40 | 31.89 | 166.38 | 427.52 | 317.76 | 447.18 |
| **7** | 55 | 30 | 40 | 60 | 52.82 | 77.40 | 31.84 | 169.83 | 408.29 | 320.24 | 455.89 |
| **8** | 55 | 45 | 30 | 60 | 52.28 | 75.58 | 31.30 | 176.98 | 402.40 | 308.32 | 445.43 |
| **9** | 60 | 45 | 30 | 80 | 53.24 | 76.89 | 29.87 | 160.20 | 400.34 | 322.01 | 450.20 |
| **10** | 55 | 45 | 40 | 80 | 55.18 | 78.95 | 31.65 | 161.28 | 420.26 | 338.37 | 457.43 |
| **11** | 50 | 45 | 50 | 80 | 59.96 | 87.89 | 34.38 | 179.16 | 445.88 | 367.98 | 480.88 |
| **12** | 55 | 60 | 40 | 100 | 57.31 | 83.49 | 33.09 | 180.19 | 439.40 | 343.47 | 483.55 |
| **13** | 55 | 45 | 30 | 100 | 54.26 | 78.96 | 30.40 | 180.38 | 423.89 | 327.01 | 453.78 |
| **14** | 50 | 45 | 30 | 80 | 53.59 | 77.93 | 31.59 | 155.81 | 418.89 | 321.22 | 429.00 |
| **15** | 55 | 45 | 40 | 80 | 54.32 | 79.01 | 31.84 | 170.38 | 401.39 | 325.77 | 457.26 |
| **16** | 55 | 30 | 30 | 80 | 52.58 | 76.63 | 30.57 | 158.19 | 417.51 | 309.66 | 436.16 |
| **17** | 60 | 60 | 40 | 80 | 54.32 | 79.09 | 30.98 | 168.16 | 446.17 | 335.61 | 440.09 |
| **18** | 55 | 60 | 30 | 80 | 55.81 | 82.04 | 31.86 | 173.51 | 424.68 | 344.16 | 455.26 |
| **19** | 60 | 30 | 40 | 80 | 53.51 | 76.76 | 31.20 | 169.73 | 424.97 | 319.49 | 430.54 |
| **20** | 50 | 45 | 40 | 60 | 54.85 | 79.11 | 31.52 | 168.91 | 418.69 | 341.09 | 464.10 |
| **21** | 55 | 30 | 50 | 80 | 56.18 | 82.96 | 32.97 | 171.54 | 441.46 | 348.01 | 472.32 |
| **22** | 55 | 30 | 40 | 100 | 53.79 | 76.21 | 29.40 | 176.56 | 412.02 | 330.37 | 445.78 |
| **23** | 55 | 45 | 50 | 100 | 59.86 | 86.43 | 33.64 | 187.49 | 448.53 | 360.12 | 494.72 |
| **24** | 60 | 45 | 40 | 100 | 54.05 | 80.56 | 31.80 | 180.37 | 436.75 | 338.13 | 464.95 |
| **25** | 50 | 30 | 40 | 80 | 52.66 | 74.98 | 29.64 | 167.12 | 417.22 | 325.52 | 439.32 |
| **26** | 55 | 45 | 50 | 60 | 58.71 | 84.99 | 32.67 | 188.49 | 440.28 | 359.48 | 498.16 |
| **27** | 55 | 60 | 50 | 80 | 58.97 | 84.84 | 33.64 | 193.14 | 455.99 | 349.42 | 503.14 |

EtOH conc.: % (v/v); Time: min; Temp.: °C; Power: W; TPC: mg GAE/g DW; TFC: mg CE/g DW; TPrC: mg CE/g DW; TSC: mg EE/g DW; DPPH, ABTS and FRAP: µM TE/g DW

**Table 2** Analysis of variance for determination of model fit

| | TPC | TFC | TPrC | TSC | Antioxidant capacity | | |
|---|---|---|---|---|---|---|---|
| | | | | | DPPH | ABTS | FRAP |
| Lack of fit | 0.63 | 0.06 | 0.02* | 0.61 | 0.71 | 0.0006* | 0.82 |
| $R^2$ | 0.95 | 0.93 | 0.90 | 0.76 | 0.83 | 0.92 | 0.88 |
| Adjusted $R^2$ | 0.89 | 0.86 | 0.77 | 0.48 | 0.62 | 0.83 | 0.73 |
| PRESS | 42.21 | 130.63 | 37.36 | 2877.99 | 10665.86 | 4587.55 | 3813.19 |
| F ratio of model | 15.44 | 12.10 | 7.33 | 2.71 | 4.06 | 10.25 | 6.01 |
| P of model > F | <.0001* | <.0001* | 0.0007* | 0.0455* | 0.0099* | 0.0018* | 0.0001* |

* Significantly different at $p < 0.05$

**Table 3** Analysis of variance for the experimental results based on predictive models

| Parameter | TPC | TFC | TPrC | TSC | DPPH | ABTS | FRAP |
|---|---|---|---|---|---|---|---|
| $\beta_0$ | <.0001* | <.0001* | <.0001* | <.0001* | <.0001* | <.0001* | <.0001* |
| $\beta_1$ | 0.0002* | 0.0108* | 0.0101* | 0.97 | 0.09 | 0.08 | 0.0126* |
| $\beta_2$ | <.0001* | 0.0002* | 0.0007* | 0.12 | 0.0042* | 0.0011* | 0.0163* |
| $\beta_3$ | <.0001* | <.0001* | <.0001* | 0.0032* | 0.0020* | <.0001* | <.0001* |
| $\beta_4$ | 0.0028* | 0.0018* | 0.41 | 0.10 | 0.0472* | 0.06 | 0.0172* |
| $\beta_{12}$ | 0.0020* | 0.0498* | 0.0012* | 0.68 | 0.08 | 0.19 | 0.98 |
| $\beta_{13}$ | 0.0180* | 0.23 | 0.31 | 0.41 | 0.73 | 0.08 | 0.0221* |
| $\beta_{23}$ | 0.79 | 0.22 | 0.68 | 0.65 | 0.79 | 0.49 | 0.07 |
| $\beta_{14}$ | 0.33 | 0.33 | 0.15 | 0.65 | 0.26 | 0.91 | 0.17 |
| $\beta_{24}$ | 0.28 | 0.0216* | 0.0294* | 0.61 | 0.77 | 0.0146* | 0.36 |
| $\beta_{34}$ | 0.63 | 0.49 | 0.23 | 0.75 | 0.63 | 0.48 | 0.29 |
| $\beta_{11}$ | 0.59 | 0.61 | 0.27 | 0.94 | 0.0343* | 0.12 | 0.59 |
| $\beta_{22}$ | 0.46 | 0.34 | 0.77 | 0.25 | 0.0055* | 0.24 | 0.80 |
| $\beta_{33}$ | 0.0038* | 0.0021* | 0.18 | 0.0269* | 0.0391* | 0.0220* | 0.0424* |
| $\beta_{44}$ | 0.36 | 0.58 | 0.89 | 0.0050* | 0.50 | 0.0397* | 0.44 |

* Significantly different at $p < 0.05$; $\beta_0$: intercept; $\beta_1$, $\beta_2$, $\beta_3$ and $\beta_4$: linear regression coefficients for ethanol concentration, time, temperature and power; $\beta_{12}$, $\beta_{13}$, $\beta_{13}$, $\beta_{23}$, $\beta_{14}$, $\beta_{24}$ and $\beta_{34}$: regression coefficients for interaction between ethanol concentration x time, ethanol concentration x temperature, time x temperature, ethanol concentration x power, time x power and temperature x power; $\beta_{11}$, $\beta_{22}$, $\beta_{33}$ and $\beta_{44}$: quadratic regression coefficients for ethanol concentration x ethanol concentration, time x time, temperature x temperature and power x power.

**Table 4** Comparison of phytochemical and antioxidant activities of *S. chinensis* root using different extraction methods

| | Decoction | CSE | UAE |
|---|---|---|---|
| **TPC** | $23.62 \pm 3.54^{a}$ | $63.04 \pm 0.84^{b}$ | $63.27 \pm 1.33^{b}$ |
| **TFC** | $32.44 \pm 5.44^{a}$ | $70.34 \pm 5.12^{b}$ | $91.75 \pm 0.62^{c}$ |
| **TPrC** | $13.82 \pm 2.72^{a}$ | $39.00 \pm 0.37^{b}$ | $41.83 \pm 0.85^{b}$ |
| **TSC** | $71.14 \pm 9.01^{a}$ | $218.44 \pm 4.59^{c}$ | $202.34 \pm 18.43^{b}$ |
| **DPPH** | $195.05 \pm 20.31^{a}$ | $450.82 \pm 21.79^{b}$ | $458.01 \pm 24.98^{b}$ |
| **ABTS** | $255.67 \pm 38.33^{a}$ | $510.68 \pm 17.18^{b}$ | $512.41 \pm 17.86^{b}$ |
| **FRAP** | $160.27 \pm 27.04^{a}$ | $344.31 \pm 19.23^{b}$ | $346.65 \pm 17.70^{b}$ |

TPC: mg GAE/g DW; TFC: mg CE/g DW; TPrC: mg CE/g DW; TSC: mg EE/g DW; DPPH, ABTS and FRAP: μM TE/g DW. The values are means ± standard deviations and those in the same row with different superscript letter are significantly different from each other ($p<0.05$)

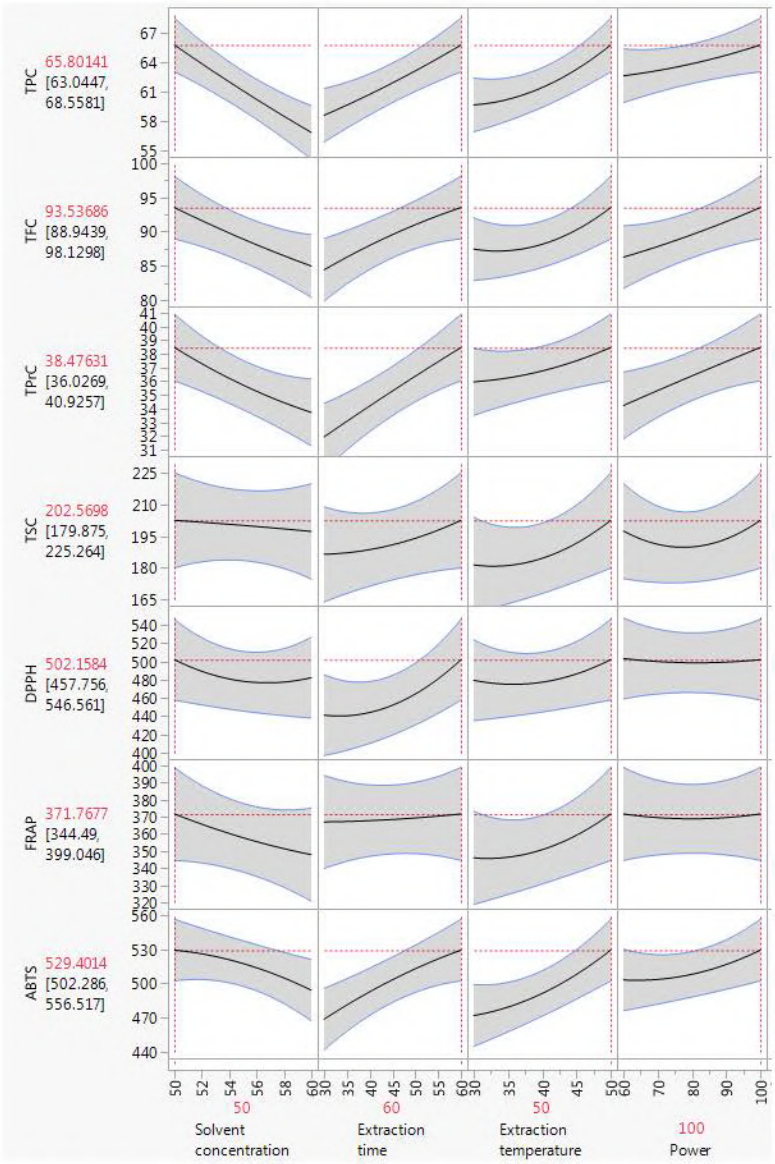
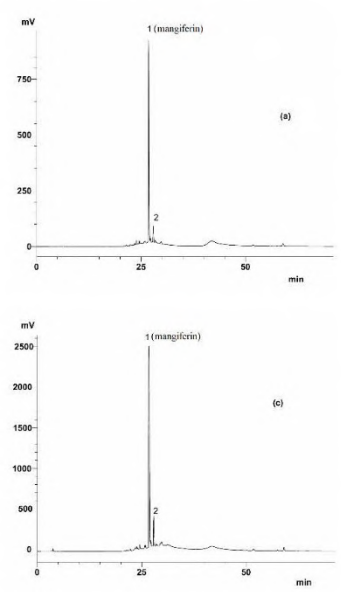



**Fig. 1** Impact of ethanol concentration, extraction time, temperature and power on TPC, TFC, TPrC, TSC and antioxidant properties (2D models)




| Peak/compound | Quantity (mg GAE/mg DW) | | |
|---|---|---|---|
| | Decoction | CSE | UAE |
| 1 (mangiferin) | $3.93 \pm 0.13^{a}$ | $34.89 \pm 1.45^{b}$ | $37.92 \pm 1.11^{c}$ |
| 2 | $0.54 \pm 0.05^{a}$ | $4.74 \pm 0.10^{b}$ | $4.77 \pm 0.11^{b}$ |
| Total | 4.47 | 39.63 | 42.69 |

**Fig. 2** Representative HPLC chromatograms detected at 254 nm and major individual compounds of *S. chinensis* root extract using different extraction methods: decoction (a), CSE (b) and UAE (c)

**Table 1** Box-Behnken design and observed responses

| Run | Ultrasonic conditions | | | | Experimental values (n = 3) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | EtOH conc. (%) | Time (min) | Tem. (°C) | Power (W) | TPC (mg GAE/g) | TFC (mg CE/g) | TPrC (mg CE/g) | TSC (mg EE/g) | Antioxidant capacity | | |
| | | | | | | | | | DPPH (μM TE/g) | ABTS (μM TE/g) | FRAP (μM TE/g) |
| **1** | 50 | 60 | 40 | 80 | 59.99 | 83.34 | 35.64 | 171.27 | 490.34 | 342.13 | 471.55 |
| **2** | 60 | 45 | 50 | 80 | 55.07 | 83.40 | 34.22 | 171.97 | 436.65 | 325.72 | 470.70 |
| **3** | 60 | 45 | 40 | 60 | 52.97 | 77.30 | 31.24 | 167.83 | 392.58 | 314.65 | 450.27 |
| **4** | 55 | 45 | 40 | 80 | 53.45 | 79.63 | 31.68 | 156.80 | 390.03 | 316.73 | 457.01 |
| **5** | 50 | 45 | 40 | 100 | 57.61 | 85.18 | 34.32 | 175.01 | 431.35 | 340.90 | 476.81 |
| **6** | 55 | 60 | 40 | 60 | 54.48 | 77.40 | 31.89 | 166.38 | 427.52 | 317.76 | 447.18 |
| **7** | 55 | 30 | 40 | 60 | 52.82 | 77.40 | 31.84 | 169.83 | 408.29 | 320.24 | 455.89 |
| **8** | 55 | 45 | 30 | 60 | 52.28 | 75.58 | 31.30 | 176.98 | 402.40 | 308.32 | 445.43 |
| **9** | 60 | 45 | 30 | 80 | 53.24 | 76.89 | 29.87 | 160.20 | 400.34 | 322.01 | 450.20 |
| **10** | 55 | 45 | 40 | 80 | 55.18 | 78.95 | 31.65 | 161.28 | 420.26 | 338.37 | 457.43 |
| **11** | 50 | 45 | 50 | 80 | 59.96 | 87.89 | 34.38 | 179.16 | 445.88 | 367.98 | 480.88 |
| **12** | 55 | 60 | 40 | 100 | 57.31 | 83.49 | 33.09 | 180.19 | 439.40 | 343.47 | 483.55 |
| **13** | 55 | 45 | 30 | 100 | 54.26 | 78.96 | 30.40 | 180.38 | 423.89 | 327.01 | 453.78 |
| **14** | 50 | 45 | 30 | 80 | 53.59 | 77.93 | 31.59 | 155.81 | 418.89 | 321.22 | 429.00 |
| **15** | 55 | 45 | 40 | 80 | 54.32 | 79.01 | 31.84 | 170.38 | 401.39 | 325.77 | 457.26 |
| **16** | 55 | 30 | 30 | 80 | 52.58 | 76.63 | 30.57 | 158.19 | 417.51 | 309.66 | 436.16 |
| **17** | 60 | 60 | 40 | 80 | 54.32 | 79.09 | 30.98 | 168.16 | 446.17 | 335.61 | 440.09 |
| **18** | 55 | 60 | 30 | 80 | 55.81 | 82.04 | 31.86 | 173.51 | 424.68 | 344.16 | 455.26 |
| **19** | 60 | 30 | 40 | 80 | 53.51 | 76.76 | 31.20 | 169.73 | 424.97 | 319.49 | 430.54 |
| **20** | 50 | 45 | 40 | 60 | 54.85 | 79.11 | 31.52 | 168.91 | 418.69 | 341.09 | 464.10 |
| **21** | 55 | 30 | 50 | 80 | 56.18 | 82.96 | 32.97 | 171.54 | 441.46 | 348.01 | 472.32 |
| **22** | 55 | 30 | 40 | 100 | 53.79 | 76.21 | 29.40 | 176.56 | 412.02 | 330.37 | 445.78 |
| **23** | 55 | 45 | 50 | 100 | 59.86 | 86.43 | 33.64 | 187.49 | 448.53 | 360.12 | 494.72 |
| **24** | 60 | 45 | 40 | 100 | 54.05 | 80.56 | 31.80 | 180.37 | 436.75 | 338.13 | 464.95 |
| **25** | 50 | 30 | 40 | 80 | 52.66 | 74.98 | 29.64 | 167.12 | 417.22 | 325.52 | 439.32 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **26** | 55 | 45 | 50 | 60 | 58.71 | 84.99 | 32.67 | 188.49 | 440.28 | 359.48 | 498.16 |
| **27** | 55 | 60 | 50 | 80 | 58.97 | 84.84 | 33.64 | 193.14 | 455.99 | 349.42 | 503.14 |

**Table 2** Analysis of variance for determination of model fit

| | TPC | TFC | TPrC | TSC | Antioxidant capacity | | |
|---|---|---|---|---|---|---|---|
| | | | | | DPPH | ABTS | FRAP |
| Lack of fit | 0.63 | 0.06 | 0.02* | 0.61 | 0.71 | 0.0006* | 0.82 |
| $R^2$ | 0.95 | 0.93 | 0.90 | 0.76 | 0.83 | 0.92 | 0.88 |
| Adjusted $R^2$ | 0.89 | 0.86 | 0.77 | 0.48 | 0.62 | 0.83 | 0.73 |
| PRESS | 42.21 | 130.63 | 37.36 | 2877.99 | 10665.86 | 4587.55 | 3813.19 |
| F ratio of model | 15.44 | 12.10 | 7.33 | 2.71 | 4.06 | 10.25 | 6.01 |
| P of model > F | <.0001* | <.0001* | 0.0007* | 0.0455* | 0.0099* | 0.0018* | 0.0001* |

* Significantly different at $p < 0.05$

**Table 3** Analysis of variance for the experimental results based on predictive models

| Parameter | TPC | TFC | TPrC | TSC | DPPH | ABTS | FRAP |
|---|---|---|---|---|---|---|---|
| $\beta_o$ | <.0001* | <.0001* | <.0001* | <.0001* | <.0001* | <.0001* | <.0001* |
| $\beta_1$ | 0.0002* | 0.0108* | 0.0101* | 0.97 | 0.09 | 0.08 | 0.0126* |
| $\beta_2$ | <.0001* | 0.0002* | 0.0007* | 0.12 | 0.0042* | 0.0011* | 0.0163* |
| $\beta_3$ | <.0001* | <.0001* | <.0001* | 0.0032* | 0.0020* | <.0001* | <.0001* |
| $\beta_4$ | 0.0028* | 0.0018* | 0.41 | 0.10 | 0.0472* | 0.06 | 0.0172* |
| $\beta_{12}$ | 0.0020* | 0.0498* | 0.0012* | 0.68 | 0.08 | 0.19 | 0.98 |
| $\beta_{13}$ | 0.0180* | 0.23 | 0.31 | 0.41 | 0.73 | 0.08 | 0.0221* |
| $\beta_{23}$ | 0.79 | 0.22 | 0.68 | 0.65 | 0.79 | 0.49 | 0.07 |
| $\beta_{14}$ | 0.33 | 0.33 | 0.15 | 0.65 | 0.26 | 0.91 | 0.17 |
| $\beta_{24}$ | 0.28 | 0.0216* | 0.0294* | 0.61 | 0.77 | 0.0146* | 0.36 |
| $\beta_{34}$ | 0.63 | 0.49 | 0.23 | 0.75 | 0.63 | 0.48 | 0.29 |
| $\beta_{11}$ | 0.59 | 0.61 | 0.27 | 0.94 | 0.0343* | 0.12 | 0.59 |
| $\beta_{22}$ | 0.46 | 0.34 | 0.77 | 0.25 | 0.0055* | 0.24 | 0.80 |
| $\beta_{33}$ | 0.0038* | 0.0021* | 0.18 | 0.0269* | 0.0391* | 0.0220* | 0.0424* |
| $\beta_{44}$ | 0.36 | 0.58 | 0.89 | 0.0050* | 0.50 | 0.0397* | 0.44 |

* Significantly different at $p < 0.05$; $\beta_o$: intercept; $\beta_1$, $\beta_2$, $\beta_3$ and $\beta_4$: linear regression coefficients for ethanol concentration, time, temperature and power; $\beta_{12}$, $\beta_{13}$, $\beta_{13}$, $\beta_{23}$, $\beta_{14}$, $\beta_{24}$ and $\beta_{34}$: regression coefficients for interaction between ethanol concentration x time, ethanol concentration x temperature, time x temperature, ethanol concentration x power, time x power and temperature x power; $\beta_{11}$, $\beta_{22}$, $\beta_{33}$ and $\beta_{44}$: quadratic regression coefficients for ethanol concentration x ethanol concentration, time x time, temperature x temperature and power x power.

**Table 4** Comparison of phytochemical and antioxidant activities of *S. chinensis* root using different extraction methods

| | Decoction | CSE | UAE |
|---|---|---|---|
| **TPC** (mg GAE/g DW) | $23.62 \pm 3.54^{a}$ | $63.04 \pm 0.84^{b}$ | $63.27 \pm 1.33^{b}$ |
| **TFC** (mg CE/g DW) | $32.44 \pm 5.44^{a}$ | $70.34 \pm 5.12^{b}$ | $91.75 \pm 0.62^{c}$ |
| **TPrC** (mg CE/g DW) | $13.82 \pm 2.72^{a}$ | $39.00 \pm 0.37^{b}$ | $41.83 \pm 0.85^{b}$ |
| **TSC** (mg EE/g DW) | $71.14 \pm 9.01^{a}$ | $218.44 \pm 4.59^{c}$ | $202.34 \pm 18.43^{b}$ |
| **DPPH** (μM TE/g DW) | $195.05 \pm 20.31^{a}$ | $450.82 \pm 21.79^{b}$ | $458.01 \pm 24.98^{b}$ |
| **ABTS** (μM TE/g DW) | $255.67 \pm 38.33^{a}$ | $510.68 \pm 17.18^{b}$ | $512.41 \pm 17.86^{b}$ |
| **FRAP**(μM TE/g DW) | $160.27 \pm 27.04^{a}$ | $344.31 \pm 19.23^{b}$ | $346.65 \pm 17.70^{b}$ |

The values are means ± standard deviations and those in the same row with different superscript letter are significantly different from each other ($p<0.05$)

Fig 1. Impact of ethanol concentration, extraction time, temperature and power on TPC, TFC, TPrC, TSC and antioxidant

Click here to access/download;line figure;Figure 1.tiff

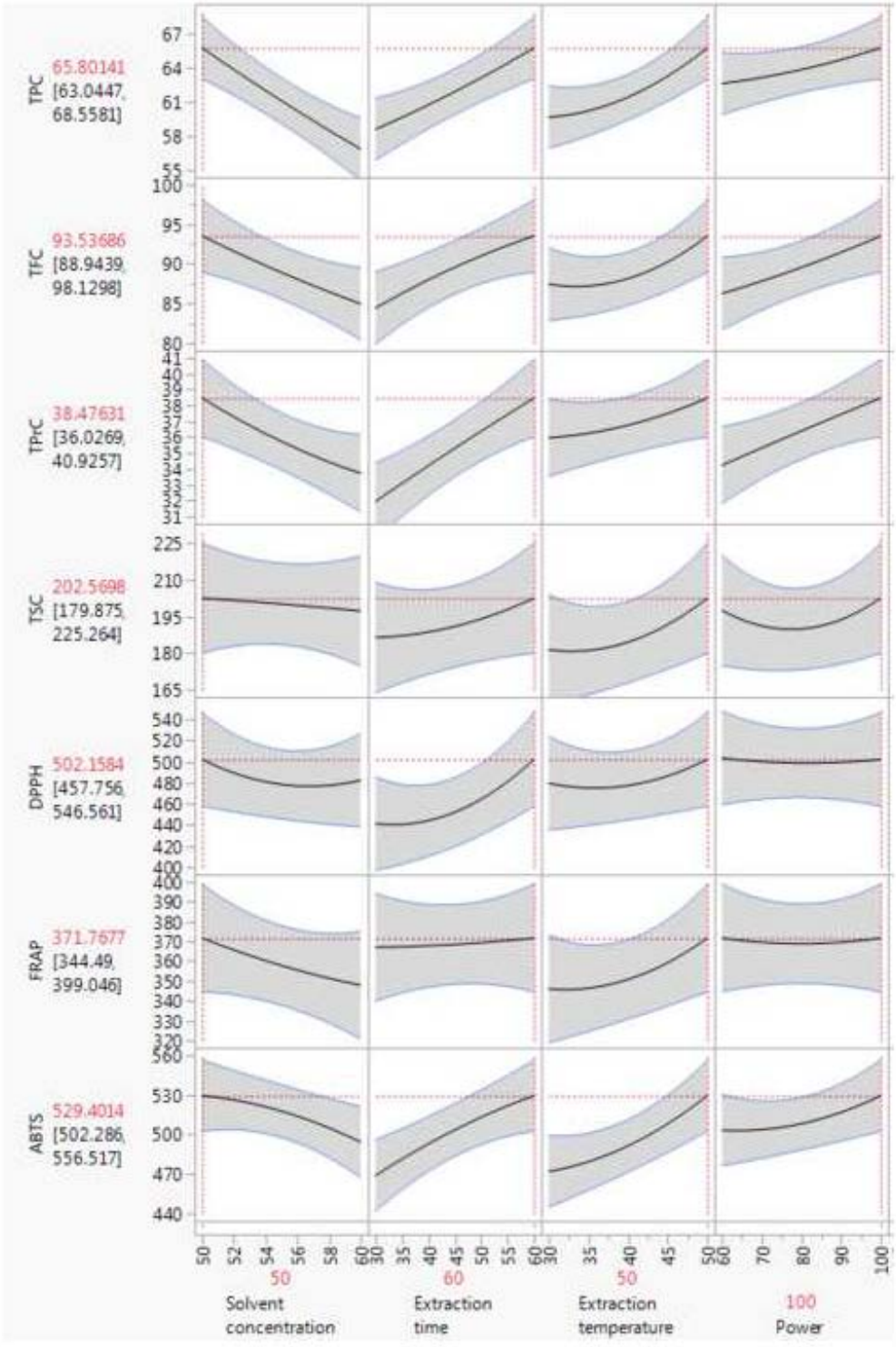
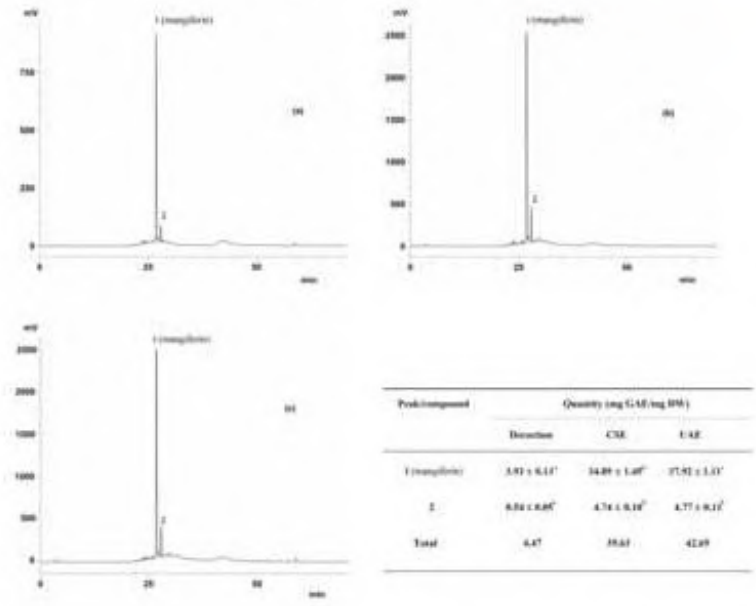


Fig 2. Representative HPLC chromatograms detected at 254 nm and major individual compounds of S. chinensis root extract using

Click here to access/download;line figure;Figure2.tif



| Peak/compound | Quantity (mg GAE/mg DW) | | |
|---|---|---|---|
| | Decoction | CSE | UAE |
| 1 (marglitin) | 3.93 ± 0.11[a] | 34.89 ± 1.49[b] | 37.92 ± 1.31[c] |
| 2 | 0.54 ± 0.05[a] | 4.74 ± 0.18[b] | 4.77 ± 0.21[b] |
| Total | 4.47 | 39.63 | 42.69 |

separations

MDPI

*Article*

# Isolation and Maximisation of Extraction of Mangiferin from the Root of *Salacia chinensis* L.

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**Abstract:** Mangiferin has been reported to exhibit anti-viral, anti-cancer, anti-diabetic, immunomodulatory and hepatoprotective properties. This study aimed to develop an HPLC method to isolate mangiferin from *Salacia chinensis* L. root; investigate the impact of solvents on yield; optimise the ultrasound-assisted extraction (UAE) technique; and compare mangiferin yield with continuously shaking extraction (CSE) and decoction techniques. The results showed that mangiferin, with a purity of over 88%, could be achieved by HPLC using a mixture of solvent A (water: acetonitrile: orthophosphoric acid, 96.8:3:0.2 (*v/v/v*)) and solvent B (acetonitrile). Solvent type significantly affected the extraction yield of mangiferin, and a mixture of acetone and water gave the highest extraction yield, as compared to other solvents or mixtures. UAE conditions, such as ultrasonic power, temperature, time and concentration of acetone significantly affected the extraction of mangiferin. Optimal UAE conditions were at an ultrasonic power of 250 W, temperature of 50 °C, acetone concentration of 40% and extraction time of 60 min. These optimal conditions could extract approximately 92 mg, whereas CSE and decoction only extracted 89.20 mg and 58.71 mg of mangiferin, respectively, from 1 g of *S. chinensis* root. Therefore, these UAE conditions are recommended for the extraction of mangiferin from *S. chinensis* root for further utilisation.

**Keywords:** *Salacia chinensis*; mangiferin; extraction; isolation; optimisation

## 1. Introduction

Mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-β-D glucoside) is a xanthone glucoside (polyphenol) found primarily in mango (*Mangifera indica*) [1]. Mangiferin is considered as a "super antioxidant", and has attracted the interest of researchers around the world. Over 450 articles have been published on its occurrence, chemical nature, synthesis and medicinal properties over the last 50 years [2,3]. It has been found to exhibit anti-viral, anti-cancer, anti-diabetic, anti-aging, immunomodulatory, hepatoprotective and analgesic properties [4].

Mangiferin-containing plant materials, such as mango, honeybush tea (*Cyclopia* spp.) and *Salacia chinensis*, have been used as traditional medicine for the prevention and treatment of numerous ailments [1]. *S. chinensis* in particular has been traditionally used for the treatment of arthritis, inflammation, diabetes, obesity, liver disorder and certain cancers [5,6], with mangiferin reported as one of the major bioactive component in the root of *S. chinensis* [5,7].

The extraction process is an essential step to separate bioactive compounds from more complex plant materials. According to literature, extraction technique is a key factor that significantly influences the yields of phytochemicals [8–12]. For example, Nayak et al. (2015) found that the employment

of different extraction methods resulted in the variation of the recovery efficiency of total phenolics, antioxidant activities as well as concentration of individual compounds in *Citrus sinensis* peels [13]. In addition, solvent type and concentration have also been reported to play an important role in the extraction process [8,14–16].

Although several extraction solvents and extraction techniques have been previously tested for the extraction of mangiferin [7,17,18], optimal conditions for maximum extraction of mangiferin from *S. chinensis* root have not been reported. Therefore, the aim of this study was to isolate and identify mangiferin in the root of *S. chinensis*; compare the impact of solvent type and concentration on the extraction efficiency of mangiferin; optimise the ultrasound assisted extraction (UAE) conditions; and finally compare the extraction efficiency of UAE, as an advanced extraction technique, with two other common conventional extraction techniques, continuously shaking extraction (CSE) and decoction.

## 2. Materials and Methods

### 2.1. Materials

*S. chinensis* L. root was collected in Nghe an province (Vietnam) and authenticated by A/Prof Vu Quang Nam, Department of Forest Plant Resources, College of Forestry Biotechnology, Vietnam National University of Forestry in March 2016. The voucher specimen of this plant material can be found in the Herbarium of Institute of Medicinal Materials, Ha Noi (SA 611/04). The root was then cut, sun-dried for 2 days and ground to a fine powder using a commercial cutter. The powder was sieved (steel mesh sieve 1.4 mm, EFL 2000; Endecotts Ltd., London, UK) and stored at −20 °C until required for analysis.

### 2.2. Methods

#### 2.2.1. Experimental Design

The overall design of this study is shown in Figure 1. Mangiferin was isolated from the UAE extract of the *S. chinensis* root using HPLC connected with an auto-fraction collector and was then confirmed via LC/MS. A range of solvents were then tested to determine the most suitable solvent for mangiferin extraction. This solvent was then used for optimising extraction conditions using ultrasound-assisted extraction with Response Surface Methodology (RSM). Optimal acetone concentration from RSM was further applied in continuously shaking extraction for comparison. A traditional extraction method, known as decoction using water, was also applied for comparison of the extraction efficiency of mangiferin with optimal ultrasonic extraction and continuously shaking extraction. The best extraction conditions to yield the greatest quantity were then identified for mangiferin from *S. chinensis* root.

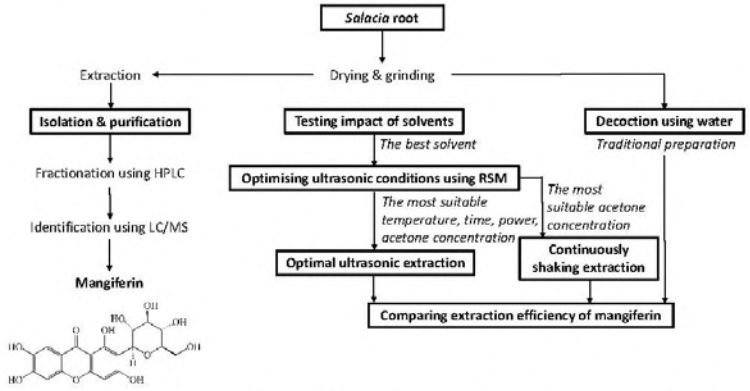


**Figure 1.** Experimental design for maximising extraction of mangiferin from *S. chinensis* root.

2.2.2. Isolation and Identification of Mangiferin in *S. chinensis* Root Extract

To identify mangiferin in *S. chinensis* root extract, the *S. chinensis* root (2 g) was extracted in 100 mL of 50% ethanol using an ultrasonic bath (Soniclean 1000HD, 220 V, 50/60 Hz and 250 W, Soniclean Pty Ltd., Thebarton, SA, Australia) set at 50 °C and 150 W for 60 min. The extract was cooled and then filtered using a Phenex Syringe filter 0.45 µm. The extract was then subjected to the Shimadzu HPLC system (M20, Shimadzu Australia, Rydalmere, NSW, Australia) connected with the EC-C18 reversed-phase column (3.0 × 150, 4 µm, Agilent Technologies Pty Ltd., Santa Clara, CA, USA). The mobile phase consisted of solvent A (a mixture of water: acetonitrile: orthophosphoric acid, 96.8:3:0.2 (*v*/*v*/*v*)) and solvent B (acetonitrile). A gradient elution schedule was used as follows: 100% A from 0 to 3 min; 100% B from 3 to 6 min; a linear gradient from 100% A to 100% B from 6 to 20 min and remained at 100% B to 25 min; and 100% A from 25 to 30 min before the next injection. The volume of injection was 20 µL, and the flow rate was 1 mL/min. The column was kept in an oven with the temperature maintained at 28 °C. The detector was set at 254 nm.

The chromatogram of the *S. chinensis* root extract with major peaks is shown in Figure 2A. Based on the retention time, peaks A and B were fractionated using an auto-fraction collector and then freeze-dried to a powder form. The fractions A and B (peaks A and B) were then identified according to the method described previously by Muraoka et al. [19] with some minor modifications using a Shimadzu LC/MS (LCMS 2020, Shimadzu Australia, Rydalmere, NSW, Australia) coupled with an electrospray ionization (ESI) interface. The mobile phase including solvents A and B as earlier described was delivered at a flow rate of 0.2 mL/min. The volume of injection was 10 µL. The mass spectrometer was operated at negative mode with selected ion monitoring (SIM) and the parameters as follows: nebulizing gas (nitrogen) flow 1.5 L/min, drying gas pressure 0.15 MPa, CDL temperature 250 °C, and block heater temperature 200 °C.

2.2.3. Impact of Different Solvents on Recovery of Mangiferin

Seven solvents were used for testing their impact on the extraction efficiency of mangiferin from *S. chinensis* root. These solvents were water (polarity index, PI 10.2), absolute methanol (PI 5.1), absolute ethanol (PI 4.3), absolute acetone (PI 5.1), methanol 50%, ethanol 50% and acetone 50%.

Extraction was performed by firstly adding 0.5 g of the sample into 25 mL of each solvent (sample/solvent ratio 1:50 g/mL), then extracting using an ultrasonic bath set at the same conditions as described in Section 2.2.2. The extract was then filtered and subjected onto the HPLC system for determination of mangiferin using the HPLC method described in Section 2.2.2. Mangiferin was quantified using a standard curve prepared from mangiferin standard (Sigma Aldrich, Australia) with different concentrations (0.25–2 mM) ($Y_{mangiferin}$ = 0.1507X − 241.368, $Y_{mangiferin}$ is the concentration of mangiferin in mM, X is the area in Volts, $R^2$ = 0.987). The best solvent (acetone 50%) was then used for optimising ultrasonic extraction.

2.2.4. Optimisation of UAE Conditions for Recovery of Mangiferin

To optimise ultrasonic extraction conditions, Response Surface Methodology (RSM) was employed to design, analyse and determine the best conditions for temperature, time, ultrasonic power and water to acetone ratio (acetone concentration). JMP Pro software (version 14.2.0, 64-bit, SAS Institute Inc., Cary, NC, USA) was employed for RSM experimental design using a three-level, four-factorial, Box–Behnken methodology design. Four factors were applied with the following ranges: acetone concentration (40–70%), time (30–60 min), extraction temperature (30–50 °C) and power (150–250 W). The sample (2 g) was extracted in 100 mL of solvent with experimental conditions as shown in Table 1.

**Table 1.** Box-Behnken design and experimental results.

| Run | Ultrasonic Conditions | | | | Experimental Values (mg/g DW, $n$ = 3) |
|---|---|---|---|---|---|
| | Power | Temp | Acetone Conc. | Time | |
| 1 | 60 | 30 | 55 | 45 | 65.55 ± 3.25 |
| 2 | 80 | 30 | 55 | 30 | 65.60 ± 2.44 |
| 3 | 80 | 30 | 70 | 45 | 64.34 ± 0.44 |
| 4 | 80 | 30 | 40 | 45 | 68.04 ± 2.71 |
| 5 | 80 | 30 | 55 | 60 | 71.27 ± 0.64 |
| 6 | 100 | 30 | 55 | 45 | 71.08 ± 0.43 |
| 7 | 60 | 40 | 55 | 30 | 73.37 ± 1.53 |
| 8 | 60 | 40 | 70 | 45 | 67.54 ± 0.74 |
| 9 | 60 | 40 | 40 | 45 | 72.10 ± 2.02 |
| 10 | 60 | 40 | 55 | 60 | 75.23 ± 2.19 |
| 11 | 80 | 40 | 40 | 30 | 78.63 ± 0.33 |
| 12 | 80 | 40 | 70 | 30 | 66.32 ± 3.55 |
| 13 | 80 | 40 | 55 | 45 | 78.20 ± 0.16 |
| 14 | 80 | 40 | 70 | 60 | 70.79 ± 0.65 |
| 15 | 80 | 40 | 40 | 60 | 80.70 ± 0.25 |
| 16 | 100 | 40 | 55 | 30 | 76.81 ± 3.31 |
| 17 | 100 | 40 | 40 | 45 | 80.15 ± 1.91 |
| 18 | 10 | 40 | 70 | 45 | 72.07 ± 4.38 |
| 19 | 100 | 40 | 55 | 60 | 85.25 ± 1.63 |
| 20 | 60 | 50 | 55 | 45 | 78.83 ± 1.92 |
| 21 | 80 | 50 | 55 | 30 | 88.25 ± 0.21 |
| 22 | 80 | 50 | 40 | 45 | 86.48 ± 0.87 |
| 23 | 80 | 50 | 70 | 45 | 71.64 ± 1.50 |
| 24 | 80 | 50 | 55 | 60 | 84.78 ± 3.32 |
| 25 | 100 | 50 | 55 | 45 | 85.90 ± 0.60 |

For the prediction of the optimal conditions, a second order polynomial Equation (1) was used to evaluate the relationship between variables and mangiferin concentration (Y):

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{14}X_1X_4 + \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{34}X_3X_4 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2 \quad (1)$$

where $X_1$: power; $X_2$: temperature, $X_3$: solvent concentration and $X_4$: time; $\beta_0$: intercept; $\beta_1$, $\beta_2$, $\beta_3$ and $\beta_4$: linear regression coefficients; $\beta_{12}$, $\beta_{13}$, $\beta_{23}$, $\beta_{14}$, $\beta_{24}$ and $\beta_{34}$: interaction regression coefficients; and $\beta_{11}$, $\beta_{22}$, $\beta_{33}$ and $\beta_{44}$: quadratic regression coefficients.

#### 2.2.5. Continuously Shaking Extraction

Continuously shaking extraction (CSE) was conducted as previously described by Chavan et al. [20] with minor modifications. Optimal acetone concentration (40%) identified from RSM was further applied in CSE. Briefly, 0.5 g of *S. chinensis* root was added into 25 mL acetone 40%. The extraction was then performed on an orbital shaker for 24 h at ambient temperature. Subsequently, the extract was filtered (Phenex Syringe filter, 0.45 µm, Phenomenex Australia, Lane Cove West NSW, Australia), collected and subjected to HPLC analysis for the determination of mangiferin concentration as described in Section 2.2.2.

#### 2.2.6. Decoction

Decoction of the *S. chinensis* L. root was conducted similar to a previous study by Karunanayake and Sirimanne [17]. Briefly, 10 g of sample was added into 500 mL DI water (sample/solvent ratio 1:50 g/mL). The mixture was then put in a small container and boiled for 3 h. Subsequently, the extract was filtered (Phenex Syringe filter, 0.45 µm, Phenomenex Australia, Lane Cove West NSW, Australia), collected and subjected to HPLC analysis for determination of the mangiferin concentration.

### 2.3. Statistical Analysis

JMP Pro version 14.2.0 (64-bit, SAS Institute Inc., Cary, NC, USA) was employed for RSM design and statistical analysis in this study. Extraction was conducted in triplicates for each experiment. Data were reported as means ± standard deviations. Differences between the mean levels of the components in the different experiments were taken to be statistically significant at $p < 0.05$ using Tukey's Honest Significant Difference test. Two-dimensional and 3D contour plots of variable responses, as well as the predictive optimal values and extraction conditions of four independent variables, were generated by the program.

## 3. Results

### 3.1. Identification of Mangiferin in S. chinensis Root Extract

Figure 2A,B shows the chromatogram and major phytochemical components as well as their major absorbances from the extract of *S. chinensis* root. These results revealed that *S. chinensis* root had two major compounds, which were referred to as peak A and peak B. The scanning of absorbance from 190 nm to 700 nm (using PDA detector, Figure 2B) indicated that these compounds had maximum absorbance values in the range of 254 nm. Peak B was then isolated using a Shimadzu auto-fraction collector collected from 8.5 min to 10 min. The fraction was then freeze-dried to get a yellow amorphous powder. Subsequently, the compound was reconstituted in methanol and subjected to LC/MS with negative polarity mode. The chromatogram (Figure 2C) and the mass spectrum (Figure 2D) showed that only a peak was detected with purity of 88.5% and *m/z* 421. From its physical property (yellow amorphous powder form) and molecular mass (422), the compound (peak B) was identified as mangiferin. Therefore, this study illustrated and confirmed that mangiferin is a major active component in *S. chinensis* [17,21].

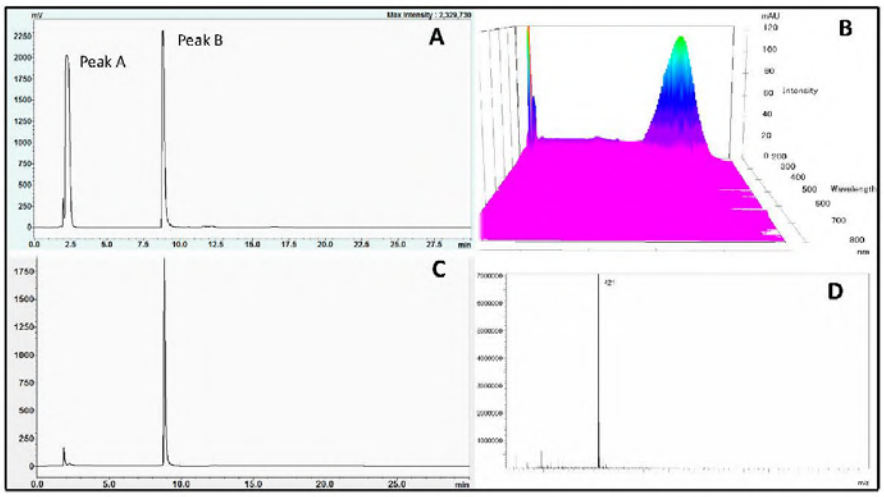


**Figure 2.** HPLC chromatogram of *S. chinensis* crude extract (**A**), wavelength spectrum (190–700 nm) (**B**), isolated mangiferin (**C**) and mass spectrum of mangiferin (**D**).

### 3.2. Impact of Type of Solvent on the Yield of Mangiferin

It was hypothesised that the extraction of mangiferin from *S. chinensis* root could be influenced by solvent type. Seven common solvents were tested, with the results (Table 2) showing that solvent type did significantly affect the extraction efficiency of mangiferin from *S. chinensis* root. In general,

the mixture of water and organic solvents (at a concentration of 50%, *v/v*) produced higher extraction efficiency than water or the organic solvents alone. A mixture of water and acetone extracted the highest content of mangiferin (78.76 mg/g of dried sample), followed by ethanol 50%, methanol 50%, methanol, ethanol, and water. By contrast, absolute acetone extracted the lowest level of concentration (10.36 mg/g of dried sample). Since acetone 50% had the highest extraction efficiency, this solvent mixture was used for further experiments. Acetone, or the combination of this organic solvent and water, has also been used effectively by other researchers. For example, while Dailey and Vuong [22] selected acetone 50% as the best solvent for recovery of total phenolics from *Macadamia tetraphylla* nut skin, absolute ethanol and acetone were chosen by Do et al. [10] for the extraction of TPC from *Limnophila aromatic*. Of note, Ngo et al. [23] reported that acetone 50% produced the highest yields of extractable solids, phenolics, flavonoids, saponins and antioxidant properties from *S. chinensis* root.

**Table 2.** Impact of type of solvent on recovery of mangiferin from *S. chinensis* root.

| Solvent | Concentration of Mangiferin (mg/g DW) |
|---|---|
| $H_2O$ | $20.45 \pm 0.85^{f}$ |
| Ethanol | $45.43 \pm 0.23^{e}$ |
| Ethanol 50% | $72.45 \pm 0.48^{b}$ |
| Methanol | $57.96 \pm 2.33^{d}$ |
| Methanol 50% | $66.10 \pm 1.49^{c}$ |
| Acetone | $10.36 \pm 0.50^{g}$ |
| Acetone 50% | $78.76 \pm 0.78^{a}$ |

Values are means ± standard deviations. Values with different superscript letters were significantly different at $p < 0.05$.

### 3.3. Optimisation of UAE Conditions for Recovery of Mangiferin

Four ultrasonic extraction factors, including power, temperature, extraction time, and acetone concentration were investigated to identify the optimal conditions using RSM. The second-order polynomial equation for estimation of mangiferin recovery yield was generated as follows:

$$Y_{\text{Mangiferin}} = -96.60 + 0.46X_1 + 3.46X_2 + 2.65X_3 - 0.07X_4 + 0.002X_1X_2 - 0.0029X_1X_3 - 0.0185X_2X_3 + 0.0055X_1X_4 - 0.0152X_2X_4 + 0.0027X_3X_4 - 0.0029{X_1}^2 - 0.0141{X_2}^2 - 0.0190{X_3}^2 + 0.0022{X_4}^2$$

To test the adequacy of the RSM mathematical model for optimisation, variance was analysed (Table 3). The results indicated that the model was significant, PRESS value was high, and the lack of fit was found to be insignificant, meaning that the model fitting was adequate. In addition, the coefficient of determination ($R^2$) was 0.898, revealing that at least 89.8% of the predicted values would match with the actual experimental values, which further confirmed the reliability of the model to predict the content of mangiferin.

As the model was reliable for prediction, the impact of individual extraction factors on extraction efficiency of mangiferin was further analysed (Table 3 and Figure 3). It was shown that all four variables significantly impact on the recovery yield of mangiferin within their tested ranges ($p < 0.05$) (Table 3). Recovery yield of mangiferin increased when increasing the ultrasonic power, temperature and time. In contrast, recovery yield of mangiferin decreased when acetone concentration raised from 40% to 70%. These findings were in agreement with our previous study which reported that while extraction time, temperature and irradiative power had a positive correlation on the recovery of total phenolic compounds from *S. chinensis* root, solvent concentration showed the opposite trend [24].

**Table 3.** Estimated regression coefficients for the model and the analysis of variance.

| Parameter | Standard Error | DF | Sum of Squares | F Value | Prob > F |
|---|---|---|---|---|---|
| $\beta_0$ | 1.53 | | | | <0.0001 * |
| $\beta_1$ | 0.44 | 1 | 373.52 | 53.27 | <0.0001 * |
| $\beta_2$ | 0.44 | 1 | 2024.85 | 288.75 | <0.0001 * |
| $\beta_3$ | 0.44 | 1 | 713.25 | 101.71 | <0.0001 * |
| $\beta_4$ | 0.44 | 1 | 90.60 | 12.92 | 0.0007 * |
| $\beta_{12}$ | 0.76 | 1 | 1.79 | 0.26 | 0.6148 |
| $\beta_{13}$ | 0.76 | 1 | 9.31 | 1.33 | 0.2538 |
| $\beta_{23}$ | 0.76 | 1 | 92.91 | 13.25 | 0.0006 * |
| $\beta_{14}$ | 0.76 | 1 | 32.57 | 4.64 | 0.0352 * |
| $\beta_{24}$ | 0.76 | 1 | 62.56 | 8.92 | 0.0041 * |
| $\beta_{34}$ | 0.76 | 1 | 4.30 | 0.61 | 0.4369 |
| $\beta_{11}$ | 0.91 | 1 | 11.12 | 1.59 | 0.2127 |
| $\beta_{22}$ | 0.91 | 1 | 16.87 | 2.41 | 0.1261 |
| $\beta_{33}$ | 0.91 | 1 | 155.24 | 22.14 | <0.0001 * |
| $\beta_{44}$ | 0.91 | 1 | 2.06 | 0.29 | 0.5895 |
| Lack of fit | 0.0996 | | PRESS | 648.44 | |
| $R^2$ | 0.898 | | RMSE | 2.648 | |
| Adjusted $R^2$ | 0.874 | | F ratio of model | 37.87 | |
| | | | Prob > F | <0.0001 * | |

Note: values with superscript * are significant ($p \leq 0.05$).

Table 3 also showed the interaction effects between four variables on the recovery yield of mangiferin. The results indicated that there were significant interactive effects between temperature and acetone concentration, power and extraction time, and temperature and time on the recovery yield of mangiferin. However, no significant interactive effect was observed between extraction power and temperature, extraction power and acetone concentration as well as acetone concentration and extraction time. These results were also confirmed by findings in our previous study which reported that total phenolics in *S. chinensis* root was influenced significantly by the interactive effect between solvent concentration and temperature, but not affected by the interaction between power and temperature as well as solvent concentration and power [24].

According to this model, the optimal conditions for recovery of mangiferin from *S. chinensis* root were predicted as follows: extraction power of 250 W, temperature of 50 °C, acetone concentration of 40% and extraction time of 60 min. To validate these predicted optimal conditions, the sample was extracted with similar conditions in triplicate. The actual results were statistically compared with the predicted data using the *t*-test. The results indicated that the actual experimental data (91.95 ± 0.55 mg/g DW) was not significantly different to the predicted data (91.43 ± 5.30 mg/g DW), revealing that the predicted conditions were reliable and thus these optimal conditions can be used for maximum extraction of mangiferin using UAE.

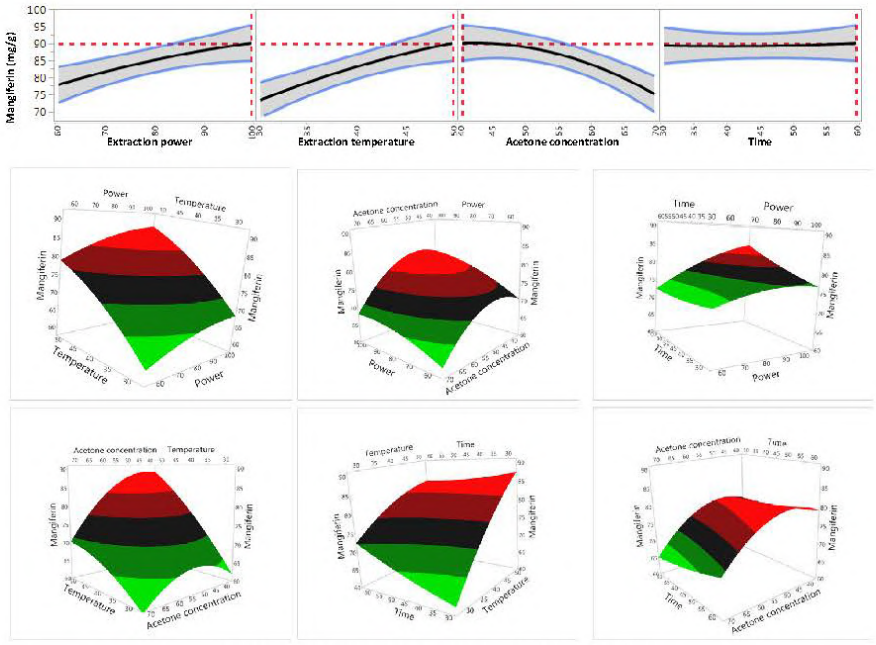


**Figure 3.** Effect of variables on the yield of mangiferin.

### *3.4. Comparison of UAE with CSE and Decoction Extraction Methods*

The results obtained from the optimal UAE conditions were further compared with two other conventional extraction methods, including decoction and continuously shaking extraction (CSE). The results are shown in Figure 4.

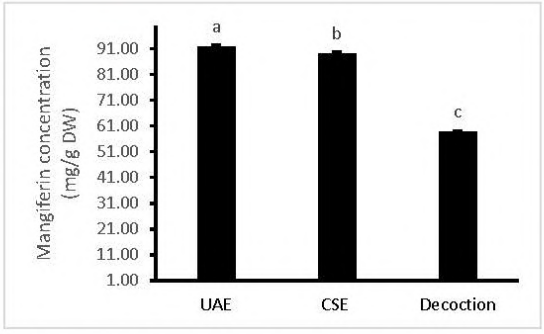


**Figure 4.** Comparison levels of mangiferin recovery using the three extraction methods of ultrasound-assisted extraction (UAE), continuously shaking extraction (CSE) and decoction. Values are means ± standard deviations. Columns with different letters were significantly different at $p < 0.05$.

It was indicated that UAE yielded the highest content of mangiferin (91.95 mg/g DW), followed by CSE (89.20 mg/g DW). Decoction extracted the lowest content of mangiferin (58.71 mg/g DW), which can be explained by the degradation of mangiferin under high temperatures for longer time periods. These results are supported by our previous study which stated that UAE produced a

significantly higher extraction yield of total bioactive compounds as well as antioxidant capacities than other extraction methods [24]. Our results were different to the findings reported by Chavan et al. [7], who found that steam bath-assisted extraction (SBAE) using dimethyl formamide 30% produced a higher yield of mangiferin than UAE, MAE (microwave assisted extraction) and CSE. Differences can be explained by the extraction techniques used in their study which were not under optimised conditions. Moreover, the concentration of acetone (70%), which was used for all methods in that study, produced the lowest yield of mangiferin in the range (40–70%) that we have found in our current research. Therefore, UAE is recommended for the extraction of mangiferin from the root of *S. chinensis*, as it is more effective, and requires less extraction time.

## 4. Conclusions

HPLC can effectively isolate mangiferin from *S. chinensis* root using EC-C18 reversed-phase column and a mixture of solvent A (water: acetonitrile: orthophosphoric acid, 96.8:3:0.2 (*v/v/v*)) and solvent B (acetonitrile). With an auto-fraction collector, mangiferin with a purity of 88.5% could be achieved. This study further confirmed that mangiferin is a major compound of *S. chinensis* root. The extraction of mangiferin was significantly affected by solvent type, and a mixture of acetone and water was more effective than absolute acetone, ethanol, methanol, water or mixtures of ethanol or methanol with water. For the application of UAE, ultrasonic power, temperature, time and acetone concentration significantly affected the extraction efficiency of mangiferin. Higher levels of mangiferin were obtained when increasing the ultrasonic power, temperature and time. In contrast, lower levels of mangiferin were achieved when increasing the acetone concentration from 40 to 70%. Optimal UAE conditions were at an ultrasonic power of 250 W, temperature of 50 °C, acetone of 40% and extraction time of 60 min. Under these optimal conditions, approximately 3% higher mangiferin levels were extracted when compared to CSE, and 57% more mangiferin was extracted when compared to the decoction. As UAE requires less time, this technique is recommended for the extraction of mangiferin from *S. chinensis* root.

**Author Contributions:** Conceptualization, T.V.N., C.J.S., M.C.B. and Q.V.V.; Formal analysis, T.V.N.; Investigation, T.V.N.; Methodology, T.V.N. and Q.V.V.; Supervision, C.J.S., M.C.B. and Q.V.V.; Writing—original draft, T.V.N.; Writing—review & editing, C.J.S., M.C.B. and Q.V.V.

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# CHAPTER 6.

# OPTIMISATION OF CONDITIONS FOR ENCAPSULATION OF *S. CHINENSIS* ROOT EXTRACT

## 6.1. Introduction

As mentioned in previous chapter, extracts prepared from *S. chinensis* root under optimal extraction conditions contains high levels of bioactive and antioxidant compounds, including mangiferin. Therefore, this extract has potential to be used as a functional ingredient. To be convenient in this application, the extract should be in powder form. However, phytochemicals within the powdered extract are generally susceptible to adverse environmental factors, including physical, chemical and biological conditions (Munin & Edwards-Levy, 2011; Papoutsis et al., 2018). In addition, the powdered extract is hygroscopic. Therefore, for commercial uses, it is necessary to encapsulate the extract to protect it from the degradation and moisture absorption. Furthermore, encapsulation increases water solubility and bioavailability, which enables easier consumption and administration (Munin & Edwards-Levy, 2011).

There are different methods applied for encapsulation, in which spray-drying and freeze-drying are two common techniques (Ballesteros, Ramirez, Orrego, Teixeira, & Mussatto, 2017; Munin & Edwards-Levy, 2011). Spray-drying is the most widely used technique in food and pharmaceutical industries due to its rapidity, continuous operation, low cost and production of particles of high quality (Fang & Bhandari, 2010; Papoutsis et al., 2018). In spray-drying, inlet temperature, feed rate, coating agent nature and concentration are the key factors that influence the physical and chemical properties of the products (Patil, Chauhan, & Singh, 2014). Therefore, to get the best quality of the encapsulated powder, these parameters need to be optimised. Freeze-drying, another technique of encapsulation, is also a method of choice as the drying process is conducted under extra low temperature which helps to minimise the degradation of bioactive components (Ballesteros et al., 2017; Munin & Edwards-Levy, 2011). However, compared to spray drying, this

technique has a higher cost and is more time consuming, meaning it is most suitable for thermolabile and high-value end-products (Punathil & Basak, 2016).

With this regards, this study aimed to optimise the conditions for encapsulation of *S. chinensis* root extract using a spray drier, and then compare the efficiency of spray drying under the optimised conditions with freeze-dried encapsulates.

### 6.2. Results and discussions

The results and detailed discussion were submitted in the form of a Research Paper to the peer reviewed journal *Microencapsulation*.

### 6.3. Conclusions

Spray drying is an effective technique for encapsulation of *S. chinensis* root extract. In the drying process, maltodextrin concentration, inlet temperature and feed rate significantly influenced the recovery yield, total phenolics, antioxidant activities as well as mangiferin content of the powder. The optimal conditions for spray drying of *S. chinensis* root extract was as follows: maltodextrin to extract ratio of 20/100 (g/mL), inlet temperature of 130ºC and feed rate of 9 mL/min. Spray drying not only produced the powder with comparable contents of total phenolics and mangiferin as well as antioxidant properties to freeze drying, but had lower moisture content, water activity, water solubility and bulk density. Therefore, spray drying is highly recommended for encapsulation of *S. chinensis* root crude extract.


# Optimising conditions for encapsulation of *Salacia chinensis* L. root extract enriched with phenolic compounds

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# Optimising conditions for encapsulation of *Salacia chinensis* root extract enriched with phenolic compounds

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# Optimising conditions for encapsulation of *Salacia chinensis* root extract enriched with phenolic compounds

***Abstract:*** This study aimed to optimise spray drying conditions and then compare with freeze-drying to identify the most suitable conditions for encapsulation of *Salacia chinensis* L. root extract. The results showed that maltodextrin concentration, inlet temperature and feed rate had significant impacts on recovery yield, phenolics, mangiferin and antioxidant activity of the spray-dried extract. The optimal spray drying encapsulation conditions were maltodextrin concentration of 20 %, inlet temperature of 130℃ and feed rate of 9 mL/min. Under these optimal conditions, the encapsulated extract had comparable solubility, total phenolics, mangiferin, and antioxidant activity, lower bulk density, moisture content, and water activity as compared to encapsulated extract made using the freeze-drying technique. These optimal spray drying conditions are recommended to encapsulate the extract of *S. chinensis* root.

Keywords: *Salacia chinensis*; encapsulation, spray drying, phenolics, mangiferin

## 1. Introduction

*Salacia chinensis* L. grows abundantly in the forests of Asian countries, such as Vietnam, China, India and Sri Lanka. People from those countries have used the extract from *S. chinensis* for treatment of ailments, such as arthritis, inflammation, obesity, diabetes, liver disorders and certain cancers (Ngo *et al.* 2017a). All parts of *S. chinensis*, including root, stem, leaf and fruit pulp contain bioactive compounds, which exhibit high antioxidant activities (Chavan *et al.* 2012, Ngo *et al.* 2017b). Among them, the root has the highest level of bioactive compounds including phenolics (Ngo *et al.* 2017b). Plant phenolics are susceptible to adverse environmental conditions due to autoxidation (Munin and Edwards-Levy 2011, Papoutsis *et al.* 2018). Therefore, for commercial uses, it is necessary to encapsulate the extract to protect it from degradation.

Encapsulation has been widely applied to increase stability of powdered extract and improve its solubility and bioavailability, which ease both the consumption and administration

(Munin and Edwards-Levy 2011). For encapsulation techniques, there are numerous methods, but spray-drying and freeze-drying are known to be the most common techniques (Ballesteros *et al.* 2017, Munin and Edwards-Levy 2011). Spray-drying has been popularly used for encapsulation due to its rapidity, continuous operation, low cost and production of particles of high quality (Fang and Bhandari 2010, Papoutsis *et al.* 2018). In spray-drying, inlet temperature, feed rate, coating agent nature and concentration are the key factors that influence the physical and chemical properties of the encapsulated products (Patil *et al.* 2014). Coating agents for encapsulation include maltodextrin, which has been widely used because it has low viscosity at a high solid ratio and is cost-effective and readily available (Madene *et al.* 2006). Freeze-drying has been commonly applied for encapsulation, especially for high value and sensitive products as the extra low temperatures of drying helps to minimise the degradation of bioactive components (Ballesteros *et al.* 2017, Munin and Edwards-Levy 2011), but it is time consuming and expensive as compared to spray drying (Punathil and Basak 2016). Due to a large number of potential operating conditions, it is necessary to optimise the parameters to obtain the best quality for the encapsulated powder.

*S. chinensis* extract enriched with phenolics is a potential food ingredient or therapeutic agent, but no previous studies have investigated the optimal conditions for encapsulation of this extract. Therefore, this study aimed to establish optimal conditions for encapsulation of *S. chinensis* root extract using spray drying technique and then compare its efficiency with freeze-drying technique.

## 2. Materials and methods

### *2.1. Materials*

*S. chinensis* L. root was taken from the forest in Nghe An province of Vietnam in March 2016. The sample was authenticated by Associate Professor Vu Quang Nam, Department of Forest

Plant Resources, College of Forestry Biotechnology, Vietnam National University of Forestry. After collection, *S. chinensis* root was washed and cut into small pieces and dried under the sun for two days, and then ground to a fine powder using a commercial cutter. The ground sample was sieved (Steel mesh sieve 1.4 mm EFL 2000; Endecotts Ltd., London, England) and stored at -20 °C for further extraction.

### *2.2. Methods*

#### *2.2.1. Experimental design*

Experimental design of the current study is shown in Figure 1. Briefly, *S. chinensis* root was extracted in 40% acetone using Ultrasonic Assisted Extraction. The extract was filtered and acetone-removed. The aqueous extract was then encapsulated using spray drying and freeze drying techniques. Optimal encapsulation of spray drying technique was established from testing the impact of the ranges of maltodextrin concentration, inlet temperature, and feed rate. Optimal concentration of maltodextrin was applied for freeze drying. Physical, total phenolics, mangiferin content and antioxidant activity of the powdered extracts prepared from the two techniques were compared to identify the best encapsulation technique for *S. chinensis* root extract.



Figure 1. Experimental design

#### *2.2.2. Preparation of S. chinensis root extract*

The *S. chinensis* root extract was prepared according to our previous study with some modification (Ngo *et al.* 2019). Briefly, 200 g of *S. chinensis* root was extracted in 4 L of acetone 40% using an ultrasonic bath (Soniclean 1000HD, 220 V, 50/60 Hz and 250 W, Soniclean Pty Ltd, Thebarton, SA, Australia) set at 50 °C and 150 W for 60 min. The extract

was filtered using Whatman No. 1 filter paper. Acetone in the extract was then removed using a rotary evaporator (Buchi Rotavapor B-480, Buchi Australia, Noble Park, VIC, Australia) set at 40 °C with reduced pressure. The aqueous extract enriched with phenolics was then used for encapsulation.

*2.2.3. Encapsulation using spray drying technique*

For investigating the impact of maltodextrin concentration, 50 mL of *S. chinensis* root extract was mixed with maltodextrin at 5 concentrations (10, 15, 20, 25 and 30 %, w/v). The mixture was homogenised (Silverson L4RT, Silverson machines Ltd. Waterside, Chesham, Bucks, England, HP5 1PQ) at 4000 rpm for 10 min and then spray dried (Mini spray dryer Buchi B-290, Noble Park, VIC, Australia). Spray drying conditions were set at inlet air temperature of 130 °C, feed rate of 20% and aspirator rate of 35 $m^3$/h. The spray dried powder was collected in a pre-weighed collection vessel.

For testing the impact of inlet air temperature, inlet temperature ranging from 115-175 °C was tested. Other spray drying conditions were set at the optimal maltodextrin concentration of 20%, feed rate of 20% and aspirator rate of 35 $m^3$/h.

For determining the impact of feed rate, the feed rate ranging from 6-15mL/min was tested. Other spray drying conditions were optimal maltodextrin concentration and inlet temperature of 20% and 130 °C, respectively, and aspirator rate of 35 $m^3$/h.

*2.2.4. Encapsulation using freeze drying technique*

*S. chinensis* root extract was mixed with maltodextrin (20% w/v). The mixture was homogenised (Silverson L4RT, Silverson machines Ltd. Waterside, Chesham, Bucks, England, HP5 1PQ) at 4000 rpm for 10 min. Subsequently, the mixture was initially frozen using liquid nitrogen and then freeze dried for 24 h (VirTis BenchTop Pro with Omnitronics, SP Scientific).

*2.2.5. Determination of physical properties*

Physical properties of the powdered extracts including recovery yield, solubility, bulk density, moisture content, and water activity were examined in the current study. Recovery yield was measured according to a previously described method of Tan *et al.* (2015) and was presented as a percentage. Solubility was expressed as a percentage and was calculated as previously described by Şahin Nadeem *et al.* (2011). Bulk density was determined using the tapping method described by Beristain *et al.* (2001) and was expressed as gram per cubic centimetre ($g/cm^3$). Moisture content of samples were measured according to a previous method of Şahin Nadeem *et al.* (2011). Water activity ($a_w$) was determined using a water activity meter (Decagon Devices, Inc., Pullman, WA) by the method described by Vuong *et al.* (2012).

*2.2.6. Determination of phytochemical properties*

Phytochemical properties including total phenolic content (TPC) and mangiferin content, which is a major phenolic compound and known as "super antioxidant"(Asif *et al.* 2016), were examined in the current study. TPC was determined similar to the method described by Ngo *et al.* (2017b). Gallic acid was used as a standard and the values were expressed milligram gallic acid equivalents per gram of powder (mg GAE/g). Mangiferin was determined using HPLC as described by Ngo *et al.* (2019). Mangiferin standard (Sigma Aldrich, Australia) was used for quantification. Values were expressed as milligram per gram of powder (mg/g).

*2.2.7. Determination of antioxidant properties*

Three antioxidant assays, including DPPH, ABTS and FRAP assays were applied to determine antioxidant activities of the powdered extracts. These assays were applied as described by Ngo *et al.* (2017b). Trolox was used as a standard and the results were expressed as millimole Trolox equivalent per gram of powder (mM TE/g).

*2.2.8. Statistical analysis*

JMP Pro version 14.2.0 (64-bit, SAS Institute Inc.) was employed for statistical analysis in this study. Each experiment was conducted in triplicate. Data were reported as the mean ± standard deviation. Differences between the mean levels of the components in the different experiments were taken to be statistically significant at $p<0.05$ using Tukey's Honest Significant Difference test.

## 3. Results and discussion

### *3.1. Impact of maltodextrin concentration*

Overall, maltodextrin concentration had a significant impact on recovery yield, total phenolics, antioxidant capacity and mangiferin content of *S. chinensis* root extract powder. The results in Table 1 showed that recovery yield increased when maltodextrin concentration increased from 10 to 20 %. These were supported by a previous observation (Tontul and Topuz 2017), which revealed that the product yield generally increased when the carrier concentration increased. However, when maltodextrin concentration exceeded 20%, the recovery yield slightly declined. Our findings were in agreement with the results of other studies (Chong and Wong 2015, Ho *et al.* 2015). This can be explained by Tonon *et al.* (2008) that too high carrier content may lead to high viscosity of the feed which would, by contrast, negatively influence the drying process. The results (Table 1) indicated that maximum recovery yield (65%) could be achieved when adding 20% of maltodextrin. This recovery yield was higher than recovery yield of lemon pomace (50%) reported by Papoutsis *et al.* (2018).

On the contrary, the opposite trend was observed on the effects of maltodextrin concentration on levels of phenolics, antioxidant capacity and mangiferin content. Phenolics, antioxidant activities and mangiferin content decreased when maltodextrin concentration increased. This could be due to the increase of maltodextrin which leads to the decrease of the

proportion of core material in the mixture. In addition, phenolics were found to be major antioxidants in *S. chinensis* root extract in our previous study (Ngo *et al.* 2017a), thus antioxidant capacity of the powder also decreased when concentration of maltodextrin increased. With the consideration of recovery yield and levels of phenolics, mangiferin content and antioxidant activities, concentration of maltodextrin of 20% was chosen for further experiments.

Table 1. Impact of maltodextrin concentration (MD) on recovery yield, total phenolics, antioxidant properties and mangiferin content

### *3.2. Impact of inlet air temperature*

Inlet air temperature is an important factor that affects the recovery yield as well as the powder quality (Tan *et al.* 2015). If the inlet temperature is too low, the droplets are not completely dried and thus stick on the drying chamber, which reduces the yield. By contrast, if the inlet temperature is too high, the particle temperature increased more rapidly, which also results in stickiness of the powder (Tan *et al.* 2015). In addition, too high temperature may destroy bioactive compounds in the extract. With the optimal maltodextrin concentration, a range of inlet temperatures from 115 to 190 °C was applied in this study. The results in Table 2 showed that the recovery yield, TPC and antioxidant activities slightly increased when inlet air temperature increased from 115 to 130 °C. This was in agreement with Tonon *et al.* (2008) who explained that the increase of inlet temperature, which creates greater efficiency of heat and mass transfer processes, leads to higher recovery yields. However, the change of temperature from 130 to 190 °C did not significantly affect the yield, total phenolics and mangiferin content as well as antioxidant properties. Therefore, for energy savings, 130 °C was chosen as the optimal inlet temperature for further investigation.

Table 2. Impact of inlet air temperature on recovery yield, total phenolics, antioxidant properties and mangiferin content

### *3.3. Feed rate*

With optimal maltodextrin concentration and inlet temperature, different feed rates ranging from 6 mL/min to 15 mL/min were tested to examine its impact on recovery yield, phenolic, mangiferin and antioxidant properties of the powder. The results (Table 3) showed that there was no significant change in the recovery yield of the powder when the feed rate increased from 6 to 9 mL/min. However, when the feed rate exceeded 9 mL/min, the yield decreased significantly. At the feed rate of 15 mL/min the yield dropped to only 18%. The negative impact of the increase of feeding rate on the recovery yield has been reported in previous studies (Can Karaca *et al.* 2016, Murugesan and Orsat 2011, Tonon *et al.* 2008) . This can be explained by the fact that in the drying chamber, the higher the flow rate, the shorter the contact time between the feed and the drying air, leading to the less efficient heat transfer and the slower water evaporation. As the result, the powder becomes wet and thus sticks on the wall of cyclone powder collector, which leads to the reduce of recovery yield (Tonon *et al.* 2008). Regarding to the chemical and antioxidant properties, except a slight decrease at the feed rate of 15 mL/min, there were no significant change in total phenolics and mangiferin as well as antioxidant activity when feed rate increased from 6 to 12 mL/min. Although both feed rates of 6 and 9 mL/min produced the highest levels of product yields and powders had high chemical and antioxidant properties, the latter is less time consuming. Therefore, the optimal encapsulation conditions for *S. chinensis* root extract using spray drying technique were maltodextrin concentration of 20%, inlet temperature of 130 °C, and feed rate of 9 mL/min. These conditions were applied to prepare powder for comparison with powder prepared using freeze drying technique.

Table 3. Impact of feed rate on recovery yield, total phenolics, antioxidant properties and mangiferin content

### *3.4. Comparison between freeze drying and spray drying techniques*

Table 4 presented the recovery yield, physical, chemical and antioxidant properties of encapsulated powder of *S. chinensis* root extract prepared by spray drying under the optimal conditions and freeze drying. The results indicated that, spray dried powder had comparable solubility, total phenolics, mangiferin content as well as antioxidant activities to those of freeze dried powder. However, freeze drying recovered more powder than spray drying. This was due to the stickiness of encapsulated powder on the wall of drying chamber in the process of spray drying. Nevertheless, the loss can be minimised when spray drying is up-scaled.

It was observed that the freeze-dried powder was more hygroscopic and it became wet and sticky after certain time exposure to ambient conditions. That was why the moisture content and water activity of freeze dried powder were 31.8% and 0.54, respectively, which were much higher than those of powder produced by spray drying. This finding was supported by Kuck and Noreña (2016) who found that spray-dried powder of grape skin had lower moisture content and water activity than freeze-dried powder. However, Papoutsis *et al.* (Papoutsis *et al.* 2018) reported the opposite result when lemon pomace was encapsulated by the two techniques. The difference could be explained by the difference of the coating materials and the nature of extract used in the two studies.

Bulk density is an important parameter to evaluate the quality of a powder. The higher bulk density the less volume it occupies (Kha *et al.* 2014). The result in Table 4 indicated that the bulk density of spray dried powder was about 2 times higher than that of freeze dried powder, which may reduce the cost for packaging and transportation. The higher bulk density of the spray dried powder also leads to less space between particles, which helps to prevent oxidation of the bioactive compounds (Goula and Adamopoulos 2008, Kurozawa *et al.* 2009). Therefore, the current study found that the spray drying technique is more suitable for encapsulation of the *S. chinensis* root extract than the freeze drying technique.

Table 4. Comparison of freeze drying and spray drying in recovery yield, physical, chemical and antioxidant properties

## 4. Conclusions

Spray drying was found to be more effective for encapsulation of *S. chinensis* root extract than freeze-drying. For the spray drying technique, maltodextrin concentration, inlet temperature and feed rate significantly influenced the recovery yield, total phenolics, antioxidant activities as well as mangiferin content of the powder. The optimal conditions for spray drying of *S. chinensis* root extract were maltodextrin concentration of 20 %, inlet temperature of 130°C and feed rate of 9 mL/min. In comparison with freeze drying, spray drying not only produced the powder with comparable phenolics, mangiferin, and water solubility as well as antioxidant properties, but lower moisture content, water activity and bulk density. Therefore, these spray drying conditions are recommended for encapsulation of *S. chinensis* root extract for further applications.

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Table 1. Impact of maltodextrin concentration (MD) on recovery yield, total phenolics, antioxidant properties and mangiferin content

| MD (%) | Recovery yield (%) | TPC (mg GAE/g) | Antioxidant properties (mM TE/g) | | | Mangiferin (mg/g) |
|---|---|---|---|---|---|---|
| | | | DPPH | ABTS | FRAP | |
| 10 | $62.6 \pm 1.3^{a}$ | $24.52 \pm 0.27^{a}$ | $133.36 \pm 1.40^{a}$ | $166.94 \pm 0.29^{a}$ | $94.22 \pm 2.21^{a}$ | $23.35 \pm 0.13^{a}$ |
| 15 | $61.7 \pm 1.1^{a}$ | $21.75 \pm 0.18^{b}$ | $130.32 \pm 0.95^{a}$ | $142.22 \pm 1.70^{b}$ | $78.38 \pm 0.45^{b}$ | $19.98 \pm 0.09^{b}$ |
| 20 | $65.3 \pm 1.1^{b}$ | $16.53 \pm 0.11^{c}$ | $121.84 \pm 1.68^{b}$ | $126.08 \pm 3.07^{c}$ | $68.42 \pm 0.83^{c}$ | $17.30 \pm 0.10^{c}$ |
| 25 | $63.0 \pm 1.4^{a}$ | $12.97 \pm 0.16^{d}$ | $89.17 \pm 3.21^{c}$ | $110.44 \pm 1.42^{d}$ | $64.15 \pm 2.06^{d}$ | $15.10 \pm 0.12^{d}$ |
| 30 | $49.9 \pm 2.1^{c}$ | $10.92 \pm 0.35^{e}$ | $81.00 \pm 2.59^{d}$ | $92.36 \pm 1.35^{e}$ | $58.81 \pm 0.45e$ | $12.68 \pm 0.06^{e}$ |

Data are means ± standard deviations. Data in the same column not sharing similar superscript letters are significantly different at $p < 0.05$.

Table 2. Impact of inlet air temperature on recovery yield, total phenolics, antioxidant properties and mangiferin content

| Inlet Temp (°C) | Recovery yield (%) | TPC (mg GAE/g) | Antioxidant properties (mM TE/g) | | | Mangiferin (mg/g) |
|---|---|---|---|---|---|---|
| | | | DPPH | ABTS | FRAP | |
| 115 | 56.67 ± 2.13$^{a}$ | 14.65 ± 0.18$^{a}$ | 117.43 ± 3.55$^{a}$ | 129.50 ± 4.52$^{a}$ | 69.76 ± 2.15$^{a}$ | 17.18 ± 0.06$^{a}$ |
| 130 | 63.6 ± 2.54$^{b}$ | 15.48 ± 0.09$^{b}$ | 119.80 ± 1.61$^{ab}$ | 136.30 ± 4.39$^{b}$ | 71.15 ± 2.11$^{ab}$ | 17.19 ± 0.15$^{a}$ |
| 145 | 64.0 ± 1.86$^{b}$ | 15.29 ± 0.14$^{b}$ | 120.06 ± 1.61$^{ab}$ | 135.98 ± 1.90$^{b}$ | 74.25 ± 2.24$^{b}$ | 17.19 ± 0.15$^{a}$ |
| 160 | 63.8 ± 1.55$^{b}$ | 15.11 ± 0.26$^{b}$ | 122.20 ± 3.58$^{b}$ | 133.18 ± 2.43$^{ab}$ | 75.34 ± 3.23$^{b}$ | 17.30 ± 0.08$^{a}$ |
| 175 | 64.3 ± 2.12$^{b}$ | 15.68 ± 0.27$^{b}$ | 123.49 ± 1.80$^{b}$ | 137.58 ± 2.83$^{b}$ | 72.94 ± 2.75$^{b}$ | 17.46 ± 0.18$^{a}$ |
| 190 | 64.1 ± 1.45$^{b}$ | 15.31 ± 0.26 | 123.82 ± 4.48$^{b}$ | 135.64 ± 4.54$^{b}$ | 72.85 ± 2.24$^{b}$ | 17.40 ± 0.14$^{a}$ |

Data are means ± standard deviations. Data in the same column not sharing similar superscript letters are significantly different at $p < 0.05$.

Table 3. Impact of feed rate on recovery yield, total phenolics, antioxidant properties and mangiferin content

| Feed rate (mL/min) | Recovery yield (%) | TPC (mg GAE/g) | Antioxidant properties (mM TE) | | | Mangiferin (mg/g) |
|---|---|---|---|---|---|---|
| | | | DPPH | ABTS | FRAP | |
| 6 | 65.4 ± 2.12[a] | 15.96 ± 0.44[a] | 124.58 ± 5.14[a] | 134.79 ± 4.62[a] | 66.28 ± 0.50[a] | 16.90 ± 0.26[a] |
| 9 | 66.3 ± 1.58[a] | 15.33 ± 0.08[a] | 122.65 ± 0.79[a] | 132.77 ± 0.91[a] | 65.66 ± 0.44[a] | 16.48 ± 0.25[a] |
| 12 | 56.4 ± 1.42[b] | 15.48 ± 0.10[a] | 124.90 ± 3.85[a] | 131.20 ± 3.74[a] | 66.01 ± 0.13[a] | 16.57 ± 0.13[a] |
| 15 | 17.87 ± 2.24[c] | 12.24 ± 0.15[b] | 113.05 ± 2.92[b] | 119.36 ± 3.59[b] | 63.88 ± 0.6[b] | 13.04 ± 0.09[b] |

Data are means ± standard deviations. Data in the same column not sharing similar superscript letters are significantly different at $p < 0.05$.

Table 4. Comparison of freeze drying and spray drying in recovery yield, physical, chemical and antioxidant properties

| | Spray drying | Freeze drying |
|---|---|---|
| Recovery yield (%) | 66.5[a] | 91.2[b] |
| Moisture content (%) | 4.52 ± 0.04[a] | 31.80 ± 7.20[b] |
| Water activity | 0.28 ± 0.04[a] | 0.54 ± 0.06[b] |
| Solubility (%) | 90.5 ± 1.3[a] | 91.5 ± 2.1[a] |
| Bulk density (g/cm$^3$) | 0.47 ± 0.01[a] | 0.27 ± 0.01[b] |
| TPC (mg GAE/g) | 16.11 ± 0.33[a] | 16.36 ± 0.67[a] |
| DPPH (mM TE/g) | 121.92 ± 2.32[a] | 119.42 ± 2.67[a] |
| ABTS (mM TE/g) | 128.34 ± 3.50[a] | 130.28 ± 2.84[a] |
| FRAP(mM TE/g) | 67.74 ± 3.91[a] | 69.63 ± 2.67[a] |
| Mangiferin (mg/g) | 17.68 ± 0.37[a] | 17.39 ± 0.72[a] |

Data are means ± standard deviations. Data in the same row not sharing similar superscript letters are significantly different at $p < 0.05$.

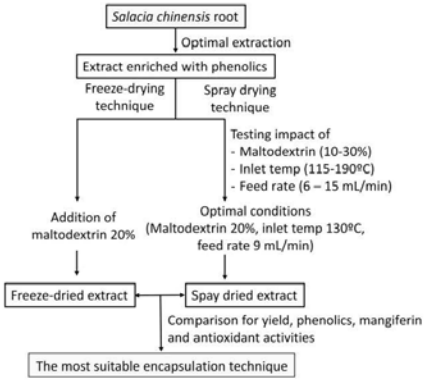


Figure 1. Experimental design

338x190mm (300 x 300 DPI)

# *CHAPTER 7.*

# GENERAL CONCLUSIONS AND FUTURE PROSPECTS

## 7.1. Conclusions

The overall aim of this project has been achieved. The root of *S. chinensis* had the highest levels of phenolic compounds, flavonoids, proanthocyanidins, saponins and antioxidant properties in comparison with other parts of *S. chinensis*. Extraction solvents played an important role in the extraction of bioactive compounds from *S. chinensis*. Among seven selected solvents, 50% ethanol and 50% acetone yielded the highest contents of phenolics, saponins as well as antioxidant properties. Although *S. chinensis* has been traditionally prepared by decoction technique, decoction was not as effective for extracting bioactive compounds including mangiferin, a major bioactive compound from *S. chinensis* as compared with continuously shaking extraction (CSE) technique. The CSE method had better extraction efficiency than decoction for total bioactive compounds and mangiferin. However, extraction efficiency using CSE was lower than ultrasound assisted extraction (UAE). UAE is more effective for extraction of total bioactive compounds and mangiferin with much less time required for extraction. However the optimal UAE conditions are different for total bioactive compounds and mangiferin. For maximum extraction of total bioactive compounds, the optimal UAE conditions are: 50% ethanol, 60 min, 50 ºC and 250 W; whereas, optimal UAE conditions for extraction of mangiferin are: acetone of 40%, temperature of 50 ºC, extraction time of 60 min, and ultrasonic power of 250 W. Extract prepared under optimal conditions for maximum extraction of total bioactive compounds was further encapsulated. This study found that spray drying is more cost effective for encapsulating of *S. chinensis* extract as compared to freeze drying. The optimal conditions for encapsulation are maltodextrin to extract ratio of 20/100 (g/mL), inlet temperature of 130 ºC and feed rate of 9 mL/min. These conditions are recommended for preparation of *S. chinensis* extract enriched with bioactive compounds for further applications.

### 7.2. Study limitations

This study has successfully compared levels of bioactive compounds in different parts of *S. chinensis*, determined the most effective solvents, and established optimal conditions for extraction of bioactive compounds, including mangiferin from *S. chinensis* root. Additionally, this study has identified the optimum conditions for encapsulation of the enriched extract of *S. chinensis*. However, due to limited time during PhD candidature, the study has following limitations:

1. The samples of *S. chinensis* (root, stem and leaf) have been collected from Nghe An province, Vietnam in May 2015 and March 2016. However, the impact of sample's ages, collected locations and seasons on the contents of the bioactive compounds were not considered in this study.
2. As mentioned previously in Literature Review, *S. chinensis* is an abundant source of phytochemicals including terpenoids, phenolics and thiosugar sulfonium sulfate compounds. However, in this study, only 01 compound (mangiferin) was identified and isolated from *S. chinensis* root.
3. Although there are many encapsulating walls, such as gum Arabic, starch and chitosan, maltodextrin was the only coating material used for optimizing the conditions for encapsulation.

### 7.3. Recommendations for future studies

Based on the limitations of the study, the candidate would like to recommend for future studies as follows:

1. To test the impact of sample's ages, collected locations and seasons on the contents of the bioactive compounds.
2. To identify and isolate more bioactive compounds from different parts of *S. chinensis*.

3. To further test the individual or synergistic effects of the compounds on microbial activities and various health benefits as potential therapeutic agents.

4. To test more conditions (encapsulating walls, other spray drying conditions) to establish optimal conditions for these individual or group of effective compounds for further applications.

5. To apply these encapsulated extracts as functional ingredients in food or therapeutic agents in the pharmaceutical industry.

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