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Volatile Constituents & Phytochemical Studies of Platycladusorientalis Grown In North Central Nigeria

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Abstract:*The hydro distilled essential oil of fresh leaves of Platycladusorientalis (L) Franco [Thujaorientalis] Family: cupressaceae was analysis by gas chromatography and gas chromatography/mass spectrometry.(GC-MS). The oil yield is (1.25%) w/w.*
Thirty two compounds representing 96.62% of the total oil were identified. The main constituents are IR-α-pinene (15.92%), α-caryophyllene (10.42%), trans-β ocimene (8.71%), limonene (8.25%), and patchoulane (7.46%).
The oil is rich in monoterpene hydrocarbons (55.04%), followed by sesquiterpene hydrocarbons (28.96%), others (7.93%), oxygenated monoterpenes (2.05%) and oxygenated sesquiterpenes 2.64%. The Phytochemical screening of the air dried leaves was also studied and found to contain medicinal active constituents like alkaloids, tannins, flavonoid, terpenoid, cardiac glycosides, sterols and anthraquinones, which justify the use of the plant in treating various infectious diseases.
Keywords: *Volatile constituents, phytochemical screening, Platycladusorientalis, hydro distillation*

I. Introduction

Scientific classification:

Botanical name:	Platycladusorientalis
Family:	cupressaceae
kingdom:	planate
Division:	coniferophyta
Subdivision:	spermatophyte
Class:	pinopsida
Order:	pinales
Genus:	Platycladus
Species:	Platycladusorientalis.

Vernacular name

English: ThujaOrientalis, Hindu: Morpankhi, Orientalisthuja, Oriental arborvitae [2].

Platycladusorientalis (L.) Franco [Thujaorientalis L.] Locally named sarv-e khomrerii or nosh is an evergreen species, which grows naturally in lran. Also, this species is widely cultivated as a common oriental plant in lran& other countries [3]. It is an evergreen monoecious trees or shrubs growing to 10-60 feet tall. The shoot are flat, leaves are scale like & arranged in flattened fan shaped growing with resinous glands [4]. It is planted in cooler areas of tropical Africa primarily as an ornamental [5].inPlatycladusorientalis was an old remedy for delayed menstruation, being also a stimulant to smooth muscles such as those of uterus and bronchial passages, so it is used for the treatment of bronchitis [6] and also cough suppressant in traditional Chinese medicine [7]. Externally it is used as a wash for infectious skin diseases such as impetigo and scabies [8]. in Platycladusorientalis is also a highly medicinal plant whose fresh leaves have been used as an anti-inflammatory [9], while the dried leaves have been used to treat flu and cough [10], high blood pressure [11], bleeding arthralgia [12], cancer [13],haemostatic [14] and used in Chinese medicine for the treatment of gout, rheumatism, diarrhea, and chronic tracheitis [15]. The leaves are anti-pyretic, astringent, diuretic, emmenagogue, emollient expectorant, refrigent and stomachic.[16,17]. Their use is said to improve the growth of hair [16].

Many chemical components from different parts of in Platycladusorientalis(L.) Franco has been extensively investigated. Monoterpenoidandsesquiterpenoids in essential oils of different parts of the plant [18, 19] flavonoids from the leaves [18, 19].

Phytochemical are bioactive compounds found in plants they are non-nutritive compound (secondary metabolites) that contributed to flavour, colour [20, 21]. They are needed by plant for purpose such as disease, pathogen defence and control.The aim of this study was to identify the volatile constituents of the leaf oil of in

Platycladusorientalisgrown in North Central Nigeria by using modern GC/MS machine [SHIMADZU GCMS-QP2010 PLUS] as well as study the phytochemical compounds of the leaf of in Platycladusorientalis.

II. Material & Methods

Collection Of Plant Leaves And Identification

Fresh matured leaves of the plant were collected in May 2014 from a village in Anyigba of kogi state Nigeria. They were identified by Dr Aina of the department of botany, kogi state university Anyigba. Voucher specimens are deposited in the herbarium of the faculty of biological science, kogi state university Anyigba, Nigeria.

Isolation Of The Volatile Oil

The method employed for the extraction of the volatile oil of the leaf of in Platycladusorientaliswas hydro distillation method according to European pharmacopoeia (2008). Oils were collected and kept in the refrigerator without further treatment before GC-MS analysis.

Preparation Of Plant Extracts Of Methanol

The collected fresh matured leaves of the plant of in Platycladusorientaliswere air dried usually at room temperature in a well aerated room; the air dried leaves were grinded into coarse powdered using a Thomas Willey machine. The aqueous extract was prepared by soaking 200g of the dried powdered samples in 400ml of methanol for 24 hrs. The extracts were filtered using Whatman filtered paper No 42 (125mm).

Screening Of Phytochemical Components

To identify the Phytochemical derivatives in the methanolic extract, standard phytochemicals screening was performed [22-24].Alkaloid test was performed by Meyer's tests, flavonoid and tannins by ferric chloride test, terpenoid and sterols by salkowski's test, saponin by frothing test, anthraquinones by borntrager's test and cardiac glycosides by Keller killiani's test.

Identification Test

The following test were carried out so as to detect active chemical constituents such as alkaloids, tannins, flavonoids, anthraquinones, steroids, cardiac glycosides, terpenoids and saponin.

Test For Alkaloids

1cm^3 of 1%HCl was added to 3cm^3 of the extract in a test tube. The extract was treated with a few drops of Meyer's reagent. A creamy white precipitate was observed indicating the presence of alkaloid.

Test For Tannins

About 0.5g of the dried powdered samples was boiled in20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue back colouration.

Test For Saponin

About 2g of the powdered samples was boiled in 20ml of distilled water in a water bath and filtered.10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously, then observed for the formation of emulsion. There was no formation of emulsion or frothing.

Test For Flavonoids

About 0.5g of the extract was boiled with 5.0ml of distilled water and then filtered .To 2.0ml of this filtrate; a few drops of 10% ferric chloride solution were added. A greenish blue or violet colouration indicated the presence of a phenolic hydroxyl group.

Test For Terpenoids

5ml of the extract was mixed in2ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of Terpenoids.

Test For Sterols

A few milligrams of the plant extract were dissolved in2ml of chloroform and then 2ml of concentrated H_2SO_4 was added from the side of the test tube. The test tube was shaken for a few minutes. Red colour development in the chloroform layer indicated the presence of sterols.

Test For Anthraquinones

An aliquot of 0.5g of the extract was boiled with 10ml of H2SO4 and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour change.

Test For Cardiac Glycosides

0.5g of the extract was diluted with 25ml of water, 2ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underplayed with 1ml of sulphuric acid. A brown ring at the interface indicates the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Gas Chromatography/Mass Spectrometry Analysis

The chemical composition of the essential oil was analysed using GC/MStechnique. The mass spectrometer was SHIMADZU GCMS-QP2010 Plus (Shimadzu Corporation, Japan) in the electron impact (EI) ionization mode (70eV) and HP-5MS (bonded 0.25µm) Capillary column (restek, Bellefonte, PA). Injector and Detector temperature were set at 250°C. The oven temperature was held at 60°C for 30minutes, then programmed to 240°C at rate of 5°C/min. Helium (99.99%) was the carrier gas at a flow rate of 1ml/min. Diluted samples (1/100 in hexane v/v) of 1.0ml were injected automatically. The linear velocity of the column was 36.8cm/sec, each peak was then analysed and assigned a number in the order that it was detected. The identification of the components was based on comparison of their mass spectra with those of NIST library mass spectra database and literature.

Table 1: Volatile constituents of the leaf oil of P.orientalis (L)

NO	RI	COMPONENTS	PERCENTAGE COMPOSITION
1	729	yclofenchene	3.87
2	894	ntolinatriene	0.48
3	896	temesiatriene	1.09
4	897	binene	0.26
5	943	nchene	4.90
6	945	mphene	1.94
7	948	-α-pinene	15.92
8	958	cimene	1.83
9	976	ans-β-Ocimene	8.71
10	978	pinene	1.76
11	990	myrcene	2.28
12	1018	monene	8.25
13	1023	Cyclohexene-4-methyl-3-(1-methylethylidene)	1.09
14	1052	Terpinolene	2.18
15	1092	Butyl-4-vinyl-1-cyclopentene	0.48
16	1131	Isopinocarveol	0.66
17	1143	α-terpineol	0.36
18	1215	8-methylenedispiro [2.0.2.5] undecane	2.03
19	1221	Copaene	0.40
20	1261	Perillic alcohol	0.34
21	1267	Ethyl-6-methylpyridine-2-carboxylate	3.87
22	1275	bbornylacetate	0.14
23	1277	rnylacetate	0.55
24	1344	cubebene	0.40
25	1386	romandendrene	3.73
26	1393	tchoulane	7.46
27	1448	9-tetradecadiyne	2.03
28		cardinene	0.40
29	1494	4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	3.73
30	1515	Germacrene D	2.43
31	1543	Cedrol	2.69
32	1579	α-caryophyllene	10.41
		Total components identified Monoterpenes hydrocarbons Sesquiterpene hydrocarbons Oxygenated monoterpenes Oxygenated sesquiterpenesOthers	96.62% 55.04% 28.96% 2.05% 2.64% 7.93%

III. Results And Discussion

The hydro distillation of the leaf oil of P.orientalis yielded 1.25% v/w essential oil. Thirty two components amounting to 96.62% were identified in the leaf oil. The identified components, their retention index and percentage composition of each component is given in table1 above. From the table it is evident that the composition of the oil is different both qualitatively and quantitatively as indicated in table 1 above.

The oil is rich in monoterpene hydrocarbons [55.04%], followed by sesquiterpene hydrocarbons [28.96%], others [7.93%], oxygenated monoterpenes [2.05%] and oxygenated sesquiterpenes [2.64%]. The main constituents of the leaf oil are IR-α-pinene [15.92%], α-caryophyllene [10.42%], Trans-β- ocimene [8.71%], limonene [8.25%] and patchoulane [7.46%].Other notable compounds found in the leaf are Fenchene [4.90%], ethyl-6-methylpyridine-2-carboxylate [3.87%], Aromandendrene [3.73%], Cedrol [2.64%], Germacrene D [2.43%] and β-myrcene [2.28%]. Constituents present in significant amount are Artemesiatriene [1.09%], ocimene [1.83%], Isopinocarveol [0.66%], Bornylacetate [0.55%], α-cubebene [0.40%], δ-cardinene [0.40] and α-terpineol [0.36%].

The Phytochemical screening of the methanolic extract was carried out in order to analyse the presence of secondary metabolites such as flavonoids, alkaloids, tannins, sterolse.t.c] by utilizing standard methods [22-24].Table 2 shows the results of the preliminary phytochemicals analysis. This study has revealed the presences of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoid, flavonoid, tannin, alkaloid, sterols, anthraquinones and cardiac glycosides were present in PlatycladusOrientalis leaves table: 2. Plants rich in terpenoid have been reported to have anti inflammatory, antimalaria, antiviral, inhibition of cholesterol synthesis and antibacterial [25], while those rich in alkaloid are used in medicine to reduce headache and fever. These are attributed for antibacterial and analgesic properties [26]. Epidemiologic studies recommended that coronary heart diseases are opposed by dietary flavonoids. [26].

Table 2: Phytochemical Constituents of the Leaf ofPlatycladusOrientalis

Phytochemicals	**Test**	**Me**
Alkaloids	Meyer's Test	+
Tannins	Ferric Chloride Test	+
Saponin	Frothing Test	_
Flavonoids	Ferric Chloride test	+
Terpenoids	Salkowski Test	+
Anthraquinones	Borntrager's Test	+
Cardiac Glycosides	Keller Killiani's Test	+
Sterols	Salkowski Test	+

Key: +=Present; -=Absent; Me= Methanolic Extract

IV. Conclusion

The results obtained in this study showed that Platycladusorientalis possess essential oil in the leaves of the plant and that their oil compositions were qualitatively different. This study represents the first to the best of my knowledge analysis of the leaves volatile constituentsof PlatycladusOrientalis by using a modern GCMS machine [SHIMADZU GCMS QP-2010 PLUS]. Further work is in progress on the identification of the root and seeds of Platycladusorientalis. The Phytochemical analysis showed the presence of effective biological compounds like alkaloids, steroids, tannins, flavonoids, cardiac glycosides, anthraquinones and terpenoid in Platycladusorientalis leaves thus providing knowledge of the Phytochemical metabolites. Observation drawn from this experiment shows clearly that the leaves of Platycladusorientalis contain Phytochemical constituents. However the present investigation showed that the studied plant is a potential good source of traditional medicine and because it's a medicinal plant ,it has commercial interest in both research institutes and pharmaceuticals company for the manufacture of new drugs in the treating of various diseases, thus we hope that the important Phytochemical properties identified by our study in the leaves of Platycladusorientalis will be helpful in cubing different diseases of this particular region of Northcentral Nigeria.

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