

Chemical Composition, Enantiomeric Distribution and Antibacterial Activity of Kernel, Mace and Pericarp Essential Oils of Nutmeg (*Myristica fragrans*) from Bali, Indonesia

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Nutmeg (*Myristica fragrans* Houtt) is a significant spice commodity employed in the culinary, medicinal and cosmetic industries. This study reports the isolation, comparative analysis of metabolites and enantiomeric distributions of essential oils from the kernel, mace and pericarp of nutmeg sourced from Bali, Indonesia. Mace essential oil contains the largest proportion of hydrocarbon monoterpenes at 88.22%, in contrast to kernel essential oil at 59.58% and pericarp essential oil at 64.66%. Moreover, nutmeg pericarp exhibited a high concentration of myristicin (14.93%) and oxygenated monoterpene (15.55%). Seven enantiomers were found from all three nutmeg essential oils, including α -pinene, sabinene, β -pinene, terpinen-4-ol and α -phellandrene. Furthermore, pericarp essential oil exhibited a significant zone of inhibition in an antibacterial disc diffusion assay against *S. aureus* and *E. coli*. The oil demonstrated only moderate antibacterial activity, with a minimum inhibitory concentration (MIC) of 125 mg/L for pericarp essential oil. To our knowledge, this study is among the first to characterize the chemical composition and enantiomeric distribution of all three nutmeg components and assess the potential of nutmeg pericarp, an under-utilized agricultural residue, as a source of high-quality essential oil.

Keywords: Nutmeg, Kernel, Mace, Pericarp, Essential oil, Enantiomer.

INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.) is a tropical evergreen tree native to the Banda Islands, Indonesia but it is widely distributed to India, Sri Lanka, South Africa and the USA [1]. Indonesia is the world's leading exporter of nutmeg, followed by India and Sri Lanka, and accounts for more than 75% of global supply. Nutmeg is an important commercial crop with applications across the food and beverage sector, essential oil and aromatherapy products, pharmaceuticals and nutraceuticals and animal feed [2,3]. Various parts of the nutmeg plant serve as sources of essential oils and oleoresins, with reported applications involving the leaves, pericarp, mace (lacy aril), rind and kernel.

Chemical compositions of essential oils extracted from leaf, mace, kernel and seed of nutmeg grown in various geographical locations have been documented. Ashokkumar *et al.* [4] reported variation of chemical composition of essential oils extracted from the leaf, mace, kernel and seed of nutmeg har-

vested from India. Mickus *et al.* [5] reported the chemical compositions of nutmeg kernel essential oil obtained from Grenada. Moreover, a study on nutmeg seed essential oil from Sulawesi island Indonesia and nutmeg mace oil from Java Indonesian were reported by Dupuy *et al.* [6] and Ansory & Putri [7], respectively. An early investigation of essential oil extracted from nutmeg pericarp obtained from Malaysia was reported by Choo *et al.* [8]. However, there has been a less literature reporting the chemical compositions of essential oil where the raw material was harvested from a distant location, Bali, Indonesia.

Each part of the nutmeg essential oils displayed a distinctive aroma due to a varied chemical composition. The essential oil from nutmeg kernel (without shell) was rich in monoterpene hydrocarbons, with sabinene, α -pinene, β -pinene, limonene and β -myrcene as the major constituents [9,10]. Nutmeg leaf oil was characterized by a high sabinene content and the presence of phenylpropanoids such as eugenol and myristicin.

Mace essential oil appeared to contain monoterpene hydrocarbons and a small portion of myristicin.

Early literature reported that the pericarp essential oil was composed of monoterpenes (60%), monoterpene alcohols (29%), aromatic ethers (7%), sesquiterpenes and esters (1%) [8]. The nutmeg pericarp, abundant during the production of the seed, is also used in food and beverage preparations [11] as well as functional food ingredients [12]. However, there has been no study carried out to investigate the chemical composition of the oils extracted from three parts of nutmeg (pericarp, mace and kernel) simultaneously. In addition, there has been also limited literature reporting the enantiomeric composition of essential oil in various parts of nutmeg. Previous studies strongly suggested that the aroma and bioactivities of essential oils were dictated by the ratios of enantiomers that composed the essential oil. The enantioselective analysis of essential oil was carried out by a GC-MS equipped with a specialized β -cyclodextrin-based column. While GC-MS profiling, chiral GC analysis and antibacterial testing of nutmeg oil are individually well established, the present study extends this work by providing, within a single study, a side-by-side comparison of the chemical composition and enantiomeric distribution of essential oils obtained from three different parts (kernel, mace and pericarp) of Bali-grown nutmeg.

EXPERIMENTAL

The fresh and mature nutmeg fruits were harvested from a nutmeg plantation in West Bali, Indonesia (8°19'54.2" S 114°39'40.7" E) in October 2024. Each fruit was separated into three parts *i.e.*, pericarps, mace and kernel, before being air-dried under the shed for one week. The voucher specimens (PL251024) were ready for inspection at the Chemistry Laboratory, Universitas Pendidikan Ganesha, Bali, Indonesia.

Isolation of essential oils: Pericarp essential oils were obtained by steam distillation of dried pericarp (10 kg) in a semi-industrial scale distiller for 8 h. The procedure was performed in duplicate and the derived oil was dried using Na_2SO_4 and kept in dark glass vial at 4 °C until subsequent analysis. An identical procedure was also carried out to produce the mace and kernel essential oils.

Qualitative and quantitative analysis: The nutmeg essential oils were analyzed by gas chromatography equipped with a flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The GC-FID analysis was performed on a Shimadzu GC-2010 plus gas chromatography apparatus equipped with FID and a Rtx-5 capillary column (30 m $\times$ 0.25 mm, film thickness 0.25 μm). The injector and detector temperatures were set at 230 °C and 220 °C, respectively. Next, the column temperature program was held at 60 °C for 1 min, increased gradually to 185 °C at 3 °C/min, then maintained for 1 min and increased gradually to 275 °C at 9 °C/min, then maintained for 2 min. Helium was used as the carrier gas at a flow rate of 1 mL/min and the sample (1 μL) was injected neat with a split ratio of 20:1. The relative percentage of individual compounds was calculated based on peak area without using a response correction factor; accordingly, all reported percentages are semi-quantitative and should be regarded as approximate relative abundances rather than abso-

lute concentrations. Retention indexes of each component were calculated based on the retention times of the homologous hydrocarbon series C9–C24 injected in the same column temperature program.

In addition, GC-MS analysis was carried out using a Shimadzu QP2010 model equipped with an autosampler (AOC-20s), an auto-injector (AOC-20i) and an RTX-5 MS column (30 m $\times$ 0.25 mm ID and 0.25 μm film thickness). The column temperature was programmed exactly the same as that in the GC experiment. In addition, the mass spectrometer was operated in the electron impact (EI) mode at 70 eV and the mass scanning range was varied over 35–500 m/z . The ion source and interface temperatures were set at 280 °C and 220 °C, respectively. The identification of the essential oil constituents was based on their retention indices and mass spectral fragmentation patterns. The obtained mass spectra were compared with reference spectra in the Wiley 09 mass spectral library and with those reported by Adams [13].

Enantioselective analysis of essential oil: The enantiomeric composition of the nutmeg essential oils was determined by GC-MS using a Shimadzu QP2010 model equipped with an autosampler (AOC-20s), an auto-injector (AOC-20i) and an RTX- β DEXsm column (30 m $\times$ 0.32 mm ID and 0.25 μm film thickness). The oven temperature program, injector and detector temperatures were set the same as those the above GC-MS program. As the authentic enantiopure reference standards were not co-injected, the absolute-configuration and elution-order assignments were prepared by comparison with published enantioselective data on the same β -cyclodextrin stationary phase and are therefore reported as tentative. Enantiomeric distributions were calculated only for peak pairs that were baseline- or near-baseline-resolved; for co-eluting or poorly resolved pairs no enantiomeric ratio was estimated.

Antibacterial activity: The antibacterial properties of the essential oils were evaluated using a disc-diffusion assay [14]. The growth inhibition of the oils was tested against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. For the antibacterial assay, 100 μL of an overnight bacterial suspension was added to 5 mL of pre-warmed growth medium in a sterile Petri dish. Each essential oil was dissolved in DMSO at 1 mg/mL and 50 μL of the solution was loaded onto sterile 6 mm diameter Whatman No. 3 filter paper discs. The discs were incubated overnight at 37 °C, after which the inhibition-zone diameters were measured. Chloramphenicol (30 $\mu\text{g}/\text{disc}$; Sigma-Aldrich) and DMSO served as the positive and negative controls, respectively. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Essential oil yields: Ripe nutmeg fruits were harvested, separated into three main parts and air-dried to avoid the loss or decomposition of heat-labile compounds. Subsequently, the nutmeg essential oils were extracted by steam distillation and hydrodistillation as they are the two most popular industrial methods due to simplicity in operational and short processing time for large quantities of biomass with reduced labour cost [2]. The essential oil yields of nutmeg mace, pericarp and kernel from this study were found to be 4.2%, 6.5% and 5.4%, resp-

ectively. The mace essential oil yield (4.2%) was smaller than that found in the Western Ghats, southern India (8.10%), as reported by Ashokkumar *et al.* [4], but it was slightly higher than that grown in Central Java, Indonesia (3.56%) (Table-1). The nutmeg kernel essential oil yield obtained in the present study was lower than the yields reported for samples from India [4,15]. The only nutmeg pericarp essential oil yield reported in the literature showed a 2-3% yield, which was much smaller than that of ours (6.5%). The highest yield of nutmeg seed essential oil was reported by Riyanto [16], who obtained 11.63% oil by using pressurized steam distillation for 24 h on a semi-industrial scale.

Variations in nutmeg essential oil yield may arise from differences in extraction technique and biomass drying conditions, with hydrodistillation, steam distillation, sun drying, air drying and freeze-drying being commonly employed methods. The application of pressurized steam distillation (~2 bar) yielded much higher oils. However, it needs a specific distiller that can retain high-pressure steam. Fresh nutmeg pericarp essential oil yielded a lower oil content compared to that obtained from air-dried material, as in this study. It was also interesting to note that we extracted nutmeg essential oils from various parts of the same nutmeg fruit simultaneously; how-

ever other studies presented essential oils from separate individual parts of the nutmeg plant, except Ashokkumar *et al.* [10], who investigated the leaf, mace, kernel and seed simultaneously. In this study, the nutmeg kernel referred to the nutmeg without the rind; however, the nutmeg seed was associated with the kernel with the rind.

The chromatograms of nutmeg kernel, mace and pericarp essential oil displayed a broadly similar pattern, with α -pinene, sabinene and β -pinene as major hydrocarbon monoterpenes shared by all three oils (Fig. 1). Myristicin was likewise prominent in kernel and pericarp essential oil, but it was only a minor constituent of mace essential oil. Present findings are in full accordance with the chemical profile nutmeg seed essential oil harvested from Sulawesi Island, Indonesia [6]. Furthermore, Trifan *et al.* [2] also reported those 5 major metabolites from the nutmeg seed essential oil, except the terpinene-4-ol was replaced by limonene as the fifth largest component [2]. However, Mickus *et al.* [5] reported a quite different GC profile of nutmeg seed essential oil for the plant grown in Grenada. In this study, the monoterpene sabinene composed more than half of the oil and myristicin was present only in a small percentage.

TABLE-1
SUMMARY OF ESSENTIAL OIL YIELDS REPORTED FOR DIFFERENT PARTS OF THE NUTMEG PLANT

No.	Essential oil yield (% v/w)					Ref.
	Leaf	Mace	Pericarp	Kernel	Seed	
1	na	4.2	6.5	5.4	na	Present study
2	na	na	na	na	7.05	[2]
3	3.16	8.10	na	6.24	5.21	[4]
4	na	na	na	na	8.4	[5]
5	na	3.56	na	na	na	[7]
6	na	na	2-3	na	na	[8]
7	na	na	na	5.93	na	[15]
8	na	na	na	na	11.63	[16]
9	na	na	na	na	6.85	[17]

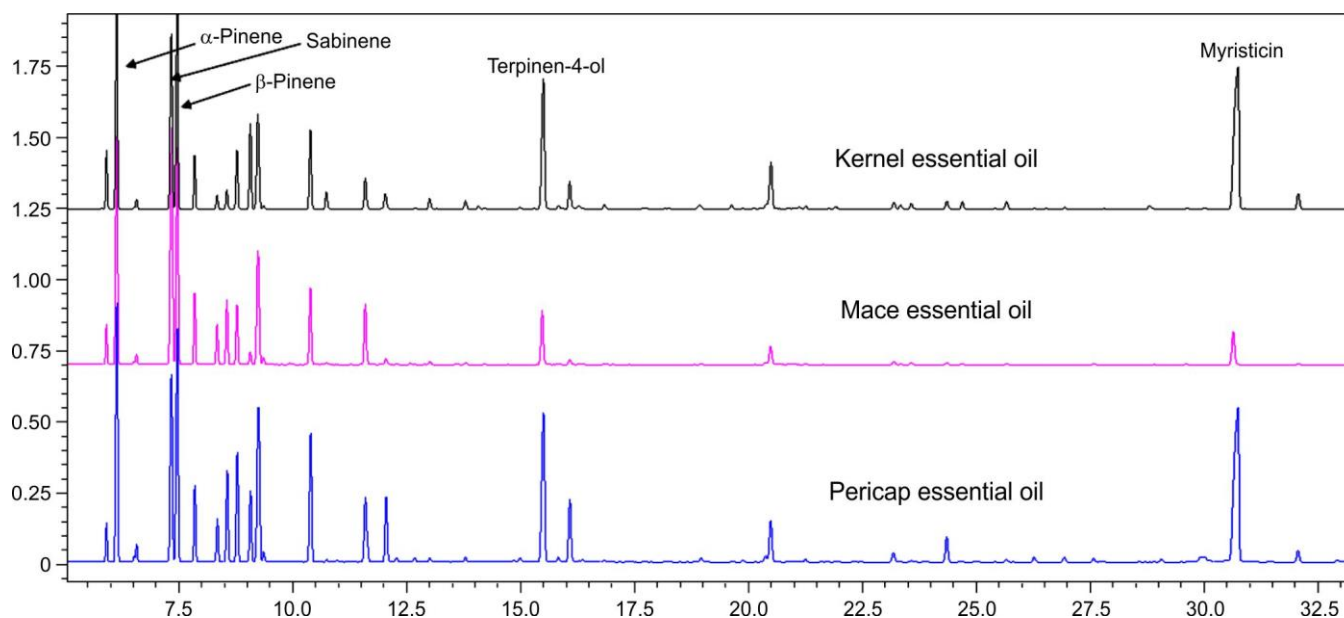


Fig. 1. GC-MS spectra of nutmeg kernel, mace, pericarp essential oils

There were 25, 28 and 29 compounds identified from the nutmeg mace, kernel and pericarp essential oil, respectively (Table-2). A small number of low-abundance peaks (each <0.5% of total area, listed as “Unknown” in Table-2) could not be assigned, as their mass spectra did not match the Wiley and Adams libraries above the acceptance threshold and no reliable retention index could be established; together these accounted for <1.5% of the total peak area in each oil and were retained in the table for completeness. The tabulated percentages are semi-quantitative FID peak-area values obtained without compound-specific response correction. Nutmeg mace essential oil consisted of the largest percentage of hydrocarbon monoterpene compounds (88.22%), followed by pericarp essential oil (64.66%) and kernel essential oil (59.58%). The presence of α -pinene, sabinene, β -pinene and limonene are examples of metabolites associated with this group of compounds and may have also influenced the aroma of the nutmeg oils. Nutmeg kernel essential oil was found to be rich in myristicin (18.52%), a signature compound of nutmeg oil which provides a spicy fragrance. The myristicin content of

nutmeg seed essential oil from various geographical origins in Indonesia ranges between 1.74% and 12.92% (Sulawesi Island) [6], 0.83% (West Java) [18] and 30.30% (Central Java) [16]. Riyanto [16] also reported that the highest percentage of myristicin in nutmeg essential oil was most likely due to using a pressurized steam distillation setup at 2 bar for 24 h. This specific treatment allowed the further breakdown of essential oil cavities in nutmeg tissue and therefore optimized the release of myristicin. Moreover, pericarp essential oil contained a significant percentage of these phenylpropanoid compounds (14.93%). This amount was much higher than that reported by Choo *et al.* [8] where the fresh and freeze-dried pericarp returned 1.0% and 3.0% myristicin, respectively.

The percentage of each volatile compound in essential oils has been associated with the physical properties and with the quality of the oils [19]. Since some essential oil constituents are chiral and their enantiomeric ratios can influence the overall aroma profile. The two enantiomers may have different odour characteristics due to their three-dimensional configurations interact differently with olfactory receptors [20]. The

TABLE-2
LIST OF CHEMICAL COMPOSITION OF ESSENTIAL OILS OBTAINED FROM NUTMEG MACE, KERNEL AND PERICARP USING GC-MS

No	Rt (min)	AI Exp.	AI Lit.	Compound	Nutmeg (%)		
					Mace	Kernel	Pericarp
1	5.909	926	921	α -Thujene	2.03	2.49	1.18
2	6.138	934	929	α -Pinene	15.61	10.68	10.69
3	6.571	949	945	Camphene	0.53	0.35	0.55
4	7.345	975	969	Sabinene	19.63	10.39	8.40
5	7.47	979	974	β -Pinene	15.60	11.42	10.23
6	7.848	991	985	b-Myrcene	4.19	2.42	2.68
7	8.339	1006	1002	α -Phellandrene	2.46	0.67	1.61
8	8.556	1012	1006	δ -3-Carene	4.15	0.91	3.60
9	8.775	1017	1012	α -Terpinene	3.92	3.07	4.45
10	9.068	1025	1020	<i>p</i> -Cymene	0.82	4.67	2.84
11	9.236	1029	1024	Limonene	9.85	6.46	8.53
12	9.357	1030	1026	1,8-Cineole	0.32	0.13	0.37
13	10.388	1059	1053	γ -Terpinene	5.43	4.56	5.81
14	10.747	1068	1065	<i>cis</i> -Sabinene hydrate	0.13	0.94	0.10
15	11.592	1091	1081	α -Terpinolene	4.61	1.92	3.49
16	12.046	1101	1095	<i>trans</i> -Sabinene hydrate	0.42	1.00	2.83
17	13.006	1120	1118	<i>cis-p</i> -Menth-2-en-1-ol	0.22	0.60	0.15
18	13.789	1132	1136	<i>trans-p</i> -Menth-2-en-1-ol	0.12	0.44	0.19
19	15.504	1180	1174	Terpinen-4-ol	4.38	10.03	8.84
20	16.077	1193	1190	α -Terpineol	0.40	1.71	3.07
21	20.497	1291	1294	Safrole	1.53	3.15	2.18
22	23.186	1376	1373	α -Ylangene	0.25	0.46	0.51
23	23.574	—	—	Unknown	0.13	0.37	nd
24	24.353	1376	1374	α -Copaene	0.15	0.52	1.30
25	25.663	—	—	Unknown	nd	0.49	nd
26	26.272	—	—	Unknown	nd	0.51	0.15
27	26.937	—	—	Unknown	nd	nd	0.26
28	27.576	—	—	Unknown	nd	nd	0.25
29	30.743	—	—	Unknown	nd	nd	0.20
30	32.061	1529	1515	Myristicin	3.10	18.52	14.93
31	24.353	1616	1616	(<i>Z</i>)-Asarone	nd	1.13	0.58
Hydrocarbon monoterpenes					88.22	60.0	64.66
Oxygenated monoterpenes					5.99	14.85	15.55
Hydrocarbon sesquiterpenes					0.40	0.98	1.81
Phenylpropanoids					4.63	22.80	17.69
Total identified					99.24	98.63	99.71

enantiomeric composition of nutmeg essential oils is therefore relevant, yet it remains insufficiently investigated. GC analysis using a β -cyclodextrin-based column revealed pairs of peaks corresponding to enantiomeric compounds. Seven constituents were detected as enantiomeric pairs across the three nutmeg essential oils, six of which were major components: α -pinene, sabinene, β -pinene, α -phellandrene, limonene and terpinen-4-ol (Fig. 2).

Separation of essential oil enantiomers by chiral GC presented a resolution challenge for several compounds. Some enantiomeric pairs appeared as well-resolved peaks, whereas

others showed partial or poor separation, preventing reliable estimation of their relative abundances from GC peak areas. Numerical enantiomeric distributions were therefore reported only for adequately resolved compounds, namely α -thujene, β -pinene, α -phellandrene, limonene and terpinen-4-ol (Table-3). The α -pinene and sabinene pairs were partially resolved or co-eluted and were consequently treated as single unresolved signals and marked with an asterisk in Table-3.

Poor resolution of α -pinene enantiomers has also been reported in previous chiral GC studies. Zimbalatti [21] observed near-overlapping (+)- and (–)- α -pinene peaks in mandarin

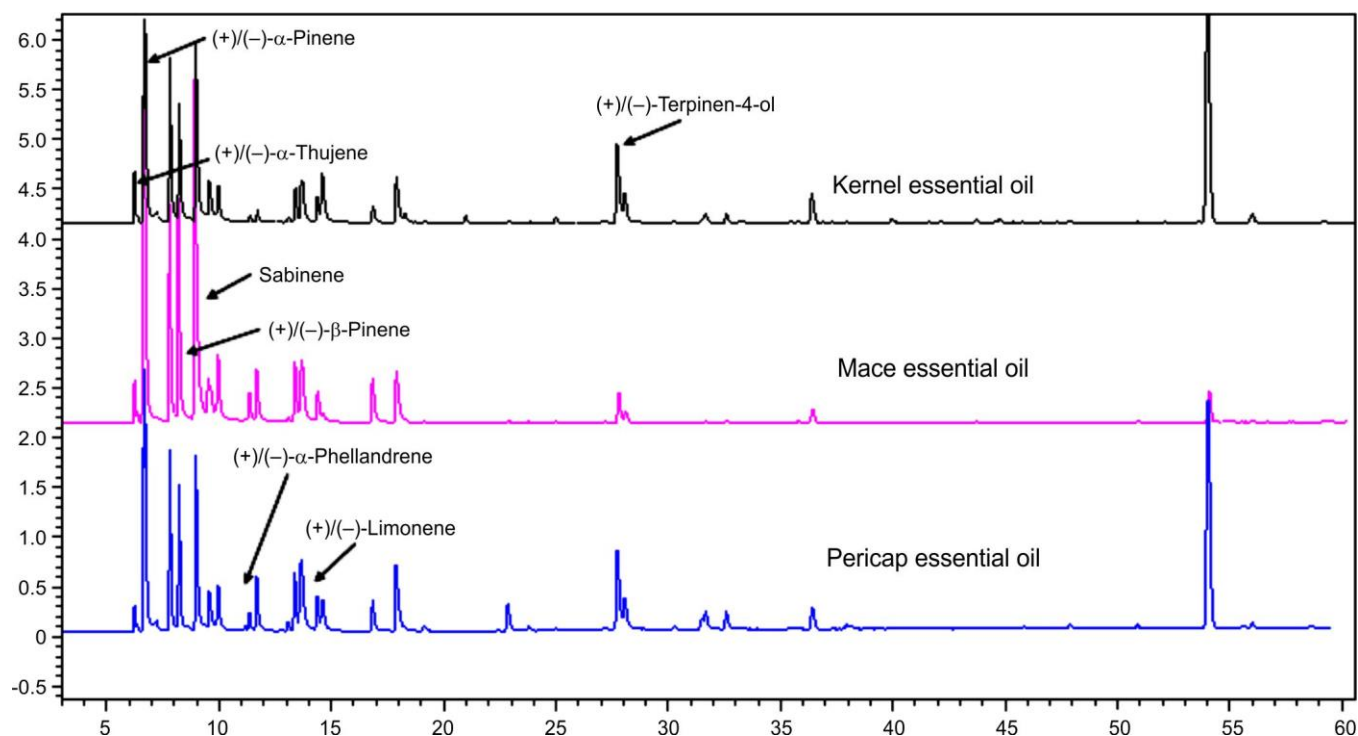


Fig. 2. Comparative enantioselective GC–MS chromatograms of nutmeg kernel, mace and pericarp essential oils with resolved enantiomeric profiles of the major chiral constituents

TABLE-3
COMPARATIVE CHIRAL PROFILES AND ENANTIOMERIC EXCESS OF MAJOR CONSTITUENTS IN NUTMEG ESSENTIAL OILS

RT (min)	Enantiomer	Enantiomeric distribution (ed, %)			Enantiomeric excess (ee, %)		
		Essential oil			Essential oil		
		Pericarp	Mace	Kernel	Pericarp	Mace	Kernel
6.233	(+)- α -Thujene	84.52	87.59	98.64	69.04	75.18	97.28
6.359	(-)- α -Thujene	15.48	12.41	1.36			
6.30	(-)- α -Pinene	100*	100*	100*	–	–	–
6.70	(+)- α -Pinene	nd	nd	nd			
7.81	(+)- β -Pinene	55.45	47.60	58.54	10.90	4.85	17.08
8.23	(-)- β -Pinene	44.55	52.40	41.46			
8.97	(+)-Sabinene	100*	100*	100*	–	–	–
nd	(-)-Sabinene	nd	nd	nd			
11.174	(-)- α -Phellandrene	17.23	3.01	15.76	65.54	93.98	68.48
11.341	(+)- α -Phellandrene	82.77	96.99	84.24			
13.392	(-)-Limonene	65.17	67.29	62.93	30.34	34.58	25.86
14.381	(+)-Limonene	34.83	32.71	37.07			
27.273	(-)-Terpinen-4-ol	78.98	77.34	79.60	57.96	54.68	59.20
28.021	(+)-Terpinen-4-ol	21.02	22.66	20.40			

*The enantiomer peaks are not well resolve and the % area are impossible to estimate.

essential oil, while Chanotiya *et al.* [22] reported moderate separation of α -pinene enantiomers in mint essential oils. These observations are consistent with the present results for the three nutmeg essential oils (Fig. 3). In contrast, terpinen-4-ol showed clear enantiomeric separation, allowing its distribution to be determined with better confidence (Fig. 4). The (+)- and (–)- β -pinene peaks were similarly well resolved (Fig. 5).

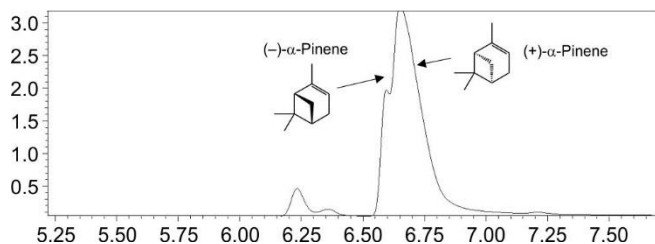


Fig. 3. Enantioselective GC-MS separation profile of (–)- α -pinene and (+)- α -pinene in nutmeg mace essential oil

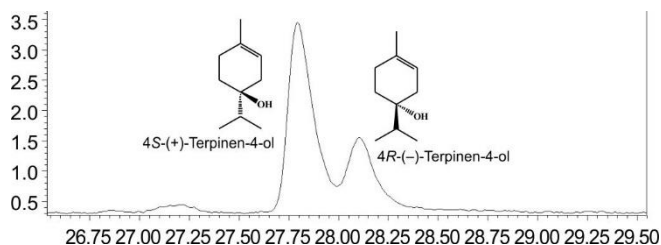


Fig. 4. Enantioselective GC-MS separation profile of 4S-(+)-terpinen-4-ol and 4R-(-)-terpinen-4-ol

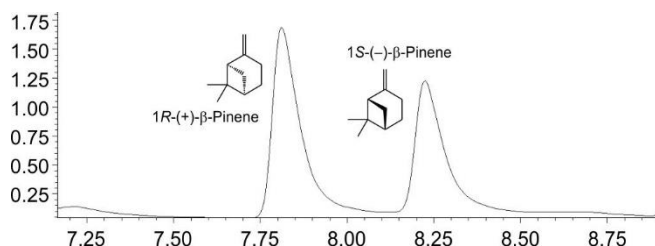


Fig. 5. Enantioselective GC-MS separation profile of 1R-(+)- β -pinene and 1S-(-)- β -pinene

Reliable enantiomeric distribution and enantiomeric excess values were calculated only for the compounds with sufficient chromatographic resolution and are shown in Table-3.

The (+)- and (–)- α -thujene enantiomers were detected in all three nutmeg essential oils. Their elution order was assigned by comparison with published chiral GC data rather than authentic enantiopure standards; therefore, the assignments should therefore be regarded as tentative. Ojha *et al.* [23] reported the elution of levorotatory α -thujene before its dextro-rotatory counterpart in commercial and laboratory-distilled frankincense oils [23], whereas Ankney *et al.* [24] reported the opposite order for *Chamaecyparis lawsoniana* and *Thuja plicata* essential oils. These differences further support the need for authentic standards to establish the absolute configuration of the α -thujene peaks in the present samples. The (+)- and (–)- β -pinene peaks were well resolved and occurred at approximately equal proportions, which is consistent with observations reported for *Piper coruscans* and *Juniperus phoenicea* essential oils [25,26].

The enantiomeric distributions can be considered in relation to monoterpene biosynthesis. Monoterpenes originate from geranyl diphosphate, with product chirality determined by the stereochemical selectivity of the corresponding terpene synthases. The near-racemic distribution of β -pinene, with an enantiomeric excess of only 5-17%, may reflect weak stereoselectivity of the responsible terpene synthase or the involvement of multiple biosynthetic pathways. In contrast, the higher enantiomeric excess values observed for α -phellandrene (66-94%) and α -thujene (69-97%) suggest stronger stereochemical selectivity. Similar elution patterns across kernel, mace and pericarp may reflect related biosynthetic pathways, whereas differences in enantiomeric excess could arise from tissue-specific variation in terpene synthase activity or expression. These interpretations remain tentative in the absence of authentic enantiomeric standards and direct enzymatic evidence.

Antibacterial activity: In the disc-diffusion assay, the three nutmeg essential oils exhibited measurable antibacterial activity against *S. aureus* and *E. coli*. Kernel essential oil produced the largest inhibition zones under the present assay conditions. The observed values were higher than those reported by Ansory & Putri [7] for nutmeg seed essential oil from Central Java, Indonesia, which showed inhibition zones of 11.7 and 9.5 mm against *S. aureus* and *E. coli*, respectively. Pericarp essential oil produced inhibition zones of 14.01 ± 0.56 mm against *S. aureus* and 12.88 ± 0.28 mm against *E. coli*.

Differences in the antibacterial activity among the three essential oils may be associated with their distinct chemical compositions. Mace essential oil contained a high proportion of hydrocarbon monoterpenes (88.22%), whereas kernel and pericarp oils contained higher proportions of oxygenated monoterpenes (14.85% and 15.55%) and phenylpropanoids (22.80% and 17.69%), respectively. Myristicin and terpinen-4-ol were prominent constituents in the latter oils. Oxygenated monoterpenes and phenylpropanoids may interact with bacterial membranes more readily than non-oxygenated hydrocarbons, which could contribute to differences in antibacterial activity. The lower MIC values observed for kernel and pericarp oils are consistent with this compositional pattern. This relationship remains associative, since the experiments were conducted with complete essential oils and did not establish the individual or synergistic contributions of their constituents.

MIC analysis classified the three essential oils as having moderate-to-weak activity against *S. aureus* and *E. coli*, with MIC values above 100 mg/L according to the criterion proposed by Kuete [27] (Table-4). Pericarp essential oil showed the lowest MIC (125 mg/L) among the samples, although this value remains within the moderate-activity range and does not support a strong antibacterial or therapeutic claim. The MIC based pattern differed from the disc-diffusion results, where kernel oil produced the largest inhibition zones. Such differences may arise from the distinct principles of the two assays: inhibition-zone diameter depends partly on diffusion through the agar matrix, whereas MIC measures inhibitory concentration under liquid-phase conditions. The present MIC values were lower than those reported by Trifan *et al.* [2], who observed MIC values above 2000 mg/L for nutmeg seed essential oil against both *S. aureus* and *E. coli*. Differences in the

TABLE-4
ZONE OF INHIBITION AND MIC VALUES OF NUTMEG ESSENTIAL OILS AGAINST SELECTED BACTERIA AT 50 µL

Sample	Zone inhibition diameter (mm)		MIC (mg/L)	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
Pericarp essential oil	14.01 ± 0.56	12.88 ± 0.28	125	125
Mace essential oil	9.75 ± 0.99	13.98 ± 4.04	250	250
Kernel essential oil	17.67 ± 4.04	19.90 ± 3.31	250	250
Chloramphenicol (2.5 mg/mL)	28.66 ± 0.55	27.99 ± 0.16	4	8

oil composition, plant material, geographical origin, extraction conditions and assay methodology may contribute to the variation among studies.

Conclusion

Nutmeg kernel and pericarp essential oils contained appreciable amounts of myristicin, whereas mace essential oil was dominated by hydrocarbon monoterpenes. The enantio-selective GC-MS identified seven enantiomeric pairs across the three oils, with differences in enantiomeric distribution among the plant parts. These compositional variations may contribute to differences in the aroma profiles and chemical characteristics of the oils. The three essential oils exhibited moderate *in vitro* antibacterial activity, with MIC values of 125-250 mg/L, which support their potential for further investigation in food-related applications rather than therapeutic use. Nutmeg pericarp, a processing residue, may serve as an additional source of essential oil and provide an opportunity for greater utilization of plantation biomass within a circular production model.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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