



## Research Article

# *Vitex agnus-castus* L.: Chemical characterization, enantiomeric distribution, and antibacterial efficacy of the essential oil from north-central Nigeria

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## Abstract

*Vitex agnus-castus* L. (Lamiaceae) is a perennial shrub tree commonly grown in tropical and sub-tropical regions. *V. agnus-castus* is used traditionally for the treatment of menstrual disorders, premenstrual dysphoric disorder, and menopausal problems. The chemical compositions of the essential oil, hydrodistilled from three different parts of the plants, were analyzed by gas chromatography and mass spectrometry as well as chiral gas chromatography. Also, hierarchical cluster analysis was performed on the essential oil composition samples from northern Nigeria as well as samples from other geographical locations. The essential oil samples were dominated by 1,8-cineole (31.6–20.6%), followed by terpinen-4-ol (8.9–2.6%), sabinene (9.4–5.8%), (*E*)- $\beta$ -farnesene (8.1–5.5%),  $\alpha$ -pinene (8.1–4.6%),  $\alpha$ -terpinyl acetate (7.7–3.0%),  $\alpha$ -terpineol (7.4–2.4%) and manoyl oxide (6.3–0.4%). The dextrorotatory enantiomers were the major stereoisomers for  $\alpha$ -pinene (88.5–83.4%),  $\alpha$ -phellandrene (95.2–88.9%), and  $\beta$ -phellandrene (86.7–81.4%), while the levorotary enantiomers were predominated by  $\alpha$ -thujene (100%), sabinene (88.3–86.1%), limonene (60.4–58.6%), terpinen-4-ol (86.8–68.9%), and  $\alpha$ -terpineol (90.6–82.9%). The cluster analysis revealed three major chemotypes: one dominated by 1,8-cineole/sabinene/(*E*)- $\beta$ -caryophyllene and other two uncommon chemotypes but rich in  $\alpha$ -pinene and 1,8-cineole/sabinene/ $\alpha$ -pinene respectively. The essential oils demonstrated antibacterial activities against seven microorganisms with minimum inhibitory concentrations (MIC) ranging from 312.5 to 1250  $\mu$ g/mL; active against *Staphylococcus aureus* and *Escherichia coli* (312.5  $\mu$ g/mL); moderately active against *Streptococcus faecalis* and *Pseudomonas aeruginosa* (625  $\mu$ g/mL), weakly active against *Bacillus subtilis*, *Proteus vulgaris* and *Salmonella typhi* (1250  $\mu$ g/mL). The antibacterial activity of *V. agnus-castus* essential oil can be attributed to the major components 1,8-cineole,  $\alpha$ -pinene, terpinen-4-ol, and  $\alpha$ -terpineol. The study shows that the essential oils of *V. agnus-castus* possess potential bacterial activities for pharmaceutical usage.

## 1. Introduction

*Vitex agnus-castus* L. (Lamiaceae), commonly known as chasteberry or monk's pepper, is a small flowering deciduous tree or shrub, that typically grows to an average of 1.5 m to 2 m tall with leaves around 7.6–10 cm in diameter, and is native to the southern Europe and Central Asia, mainly the Mediterranean region [1]. The ethnopharmacology and phytochemistry of *V.*

*agnus-castus* has have been reviewed [2–7]; the plant has been used to treat various female conditions such as menstrual disorders, premenstrual dysphoric disorder, corpus luteum deficiency, and menopausal problems [1,8]. The important constituents of *V. agnus-castus* essential oil are 1,8-cineole, sabinene,  $\alpha$ -pinene, (*E*)- $\beta$ -farnesene, (*E*)- $\beta$ -caryophyllene, and

$\alpha$ -terpinyl acetate [4, 9]. As part of our ongoing interest in essential oils of aromatic and medicinal plants of Nigeria, this study is aimed at investigating to investigate the chemical characterization, enantiomeric distribution, and antibacterial efficacy of the essential oil of *V. agnus-castus* growing in north-central Nigeria.

## 2. Materials and methods

### 2.1. Plant sample collection and identification

The fresh plant of *Vitex agnus-cactus* was collected in July 2023 along new Jos Road, Zaria (11° 5' 0.2544"N, 7° 42' 48.726" E), located in Kaduna South Local Government area of Kaduna State, Nigeria. The plant was authenticated by Mr. Namadi Sunusi of the Botany Department, Ahmadu Bello University, Zaria, with voucher number ABU0841. The fresh aerial parts, leaves, and seeds of the plant were air-dried in the shade for seven days and then pulverized using an electric blender before extraction.

### 2.2 Hydrodistillation of the essential oil

Each of the air-dried plant samples (500g) was placed in a 5-L flask, and distilled water was added to cover the sample. Hydrodistillation was carried out for four hours in an all-glass Clevenger apparatus in accordance with the British Pharmacopoeia. The distillate was extracted with *n*-hexane, transferred to a pre-weighed amber sample vial, and dried with anhydrous sodium sulfate to remove any remaining water. The essential oil yields ranged from 1.20 to 4.5% (v/w) with a yellowish colouration. The oils were refrigerated at 4 °C until ready for analysis.

### 2.3. Gas chromatography– mass spectral analysis

The essential oils were analyzed by GC-MS as reported previously [10]: Shimadzu GCMS-QP2010 Ultra (Shimadzu Scientific Instruments, Columbia, MD, USA), electron impact (EI) mode (electron energy = 70eV), scan range= 40-100 atomic mass units, scan rate=3.0 scan/s, ZB-5 fused silica capillary GC column (30m×0.25mm× 0.25 µm film); He carrier gas, column head pressure = 553 kPa, flow rate =1.37 mL/min; injector temperature =250°C, ion source temperature = 200 °C;GC oven temperature program, 50 °C initial temperature, increased to 260 °C at 2°C/min. A 5% w/v solution of each essential oil in CH<sub>2</sub>Cl<sub>2</sub> was prepared and 0.1 µL was injected, splitting mode = 30:1. Identification of the volatile oil constituents was

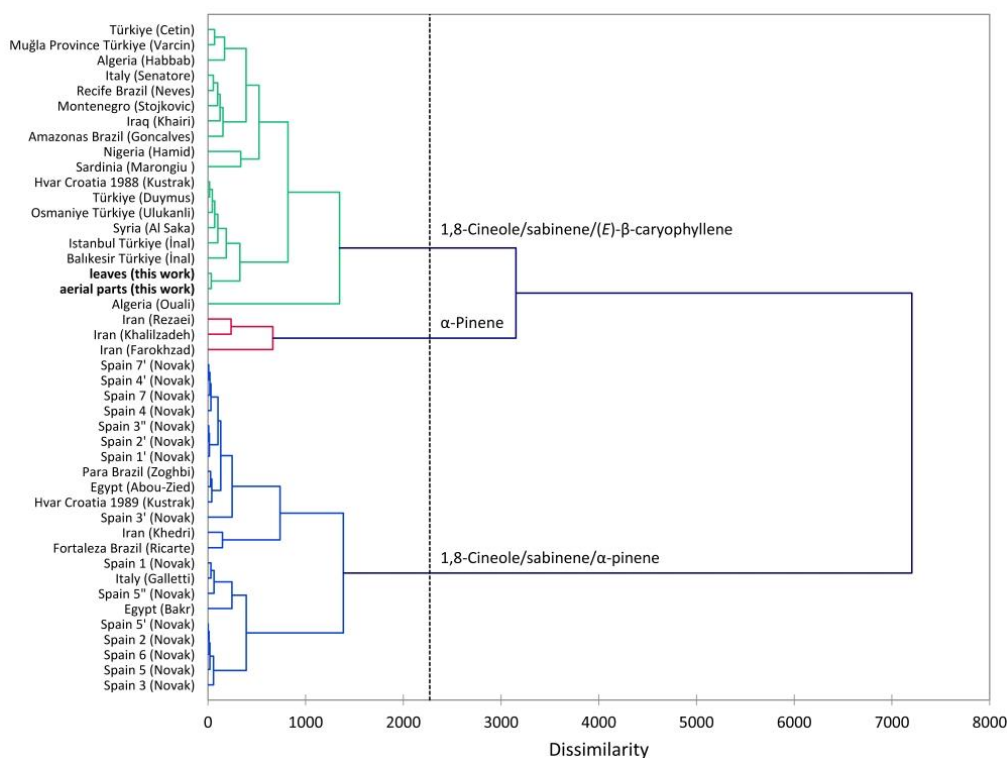
achieved based on their retention indices and by comparison of their mass spectral fragmentation pattern with those reported in databases [11–14].

### 2.4. Chiral gas chromatography – mass spectral analysis

Chiral GC-MS of the essential oils of *Vitex agnus-cactus* carried out as previously reported [10] : Shimadzu GCMS-QP2010S (Shimadzu Scientific Instruments, Columbia, MD, USA), EI mode (electron energy=70 eV), scan range = 40–400 amu, scan rate = 3.0 scans/s, Restek B-Dex 325 capillary column (Restek Corp., Bellefonte, PA, USA) (30m×0.25mm ID×0.25µm film). The oven temperature program, 50 °C initial temperature, increased to 120 °C at 1.5 °C/min, increased to 200 °C at 2 °C/min, kept at 200 °C for 5 min; He carrier gas, flow rate = 1.8 mL/min. Essential oil samples were diluted to 3% w/v with CH<sub>2</sub>Cl<sub>2</sub>, and a 0.1µL was injected, split mode = 1:45. The terpenoid enantiomers were identified by comparison of retention indices with authentic samples obtained from Sigma-Aldrich (Milwaukee, WI, USA). Relative enantiomer percentages were determined based on peak areas.

### 2.5. Antibacterial screening

The *A. agnus-castus* leaf essential oil was screened for antibacterial activity using the microbroth dilution assay as previously reported [10] : *Staphylococcus aureus* (ATCC No. 25923), *Bacillus subtilis* (ATCC No.6633), *Streptococcus faecalis* (ATCC No.9790), *Salmonella typhi* (ATCC No. 6539), *Proteus vulgaris* (ATCC No. 6380), *Escherichia coli* (ATCC No.25922), and *Pseudomonas aeruginosa* (ATCC No. 27853); a 1% stock solution of the essential oil in DMSO (50 µL) and 50 µL of cation-adjusted Mueller Hinton broth (CAMHB) (Sigma-Aldrich, St. Louis, MO) was serially diluted in a 96-well microdilution plate (essential oil concentrations = 2500, 1250, 625, 312.5, 156.3, 78.1, 39.1, and 19.5 µg/mL); bacteria were added to each well at concentrations of 1.5 × 10<sup>8</sup> CFU/mL; plates incubated at 37°C for 24 h; minimum inhibitory concentration (MIC) was determined as the lowest concentration with no turbidity; positive antibiotic control = streptomycin (Sigma-Aldrich, St. Louis, MO), negative control = DMSO (50 µL DMSO diluted in 50 µL broth medium, serially diluted. 1,8-cineole and  $\alpha$ -terpineol (Sigma-Aldrich, St. Louis, MO) were individually screened for activity.



**Figure 1.** Dendrogram obtained by hierarchical cluster analysis of 44 essential oil samples (aerial parts or leaves) of *Vitex agnus-castus*. Cetin [19], Varcin [39], Habbab [23], Senatore [36], Neves [31], Stojkovic [37], Khairi [26], Goncalves [17], Hamid [24], Marongiu [30], Kustrak [29], Duymus [20], Ulukanli [38], Al Saka [16], Inal [25], Ouali [33], Rezaei [34], Khalilzadeh [27], Farokhzad [21], Novak [32], Zoghbi [40], Abou-Zied [15], Khedri [28], Ricarte [35], Galletti [22], Bakr [18].

## 2.6. Hierarchical cluster analysis

Hierarchical cluster analysis (HCA) analysis was carried out on the *V. agnus-castus* essential oils using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). The HCA was performed using the concentrations of the 12 most abundant components (1,8-cineole, sabinene,  $\alpha$ -pinene, (*E*)- $\beta$ -farnesene, (*E*)- $\beta$ -caryophyllene,  $\alpha$ -terpinyl acetate, terpinen-4-ol,  $\alpha$ -terpineol, bicyclogermacrene, caryophyllene oxide, limonene, and  $\tau$ -cadinol) from this current work as well as those previously reported compositions from the literature [15–40]. Dissimilarity was used to determine clusters, considering Euclidean distance, and Ward's method was used to define agglomeration.

## 3. Results and discussion

### 3.1. Essential oil compositions

The essential oils of the aerial parts, leaves, and seeds of *V. agnus-castus* were analyzed by GC-MS (Table 1). The major components in the aerial parts essential oil were 1,8-cineole (26.4%), terpinen-4-ol (8.9%),  $\alpha$ -terpineol (7.4%), sabinene (5.8%), and (*E*)- $\beta$ -farnesene (5.5%). The leaf essential oil showed 1,8-cineole

(31.6%), sabinene (9.4%),  $\alpha$ -pinene (8.1%), terpinen-4-ol (7.5%), and  $\alpha$ -terpineol (5.7%) as major compounds. The essential oil from the seeds of *V. agnus-castus* were rich in 1,8-cineole (20.6%), (*E*)- $\beta$ -farnesene (8.1%),  $\alpha$ -terpinyl acetate (7.7%), sabinene (7.3%),  $\tau$ -cadinol (6.9%), manoyl oxide (6.3%), and  $\alpha$ -pinene (5.5%).

There have been numerous previous examinations of essential oils of *V. agnus-castus* [15–40]. In order to place the present study into perspective, a hierarchical cluster analysis (HCA) was carried out based on the major essential oil components (Fig. 1). The HCA shows three well-defined clusters for the leaf and aerial parts of the essential oils of *V. agnus-castus*: (1) a 1,8-cineole/sabinene/(*E*)- $\beta$ -caryophyllene cluster, which includes the samples from this work; (2) an  $\alpha$ -pinene cluster, which includes samples from Iran with little or no 1,8-cineole; and (3) a 1,8-cineole/sabinene/ $\alpha$ -pinene cluster.

### 3.2. Enantiomeric Distribution

The *V. agnus-castus* essential oils were subjected to enantioselective GC-MS in order to determine the enantiomeric distribution of chiral monoterpenoid components (Table 2). The (+)-enantiomers were the major stereoisomers for  $\alpha$ -pinene,  $\alpha$ -phellandrene,

**Table 1.** Chemical compositions (percent) of essential oils of *Vitex agnus-castus* from North-Central Nigeria.

RI <sub>calc</sub>	RI <sub>db</sub>	Compounds	Aerial parts	Leaves	seeds
925	925	$\alpha$ -Thujene	0.4	0.8	0.3
929	---	3-Hexyl acetate	0.2	0.7	0.4
932	932	$\alpha$ -Pinene	4.6	8.1	5.5
972	972	Sabinene	5.8	9.4	7.3
978	978	$\beta$ -Pinene	1.0	2.0	0.9
989	989	Myrcene	1.4	2.7	1.0
1007	1007	$\alpha$ -Phellandrene	0.3	0.4	0.2
1017	1017	$\alpha$ -Terpinene	1.1	2.2	0.4
1025	1025	<i>p</i> -Cymene	0.7	0.2	0.5
1029	1030	Limonene	1.9	0.4	1.4
1031	1031	$\beta$ -Phellandrene	1.4	0.5	0.9
1033	1032	1,8-Cineole	26.4	31.6	20.6
1035	1034	( <i>Z</i> )- $\beta$ -Ocimene	0.1	-	0.1
1036	1035	2,2,6-Trimethylcyclohexanone	tr	tr	tr
1046	1046	( <i>E</i> )- $\beta$ -Ocimene	0.5	0.7	0.3
1058	1058	$\gamma$ -Terpinene	2.3	3.8	0.7
1071	1069	<i>cis</i> -Sabinene hydrate	-	-	tr
1085	1086	Terpinolene	0.6	0.8	0.2
1100	1101	Linalool	0.3	0.2	0.2
1102	1101	<i>trans</i> -Sabinene hydrate	-	-	tr
1106	1107	Nonanal	tr	tr	tr
1119	1118	3-Octyl acetate	0.2	0.2	0.3
1127	1124	<i>cis-p</i> -Menth-2-en-1-ol	0.3	0.2	0.1
1142	1141	<i>trans</i> -Pinocarveol	-	-	tr
1145	1142	<i>trans-p</i> -Menth-2-en-1-ol	0.2	0.2	0.1
1172	1170	$\delta$ -Terpineol	1.0	0.8	0.4
1182	1180	Terpinen-4-ol	8.9	7.5	2.6
1197	1195	$\alpha$ -Terpineol	7.4	5.7	2.4
1227	1227	Citronellol	-	-	0.2
1269	1268	Geranial	tr	tr	-
1284	1285	Bornyl acetate	0.1	0.1	0.1
1288	1287	Dihydroedulan IA	0.1	tr	-
1293	1294	Dihydroedulan IIA	tr	tr	-
1300	1300	Tridecane	0.1	-	-
1312	1312	$\delta$ -Terpinyl acetate	-	-	0.1
1331	1335	$\delta$ -Elemene	0.1	0.1	0.1
1339	1337	2-Hydroxycineol acetate	tr	tr	0.1
1347	1346	$\alpha$ -Terpinyl acetate	3.3	3.0	7.7
1349	1350	Citronellyl acetate	0.2	-	0.3
1357	1355	<i>iso</i> - $\alpha$ -Terpinyl acetate	0.1	0.1	0.1
1358	1361	Neryl acetate	tr	tr	tr
1375	1375	$\alpha$ -Copaene	tr	-	tr
1378	1378	Geranyl acetate	-	-	0.1
1378	1379	( <i>E</i> )- $\beta$ -Damascenone	tr	tr	-
1383	1382	$\beta$ -Bourbonene	0.1	tr	0.1
1388	1390	<i>trans</i> - $\beta$ -Elemene	0.1	tr	0.1
1406	1406	$\alpha$ -Gurjunene	0.2	0.1	0.2
1419	1417	( <i>E</i> )- $\beta$ -Caryophyllene	2.7	1.9	2.2
1429	1430	$\beta$ -Copaene	tr	tr	0.1
1432	1432	<i>trans</i> - $\alpha$ -Bergamotene	0.2	tr	0.2
1438	1438	Aromadendrene	tr	tr	tr

Table 1. (Continued)

RI <sub>calc</sub>	RI <sub>db</sub>	Compounds	Aerial parts	Leaves	seeds
1439	1439	Isoamyl benzoate	tr	0.1	0.2
1439	1439	(Z)- $\beta$ -Farnesene	0.1	-	-
1452	1452	(E)- $\beta$ -Farnesene	5.5	3.3	8.1
1455	1454	$\alpha$ -Humulene	0.1	tr	0.1
1459	1458	<i>allo</i> -Aromadendrene	0.4	0.3	0.4
1461	1463	<i>cis</i> -Muurolo-4(14),5-diene	tr	tr	tr
1478	1481	(E)- $\beta$ -Ionone	tr	tr	-
1480	1480	Germacrene D	0.5	0.3	1.0
1490	1491	Viridiflorene	0.1	0.1	-
1495	1497	Bicyclgermacrene	2.1	1.1	1.8
1513	1512	$\gamma$ -Cadinene	0.1	0.1	0.2
1514	1510	1,11-Oxidocalamenene	0.1	tr	tr
1517	1518	$\delta$ -Cadinene	0.1	0.1	0.1
1559	1562	(E)-Nerolidol	-	tr	0.1
1570	1568	Palustrol	0.1	0.1	0.1
1577	1576	Spathulenol	-	0.5	1.2
1582	1587	Caryophyllene oxide	0.6	0.1	0.4
1589	1590	Globulol	0.4	0.3	0.4
1593	1592	Viridiflorol	-	0.1	0.1
1606	1605	Ledol	0.5	0.2	0.5
1632	1629	<i>iso</i> -Spathulenol	-	0.1	0.1
1646	1643	$\tau$ -Cadinol	3.3	2.3	6.9
1654	1655	$\alpha$ -Cadinol	-	-	0.1
1858	1862	$\alpha$ - <i>iso</i> -Methylionone	4.2	2.4	3.7
1883	1886	Sclareol oxide	-	tr	0.2
1889	---	Unidentified <sup>a</sup>	1.5	0.8	1.4
1905	1907	Isopimara-9(11),15-diene	0.2	0.1	0.2
1907	1910	Methyl (2E,6E)-3,7,11-trimethyl-2,6,10-dodecatrienyl carbonate	-	tr	0.1
1945	1943	Beyerene isomer	1.0	0.5	1.0
1949	1948	$\beta$ - <i>iso</i> -Methylionone	0.6	0.4	0.9
1958	1958	Palmitic acid	0.1	-	-
1974	1978	Manool	1.1	0.8	1.7
1981	---	Unidentified <sup>b</sup>	0.3	0.4	1.3
1992	1994	Manoyl oxide	0.9	0.4	6.3
2007	---	Unidentified <sup>c</sup>	0.7	0.4	1.0
<b>Compound Classes</b>					
		Monoterpene hydrocarbons	22.0	31.9	19.5
		Oxygenated monoterpenoids	48.2	49.2	35.0
		Sesquiterpene hydrocarbons	12.4	7.3	14.8
		Oxygenated sesquiterpenoids	5.0	3.8	10.2
		Diterpenoids	3.2	1.8	9.2
		Benzenoid aromatics	tr	0.1	0.2
		Others	5.4	3.8	5.2
		Total identified	96.1	97.9	94.1

RI<sub>calc</sub> = Retention index calculated with respect to a homologous series of *n*-alkanes on a ZB-5ms column [41]. RI<sub>db</sub> = Reference retention index from the databases [11–14]. tr = trace (< 0.05%). <sup>a</sup> MS(EI): 272(1%), 257(3%), 203(3%), 191(58%), 189(29%), 177(10%), 175(15%), 149(9%), 147(10%), 136(31%), 121(59%), 119(76%), 107(38%), 105(26%), 95(27%), 93(39%), 91(26%), 80(100%), 69(22%), 67(19%), 55(27%), 41(37%). <sup>b</sup> MS(EI): 272(1%), 257(2%), 191(100%), 189(22%), 136(34%), 121(49%), 119(53%), 107(34%), 95(26%), 93(32%), 91(19%), 80(98%), 71(29%), 55(32%), 43(33%), 41(28%). <sup>c</sup> MS(EI): 320(5%), 257(6%), 217(7%), 189(43%), 175(16%), 161(14%), 159(15%), 147(19%), 135(52%), 122(63%), 121(52%), 120(64%), 119(60%), 109(74%), 107(88%), 105(69%), 95(100%), 93(66%), 91(50%), 81(60%), 79(57%), 69(50%), 67(49%), 55(64%), 43(52%), 41(74%).



**Table 2.** Enantiomeric distribution (percent of each enantiomer) of chiral monoterpenoids in *Vitex agnus-castus* essential oils.

Compounds	RI <sub>lab</sub>	RI <sub>calc</sub>	Aerial parts	Leaves	Seeds
(+)- $\alpha$ -Thujene	950	n.o.	0.0	0.0	0.0
(-)- $\alpha$ -Thujene	951	954	100.0	100.0	100.0
(-)- $\alpha$ -Pinene	976	977	16.6	11.5	13.9
(+)- $\alpha$ -Pinene	982	981	83.4	88.5	86.1
(+)-Sabinene	1021	1019	12.1	11.7	13.9
(-)-Sabinene	1030	1027	87.9	88.3	86.1
(+)- $\beta$ -Pinene	1027	1025	13.3	11.8	19.9
(-)- $\beta$ -Pinene	1031	1030	86.7	88.2	80.1
(-)- $\alpha$ -Phellandrene	1050	1050	8.2	11.1	4.8
(+)- $\alpha$ -Phellandrene	1053	1051	91.8	88.9	95.2
(-)-Limonene	1073	1075	58.6	60.4	60.1
(+)-Limonene	1081	1082	41.4	39.6	39.9
(-)- $\beta$ -Phellandrene	1083	1086	17.9	18.6	13.3
(+)- $\beta$ -Phellandrene	1089	1090	82.1	81.4	86.7
(-)-Linalool	1228	1228	51.3	47.9	56.2
(+)-Linalool	1231	1232	48.7	52.1	43.8
(+)-Terpinen-4-ol	1297	1296	29.3	13.2	31.1
(-)-Terpinen-4-ol	1300	1298	70.7	86.8	68.9
(-)- $\alpha$ -Terpineol	1347	1347	90.1	82.9	90.6
(+)- $\alpha$ -Terpineol	1356	1357	9.9	17.1	9.4

RI<sub>lab</sub> = Retention index from our in-house database based on commercially available compounds available from Sigma-Aldrich and augmented with our own data. RI<sub>calc</sub> = Calculated retention index based on a series of *n*-alkanes on a Restek B-Dex 325 capillary column. n.o. = not observed.

and  $\beta$ -phellandrene, while the (–)-enantiomers predominated for  $\alpha$ -thujene, sabinene, limonene, terpinen-4-ol, and  $\alpha$ -terpineol. As far as we are aware, there have been no previous chiral gas chromatographic examinations of *V. agnus-castus* or any *Vitex* essential oils.

3.3. Antibacterial Activity

The *V. agnus-castus* leaf essential oil was screened for antibacterial activity against a panel of Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*) and Gram-negative (*Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*) organisms (Table 3). The essential oil showed strong antibacterial activity (MIC = 312.5  $\mu$ g/mL) against *S. aureus* and *E. coli* and moderate activity (MIC = 625  $\mu$ g/mL) against *S. faecalis* and *P. aeruginosa* [42, 43]. Of the major components, 1,8-cineole and  $\alpha$ -terpineol showed antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. typhi* (Table 3). Previous work has shown sabinene to be relatively inactive as an antibacterial [44, 45]. However,  $\alpha$ -pinene has shown antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, and *S. typhi* [46, 47]; and

terpinen-4-ol has shown activity against *B. subtilis*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, and *S. aureus* [44,48]. Thus, the antibacterial activity of *V. agnus-castus* leaf essential oil can be attributed to the major components 1,8-cineole,  $\alpha$ -pinene, terpinen-4-ol, and  $\alpha$ -terpineol. Consistent with the antibacterial observations in this report, there have been previous reports on the antibacterial activities of *V. agnus-castus* essential oils, including activities against *B. subtilis* [18], *Streptococcus mutans* [17], *P. aeruginosa* [23], *Bacillus cereus* [28], and *Salmonella enterica* serovar Typhimurium [37].

4. Conclusions

The essential oil compositions and antibacterial activities of *V. agnus-castus* from North Central Nigeria are comparable to compositions (largely dominated by oxygenated monoterpenoids and monoterpene hydrocarbons) and antibacterial activities (good antibacterial activity against *S. aureus* and *E. coli*) from other geographical locations. This is the first report on the enantiomeric distributions of chiral monoterpenoids in *V. agnus-castus* essential oils,

**Table 3.** Antibacterial activity of *Vitex agnus-castus* leaf essential oil, 1,8-cineole, and  $\alpha$ -terpineol.

Organism	<i>Vitex agnus-castus</i> EO	1,8-Cineole	$\alpha$ -Terpineol	Streptomycin
<i>Bacillus subtilis</i>	1250	Nt	nt	<19.5
<i>Staphylococcus aureus</i>	312.5	312.5	312.5	<19.5
<i>Streptococcus faecalis</i>	625	Nt	nt	<19.5
<i>Escherichia coli</i>	312.5	312.5	625	< 19.5
<i>Proteus vulgaris</i>	1250	Nt	nt	< 19.5
<i>Pseudomonas aeruginosa</i>	625	312.5	312.5	< 19.5
<i>Salmonella typhi</i>	1250	312.5	156.3	<19.5

however, this adds to our knowledge on this important medicinal plant. Additional research is needed on the enantioselective GC-MS of other *Vitex* essential oils to identify any trends in enantiomeric distributions.

Authors’ contributions

Conceptualization, M.S.O; Methodology, D.S.R.O., M.S.O., N.A.F., P.S., and W.N.S.; Software, P.S.; Validation, L.A.O. and W.N.S., Formal Analysis, A.P. and W.N.S.; Investigation, D.S.R.O., M.S.O., P.S., A.P., E.A.O. and W.N.S.; Resources, D.S.R.O., M.S.O., N.A.O., P.S. and W.N.S.; Data Curation, W.N.S.; Writing – Original Draft Preparation, M.S.O., E.A.O. and W.N.S.; Writing – Review & Editing, M.S.O. and W.N.S.; Project Administration, D.S.R.O. E.A.O., N.A.F. and M.S.O.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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