Phytochemical Analysis and *in vitro* Antioxidant Activities of Three Species of *Premna* L. from Kerala

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ABSTRACT

Aim: Premna, a genus belonging to the family Lamiaceae, is known for its immense bioactivity and has a significant role in traditional medicine and modern pharmacological research. The present study sought to look into the bioactive compounds present in the methanolic extracts and the antioxidant activities of three selected species of Premna from different localities of Kerala State, India. Materials and Methods: This study offers a thorough comparison of the phytochemical composition, antioxidant activity, and Gas Chromatography-Mass Spectrometry (GC-MS) profiling of three species of Premna such as Premna scandens Roxb., Premna mollissima Roth. Premna glaberrima Wight. from Kerala, India. Results: Qualitative analysis revealed the presence of major phytochemical compounds such as alkaloids, phenolics, flavonoids, terpenoids and steroids in the three species and the quantitative assays indicated considerable differences in their concentrations in these species. The antioxidant activity experiments revealed varied degrees of free radical scavenging ability, which corresponded to the observed phytochemical diversity. Several bioactive compounds were identified by GC-MS investigation of three different Premna species. Among them P. glaberrima, showed peaks indicating the presence of 22 compounds, whereas P. scandens and P. mollisima were confirmed with the presence of 17 and 16 compounds respectively some of which may contribute to the reported antioxidant activity. Conclusion: Thus, a variety of phytochemicals present in the three Premna species can potentially enhance their antioxidant activity. The ability to neutralize free radicals and lessen oxidative stress was indicated by the antioxidant capacity measured using the DPPH, nitric oxide scavenging, and superoxide scavenging procedures.

Keywords: Antioxiodant activity, Premna mollissima, P. glaberrima, P. scandens.

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INTRODUCTION

Premna is one among the old age genera of medicinal plants which has been used in various systems of Indian medicinal practices such as Ayurveda and folk medicine and also in Vedic rituals. The fruits of *P. barbata* are used as an effective remedy for fever and eczema whereas the leaves of *P. bengalensis* are used to improve the functioning of the immune system. The root of *P. esculenta* is used

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for the treatment of urinary disorders.^[1] The woundhealing property of the stem bark of *P. latifolia* is exploited by the people of Eastern Ghats in India.^[2] The shoots of *P. pyramidata* is applied externally on the abdomen to control the growth of worms.^[1] Several ethnobotanically important species of *Premna* have been studied for their pharmacological properties. The leaf extract of *P. barbata* showed antimicrobial properties. The essential oils and leaf extracts of *P. quadrifolia*, *P. cordifolia*, *P. esculenta* and *P. integrifolia* are found to be with potent antioxidant activities. The anti-diabetic, anti-hyperlipidemic, and hepatoprotective properties of *P. tomentosa*, *P. obtusifolia*, *P. esculenta*, *P. corymbosa* were also proved scientifically and all these studies justify the traditional medicinal use of these species.^[3] The docking studies conducted in *P. integrifolia* confirmed the presence of two hepatoprotective lead compounds, stigmasterol and campesterol.^[4] It is clear from the previous studies that the roots and leaves of *Premna* plants contains phytochemically active compound with wide application in the field of pharmacology. The present study sought to look into the bioactive compounds present in the methanolic extracts and the antioxidant activities of three selected species of *Premna* from different localities of Kerala State, India. The present study will provide an insight for understanding the phytochemical constituents and biological activity of three un explored species such as *Premna scandens* Roxb., *Premna mollissima* Roth. *Premna glaberrima* Wight.

MATERIALS AND METHODS

Collection and Processing of Plant material

Fresh leaves of the three species such as *Premna scandens* Roxb., *Premna mollissima* Roth. *Premna glaberrima* Wight. were collected from the different localities in Kerala, India in June 2022 (Figures 1-3). Photographs of the plants were taken by using digital camera (Sony Alpha SLT A57K). The plant specimens were identified by using the available literature. The herbarium sheets were prepared and deposited in the herbarium of Department of Botany, University of Kerala, Thiruvananthapuram (KUBH 11287, 11288,11289) The samples were washed with sterile distilled water. After being chopped and shade-dried for ten days at room temperature, the leaves were powdered finely and kept in an airtight bottle until needed. Samples that had been dried and powdered were extracted using Soxhlet with methanol serving as the solvent. For the extraction, 20 g of powder per species were added to 200 mL of methanol. The percentage yield was determined by weighing the extract.^[5] For further assessment of potential *in vitro* antioxidant properties and secondary metabolite quantification, the dried leaf extracts were kept at 4°C.

$$\%$$
 of yield = $\frac{\text{weight of extract}}{\text{Weight of sample}} \times 100$

Phytochemical Analysis Preliminary Phytochemical Analysis

The different qualitative chemical tests were done for establishing the profile of phytoconstituent of methanolic leaf extracts of three *Premna* species as per reported method.^[6]

Quantitative Estimation of Secondary Metabolites

The total amount of phenolic content was determined using the Folin- Ciocalteu method.^[7] The alkaloid content was estimated as per reported protocol.^[8] The aluminum chloride technique was used to estimate the total flavonoid content.^[9]

GCMS Analysis

GCMS analysis of methanolic extract was performed using GCMS QP 2010, Shimadzu Tokyo Japan) equipped with a VF 5 mm fused silica capillary column diameter and .25µm film thickness. The components were separated using helium gas as carrier at a constant rate of 1.2m/min. The 2µl sample extract injected into the instrument. Injector and mass transfer line temperature set at 200°C and 255°C respectively. GC



Figure 1: *Premna scandens* Roxb. Figure 2: *Premna mollissima* Roth. Figure 3: *Premna glaberrima* Wight.

oven temperature started at 70°C and was held at 300°C and then for 10 min with program rate 4°C/min for 9 min. The components were identified by comparing their mass spectra with those available in the National Institute of Standards and Technology (NIST) database, (G1036A, revision D.01.00)/Chem station data system (D.02.00.275, version 2.0d).

Determination of Antioxidant property DPPH Radical Scavenging Assay

The DPPH free radical scavenging assay were done according to the method of Blois, M. S.^[10] A solution of 0.1 mM DPPH (4 mg in 100 mL) in methanol was combined with 1 mL of plant extract varying in concentration (20 μ g-100 μ g) of the methanolprepared sample to create a volume of 2 mL. After giving the mixture a good shake and letting it stand for 30 min at room temperature in the dark, the absorbance was measured at 517 nm (UV-1700-(E) 23OEC- Shimadzu). The following formula was used to calculate the free radical scavenging ability (%) of the DPPH radical.

$$(\%) = \left[\frac{\mathbf{A}_0 - \mathbf{A}_1}{\mathbf{A}_0}\right]$$

Where A_0 and A_1 represent the absorbance of values of the control and of the test sample, respectively. Ascorbic acid was used as reference.

Nitric Oxide Scavenging Activity

Different concentrations of extracts dissolved in standard phosphate buffer (pH 7.4) were incubated with sodium nitroprusside (5 mM) in standard phosphate buffer solution, and the tubes were incubated at 25°C for 5 hr. The incubated solution (0.5 mL) was taken out after 5 hr and diluted using 0.5 mL of Griese reagent. At 546 nm, the absorbance of the generated chromospheres was measured.^[11]

Super Oxide Radical Scavenging Assay

The radical scavenging assay was conducted utilizing the protocol developed by Kunchandy, E., & Rao, M. N. A^[12] 0.1 mL of NBT (mg/mL solution in DMSO), different concentrations (20-100µg) of plant extract, and standard ascorbate in DMSO comprise the reaction mixture. Using DW, it will be made up to 100 µL. A spectrophotometer (UV-1700-(E) 23OEC- Shimadzu) was used to measure the absorbance at 560 nm after a volume of 1.4 mL of DMSO (1 mL DMSO containing 5 mM NaOH in 100 µL DW) was added. Both the percentage of inhibition and the reduced wavelength were measured.

Table 1: Extract yield of three different species of Premna L. (PM, PG and PSC).				
Plant Species	PM	PG	PSC	
Dry Weight (g)	20	20	20	
Methanol Fraction Yield (mL)	200	200	200	
Methanol Extract Final Yield (g)	3.45	2.23	3.87	

RESULTS

Yield of three Different Species of *Premna* L. extraction

Extraction of three different species of *Premna* L. such as *Premna mollissima* (PM) *Premna glaberrima* (PG) and *Premna scandens* (PSC) of 20 g dry weight were carried out using methanol. The fractions were dried and quantified. PM, PG and PSC fractions yielded 3.45 g, 2.23 g and 3.87 g respectively (Table 1).

Qualitative Phytochemical analysis of three Different Species of *Premna* L. Extracts

All three extracts of the species of *Premna* L., namely *Premna mollissima* (PM) *Premna glaberrima* (PG) and *Premna scandens* (PSC) were subjected to various qualitative phytochemical assays which revealed the presence of different kinds of phytochemical groups that are summarised in Table 2.

Quantitative Phytochemical analysis of three Different Species of L.

Quantitative phytochemical assays for flavonoids, phenolic compounds, alkaloids, terpenoids and steroids of the three species showed that they contain significant amount of phytochemicals. Compared to the others, flavonoids content was high in *Premna scandens*, but the amount of other phytochemicals like phenols, alkaloids and terpenoids were high in *Premna glaberrima* species (Figures 4-6).

Table 2: Qualitative phytochemical screening of three different species of <i>Premna</i> L.				
SI. No.	Phytochemicals	PSC		
1	Alkaloids + +		+	
2	Phenols	Phenols + +		+
3	Flavonoids +		+	+
4	Terpenoids +		+	+
5	Saponins	Saponins + -		+
6	Tannins	Tannins - + +		+
7	Coumarin	Coumarin + _ +		+
8	Anthraquinones	nthraquinones		-
9	Steroids	Steroids + + +		+



Different species of Premna L.

Figure 4: Amounts of flavonoids in different species of *Premna* L. Values are expressed as mean±SD of triplicate readings, a; significantly different from *P. mollissima*, b; significantly different from *P. glaberrima*. Abbreviations: *Premna mollissima* (PM) *Premna glaberrima* (PG) and *Premna scandens* (PSC).

GCMS Analysis

GCMS is among the most effective chromatographic techniques now in use for identifying bioactive components found in plant extracts, including long chain hydrocarbons, alcohols, fatty acids, esters, and steroids. A variety of bioactive chemicals were found in three distinct *Premna* species, according to GCMS analysis. Among them, *P. glaberrima*, showed peaks indicating the presence of 22 compounds (Table 3, Figure 7), whereas *P. scandens and P. mollisima* were confirmed with the presence of 17 and 16 compounds respectively. (Tables 4 and 5, Figures 8 and 9).



Different species of Premna L.

Figure 5: Amounts of phenolic compounds in different species of *Premna* L. Values are expressed as mean±SD of triplicate readings, a; significantly different from PM, b; significantly different from PG. Abbreviations: *Premna mollissima* (PM) *Premna glaberrima* (PG) and *Premna scandens* (PSC).



Different species of Premna L.

Figure 6: Amounts of alkaloids in different species of *Premna* L. Values are expressed as mean±SD of triplicate readings, a; significantly different from PM, b; significantly different from PG. Abbreviations: *Premna mollissima* (PM) *Premna glaberrima* (PG) and *Premna scandens* (PSC).

The major bioactive compounds identified were squalene, phytol, γ -sitosterol, α amyrin, coumarin, Vitamin E, Emicymarin, Cedral diol, Pluchidol, Loliolate. Squalene and phytol are the compounds common to all the three species, but γ -sitosterol was present only in *P. glaberrima*.

In vitro antioxidant activity of three Different Species of *Premna* L.

Estimation of DPPH Radical Scavenging Activity

Extracts of three different species of *Premna* L., namely PM, PG and PSC were evaluated for DPPH radical scavenging activity and the effect was compared to the

Table 3: Compounds Identified by GCMS Analysis of Methanolic Extract of Premna scandens Roxb.				
Peak	R time	Name	Molecular formula	Area %
1	23.537	Dodecyl acrylate	C ₁₅ H ₂₈ O ₂	4.10
2	26.625	Phytol acetate	$C_{22}H_{42}O_{2}$	7.87
3	26.739	Hexahydrofarnesol	C ₁₅ H ₃₂ O	1.23
4	27.503	3,7,11,15-Tetramethyl-2-hexadecen1-ol	$C_{20}H_{40}O$	2.44
5	28.433	Methylpalmitate	C ₁₇ H ₃₄ O ₂	3.81
6	31.545	n-Heptadecanol	C ₁₇ H ₃₆ O	0.78
7	31.637	Methyl 9,12-octadecadieonoate	$C_{19}H_{34}O_{2}$	1.57
8	31.751	Alpha Linolenic acid methyl ester	$C_{19}H_{32}O_{2}$	2.76
9	32.023	Phytol	$C_{20}H_{40}O$	31.31
10	32.271	Methyl stearate	C ₁₉ H ₃₈ O ₂	1.36
11	35.189	Methyl undecylenate	$C_{12}H_{22}O_{2}$	1.08
12	35.788	Methyl arachidate	$C_{21}H_{42}O_{2}$	0.93
13	39.047	1,2-Benzene dicarboxylic acid	$C_8H_6O_4$	2.56
14	41.584	1-Dotriacontanol	C ₃₂ H ₆₆ O	8.84
15	43.184	Squalene	C ₃₀ H50	7.63
16	44.487	1-Heneicosanol	C ₂₁ H ₄₄ O	21.22
17	44.700	Trans-Geranylgeraniol	C ₂₀ H ₃₄ O	0.51
				100.00



Figure 7: GCMS Chromatogram of Methanolic Extract of Premna glaberrima Wight.

reference antioxidant ascorbic acid. All the extracts exhibited radical scavenging activities, but the effect was found to be superior in PG, when compared to the other extracts (Figure 10). The IC_{50} values are expressed in Table 6.

In vitro radical scavenging assay was carried out with different concentrations (50-250 μ g/mL) of extracts and reference standard (ascorbate). Values are expressed as mean \pm SD of triplicate readings.

Estimation of Nitric oxide radical scavenging activity

In vitro nitric oxide radical scavenging assay revealed that the effect of PG was superior when compared to other extracts. Ascorbate was used as the positive standard (Figure 11). The IC_{50} values are expressed in Table 6.

In vitro radical scavenging assay was carried out with different concentrations (50-250 μ g/mL) of extracts and reference standard (ascorbate). Values are expressed as mean \pm SD of triplicate readings.

Estimation of Superoxide radical scavenging activity[12]

The superoxide anion scavenging activity of *Premna* mollissima (PM) *Premna glaberrima* (PG) and *Premna* scandens (PSC) was found to be comparable to that of the reference standard ascorbic acid, but the effect was found to be superior in *P. glaberrima* when compared to the other extracts (Figure 12). The IC_{50} values are expressed in Table 6.

In vitro radical scavenging assay was carried out with different concentrations (50-250 μ g/mL) of extracts and reference standard (ascorbate). Values are expressed as mean \pm SD of triplicate readings.

DISCUSSION

Alkaloids, phenols, flavonoids, terpenoids, and steroids were identified in the preliminary phytochemical investigation, and these compounds are responsible

Tabl	Table 4: Compounds Identified by GCMS Analysis of Methanolic Extract of Premna mollissima Roth.				
Peak	R time	Name	Molecular formula	Area %	
1	9.552	Pyranone	$C_{10}H_5NO_3$	1.85	
2	11.780	Coumaran	C ₈ H ₈ O	2.85	
3	14.413	2-methoxy 4-vinylphenol	C ₉ H ₁₀ O ₂	2.24	
4	22.025	3-Hydroxy-beta-damascone	C ₁₃ H ₂₀ O ₂	1.42	
5	23.019	7,8- Dihydro beta ionone	C ₁₃ H ₂₂ O	1.87	
6	23.481	1,3,2 Oxazaborolane,2-butyl-4-methyl	C ₈ H ₁₆ O ₂	3.52	
7	24.567	Benzeneacetic acid,4-hydroxy-3-methoxy, methyl ester	C ₁₀ H ₁₂ O	2.78	
8	25.512	Loliolide	C ₁₁ H ₁₆ O ₃	3.92	
9	25.953	Pluchidiol	C ₁₃ H ₂₀ O ₂	3.87	
10	26.667	3-Ethyl-2 thiomethylpyrazine		3.12	
11	26.710	Neophytadiene	C ₂₀ H ₃₈	3.43	
12	28.338	4-(1,3,3-Trimethyl-7-oxabicyclo [4.1.0] hept-2yl)-2-pentanone	$C_{14}H_{24}O_{2}$	6.69	
13	32.040	Phytol	C ₂₀ H ₄₀ O	7.20	
14	39.316	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	3.06	
15	43.318	Squalene	C ₃₀ H50	28.58	
16	48.483	Vitamin E	$C_{29}H_{50}O_{2}$	23.60	
				100.00	

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Peak	R time	Name	Molecular formula	Area %
1	9.567	Pyranone	$C_{10}H_5NO_3$	2.13
2	10.575	1,3,2- Dioxaborolan-4-one, 2-ethyl-5-methyl	$C_{5}H_{9}BO$	2.68
3	11.778	Coumaran	C ₈ H ₈ O	5.21
4	14.420	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	15.99
5	18.027	2-Hydroxy 5-Methyl isophthalaldehyde	$C_9H_8O_3$	7.32
6	18.275	Alpha Bergamotene	$C_{15}H_{24}$	2.72
7	19.650	Beta D-Glucopyranose, 1,6-Anhydro	$C_{6}H_{10}O_{5}$	3.83
8	20.869	4-methyl-2,5-dimethoxybenzaldehyde	C ₁₀ H ₁₂ O ₃	3.44
9	21.218	Megastigmatrienone 4	C ₁₃ H ₁₈ O	1.99
10	22.034	3-Hydroxy-beta-damascone	C ₁₃ H ₂₀ O ₂	2.50
11	22.315	Megastigmatraienone	C ₁₃ H ₁₈ O	4.45
12	22.757	3- Oxo-alpha-ionol	C ₁₃ H ₂₀ O ₂	2.12
13	24.841	4((1E)-3-3Hydroxy-1-propenyl)2-methoxyphenol	C ₁₀ H ₁₂ O ₃	12.61
14	25.531	Lolilode	C ₁₁ H ₁₆ O ₃	2.64
15	25.714	Bicyclo(2-2-2)octane,1-methoxy-4-methyl	C ₁₀ H ₁₈ O	3.76
16	25.967	Pluchidiol	C ₁₃ H ₂₀ O ₂	5.35
17	26.708	3,7,11,15-Tetramethyl-2-hexadecen1-ol	$C_{20}H_{40}O$	1.96
18	28.363	2-Pentanone,4-(1,3,3-trimethyl-7 oxabicyclo[4.1.0]hep-2-yl	C ₁₄ H ₂₄ O ₂	4.84
19	30.025	Benzoic acid,2-hydroxy-4methoxy-3,5,6 trimethyl	C ₁₉ H ₂₀ O ₇	5.86
20	32.040	Phytol isomer	C ₂₀ H ₄₀ O	3.68
21	40.866	Gamma sitosterol	C ₂₉ H ₅₀ O	3.61
22	43.317	Squalene	$C_{30}H_{50}$	1.32
				100.00



Figure 8: GCMS Chromatogram of Methanolic Extract of Premna mollissima Roth.



Figure 9: GCMS Chromatogram of Methanolic Extract of Premna scandens Roxb



Figure 10: Estimation of DPPH radical scavenging ability of PM, PG and PSC.

for antioxidant properties. Quantification assays of phytochemical groups showed significant differences in the quantity of the phenolic compounds, alkaloids, and flavonoids among the three species. GC-MS analysis provides precise insight into the chemical makeup, identifying particular chemicals present in the sample.

The species of *Premna* showed significant variations in antioxidant activities. *P. scandens* showed more scavenging activity, which was followed by *P. mollissima* and *P. glaberrima*. Antioxidant activity of plant extract is primarily attributed to a diverse array of phytocompounds, such as phenolic compounds, carotenoids, vitamins, selenium, glutathione, polyphenols, terpenoids, and alkaloids. Phenolic compounds, which are used as antioxidants, are abundantly available from all three species. Its antioxidant action is advantageous and helpful for a number of illnesses due to the presence of hydroxyl groups, which are crucial to their capacity to scavenge. ^[13] Hence, they have the ability to react with hydroxyl

radicals and other active oxygen radicals.^[14] Fruits and vegetables provide many health benefits because of flavonoids, which are polyphenolic compounds that include several phenolic groups.^[15] Owing to their redox properties, they actively contribute to the scavenging of free radicals.^[16] High molecular weight polyphenolic compounds are known for their best qualities and are used as antioxidants. Antioxidants are mostly used in the prevention of diseases like arthritis, cancer, dementia, coronary heart disease, and Alzheimer's disease.^[17-19] Antioxidants have a wide range of industrial applications, including food preservation and cosmetics.

GCMS analysis revealed the presence of bioactive constituents in three different species. *P. scandens* was confirmed with the presence of 17 compounds, *P. mollissima* and *P. glaberrima* showed peaks for 16 and 22 bioactive compounds. Many pharmacological properties are possessed by the identified compounds. Fatty acids with a broad range of biological actions, such

Table 6: IC ₅₀ values of DPPH, Nitric oxide and Superoxide radical scavenging activities of Ascorbic acid, Premna mollissima (PM) Premna glaberrima (PG) and Premna scandens (PSC).					
Sample	IC ₅₀ (μg/mL)				
	DPPH Radical scavenging (μg/mL)	Nitric-oxide radical scavenging (μg/mL)	Superoxide Radical scavenging (μg/mL)		
Ascorbic acid	134.30±5.67µg/mL	129.68±8.13 μg/mL	139.30±4.09µg/mL		
PM	236.15±11.21µg/mL	226.37±11.89 µg/mL	223.15±13.39µg/mL		
PG	193.59±8.01µg/mL	169.47±5.09µg/mL	150.02±10.65µg/mL		
PSC	242.65±15.95µg/mL	233.37±11.95µg/mL	243.60±16.38µg/mL		

NITRIC OXIDE RADICAL SCAVENGING ASSAY



Figure 11: Estimation of nitric oxide radical scavenging ability of PM, PG and PSC.

as antifungal, antioxidant, anti-androgenic, hemolytic, 5-alpha reductase inhibitor, and anti-microbial activity, including methyl ester of hexadecenoic acid and methyl ester of 9,12-octadecanoic acid.^[20] Hexadecanoic acid was reported from the ethyl acetate extract of *P. integrifolia*.^[21] 9,12-octadecanoic acid methyl ester was discovered to possess possible anti-inflammatory, antiarthritic, and cancer-prevention properties.^[20] That was proved by Mangunwidjaja *et al.* 2006.^[22] In the study carried out in *Croton tiglium* seed. Phytol is a diterpene compound with numerous biological activities. It is widely used as a fragrant ingredient as an essential oil and also has significance in the field of pharmaceuticals. Many investigations have demonstrated the enormous pharmacological potential of phytol, which includes immunological modulatory, autophagic, anti-inflammatory, antibacterial, and apoptosis-inducing properties.^[18] Squalene is a triterpene with anti-cancer, chemopreventive, antitumour, antioxidant, hepatoprotective, and sunscreen



SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Figure 12: Estimation of Superoxide Radical Scavenging ability of PM, PG and PSC.

properties.^[23] Antimicrobial activity of α amyrin was also reported.^[24]

Vitamin E refers to a group of compounds known as tocopherols which possess immense properties and has major role in various biological processes. One of the major compounds found in the methanol extract used in this investigation is Vitamin E, which has been shown to have anti-inflammatory, antibacterial, and antioxidant properties.^[25] In addition to its antioxidant properties and ability to suppress platelet aggregation, vitamin E also has immune-stimulating properties and inhibits lipid peroxidation.^[26]

Coumarin, a class of organic compounds are widely distributed in plants. Coumarin isolated from various plant sources can be used as a source of biofumigant for the effective and eco-friendly control of pests in stored grains and pulses.^[27] γ sitosterol is a sterol found in many plant parts with a similar structure to human cholesterol. According to Sundarraj *et al.*,^[28] γ sitosterol is useful in preventing cancer cell lines from proliferating and halting their cell cycle. Similar studies on colon and liver cancer cell lines also revealed the cytotoxic effect of γ sitosterol^[29] The antidiabetic properties of γ sitosterol make them ideal candidates for the development of a potent antidiabetic drug.^[30]

CONCLUSION

In various traditional medicine systems, extracts from different species of Premna have been used for their medicinal properties, which may include antioxidant effects. However, it's essential to note that traditional use does not always align with scientific evidence, and further research is often needed to validate these claims. The study reports the phytochemical constituents of three different species of Premna viz, Premna scandens, Premna mollissima, Premna glaberrima and evaluation of its antioxidant activity. Several species of Premna have been investigated for their potential antioxidant activity due to their phytochemical composition. The specific antioxidant activity of Premna species can vary depending on factors like the plant part used and environmental conditions etc. Premna species contain a range of phytochemicals that can contribute to their antioxidant activity. Some of these that are well-known for their capacity to scavenge free radicals and lessen oxidative stress are polyphenols, flavonoids, phenolic acids, and terpenoids. The qualitative and quantitative analysis ascertain the same. Further, GCMS analysis revealed variety of phytochemicals having potential to scavenge free radicals. In conclusion, the antioxidant activity of various Premna species has been investigated

in several studies, and the results suggest that they may indeed possess antioxidant properties and the results suggest that they may indeed possess antioxidant properties. However, the extent and effectiveness of this activity can vary among different species and parts of the plant. Further research, including clinical trials and more extensive biochemical studies, is needed to better understand the specific antioxidant mechanisms and potential health benefits associated with *Premna* species.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Not Applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not Applicable.

PATIENT CONSENT

Not Applicable.

ABBREVIATIONS

PM: Premna mollissima; **PSC:** Premna scandens; **PG:** Premna glaberrima.

SUMMARY

The study reveals the phytochemical constituents of three different species of *Premna* viz, *Premna scandens*, *Premna mollissima*, *Premna glaberrima* and their antioxidant potential. To fully comprehend the unique antioxidant mechanisms and potential health advantages linked with *Premna* species, further research has to be done. This involves carrying out clinical trials along with more extensive biochemical evaluations.

REFERENCES

 Quattrocchi U. CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology (5 volume Set). CRC press; 2012.

- Jeevan Ram A, Bhakshu LM, Venkata Raju RR. *In vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. J Ethnopharmacol. 2004;90(2-3):353-7. doi: 10.1016/j.jep.2003.10.013, PMID 15013201.
- Dianita R, Jantan I. Ethnomedicinal uses, phytochemistry and pharmacological aspects of the genus *Premna*: a review. Pharm Biol. 2017;55(1):1715-39. doi: 10.1080/13880209.2017.1323225, PMID 28486830.
- Singh C, Upadhyay R, Tiwari KN. Comparative analysis of the seasonal influence on polyphenolic content, antioxidant capacity, identification of bioactive constituents and hepatoprotective biomarkers by *in silico* docking analysis in *Premna integrifolia* L. Physiol Mol Biol Plants. 2022;28(1):223-49. doi: 10.1007/s12298-021-01120-0, PMID 35221581.
- Kalaisezhiyen P, Uddandrao VS, Saravanan G, Sasikumar V. Therapeutic potentiality of *Kedrostis foetidissima* (Jacq.) Cogn., leaf extracts on free radicals induced oxidative damage in the biological system. Oxid. Antioxid. Med Sci. 2016;6(1):1-5.
- Mani B, Thomas S. Variation in antioxidant activity at two ripening stages of wild mango, *Spondias pinnata* (L.f.) Kurz an underutilized fruit. Plant Sci Today. 2020;7(4):534-41. Doi: 10.14719/pst.2020.7.4.863.
- Malik ZA, Singh M, Sharma PL. Neuroprotective effect of *Momordica charantia* in global cerebral ischemia and reperfusion induced neuronal damage in diabetic mice. J Ethnopharmacol. 2011;133(2):729-34. Doi: 10.1016/j.jep.2010.10.061, PMID 21056650.
- Shamsa F, Monsef H, Ghamooshi R, Verdian-rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci. 2008;32(1):17-20.
- Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3).
- Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-200. Doi: 10.1038/1811199a0.
- Marcocci L, Maguire JJ, Packer L. Nitric oxide scavenging activity of nitecapone. Free Radic Biol Med. 1993;15(5):498. Doi: 10.1016/0891-5849(93)90289-7.
- 12. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm. 1990;58(3):237-40. Doi: 10.1016/0378-5173(90)90201-E.
- Shahidi F, Ambigaipalan P. Phenolics and Polyphenolics in foods, beverages and spices: antioxidant activity and health effects-A review. J Funct Foods. 2015;18:820-97. Doi: 10.1016/j.jff.2015.06.018.
- Brewer MS. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Compr Rev Food Sci Food Saf. 2011;10(4):221-47. Doi: 10.1111/j.1541-4337.2011.00156.x.
- Han RM, Zhang JP, Skibsted LH. Reaction dynamics of flavonoids and carotenoids as antioxidants. Molecules. 2012;17(2):2140-60. Doi: 10.3390/ molecules17022140, PMID 22354191.
- Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J Agric Food Chem. 2003;51(3):609-14. Doi: 10.1021/jf020782a, PMID 12537430.

- Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet. 1993;342(8878):1007-11. Doi: 10.1016/0140-6736(93)92876u, PMID 8105262.
- Moure A, Cruz JM, Franco D, Domínguez JM, Sineiro J, Domínguez H, *et al.* Natural antioxidants from residual sources. Food Chem. 2001;72(2):145-71. Doi: 10.1016/S0308-8146(00)00223-5.
- Holiman PCH, Hertog MGL, Katan MB. Analysis and health effects of flavonoids. Food Chem. 1996;57(1):43-6. Doi: 10.1016/0308-8146(96)00065-9.
- Sweetlin P, Daniel RR. Determination of bioactive copmounds in ethanolic extract of callus derived from *Mucuna pruriens* using gas chromatography and mass spectroscopic technique. J Nat Rem. 2020;21(7):11-6.
- 21. Sermakkani M, Thangapandian V. GC-MS analysis of *Cassia italica* leaf methanol extract. Asian J Pharm Clin Res. 2012;5(2):90-4.
- Mangunwidjaja D, Raharja S, Kardono L, Iswantini D, DI. Gas chromatography and gas chromatography-mass spectrometry analysis of Indonesian Croton Tiglium seeds. J Appl Sci. 2006;6(7):1576-80. Doi: 10.3923/jas.2006.1576.1580.
- Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. GC-MS analysis of bioactive compounds from the whole plant ethanolic extract of *Evolvulus alsinoides* (L.) L. J Food Sci Technol. 2015;52(2):1212-7. Doi: 10.1007/ s13197-013-1105-9, PMID 25694742.
- Saeed MA, Sabir AW. Antibacterial activity of *Caesalpinia bonducella* seeds. Fitoterapia. 2001;72(7):807-9. Doi: 10.1016/s0367-326x(01)00292-1, PMID 11677020.
- Ramya R. GC-MS analysis of bioactive compounds in ethanolic leaf extract of *Hellenia speciosa* (J. Koenig) SR Dutta. Appl Biochem Biotechnol. 2022;194(1):176-86.
- Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The role of Vitamin E in human health and some diseases. Sultan Qaboos Univ Med J. 2014;14(2):e157-65. PMID 24790736.
- Rajashekar Y, Vijay Kumar HV, Ravindra KV, Bakthavatsalam N. Isolation and characterization of biofumigant from leaves of *Lantana camara* for control of stored grain insect pests. Ind Crops Prod. 2013;51:224-8. Doi: 10.1016/j.indcrop.2013.09.006.
- Sundarraj S, Thangam R, Sreevani V, Kaveri K, Gunasekaran P, Achiraman S, *et al.* γ-sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. J Ethnopharmacol. 2012;141(3):803-9. doi: 10.1016/j.jep.2012.03.014, PMID 22440953.
- Endrini S, Rahmat A, Ismail P, Taufiq-Yap YH. Cytotoxic effect of γ-sitosterol from Kejibeling (*Strobilanthes crispus*) and its mechanism of action towards c-myc gene expression and apoptotic pathway. Med J Indones. 2014;23(4):203-8. Doi: 10.13181/mji.v23i4.1085.
- Balamurugan R, Stalin A, Ignacimuthu S. Molecular docking of γ-sitosterol with some targets related to diabetes. Eur J Med Chem. 2012;47(1):38-43. doi: 10.1016/j.ejmech.2011.10.007, PMID 22078765.

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