

Phytochemical, antioxidant and antimicrobial activity of *Livistona chinensis* (Jacq.) R.Br. ex Mart. seeds

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The seeds of *Livistona chinensis* (Jacq.) R.Br. ex Mart. (Family: Arecaceae) plant traditionally find application in the treatment of various diseases. Therefore, in the present study, phytochemical screening and biological profiling of *Livistona chinensis* seed extracts were conducted. The bioactive components were analysed using GC-MS and UHPLC-QTOF-MS and the fatty acid composition was determined using FAME analysis. GC-MS analysis results indicated the presence of oleic acid (35.71%) and palmitic acid (30.7%) as major components in the seed extract prepared in n-hexane. UHPLC-QTOF-MS analysis revealed the presence of 25 secondary metabolites, including isobutyric acid, acetoin, 9-oxononanoic acid, piperidine, umbellic acid, furaneol, and ketopantolactone in the methanolic extract of seeds. FAME analysis results suggested the presence of oleic acid (18.44%), lauric acid (11.66%), linoleic acid (11.30%), linolenic acid (10.20%), and palmitic acid (9.96%) in abundance. In biological profiling, *Livistona chinensis* seed extracts were subjected to *in vitro* antimicrobial, and antioxidant activity. The aqueous extract exclusively showed antimicrobial activity, and exhibited inhibition zone of 16.1 mm only against gram-negative bacteria (*Klebsiella pneumonia*). The methanolic extract demonstrated maximum antioxidant activity, at sample concentration of 200 μ L. These findings suggest that *Livistona chinensis* seeds might be a potential source of bioactive metabolites, antimicrobial agents, and antioxidant agents that may be utilised in pharmaceutical, cosmetics, and food industries.

Keywords: Antimicrobial activity, Antioxidant activity, Elemental analysis, *Livistona chinensis*, Phytochemical analysis, UHPLC-QTOF-MS

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Nutraceutical and functional foods are essential in managing numerous lifestyle-related illnesses like diabetes, obesity, and heart disease. Due to the abundance of health-promoting ingredients present today, goods from plant sources are attracting a lot of interest. A huge attention has shifted towards medicinal seeds in particular due to their rich phytochemistry and preventive properties¹.

Livistona chinensis (*L. chinensis*, Family: Arecaceae) is a decorative plant and is recognised as Chinese Fountain palm or Chinese Fan palm. Its native habitats are Taiwan, Southern Japan, East Asia, Australia, and several islands in the Southern China Sea². The fruits of *L. chinensis* are oval in shape, with a hard outer covering, and black in colour. According to the previous reports, *L. chinensis* fruits exhibited anti-cancer, anti-microbial, and anti-HIV-1 properties³. Historically, the analgesic and haemostatic properties of their fruits have been

utilised to treat nasopharyngeal carcinoma, choriocarcinoma, oesophageal cancer, gastric cancer, and leukemia⁴. People in China and East Asia have utilised the *L. chinensis* fruit as an herbal ingredient in soups to treat hepatitis and many types of liver cancer⁵. *L. chinensis* seeds have a long history of usage in Traditional Chinese Medicine, as an anti-cancer agent. Also, various experimental studies have shown the anti-proliferative and anti-angiogenic properties of aqueous extract from their seeds and fruits⁶.

Moreover, the presence of phenolic compounds in fruits of *L. chinensis* has been reported to possess haemolytic activity⁷. Chinese Fan palm seeds' biochar were also found to absorb the Malachite Green, which is an effluent from the textile dye⁸.

Phytochemicals, mainly found in plants are secondary metabolites and are responsible for their different medicinal properties. The preliminary screening of phytochemicals was crucial in discovering the different phytoconstituents present in

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medicinal plant extracts, providing an insight into future drug discovery and development⁹. The chemical constituents present in plant parts are considered as biologically active compounds as they possess diverse activities like anti-analgesic, anti-cancer, anti-viral, and anti-microbial¹⁰. The phytoconstituents like alkaloids, glycosides, saponins, resins, and oils have a protective or disease-preventive effect¹¹.

Medicinal plants are now gaining more credibility due to the growing demand for their antioxidant properties. In the food industry, anti-oxidants are used to delay the oxidation process. Natural substances are the possible substitute for the widely used synthetic anti-oxidants. In this paper, the anti-oxidant activity of the seeds extract was evaluated using free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which absorbs strongly at 517 nm, and its absorbance decreases with the addition of any antioxidant¹².

Fatty acid composition controls functional and nutritional values. Polyunsaturated fatty acids (PUFA) like linoleic (omega-6) and linolenic acids (omega-3) are crucial for human metabolism due to a shortage of the enzymes needed for their formation. PUFA are regarded as desirable substances in the human diet due to their ability to lower the risk of cancer and cardiovascular disease¹³. The novelty in this study is the application of soxhlet extraction of seeds of *L. chinensis* followed by the use of ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) method to analyse the secondary metabolites present in the isolated extract.

In view of the above, the current study investigated the evaluation of the bioactive compounds of *L. chinensis* seeds cultivated in India by UHPLC-QTOF-MS and GC-MS analysis, elemental analysis by ICP-MS analysis, antimicrobial property against *Klebsiella pneumoniae* (gram-negative) and *Staphylococcus aureus* (gram-positive), and antioxidant property by DPPH free radical scavenging method.

Material and Methods

Plant material collection and authentication

Livistona chinensis fruits were collected from the trees growing at Delhi Technological University, New Delhi, India. The botanical specimens of the *Livistona chinensis* fruits were authenticated by CSIR-NIScPR, Raw Materials Herbarium, and Museum, Delhi (RHMD), India and the voucher specimen

(NIScPR/RHMD/Consult/2023/4482-83) was deposited at the RHMD, Delhi. The collected *L. chinensis* fruits were cleaned and their seeds were removed manually from the fruits. The seeds were sun dried for 2-3 days and coarsely grounded by grinder for their extraction.

Preparation of extracts

L. chinensis (50 g) grounded seeds were extracted sequentially with n-hexane, dichloromethane (DCM), methanol, and deionised water for 6-8 h, using Soxhlet apparatus. After successful extraction, the solvents were evaporated using a rotary evaporator. The concentrated extracts obtained were kept at 4°C for further use¹⁴.

Phytochemical analysis

The qualitative phytochemical analysis of methanol extract of *L. chinensis* seeds was done following the standard methods¹⁵⁻¹⁷. Various phytoconstituents identification tests for tannins, alkaloids, glycosides, anthraquinones, steroids, proteins, flavonoids, terpenoids, reducing sugars, anthocyanins, emodins, coumarins, phlobatannins, and phenols were performed.

GC-MS (Gas Chromatography-Mass Spectrometry) analysis

The determination of volatile compounds present in the crude n-hexane extract of *L. chinensis* seeds was carried out using GC-MS (Shimadzu, GCMS-QP2010). The sample was injected into GC-MS, using Helium as a carrier gas in split mode at 260°C. The column flow rate was kept at 1.21 mL/min and the ionization mode was electron ionisation (EI). The ion source and interface temperature were 220°C and 270°C, respectively. A solvent delay time of 3.50 min, the temperature of the oven was set at 80°C for 2 min to 300°C for 21 min. To identify the separated peaks, NIST mass spectral data-base (National Institute of Standards and Technology) was used¹⁸.

UHPLC-QTOF-MS analysis

For the UHPLC and mass spectrometric analysis of the methanolic extract of *L. chinensis* seeds, HPLC (Waters, SYNAPT-XS HDMS, UK) fitted with an AD pump, AD auto sampler controller, degasser, AD column, coupled with a quadrupole time-of-flight mass spectrometer (QTOF-MS) was used. It was used for the identification of secondary metabolites in the DCM and methanol extracts of the seeds. Both the extracts were mixed with formic acid (1%, 10 mL) in water and set aside for 10 min. 10 mL acetonitrile and

10 mL methanol were mixed and vortexed for 1 min then centrifuged at 5000 rpm for 5 min. Diluting the sample with acidified water, it was injected (5 µL) into the instrument. 100 mm x 2.1 mm column C18 (Waters, Acquity BEH 2.1) was used for the chromatographic separation of the samples. The mass spectroscopic analysis was done in only positive mode (ESI+) with the following mentioned conditions: desolvation gas flow (950 L/h), source temperature, (120°C), capillary voltage (3.22 keV), cone gas flow (50 L/h). For eluting the secondary metabolites, a binary mobile phase at a flow rate of 0.2 mL/min was used, where solvent A was LC-MS grade water containing 1% formic acid and solvent B was 1% formic acid with acetonitrile. ChemSpider software was used to perform data acquisition and processing¹⁹.

ICP-MS (Inductively Coupled Plasma Mass Spectrometry) analysis

About 0.1 g of the *L. chinensis* grounded seeds were taken in digestion vessel. 1 mL H₂O₂, 1 mL distilled water, and 2 mL H₂SO₄ (conc.) was added. After 30 min, the digestion vessel was closed and kept within the digester at 483 K. The similar method was used to examine blank sample and NIST standards. The instrument calibration was evaluated using three NIST-certified samples²⁰.

FAME (Fatty Acid Methyl Ester) analysis

The fatty acid content in the *L. chinensis* seeds were evaluated using Fatty acid methyl ester (FAME) analysis by taking 0.2 mL of the extracted oil sample. 200 µL of 2N alcoholic potassium hydroxide (KOH) was used to saponify the oil obtained from the seeds and vortexed for 2 min. This mixture was heated for 10 min in a water bath at 60°C, and then cooled off. To obtain mixed fatty acids (MFAs), the aqueous layer was then acidified with 1 mL of 5% hydrochloric acid (HCl) solution, heated for 10 min at 70°C. The mixture obtained was cooled and vortexed for 2 min after addition of 2 mL of petroleum ether. The separated upper layer was examined by GC-MS (Shimadzu, GC-2010).

Antioxidant activity

DPPH radical scavenging activity

The method used for antioxidant activity described by Velazquez *et al.*, was applied with some modifications¹³. The methanolic extract of the sample at different concentrations (60 µL, 80 µL, 100 µL, 120 µL, 140 µL, 160 µL, 180 µL, and 200 µL) were

made and 1 mL of each sample was mixed with 3 mL of DPPH radical, with methanol serving as the blank sample. The samples were incubated for 30 min at 37°C, and after that the absorbance was measured for each sample at 517 nm. The % radical scavenging was measured using the following formula¹²:

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

Antimicrobial activity

The antimicrobial activity of aqueous and methanol extracts of *L. chinensis* were done using the disc diffusion method²¹. 20 mL of the nutrient media was poured into sterilised petri dishes, which was then kept to solidify. 20 µL of the extract was tested against following bacterial strain: gram-negative bacteria, *Klebsiella pneumoniae* and gram-positive bacteria, *Staphylococcus aureus*. The bacterial strains were cultivated at 37°C in incubator and left for 24 h. After the incubation period, zone of inhibition was measured.

Results and Discussion

Phytochemical analysis of *L. chinensis* seeds extract

The data of phytochemical analysis revealed that tannins, glycosides, alkaloids, phenols, and emodins were present in water and methanol extract, while flavonoids, terpenoids, reducing sugars, steroids, anthraquinones, and coumarins were only identified in the methanol extract (Table 1). Proteins, anthocyanins, and phlobatannins were not identified in any of the extracts. The phytoconstituents present in both the extracts were identified as biologically active compounds and they have been responsible for

Table 1 — Phytochemical analysis of *L. chinensis* seeds extracts

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. Coumarins	+	-
14. Phenols	++	++

+++ = strongly presence of phytochemicals, + = presence of phytochemicals, - = absence of phytochemicals

various activities. Phenolic compounds are well-known for antiviral, antioxidant, anti-mutagenic, antimicrobial activities. Terpenoids also show antibacterial, anti-malarial, anti-inflammatory, antiviral, anti-cancer, and antimicrobial activities¹⁰. Hifnawy *et al.*²², analysed similar results for the phytochemical screening of seeds extract of *L. chinensis*.

Gas chromatography-mass spectrometry analysis of *L. chinensis* seeds extract

Fifty-five volatile components were characterised in the n-hexane extract of *L. chinensis* seeds by

GC-MS studies (Table 2). The major constituents were found to be oleic acid (35.71%) and palmitic acid (30.7%). These phytochemicals may be responsible for diverse pharmacological activities like hepatoprotective, antioxidant, wound-healing, and antibacterial activities.

UHPLC-QTOF-MS analysis

UHPLC-QTOF-MS analysis was done to evaluate secondary metabolites present in the DCM and methanol extract of *L. chinensis* seeds. UHPLC-QTOF-MS data of methanol and DCM extract as shown in

Table 2 — Bioactive compounds identified by GC-MS analysis of n-hexane extract of *L. chinensis* seeds

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-(Trimethylsilyloxy) 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 ₅ Si
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
Diocetyl 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
Tributylacetyl citrate	18.604	0.08	402	C ₂₀ H ₃₄ O ₈
Trimethylsilyl stearate	18.531	1.01	356	C ₂₁ H ₄₄ O ₂ Si
Oleic acid, trimethylsilyl ester	18.342	35.71	354	C ₂₁ H ₄₂ O ₂ Si
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 ₂ Si
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 ₂ Si
Palmitoleic acid 1tms	16.536	0.17	326	C ₁₉ H ₃₈ O ₂ Si
Octadecane	16.333	0.17	254	C ₁₃ H ₂₈
Ethyl undecanoate	16.277	0.04	214	C ₁₃ H ₂₆ O ₂
1-Trimethylsilyloxyhexadecane	15.900	0.18	314	C ₁₉ H ₄₂ OSi
Trimethylsilyl pentadecanoate	15.783	0.15	314	C ₁₈ H ₃₈ O ₂ Si
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 ₂ Si
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 ₂ Si
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 ₂ Si
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 3 confirms the presence of chloroneb, isobutyric acid, acetoin, 9-oxononanoic acid, piperidine, umbellic acid, L-(+)-mandelic acid, 2-hydroxyphenylacetic acid, vanillin, 3-hydroxyphenyl-acetic acid, 3,4-dihydroxyphenylacetaldehyde, artemisinic aldehyde, zerumbone, solavetivone, 4-hydroxyphenylpyruvate, trans-caffeic acid, 4-hydroxyphenylacetic acid, butyl propionate, (9E)-9-octadecenedioate, 1-naphthyl β -D-glucopyranoside, 1,2-dihydropyrimidine, 5',5'-dimethyl-1,1'-bi(cyclohexane)-1',3'-diene-4-carbaldehyde, 2-(2-methylenecyclopropyl)-3-oxo-succinic acid, furaneol, and ketopantolactone.

According to the reported study, isobutyric acid plays an important role in maintaining improving intestinal barrier function, reducing risk of chronic diseases (inflammatory bowel disease, colorectal cancer, and type 2 diabetes) because of its anti-inflammatory and antioxidant properties. It also enhances the activity of key enzymes involved in the

synthesis of tight junction proteins²³. Acetoin shows antioxidant and anti-inflammatory properties useful for maintaining cellular health and preventing disease. It is an intermediate in the biosynthesis of several amino acids, including valine, leucine, and isoleucine. It is used as a flavouring agent in food industry and used for fragrance and fixative in the perfume industry²⁴. 9-oxononanoic acid has antimicrobial and anticancer properties. Piperidine acts as a neurotransmitter, and exhibits analgesic, antibacterial, antifungal, anti-inflammatory, and antitumour activities having wide applications in the field of pharmaceuticals and agrochemicals²⁵.

ICP-MS analysis of *L. chinensis* seeds

The elemental analysis of *L. chinensis* seeds, as presented in Table 4, showed the presence of different types of minerals at different concentrations. The main elements in *L. chinensis* seeds are iron, zinc and calcium. Calcium was the most abundant macro-

Table 3 — UHPLC-QTOF-MS of DCM and methanol extract of *Livistona chinensis* seeds

Peak No.	Tentative metabolites	RT (min)	MF	MM (gmol^{-1})	[M-H] ⁺	Error (ppm)	Compound ID
DCM extract							
1.	Chloroneb	9.389	C ₈ H ₈ Cl ₂ O ₂	205.989	206.997	0.085	CSID16623
2.	Isobutyric acid	10.38	C ₄ H ₈ O ₂	88.0522	89.06	3.44	CSID6341
3.	Acetoin	10.97	C ₄ H ₈ O ₂	88.0522	89.06	3.38	CSID21105851
4.	9-Oxononanoic acid	11.44	C ₉ H ₁₆ O ₃	172.10	173.1171	4.53	CSID68222
5.	Piperidine	11.83	C ₅ H 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 ₉ H ₈ O ₄	152.0473	153.0551	3.74	CSID124
11.	3,4-Dihydroxyphenylacetaldehyde	12.40	C ₉ H ₈ O 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 ₉ H ₈ O ₄	180.0424	181.0502	4.38	CSID954
18.	Trans-caffeic acid	12.95	C ₉ H ₈ O ₄	180.0424	181.0502	4.38	CSID600426
19.	Butyl propionate	18.50	C ₇ H 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 ₄ H ₆ N ₂	82.0526	83.0604	0.49	CSID1027
23.	2-(2-Methylenecyclopropyl)-3- oxosuccinic acid	20.55	C ₈ H ₈ O ₅	184.0373	185.0451	4.15	CSID81407866
24.	Furaneol	27.02	C ₆ H ₈ O ₃	128.0202	129.028	1.61	CSID18218
25.	Ketopantolactone	27.02	C ₆ H ₈ O ₃	128.0202	129.028	1.61	CSID38

MM = Monoisotopic mass

Table 4 — ICP-MS analysis of *L. chinensis* seeds

S.No.	Elements	Sample concentration (ppm)
1	Fe	15.95
2	Zn	10.86
3	As	bdl
4	Co	bdl
5	Cu	3.37
6	Mn	2.97
7	Ni	0.16
8	Pb	0.31
9	Rb	3.38
10	Se	0.09
11	Sr	5.61
12	U	0.06
13	V	bdl
14	Ca	77.09
15	Al	0.37

bdl: below detection limit

Table 5 — Fatty acid composition of *L. chinensis* seeds

S.No.	Type of fatty acid	Area %
1.	Caproic acid (C6:0)	0.14
2.	Caprylic acid (C8:0)	0.25
3.	Capric acid (C10:0)	0.28
4.	Lauric acid (C12:0)	11.66
5.	Myristic acid (C14:0)	5.20
6.	Pentadecanoic acid (C15:0)	0.05
7.	Palmitic acid (C16:0)	9.96
8.	Palmitoleic acid (C16:1)	0.55
9.	Heptadecanoic acid (C17:0)	0.19
10.	Cis-10-pentadecanoic acid (C15:1)	0.69
11.	Stearic acid (C18:0)	2.08
12.	Elaidic acid (C18:1 Δ9t)	10.36
13.	Oleic acid (C18:1 Δ9c)	18.44
14.	Linolelaidic acid (C18:2 Δ6t)	0.50
15.	Linoleic acid (C18:2)	11.30
16.	Arachidic acid (C20:0)	0.61
17.	Linolenic acid (C18:3)	10.20
18.	Cis-11-eicosenoic acid (C20:1)	0.21
19.	Henicosanoic acid (C21:0)	0.32
20.	Cis-8,11,14-eicosadienoic acid (C20:3 Δ6)	0.53
21.	Behenic acid (C22:0)	0.66
22.	Cis-11,14-eicosadienoic acid (C20:2)	0.36
23.	Erucic acid (C22:1 Δ9c)	6.60
24.	Methyl cis-5,8,11,14-eicosatetraenoic acid (C20:4)	0.47
25.	Cis-13,16-docosadienoic acid (C22:2)	4.80
26.	Lignoceric acid (C24:0)	0.46
27.	Nervonic acid (C24:1)	3.15

element with 77.09 ppm concentration in the *L. chinensis* seeds. All the minerals present in *L. chinensis* seeds are extremely beneficial to human health. For example, calcium can be beneficial to build bones, iron metal is required in the production of haemoglobin. Zinc, copper, and manganese are important cofactors in the bone metabolism²⁶.

FAME analysis

The fatty acid composition of *L. chinensis* seeds is shown in Table 5. The highest quantity of fatty acid was found for oleic acid (18.44%), followed by lauric acid (11.66%), linoleic acid (11.30%), linolenic acid (10.20%), and palmitic acid (9.96%). Oleic acid reduces the risk of cardiovascular disease and improves insulin sensitivity²⁷. Banerjee *et al.*²⁸ has concluded that this fatty acid has a future therapeutic effect associated with human cell apoptosis. It also shows anti-inflammatory and antioxidant properties.

Lauric acid has antimicrobial and anti-viral properties making it a useful natural alternative to conventional antibiotics and antiviral drugs. It contains a number of health benefits, including improved heart health, lower cholesterol level, increased levels of high-density lipoprotein (HDL), and weight loss²⁹.

Palmitic acid helps in regulating glucose metabolism and the development of insulin resistance in type 2 diabetes³⁰. Linoleic acid that cannot be synthesised by the human body is an essential omega-6 PUFA. It has various biological effects over human body cells such as cell membrane flexibility, reduced cardiovascular disease, lower cholesterol levels, immune system function, and inflammation regulation³¹. Another essential omega-3 polyunsaturated fatty acid, linolenic acid is an important component of cell membranes involved in many biological processes in the body. It shows anti-inflammatory effects and prevention as well treatment of chronic inflammatory diseases³².

Antioxidant activity

DPPH radical scavenging assay

The anti-oxidant activity of methanolic extract of *L. chinensis* seeds was examined by DPPH radical scavenging test. DPPH radical assay is widely used for screening antioxidants for the majority of the plant products and plant extract's activity to quench free radicals. The antioxidants present in the extract, decolourise DPPH radical, this free radical decolourisation follows the mechanism of electrons or hydrogen atoms to neutralise DPPH^{14,20}. The result of the free radical scavenging activity is displayed in Figure 1 which shows that the maximum antioxidant activity was shown by the solution with a sample concentration of 200 µL with a colour change from purple to yellow, confirming interaction of the antioxidants in the sample with the free radicals.

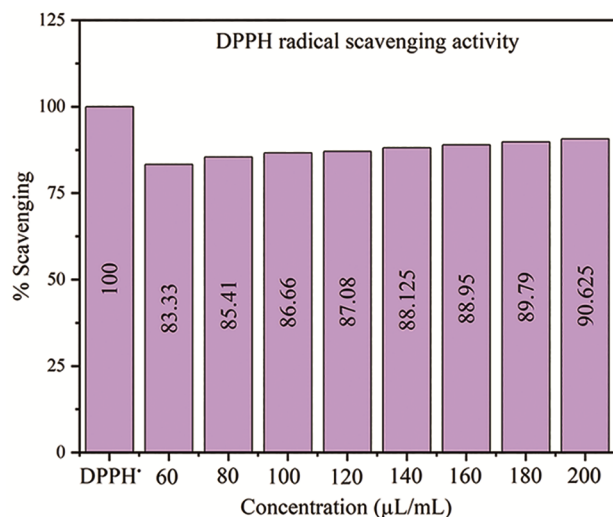


Fig. 1 — Antioxidant activity of methanol extract of *Livistona chinensis* seeds extract

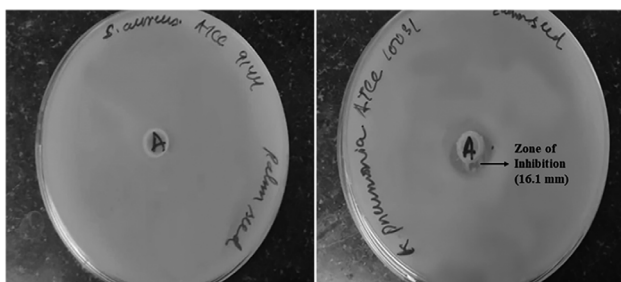


Fig. 2 — Antimicrobial activity of *Livistona chinensis* seeds extract

Antimicrobial activity

The results of the antimicrobial activity revealed that among methanol and aqueous extracts of *L. chinensis*, only the aqueous extract showed the anti-microbial activity against gram-negative bacteria (*Klebsiella pneumoniae*) with inhibition of 16.1 mm while no activity was observed for the same extract for gram-positive bacteria (*Staphylococcus aureus*) as shown in Figure 2. *L. chinensis* inhibits the bacteria growth. From the above results, we can conclude that the aqueous extract of *L. chinensis* could be a source of antimicrobial agent in cosmetic and pharmaceutical formulation.

Conclusion

This study focused on the extraction and exploring the significance of the phytoconstituent of the Indian variety of *L. chinensis* seeds previous studies had shown their effectiveness in various therapeutic properties like anti-cancer, anti-microbial, and anti-oxidant agent. Our study investigated the presence of significant amount of flavonoids,

alkaloids, and phenolic compounds, which possess various biological activities, while proteins, anthocyanins, and phlobatannins were absent. GC-MS and UHPLC-QTOF-MS analysis confirmed the presence of varied classes of phytoconstituents that may have therapeutic application for the treatment of various diseases. The presence of a high percentage of fatty acids in the seeds extract such as oleic acid, linoleic acid, and palmitic acid make *L. chinensis* application more prominent. The methanolic extract showed significant antioxidant activity. The best antimicrobial activity was only shown by aqueous extract of the seeds with inhibition of 16.1 mm. The above results of phytochemicals examination, antioxidant, and antimicrobial profiling of *L. chinensis* seeds concluded that the seeds may be beneficial for new researchers for further research on the application of investigated phytochemicals.

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Conflict of Interest

The authors declare that there is no competing or conflict of interest.

Author Contributions

TB: Methodology, writing- original draft; RS: Conceptualization, review, editing, data analysis; RKG: Conceptualization, supervision, review & editing.

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