

# HPTLC and GC-MS Analyses of Biologically Active Extracts and fractions from *Premna latifolia* Roxb Leaves reveal Anti-inflammatory and Analgesic Effects

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## ABSTRACT

**Introduction:** The goal of this study was to look at the various phyto-constituents in the ethyl acetate fraction of *Premna latifolia* methanolic leaves extract (MEPL) and see if they might be used for central/peripheral analgesia and inflammation.

**Method:** The goal of this study was to look at the various phyto-constituents in the ethyl acetate fraction of *Premna latifolia* methanolic leaves extract (MEPL) and see if they might be used for central/peripheral analgesia and inflammation. The analysis was carried out using GC-MS and HPTLC equipment. *Premna latifolia* methanolic leaves extract was tested for various activities at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg.

**Results:** Squalene (13.57%), Ergosta-5,7,9(11), 22-tetraen-3-ol, (3-beta, 22 E)-(0.15%), Stigmasterol(3.73%), gamma-Sitosterol (10.13%), Lupeol (0.33), beta-Amyrin

(2.27%), alpha-Amyrin (2.05%), gamma (0.72%), GC-MS analysis revealed that they were prominent secondary metabolites. *Premna latifolia* dosages reduced central analgesia, as well as carrageenan and histamine-induced inflammation, in a dose-dependent manner ( $p < 0.05$ ).

**Conclusion:** In numerous animal models, key phyto-constituents of MEPL have been found to have significant analgesic and anti-inflammatory effects.

**Keywords:** *Premna latifolia*, HPTLC analyses, GC-MS analyses, Anti-inflammatory effect, Analgesic effect

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## INTRODUCTION

Phytomedicines have been used by people all over the world since ancient time (Pan SY, *et al.*, 2014). Plant-based crude medications have been used by Northern Indian tribes to cure a variety of ailments (Debbarma M, *et al.*, 2017). In Himachal Pradesh's local areas, unregistered individuals known as vaidas employ crude medications to treat pathological conditions. This uncommon value, which lacks study data, requires rigorous scientific research to be validated. *Premna latifolia* is a member of the Verbenaceae family and is locally known as Agnimantha (Dianita R, *et al.*, 2017). *Premna* contains a large number of species, with 35-40 of them found in India (Mali PY, 2015). *Premna latifolia* has traditionally been used as an antihistamine and antipyretic (Aye MM, *et al.*, 2019). It's been used to treat numerous illnesses as an anti-diuretic (Krishnamoorthi R, *et al.*, 2015). Additionally, sinusitis and pharyngitis have been treated with it (Hoi HT, 2015). In this study, we used modern instruments like GC-MS and HPTLC to analyze the various phytoconstituents in the ethyl acetate fraction of *Premna latifolia* methanolic leaves extract. The major goal was to determine the pharmacological activity of the diterpenes and triterpenes found in their leaves. Furthermore, numerous animal models were used to assess its use as a peripheral analgesic/central analgesic and anti-inflammatory to create scientific data.

## MATERIALS AND METHODS

### Animals

Wistar rats (200-250 g) and Swiss albino mice (20-25 g) of either sex were supplied by the Central Animal House, Institute of Medical Sciences, and Banaras Hindu University. Temperature (24°C), relative humidity (45%-55%), light and dark cycle (12-12 hours), and free access to feed pellets and water were all used to keep the animals in a departmental animal house. The experimental protocol was approved by the Institutional Animal Ethics Committee (Dennis/2020/IAEC/2212).

### Drugs, chemicals and reagents

Carrageenan (Sigma Aldrich, USA), histamine hydrochloride (Analab Fine Chemicals, Mumbai), ibuprofen (Loba Chemie, Mumbai), and distilled water (In-house), indomethacin, and morphine (Cipla Healthcare Ltd., India) were purchased.

### Preparation of methanolic extract of *Premna latifolia* leaves (MEPL)

Plant leaves collected in the Himachal Pradesh district of Bilaspur. Department of Forest Products, Dr. Y. S. Parmar University of Horticulture and Forestry, Nouni, Himachal Pradesh, with reference number 12436, performed the authentication. Plant leaves were collected, dried at room temperature, and ground to a fine powder. The dry powder (1000 g) of leaves was extracted using a 72-hour cold maceration with 95 percent methanol. The methanolic extract was filtered and concentrated in a rotary vacuum evaporator, yielding a semi-solid extract that was kept at 2°C-8°C.

### Phytochemical analysis of MEPL

MEPL was analyzed for the presence of alkaloids, saponins, flavonoids, tannins, and proteins, as well as spectrum analyses such as HPTLC and GC-MS.

### High Pressure Thin Layer Chromatography (HPTLC)

TLC aluminium sheets (silica gel 60 F<sub>254</sub>) were used for HPTLC (Merck). The mobile phase was Toluene: Chloroform: Ethyl acetate (4:4:1) and the sample injection volume was 30:1. The CAMAG TLC Scanner was used to analyze 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 plates were computed after they were analyzed.

### Gas chromatography mass spectrometry

GC-MS analysis was performed on the PLEA fraction produced

following partition chromatography. For the GC programme, instrument conditions such as ion source temperature 2200°C, interface temperature 2600°C, flow control mode-linear velocity, pressure-90.4 kPa, 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, start m/z 40.00, end time m/z 650.00 parameters were employed. The National Institute of Standards and Technology (NIST) library was used to compare the peaks of components on chromatograms derived in the form of area percent at a certain RT.

#### **Histamine induced paw edema**

Six groups of rats were created. Groups I and II were given CMC (p.o.), whereas groups III, IV, V, and VI were given MEPL (100, 200, and 300 mg/kg, p.o.) and ibuprofen (50 mg/kg, p.o.) (p.o.=by mouth). 30 minutes before the injection of 0.1 ml histamine (1 percent w/v) into the planter region of each rat's right paw, the MEPL and ibuprofen were given. Using a plethysmometer, the paw volume was measured at 0 minutes, 30 minutes, 60 minutes, 120 minutes, and 180 minutes. The difference between the treatment and control groups in terms of paw edema was calculated (Tamaddonfard E, *et al.*, 2012).

#### **Carrageenan induced paw edema**

Six groups of rats were formed. Groups I and II were given CMC (p.o.), whereas groups III, IV, V, and VI were given MEPL (100, 200, and 300 mg/kg, p.o.) and Indomethacin (20 mg/kg, p.o.). MEPL and Indomethacin were given 30 minutes before injecting 0.1 ml Carrageenan (1 percent w/v) into the planter region of each rat's right paw. A plethysmometer was used to measure the paw volume at 0 minutes, 30 minutes, 60 minutes, 120 minutes, and 180 minutes. The difference between the treatment and control groups in terms of paw edema reduction was calculated (Mansouri MT, *et al.*, 2015).

#### **Tail flick method**

Five sets of wister rats of both sexes were created. Group I was given CMC (p.o.), whereas Groups II, III, IV, and V were given MEPL (100, 200, and 300 mg/kg, p.o.) and morphine (5 mg/kg, i.p.). The end point is the tail withdrawal from the heat (flicking response). Analgesimeter was used to record the reaction time at 5 minutes, 15 minutes, 30 minutes, 60 minutes, and 90 minutes following drug administration (Techno). At each time period, the percentage increase in reaction time (index of analgesia) was calculated (Deuis JR, *et al.*, 2017).

#### **Hot plate method**

Rats were separated into five groups to perform the analgesic effect using the hot plate. MEPL (100, 200, and 300 mg/kg, p.o.) and Morphine (5 mg/kg, i.p.) were used in groups II, III, IV, and V, respectively. When placed on a hot plate kept at a constant temperature (55°C), the end point is paw licking or jump response (whichever occurs first). Eddy's hot plate was used to record the reaction time 15 minutes, 30 minutes, 60 minutes, 90 minutes,

and 120 minutes after the drug was administered (Techno). At each time interval, the percent increase in reaction time (analgesia index) was measured (Fan SH, *et al.*, 2014).

#### **Acetic acid writhing test**

For the acetic acid writhing test, mice were split into five groups. Group I was given CMC (p.o.), whereas Groups II, III, IV, and V were given MEPL (100, 200, and 300 mg/kg, p.o.) and Diclofenac sodium (20 mg/kg, i.p.) accordingly. Following 60 minutes of drug administration, acetic acid (1 percent v/v and 1 ml/100 g) was injected. For observation, each animal was placed in its own glass jar. For a period of 20 minutes, the number of writhing (abdominal contractions, trunk twist reaction, and hind limb extension) was measured. The inhibition in terms of the number of writhing was calculated and compared to the control group (Saha A, *et al.*, 2008).

#### **Formalin Test**

Male rats were separated into five groups to undergo central analgesia using Formalin induction. Group I was given CMC (p.o.), whereas groups II, III, IV, and V were given MEPL (100, 200, and 300 mg/kg, p.o.) and Morphine (5 mg/kg, i.p.). After 60 minutes, formalin (0.05 ml of 10% v/v) was administered into the dorsal region of the front paw. Individual animals were placed in glass jars for observation. After formalin injection, the duration of reaction time (paw licking or biting) was measured at 0-5 minutes (first phase) and 15-30 minutes (second phase). The animals' reaction times were compared to those of the control group and represented as percent inhibition (Abadi SS, *et al.*, 2012).

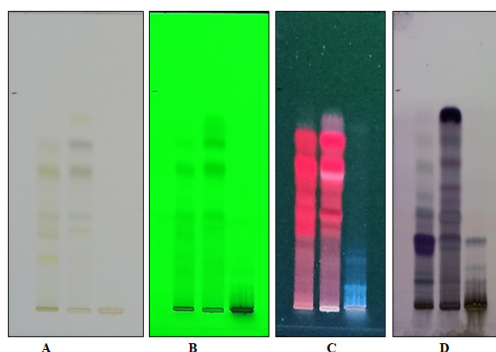
## **RESULTS**

### **High Pressure Thin Layer Chromatography (HPTLC)**

After partition chromatography, the three fractions were used to inject into the HPTLC device. The results are depicted in *Figures 1-2*, which clearly demonstrate the many peaks, their R<sub>f</sub> values, and the percentage of area occupied by each peak. The most of peaks are found in PLPE and PLEA as compared to PLAQ. The peaks of all three fractions were also clearly visible in the densitogram generated following the analysis, with good intensity (*Figure 3*). Some peaks were more evident following derivatization and when seen at a wavelength of 366 nm. The peak's intensity was clearly reflected by the area percentage.

### **Gas Chromatography mass spectrometry**

The presence of phytochemical components was confirmed by GC-MS Spectra analysis of the PLEA (*Figures 4-5; Table 1*). The 10 phyto-compounds were identified and characterised by comparing their mass spectra to those of the National Institute of Standards and Technology (NIST library). Squalene (13.57 percent), Ergosta-5,7,9,(11),22-tetraen-3-ol, (3-beta, 22-E)-(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 the most prevailing compounds.



**Figure 1: High Pressure Thin Layer Chromatography (HPTLC): A (Visible light), B (254 nm, UV light), C (366 nm, UV light) and D (366 nm, Post Derivatization, UV light)**

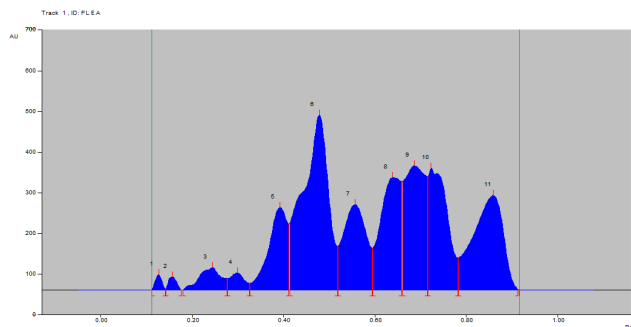


Figure 2A: High Pressure Thin Layer Chromatography (HPTLC) Peaks of Different Phytoconstituents: (PLPE: *Premna latifolia*, Petroleum Ether Fraction)

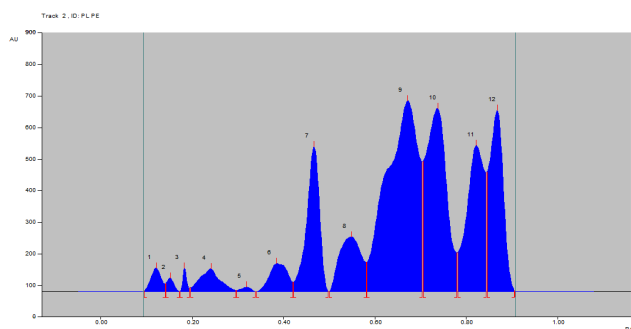


Figure 2B: High Pressure Thin Layer Chromatography (HPTLC) Peaks of Different Phytoconstituents: (PLEA: *Premna latifolia*, Ethyl Acetate Fraction)

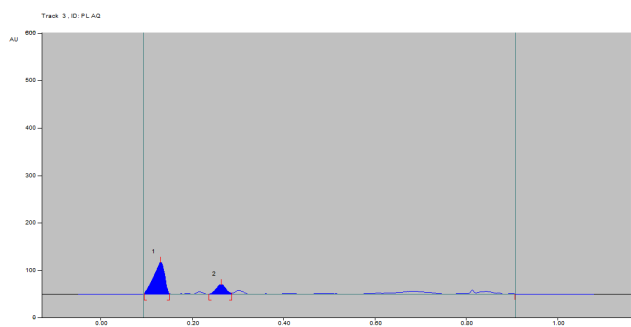


Figure 2C: High Pressure Thin Layer Chromatography (HPTLC) Peaks of Different Phytoconstituents: (PLAQ: *Premna latifolia*, aqueous fraction)

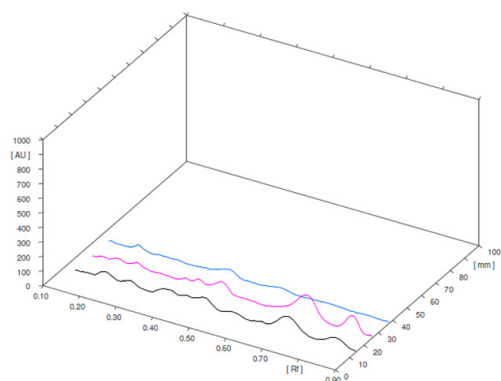


Figure 3: High Pressure Thin Layer Chromatography (HPTLC) Densitogram at 254 nm different peaks of PLPE, PLEA and PLAQ

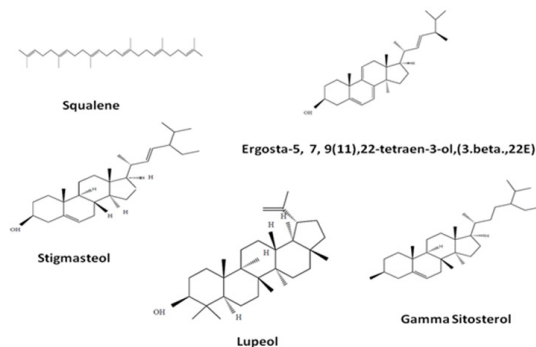


Figure 4A: Structure of phytoconstituents obtained after Gas Chromatography Mass Spectrometry (GC-MS) analysis

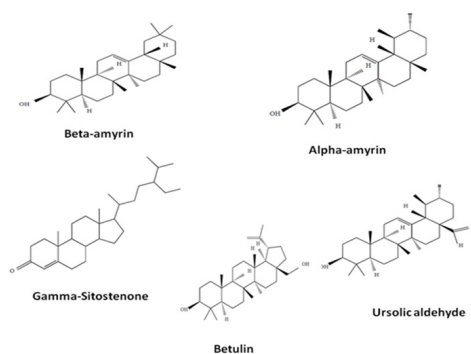
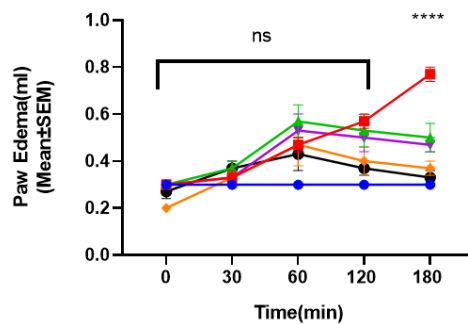


Figure 4B: Structure of phytoconstituents obtained after Gas Chromatography Mass Spectrometry (GC-MS) analysis



(●) Control, (■) IC, (▲) PL 100, (▼) PL 200, (◆) PL 300, (●) IPN

Figure 5: Effect of different concentration of methanolic extract of *Premna latifolia* and ibuprofen on histamine induced paw edema in rats

Note: Values are expressed as Mean ± SEM (n=6). \*Denotes statistically significant values (Two Way ANOVA followed by Dunnett Post Test) relative to control group (ns=non-significant; \*\*\*\*=p<0.0001). PL 100-*Premna latifolia* (100 mg/kg), PL 200-*Premna latifolia* 200 mg/kg, PL 300-*Premna latifolia* (300 mg/kg) and IPN-ibuprofen (50 mg/kg, p.o.)

Table 1: On comparison of the mass spectra of the constituents with the NIST library, the ten phyto-compounds were characterized and identified

Peak	Retention time	Area	Area%	Compound name
1	21.129	44026008	13.57	Squalene
2	22.48	472805	0.15	Ergosta-5,7,9(11),22-tetraen-3-ol, (3-beta,22E)-
3	25.844	12111883	3.73	Stigmasterol
4	26.832	32875166	10.13	Gamma sitosterol
5	27.218	1056872	0.33	Lupeol
6	27.542	7351896	2.27	Beta amyrin
7	28.401	6639782	2.05	Alpha amyrin
8	29.156	1121292	0.35	Gamma sitostenone
9	33.86	3264493	1.01	Ursolic aldehyde
10	36.191	2350697	0.72	Betulin

Note: NIST: National Institute of Standards and Technology

### Histamine induced paw edema

The sub-plantar injection of histamine in the subacute model of histamine-induced paw edema resulted in a time-dependent elevation in paw size, which peaked at 3 hours and remained elevated for the next 24 hours. At 3 hours following histamine injection, pre-treatment with MEPL (100 mg/kg, 200 mg/kg, and 300 mg/kg, p. o.) significantly ( $p < 0.05$ ) reduced paw edema formation in a dose-dependent manner (Figure 6). MEPL was found to be most active at a dose of 300 mg/kg, with activity comparable to that of the conventional anti-inflammatory medicine ibuprofen (50 mg/kg, p. o.).

### Carrageenan induced paw edema

Carrageenan injection in the planter region caused paw edema, which resulted in an increase in paw volume that peaked at 3 hours and lasted for 48 hours. When given orally, MEPL at different doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg reduced paw edema, which was evaluated by measuring paw volume, in a dose-dependent fashion. At 3 hours after the Carrageenan injection, the effects are significant ( $p < 0.05$ ). MEPL (300 mg/kg) and ordinary Indomethacin (20 mg/kg, p.o.) were observed to be more effective, as shown in Figure 6.

### Tail flick method

After test drug and conventional drug administration, tail withdrawal from heat (Flicking response) was assessed as an end point, and the findings were compared to determine the significance of the results in different groups. The data displayed in Figure 7 clearly showed that MEPL (100 mg/kg, 200 mg/kg, and 300 mg/kg, p.o.) significantly ( $p < 0.05$ ) increased reaction time (sec) after 30 minutes of drug administration as compared to the control group. Morphine (5 mg/kg, i.p.) was shown to be more effective in increasing the reaction time for tail withdrawal from 5 to 90 minutes.

### Hot plate method

Paw licking or jump response (whichever occurs first) as reaction time (in sec) when placed on a hot plate kept at a constant temperature (55°C) was evaluated as an end point, and the findings were compared to determine the significance of the results in different groups. MEPL (100 mg/kg, 200 mg/kg, and 300 mg/kg, p. o.) increased reaction time (sec) considerably ( $p < 0.05$ ) after 15 and 30 minutes of drug administration as compared to the control group. Morphine (5 mg/kg, i.p.) was shown to be more effective at increasing reaction time, as illustrated in Figure 8.

### Acetic acid writhing test

In various groups of rats, intra-peritoneal injections of acetic acid (1 percent v/v and 1 ml/100 g) produced writhing, i.e. abdominal contractions; trunk twists reaction, and hind limb extension. As shown in Figure 9, MEPL (100 mg/kg, 200 mg/kg, and 300 mg/kg, p. o.) significantly ( $p < 0.05$ ) reduced writhing in a dose-dependent manner when compared to the control group. MEPL, at a dose of 300 mg/kg, successfully reduced the writhing response, indicating that the drug has analgesic properties. Diclofenac sodium (20 mg/kg i. p.) reduced the number of writhing substantially, and its efficacy was comparable to that of 300 mg/kg MEPL.

### Formalin Test

The injection of formalin (0.05 ml of 10% v/v) into the dorsal area of the front paw produced paw licking or biting, indicating a pain response. Figure 10 depicts the outcomes of two phases I and II (in minutes). In phase II, MEPL (100, 200, and 300 mg/kg, p.o.) significantly ( $p < 0.05$ ) reduced formalin-induced paw licking, whereas in phase I, all results were non-significant ( $p > 0.05$ ). MEPL (100 mg/kg) effectively reduced the paw licking, indicating that the medication has analgesic properties. In phase II of the analgesic effect observation, the standard drug Morphine (5 mg/kg, i.p.) greatly reduced the number of writhing, demonstrating its greater efficiency.

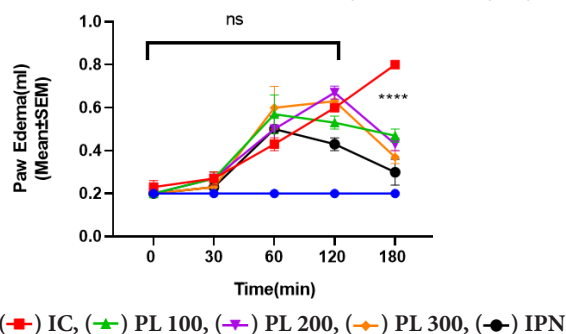


Figure 6: Effect of different concentration of methanolic extract of *Premna latifolia* and Indomethacin on Carrageenan induced paw edema in rats. Note: Values are expressed as Mean  $\pm$  SEM (n=6). \*Denotes statistically significant values (Two Way ANOVA followed by Dunnett Post Test) relative to control group (ns=non-significant; \*\*\*\*= $p < 0.0001$ ). PL 100-*Premna latifolia* (100 mg/kg), PL 200-*Premna latifolia* (200 mg/kg), PL 300-*Premna latifolia* (300 mg/kg) and IM- Indomethacin (20 mg/kg, p.o.)

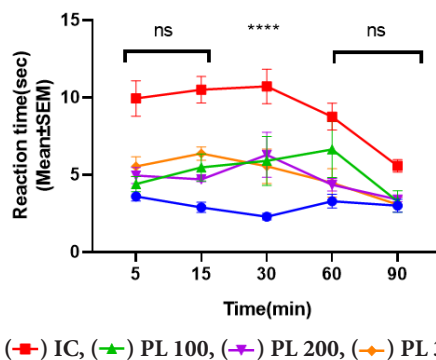
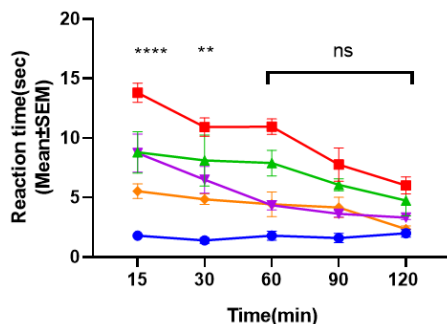


Figure 7: Analgesic effect of different concentration of methanolic extract of *Premna latifolia* and Morphine via tail flick method in rats. Note: Values are expressed as Mean  $\pm$  SEM (n=6). \*Denotes statistically significant values (Two Way ANOVA followed by Dunnett Post Test) relative to control group (ns=non-significant; \*\*\*\*= $p < 0.0001$ ). PL 100-*Premna latifolia* (100 mg/kg), PL 200-*Premna latifolia* (200 mg/kg), PL 300-*Premna latifolia* (300 mg/kg) and MPN-Morphine (5 mg/kg, i.p.)



(●)Control, (■) IC, (▲) PL 100, (▼) PL 200, (◆) PL 300

Figure 8: Analgesic effect of different concentration of methanolic extract of *Premna latifolia* and Morphine via hot plate method in rats. Values are expressed as Mean  $\pm$  SEM (n=6). \*Denotes statistically significant values (Two Way ANOVA followed by Dunnett Post Test) relative to control group (ns=non-significant; \*\* =p<0.01; \*\*\*\* =p<0.0001). PL 100-*Premna latifolia* (100 mg/kg), PL 200-*Premna latifolia* 200 mg/kg, PL 300-*Premna latifolia* (300 mg/kg) and MPN-Morphine (5 mg/kg,i.p.)

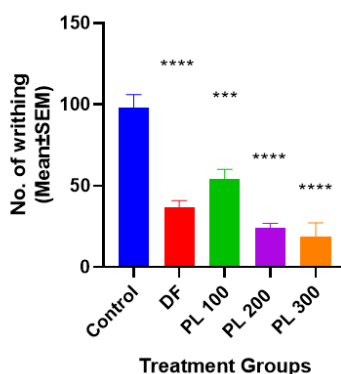
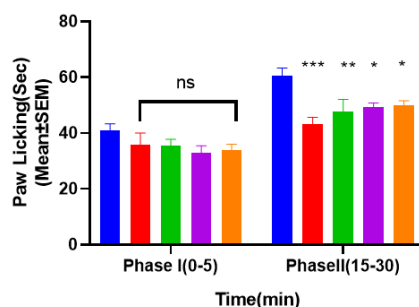


Figure 9: Analgesic effect of different concentration of methanolic extract of *Premna latifolia* and Diclofenac sodium via Acetic acid induced writhing test. Values are expressed as Mean  $\pm$  SEM (n=6). \*Denotes statistically significant values (One Way ANOVA followed by Dunnett Post Test) relative to control group (\*\*=p<0.001; \*\*\*\*=p<0.0001). PL 100-*Premna latifolia* (100 mg/kg), PL 200-*Premna latifolia* 200 mg/kg, PL 300-*Premna latifolia* (300 mg/kg) and DF-Diclofenac sodium (20mg/kg i.p.)



(■)Control, (■) IC, (■) PL 100, (■) PL 200, (■) PL 300

Figure 10: Analgesic effect of different concentration of methanolic extract of *Premna latifolia* and Morphine via Formalin induced paw licking. Values are expressed as Mean  $\pm$  SEM (n=6). \*Denotes statistically significant values (Two Way ANOVA followed by Dunnett Post Test) relative to control group (ns=non-significant; \* = p<0.05; \*\* =p<0.01; \*\*\*=p<0.001). PL 100-*Premna latifolia* (100 mg/kg), PL 200-*Premna latifolia* 200 mg/kg, PL 300-*Premna latifolia* (300 mg/kg) and MPN-Morphine (5 mg/kg,i.p.)

## DISCUSSION

The MEPL has significant analgesic and anti-inflammatory benefits, as well as a reasonable safety profile, according to the findings of the current study. Anti-inflammatory properties have already been demonstrated in phytoconstituents such as betulinic acid, oleanolic acid, betulin, and lupeol (Naik SR, *et al.*, 2013). When testing pharmaceutical compounds for their analgesic properties, acetic acid-induced writhing is a well-recommended method (Ilmi H, *et al.*, 2021). The release of endogenous chemicals, as well as some additional pain mediators such arachidonic acid via cyclooxygenase and prostaglandin production, causes pain induction (Ricciotti E, *et al.*, 2011). Because of its sensitivity and reactivity to drugs at a level that is not

effective in other approaches, this pain paradigm is commonly utilised for the testing of peripheral analgesic action (Kim KH, *et al.*, 2014). Writhings in the abdomen may be caused by the local peritoneal receptor (Yin ZY, *et al.*, 2016). In the acetic acid induced writhing paradigm, pain sensation is elicited by a localised inflammatory response caused by the release of free arachidonic acid from tissue phospholipids via Cyclo-Oxygenase (COX) and the production of prostaglandins, specifically PGE2 and PGF2 (Jang Y, *et al.*, 2020). The level of lipoxygenase products may also increase in peritoneal fluids (Irvin CG, *et al.*, 1997). By increasing capillary permeability, these prostaglandin and lipoxygenase products trigger inflammation and discomfort (Edwards SH, 2014). The chemical that stops the writhings will have an analgesic impact, preferably via suppressing prostaglandin synthe-

sis, which is a peripheral pain-blocking mechanism (Gunaydin C, *et al.*, 2018). Our extract showed a significant reduction of writhing reflex in an acetic acid-induced abdominal constriction assay. These findings strongly suggest that MEPL has peripheral analgesic efficacy, with mechanisms of action involving inhibition of local peritoneal receptors and maybe cyclooxygenase inhibition potential. MEPL's potent analgesic action could be attributed to their active principles interfering with the release of pain mediators.

To assess central analgesic activity, thermal nociception models such as the hot plate and tail flick tests were used. In both the hot plate and tail flick tests, MEPL had a substantial ( $P < 0.05$ ) analgesic effect, involving both spinal and supraspinal analgesic mechanisms. Morphine reduced pain levels in these pain models within 30 minutes of dosing. MEPL, on the other hand, had the greatest analgesic impact after 30 minutes, although at smaller doses than the usual medication. This discrepancy in the highest analgesic point could be explained by differences in each drug's metabolism rate or potency, as morphine has a higher analgesic potential (300 mg/kg) than MEPL.

Analgesic agents have been examined in rodents, mostly mice, using the formalin-induced nociception model (Barua CC, *et al.*, 2011). This test causes painful sensitivity in two stages. The first phase (neurogenic nociceptive response) occurs within the first 5 minutes after formalin injection and is marked by direct stimulation of nociceptors on afferent C and, to a lesser extent, fibres (glutamate, bradykinin, and substance P release). The second phase (inflammatory nociceptive response) takes place 15 to 30 minutes after the formalin injection and is linked to the production of inflammatory mediators like nitric oxide, adenosine, bradykinin, histamine, prostaglandin, and serotonin (Shibata M, *et al.*, 1989). Medications that act centrally, such as narcotics and morphine, block both phases, but drugs that act peripherally, such as NSAIDs and corticosteroids, only block the second phase (Ghlichloo I and Gerriets V, 2021). MEPL was able to reduce nociceptive response in both phases of the formalin test, indicating that its anti-nociceptive impact was attributable to its central action. However, the results are only significant in the second phase.

The Carrageenan-induced paw edema is a well-known animal model for evaluating the anti-inflammatory effects of natural and synthesised chemical substances (Patil KR, *et al.*, 2019). Carrageenan-induced edema in the paw occurs in two phases, lasting 1-5 hours. The first phase (1-1.5 hours) is primarily non-phagocytic, followed by a second phase (2-5 hours) with enhanced edema production that lasted up to 5 hours (Posadas I, *et al.*, 2004). The action of mediators including histamine, serotonin, and bradykinin on vascular permeability caused the initial phase to occur (Zhao J, *et al.*, 2018). Overproduction of prostaglandins has been proven to cause late phase or second phase edema (Dzoyem JP, *et al.*, 2017). The extract (100, 200, and 300 mg/kg i.p.) is beneficial in the late phase of inflammation caused by prostaglandin release, according to the results of MEPL pre-treatment. At the third hour of the experiment, the extract had a strong anti-inflammatory impact. In the late stages of the study, MEPL have shown considerable efficacy against histamine-induced edema. Important medicinal components such as glycosides, diterpenes, triterpenes, flavonoids, tannins, and others were identified during preliminary phytochemical screening of the crude extract. As a result, these categories of chemical substances can be blamed for the current findings. MEPL will require more research to determine the exact mechanism of action for its analgesic and anti-inflammatory properties.

## CONCLUSION

Finally, experimental results on *Premna latifolia* leaves extract supported the use of MEPL for analgesic and inflammation treatment. The discovery was made through a series of trials, and it has been shown to be a safe treatment for a variety of pain conditions. MEPL has analgesic effects in

both the central and peripheral nervous systems, which could be related to the presence of several phytoconstituents in the crude extract. The isolation and identification of numerous metabolites from crude drugs will aid in determining the precise mechanism of action. The goal of metabolite identification is to aid therapeutic applications in the treatment of a variety of illnesses.

## FUNDING

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## AUTHOR'S CONTRIBUTION

All authors have equally contributed in experimental designing, phyto-chemical analysis, *in-vivo* studies and writing the manuscript.

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