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ORIGINAL ARTICLE

Chemical Constituents of Leaf Essential Oil of North-central Nigerian Grown *Vitex Agnus-castus* l

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ABSTRACT

Pulverized leaf of *vitex agnus-castus* on hydrodistillation, afforded oil in the yield of 0.8%v/w. GC, GC/MS analyses of the oil revealed the abundance of hydrocarbon and oxygenated monoterpenes (53.2 and 24.5% respectively). The major constituents of the oil were; β-pinene (20.0%), viridiflorol (9.8%), α-pinene (9.1%), cis-ocimene (8.4%), 1,8-cineole (6.7%), β-farnesene (5.4%), terpinen-4-ol (4.2%), α-terpineol (4.1%) and β-phellandrene (4.1%).

Key words: *Vitex agnus-castus*, verbenaceae, β-pinene, α-pinene, cis-ocimene, viridiflorol and 1,8-cineole.

Introduction

Vitex agnus-castus L. (Verbenaceae) is a perennial grey shrub, with a strong aromatic odour [1]. It is grown as ornamental plant in Nigeria and in different parts of the world. The plant is used in folk medicine for the treatment of premenstrual syndrome (PMS), gynecological and digestive complaints, infertility, stomachache, headache, influenza, diarrhea and syphilis [2-11].It is also used as carminative, antiseptic, a diuretic and an anaphrodisiac [7,9]. The various biological activities of the plant such as immunodulatory, antimicrobial and antioxidant properties justified its use in traditional medicine [12-14]. The insecticidal property of the plant has also been reported [15].

Phytochemical studies of the plant revealed the presence of casticin, eupatorin, penduletin, vitexin, orientin, vitetrifolin B and C, rotundifuran, vitexilactone and spathulenol [12-14]. Earlier work on leaves, inflorescences and fruits essential oils of *vitex agnus-castus* grown in Southern Italy has led to the identifications of 1,8-cineole, α-terpineol, sabinene, β-selinene, β-caryophyllene and cis-β-farnesene as the main constituents of the oils [16]. Sabinene, β-farnesene and 1,8-cineole were the most abundant constituents of the leaf, flower and fruit essential oils of North Brazilian grown *V.agnus-castus* [17]. α-pinene, β-pinene, limonene, sabinene, 1,8-cineole and terpineol were identified as the principal constituents of the leaf oil of South-west Nigerian grown *V.agnus-castus* [13].

In continuation of our studies of essential oil from medicinal plants used in folk medicine in North-central Nigeria, we investigated the leaf essential oil of *Vitex agnus-castus*.

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Experimental

Plant Materials

The fresh leaves of *V. agnus-castus* were obtained in Ilorin, Kwara State, North Central Nigeria. Identification was carried out at the herbarium of the Department of plant Biology University of Ilorin, where voucher specimens were deposited

Oil Isolation

Pulverized leaves were hydrodistilled for 3h in a Clevenger-type apparatus, according to the British Pharmacopoea Specification [18]. The resulting oil was collected, preserved in a sealed sample tube and stored under refrigeration until analysis.

Gas Chromatography

GC analysis were performed on an orion micromat 412 double focusing gas chromatography system fitted with two capillary columns coated with CP-Sil 5 and CP-Sil 19 (fused silica, 25m × 0.25mm, 0.15μm film thickness) and flame ionization detector (FID). The volume injected was 0.2μL and the split ratio was 1:30. Oven temperature was programmed from 50°C-230°C respectively. Qualitative data were obtained by electronic integration of FID area percents without the use of correction factors.

Gas Chromatography/mass Spectrometry

A Hewlett Packard (HP 5890A) GC interfaced with a VG Analytical 70-250S double focusing mass spectrometer was used. Helium was the carrier gas at 1.2ml/min. The MS operating conditions were: ionization voltage 70ev, ion source temperature 230°C. The GC was fitted with a 25m×0.25mm, fused silica capillary column coated with CP-Sil 5.

The film thickness was 0.15μm. the GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by online desktop computer equipped with disk memory. The percentage compositions of the oil were computed in each case from GC peak areas.

The identification of the components was based on the retention indices (determined relative to the retention times of series of n-alkanes) and mass spectra with those of authentic samples and with data from Literature [19-21].

Results and Discussion

Pulverized leaf of *V. agnus-castus*, on hydrodistilation afforded oil in the yield of 0.8%v/w. The yield compared favourably with the yield of South Italian and North Brazilian grown *V. agnus-castus* [16, 17]. Table 1 shows retention indices, relative percentages and the identities of the constituents of the oil.

A total of 34 compounds representing 98.5% of the oil were identified from their retention indices and mass spectra.

Hydrocarbon and oxygenated monoterpenes constituted 53.2 and 25.8% of the oil respectively. Percentage composition of hydrocarbon and oxygenated sesquiterpenes in the oil were 8.3 and 9.9% respectively. β-pinene (20.0%), α-pinene (9.1%), cis-ocimene (8.4%) and β-phellandrene (4.1%) were the abundant hydrocarbon monoterpenes in the oil. Other notable hydrocarbon monoterpenes were; γ-terpene (3.7%) and β-myrcene (3.2%). Each of the following hydrocarbon monoterpenes constituted less than 0.2% of the oil: Car-2-ene (1.7%), alloocimene (1.7%) and α-thujene (1.3%).

The most abundant oxygenated monoterpenes in the oil were 1,8-cineole (6.7%), terpinen-4-ol (4.2%) and α-terpineol (4.1%). Borneol acetate (2.0%), β-citronellol (1.6%), neral (1.3%), β-linalool (1.2%) and geranial (1.2%) existed in appreciable quantities. The most abundant hydrocarbon sesquiterpene in the oil was β-farnesene (5.4%), while β-caryophyllene (1.1%) was found in appreciable proportion. β-bisabolene (0.9%), germacrene D (0.6%) and β-elemene (0.3%) existed as minor constituents. Viridiflorol (9.8%) was the predominant oxygenated sesquiterpene in the oil.

The qualitative and quantitative composition of the oil was found to be quite different from the leaf essential oils of South-west Nigerian, South Italian and North Brazilian grown *V. agnus-castus* [13, 16, and 17]. For instance, some of the principal constituents in this study such as viridiflorol, β-farnesene and β-phellandrene did not exist as principal constituents in the oil of south-west Nigerian grown *V. agnus-castus*. Limonene and sabinene which existed as major constituents in the South-west Nigerian grown *V. agnus-castus* were not found in the North-central Nigerian grown *V. agnus-castus* [14]. However, the most abundant constituent of North-central Nigerian grown *V. agnus-castus*, β-pinene, existed in appreciable amount in the oil obtained from South Italian grown *V. agnus-castus*, but was not found in the oil of North Brazilian grown *V. agnus-castus*. Hence, the oil of North-central Nigerian grown *V. agnus-castus* was of β-pinene chemotype. Similarly, 1,8-cineole, the most abundant constituent in the leaf oils of both the South Italian and North Brazilian grown *V. agnus-castus* existed in appreciable amount in the oil of North-central Nigerian grown *V. agnus-castus*. Thus, the oils obtained from Southern Italy and Northern Brazil were of 1, 8-cineole chemotypes [16, 17].

Table 1: Chemical composition(%) of Leaf oil of Vitex agnus-castus.

Compounda	RIb	%Composition
-thujene	923	1.3
-pinene	931	9.1
-pinene	974	20.0
-myrcene	988	3.2
Car-2-ene	1001	1.7
-phellandrene	1026	4.1
1,8-cineole	1029	6.7
Cis-ocimene	1034	8.4
Alloocimene	1046	1.7
-terpinene	1057	3.7
Isoartemisia	1057	0.5
-linalool	1097	1.2
Lavandulol	1146	1.1
Borneol	1163	tr.
Terpinen-4-ol	1176	4.2
-terpinrol	1187	4.1
-citronellol	1226	1.6
Thymyl methy ether	1233	tr.
Neral	1237	1.3
Trans-geraniol	1253	1.1
Geranial	1267	1.2
Borneol acetate	1284	2.0
Eugenol	1353	0.8
-copane	137	4tr.
-elemene	1393	0.3
-caryophyllene	1417	1.1
-farnesene	1456	5.4
Ethyl cinnamate	1461	1.3
Germacrene D	1480	0.6
-bisabolene	1501	0.9
Elemicin	1553	tr.
Viridiflorol	1590	9.8
Torrevol	1645	0.1
Benzyl benzoate	1759	tr.
	Total	98.5

aCompounds are listed in order of elution from silica capillary column coated in CP-Sil 5; bretention indices on fused capillary column coated with CP-Sil 5; tr=trace (<0.1%).

Sabinene, one of the main components in the essential oils of Italian and Brazilian grown *V. agnus-castus,* was not found in this study. Furthermore, viridiflorol, the most abundant sesquiterpenoid in the oil of North-central Nigerian grown *V. agnus-castus,* did not exist in the oils of Italian and Brazilian grown *V. agnus-castus*. Cis-ocimene, which existed in appreciable amount in our study, was found as minor constituents in the oils obtained from Southern Italy and Northern Brazil.

However, the oil shared similar composition pattern with respect to the notable constituents like α-pinene and β-farnesene in the leaf essential oils of *V. agnus-castus* grown in Southern Italy and Northern Brazil.The qualitative and quantitative variations in the constituents of the oils from these four locations may be due to their agroclimatic and geographical conditions.

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