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Original Article

GC-MS analysis of Phytocomponents in the Methanol Extract of *Premna latifolia* Roxb

Rajesh Kumar, Brijesh Kumar*, Ashutosh Kumar, Ajay Kumar, Manish Singh

ABSTRACT

Background: According to existing literature, the genus *Premna* has a variety of biologically active secondary metabolites, but there are few findings on phytochemical screening of *P. latifolia* Roxb. Methanolic leaves extract. **Objectives:** Phytochemical screening of methanolic leaves extract of *P. latifolia* was proposed for this study. **Materials and Methods:** UV-chamber, HPTLC instrument (CAMAG TLC Scanner), and GC-MS instrument (Perkin-Elmer GC- Clarus) were used to analyse methanolic leaves extract of *P. latifolia*. To compare the peaks of components on chromatograms, the NIST library was employed. **Results:** The current study used GC-MS to identify probable chemical components of *P. latifolia*. The GC-MS analysis and NIST library comparison revealed that the methanolic extract of *P. latifolia* contained mainly Squalene (13.57 percent), Ergosta-5, 7, 9 (11), 22-tetraen-3-ol, (3. beta, 22E)-(0.15 percent), Stigmasterol (3.73 percent), gamma-Sitosterol (10.13 percent), Lupeol (0.33), beta-Amyrin (2.27 percent), alpha-Amyrin (2.05 percent), gamma-Sitostenone (0.35 percent), Ursolic aldehyde (1.01 percent) and Betulin (0.72 percent). The biological actions of the majority of the identified components have been reported. While scientific evidence of gamma-sitostenone's biological function is still not available. **Conclusion:** According to the findings, *P. latifolia* contains a variety of biologically active components, the majority of which are tri-terpenoids and phytosterols.

Key words: *Premna latifolia*, GC-MS analysis, Phytocomponents TLC,HPTLC, Methanolic leaves extract.

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INTRODUCTION

Plants provide over 70% of the strong medications, so they can be regarded a significant source of drugs in various countries.[1] People have been getting drugs from plants since the dawn of humanity.[2] As a result, herbs are well-known for their traditional uses as folk medicines.[3] Phytochemical screening is a crucial step in determining the active pharmacological components.[4] Preliminary screening procedures for plant extracts have been suggested as a way to identify their beneficial pharmacological components.[5] These pharmacological active components lead to the development of novel drug moieties using *in vitro* and *in vivo* approaches.[6]

Premna is a genus of more than 40 species in the verbinaceceae family.[7] *Premna latifolia* is a North Indian plant that is best known for its anti-inflammatory and anti-allergic properties in traditional medicine.[8] However, no scientific evidence exists to date that it has a powerful anti-pathological effect. *Premna's* diverse biological activities can be attributed to the presence of alkaloids, flavonoids, tannins, resins, enzymes, and physiologically important secondary metabolites in various species.[9] According to the available data, there haven't been many reports on *P. latifolia's* phytochemical components or biological activity. The goal of this study was to use a sophisticated instrument called GC-MS to look into the biological active components of *P. latifolia*.

MATERIALS AND METHODS

Plant material

Dr. Y. S. Parmar University of Horticulture and Forestry, Nouni, Himachal Pradesh, identified *P. latifolia* gathered from Dol Lasawa, Jhandutta, Bilaspur District, and Himachal Pradesh, India. A herbarium sheet of the same plant sample was sent to the Department using UHF-herbarium Field Book No. 12436.

Preparation of extract

To avoid degradation, the *P. latifolia* plant material was dried in the shade and the size was reduced with a grinder. The extraction procedure with methanol (95 percent) was used after a 7-day cold maceration. Whatman No. 41 filter paper was used to filter the retrieved material. The filtrate was dried with liquid nitrogen in a nitrogen evaporator at 30°C. For subsequent examination, the dried methanol extract of *P. latifolia* (MEPL) was kept in the refrigerator at 2-8°C.

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Partition Chromatography

By using 150ml each of petroleum ether (PE), ethyl acetate (EA), and water (AQ), the MEPL main extract was separated into three fractions: PLPE, PLEA, and PLAQ. At room temperature, the separated fractions were dried.

Thin layer chromatography (TLC)

Using a solvent system, TLC was done on pre-coated plates of silica gel 60 F_{254} (Merck) (5 percent Ethyl Acetate in Hexane). Spraying a combination of Anisaldehyde sulphate on a chromatographic plate separated by running reference compounds was used to identify the phyto-constituents, which were then scanned at (short wave - 254 nm and long wave - 366 nm) and spotted. The retention factor (R_F) values were calculated using the compounds collected on the TLC plate.

High Pressure Thin Layer Chromatography (HPTLC)

TLC aluminium sheets silica gel 60 F_{254} (Merck) were used for HPTLC. The mobile phase was Toluene: Chloroform: ethyl acetate (4:4:1) and the sample injection volume was 30 µl. The CAMAG TLC Scanner was used to analyse the PLPE, PLEA, and PLAQ fractions using the CATS Planer Chromatography Manager Software (2.01.02). Scan wavelengths were 254 nm and 366 nm. The retention factor and area percent of the individual bands on the TLC plate were computed after they were analysed.

GC-MS analysis

The PLEA fraction generated after partition chromatography was subjected to GC-MS analysis. Instrument conditions for the GC programme include Ion source temperature 2200°C, Interface temperature 2600°C, Flow control mode-Linear velocity, Pressure-90.4kPa, solvent cut time 2.5 min, Detector gain mode-Relative, Threshold-1000, and for the MS table, start time 3.0 min, end time 39.98 min, ACQ mode- Scan, Event time 0.20 sec, Scan Speed 3333, and solvent cut time 2.5 min. The settings of start *m/z* 40.00 and finish time *m/z* 650.00 were used.

RESULTS AND DISCUSSION

The MEPL extract (10 g) was separated using a separating funnel and various solvents based on polarity, yielding three different fractions (PLPE, PLEA, and PLAQ), which were then dried at room temperature. The yields were determined to be 1.8, 2.4, and 1.6 percent, respectively. When run through the TLC plate using the solvent system, the TLC profile clearly demonstrates the presence of distinct phyto-constituents determined on the basis of colour density in three different fractions (PLPE, PLEA, and PLAQ). When spotted on a TLC plate following derivatization, the R_f values found in three fractions were 0.57, 0.56, and 0.46, respectively as shown in Figure 1. After partition chromatography, the three fractions were used for a 5l injection on an HPTLC device. The results are depicted in Figures 2 and 3, which clearly demonstrate the various peaks, their R_f values, and the area percentage of each peak. In comparison to PLAQ, PLPE and PLEA have the most peaks. Some peaks were more evident following derivetization and when seen at a wavelength of 366 nm. The peak's intensity was clearly reflected by the area percentage.

GC-MS Spectra analysis of the PLEA indicated the existence of various phytochemical components (Figures 4 and 5). By comparing their mass spectra to those in the NIST libraries, (Table 1) the 10 phyto-compounds were identified and characterised. Table 2 lists the various phytocomponents as well as their biological activities. Squalene (13.57 percent), Ergosta-5, 7, 9 (11), 22-tetraen-3-ol, (3. beta, 22E)-(0.15 percent), Stigmasterol (3.73 percent), gamma-Sitosterol (10.13 percent), Lupeol (0.33), beta-Amyrin (2.27 percent), alpha-Amyrin (2.05 percent), gamma-Sitostenone (0.35 percent), Ursolic aldehyde (1.01 percent) and

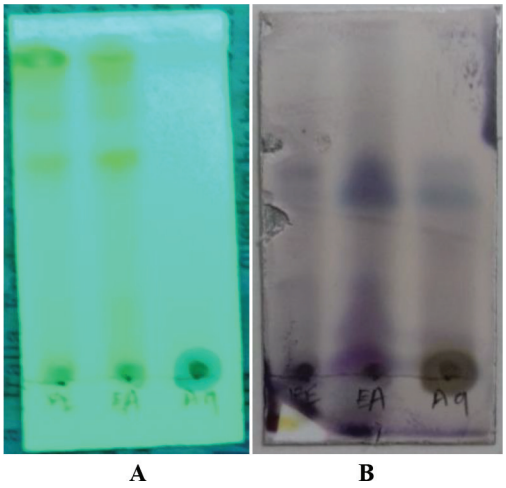

Figure 1: Thin Layer Chromatography. *A (Before derivetization), B (After derivetization), spotted at 254 nm and 366 nm)*

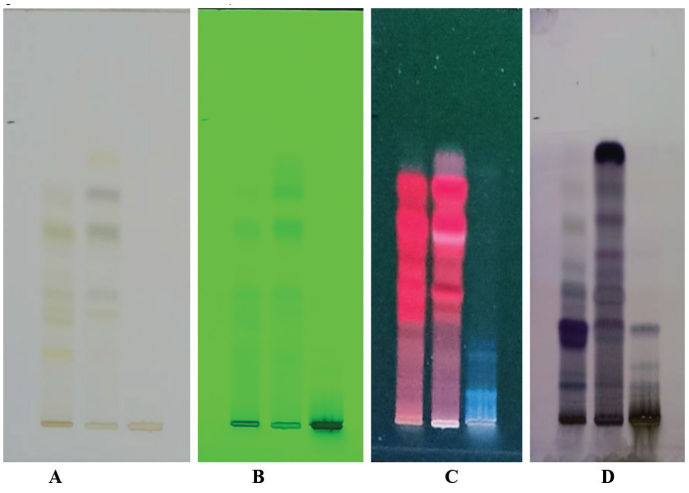

Figure 2: High Pressure Thin Layer Chromatography: *A (Visible light), B (254 nm, UV light), C (366 nm, UV light) and D (366 nm, Post Derivetization, UV light)*

Betulin (0.72 percent) were the most prevailing compounds (Figure 6). Squalene is a triterpene that has been shown to have anticancer properties.[10] It also hydrates the skin by acting as an emollient and antioxidant.[11] Triterpines are phenolic compounds found in the latex of some plants that function as secondary metabolites in the defence against infections that cause human and animal diseases.[12] Stigmasterol is a steroidal compound with anti-osteoarthritic activity, which is due to properties of their functional groups and their aqueous solubility.[13] Ergosta-5, 7, 9 (11), 22-tetraen-3-ol, (3. beta. 22E) - has been justified as a phytosterol for its antitumor activity.[14] Lupeol is a triterpenoid compound that has been used to treat prostate and skin cancers as well as an anti-inflammatory drug.[15] Beta amyrin and alpha amyrin are two compounds that exhibit analgesic, anti-inflammatory, anticonvulsant, antidepressive, gastroprotective, hepatoprotective, antipancreatitic, anticholytic, antihyperglycemic, and hypolipidemic properties.[16] Gamma.-Sitostenone, on the other hand, has no known activity. Ursolic aldehyde is a triterpenoid that has anti-glycative properties.[17] Finally, Betulin demonstrates antimalarial, anti-inflammatory, and antifungal properties.[18]

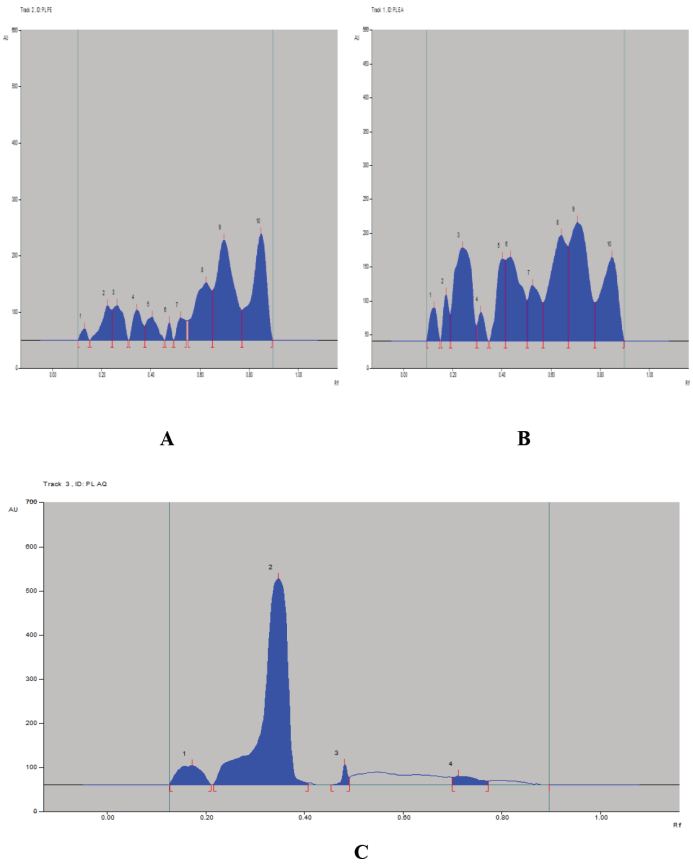

Figure 3: HPTLC Peaks of Different Phytoconstituents: *A (PLPE), B (PLEA) and C (PLAQ).*
PLPE (*Premna latifolia*, petroleum ether fraction), PLEA (*Premna latifolia*, ethyl acetate fraction) and PLAQ *Premna latifolia*, aqueous fraction)

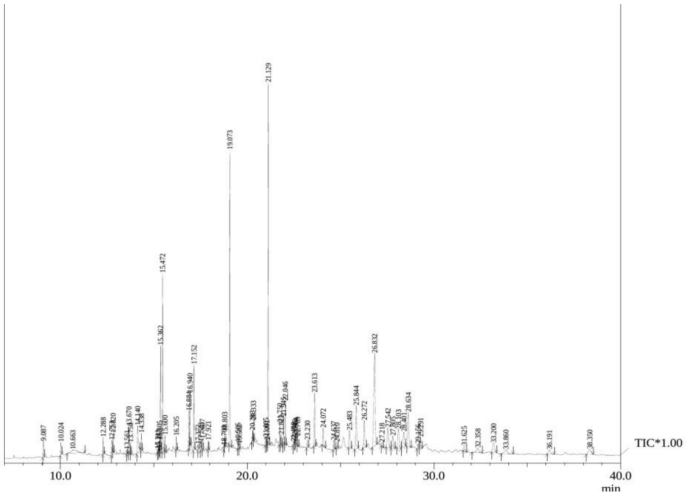

Figure 4: GC-MS chromatogram of ethyl acetate of *P. latifolia* methanolic extracts.

The presence of several biological active components in *P. latifolia* justifies traditional practitioners' use of it for a variety of diseases. However, the tedious task of isolating individual components and subjecting them to their biological activity will yield useful data. Finally, the results of the study show that *P. latifolia* contains a variety of biologically active components. As a result, this plant is indicated as a valuable therapeutic herb with pharmacological value.

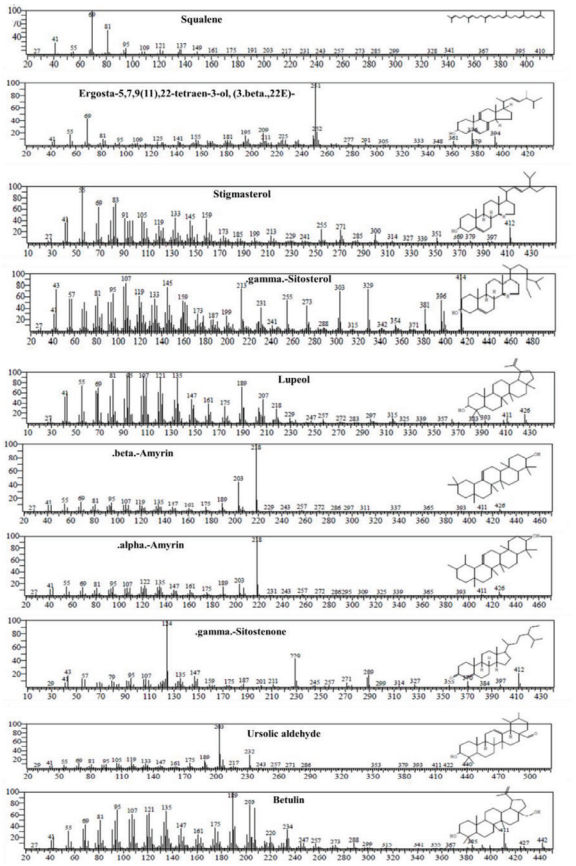

Figure 5: Mass spectrums of phyto-components identified by GC-MS in ethyl acetate fraction of methanolic extract of *P. latifolia*.

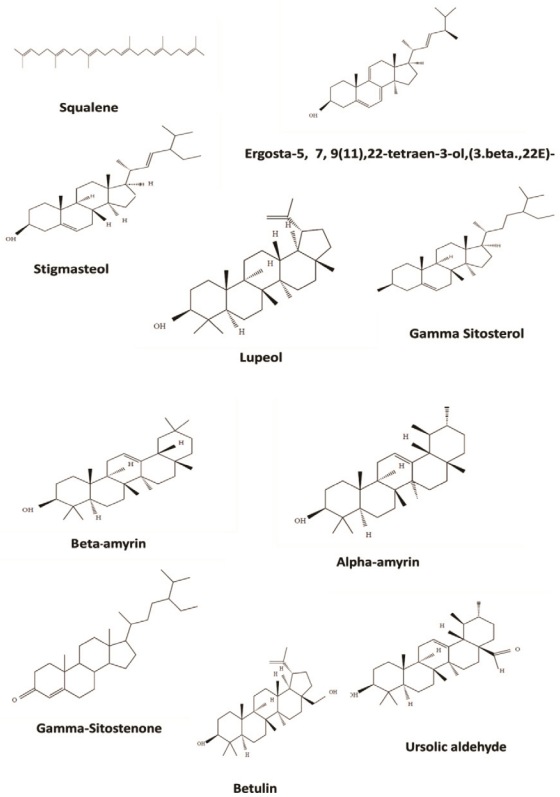

Figure 6: Structure of phytoconstituents obtained after GC-MS analysis.

Table 1: Phyto-components identified in the methanolic extract of *P. latifolia* by GC-MS, MW: Molecular weight (g), RT: Retention Time.

No.	R_T	Name of the compound	Molecular formula	MW	Peak area %
1.	21.129	Squalene	C30H50	410.7	13.57
2.	22.480	Ergosta-5,7,9(11), 22-tetraen-3-ol, (3.beta.,22E)-	C28H42O	394.6	0.15
3.	25.844	Stigmasterol	C29H48O	412.7	3.73
4.	26.832	.gamma.-Sitosterol	C29H50O	414.7	10.13
5.	27.218	Lupeol	C30H50O	426.7	0.33
6.	27.542	.beta.-Amyrin	C30H50O	426.7	2.27
7.	28.401	.alpha.-Amyrin	C30H50O	426.7	2.05
8.	29.156	.gamma.-Sitostenone	C29H48O	412.7	0.35
9.	33.860	Ursolic aldehyde	C30H48O2	440.7	1.01
10	36.191	Betulin	C30H50O2	442.8	0.72

Table 2: Nature and biological activity of phyto-components identified in the methanolic extract of *P. latifolia* by GC-MS, RT: Retention Time

No.	RT	Name of the compound	Nature of compound	Biological activity
1.	21.129	Squalene	Triterpene	Skin emollient, antioxidant Antitumor
2.	22.480	Ergosta-5,7,9(11), 22-tetraen-3-ol, (3.beta.,22E)-	Phytosterol	Antitumor
3.	25.844	Stigmasterol	Steroid	Anti-osteoarthritic
4.	26.832	.gamma.-Sitosterol	Phytosterol	Antidiabetic
5.	27.218	Lupeol	Triterpenoid	Prostate and Skin cancers, Anti-inflammatory
6.	27.542	.beta.-Amyrin	Triterpene	Analgesic, Anti inflammatory, Anticonvulsant, Antidepressive, Hepatoprotective, Antihyperglycemic Hypolipidemic
7.	28.401	.alpha.-Amyrin	Triterpenoid	Anti-inflammatory, Antinociceptive, Antioxidant, Antipruritic, Gastroprotective
8.	29.156	.gamma.-Sitostenone	Phytosterol	
9.	33.860	Ursolic aldehyde	Triterpenoid	Anti-glycative
10	36.191	Betulin	Triterpene	Antimalarial, Anti-inflammatory, Antifungal

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MEPL: Methanol extract of *Premna latifolia*; **TLC:** Thin layer chromatography; **HPTLC:** High Pressure Thin Layer Chromatography; **GCMS:** Gas Chromatography mass; **PLPE:** (*Premna latifolia*, petroleum ether fraction); **PLEA:** (*Premna latifolia*, ethyl acetate fraction); **PLAQ:** *Premna latifolia*, aqueous fraction).

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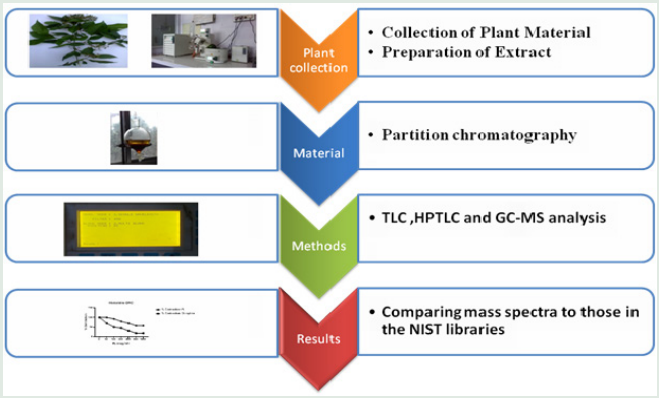
GRAPHICAL ABSTRACT



SUMMARY

The results obtained from phytochemical screening of *Premna latifolia* Roxb. identified the presence of glycosides, terpenoids, diterpenes and phenols. In GC-MS analysis; 66 different compounds with 10 major components Squalene (13.57 percent), Ergosta-5, 7, 9 (11), 22-tetraen-3-ol, (3. beta, 22E)-(0.15 percent), Stigmasterol (3.73 percent), gamma-Sitosterol (10.13 percent), Lupeol (0.33), beta-Amyrin (2.27 percent), alpha-Amyrin (2.05 percent), gamma-Sitostenone (0.35 percent), Ursolic aldehyde (1.01 percent) and Betulin (0.72 percent) were found. These compounds possess important biological activity and strongly support the pharmacological potency of *Premna latifolia* Roxb.

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Prof Brijesh Kumar is working as faculty in Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University Varanasi, Uttar Pradesh, India.

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