RESEARCH PAPER

Characterization of Betel Leaf Essential Oil: Evaluating Temperature-Dependent Variations in GC-MS Volatile Fingerprints

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Abstract
Piper betle L. is well known for its aromatic leaves that are rich in bioactive essential oils used in food preservation, food flavoring, and traditional medicine. Although important, systematic studies on the effects of hydro distillation parameters on the yield, composition, and physicochemical characteristics of hydro distillates remain scarce. The extraction temperature (80–100°C) and time (180–300 min) were investigated in this study to assess their effects on the yield and composition of the essential oil. The volatile fraction was profiled using gas chromatography–mass spectrometry (GC–MS), and standard physicochemical analyses were performed. Hydro distillation was performed under four conditions, and the oils were characterized in detail. The results showed that the yield of essential oil increased gradually with an increase in temperature from 0.86 to 1.15% at 100°C/300 min, indicating more volatilization and mass transfer at high temperatures and long periods. GC–MS analysis revealed 20 compounds, of which eugenol (31.7%) was the major compound, followed by chavicol (c. 19%) and chavicol acetate (15.1–16.8%), which are phenylpropanoids, as previously reported. The physicochemical properties were moderately different among treatments like specific gravity 0.985 to 1.025, compositions that vary with extraction severity were observed, ranging from 1.025 to 1.495 refractive index.The results indicate that the yield and chemical composition of P.betle essential oil can be controlled under optimal hydro distillation conditions, which can be beneficial for optimizing its application in food and flavor formulation processes, where compositional uniformity is required.

Keywords: Betel Leaf, Essential Oil, hydrodistillates, gas chromatography–mass spectrometry, chavicol acetate.

1. Introduction

Piper betle L. popularly known as betel leaf is a special aromatic tropical plant with long history of cultural, medicinal and economic values. The plant is also known as “Green gold” due to its shiny and heart shaped leaves which were referred in ancient Sanskrit scriptures for its medicinal and ceremonial uses (Guha 2006). The cultivation of P. betle is widespread in South and South East Asia and is traditionally used in India, Bangladesh, Sri Lanka, Thailand and Indonesia. Leaves are not only ethno botanical important but also contain a number of nutrients, such as vitamins (ascorbic acid and niacin) and minerals (calcium and iron) which make them relevant in terms (Guha, 2006). The nutritional value of P. betle has been an important aspect of interest as a functional food and source of bioactive compounds.

The phytochemical profile of P. betle has been extensively studied and it contains a complex mixture of phenolics and terpenoids responsible for various biological activities. Some of the most often reported major compounds responsible for the antioxidant and antimicrobial properties of these plants are eugenol, chavicol, hydroxychavicol and chavibetol (Prakash et al., 2010). The essential oil fraction also contains different amounts of monoterpenes and sesquiterpenes, whose distribution depends on geographical location and cultivation conditions, leading to different chemotypes (Jirovetz et al.,2002 & Pradhan et al 2013). This compositional variability has been linked with diverse biological activities like anti-inflammatory and antimicrobial activities both in vitro and in vivo (Prakash et al., 2010). This chemical diversity emphasises the necessity of detailed compositional analyses for the evaluation of functional potential of P. betle extracts.

The importance of essential oils derived from aromatic plants is increasing in the domain of food systems because of their use as flavouring and natural preservatives. Taste is a major factor in consumer acceptance and the use of plant volatiles may be a method to improve the sensory properties while stabilising the product. P. betle essential oil has shown antimicrobial activities against food borne pathogens (Prakash et al., 2012). P. betle is of interest due to its unique aroma. The use of these oils in foods has been associated with a longer shelf life and the requirement of less synthetic additives in the food products. The efficacy of essential oils depends very much on the chemical composition of the plants, but strong analytical tools like gas chromatography–mass spectrometry (GC–MS) are needed to develop a clear and reliable volatile fingerprint. Such profiling can be used to standardise, quality-check and compare products from different extraction conditions and sources (Burt, 2004; Hyldgaard et al., 2012). Hydrodistillation is still a common technique for extraction of essential oils, however, the effects of various process conditions such as temperature and

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extraction time on yield and chemical profile of P. betle essential oil have not yet been systematically characterised. It was found that thermal conditions applied in the process significantly affect the amount of volatiles and their composition from other aromatic plants. For example, variations7 in drying. (Nabi et al., 2025)

The extraction temperature can affect the relative abundance of main components of basil and thyme essential oils, and it can degrade or even transform some thermolabile components, causing measurable changes of GC–MS profiles (Khangholil and Rezaeinodehi, 2008). In addition, basil was found to be sensitive to thermal processing during distillation as with other species with significant changes in the composition of volatiles and the nature of the aroma (Farahbakhsh et al., 2020). Similar observations indicate that there are limited comparisons for P. betle . Hence, there is a need to systematically investigate the temperature-dependent changes in this species.

To fill this gap, the present work focused on the study of the change in composition and yield of essential oil of essential oils under controlled hydro distillation conditions with a view of studying the effect of temperature on the essential oil. In this study, physicochemical characterization was coupled with GC–MS profiling to provide an extended evaluation. This investigation aims to understand the conditions that will lead to the best oil recovery and gain insight into the effect of extraction severity on the physicochemical properties and volatile fingerprints of the oil. The development of such correlations can help in process standardization and aid in the use of P. betle essential oil in a specific manner in food and flavor systems.

2. Materials and Methods

2.1 Plant Material

Fresh leaves of the Kolkata variety of Piper betle L. were collected from the MGM Hills, Gandheli Campus, Chhatrapati Sambhajinagar, Maharashtra, India. The plant material was identified by a competent botanist, and a voucher specimen was kept in the herbarium of MGM University for future studies. The leaves were collected, thoroughly washed in distilled water to remove dust and other contaminants, and then shade-dried at room temperature to reduce the thermal degradation of the volatiles. The dried leaves were then used for the essential oil extraction. A similar protocol has been described in previous studies on medicinal and aromatic plants regarding plant authentication and voucher specimen deposition (Sasidharan et al., 2011).

2.2 Extraction of Essential Oil

Essential oil was extracted using Clevenger-type hydrodistillation apparatus. In aromatic plants, essential oil extraction has been broadly performed using the hydrodistillation method with a Clevenger apparatus (Burt, 2004). 100gm of shade dried leaf material was added to 400 ml of distilled water, and the solid-to-liquid ratio was maintained at 1:4 (w/v). Four different temperature and time conditions were used for hydro distillation. Trial-1 essential oil was extracted at 80°C for 180 min. This resulted in 0.86% of essential oil. Trial 2 was conducted at a temperature of 90°C for 210 min. (Olascuaga-Castillo et al., 2024). This resulted in a yield of 0.90%. In Trial 3, the extraction was performed at 100 °C for 240 min, resulting in 1.10 % oil extraction, while in Trial 4, the extraction time was increased to 300 min at 100 °C, yielding 1.15 % oil extraction. The extracted oil was collected, dried over anhydrous sodium sulfate, and stored in airtight amber vials at 4°C for further analyses.

2.3 Physicochemical Analysis

The physicochemical properties of the extracted essential oils were determined using standard procedures. Specific gravity was determined using a calibrated pycnometer at room temperature. The refractive index was measured and recorded using handheld refractometer. Acid value was determined by titration method with standardised potassium hydroxide solution to evaluate the free fatty acid content of the essential oil. Further, organoleptic evaluation was performed for characteristics like colour, appearance and odour. These analyses of samples were analysed by standard analytical methods recommended by the AOAC, 2016.

2.4 GC–MS Analysis

Gas chromatography mass spectrometry (GCMS) was used to analyze the chemical composition of the essential oil samples using a Shimadzu GCMS-TQ8050 Plus system (Shimadzu Corporation, Kyoto, Japan) with a headspace sampler (HS-20) (Shimadzu Corporation). An HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness) was used for separation. The carrier gas used was helium at a constant flow rate of 1 ml/min. Samples were introduced in split mode, and the temperature of the injector was maintained at 250 °C. The oven temperature program was set from 60°C (held for 2 min) to 250°C (ramped at 10°C/min) and then held for 10 min. Detection was performed using mass spectrometry in electron ionization mode at 70 eV and in the mass range of m/z 40–450. Volatile compounds were identified by matching the mass spectra with the NIST library and/or retention index matching methods. The retention indices were determined using a homologous series of n-alkanes (C8–C32) under the same conditions as described by Adams (2007).

2.5 Statistical Analysis

The results are presented as mean ± standard deviation (SD) under the controlled conditions. One-way ANOVA was used for statistical analysis, and differences were deemed significant at $p < 0.05$.

3. Results

3.1 Essential Oil Yield

Table 1 shows the yields of essential oils extracted under different hydrodistillation conditions. The yield from the extraction at 80°C for 180 min (Trial 1) was 0.86%, whereas it was 0.90% at 90°C for 210 min (Trial

2). At 100°C for 240 min (Trial 3), the yield of 1.10% was significantly increased. The maximum yield of 1.15% was recorded for the extraction carried out at 100°C for 300 min (Trial 4). In general, the data showed a steady rise in oil yield in the four trials, with an overall increase of approximately 34% over these four trials (Sarkar, U. 2023). The differences in yields under different extraction conditions are summarized in the following table:

The essential oil yields under different extraction conditions were statistically different ($p < 0.05$), as revealed by one-way ANOVA.

Table 1: Essential oil yield of *Piper betle* L. under different hydrodistillation conditions

Sr. No.	Temperature (°C)	Time (min)	Oil Yield (mL)	Oil Yield (%)
1	80	180	0.86 ± 0.01	0.86 ± 0.01
2	90	210	0.90 ± 0.01	0.90 ± 0.01
3	100	240	1.10 ± 0.02	1.10 ± 0.02
4	100	300	1.15 ± 0.02	1.15 ± 0.02

3.2 Physicochemical Properties

The physicochemical data of the extracted essential oils are summarized in Table 2. The specific gravity of the oils increased gradually from Trial 1 (0.985) to Trial 7 (1.025). Similarly, the refractive index values were in the range of 1.495–1.512 for all extraction conditions. The acid value in all the trials increased with increasing trial numbers from 1.02 mg KOH/g (Trial 1) to 1.83 mg KOH/g (Trial 4).Changes in organoleptic properties were also observed. In Trial 1, the essential oil was light yellow, whereas in Trial 4, it turned brownish-yellow. Moreover, the smell of the oils was different depending on the extraction conditions: the oil extracted at a lower temperature and for a shorter duration was described as fresh and sharp, whereas the oil obtained at a higher temperature and for a longer duration was described as heavier and denser (Hoang, S 2025).. The physicochemical and sensory changes are presented in Table 2.

Table 2: Physicochemical properties of Piper betle L. essential oil under different extraction conditions

Parameter	Trial 1 (80°C / 180 min)	Trial 2 (90°C / 210 min)	Trial 3 (100°C/240 min)	Trial 4 (100°C/300 min)
Appearance	Clear, highly mobile liquid	Clear liquid	Clear liquid	Clear to slightly viscous liquid
Color	Pale yellow	Light yellow	Golden / Yellowish-brown	Dark brownish-yellow
Odor	Fresh, sharp, slightly spicy	Warm, mildly pungent	Intensely pungent, spicy, clove-like	Heavy, dense, deeply pungent
Specific Gravity (at 20°C)	0.985 ± 0.002	0.995 ± 0.002	1.015 ± 0.003	1.025 ± 0.003
Refractive Index (at 20°C)	1.495 ± 0.001	1.502 ± 0.001	1.508 ± 0.001	1.512 ± 0.001
Acid Value (mg KOH/g)	1.02 ± 0.03	1.24 ± 0.03	1.56 ± 0.04	1.83 ± 0.04

3.3 GC–MS Volatile Composition

Table 3 shows the volatile composition of Piper betle essential oil (PBE) obtained via GC–MS analysis. A total of 20 compounds were identified under all extraction conditions, most of which were phenolic compounds**.** The relative abundance of phenolic constituents varied from approximately 60-70% of the total composition. Eugenol was the principal component, and its amount was the highest in Trial 4 at 31.7% compared to Trial 1 at 29.8%. In the case of chavicol, there was an increase in the trend from 16.9% to 19.0% under similar conditions. However, the decrease in chavicol acetate was from 16.8% (Trial 1) to 15.1% (Trial 4) in the present study. The amount of methyl chavicol decreased slightly from 3.1% to 2.6%, whereas that of eugenyl diacetate increased from 2.5% to 2.8% (Sarkar, U. 2022).

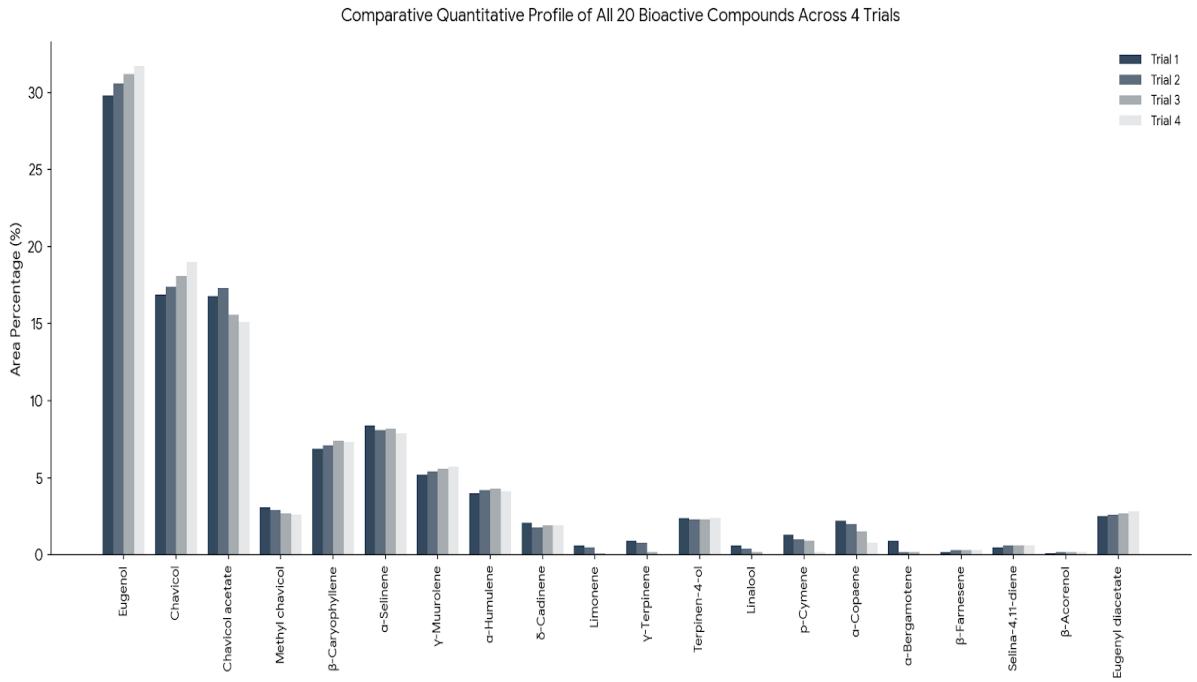
The sesquiterpene fraction exhibited moderate differences among the extraction conditions, with the percentage of β- Caryophyllene being slightly higher in Trial 4 (7.3%) than in Trial 1 (6.9). Both α-humulene (4.0% to 4.1%) and exhibited an increase (5.2% to 5.7%). α-selinene on the contrary slightly decreased, proportionately from 8.4 % in Trial 1 to 7.9 % in Trial 4. The changes in sesquiterpene composition are summarized in Table 3. (Gupta, 2023).

The relative concentrations of the monoterpene compounds were low and exhibited a downward trend with the extraction conditions. Limonene was detected in Trial 1 (0.6%) but not detected in Trial 4. Similarly, γ-terpinene was reduced from 0.9% in Trial 1 to zero in Trial 4. The final extraction condition in Trial 1 was 0.6% linalool, which was not detected (Sarkar, U. (2022). These monoterpenes were not detected in Trial 4 (Table 3).

Table 3: GC–MS volatile composition of *Piper betle* L. essential oil under different extraction conditions

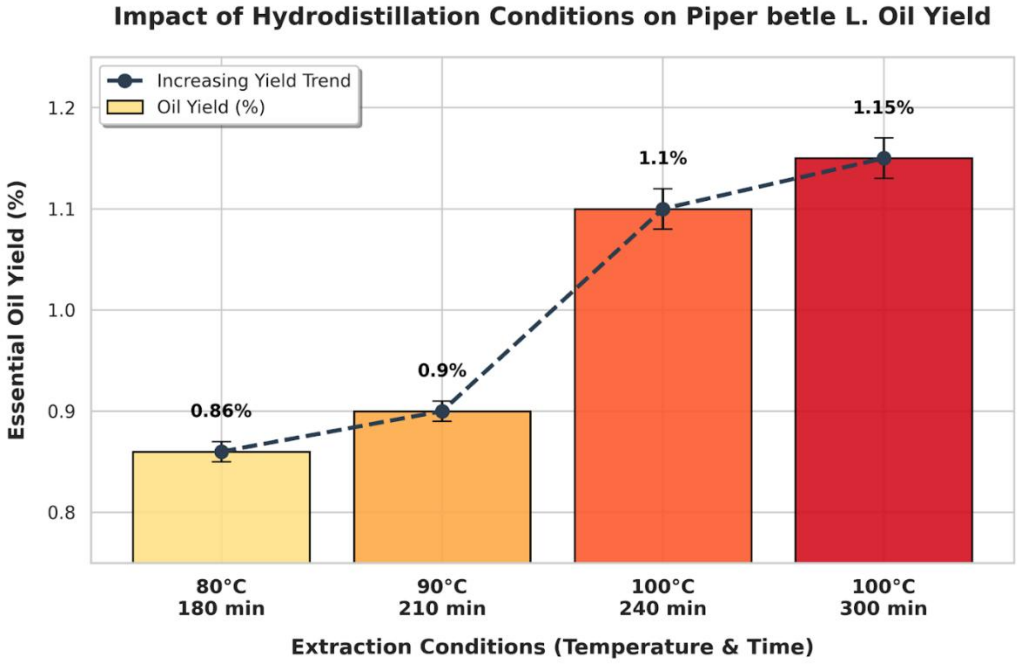
Sr. No.	Compound Name	RT (min)	RI	Area % (Trial 1)	Area % (Trial 2)	Area % (Trial 3)	Area % (Trial 4)
1	Eugenol	17.45– 17.62	1356	29.8 ± 0.4	30.6 ± 0.4	31.2 ± 0.5	31.7 ± 0.5
2	Chavicol	14.82– 15.01	1254	16.9 ± 0.3	17.4 ± 0.3	18.1 ± 0.4	19.0 ± 0.4
3	Chavicol acetate	18.92– 19.11	1368	16.8 ± 0.3	17.3 ± 0.3	15.6 ± 0.3	15.1 ± 0.3
4	Methyl chavicol	12.43– 12.58	1196	3.1 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.6 ± 0.1
5	β-Caryophyllene	20.32– 20.58	1417	6.9 ± 0.2	7.1 ± 0.2	7.4 ± 0.2	7.3 ± 0.2
6	α-Selinene	22.11– 22.36	1489	8.4 ± 0.2	8.1 ± 0.2	8.2 ± 0.2	7.9 ± 0.2
7	γ-Muurolene	21.48– 21.70	1476	5.2 ± 0.2	5.4 ± 0.2	5.6 ± 0.2	5.7 ± 0.2
8	α-Humulene	20.95– 21.10	1452	4.0 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.1 ± 0.1
9	δ-Cadinene	23.44– 23.68	1522	2.1 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
10	Limonene	9.42– 9.55	1031	0.6 ± 0.05	0.5 ± 0.05	0.1 ± 0.01	ND
11	γ-Terpinene	10.88– 11.02	1064	0.9 ± 0.05	0.8 ± 0.05	0.2 ± 0.02	ND
12	Terpinen-4-ol	13.65– 13.82	1174	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1
13	Linalool	10.23– 10.40	1098	0.6 ± 0.05	0.4 ± 0.05	0.2 ± 0.02	ND
14	p-Cymene	8.95– 9.10	1025	1.3 ± 0.1	1.0± 0.1	0.9± 0.05	0.2± 0.02
15	α-Copaene	18.50– 18.72	1374	2.2 ± 0.1	2.0 ± 0.1	1.5 ± 0.1	0.8 ± 0.05
16	α-Bergamotene	19.75– 19.94	1438	0.9 ± 0.05	0.2 ± 0.02	0.2 ± 0.02	ND
17	β-Farnesene	22.92– 23.14	1505	0.2 ± 0.02	0.3 ± 0.02	0.3 ± 0.02	0.3 ± 0.02
18	Selina-4,11-diene	21.75– 21.96	1494	0.5 ± 0.05	0.6 ± 0.05	0.6 ± 0.05	0.6 ± 0.05
19	β-Acorenol	24.30– 24.58	1560	0.1 ± 0.01	0.2 ± 0.02	0.2 ± 0.02	0.2 ± 0.02
20	Eugenyl diacetate	19.98– 20.20	1389	2.5 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.8 ± 0.1

*ND = Not Detected



4. Discussion

4.1 Impact of Hydrodistillation Conditions on Piper betle L. Oil Yield



The increase in oil yield at higher temperatures can be explained by the increase in cell wall disruption and the increase in the solubility of volatile compounds in the steam phase, as reported in previous hydrodistillation optimization studies (Mebrate, S. B.,et al.,2026). At 80°C, the lower thermal energy might not be sufficient to completely break the oil glands in *P. betle* leaves, leading to lower extraction efficiency. The 100°C condition provided optimal thermal energy for effective oil release in accordance as higher temperatures allow better disruption of plant cell structures and enhancement of essential oil diffusion from intracellular sites (Sarkar , U. (2023).

The yield was further enhanced with an increase in distillation time from 180 to 300 min, where Trial 4 (100 °C/300 min) resulted in a maximum yield of 1.15%. This indicates that the complete removal of essential oil from the plant matrix through exhaustive hydrodistillation requires an adequate period, while 5 h (300 min) hydrodistillation is optimum for P. betle leaf extraction (Mondal,B., 2022). . The progressive increase in yield from 0.86% to 1.15% demonstrates that the extraction process had not reached equilibrium at 180 min and that further extraction up to 300 min was necessary to achieve maximum oil recovery (Cang et al., 2020). The optimized conditions (100°C/300 min) had a superior extraction efficiency. The yield range achieved in this study (0.86–1.15%) is consistent with the usual range of 0.15–2.0% reported for P. betle essential oil, which is influenced by factors such as geographical location, cultivar, leaf age, and extraction method (Gupta & Guha, P. (2023).

The slightly higher yields observed in Trials 3 and 4 may be attributed to the use of fresh leaves, optimized extraction parameters, and exhaustive hydrodistillation, which ensured complete oil recovery. maximum yield using optimization was reported at 100°C with 6 h extraction time for fresh betel leaves, supporting our findings that higher temperature and prolonged distillation enhance yield (Madhumita, M.,. et al., 2019)

4.2 Effect of on the Physicochemical Properties of *Piper betle* L. Essential Oil

The results for physicochemical properties of an essential oil were presented in table no 2and are direct reflections of its chemical composition table no 3 and the extraction conditions applied table no 1. As the hydrodistillation temperature and time increased from Trial 1 (80°C for 180 min) to Trial 4 (100°C for 300 min), the *P betle* essential oil underwent significant physical and chemical transformations. Here is an analysis of the data in Table 2, supported by the yield and chemical composition data.

4.2.1. Appearance and Color

The oil transitions from a "clear, highly mobile liquid" with a "pale yellow" color in Trial 1 to a "slightly viscous" and "dark brownish-yellow" liquid in Trial 4. Extraction at higher temperatures (100°C) for prolonged periods (300 min) often leads to the thermal degradation or oxidation of sensitive plant metabolites this results were nearby similar to Biswas et al., 2022; Das et al., 2016. Furthermore, extended extraction times allow for the exhaustive release of heavier, more complex lipophilic (fat-soluble) compounds that are denser and naturally darker as cited by Amaresh et al., 2017. As the lighter, highly volatile compounds evaporate or degrade under sustained heat, the remaining oil becomes more concentrated with heavier molecules, increasing its viscosity and darkening its color(Basak, S., & Guha, P. 2017).

4.2.2. Odor Profile

The aroma shifts from "fresh, sharp, slightly spicy" in trial 1 at temperature of 80° C for to "heavy, dense,

deeply pungent" and "clove-like" in trials 3 and 4. Trial 1 "Fresh" and "sharp" aromas are typically provided by highly volatile, lightweight monoterpenes like limonene, linalool, and p-cymene. As the temperature reaches 100°C in Trials 3 and 4, these lighter compounds are either lost to evaporation or thermally degraded, becoming "Not Detected" (ND) results are closed to finding by Joshi, R. K., et al., 2016. Simultaneously, the concentration of heavier phenolic compounds like eugenol (known for its distinct clove-like aroma) and chavicol (highly pungent) increases from 29.8% to 31.7% and 16.9% to 19.0%, respectively reported by Alam, M. B., et al.,2023. This accumulation of heavy phenols creates a much denser, spicier scent characteristic of high-eugenol chemotypes as seen in the reports shown by Prakash et al., 2010; Sugumaran et al., 2011.\

4.2.3. Specific Gravity and Refractive Index

The result for specific gravity and refractive index depicted in table no 02.Specific gravity (0.985 to 1.025) and refractive index (1.495 to 1.512) both increases progressively from Trial 1 to Trial 4. Due to this oil, contain heavy phenolic compounds i.e. eugenol and chavicol that have higher molecular weights, higher densities, and higher refractive indices compared to lighter monoterpenes this results were nearby similar to Fachriyah,et al., 2019. Especially high heat is used in Trial 3 and trail 4 that causes the loss of lightweight terpenes, which are presented in table no Table 2,which retained a higher percentage of the heavy phenolic compounds like eugenol, chavicol, that's leads to increase in overall density and light-bending capacity of the essential oil naturally. This result of specifica gravity and refractive index are in close agreement with the results reported by Guha, P., & Zari, S. R. (2017).

4.2.4. Acid Value

The acid value increases steadily from 1.02 mg KOH/g in Trial 1 to 1.83 mg KOH/g in Trial 4. During prolonged hydrodistillation at high temperatures, esters in the essential oil undergo a chemical reaction with water called hydrolysis, which breaks them down into free acids and alcohols the observed result are highly comparable with Madhumita et al., 2019 Looking at Table 3, the concentration of chavicol acetate (an ester) decreases from 16.8% in Trial 1 to 15.1% in Trial 4. The thermal hydrolysis of chavicol acetate and similar esters produces free acids over the 300-minute extraction, which directly explains the sharp rise in the acid value observed in table 2 the data obtained in the study closely matches to Amaresh et al., 2017).

4.2 GC–MS Volatile Composition under Different Extraction Conditions

4.2.1 Phenylpropanoids

Gas chromatography–mass spectrometry (GC–MS) analysis revealed that the essential oil of *Piper betle* L. contained 20 volatile compounds, which comprised approximately 98–99% of the total composition (Table 3). The volatile fraction was mainly composed of phenylpropanoids (eugenol, chavicol, and chavicol acetate) 63-65% of the total composition, followed by sesquiterpenes (29.8-30.8%) and minor amounts of monoterpenes (limonene, γ-terpinene, linalool, and p-cymene) (1.5-2.8%) these outcome agree with results of Guha, P., & Nandi, S 2019. This compositional pattern is in line with earlier studies Sarkar, U.2022 and Mondal, B.2023 and Biswas, P et al., 2022. Reporting phenylpropenoids, sesquiterpenes, and monoterpenes as the three major chemical groups in *P. betle* essential oil, accounting for approximately 90.3% of the total composition. The essential oil of P. betle L. showed 98.4% of volatile compounds in fresh leaves and 97.34% in cured leaves, confirming the authenticity of the volatile profile of this species reported by Sathiamoorthy, V., et al., 2022 .The findings are in accordance with the comparative metabolome profiling of popular betel cultivars, which identified chavicol, chavibetol, methyl chavicol, and β-caryophyllene as the major components in the most widely grown cultivars (Mondal B. and Kothari 2025).

Eugenol was the major constituent in all trials, increasing progressively from 29.8 ± 0.4% (Trial 1) to 31.7 ± 0.5% (Trial 4) in the final product after distillation. This is a 6.4% relative increase with increasing temperature and distillation time. The recorded eugenol value is in agreement with previous reports that eugenol is the major constituent in *P. betle* essential oil, with values of 29.85-33.88%. Chromatographic analysis has shown that *P. betle* leaf essential oils contain eugenol at a concentration of approximately 40%, which is a high eugenol content that is typical for this species. Some studies on Vietnamese P. betle reported by Minh, P. T., et al., 2025 followed by eugenol, indicating geographical and chemotypic variations in the phenylpropanoid composition. The increased percentage of eugenol at higher extraction temperatures indicates a better release of this phenylpropanoid from the plant matrix, probably due to better cell wall disruption and higher volatility at 100°C our results share their similarity with Gupta, C. 2023. This result is consistent with that found in other essential oil-producing plants, where higher temperatures in hydrodistillation enhance the extraction efficiency of phenylpropanoids our results parallel the reports of Karak, S., 2018. The eugenol content in P. betle is found to varies in different Indian varieties with eugenol as major component in most of the chemotypes confirming its characteristic presence in different geographical regions and findings are strongly agree with Slama, et al., 2025.

Chavicol was the second major compound with the increasing trend similar to the Trial 1 (16.9 ± 0.3%) to Trial 4 (19.0 ± 0.4%) with a relative increase of 12.4% in its content. During the trials, a continuous increase was observed, demonstrating that the efficiency of chavicol extraction depends on time and temperature. P. betle var. The content of chavicol in this study was reported for Bangla, where chavicol was found to be a volatile compound with high abundance. Chromatographic analysis has shown that essential oils

of P. betle leaf contain chavicol upto 40% concentration, which indicates its importance as a major phenolic compound(Biswas,Petal.,2022) Whereas chavicol acetate showed a decreasing trend from 16.8 ± 0.3% (Trial 1) to 15.1 ± 0.3% (Trial 4) with a relative decrease of 10.1%. The reduction at higher temperature (100°C) and longer time (300 min) indicates the thermal degradation or hydrolysis of chavicol acetate into chavicol under more severe extraction conditions. The trend of ester hydrolysis is in line with the thermal degradation of acetate esters reported in essential oils during long hydrodistillation (Slama et al., 2025).

The methyl chavicol (estragole) content gradually decreased from 3.1 ± 0.1% to 2.6 ± 0.1%, which is in agreement with previous reports that monoterpenoid phenylpropanoids are more susceptible to thermal degradation at higher temperatures. Comparative metabolomic profiling studies have also reported the presence of methyl chavicol in P. betle essential oil and shown it to be a major component in the most commonly grown cultivars of this plant. Eugenyl diacetate showed a small increase from 2.5 ± 0.1% to 2.8 ± 0.1%, indicating improved formation or extraction of this compound at higher temperatures, possibly due to increased acetylation or better extraction efficiency of this less-volatile ester (Mondal & Kothari, S. K. (2025).

4.2.2 Sesquiterpenes

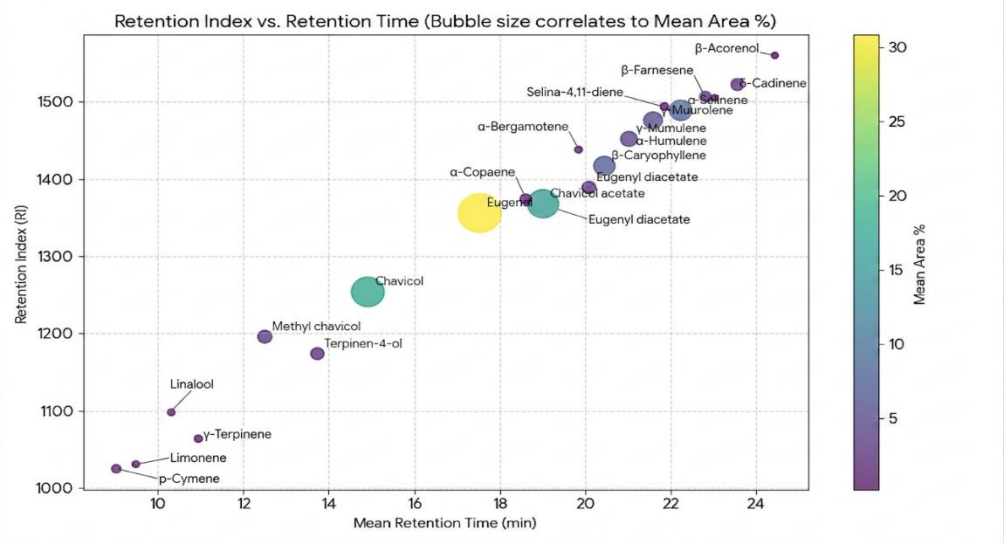
The concentration of β-caryophyllene increased from 6.9 ± 0.2% (Trial 1) to 7.4 ± 0.2% (Trial 3) and decreased slightly to 7.3 ± 0.2% in Trial 4. This relatively stable profile indicates that sesquiterpenes are more thermally stable than monoterpenes. The caryophyllene content is in accordance with previous GC–MS analyses, which reported 5.57–7.4% in *P. betle* essential oil. The presence of caryophyllene in P. betle leaf essential oil has been reported in studies by Ghosh K. and Bhattacharya (2005), with other sesquiterpenes such as cadinene well characterized in *P. betle* leaves.

α-Selinene decreased slightly from 8.4 ± 0.2% (Trial 1) to 7.9 ± 0.2% (Trial 4), whereas γ-muurolene increased from 5.2 ± 0.2% to 5.7 ± 0.2% in the final trial. α-Humulene was relatively stable at 4.0-4.3% These differences are due to the different extraction efficiencies and thermal stabilities of the sesquiterpene isomers. In the trials, the total sesquiterpene content varied between 29.8% and 30.8%, which shows a relatively stable sesquiterpene profile this data support previous research finding Al-Sayed, et al., 2021.

α-Copaene decreased significantly from 2.2 ± 0.1% (Trial 1) to 0.8 ± 0.05% (Trial 4), a relative decrease of 63.6%, suggesting its thermal degradation at higher temperatures. α-bergamotene also decreased significantly from 0.9 ± 0.05% to ND in Trial 4, indicating its high temperature sensitivity. These results are consistent with the known thermal instability of some sesquiterpene hydrocarbons under prolonged heating these results are closely aligned with (Slama et al. (2025).

4.2.3 Monoterpenes

The greatest temperature-dependent change was observed for monoterpenes. Limonene was reduced from 0.6 ± 0.05 (Trial 1) to ND (Trial 4) in the present study. γ-Terpinene decreased from 0.9 ± 0.05% to ND, and linalool decreased from 0.6 ± 0.05% to ND. p-Cymene decreased from 1.3 ± 0.1% to 0.2 ± 0.02%. The results clearly demonstrate that monoterpenes are highly volatile and thermally unstable compounds that degrade or evaporate significantly at 100°C after prolonged exposure (240-300 min) these outcome accordance with the trends identified by Madhumita M. et al., 2019



4.3 Relationship between Extraction Conditions and GC-MS Chemical Profile

The data reveal a clear relationship between hydrodistillation conditions and the essential oil composition. As the temperature and time increased:

4.3.1 Oil yield increased (0.86% → 1.15%) owing to enhanced cell wall disruption and improved extraction efficiency this data aligns perfectly with the literature of Madhumita, M., et al., 2019 and Chemat et al., 2020.

4.3.2 Phenylpropanoids (eugenol, chavicol) increased, suggesting that these compounds are more efficiently extracted at higher temperatures and are thermally stable, consistent with exhaustive hydrodistillation optimization studies our results are consistent with previous observation mabe by Golmakani et al., 2017 and K Ghosh , T K Bhattacharya 2005

4.3.3 Monoterpenes decreased or disappeared, indicating high thermal volatility and susceptibility to degradation at 100°C the observed results are highly comparable to Slama, M., v 2025 and Fotsing Yannick Stephane & Kezetas Jean Jules, 2020.

4.3.4 Sesquiterpenes remained relatively stable, consistent with their higher molecular weight and thermal stability compared to monoterpenes. These outcomes bear a strong resemblance to the finding of K Ghosh 1, T K Bhattacharya 2005 and Oliveira et al., 2017.

4.3.5 Chavicol acetate decreased, likely due to hydrolysis to chavicol under prolonged heating. Our results further substantiate the clams made by Madhumita, M., et al., 2019 and Fotsing Yannick Stephane & Kezetas Jean Jules, 2020 and Golmakani et al., 2017.

5. Conclusion

The best essential oil yield i.e. 15% and the richest phenolic profile were achieved by the distillation procedure at 100°C for 300 min. It has been concluded that, the extraction temperature and time had a significant effect on the production of volatile fingerprints of *Piper betle* essential oil. The high extraction temperature significantly increased the high-boiling volatile compounds such as eugenol and chavicol, while the low-boiling volatile compounds such as limonene, linalool, and γ-terpinene significantly decreased during the extraction period. Thermal hydrolysis of ester compounds was a characteristic change in the composition, because the consistent decrease in chavicol acetate was observed at high temperature under long conditions.The overall physicochemical parameters revealed an increasing predominance of aromatic and phenolic components with the increasing intensity of extraction. The optimized essential oil was found to have a composition suitable for food applications andflavoring or as a natural preservative. The possible biological activities of these compounds including antimicrobial and antioxidant activities are worth investigating as they provide validation for their functional potential. Moreover, the development of delivery systems such as microencapsulation has increased their stability and controlled release increasing their applicability in food systems.

Conflict of Interest declaration: The authors declare no conflicts of interest.

Author Contributions: Conceptualization, methodology, data collection, formal analysis, writing original draft. R.V.S.: Supervision, conceptualization, resources, writing, review, and editing. Both authors have read and approved the final manuscript.

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