Trop J Nat Prod Res, September 2025; 9(9): 4304 - 4309 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Chemical Composition and Anti-Inflammatory Activity of Essential Oils from *Piper betle* Leaves Growing in Vietnam

Pham T. H. Minh[1,2], Vu T. Ha[1], Hoang T. Bich[1], Dinh T. T. Thuy[1], Pham M. Quan[1], Vu T. Dat[1], Nguyen T. Hue[1], Nguyen N. Linh[3], Nguyen T. Tuan[4], Do T. Lam[1,2]*

[1]*Institute of Chemistry, VAST, 18 Hoang Quoc Viet, Nghia Do, Hanoi, 10000, Vietnam*
[2]*Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Nghia Do, Hanoi, 10000, Vietnam*
[3]*Hanoi-Amsterdam High School for the Gifted, Hoang Minh Giam, Yen Hoa, Hanoi, 10000, Vietnam*
[4]*Institute of Life Science, Thai Nguyen University of Agriculture and Forestry, TNU, Quyet Thang, Thai Nguyen, 250000, Vietnam*

ARTICLE INFO

Article history:
Received 04 July 2025
Revised 28 July 2025
Accepted 01 August 2025
Published online 01 September 2025

ABSTRACT

Piper betle commonly called betel, is a medicinally important plant used traditionally for the treatment of inflammatory conditions, gastrointestinal disorders, hepatic diseases, and microbial infections. *Piper betle* leaves are particularly rich in essential oil with numerous bioactive constituents. This study aimed to investigate the chemical composition and anti-inflammatory activity of essential oils (EOs) from *Piper betle* leaves. The EOs were extracted via steam distillation from fresh and dried leaves collected at three distinct time points in 2022 (January, May, and September). The chemical profile of the EOs were determined by gas chromatography-mass spectrometry (GC-MS). The anti-inflammatory activity of the EOs was evaluated by the inhibition of lipopolysaccharide (LPS)-stimulated nitric oxide (NO) production in murine RAW 264.7 macrophage cells. GC-MS analysis identified chavibetol and eugenol acetate as major EO components, with varying numbers of compounds detected across samples and collection periods. Chemical profiling consistently revealed significant differences between fresh and dried leaf EOs, as well as notable variations across collection times. Phenylpropanoids (44.56 – 84.14%) and hydrocarbon sesquiterpenes (6.86–39.71%) were the dominant compound classes in all EOs. Regarding anti-inflammatory activity, the January fresh EO exhibited the highest potency with IC_{50} value of 26.33 ± 1.51 µg/mL), followed by the September fresh EO (IC_{50} = 35.71 ± 2.05 μg/mL). These findings highlight the variability in *Piper betle* EO composition based on drying and collection time, underscoring their potential as a natural source of anti-inflammatory agents.

Keywords: Piper, *Piper betle*, Essential Oils, Anti-Inflammatory, Steam Distillation.

Introduction

Piper betle L. (Family Piperaceae), commonly known as betel, is a medicinally important plant indigenous to Southeast Asia, India, and Sri Lanka. In Vietnam, it is cultivated nationwide. Traditionally, the leaves are used as a mouth freshener due to their antibacterial efficacy against halitosis, while leaf chewing exhibits stimulatory effects on both nervous and cholinergic systems.[1-3] Phytochemical studies have revealed that *P. betle* leaves contain diverse bioactive compounds, primarily volatile oils, glycosides, saponins, flavonoids, and tannins.[4,5] These phytoconstituents underlie the plant's well-established pharmacological activities, including anti-inflammatory, gastroprotective, hepatoprotective, antibacterial, and antiseptic properties. As such, *Piper betle* has been widely utilized in traditional medicine for treating inflammatory conditions, gastrointestinal disorders, hepatic diseases, and microbial infections.[6,7] *Piper betle* leaves typically yield approximately 0.2% w/w of EOs, which consist predominantly of monoterpenes, sesquiterpenes, phenylpropanoids, and aldehydes.

*Corresponding author. Email: dotienlam198@gmail.com
Tel: (+84)989856515

Citation: Minh PTH, Ha VT, Bich HT, Thuy DTT, Quan PM, Dat VT, Hue NT, Linh NN, Tuan NT, Lam DT. Chemical composition and anti-inflammatory activity of essential oils from *Piper betle* leaves growing in Vietnam. Trop J Nat Prod Res. 2025; 9(9): 4304 – 4309 https://doi.org/10.26538/tjnpr/v9i9.28

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The chemical profile of these EOs demonstrates substantial variation influenced by factors such as plant origin, developmental stage, and harvest time. Importantly, these volatile constituents collectively govern both the organoleptic characteristics (aroma and flavour profile) and bioactivity of *Piper betle* EOs.[8,9]

Chromatographic analysis demonstrated that the EOs derived from *Piper betle* leaves contain eugenol (40%) and chavicol (up to 40%) as primary components, along with other significant constituents like α-terpinene, methyl chavicol, *p*-cymene, α-copaene, anethole, β-caryophyllene, α-humulene, and chavibetol.[10-12] Pharmacological investigations of *Piper betle* EOs have revealed their anti-inflammatory activity,[13] as well as distinctive broad-spectrum antibacterial activity against both Gram-negative and Gram-positive bacteria.[14-18] Particularly noteworthy was their ability to inhibit biofilm formation and extracellular matrix production in *Pseudomonas aeruginosa*.[17] Both *in vivo* and *in vitro* studies have confirmed the anti-inflammatory properties of chavibetol, chavibetol acetate, and chavicol isolated from *Piper betle* EOs.[19,20] Notably, hydroxychavicol demonstrated significant suppression of lipid peroxidation and TNF-α production in human neutrophils, while effectively reducing edema and swelling through downregulation of pro-inflammatory cytokines.[21-24] In addition, hydroxychavicol and eugenol from *Piper betle* leaves EOs have shown potent inhibition of key inflammatory enzymes, including xanthine oxidase and lipoxygenase. [25] This study aimed to investigate the seasonal variation in chemical composition and *in vitro* anti-inflammatory activity of EOs extracted from *Piper betle* leaves in Vietnam. The EOs were obtained through steam distillation and analyzed using gas chromatography-mass spectrometry (GC-MS) to characterize their phytochemical profiles across different harvesting periods.

Materials and Methods

Plant collection and identification

The leaves of *Piper betle* L. were collected from Thai Nguyen city, Thai Nguyen province, Vietnam (21°35'41.7"N, 105°48'39.2"E) during three seasonal periods: January 2022 (PB1), May 2022 (PB2), and September 2022 (PB3). The plant was authenticated at the herbarium of the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), where herbarium specimens with voucher numbers PB1.202201, PB2.202205, and PB3.202209, for PB1, PB2, and PB3, respectively, were deposited.

Essential oil extraction

The leaves of *Piper betle* (500 g/species/time) were subjected to hydro-distillation for 7 hours using a Clevenger-type apparatus (Techlab, Vietnam). The Eos obtained were pooled for component identification and *in vitro anti*-inflammatory activity.

Phytochemical analysis of essential oils

The qualitative analysis of the essential oils was carried out on an Agilent Technologies HP7890A GC equipped with a mass spectrum detector (MSD) Agilent Technologies HP5975C and a HP5-MS column (60 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies). The injector and detector temperatures were set at 250 and 280ºC, respectively. The column temperature was programmed to start at 60ºC, and increase to 240ºC at 4ºC/min. Helium was used as the carrier gas at a flow rate of 1 mL/min. Essential oils samples were injected by auto sampling in 1 µL volume in split mode at a split ratio of 100:1. The MSD conditions were as follows: ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range 35 - 450 amu under full scan. A homologous n-alkane series was used as the standard to calculate retention time indices (RI) of each component. MassFinder 4.0 software connected to the HPCH1607, W09N08 libraries, and the NIST Chemistry WebBook was used to match mass spectra and retention indices. The results were further confirmed by comparison with data of authentic compounds reported in the original literature.

The quantitative analysis of the essential oils was carried out on an Agilent Technologies HP7890A GC equipped with a flame ionization detector (FID) Agilent Technologies and a HP5-MS column (60 m x 0.25 mm, film thickness 0.25 µm, Agilent Technologies) with same condition above. The relative amounts of individual components were calculated based on the GC peak area (FID response) without correction.[26]

Determination of anti-inflammatory activity

The anti-inflammatory activity of the EOs was evaluated by the inhibition of lipopolysaccharide (LPS)-stimulated nitric oxide (NO) production in murine RAW 264.7 macrophage cells (American Type Culture Collection, Manassas, VA, USA). *Piper betle* EOs were tested at various concentrations to determine their dose-dependent inhibitory effect against NO production. All experiments were performed at the Institute of Chemistry, VAST.

RAW 264.7 cells were cultured in DMEM supplemented with 10% FBS at 37°C in a 5% CO_2 atmosphere for 48 hours, and then seeded (2.5×10^5 cells/well) in 96-well plates. Following adherence, cells were stimulated with LPS (0.1 mg/mL) in the presence or absence of test compounds at various concentrations, with cardamonin included as a positive control. After 24 hours of incubation, nitric oxide levels in the culture supernatants were quantified using Griess reagent. Absorbance was measured at 570 nm, nitric oxide concentration was estimated from sodium nitrite ($NaNO_2$) standard curve.[27]

Cell survival rate was calculated using the formula shown in Equation 1:

$$\text{Cell survival rate (\%)} = \left(\frac{\text{OD [sample]}}{\text{OD [control (−)]}}\right) x\ 100 \quad \ldots\ldots\ldots\ldots\ldots \quad (1)$$

Statistical analysis

Data were presented as mean ± standard deviation (SD) of triplicate determination. SD was calculated as the square root of variance using the STDEV function in Excel 2016 (Microsoft Corporation, Redmond, WA).

Results and Discussion

Chemical constituents of Piper betle EOs

The EOs extracted from both fresh and dried *Piper betle* leaves collected in January (PB1), May (PB2), and September (PB3) 2022 were analyzed by GC-MS. The chemical compositions and relative abundances of identified compounds are presented in Table 1 and Figure 1.

For samples collected in January (PB1), 28 compounds were identified in the fresh sample, accounting for 96.51% of the total EO weight. The composition of the fresh leaf EO comprised: 5 oxygenated monoterpenes (17.86%), 13 hydrocarbon sesquiterpenes (42.86%), 5 oxygenated sesquiterpenes (17.86%), and 5 benzene derivatives (14.29%). For the dried leaves, 29 compounds were identified, representing 98.41% of the total EO weight, consisting of 8 oxygenated monoterpenes (27.59%), 10 hydrocarbon sesquiterpenes (31.03%), 5 oxygenated sesquiterpenes (17.24%), and 6 benzene derivatives (17.24%).

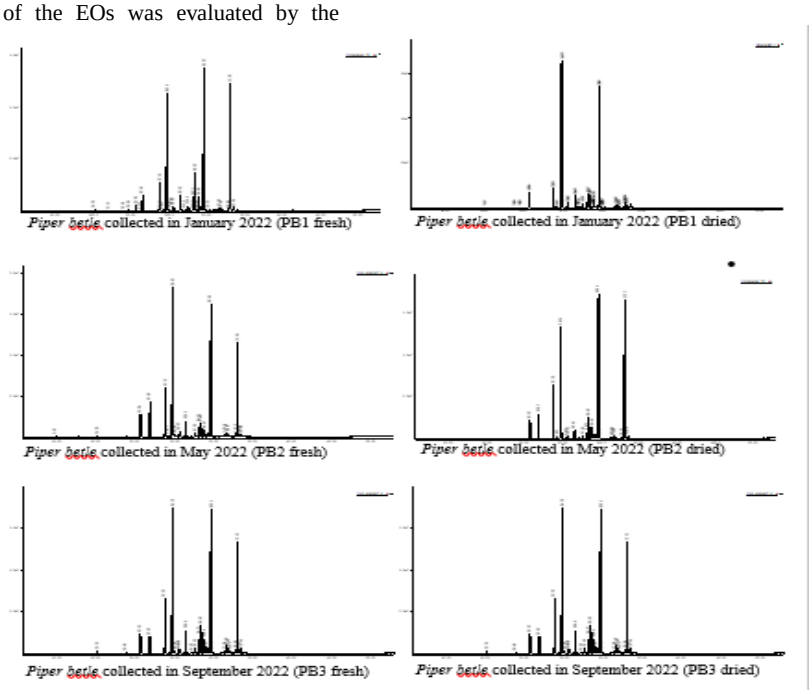

Figure 1: The GC-MS chromatograms of *Piper betle* EOs

Table 1: Chemical composition of *Piper betle* EOs

No.	Chemical name	Formula	PB1 (Fresh)			PB1 (Dried)			PB2 (Fresh)			PB2 (Dried)			PB3 (Fresh)			PB3 (Dried)		
			Time	*RI*	*%FID*	*Time*	*RI*	*%FID*	*Time*	*RI*	*%FID*	*Time*	*RI*	*%FID*	*Time*	*RI*	*%FID*	*Time*	*RI*	*%FID*
1	*α*-Pinene	$C_{10}H_{16}$	-	-	-	-	-	-	9.81	938	0.16	-	-	-	-	-	-	-	-	-
2	Eucalyptol	$C_{10}H_{18}O$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12.93	1036	0.27
3	(*E*)-*β*-Ocimene	$C_{10}H_{16}$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13.33	1048	0.17
4	Linalool	$C_{10}H_{18}O$	15.17	1102	0.20	15.16	1101	0.29	15.16	1101	0.25	-	-	-	15.15	1101	0.34	15.15	1101	0.73
5	Citronellal	$C_{10}H_{18}O$	-	-	-	17.03	1155	0.11	-	-	-	-	-	-	-	-	-	-	-	-
6	Methyl Chavicol	$C_{10}H_{12}O$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18.75	1204	0.14
7	Decanal	$C_{10}H_{20}O$	18.86	1207	0.23	18.85	1206	0.16	-	-	-	-	-	-	18.84	1206	0.23	18.84	1206	0.43
8	Citronellol	$C_{10}H_{20}O$	19.63	1229	0.21	19.62	1229	0.31	-	-	-	-	-	-	-	-	-	-	-	-
9	Chavicol	$C_9H_{10}O$	20.64	1259	3.03	20.64	1259	1.15	20.64	1258	2.70	20.63	1258	2.39	20.63	1258	2.07	20.63	1258	2.47
10	Geranial	$C_{10}H_{16}O$	-	-	-	21.17	1274	0.16	-	-	-	-	-	-	-	-	-	-	-	-
11	Perilla aldehyde	$C_{10}H_{14}O$	-	-	-	21.50	1284	2.66	-	-	-	-	-	-	-	-	-	-	-	-
12	(*E*)- Anethole	$C_{10}H_{12}O$	-	-	-	-	-	-	21.82	1293	3.16	21.81	1292	2.28	21.81	1292	1.27	21.81	1292	1.90
13	*δ*-Elemene	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23.59	1346	0.17
14	Chavicol acetate	$C_{11}H_{12}O_2$	23.76	1351	2.59	23.75	1351	3.15	23.77	1351	4.72	23.77	1351	5.18	23.77	1351	4.71	23.76	1351	4.73
15	Eugenol	$C_{10}H_{12}O_2$	24.24	1365	0.28	24.23	1365	0.19	24.23	1365	0.30	24.23	1365	0.13	-	-	-	24.23	1365	0.15
16	Chavibetol	$C_{10}H_{12}O_2$	24.79	1382	47.79	24.74	1380	24.41	24.81	1383	39.62	24.75	1381	21.73	24.79	1382	32.27	24.74	1381	28.17
17	*α*-Copaene	$C_{15}H_{24}$	24.99	1388	0.13	24.97	1388	0.19	24.98	1388	0.23	-	-	-	24.98	1388	0.19	24.97	1387	0.33
18	*cis*-*β*-Elemene	$C_{15}H_{24}$	-	-	-	25.43	1402	0.64	-	-	-	25.43	1402	0.26	25.44	1402	0.36	25.43	1402	0.61
19	Methyl eugenol	$C_{11}H_{14}O_2$	25.62	1407	0.99	25.61	1407	0.58	25.61	1407	0.75	25.61	1407	0.31	25.61	1407	0.42	25.61	1407	0.32
20	*α*-*cis*-Bergamotene	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26.12	1423	0.16
21	(*E*)-Caryophyllene	$C_{15}H_{24}$	26.49	1435	1.89	26.47	1434	2.10	26.49	1435	1.76	26.47	1434	0.98	-	-	-	26.48	1435	3.46
22	*β*-Gurjunene	$C_{15}H_{24}$	26.75	1443	0.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	*α*-*trans*-Bergamotene	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26.77	1444	0.17
24	Aromadendrene	$C_{15}H_{24}$	27.10	1455	0.42	27.09	1454	0.25	-	-	-	-	-	-	-	-	-	27.09	1454	0.16
25	(Z)-*β*-Farnesene	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27.25	1459	0.16
26	*α*-Humulene	$C_{15}H_{24}$	27.57	1469	3.62	27.56	1469	0.82	27.56	1469	0.92	27.55	1469	0.68	27.56	1469	0.55	27.56	1469	1.03
27	*δ*-Muurolene	$C_{15}H_{24}$	28.17	1489	2.10	28.17	1488	1.83	28.18	1489	1.46	28.17	1488	0.77	28.17	1488	1.67	28.17	1488	1.98
28	Germacrene D	$C_{15}H_{24}$	28.41	1496	1.47	28.41	1496	4.70	28.41	1496	1.56	28.41	1496	2.31	28.41	1496	2.41	28.40	1496	4.02
29	*α*-Zingiberene	$C_{15}H_{24}$	28.60	1502	0.23	28.59	1502	0.25	-	-	-	-	-	-	28.59	1502	0.29	28.59	1502	0.28
30	Viridiflorene	$C_{15}H_{24}$	28.83	1510	1.25	-	-	-	-	-	-	-	-	-	28.81	1509	0.47	-	-	-
31	Bicyclogermacrene	$C_{15}H_{24}$	28.88	1512	1.19	28.88	1511	4.34	28.87	1511	2.54	28.87	1511	3.44	28.88	1511	1.51	28.88	1511	1.25
32	*β*-Bisabolene	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	-	-	-	-	29.00	1516	1.44	29.01	1516	2.90
33	Eugenol acetate	$C_{12}H_{14}O_3$	29.61	1536	25.17	29.61	1536	25.91	29.64	1537	24.31	29.65	1537	28.75	29.65	1537	26.67	29.60	1536	21.04
34	(*E*)-*δ*-Bisabolene	$C_{15}H_{24}$	29.76	1541	0.34	29.75	1541	0.26	-	-	-	29.75	1541	0.15	29.75	1541	0.31	29.74	1540	0.42
35	*trans*-Cadina-1,4-diene	$C_{15}H_{24}$	29.91	1546	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	*α*-Cadinene	$C_{15}H_{24}$	30.06	1551	0.11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	Elemicine	$C_{12}H_{16}O_3$	-	-	-	30.31	1559	0.14	-	-	-	-	-	-	-	-	-	-	-	-

38	Spathulenol	$C_{15}H_{24}O$	-	-	-	-	-	-	31.39	1595	0.40	31.39	1595	0.17	31.39	1595	0.28	31.37	1595	0.18
39	Caryophyllene oxide	$C_{15}H_{24}O$	-	-	-	31.59	1603	0.19	31.59	1602	0.49	31.59	1602	0.28	31.59	1603	0.73	31.59	1602	0.55
40	Guaiol	$C_{15}H_{26}O$	31.60	1603	0.61	31.84	1611	0.38	31.85	1612	0.18	31.85	1611	0.21	31.85	1612	0.51	31.84	1611	0.46
41	Cubeban-11-ol	$C_{15}H_{26}O$	31.89	1613	0.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
42	Rosifoliol	$C_{15}H_{26}O$	32.10	1620	0.16	-	-	-	-	-	-	-	-	-	32.09	1620	0.18	32.08	1620	0.15
43	1-*epi*-Cubenol	$C_{15}H_{26}O$	32.80	1645	0.91	32.79	1644	0.15	32.79	1644	0.24	32.79	1644	0.16	32.79	1645	0.27	32.78	1644	0.23
44	1,2-Diacetoxy-4-allylbenzene	$C_{13}H_{14}O$	32.95	1650	0.51	33.06	1654	21.79	33.05	1654	13.02	33.11	1656	29.05	33.07	1654	16.54	33.05	1658	0.38
45	*epi*-α-Cadinol	$C_{15}H_{26}O$	33.18	1658	0.36	33.13	1657	0.35	33.21	1659	0.18	-	-	-	33.17	1658	0.46	33.16	1658	0.38
46	α-Muurolol	$C_{15}H_{26}O$	-	-	-	-	-	-	33.27	1661	0.29	33.27	1662	0.14	33.27	1661	0.32	33.27	1661	0.27
47	α-Cadinol	$C_{15}H_{26}O$	-	-	-	33.54	1671	0.39	33.54	1671	0.22	33.55	1671	0.24	33.55	1671	0.51	33.54	1671	0.42
Monoterpene hydrocarbons (%)			**-**			**-**			**4.35**			**-**			**-**			**2.78**		
Oxygenated monoterpenes (%)			**17.86**			**27.59**			**17.39**			**19.05**			**11.11**			**19.44**		
Sesquiterpene hydrocarbons (%)			**42.86**			**31.03**			**21.74**			**33.33**			**29.63**			**36.11**		
Oxigenated sesquiterpens (%)			**17.86**			**17.24**			**30.43**			**23.81**			**25.93**			**19.44**		
Derivatives of benzene (%)			**14.29**			**17.24**			**21.74**			**23.81**			**18.52**			**16.67**		

(-): Not available, RI: Retention index, %FID: % peak area contribution by the flame ionization detector

For the samples collected in May (PB2), 23 compounds were detected in the fresh sample, constituting 99.44% of the total EO weight. The fresh sample composition featured 1 hydrocarbon monoterpene (4.35%), 4 oxygenated monoterpenes (17.39%), 6 hydrocarbon sesquiterpenes (21.74%), 7 oxygenated sesquiterpenes (30.43%), and 5 benzene derivatives (21.74%). The dried sample contained 21 compounds constituting 99.60% of the total EO weight, with 4 oxygenated monoterpenes (19.05%), 7 hydrocarbon sesquiterpenes (33.33%), 5 oxygenated sesquiterpenes (23.81%), and 5 benzene derivatives (23.81%).

For the September samples (PB3), 27 compounds were identified in the fresh sample (99.40% of total EO weight), including 4 oxygenated monoterpenes (10.34%), 10 hydrocarbon sesquiterpenes (29.63%), 8 oxygenated sesquiterpenes (25.93%), and 5 benzene derivatives (18.52%). The dried sample yielded 36 compounds (98.56% of total EO weight), comprising 1 hydrocarbon monoterpene (2.78%), 7 oxygenated monoterpenes (19.44%), 15 hydrocarbon sesquiterpenes (36.11%), 8 oxygenated sesquiterpenes (19.44%), and 5 benzene derivatives (16.67%).

There are clear differences in the chemical composition of EOs obtained from *Piper betle* across different seasons.

For PB1, the dried samples contained higher levels of oxygenated monoterpenes (27.59%) than the fresh samples (17.86%). The hydrocarbon sesquiterpene content differed significantly between fresh (42.86%) and dried samples (31.03%), while oxygenated sesquiterpenes showed minimal variation (17.86% in fresh versus 17.24% in dried samples).

For PB2, fresh samples exhibited greater amounts of both hydrocarbon monoterpenes (4.35%) and oxygenated sesquiterpenes (30.43%) compared to dried samples (0% and 23.81%, respectively). The most notable compositional differences appeared in oxygenated monoterpenes (dried: 19.05%; fresh: 17.39%) and hydrocarbon sesquiterpenes (dried: 33.33%; fresh: 21.74%).

For PB3, dried samples again demonstrated higher concentrations of hydrocarbon monoterpenes (2.78% vs. 0% in fresh) and oxygenated sesquiterpenes (25.93% vs. 19.44% in fresh). The most substantial variations occurred in oxygenated monoterpenes (dried: 19.44%; fresh: 10.34%) and hydrocarbon sesquiterpenes (dried: 36.11%; fresh: 29.63%).

These compositional variations in *Piper betle* EOs likely result from multiple factors including environmental conditions, plant growth stage, harvesting methods, genetic variability, and seasonal physiological changes. The observed chemical diversity underscores how growth conditions and processing methods significantly influence both the composition and properties of EOs.

The EO composition of *Piper betle* leaves exhibited marked seasonal variations between fresh and dried samples.[28,29] January collections revealed chavibetol as the dominant component in fresh leaves (47.79%), contrasting with eugenol acetate (25.91%) in dried samples. This pattern persisted in May, with fresh leaves containing 39.62% chavibetol versus 28.75% eugenol acetate in dried samples. September samples showed similar trends, though at reduced concentrations (32.27% chavibetol in fresh versus 21.04% eugenol acetate in dried). Trace components including α-cadinene (0.11-0.32%), citronellal (0.11-0.45%), α-pinene (0.08-0.22%), α-copaene (0.15-0.41%), and methyl chavicol (0.09-0.38%) were consistently present across all samples. These compositional differences likely reflect complex interactions between seasonal environmental factors (temperature fluctuations, precipitation patterns), plant physiological changes during growth, and post-harvest processing methods. The consistent predominance of chavibetol in fresh leaves versus eugenol acetate in dried samples suggests distinct biochemical transformation pathways during the drying process, while the seasonal concentration variations indicate environmental influences on secondary metabolite production.[30,31]

The EO of the *Piper betle* not only contains chavibetol and eugenol acetate but also several other components that vary in quantity with season.[32] These components include: guaiol, chavicol, chavicol acetate, methyl eugenol, α-humulene, δ-muurolene, germacrene D, bicyclogermacrene, 1-*epi*-cubenol, and 1,2-diacetoxy-4-alkylbenzene. Worldwide studies have classified these chemical components into five main groups: phenylpropanoid, hydrocarbon monoterpene, oxygenated monoterpene, hydrocarbon sesquiterpene, and oxygenated sesquiterpene. Among these, phenylpropanoid (44.56-84.14%) predominates in all EO samples, followed by hydrocarbon sesquiterpene (6.86-39.71%).

While chavibetol and eugenol acetate have been established as the primary bioactive compounds in Indian *Piper betle* EOs, Vietnamese specimens exhibit a distinct phytochemical profile. Our research in Vietnam has identified significant sesquiterpenoid constituents including γ-elemene, valeranone, and ishwarone in the EO composition. Seasonal analyses revealed consistent chemical constituents across

different collection periods, with variations primarily manifested in oil yield rather than qualitative composition. These observed differences may be attributed to multiple influencing factors including geographical variation, local geological conditions, and region-specific harvesting and processing techniques. This study not only broadens the knowledge on documented phytochemical spectrum of *Piper betle* EOs but also provides valuable insights into the seasonal dynamics of oil production, highlighting how environmental and anthropogenic factors can influence secondary metabolite expression in medicinal plants.

Anti-inflammatory activity of Piper betle EOs

The *in vitro anti*-inflammatory activity of EOs obtained from the leaves of *Piper betle* were evaluated through inhibition of NO production in the RAW 264.7 macrophage cells. The experimental data were recorded and shown in Figure 2.

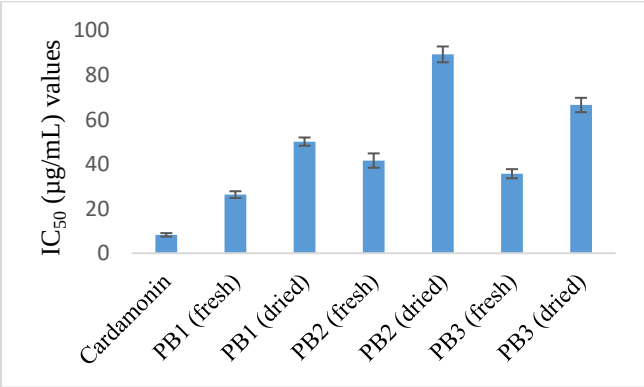

Figure 2: The IC_{50} (µg/mL) values of inhibition of NO production by EOs obtained from the leaves of *Piper betle*. Data are mean ± SD, n = 3. Cardamonin was used as positive control

The results presented in Figure 2 demonstrate significant NO production inhibition in RAW 264.7 macrophages by all tested EO samples, with IC_{50} values ranging from 26.33 to 89.32 µg/mL across the investigated concentration range compared to the positive control cardamonin (IC_{50} = 8.25 ± 0.79 µg/mL). Notably, fresh leaves EOs exhibited superior anti-inflammatory activity, with PB1 (fresh) showing the strongest inhibition (IC_{50} = 26.33 ± 1.51 µg/mL), followed by PB3 (fresh, IC_{50} = 35.71 ± 2.05 µg/mL) and PB2 (fresh, IC_{50} = 41.63 ± 3.22 µg/mL). In contrast, dried leaves EOs demonstrated markedly reduced potency, displaying IC_{50} values of 50.14 ± 1.85 µg/mL (PB1 dried), 66.58 ± 3.23 µg/mL (PB3 dried), and 89.33 ± 3.56 µg/mL (PB2 dried). These findings indicate that fresh *Piper betle* leaves yield EOs with significantly enhanced anti-inflammatory properties (IC_{50} range: 26.33 - 41.63 µg/mL) compared to those from dried leaves (IC_{50} range: 50.14 - 89.33 µg/mL). The observed 1.9- to 3.4-fold difference in efficacy between fresh and dried preparations suggests substantial bioactive compound degradation or transformation during the drying process, highlighting the importance of processing methods for preserving the phytotherapeutic potential of *Piper betle* EOs.

Conclusion

This study demonstrates the variability in *Piper betle* L. EOs composition influenced by collection time and processing (fresh versus dried), with chavibetol and eugenol acetate consistently identified as major components. All EOs exhibited significant anti-inflammatory activity, notably with fresh samples showing superior potency (e.g., PB1 fresh EO with the highest activity). These findings not only enhance the understanding of *Piper betle* phytochemical diversity but also underscore its promising potential as a natural source of anti-inflammatory agents. Future research should focus on isolating and elucidating the mechanisms of action of the most active compounds, optimizing harvest and processing protocols based on seasonal variations, and conducting *in vivo* studies to validate their therapeutic applications.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research was funded by Grant for senior research 2022-2023 under code number NCVCC.07.06/22-23.

References

1. Ni MDMWN, Maria MVS, Dwi AS, Putu ESKY, Ni LKAAD, Erna C, Rika H. *Piper betle* (L): Recent Review of Antibacterial and Antifungal Properties, Safety Profiles, and Commercial Applications. Molecules. 2021; 26(8):2321.
2. Fazal F, Mane PP, Rai MP, Thilakchand KR, Bhat HP, Kamble PS, Palatty PL, Baliga MS. The Phytochemistry, Traditional Uses and Pharmacology of *Piper Betel*. Linn (Betel Leaf): A Pan-Asiatic Medicinal Plant. Chin J Integr Med. 2014; 20(10):820–830.
3. Kaypetch R and Thaweboon S. Antifungal Property of *Piper Betle* Leaf Oil against Oral Candida Species. Matec Web Conf. 2018; 242:1–4.
4. Joesoef MR, Sumampouw H, Linnan M, Schmid S, Idajadi A, St Louis ME. Douching and Sexually Transmitted Diseases in Pregnant Women in Surabaya, Indonesia. Am J Obs Gynecol. 1996; 174(1):115–119.
5. Chowdhury U, Baruah PK. Betelvine (*Piper Betle* L.): A Potential Source for Oral Care. Curr. Bot. 2020; 11:87–92.
6. Arambewela L, Arawwawala M, Withanage D, Kulatunga S. Efficacy of Betel Cream on Skin Ailments. J Complement Integr Med. 2010; 7(1):1–11.
7. Breijyeh Z, Jubeh B, Karaman R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules. 2020; 25(6):1340.
8. Hafidh RR, Abdulamir AS, Vern LS, Abu Bakar F, Abas F, Jahanshiri F, Sekawi Z. Inhibition of Growth of Highly Resistant Bacterial and Fungal Pathogens by a Natural Product. Open Microbiol J. 2011; 5(1):96–106.
9. Akpan A and Morgan R. Oral Candidiasis. Postgrad Med J. 2002; 78(922):455–459.
10. Benedict K, Chiller TM, Mody RK. Invasive Fungal Infections Acquired from Contaminated Food or Nutritional Supplements: A Review of the Literature. Foodborne Pathog Dis. 2016; 13(7):343–349.
11. Pawar S, Kalyankar V, Dhamangaonkar B, Dagade S, Waghmode S, Cukkemane A. Biochemical Profiling of Antifungal Activity of Betel Leaf (*Piper Betle* L.) Extract and Its Significance in Traditional Medicine. J Adv Res Biotechnol. 2017; 2(1):1–4.
12. Hafidh RR, Abdulamir AS, Vern LS, Abu Bakar F, Abas F, Jahanshiri F, Sekawi Z. Inhibition of Growth of Highly Resistant Bacterial and Fungal Pathogens by a Natural Product. Open Microbiol J. 2011; 5(3):96–106.
13. Saeed S, Farnaz S, Simjee R, Malik A. Triterpenes and *β*-sitosterol from *Piper betle*: Isolation, antiplatelet and *anti*-inflammatory effects. Biochem Soc Trans. 1993; 21(4):462S.
14. Devyani CK. An overview of betel leaf: Green gold of India. J Pharm Phyto. 2024; 13(2):240-248.
15. Tan YP and Chan EWC. Antioxidant, antityrosinase and antibacterial properties of Fresh and processed leaves of *Anacardium occidentale* and *Piper betle*. Food Biosci. 2014; 6:17–23.
16. Phumat P, Khongkhunthian S, Wanachantararak P, Okonogi S. Potential of *Piper betle* extracts on inhibition of oral pathogens. Drug Discov Ther. 2017; 11(6):307-315.
17. Teanpaisan R, Kawsud P, Pahumunto N, Puripattanavong J. Screening for antibacterial and antibiofilm activity in Thai

medicinal plant extracts against oral microorganisms. J Tradit Complement Med. 2017; 7(2):172-177.
18. Valle DL, Andrade JI, Puzon JJM, Cabrera EC, Rivera WL. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. Asian Pac J Trop Biomed. 2015; 5(7):532–540.
19. Khozirah S, Ling SK, Nik M. Evaluation of the anti-inflammatory properties of *Piper betle* L. Herba Pol. 2000: 46(4):308–318.
20. Ganguly S, Mula S, Chattopadhyay S, Chatterjee M. An ethanol extract of *Piper betle* Linn. mediates its *anti*-inflammatory activity via down-regulation of nitric oxide. J Pharm Pharmacol. 2007; 59(5):711–718.
21. Vaghasiya Y, Nair R, Chanda S. Investigation of Some *Piper* Species for *anti*-bacterial and *anti*-inflammatory Property. Inter J Pharm. 2007; 3(5):400–405.
22. Sarkar D, Saha P, Gamre S, Bhattacharjee S, Hariharan C, Ganguly S, Sen R, Mandal G, Chattopadhyay S, Majumdar S, Chatterjee M. Anti-inflammatory effect of allylpyrocatechol in LPS-induced macrophages is mediated by suppression of iNOS and COX-2 via the NF-κB pathway. Int. Immunopharmacol. 2008; 8(9):1264–1271.
23. Noor AM, Amirah AR, Siti HSAK. Hydroxychavicol as a potential anticancer agent (Review). Oncology Lett. 2023; 25(34):1–12.
24. Pandey A, Bani S, Dutt P, Suri KA, Modulation of Th1/Th2 cytokines and inflammatory mediators by hydroxychavicol in adjuvant induced arthritic tissues. Cytokine. 2010; 49(1):114–121.
25. Pin KY, Chuah AL, Rashih AA, Mazura MP, Fadzureena J, Vimala S, Rasadah MA. Antioxidant and *anti*-inflammatory activities of extracts of betel leaves (*Piper betle*) from solvents with different polarities. J Trop Sci. 2010; 12(4):448–455.
26. Falodun A, Siraj R, Choudhary MI. GC-MS Insecticidal Leaf essential oil of *P. staudtii* Hutch and Dalz (Icacinaceae). Trop J Pharm Res. 2009; 82(2):139-143.
27. Minh PTH, Tuan NT, Van NTH Bich HT, Lam DT. Chemical Composition and Biological Activities of Essential Oils of Four *Asarum* Species Growing in Vietnam. Molecules. 2023; 28(6):2580. https://doi.org/10.3390/molecules28062580.
28. Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afr J Trad Compl Altern Med. 2014; 11(3):221-227. Doi: 10.4314/ajtcam. v11i3.31
29. Kumar S. ExVivo, Studies On Gluconeogenes is Inhibitory Activity of *Piper betle* Inflorescence. Int J Engineer Appl Sci Technol. 2020; 5(2):264-268.
30. Dai DN, Pham TV, Dinh Luyen N, Dung VT, Huong LT, Son NT. Essential Oils of Two Vietnamese Plants Piper betle f. densum (Piperaceae) and Disepalum plagioneurum (Annonaceae): Chemical Composition, Antimicrobial, and Cytotoxic Activities. Nat Prod Commun. 2023; 18(7):1-7.
31. Saeid H, Mostafa G, Mojde S, Arman BK. A comparative study of essential oil profile, antibacterial and antioxidant activities of two cultivated *Ziziphora* species (*Z. clinopodioides* and *Z. tenuior*). Ind Crops Prod. 2020; 157(2):112942. Doi:10.1016/j.indcrop.2020.112942.
32. Kesavan S, Rajendran S, Sowmya M, Geetha GT, Vppalayam SP, Nandagopal B, Muthu GM, Sengottayan SN, Karamankodu JD, Sekarappa B, Chandish B. Toxicity, behavioural and biochemical effect of *Piper betle* L. essential oil and its constituents against housefly, *Musca domestica* L. Pestic Biochem Physiol. 2021; 174(2):104804. DOI:10.1016/j.pestbp.2021.104804.