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## Exploring the phytochemical wealth of *Platycladus orientalis* (L.) franco through solvent-based and essential oil extracts

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### Abstract

*Platycladus orientalis* (L.) Franco, an evergreen monoecious conifer of the Cupressaceae family, is native to China and widely distributed across Asia and Europe. This study examined the phytochemical composition and pharmacological potential of methanolic leaf extract (MLE), n-hexane leaf extract (HLE), and essential oil (EO) from its leaves. Soxhlet extraction and hydro-distillation were used to obtain the extracts, followed by phytochemical screening and quantitative analysis of total phenolic, flavonoid, alkaloid and tannin contents. Phenols, flavonoids, terpenes, glycosides, proteins, and amino acids were detected in all extracts, with MLE showing the highest phenolic ( $507.01 \pm 14.20$  mg/g), flavonoid ( $283.17 \pm 8.21$  mg/g), and alkaloid ( $135.40 \pm 5.16$  mg/g) levels. GC-MS profiling of the extracts and oil revealed major bioactive compounds. Identified compounds included  $\alpha$ -Atlantone, cis-Thujopsene, 10, 12-pentacosadiynoic acid, and 3-methyl-5-(propan-2-yl) phenol, indicating antioxidant, anticancer, anti-inflammatory, and antimicrobial potential. These findings support the species traditional medicinal uses.

**Keywords:** *Platycladus orientalis*, essential oil, phytochemistry, GC-MS

### 1. Introduction

The genus name, *Platycladus orientalis* (L) Franco, is derived from the Greek word "platys" meaning broad and "klados" meaning a branch, referring to the two-ranked branchlets in the vertical plane [1]. *Oriental arborvitae*, also known as *Platycladus orientalis*, is a needled evergreen Gymnosperm tree or shrub (Figure 1A) that belongs to the Cupressaceae family. It is indigenous to Russia, Korea, and China [2]. This plant grows to the height of 18 to 25 feet tall, with branches spreading around 10 to 15 feet wide. It normally develops as a thick, conical to columnar tree, although it can also grow as a huge shrub. It is a slow growing plant and tends to open up with age. The leaves are triangular yellowish green, scaly like, flattened & become darker green with ageing (Figure 1B) [3]. It bears male & female cones, with the males often being smaller than the females. smaller than the female cones. Cones are usually of oval shape with 3 to 4 mm long (Figure 1C & 1D). The bark varies from red to brown color. The seeds are inside the cone which is the source of propagation. The synonymous of *P. orientalis* are *Thuja orientalis* and *Biota orientalis* [4].

In general, pharmacological, biological, and chemical processes depend heavily on aromatic and therapeutic plants [5]. Because of its medicinal and healing properties, they have been utilized from ages to treat a wide range of illnesses and conditions [6]. A range of organic compounds can be found in essential oils, which are complex natural organic compounds [7]. The ability of the plants to dissolve in fats is called "oils," and the distinctive scent that they produce is called "essential" [11]. Glandular trichomes (in the Lumnaceae), glandular pockets (in the Myrtaceae), and secretory ducts (in the Apiaceae) are examples of the specialized structures that aromatic plants use to biosynthesize EOs, which are secondary metabolites. In addition to being involved in EO storage, these structures differ according to the plant organ [8].

EO extraction is a crucial process for isolating volatile, fragrant chemicals from various plant components, such as leaves, flowers, seeds, bark, and roots. These oils contain a complex mixture of terpenes, alcohols, esters, and other bioactive components responsible of the plant's characteristic aroma and therapeutic benefits [9]. The most bioactive elements of *P. orientalis* oil are camphor, fenchone, isothujone, and thujone [10]. More than 80% of people in Asia and Africa utilize plant extracts to treat a range of diseases.

Like many other members of the Cupressaceae family, *P. orientalis* can cause allergic reactions that include breathing issues, skin and eye. The leaves are used to treat a range of diseases, including those that are antibacterial, stomachic, antipyretic and diuretic [10]. The tree is known as arbour vitae or the "tree of life" because of its vitamin C-rich leaves and twigs, which are beneficial in preventing or treating scurvy [11]. It has been used externally to cure fungal skin illnesses including ringworm and thrush (fungal infection that develops in mouth), reduce the discomfort associated with arthritis and rheumatism, and eradicate anal or vaginal warts. *P. orientalis* leaf extracts were reported to have diuretic, antioxidant, antidiabetic, fungitoxic, antibacterial and neuroprotective effects and could even be used in cosmetics [13]. Phytochemistry studies of *P. orientalis* revealed several chemical compounds such as diterpenes and flavonoids from

essential oils Harborne JB [1973]. These constituents may be effective agents and have pharmacological bioactivity. Furthermore, essential oils and flavonoids from this tree showed cytotoxicity against cancer cells [12]. Extensive research has been conducted on *P. orientalis* in various countries, but studies in India are limited. Given the significant impact of environmental conditions on plant composition, seasonal variations necessitate updated profiling to identify novel or potent constituents from the plants grown in India. Therefore, our study sought to examine the chemical composition of MLE, HLE, EO through GCMS and to determine the total phenolic, flavonoid, alkaloid and tannin content of *P. orientalis* leaves extracts and essential oil which may provide data and insights for its utilization in several domains.



Fig 1: *Platycladus orientalis* (L) Franco. A) Habit-Small coniferous shrub; B) Leaf arrangement; C)-D). Immature seed cones.

## 2. Materials and Methods

### 2.1 Collection of Plant material

Fresh leaves of *P. orientalis* have been obtained from a cultivated ornamental layout located in Channagiri Taluk, Davangere District, Karnataka, India (14.47° N and 75.98° E). The plant was taxonomically identified and validated by Dr. Haleshi C, the taxonomist in Department of Studies in Botany, Davangere University, Davangere. A voucher specimen (ID: HDUD517) was prepared and deposited in the herbarium of the Department of Studies in Botany, Davangere University, for future reference.

### 2.2 Plant preparation for solvent extraction

The collected leaves of *P. orientalis* are thoroughly washed with distilled water, and shade-dried for ten days to preserve thermolabile constituents. The dried leaves were pulverized with a mechanical grinder to create a fine powder, and stored in sterile polythene bags at 4 °C until further use. For soxhlet extraction, 25 g of powdered leaf material was loaded into the thimble of a soxhlet apparatus [13]. Methanol (HPLC grade) was used as a polar solvent, and n-hexane (HPLC grade) served as a non-polar solvent, maintaining a solvent-to-sample ratio of 14:1 (v/w) with 350 mL of solvent for each extraction. The extraction procedure took six hours, comprising approximately 10-12 cycles. The resulting extracts were concentrated in a hot air oven at 35 °C until complete solvent evaporation. The dried extracts were then transferred to labelled, airtight vials and stored at 4 °C until further phytochemical analysis.

### 2.3 Extraction of essential oil (EO)

Fresh leaves of *P. orientalis* were collected, chopped into little pieces after being thoroughly washed under running water. A total of 200 g of fresh leaf material was placed in a

round bottom flask having 700 ml of distilled water as the solvent. EO extraction was performed using a Clevenger apparatus through the hydro-distillation method [8, 14]. The extraction process was carried out at a temperature maintained between 85°C and 90°C for a duration of 4 hours per cycle, yielding approximately 1 ml of essential oil per cycle. The obtained oil was collected and stored in an aluminum container in a refrigerator for subsequent analysis.

### 2.4 Preliminary qualitative analysis

Preliminary phytochemical screening was carried out on the leaf extracts to determine the presence of various secondary metabolites, including proteins and amino acids, flavonoids, tannins, phenols, cholesterol, fixed oils and fats, alkaloids, carbohydrates, glycosides, terpenes, and steroids (Table 1) following standard methods described by Harborne (1973) and Shaikh (2020).

### 2.5 Quantitative analysis

#### 2.5.1 Total phenols estimation

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method [15] with minor modifications. Briefly, 3 ml of extract was mixed with 0.5 ml of 50% Folin-Ciocalteu reagent, followed by 2 ml of 7% sodium nitrate after 10 min. The volume was adjusted to 10 ml with distilled water and incubated in the dark for 30 min at room temperature. Absorbance was measured at 760 nm using spectrophotometer (Shimadzu UV-1800). The results were expressed as mg gallic acid equivalents (GAE)/g extract.

#### 2.5.2 Total flavonoid estimation

The total flavonoid content was estimated using the method of Delcour and Varebeke [1985] [16] with slight modifications. Briefly, 200 mg of each extract was mixed with 5 ml of

chromogen reagent (0.1% cinnamaldehyde in a cooled mixture of 75 ml methanol and 25 ml concentrated HCl) and incubated for 15 min. Absorbance was measured at 640 nm using spectrophotometer against a blank (all the reagents excluding test samples) and results were expressed as mg catechin equivalents (CE)/g extract.

### 2.5.3 Total alkaloid estimation

The total alkaloid content was determined following the method described by Shamsa [17] with slight modifications. Briefly, 200 mg of extract was dissolved in 1 ml of HCl (2N), filtered, and washed with 10 ml of chloroform. Similarly, atropine (Sigma Chemical, USA) standard solutions (20-100 µg/ml) were prepared in the same manner. To each sample, 5 ml of bromocresol green (BCG) solution [prepared by heating 69.8 mg of BCG with 3 ml NaOH (2N)] and phosphate buffer (pH 4.7) was added, followed by dilution to 10 ml with distilled water. The absorbance of the chloroform complex was measured at 470 nm using spectrophotometer against a blank prepared without atropine or extract. Results were expressed as mg atropine equivalents (AE)/g extract.

### 2.5.4 Tannins estimations

The total tannin content was estimated according to the method described by Makkar (2003) [18], with slight modifications. Briefly, 0.05 ml of the extract was transferred into a test tube, and the volume was adjusted to 0.5 ml with distilled water. Subsequently, 0.25 ml of Folin-Ciocalteu reagent (1 N) and 1.25 ml of sodium carbonate solution ( $\text{Na}_2\text{CO}_3$ , 20%) were added. The mixture was incubated for 40 minutes, after which the samples were vortexed, and absorbance was recorded at 725 nm using spectrophotometer. The total tannin content was expressed as milligrams of tannic acid equivalents (TAE) per gram of extract (mg TAE/g extract).

## 2.6 GCMS analysis

Phytochemicals in *P. orientalis* MLE, HLE and EO were analyzed using a GC-MS QP2010 Plus system (Shimadzu, Japan) at SAIF, Dharwad, following standard protocols. GC-MS was performed on a QP2010 with a Thermal Desorption System (TD-20) and a Restek XTI-5 column (60 m × 0.25 mm). Ionization voltage was 70 eV. The oven temperature was programmed from 80 °C (1 min) to 220 °C at 70 °C  $\text{min}^{-1}$  (3 min hold), then to 290 °C at 10 °C  $\text{min}^{-1}$  (10 min hold). Injector and interface temperatures were 290 °C. Samples were injected in split mode using helium as carrier gas (1.2 ml  $\text{min}^{-1}$ ). Mass spectra were recorded in scan mode (3-37 min, event time 0.5 s, scan speed 1428, m/z 40-700). Compounds were identified by comparing retention times and mass spectra with library data. The peaks were detected on the total ion chromatogram and mass chromatograms and the detected peaks were identified using the GC/MS Metabolites Spectral Database and NIST05 mass spectral library for MLE, HLE and NIST23s.LIB database for essential oil.

## 3. Results and Discussion

### 3.1 Preliminary qualitative analysis

The results of the preliminary phytochemical screening of solvent extracts and EO of *P. orientalis* are presented in Table 1. A greater diversity of phytoconstituents was observed in

the EO compared to the MLE and HLE. The qualitative screening revealed the presence of proteins, phenols, tannins, fixed oils and fats, flavonoids, and terpenoids in all tested samples. Carotenoids were absent in the MLE, carbohydrates and glycosides were not detected in the HLE, and both MLE and HLE lack steroids, suggesting a solvent-dependent extraction pattern for these compounds. Cholesterol was found to be absent in all test samples. This observation is consistent with the biochemical nature of plant sterols, as cholesterol is a primary sterol predominantly found in animals, whereas plants synthesize phytosterols such as  $\beta$ -sitosterol, stigmasterol, and campesterol [19]. These phytosterols, which differ structurally and functionally from cholesterol, are characteristic of gymnosperms. Previous phytochemical reports on *P. orientalis* also confirmed the presence of terpenoids, flavonoids, alkaloids, and phytosterols, while consistently noting the absence of cholesterol in both polar and non-polar extracts [20, 21].

The predominance of bioactive constituents in EO indicates that volatile and semi-volatile compounds are major secondary metabolites in *P. orientalis*, with terpenoids and flavonoids being the principal classes. These metabolites are widely recognized for their antimicrobial, anti-inflammatory, and antioxidant activities, which may explain several of the plant's traditional therapeutic applications [11]. The detection of phenolic compounds and tannins in all extracts further reinforces the antioxidant potential of the species, as these compounds are known to scavenge free radicals and inhibit oxidative stress. The absence of steroids in both MLE and HLE could be attributed to low abundance or the inefficiency of the selected solvents to extract these less polar metabolites. The presence of fixed oils and fats in the methanol and n-hexane extracts of *Platycladus orientalis* (L.) Franco can be justified based on solvent polarity and extraction characteristics. Fixed oils and fats are primarily lipophilic compounds composed of triglycerides and fatty acids, which are readily soluble in non-polar solvents such as n-hexane. Therefore, their detection in the n-hexane extract is expected and confirms the lipid-rich nature of the *P. orientalis*. Although methanol is a polar solvent, it is known to extract a wide range of phytoconstituents, including certain non-polar or weakly polar compounds. Fatty acids, partial glycerides, and phospholipids may show limited solubility in methanol or may be co-extracted along with polar constituents during Soxhlet extraction. The occurrence of fixed oils and fats in both extracts indicates that *P. orientalis* contains phytochemicals spanning a broad polarity range, supporting its pharmacological relevance and traditional medicinal use [22, 23]. Interestingly, the consistent detection of phytosterols and terpenoids in the EO and leaf extracts supports earlier studies describing *P. orientalis* as a rich source of bioactive terpenoid derivatives [20]. These compounds have been implicated in diverse pharmacological effects, including cytotoxic, anti-inflammatory, and hepatoprotective activities. Thus, the current findings not only confirm the established phytochemical profile of *P. orientalis* but also highlight the influence of solvent polarity on metabolite extraction efficiency. The results suggest that the essential oil, being rich in terpenoids and flavonoids, represents a particularly valuable fraction for further bioactivity-guided studies aimed at identifying compounds with therapeutic potential.

**Table 1:** Preliminary qualitative phytochemical screening of *P. orientalis* leaf extracts

Phytochemical tests		MLE	HLE	EO
Proteins & Amino acids	Millon's test			
	Xanthoproteic test	+	+	+
	Ninhydrin test			
Phenolics	Potassium dichromate test	+	+	+
	Hot water test			
Carotenoids		-	+	+
	10% NaOH test	+	+	+
	Bromine water test			
Cholesterol	Turbidity test	-	-	-
Fixed oils fats	Spot test	+	+	+
	Saponification test			
Alkaloids				
	Mayer's test, Dragendorff's test, Wager's test	+	+	-
Carbohydrates	Barfoed's test			
	Molisch's test	+	-	+
	Seliwanoff's test			
Flavonoids	Ferric Chloride test	+	+	+
	Alkaline test			
Glycosides	Keller-Kiliani	+	-	+
Terpenoids	Salkowski's test	+	+	+
Steroids	Liebermann-Burchard test	-	-	+

**Note:** The symbol '+' indicates presence of phytochemical compounds, '-' indicates the absence of phytochemical compounds

### 3.2. Total phenol content

The total phenolic content (TPC) of MLE, HLE and EO is presented in Figure 2, with corresponding quantitative data summarized in Table 2. Among the extracts, the MLE exhibited the highest phenolic content ( $507.01 \pm 14.20$  mg GAE/g), followed by the HLE ( $381.58 \pm 15.51$  mg GAE/g) and the EO ( $103.58 \pm 4.06$  mg GAE/g). These results clearly indicate that methanol is a more effective solvent for extracting phenolic compounds than HLE and EO, likely due to its higher polarity and ability to solubilize a wide range of polyphenolic constituents. Phenolic compounds are known for their strong antioxidant potential and play a vital role in the defense mechanisms of plants against oxidative stress. The elevated TPC observed in MLE suggests that methanol efficiently extracts bioactive phenolic constituents responsible for the antioxidant, anti-inflammatory, and antimicrobial activities attributed to *P. orientalis*. The comparatively lower phenolic content in the EO is consistent with its volatile nature, as essential oils are typically dominated by terpenoids rather than polyphenolic compounds<sup>[24]</sup>.

Recent studies have similarly reported *P. orientalis* leaf extracts to be rich in phenols. Khammassi *et al.* (2022) found that ethyl acetate and ethanolic crude leaf extracts contained 62.69 mg GAE/g and 61.44 mg GAE/g, respectively<sup>[24]</sup>. Likewise, a study conducted in Northern Iran comparing water, methanol, ethanol, and ethyl acetate extracts reported that the methanolic extract exhibited the highest phenolic content, with a strong positive correlation between total phenol/flavonoid levels and antioxidant activity<sup>[26]</sup>. Furthermore, a chemometric study of *P. orientalis* and related Cupressaceae taxa demonstrated moderate phenolic content in the EO, as determined by the Folin-Ciocalteu method, further supporting the current findings<sup>[25]</sup>. Overall, these results reaffirm that *P. orientalis* is a rich natural source of phenolic compounds, particularly in methanol-based extracts. The observed solvent-dependent variation in phenolic yield underscores the importance of solvent selection for optimizing extraction efficiency and maximizing the recovery

of bioactive phytochemicals with potential therapeutic applications.

### 3.3. Total flavonoid content

The total flavonoid content of MLE, HLE and EO was estimated using Catechin as a standard. MLE showed the highest flavonoid content ( $283.17 \pm 8.21$  mg/g), followed by HLE ( $171.74 \pm 4.29$  mg/g) and EO ( $72.67 \pm 3.18$  mg/g) (Table 2, Figure 2). These results indicate that solvent polarity strongly affects flavonoid extraction efficiency. The higher yield in MLE can be attributed to methanol's polarity, which enhances penetration into plant tissues and solubilization of flavonoids. In contrast, the low values in HLE and EO reflect the limited ability of non-polar solvents to extract polar flavonoids. A similar pattern was reported in northern Iran, where methanol extracts of *P. orientalis* exhibited the highest flavonoid content and strong correlation with antioxidant activity<sup>[26]</sup>. This supports the established role of flavonoids as potent antioxidants that scavenge free radicals and chelate metal ions. Overall, methanol proved to be the most effective solvent for obtaining flavonoid-rich extracts from *P. orientalis*. The high flavonoid content in MLE likely contributes to its superior antioxidant potential, emphasizing its value for developing natural antioxidant and therapeutic formulations.

### 3.4. Total alkaloid content

The total alkaloid content of MLE, HLE and EO was determined using atropine as a standard. As shown in Table 2 and Figure 2, the MLE exhibited the highest alkaloid content ( $135.40 \pm 5.16$  mg/g), followed by HLE ( $85.59 \pm 4.16$  mg/g), while the EO showed negligible amounts. This indicates that methanol is a more efficient solvent for extracting alkaloids due to its polarity, which enhances solubility of nitrogen-containing compounds. The minimal alkaloid content in EO is expected, as most alkaloids are non-volatile and thermolabile, thus not recoverable by distillation.

These results align with previous qualitative reports confirming the presence of alkaloids in *P. orientalis* (Khammassi *et al.*, 2022) and related *Platycladus* species such as *P. harmala*. However, quantitative data remain limited, highlighting the present findings as valuable evidence of alkaloid richness in *P. orientalis*. The higher concentration in MLE may underlie the plant's reported pharmacological effects, including antimicrobial, analgesic, and anti-inflammatory activities, since alkaloids are known to contribute to such bioactivities. Overall, the study confirms both the presence and solvent-dependent variability of alkaloid content in *P. orientalis*. Further work should focus on isolation, structural elucidation, and biological evaluation of individual alkaloids using advanced analytical techniques such as LC-MS/MS and NMR to better understand their therapeutic potential.

### 3.5. Total tannin content

The total tannin content in the MLE, HLE and EO of *P. orientalis* was quantified using tannic acid as the standard. As shown in Table 2 and Figure 2, tannin levels were negligible across all extracts. Although qualitative tests confirmed trace presence, the low quantitative values suggest that methanol and *n*-hexane were ineffective in extracting significant tannin amounts. Environmental factors such as seasonal variation, soil composition, and geographic location may also have influenced the reduced tannin accumulation. These findings demonstrate the crucial significance of solvent polarity and

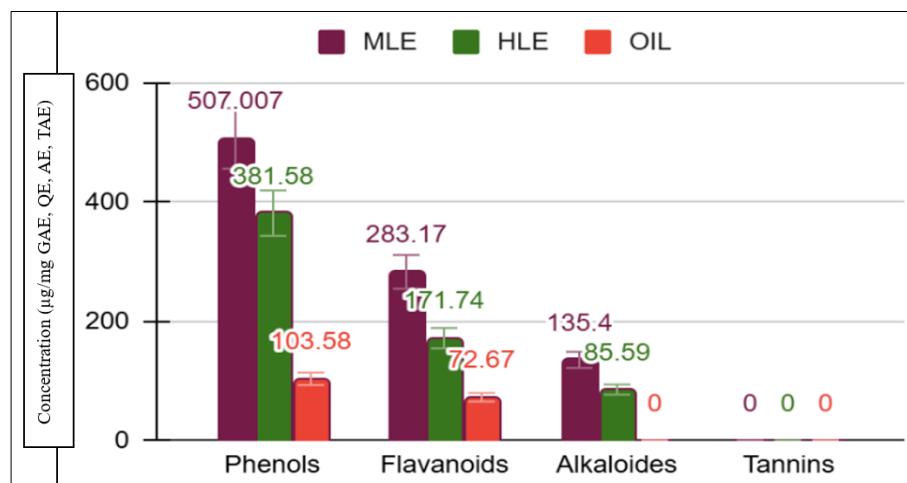
extraction conditions in recovering polyphenols, in contrast to previous research that reported higher tannin levels (1.03%) using 70% ethanol [27]. Ethanol's higher polarity and hydrogen-bonding ability likely enhance tannin solubility, leading to better extraction efficiency. The minimal tannin content found here indicates that *P. orientalis* may not be a

rich tannin source under current conditions, and other phytochemicals such as flavonoids or terpenoids might contribute more to its bioactivity. Optimizing extraction techniques, particularly with hydroalcoholic or assisted methods, could improve tannin recovery and clarify their pharmacological role in this species.

**Table 2:** Total phenols, flavonoids, alkaloids and tannin contents *P. orientalis*

Sl. No.	Samples	Phenols (µg/mg GAE)	Flavonoids (µg/mg QE)	Alkaloids (µg/mg AE)	Tannins (µg/mg TAE)
1	MEL	507.007±14.20	283.17±8.21	135.40±5.16	---
2	HLE	381.62±15.51	171.74±4.29	85.59±4.16	---
3	EO	103.58±4.06	72.67±3.18	---	---

**Note:** The symbol '---' indicates no activity at tested concentrations (MLE-Methanol leaf extract, HLE-Hexane leaf extract, EO - Essential oil)



**Fig 2:** Total phenols, flavonoids, alkaloids, tannins contents *P. orientalis*

### 3.6 GCMS analysis

The GC-MS analysis enabled precise identification of phytoconstituents through characteristic retention times and mass fragmentation patterns. This analytical approach enables reliable identification of both major and minor constituents within the extracts. The chromatographic profiles of MLE (Figure 3), HLE (Figure 4), and EO (Figure 5) of *P. orientalis* demonstrated the presence of nineteen, eleven, and forty-five distinct phytochemical constituents, respectively. Based on the identification results, the quantitation ion, reference ion(s), retention time, mass spectrum and retention index for each compound were registered in the Table 3. Multivariate Data Analysis was conducted using SIMCA-P software [28].

The GC-MS analysis of MLE (Table 3) revealed a diverse range of 19 bioactive compounds, including aldehydes, terpenoids, carbohydrates, long-chain fatty acids, sterol chloroformates, diterpenoid lactones, fatty aldehydes, and diacetylenic fatty acids. The major constituents of the MLE (Table 3) included Caryophyllene oxide, known for its anti-inflammatory, antifungal, and anticancer activities and often contributing to the aroma and therapeutic potential of EO [28]; 3-Cyclohexen-1-carboxaldehyde, a volatile compound responsible for fragrance with potential antimicrobial activity and involvement in plant defense mechanisms [29]; 3-O-Methyl-D-glucose, indicative of carbohydrate modification that may influence bioactivity [30]; Phytol, an acyclic diterpene alcohol with antioxidant, antimicrobial, and anticancer effects, also serving as a precursor for vitamins E and K1 [31]; 10, 12-Pentacosadiynoic acid, a long-chain fatty acid possessing antimicrobial and anti-inflammatory properties relevant to lipid metabolism [32];  $\gamma$ -Elemene, a sesquiterpene known for its anticancer activity and traditional use in tumor therapy [33]; 1-Heptatriacotanol, a long-chain fatty alcohol

with moisturizing and antimicrobial properties commonly found in plant waxes [34]; Tetratetracontane, a long-chain alkane contributing to plant cuticle hydrophobicity and environmental stress protection [35]; and n-Hexadecanoic acid, a saturated fatty acid widely distributed in plants, exhibiting antimicrobial and anti-inflammatory properties and playing a crucial role in membrane structure [36].

Overall, the findings confirm the predominance of terpenoid compounds in *P. orientalis*, underscoring the plant's chemical richness and the significance of its terpenoid metabolites.

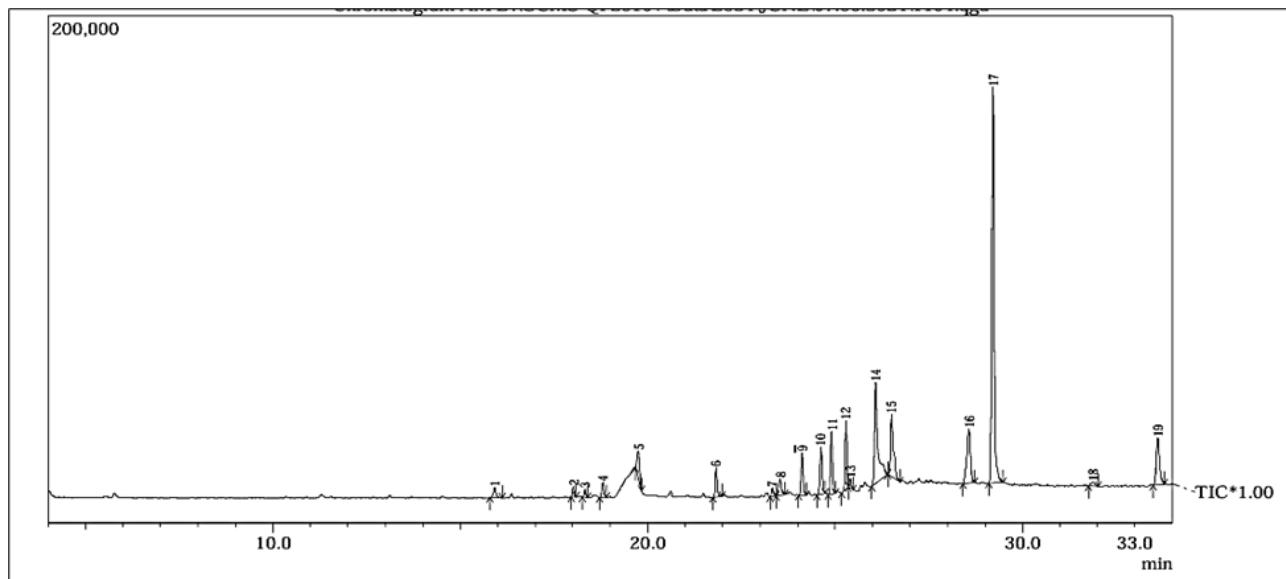
The GC-MS analysis of the HLE (Table 4) revealed the presence of diverse (11) bioactive compounds, primarily terpenoids such as 1, 3, 6, 10-Cyclotetradecatetraene, 3, 7, 11-trimethyl-14-(1-methylethyl)-, [S-(E, Z, E, E)], which exhibits antioxidant activity, along with predominant diterpenoids and sesquiterpene hydrocarbons [37], also commonly reported in other *Thuja* species. The analysis further identified labdane diterpenoids such as Andrographolide (anti-inflammatory), flavonoids such as 10, 12-Pentacosadiynoic acid (anti-inflammatory), and aromatic metabolites including 2-Phenanthrenol (anti-cancer, antimicrobial). Hydrophilic volatile metabolites like 1, 3, 6, 10-Cyclotetradecatetraene contribute to the extract's scent and flavor, while xenobiotic metabolites of fungal and bacterial origin such as 1-Naphthalenecarboxylic acid and Lambertianin acid were also detected. Alcohols and esters, including heptadecyl ester, were identified as minor components. These findings highlight the efficacy of n-hexane a non-polar solvent in selectively extracting high molecular weight, non-volatile compounds such as diterpenoids, which might otherwise be underrepresented in polarity-driven extraction methods.

Similarly, the GC-MS analysis of EO (Table 5) revealed the presence of 45 bioactive compounds acyclic monoterpenes ( $\beta$ -

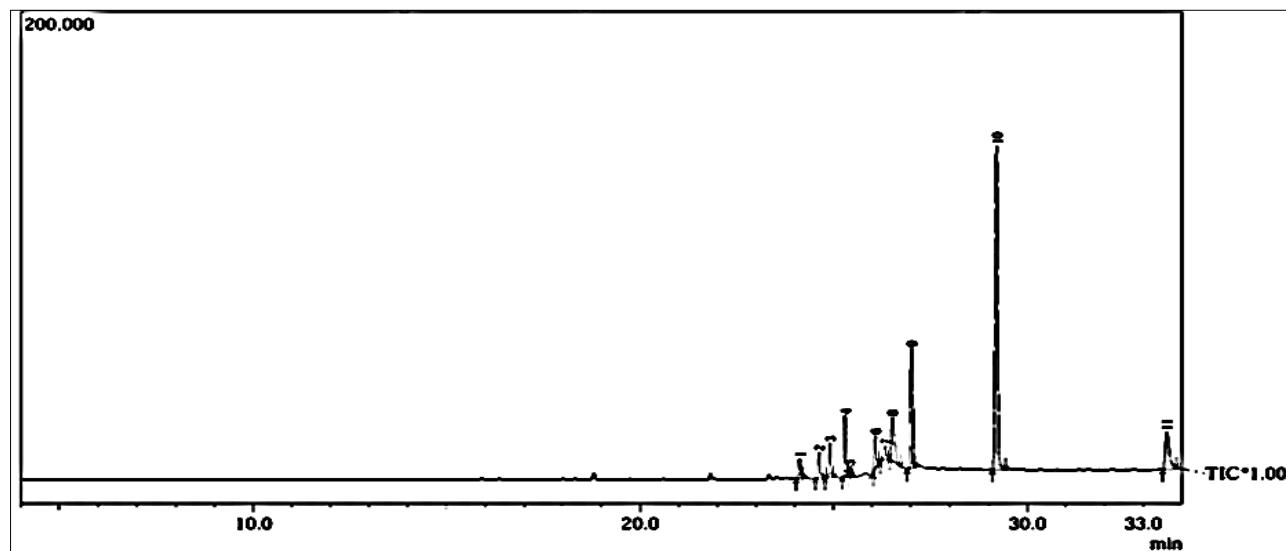
Myrcene), alkenes (Cyclohexene), and alkynes (Bicyclo [3.1.1] heptane, 6, 6-dimethyl-2-methylene-, (1S)-), which significantly contribute to the oil's fragrance, volatility, and reactivity [38]. Major monoterpene constituents, including  $\alpha$ -Pinene and  $\delta$ -Carene, are characteristic of *P. orientalis* [38] and are known for their antioxidant, anti-inflammatory, and antimicrobial properties. Ketones such as 4-Acetyl-1-methylcyclohexene impart mild, spicy, or green aromatic tones, whereas esters, particularly  $\alpha$ -terpinyl acetate, contribute to long-lasting fruity and floral fragrances [39]. Phenolic compounds (e.g., 3-Methyl-5-(propan-2-yl) phenol) exhibited strong antioxidant, antimicrobial, and antianesthetic activities. Sesquiterpenes, including Cedrol, Caryophyllene, and  $\alpha$ -Caryophyllene, represented major fractions of the essential oil. Additionally, various volatile metabolites alcohols, aldehydes, and minor terpenoids enhance the oil's olfactory complexity and potential applications in aromatherapy and perfumery. Prominent volatile compounds identified included Camphene, D-Limonene, Copaene,

Cedrol, cis-Thujopsene, 3-Carene,  $\beta$ -Myrcene, p-Cymene,  $\gamma$ -Terpene, 1, 3, 7, 11-Cyclotetradecatetraene, 2-Methyl,  $\epsilon$ -Atlantone, and Squamulosone (Table 5).

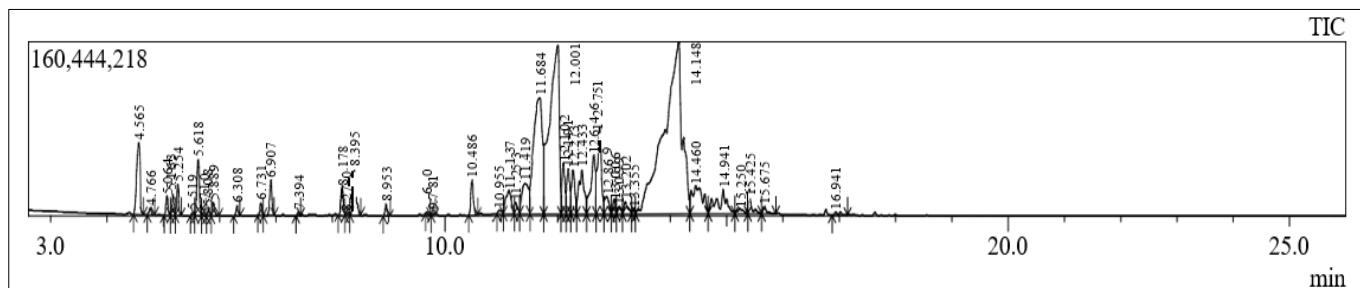
GC-MS separates compounds based on volatility and their interaction with the column's stationary phase, expressed as retention time (RT). Occasionally, compounds sharing identical molecular weights may elute at distinct RT, appearing as different entities. This phenomenon arises from the existence of isomers molecules with identical molecular formulas but distinct structural arrangements. GC separates these isomers according to their physicochemical characteristics, including polarity, boiling point, and molecular geometry, resulting in distinct retention profiles. However, their mass spectra may remain similar or identical due to shared molecular weights, complicating unambiguous identification. Moreover, overlapping or indistinct fragmentation patterns can further obscure differentiation, emphasizing the necessity of comparing retention times with authentic standards for reliable compound confirmation [39].



**Fig 3:** GCMS Chromatogram of *P. orientalis* methanol leaf extract (MLE)



**Fig 4:** GCMS Chromatogram of hexane leaf extract (HLE) of *P. orientalis*

Fig 5: GCMS Chromatogram of *P. orientalis* essential oil (EO)Table 3: Bioactive compounds identified in MLE of *P. orientalis*

Si. No	RT	Compound detected	M.F	M.W (g/mol)	Phytochemical group
01.	15.912	1.12 Bicyclo [7.2.0] undec-4-ene, 4, 11, 11-trimethyl-8-methylene-, [1R-(1R*, 4Z, 9S*)]-	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenoids
02.	18.018	Caryophyllene oxide	C <sub>15</sub> H <sub>24</sub> O	220	Sesquiterpenoid oxide
03.	18.328	3-Cyclohexen-1-carboxaldehyde, 3, 4-dimethyl-	C <sub>9</sub> H <sub>14</sub> O	138	Aldehydes
04.	18.806	2-Naphthalenemethanol, decahydro -. alpha.,alpha., 4a-trimethyl-8-methylene-, [2R-(	C <sub>15</sub> H <sub>26</sub> O	222	Terpenoids
05.	19.749	3-O-Methyl-d-glucose	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194	Carbohydrate,
06.	21.825	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	Long-chain fatty acids
07.	23.332	Phytol	C <sub>20</sub> H <sub>40</sub> O	296	Acyclic diterpenoid
08.	23.532	9-Octadecenal, (Z)-	C <sub>18</sub> H <sub>34</sub> O	266	Fatty aldehydes
09.	24.117	Cholest-5-en-3-ol (3. beta.)-, carbonochloridate	C <sub>28</sub> H <sub>45</sub> ClO <sub>2</sub>	448	Sterol chloroformates
10.	24.624	Andrographolide	C <sub>20</sub> H <sub>30</sub> O <sub>5</sub>	350	Diterpenoid lactones
11.	24.906	10-12-Pentacosadiynoic acid	C <sub>25</sub> H <sub>42</sub> O <sub>2</sub>	374	Di-acetylenic fatty acids
12.	25.294	2-Phenanthrenol, 4b, 5, 6, 7, 8, 8a, 9, 10-octahydro-4b, 8, 8-trimethyl-1-(1-methylethyl)-,	C <sub>20</sub> H <sub>30</sub> O	286	Unsaturated fatty acids
13.	25.405	Podocarp-7-en-3-one, 13. beta. -methyl-13-vinyl- \$\$ Pimara-7, 15-dien-3-one	C <sub>20</sub> H <sub>30</sub> O	286	Diterpenes
14.	26.085	Tricyclo [20.8.0.0(7, 16)] triaconta-1(22), 7(16), 9, 13, 24, 28-hexaene	C <sub>30</sub> H <sub>44</sub>	404	
15.	26.508	1-Heptatriacotanol	C <sub>37</sub> H <sub>76</sub> O	536	Long-chain fatty alcohol
16.	28.575	Tetratetracontane	C <sub>44</sub> H <sub>90</sub>	618	Long-chain alkane
17.	29.220	Gamma. -Elemene	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenoids
18.	31.883	Tridecane, 1-iodo-	C <sub>13</sub> H <sub>27</sub> I	310	Organic-iodine compounds
19.	33.607	1-Hentetracontanol	C <sub>41</sub> H <sub>84</sub> O	592	

Note: RT: Retention time, MF: Molecular formula, MW: Molecular weight

Table 4: Bioactive compounds identified in HLE of *P. orientalis*

SI. No.	R. T	Compound detected	M.F	M.W (g/mol)	Phytochemical group
01.	24.121	1, 3, 6, 10-Cyclotetradecatetraene, 3, 7, 11-trimethyl-14-(1-methylethyl)-, [S-(E, Z, E, E)]	C <sub>20</sub> H <sub>32</sub>	272	Terpenoid
02.	24.625	Andrographolide	C <sub>20</sub> H <sub>30</sub> O <sub>5</sub>	350	Labdane diterpenoid
03.	24.907	10-12-Pentacosadiynoic acid	C <sub>25</sub> H <sub>42</sub> O <sub>2</sub>	374	Flavonoids
04.	25.294	2-Phenanthrenol, 4b, 5, 6, 7, 8, 8a, 9, 10-octahydro-4b, 8, 8-trimethyl-1-(1-methylethyl)-,	C <sub>20</sub> H <sub>30</sub> O	286	Aromatic metabolites
05.	25.415	Podocarp-7-en-3-one, 13. beta. -methyl-13-vinyl-	C <sub>20</sub> H <sub>30</sub> O	286	Diterpenoid
06.	26.083	1, 3, 6, 10-Cyclotetradecatetraene, 3, 7, 11-trimethyl-14-(1-methylethyl)-, [S-(E, Z, E, E)]	C <sub>20</sub> H <sub>32</sub>	272	Hydrophilic volatile metabolites
07.	26.343	1-Naphthalenecarboxylic acid, 5- [2-(3-furanyl) ethyl] decahydro-1, 4a-dimethyl-6-me	C <sub>20</sub> H <sub>28</sub> O <sub>3</sub>	316	Fungal and bacterial xenobiotic metabolite, Lambertian acid
08.	26.517	9, 19-Cycloergost-24(28)-en-3-ol, 4, 14-dimethyl-, acetate, (3. beta., 4. alpha., 5. alpha.)	C <sub>32</sub> H <sub>52</sub> O <sub>2</sub>	468	Alcohol, esters
09.	27.015	1, 2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	Carboxylic acid and ester
10.	29.217	. gamma. -Element	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
11.	33.611	Pentafluoropropionic acid, heptadecyl ester	C <sub>20</sub> H <sub>35</sub> F <sub>5</sub> O <sub>2</sub>	402	Ester

Note: RT: Retention time, MF: Molecular formula, MW: Molecular weight

**Table 5:** Bioactive compounds identified in EO of *P. orientalis*

Si No.	R TIME	Compound detected	M.F	M.W (g/mol)	Phytochemical group
01.	4.565	Cyclohexene, 1-methyl-4-(1-methyl ethylidene)-	C <sub>10</sub> H <sub>16</sub>	136	Alkene
02.	4.766	Camphene	C <sub>10</sub> H <sub>16</sub>	136	Monoterpene
03.	5.064	Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-	C <sub>10</sub> H <sub>16</sub>	136	Alkene
04.	5.163	Bicyclo [3.1.1] heptane, 6, 6-dimethyl-2-methylene-, (1S)-	C <sub>10</sub> H <sub>16</sub>	136	Alkyne
05.	5.254	beta. -Myrcene	C <sub>10</sub> H <sub>16</sub>	136	Acyclic monoterpene
06.	5.519	Cyclohexane, 1-methylene-4-(1-methylethenyl)-	C <sub>10</sub> H <sub>16</sub>	136	Alkene
07.	5.618	3-Carene	C <sub>10</sub> H <sub>16</sub>	136	Bicyclic monoterpene
08.	5.710	p-Cymene	C <sub>10</sub> H <sub>14</sub>	134	Alkylbenzene
09.	5.808	p-Cymene	C <sub>10</sub> H <sub>14</sub>	134	Alkylbenzene
10.	5.889	D-Limonene	C <sub>10</sub> H <sub>16</sub>	136	Terpenes
11.	6.308	gamma. -Terpinene	C <sub>10</sub> H <sub>16</sub>	136	Monoterpene
12.	6.731	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	C <sub>10</sub> H <sub>16</sub>	136	Alkene
13.	6.907	Bicyclo [3.1.1] heptane, 6, 6-dimethyl-2-methylene-, (1S)-	C <sub>10</sub> H <sub>16</sub>	136.234	Alkyne
14.	7.394	4-Acetyl-1-methylcyclohexene	C <sub>9</sub> H <sub>14</sub> O	138.21	Ketone
15.	8.178	4-Terpinenyl acetate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	196.286	Ester (acetate)
16.	8.270	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, epsilon-	C <sub>10</sub> H <sub>16</sub>	136	Alkene
17.	8.395	alpha. -Terpineol	C <sub>10</sub> H <sub>18</sub> O	154	Monoterpenoids
18.	8.953	Benzene, 1-methoxy-4-methyl-2-(1-methylethyl)-	C <sub>11</sub> H <sub>16</sub> O	164	Ether
19.	9.680	Camphene	C <sub>10</sub> H <sub>16</sub>	136	Monoterpene
20.	9.781	3-Methyl-5-(propan-2-yl) phenol	C <sub>10</sub> H <sub>14</sub> O	150	Phenol
21.	10.486	D-Limonene	C <sub>10</sub> H <sub>16</sub>	136	Terpene
22.	10.955	Copaene	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
23.	11.137	Di-epi-. Alpha -cedrene-(I)	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
24.	11.253	2H-2, 4a-Methanonaphthalene, 1, 3, 4, 5, 6, 7-hexahydro-1, 1, 5, 5-tetram	C <sub>15</sub> H <sub>24</sub>	204.3511	Sesquiterpenes
25.	11.419	cis-Thujopsene	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
26.	11.684	(1R, 4R, 5S)-1, 8-Dimethyl-4-(prop-1-en-2-yl) spiro [4.5] dec-7-ene	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
27.	12.001	cis-Thujopsene	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
28.	12.102	1H-Benzocycloheptene, 2, 4a, 5, 6, 7, 8, 9, 9a-octahydro-3, 5, 5-trimethyl-	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
29.	12.191	(1R, 4R, 5S)-1, 8-Dimethyl-4-(prop-1-en-2-yl) spiro [4.5] dec-7-ene	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
30.	12.273	(4As, 9Ar)-3, 5, 5, 9-Tetramethyl 2, 4a, 5, 6, 7, 9a-hexahydro-1H-benzo [7	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
31.	12.433	Spiro [5.5] undec-2-ene, 3, 7, 7-trimethyl-11-methylene-, (-)-	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
32.	12.646	1H-Benzocycloheptene, 2, 4a, 5, 6, 7, 8-hexahydro-3, 5, 5, 9-tetramethyl-	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
33.	12.751	Spiro [5.5] undeca-1, 8-diene, 1, 5, 5, 9-tetramethyl-, epsilon-	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
34.	12.869	(1S, 2E, 6E, 10R)-3, 7, 11, 11-Tetramethylbicyclo [8.1.0] undeca-2, 6-die	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
35.	13.006	1H-Benzocycloheptene, 2, 4a, 5, 6, 7, 8-hexahydro-3, 5, 5, 9-tetramethyl-	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
36.	13.099	Valeren-4, 7(11)-diene	C <sub>15</sub> H <sub>24</sub>	204	Bicyclic sesquiterpenes
37.	13.202	epsilon-3-Methylene-6-((S)-1, 2, 2-trimethylcyclopentyl) cyclohex-1-ene	C <sub>15</sub> H <sub>24</sub>	204	alkene
38.	13.355	Eremophila ketone	C <sub>15</sub> H <sub>24</sub> O	220	Ketone
39.	14.148	Cedrol	C <sub>15</sub> H <sub>26</sub> O	222	Sesquiterpene alcohol (Terpinol)
40.	14.460	cis-Thujopsene	C <sub>15</sub> H <sub>24</sub>	204	Sesquiterpenes
41.	14.941	(1As, 4As, 8Ar)-4a, 8, 8-Trimethyl-2-methylene-1, 1a, 2, 4a, 5, 6, 7, 8-octa	C <sub>15</sub> H <sub>22</sub>	202	methylene
42.	15.250	1, 3, 7, 11-Cyclotetradecatetraene, 2-methyl-	C <sub>15</sub> H <sub>22</sub>	202	Terpene
43.	15.425	epsilon-Atlantone	C <sub>15</sub> H <sub>22</sub> O	218	Aldehyde
44.	15.675	Squamulosone	C <sub>15</sub> H <sub>22</sub> O	218	Sesquiterpenes
45.	16.941	((4S, 5S, 8S, 9R)-4-Isopropyl-6, 8, 9-trimethyl-3-oxabicyclo [3.3.1] non	C <sub>17</sub> H <sub>28</sub> O <sub>3</sub>	280	Monoterpene

**Note:** RT: Retention time, MF: Molecular formula, MW: Molecular weight

#### 4. Conclusion

This study provides a comprehensive phytochemical assessment of *P. orientalis* leaves using methanol (MLE), n-hexane (HLE), and essential oil extracts (EO). Distinct extraction methods yielded varied phytochemical profiles and bioactivities. MLE showed the highest phenolic content (507.01 mg GAE/g), followed by HLE (381.62 mg/g) and EO (103.58 mg/g). GC-MS analysis of the oil revealed diverse compounds, particularly sesquiterpenes such as cedrol and caryophyllene contributing to antioxidant, antimicrobial, and anti-inflammatory activities. MLE is rich in polar antioxidants, HLE in non-polar diterpenoids and sesquiterpenes, and EO demonstrated strong antimicrobial and aromatic potential. Future work should focus on isolating major compounds and evaluate their pharmacological efficacy to optimize *P. orientalis* for therapeutic and industrial applications.

#### 5. Conflict of interest

The authors have no conflict of interest to disclose and publish this research work.

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#### 7. Authors' Contributions

All the authors are equally contributed in this research work

#### 8. Data Availability

Data made available on request to the corresponding author.

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