Phytochemical Investigation and biological profiling of *Livistona chinensis* (Chinese fan palm) seeds

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2K21/MSCCHE/43

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CANDIDATE'S DECLARATION

I, Tamanna Beniwal (2K21/MSCCHE/43) student of M.Sc Chemistry, hereby declare that the project Dissertation titled "Phytochemical Investigation and biological profiling of Livistona chinensis (Chinese fan palm) seeds" which is submitted by me to the Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the Project Dissertation title "Phytochemical Investigation and biological profiling of Livistona chinensis (Chinese fan palm) seeds" which is submitted by Tamanna Beniwal (2K21/MSCCHE/43), Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi **Prof. Rajinder Kumar Gupta**

Date: **(Supervisor)**

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Tamanna Beniwal

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ABSTRACT

Livistona chinensis (L. chinensis) seeds have been used traditionally in treating various diseases. The qualitative analysis of the seeds extract showed the presence of different types of bioactive secondary metabolites like tannins, alkaloids, flavonoids, glycosides, steroids, anthraquinones, reducing sugars, emodins, terpenoids, phenols, and coumarics. The antioxidant activity was checked using DPPH free radical scavenging activity, showing a high antioxidant property. The GC-MS fatty acid composition of the seeds extract showed majorly the presence of Oleic acid (18.44%), Lauric acid (11.66%), Linoleic acid (11.30%), Linolenic acid (10.20%), and Palmitic acid (9.96%). UHPLC-QTOF-MS analysis showed the presence of 25 non-volatile compounds present in the seeds extract including isobutyric acid, acetoin, 9-oxononanoic acid, piperidine, umbellic acid, furaneol, and ketopantolactone. The antimicrobial activity was shown only by the aqueous extract against the gram-negative bacteria (*Klebsiella pneumonia)* with an inhibition of 16.1 mm.Thus, the results from the current study reveals that the novel methanolic extract of *L. chinensis* have potential application in the pharmaceutical, cosmetics, and food industry.

GRAPHICAL ABSTRACT

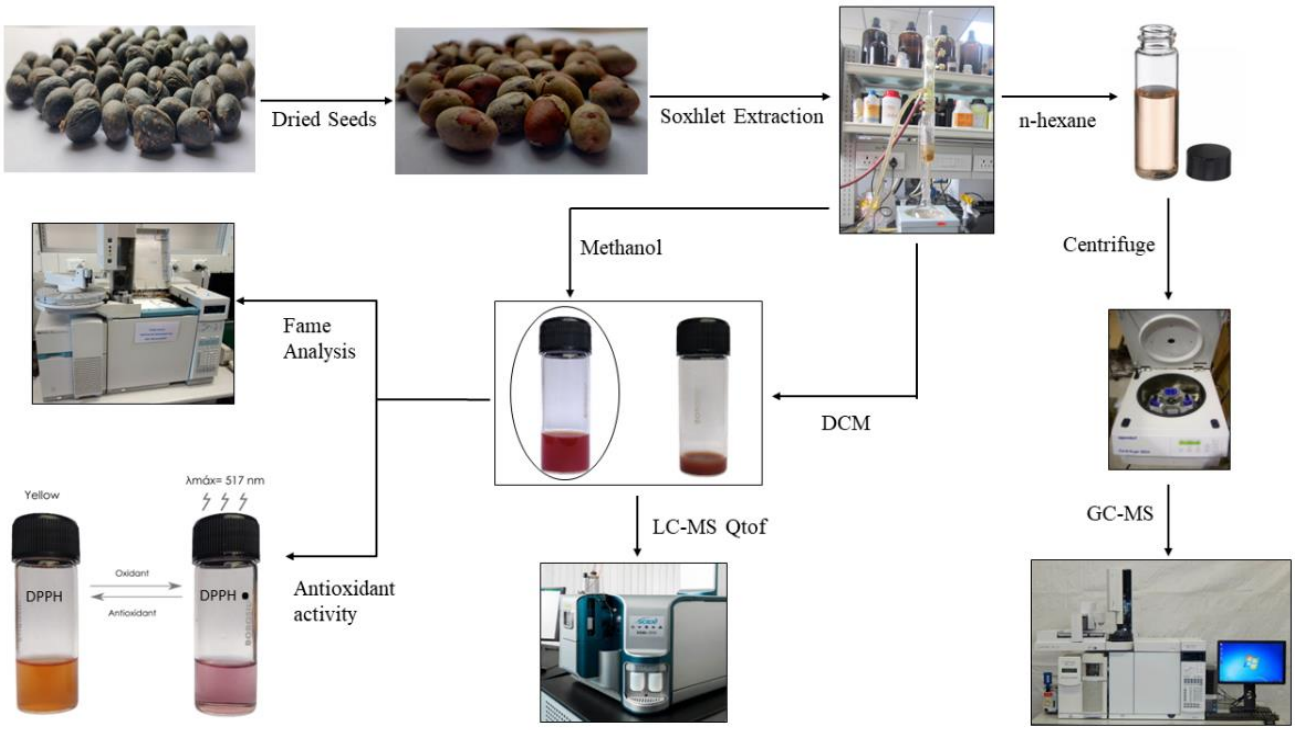

Fig. 2 ─ Graphical representation

The outer shell was removed to obtain the dried seeds for which sochlet extraction was done with three different solvents in order of increasing polarity: n-hexane, DCM, and methanol. The n-hexane extract was used for the GC-MS analysis to identify the volatile components present. The DCM and methanol extract were used for the UHPLC-QTOF-MS analysis for the non-volatile components identification. Methanol extract was also used for the antioxidant activity.

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List of Symbols and Abbrevations

1. *Livistona chinensis*	*L. chinensis*
2. Dichlromethane	DCM
3. Retention time	RT
4. Molecular Formula	MF
5. Monoisotopic mass	MM
6. degree Celcius	° C
7. Dimethyl sulfoxide	DMSO

CHAPTER 1
INTRODUCTION

In order to effectively cure a variety of lifestyle-related diseases like diabetes, obesity, and heart disease, functional and nutraceutical foods are crucial. Products made from plant sources are becoming increasingly popular due to the quantity of health-promoting components on the market today. Due to their rich phytochemistry and preventative effects, medicinal seeds in particular have gained a lot of interest. The beautiful plant *Livistona chinensis* (*L. chinensis*) is a member of the Arecaceae family of palms. Chinese Fan Palm and Chinese Fountain Palm are other names for it. Taiwan, Southern Japan, East Asia, Australia, and a number of islands in the Southern China Sea are its natural habitats. The L. chinensis fruit is oval in shape, has a tough exterior, and is dark in colour (Fig. 1.1) [1].

L. chinensis fruit has anti-cancer, anti-microbial, and anti-HIV-1 effects, according to earlier reports. Their fruits have historically been used to cure leukaemia, esophageal cancer, gastric cancer, choriocarcinoma, and nasopharyngeal carcinoma thanks to their analgesic and hemostatic qualities [2]. *L. chinensis* fruit has been used as a herbal component in soups by people in China and East Asia to cure hepatitis and many forms of liver cancer [3]. Additionally, it has been noted that phenolic chemicals found in the fruits of *L. chinensis* have hemolytic action [4]. Biochar made from Chinese Fan palm seeds has been proven effective in absorbing the Malachite Green dye effluent [5].

Fig. 1.1. Seeds of *Livistona chinensis*

Secondary metabolites, also known as bioactive substances, or phytochemicals, are mostly found in plants and have antioxidant capabilities. The identification of the many phytoconstituents present in medicinal plant extracts, which offered insight into future drug discovery and development, was made possible by the preliminary screening of phytochemicals. Plant parts include phytochemical components that are thought to be physiologically active substances because they have a variety of properties, including anti-analgesic, anti-cancer, anti-viral, and anti-microbial effects. Alkaloids, glycosides, saponins, resins, and oils are phytoconstituents with a protective or disease-preventive action [6].

Due to the rising demand for their antioxidant effects, medicinal plants are currently getting increasing respect. Antioxidants are employed in the food business to postpone the oxidation process. Alternatives to the common synthetic antioxidants include natural compounds. In this study, the anti-oxidant activity of the seed extract was assessed using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which absorbs significantly at 517 nm and loses absorbance when any antioxidant is added. The antioxidant property was determined by calculating the % scavenging activity given by the following formula [7]:

$$\%\ \text{Scavenging activity} = \frac{\text{Absorbance of Control} - \text{Absorbance of sample}}{\text{Absorbance of Control}} \text{x } 100$$

Functional and nutritional benefits are controlled by fatty acid composition. Due to a lack of the enzymes required for their production, polyunsaturated fatty acids (PUFA) like linoleic (omega-6) and linolenic acids (omega-3) are essential for human metabolism. Due to their capacity to reduce the risk of cardiovascular disease and cancer, PUFA are recognised as beneficial nutrients in the human diet [8]. The application of soxhlet extraction of L. chinensis seed and subsequent use of the UHPLC-QTOF-MS technology to analyse the secondary metabolites are innovative aspects of this work.

Given the foregoing, the current study's objectives are to assess the bioactive components of *L. chinensis* seeds grown in India using GC-MS and UHPLC-QTOF-MS analysis, elemental analysis using ICP-MS analysis, antimicrobial activity against *Klebsiella pneumoniae* (gram-negative) and *Staphylococcus aureus* (gram-positive), and antioxidant activity using DPPH free radical scavenging method.

CHAPTER 2

LITERATURE REVIEW

Livistona chinensis (*L. chinensis)* was used traditionally in China for treating hepatitis. The protective effect of this plant was because of flavanoids from *L. chinensis* fruits [3].

In a study conducted by Yuwei Wang et. al, thorough phytochemical experiments were conducted to determine FLC's potential for edibility. Following that, a unique antidiabetic effect for FLC was predicted using network pharmacology and chemical profiles. The network pharmacology study also showed that FLC's biological action against diabetes may include the control of inflammatory response, oxidation-reduction process, glucose metabolic process, insulin signalling route, and other processes. The antioxidant, anti-inflammatory, and inhibitory effects of the extract, fractions, and all identified substances on -glucosidase and PTP1B were therefore evaluated. Further research was done on polyphenolic PTP1B inhibitors produced from FLC and its effect on glucose absorption in insulin-resistant HepG2 cells [2].

Phenolics present in the *L. chinensis* fruits show hemolytic activity. It was seen that proteins and lipids inhibit the hemolytic process, suggesting that astringent and membrane-damaging processes are the causes of hemolysis. It was thus concluded from this study that the haemolytic activity of phenolics present is due to astringent and membrane-damaging activities [4].

The high content of phenolic compounds of the fruits of *L. chinensis* showed antibacterial activity [9].

Another study done by Xiaobin Zeng et. al, examined the phenolics extracted from the fruits of Livistona chinensis for their cytotoxic and antioxidant properties. Based on the results of this investigation, it was suggested that the fruits of L. chinensis could be useful sources of potent antioxidant and anticancer compounds for a variety of uses [10].

The oil from *Livistona chinensis* has a low oil content, according to research on its physicochemical qualities. The results also indicated that the oil contains a high concentration of saturated fatty acids and free fatty acids, low thermal stability, and good oxidative stability. These implied that the oil would be better suited for industrial uses rather than culinary ones (until refined). It was also shown that the crude protein, lipid, and carbohydrate content of raw *L. chinensis* seed is comparatively low, however the ash and crude fibre content is rather high.

Therefore, it can be inferred that it can provide animals with a good amount of fibre and roughage [11].

Antioxidant screening of fruits of *L. chinensis* identified six antioxidative flavonoids: orientin, isovitexin, isoorientin, tricin, vitexin, and isohamnetin-3-O-glucoside. Their free radical scavenging activity was further determined using off-line DPPH assay. It was found that orientin and isoorientin are even stronger antioxidants than vitamin C and baicalin [12].

Another study was conducted to evaluate the amino acids, fatty acids, and minerals composition of *L. chinensis*. Amino acid profile, mineral composition, and fatty acid analysis revealed the following results [13]:

Essential Amino acid	Amino acid profile (g/100g)
Lys	2.80
Val	3.04
Thr	2.10
His	1.78
Met	0.91
Leu	5.28
Phe	3.52
Ile	2.12
Total	**21.55**

Table 2.1. Amino acid analysis of *L. chinensis*

Mineral	Mineral composition (mg/L)
Ca	0.00±0.023
Fe	1.14±0. 041
Cu	0.16±0. 001
Zn	0.71±0. 016

Table 2.2. Mineral composition of *L. chinensis*

Fatty acid	Fatty acid composition (%)
Lauric acid	2.14
Myristic acid	2.62
Palmitic acid	14.28
Palmitoleic acid	0.14
Heptadecanoic acid	0.07
Stearic acid	3.56
Oleic acid	52.93
Linoleic acid	23.38
Linolenic acid	0.45

Table 2.3. Fatty acid composition of *L. chinensis*

Seeds of *L. chinensis* inhibits the tumor angiogenesis by suppression of the Notch pathway [14].

The investigation of a 70% ethanol extract of Livistona chinensis roots resulted in the extraction of 13 chemicals, including a novel ceramide, (2S,3S,4R,9Z)-2-[(2R)-2-hydroxytricosanoylamino]- 9-octadecene-1,3,4-triol, novel glycosyl ceramide 1-O-β-D-glucopyranosyl-(2S,3S,4R,9Z)-2-[(2R)-2-hydroxydocosanoylamino], three novel monoacylglycerols, a new diacylglycerol, and a new diacylglycerol aminoglycoside [15].

Study of phenolic compounds from the fruits of *Livistona chinensis* showed cell protective activity. These were two new depsidones, a new benzofurane, four steroids, three stilbenes, three flavan-3-ols, and an alkaloid [16].

A study conducted in Nigeria on fruits of *Livistona chinensis* for its phytoconstituents, antimicrobial activities showed the following results [17]:

Test	Result
Saponins	+++
Phlobatannins	+++
Deoxy-sugars	+++
Tannins	+++
Phenols	+++
Anthroquinones	++
Cardiac glycosides	-
Reducing Sugars	-
Terpenes	-
Alkaloids	++
+++: High, ++: Moderate, +: Trace, -: Not detected	

Table 2.4. Phytochemical composition of *L. chinensis* nuts

Phytochemical (mg/g)	**Result**
Polyphenols	245.0±0. 02
Tannins	78.5±0. 18
Flavanoids	25.5±0. 15

Table 2.5. Phenolic contents of *L. chinensis* nuts extracts

CHAPTER 3

MATERIALS AND METHOD

3.1 Plant material collection and authentication

Fruits from *Livistona chinensis* trees were collected at Delhi Technological University in New Delhi, India. The *Livistona chinensis* fruit botanical specimens were verified by the CSIR-NIScPR, Raw Materials Herbarium, and Museum, Delhi (RHMD), India. The fruits were cleaned up after collection, and the seeds were manually removed. The seeds were exposed to the sun for two to three days before being coarsely grounded in a grinder to begin the extraction process.

3.2 Preparation of extracts

Using a Soxhlet apparatus, 50 g of crushed *L. chinensis* seeds were extracted for 6 to 8 hours with n-hexane, DCM, deionized water, and methanol. The solvents were evaporated using a rotary evaporator after a successful extraction. The resulting concentrated extracts were stored at 4° C for further use [18].

3.3 Phytochemical Analysis

For the phytochemical analysis following tests were performed [19], [20]:

Tannins:

Braymer's Test: 1 mL of the water extract prepared was taken in a test tube. To this 2 mL of distilled water was added. 2-3 drops of 5% $FeCl_3$ were added to the aqueous extract.

Alkaloids:

Hager's Test: Few drops of Hager's reagent were added to about 2 mL of extract.

Flavonoids:

To 10 mL of extract solution, 5 mL of dilute ammonia solution was added. To it 1 mL of conc. H_2SO_4 was added.

Glycosides:

Liebermann's Test: To 2 mL of water extract, 2 mL chloroform ($CHCl_3$) then 2 mL acetic acid were added.

Terpenoids:

10 mL of methanol was added to 0.8 g of plant sample, which was shaken and filtered. 5 mL of this extract was taken and 2 mL of chloroform was added, followed by 3 mL of sulphuric acid.

Reducing Sugar:

To 2 mL of water extract, 1 mL of Benedict's reagent was added.

Proteins:

Xanthoproteic Test: 1 mL of conc. H_2SO_4 was added to 1 mL of water extract from the seeds.

Anthocyanins:

2 mL of water extract was taken in a test tube, and 2 mL of 2N HCl was added to it. To the contents of the test tube, dropwise NH_3 was added.

Emodins:

To 2 mL of extract, 2 mL NH_4OH followed by 3 mL Benzene were added.

Steroids:

Salkowski Test: To about 2 mL of the extract, 2 mL $CHCl_3$ was added, followed by 2 mL conc. H_2SO_4.

Anthraquinones:

Borntrager's Test: To 3 mL of extract, 2 mL HCl and around 5 mL of NH_3 were added.

Coumarins:

To 2 mL of extract, 3 mL of 10% NaOH was added.

Phlobatannins:

2 mL 1% HCl was added to 2 mL extract solution. This solution was then heated.

Phenols:

Liebermannn's test: To 1 mL of extract, 1 mL of sodium nitrite was added, followed by a few drops of dilute sulphuric acid and 2 mL of diluted NaOH.

3.4 Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS (Shimadzu, GCMS-QP2010) was used to determine the volatile components in crude n-hexane extract from *L. chinensis* seeds. Helium was used as a carrier gas in split mode at 260° C, when the sample was injected into the GC-MS. When electron ionisation (EI) was employed as the ionisation method, the column flow rate was maintained at 1.21 mL/min. Temperatures at the contact and the ion source were 220° C and 270° C, respectively. The oven temperature was adjusted at 80° C (2 min) to 300° C (21 min), with a solvent delay time of 3.50 min. The NIST (National Institute of Standards and Technology) mass spectral data-base was utilised to detect the separated peaks [21].

3.5 Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) analysis

The methanolic extract of *L. chinensis* seeds was analysed using UHPLC and mass spectrometry using HPLC (Waters, SYNAPT-XS HDMS, UK) equipped with a controller, AD pump, degasser, AD auto sampler, AD column, and quadrupole time-of-flight mass spectrometer (QTOF-MS). It was used to the dichloromethane and methanol extracts of the seeds to identify secondary metabolites. Both extracts were combined with 10 mL of water containing 1% formic acid before being set aside for 10 min. 10 mL of methanol and 10 mL of acetonitrile were combined, vortexed for one minute, and then centrifuged at 5000 rpm for five minutes. The sample was injected (5 L) into the device after being diluted with acidified water. The materials were separated by chromatography using 100 mm x 2.1 mm column C18 (Waters, Acquity BEH 2.1). The following parameters were used for the positive mode (ESI+) mass spectroscopic analysis: capillary voltage of 3.22 keV, source temperature of 120° C, desolvation gas flow of 950 L/hr, and cone gas flow of 50 L/hr. For the elution of the secondary metabolites, a binary mobile phase with a flow rate of 0.2 mL/min was utilised, with solvent A being LC-MS grade water with 1% formic acid and solvent B being 1% formic acid with acetonitrile. Data analysis and data capture were carried out using ChemSpider software [22].

3.6 Antioxidant activity

- **DPPH radical scavenging activity**

With slight adjustments, the procedure for determining antioxidant activity as reported by Velazquez et al. was followed. 60, 80, 100, 120, 140, 160, 180, and 200 µL/mL of the sample's methanolic extract were prepared, and 1 mL of each sample was combined with 3 mL of DPPH radical, with methanol acting as the control sample. The samples were incubated for 30 minutes at 37°C, after which the absorbance of each sample was determined at 517 nm.

3.7 Antimicrobial activity

The disc diffusion technique was used to test the *L. chinensis* methanol extract's antibacterial properties. In order for the nutritional media to harden, 20 mL of it was placed onto clean petri plates. *Staphylococcus aureus*, a gram-positive bacteria, and *Klebsiella pneumoniae*, a gram-negative bacteria, were both tested using 20 µL of the extract. The bacterial strains were grown for 24 hours in an incubator at 37° C. Zone of inhibition was assessed following the incubation period.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Estimation of Oil Content

The fat analysis results in the *L. chinensis* seeds are presented in Table 1.

S.No	Chemical Parameters	Result
1.	Fat (gm/100gm)	4.12
2.	Saturated Fat (%)	1.31
3.	MUFA (%)	1.22
4.	PUFA (%)	1.14
5.	Trans Fat (%)	0.45

Table 4.1. Fat present, Saturated Fat, MUFA, PUFA, and Trans Fat

4.2 Phytochemical analysis

The phytochemical screening was performed on methanol as well as water extract. The results revealed the presence of flavonoids, terpenoids, tannins, glycosides, reducing sugars, alkaloids, steroids, emodins, anthraquinones, and phenols. However, proteins, anthocyanins, and phlobatannins were absent. The results are presented in Table 4.1.

These biologically active compounds have various properties like antimicrobial, antifungal, antioxidant, and anticancer. Phenols show antioxidant, anti-inflammatory, antimicrobial, antiviral activities.

S. No.	Phytochemicals	Result	
		Methanol Extract	Water Extract
1.	Tannins	++	++
2.	Flavonoids	+	-
3.	Glycosides	++	+
4.	Terpenoids	+	-
5.	Reducing Sugar	+	-
6.	Proteins	-	-
7.	Anthocyanins	-	-
8.	Steroids	+	-
9.	Anthraquinones	+	-
10.	Emodins	+	+
11.	Alkaloids	+++	+
12.	Phlobatannins	-	-
13.	Coumarics	+	-
14.	Phenols	++	++
+++ = strongly present, + = present, - = absent			

Table 4.2. Phytochemical analysis of *L. chinensis* seeds extracts

4.3 Fatty acid composition of *L. chinensis* seeds

The FAME analysis of the petroleum ether extract of *L. chinensis* seeds showed the presence of diverse fatty acids as shown in Table 3. The fatty acids are as follow: Caprylic acid, Caproic acid, Capric acid, Lauric acid, Myristic acid, Pentadecanoic acid, Palmitic acid, Palmitoleic acid, Heptadecanoic acid, Cis-10-Pentadecanoic acid, Stearic acid, Elaidic acid, Linolelaidic acid, Oleic acid, Linoleic acid, Arachidic acid, Linolenic acid, Cis-11-Eicosenoic acid, Heniecosanoic acid, Cis-8,11,14-Eicosadienoic acid, Behenic acid, Cis-11,14-Eicosadienoic acid, Erucic acid, Methyl Cis-5,8,11,14-Eicosatetraenoic acid, Cis-13,16-Docosadienoic acid, Lignoceric acid, Nervonic acid.

Oleic acid was present in the highest quantity (18.44%). Good amount of Lauric acid (11.66%), Linoleic acid (11.30%), Linolenic acid (10.20%), and Palmitic acid (9.96%) were also present.

S.No.	Type Of Fatty Acid	Area %
1	C6 Caproic Acid	0.14
2	C8 Caprylic Acid	0.25
3	C10 Capric Acid	0.28
4	C12 Lauric Acid	11.66
5	C14 Myristic Acid	5.20
6	C15 Pentadecanoic Acid	0.05
7	C16 Palmitic Acid	9.96
8	C16:1 Palmitoleic Acid	0.55
9	C17 Heptadecanoic Acid	0.19
10	C15:1 Cis-10-Pentadecanoic Acid	0.69
11	C18 Stearic Acid	2.08
12	C18:1 Δ9t Elaidic Acid	10.36
13	C18:1 Δ9c Oleic Acid	18.44
14	C18:2 Δ6t Linolelaidic Acid	0.50
15	C18:2 Linoleic Acid	11.30
16	C20 Arachidic Acid	0.61
17	C18:3 Linolenic Acid	10.20
18	C20:1 Cis-11-Eicosenoic Acid	0.21
19	C21 Heniecosanoic Acid	0.32
20	C20:3 Δ6 Cis-8,11,14-Eicosadienoic Acid	0.53
21	C22 Behenic Acid	0.66
22	C20:2 Cis-11,14-Eicosadienoic Acid	0.36
23	C22:1 Δ9c Erucic Acid	6.60
24	C20:4 Methyl Cis-5,8,11,14-Eicosatetraenoic Acid	0.47
25	C22:2 Cis-13,16-Docosadienoic Acid	4.80
26	C24 Lignoceric Acid	0.46
27	C24:1 Nervonic Acid	3.15

Table 4.3. Fatty acid composition of *L. Chinensis* seed extract

The data is also represented in a pie chart representation as shown in Fig. 3.1.

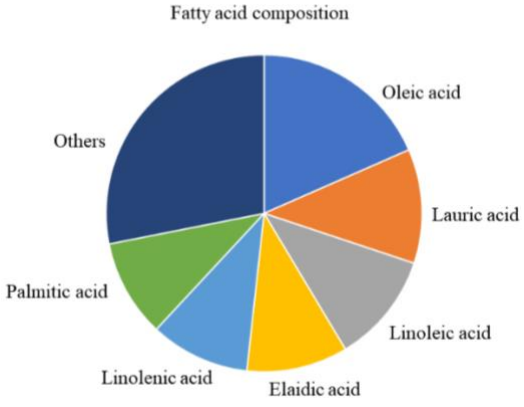

Fig. 3.1. Graphical representation of Fatty Acid Composition

4.4 Ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) analysis

The results from the UHPLC-QTOF-MS revealed the presence of following compounds in the dichloromethane extract of the seeds: Chloroneb, Acetoin, Isobutyric acid, 9-Oxononanoic acid, Piperidine, Umbellic acid, L-(+)-Mandelic acid, 2-Hydroxyphenylacetic acid, 3-Hydroxyphenylacetic acid, 4-Hydroxyphenylacetic acid, 3,4-Dihydroxyphenylacetaldehyde, Vanillin, Artemisinic aldehyde, Zerumbone, Solavetivone, 5',5'-Dimethyl-1,1'-bi(cyclohexane)-1',3-diene-4-carbaldehyde, 4-Hydroxyphenylpyruvate, Trans-caffeic acid, Butyl propionate, (9E)-9-Octadecenedioate, 1-Naphthyl β-D-glucopyranoside, and the compounds present in the methanol extract are:1,2-Dihydropyrimidine, 2-(2-Methylenecyclopropyl)-3-oxosuccinic acid, Furaneol, Ketopantolactone. This data is represented in Table 4.4.

Peak No.	Tentative metabolites	RT (min)	MF	MM ($gmol^{-1}$)	$[M-H]^+$	Error (ppm)	Compound ID
DCM extract							
1.	Chloroneb	9.389	$C_8H_8Cl_2O_2$	205.989	206.997	0.085	CSID16623
2.	Isobutyric acid	10.38	$C_4H_8O_2$	88.0522	89.06	3.44	CSID6341
3.	Acetoin	10.97	$C_4H_8O_2$	88.0522	89.06	3.38	CSID21105851
4.	9-Oxononanoic acid	11.44	$C_9H_{16}O_3$	172.10	173.1171	4.53	CSID68222
5.	Piperidine	11.83	$C_5H_{11}N$	85.0886	86.0964	-0.71	CSID7791
6.	Umbellic acid	12.40	$C_{15}H_{22}O$	180.0422	181.05	3.36	CSID393925
7.	L-(+)-Mandelic acid	12.40	$C_{15}H_{22}O$	152.0473	153.0551	3.74	CSID388690
8.	2-Hydroxyphenylacetic acid	12.40	$C_{15}H_{22}O$	152.0473	153.0551	3.74	CSID11476
9.	3-Hydroxyphenylacetic acid	12.40	$C_{15}H_{22}O$	152.0473	153.0551	3.74	CSID11624
10.	4-Hydroxyphenylacetic acid	12.40	$C_9H_8O_4$	152.0473	153.0551	3.74	CSID124

11.	3,4-Dihydroxyphenylacetaldehyde	12.40	$C_9H_8O_4$	152.0473	153.0551	3.74	CSID106504
12.	Vanillin	12.40	$C_{23}H_{24}O_8$	152.0473	153.0551	3.74	CSID13860434
13.	Artemisinic aldehyde	12.48	$C_{15}H_{22}O$	218.0372	219.045	0.10	CSID13115339
14.	Zerumbone	12.48	$C_{15}H_{22}O$	218.0372	219.045	0.10	CSID13450367
15.	Solavetivone	12.48	$C_{15}H_{22}O$	218.0372	219.045	0.10	CSID390842
16.	5',5'-Dimethyl-1,1'-bi(cyclohexane)-1',3-diene-4-carbaldehyde	12.48	$C_{15}H_{22}O$	218.0372	219.045	0.10	CSID58829872
17.	4-Hydroxyphenylpyruvate	12.95	$C_9H_8O_4$	180.0424	181.0502	4.38	CSID954
18.	Trans-caffeic acid	12.95	$C_9H_8O_4$	180.0424	181.0502	4.38	CSID600426
19.	Butyl propionate	18.50	$C_7H_{14}O_2$	130.0993	131.1071	4.28	CSID11045
20.	(9E)-9-Octadecenedioate	19.5	$C_{18}H_{30}O_4$	310.2148	311.2226	-0.33	CSID15077503
21.	1-Naphthyl β-D-glucopyranoside	21.36	$C_{16}H_{18}O_6$	307.1189	306.111	4.72	271.0979
Methanol extract							
22.	1,2-Dihydropyrimidine	12.14	$C_4H_6N_2$	82.0526	83.0604	0.49	CSID1027
23.	2-(2-Methylenecyclopropyl)-3-oxosuccinic acid	20.55	$C_8H_8O_5$	184.0373	185.0451	4.15	CSID81407866
24.	Furaneol	27.02	$C_6H_8O_3$	128.0202	129.028	1.61	CSID18218
25.	Ketopantolactone	27.02	$C_6H_8O_3$	128.0202	129.028	1.61	CSID38
MM = Monoisotopic mass							

Table 4.4. UHPLC-QTOF-MS of DCM and methanol extract of *Livistona chinensis* seeds

4.5 DPPH radical scavenging assay

The DPPH radical scavenging assay showed that the methanolic extract of *L. chinensis* seeds showed good antioxidant properties. DPPH free radical strongly absorbs in the range 515-520 nm, and this absorbance decreases when any antioxidant is added to it. The radical scavenging activity was performed with different concentrations of the methanolic extract (60, 80, 100, 120, 140, 160, 180, and 200 µL/mL). The highest antioxidant activity was observed with the solution with 200 µL/mL solution. The results are shown in Fig. 3.2.

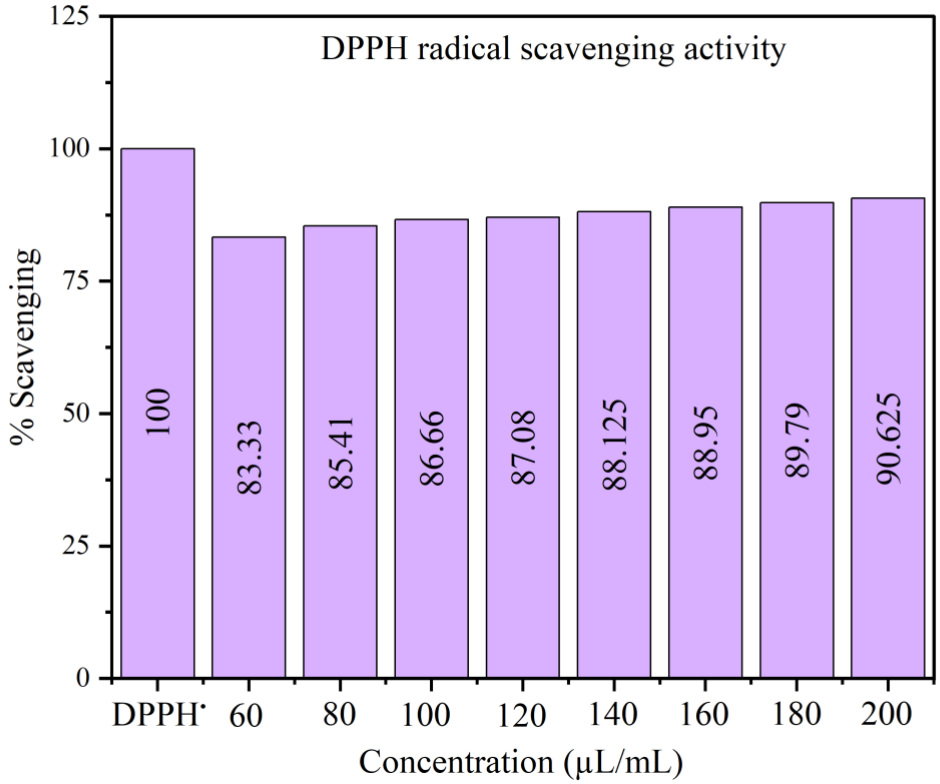

Fig. 3.2. Antioxidant activity

The color change from violet to yellow was observed when the methanolic extract samples were added to DPPH· as shown in Fig 3.3.

Fig.3.3. Color change observed in antioxidant property

4.6 Gas Chromatography- Mass Chromatography analysis of *L. chinensis* seeds extract

The GC-MS analysis of n-hexane extract of *L. chinensis* seeds showed the preence of following volatile compounds: 3β-(Trimethylsiloxy) stigmasta-5,22-diene, 3-Trimethylsilyloxy-24-ethylcholesta-5,22-diene, 1-(Trimethylsilyloxy)octacosane, 2-(Dodecanoyloxy)-1-([(trimethylsilyl)oxy]methyl)ethyl laurate, 2-Tetradecyloxirane, Pentatriacontane, Squalene, Carbonic acid, eicosyl prop-1-en-2-yl ester, Octacosane, Dioctyl phthalate, 2-Methyleicosane, (3Z,13Z)-2-Methyl-3,13-octadecadien-1-ol, Tricosane, 2-Butyloctanol, Tributylacetylcitrate, Trimethylsilyl stearate, Oleic acid, trimethylsilyl ester, Octadecyl trimethylsilyl ether, Heptadecanoic acid, trimethylsilyl ester, Methyl linolelaidate, Palmitic acid, trimethylsilyl ester, Palmitoleic acid 1tms, Octadecane, Ethyl undecanoate, 1-Trimethylsiloxyhexadecane, Trimethylsilyl pentadecanoate, Methyl hexadecanoate, 17-Octadecynoic acid, trimethylsilyl ester, n-Decyl fluoride, Trimethylsilyl myristate, Isopropyl tetradecanoate, Hexadecane, Trimethylsilyl laurate, Heptadecane, Tridecane, Dodecane, Trimethylenenorbornane, Di hydrodicyclopentadiene.

Oleic acid was present in the highest quantity with area% of 35.71. Followed by Palmitic acid (30.7%). The data is also represented graphically in Fig. 3.4.

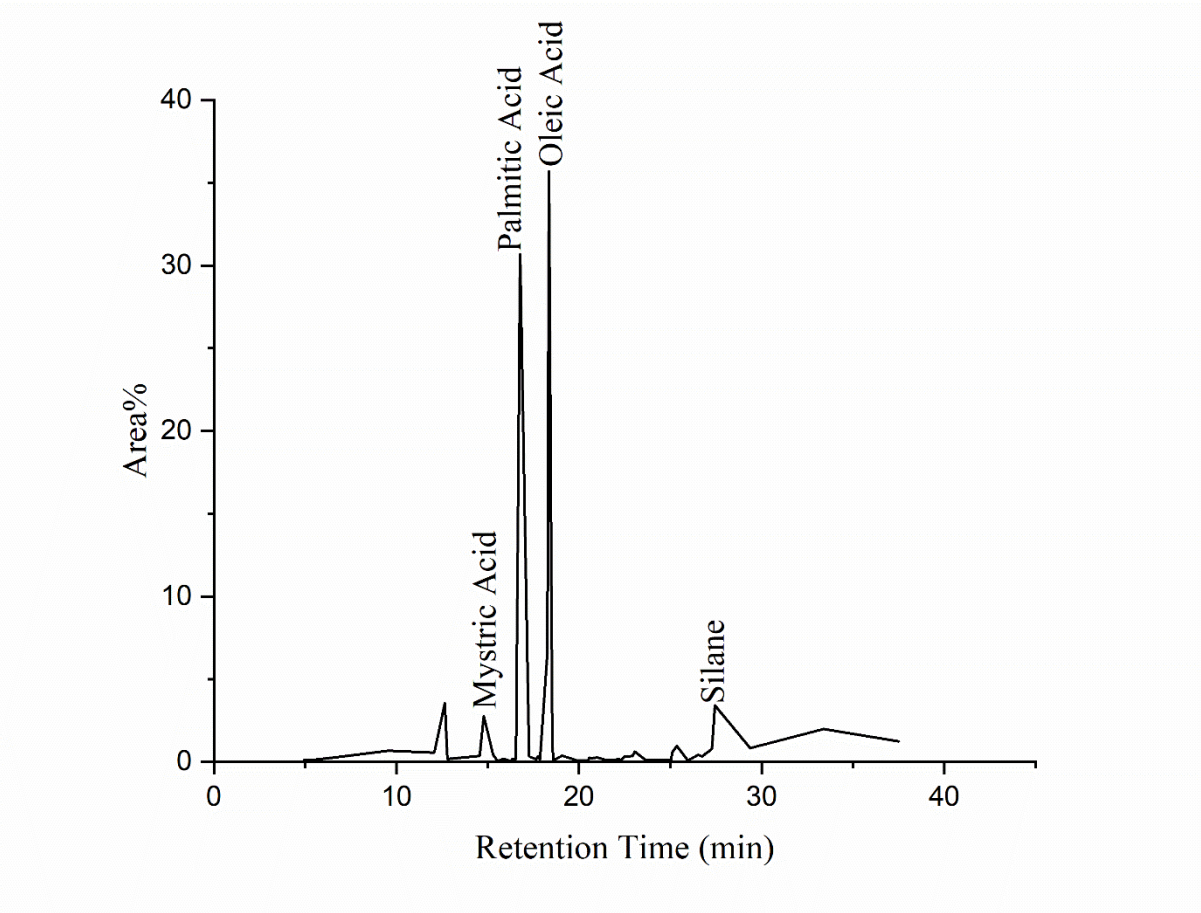
Palmitic Acid
Oleic Acid
Mystric Acid
Silane
Area%
Retention Time (min)
0
10
20
30
40

Fig. 3.4. GC-MS

Name of Compounds	RT (min)	Area %	MW ($gmol^{-1}$)	MF
3-Trimethylsilyloxy-24-ethylcholesta-5,22-diene	27.437	3.41	484	$C_{32}H_{56}OSi$
3β-(Trimethylsiloxy) stigmasta-5,22-diene	26.748	0.34	484	$C_{32}H_{56}OSi$
1-(Trimethylsilyloxy)octacosane	25.343	0.97	482	$C_{31}H_{66}OSi$
2-(Dodecanoyloxy)-1-([(trimethylsilyl)oxy]methyl)ethyl laurate	25.11	0.57	528	$C_{32}H_{60}O_5Si$
2-Tetradecyloxirane	24.791	0.14	240	$C_{16}H_{32}O$
Pentatriacontane	24.298	0.41	450	$C_{32}H_{66}$
Squalene	23.039	0.62	410	$C_{30}H_{50}$
Carbonic acid, eicosyl prop-1-en-2-yl ester	22.923	0.35	382	$C_{24}H_{46}O_3$
Octacosane	21.462	0.11	394	$C_{28}H_{58}$
Dioctyl phthalate	20.993	0.27	390	$C_{24}H_{38}O_4$
2-Methyleicosane	20.69	0.21	296	$C_{21}H_{44}$
(3Z,13Z)-2-Methyl-3,13-octadecadien-1-ol	20.545	0.24	280	$C_{19}H_{36}O$
Tricosane	19.89	0.09	324	$C_{23}H_{48}$
2-Butyloctanol	19.059	0.38	186	$C_{12}H_{26}O$
Tributylacetylcitrate	18.604	0.08	402	$C_{20}H_{34}O_8$
Trimethylsilyl stearate	18.531	1.01	356	$C_{21}H_{44}O_2Si$
Oleic acid, trimethylsilyl ester	18.342	35.71	354	$C_{21}H_{42}O_2Si$
Octadecyl trimethylsilyl ether	17.753	0.36	342	$C_{21}H_{46}OSi$
Heptadecanoic acid, trimethylsilyl ester	17.654	0.16	342	$C_{20}H_{42}O_2Si$
Methyl linolelaidate	17.253	0.33	294	$C_{19}H_{34}O_2$
Palmitic acid, trimethylsilyl ester	16.766	30.7	328	$C_{19}H_{40}O_2Si$
Palmitoleic acid 1tms	16.536	0.17	326	$C_{19}H_{38}O_2Si$
Octadecane	16.333	0.17	254	$C_{13}H_{28}$
Ethyl undecanoate	16.277	0.04	214	$C_{13}H_{26}O_2$
1-Trimethylsiloxyhexadecane	15.900	0.18	314	$C_{19}H_{42}OSi$
Trimethylsilyl pentadecanoate	15.783	0.15	314	$C_{18}H_{38}O_2Si$
Methyl hexadecanoate	15.621	0.09	270	$C_{17}H_{34}O_2$
17-Octadecynoic acid, trimethylsilyl ester	15.496	0.10	352	$C_{21}H_{40}O_2Si$
n-Decyl fluoride	15.29	0.42	160	$C_{10}H_{21}F$
Trimethylsilyl myristate	14.782	2.79	300	$C_{17}H_{36}O_2Si$
Isopropyl tetradecanoate	14.559	0.35	270	$C_{17}H_{34}O_2$
Hexadecane	14.308	0.33	226	$C_{16}H_{34}$
Trimethylsilyl laurate	12.64	3.55	272	$C_{15}H_{32}O_2Si$
Heptadecane	12.069	0.55	240	$C_{17}H_{36}$
Tridecane	9.563	0.68	184	$C_{13}H_{28}$
Dodecane	6.746	0.29	170	$C_{12}H_{26}$
Trimethylenenorbornane	5.347	0.11	136	$C_{10}H_{16}$
Di hydrodicyclopentadiene	4.954	0.13	134	$C_{10}H_{14}$
RT = Retention time MF = Molecular formula				

Table 4.5. Bioactive compounds identified by GC-MS analysis of n-hexane extract of *L. chinensis* seeds

4.7 Antimicrobial activity

The antimicrobial activity was tested for four different solvents: DMSO, methanol, petroleum ether, and water extract. However, the activity was obtained only by aqueous extract against gram –ve bacteria *Klebsiella pneumoniae* with inhibition area of 16.1 mm.

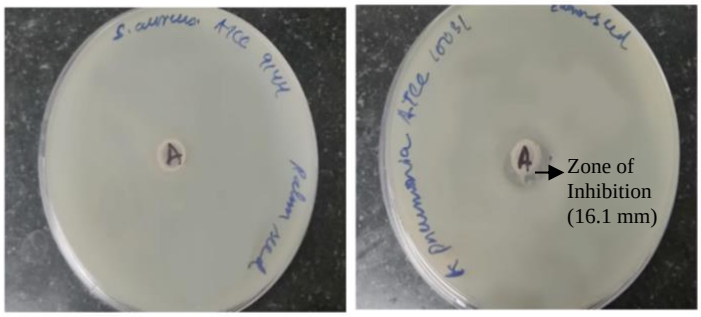

Fig. 3.5. Antimicrobial activity of *Livistona chinensis* seeds extract

CHAPTER 5

CONCLUSION

In this work, the phytoconstituent of the Indian variety of *L. chinensis* was extracted and its relevance was examined. The results of the phytochemical examination showed the absence of proteins, anthocyanins, and phlobatannins but a high amount of the biologically active flavonoids, alkaloids, and phenolic compounds. The existence of numerous classes of phytoconstituents that may have therapeutic applications for the treatment of various illnesses was confirmed by GC-MS and UHPLC-QTOF-MS analysis. Because oleic acid, linoleic acid, and palmitic acid make up a large portion of the extract from the seeds, *L. chinensis* is used more frequently. Significant antioxidant activity was shown in the methanolic extract. The seeds' aqueous extract had the strongest antibacterial action, with an inhibition of 16.1 mm. According to the aforementioned phytochemical, antioxidant, and antimicrobial profiles of *L. chinensis* seeds, the seeds may be useful for new researchers doing more studies on the use of the phytocompounds under investigation.

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