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Phytochemical and Therapeutic Studies of the Fruit Essential Oil of *Thuja orientalis* from Nigeria

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Keywords: Thuja orientalis, fruit essential oil, phytochemical, antioxidant, antibacterial.

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Phytochemical and Therapeutic Studies of the Fruit Essential Oil of *Thuja orientalis* from Nigeria

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Abstract- The aim of this study was to establish correlations between the identified phytocompounds in the fruit essential oil of Thuja orientalis from Nigeria and their medicinal properties (free radical scavenging, antioxidant and antimicrobial). Forty-seven compounds were identified in the fruit essential oil of T. orientalis making up 97.5% of the total percentage composition. The essential oil was predominantly made up of monoterpenoids (62.2%). The most abundant components was 1R-a-pinene (15.2%) followed by a-pinene (9.6%), 1S-α-pinene (5.6%), cyclofenchene (5.6%), (+)-3carene (4.5%), DL-pinene (4.5%) and *trans-6-*ocimene (4.0%). The high amount of terpenoids leads to more potent radical scavenging, antioxidant and antibiotic properties. The essential oil showed high potential as natural antioxidant and free radical inhibitor with IC₅₀ value: 2.5µgml⁻¹, the percentage inhibition of free radical ranged between 68-70%. Screening of the fruit essential oil for antimicrobial activities using the agarwell diffusion assay showed that the oil had high antibacterial properties against all bacteria isolates tested with zones of inhibition ranging from 10-30 mm. This study confirms that the fruit essential oil of this plant contains phytochemicals that can form the basis for the development of a potential natural antioxidant and antibiotic which are safe, cheap, and readily available at a large scale for pharmaceutical industries for further investigation and processing.

Keywords: Thuja orientalis, fruit essential oil, phytochemical, antioxidant, antibacterial.

I. INTRODUCTION

ssential oil is one of the plant based secondary metabolites that are used as the basis of many of the modern pharmaceuticals used today to treat some major diseases (Buchbauer, 2000). Essential oils are more environmentally friendly than synthetic products because they are biodegradable and have no residual effect. The impacts of essential oils and aromachemicals on the nervous system, gastrointestinal system, immune system, respiratory system, antimicrobial and antifungal activities have in recent years been the area of interest for researchers. Currently, there is global interest in finding new and safe antioxidants and antibiotics from natural sources, to prevent oxidative deterioration of foods and to minimize oxidative effect on living cells. The antioxidant and

antimicrobial potentials of several essential oils extracted from odoriferous medicinal plants are very important to human because antioxidants inhibit free oxygen radicals and free radicals formed from the substrate by donating hydrogen atoms or electrons (Prior et al., 2005). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raise serious concern of health delivery and accessibility due to untreatable infections. There is therefore the needed urgency to the search for safe and more active antibiotics. Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents (Savithramma et al., 2011; Ibrahim et al., 2012).

Thuja orientalis (*Cupressaceae*) commonly known as arborvitae, is a medicinal plant whose leaves have been locally used to treat flu and cough, high blood pressure, bleeding arthralgia, cancer, haemostatic, gout, rheumatism, diarrhoea, and chronic tracheitis (Zhu *et al.*, 2004). Homeopathic preparations of *T. orientalis* include pills, granules, oils, ointments and liquid dilutions. Due to its scent it has been traditionally used for clothing protection from moths and nowadays is added to pest repellant sprays and paints to protect against mites, moths, and rodents.

To the best of our knowledge, there is paucity of information on the phytochemicals, free radical scavenging, antioxidant and antimicrobial potentials of fruit of this plant from Nigeria. Therefore present study was undertaken for the first time with the aim at looking into these quantitative and qualitative parameters in the fruit of *T. orientalis grown in Nigeria*.

II. MATERIAL AND METHODS

a) Identification, Collection of Plant Material and Isolation of the Essential Oil

Fresh fruits of the plant were collected from its natural habitat in Ota, Nigeria and it was authenticated as *Thuja orientalis* (*Cupressaceae*). Fruit of the plant was subjected to hydro-distillation for 2 hours using a Clevenger-type apparatus and the essential oil extracted was stored at -4° C in a refrigerator (European Pharmacopoeia, 2004).

b) GC and GC-MS Analyses

Analysis of the fruit essential oil of *T. orientalis* was performed using multi-dimensional gas

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chromatograph coupled with Gas Chromatography-Mass Spectrophotometer (Shimadzu, Japan) equipped with non-polar and polar double capillary columns (25.0 m x 0.25 µm i.d., 0.25 µm df). High purity helium was used as the carrier gas at a constant flow rate of 0.99 ml/min. A total of 1 μ l sample was injected (split ratio 100:1) into GC and GCMS using AOC20i auto injector for analysis. The initial temperature was set at 60°C, heated at a rate of 3°C/minutes to 280°C and held isothermally for 6 minutes. Ion source temperature for these analyses was set at 200°C, while the interface temperature was set at 250°C, solvent cut time was 3.0 minutes and the mass spectrometer was set to operate in electron ionization mode with an ionizing energy of 70 eV as acquisition mass range from 40-700 a.m.u. at 0.50 scan/s. The constituents were identified by comparison of their retention indices with those of the literature. The retention indices were determined in relation to a homologous series of *n*-alkanes under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in National Institute for Standards and Technology (NIST) and with mass spectra from literature.

c) Free Radical Scavenging and Antioxidant Capacity

The free radical scavenging and antioxidant activities of the fruit essential oil against the stable free radical DPPH were measured using different concentrations (1000, 100 and 10 μ gml⁻¹) of the essential oil in methanolic solution of DPPH. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured at 517nm spectrophotometrically. Ascorbic acid was used as reference compound. The assay was carried out in triplicate. The percentage inhibition (1%) for each concentration was calculated by using the absorbance (A) values according to the following formula:

$I\% = [(A_{blank} - A_{eo})/A_{blank}] \times 100$

Where: A_{blank} is the absorbance of blank solution and A_{eo} is the absorbance of different concentrations of the essential oil. The dose-response curve was plotted and IC₅₀ value for the essential oil and the standard were calculated (Formagio *et al.*, 2011).

d) Antimicrobial Activity

Antibacterial activities of the fruit essential oil of *T. orientalis* were measured against Grampositive bacteria (*Streptococcus agalactiae*, *Streptococcus species and Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa and Salmonella typhimurium*) using agar-well diffusion method. Briefly, Petri dishes containing 20 ml of nutrient agar medium were swabbed using cotton applicator with an overnight starter culture of the bacteria isolates which were prepared in dilution to match the turbidity

intensity of the 0.5 McFarland standards. Wells (6mm diameter) were punched in the agar and filled with 10µl of different concentrations of the essential oil. The plates were incubated at 37°C for 24 hours. The quantification of microbial growth inhibition was determined by measuring the diameters (mm) of clear zones of microbial growth including the well itself. Gentamicin (GEN) and augmentin (AUG) were used as control (Pimentel *et al.*, 2013).

III. Results and Discussion

a) Essential Oil Composition

Hydrodistillation of the fruits of *T. orientalis gave* a crude essential oil with pleasant odour. Forty-seven compounds were identified from the GC and GC-MS analyses of the fruit essential oil amounted to 97.5% of the percentage total composition (Table 1). The most abundant components was $1R-\alpha$ -pinene (15.2%) followed by α -pinene (9.6%), $1S-\alpha$ -pinene (5.6%), cyclofenchene (5.6%), (+)-3-carene (4.5%), DL-pinene (4.5%) and trans- β -ocimene (4.0%). The principal classes of organic compounds in the fruit essential oil were monoterpenes (62.2%), sesquiterpenes (8.3%), sesterpenes (5.4%) and diterpenes (1.6%).

Comparatively, the chemical composition of this fruit essential oil was different from those reported in other studies such as foliage essential oils of four varieties of Thuja species from Poland which are T. occidentalis 'globosa', T. occidentalis 'aurea', T. plicata and T. plicata 'gracialis'. The study of the four samples resulted in the identification of thirty-one compounds in the essential oil of T. occidentalis "globosa" (96.92%) while in the oil of T. occidentalis "aurea" twenty-seven constituents have been identified (94.34%), major constituents in both were: α -thujone (50.14 and 51.60%, beverene (8.54% respectively). and 11.28%. respectively), sabinene (4.55% and 3.43% respectively) and camphor (4.47 and 3.09 % respectively). The characteristic difference between them is that T. occidentalis "globosa" has a high content of the ketones (6-thujone and fenchone), while T. occidentalis "aurea" has high levels of the diterpene (rimuene). The chemical profiles of the oils of the two T. plicata were also comparable, as thirty-two compounds have been identified in T. plicata (94.75%) and thirty in the oil of T. plicata "gracialis" (96.36%) having also α -thujone (62.12% and 54.48%, respectively), β-thujone (7.06% and 6.39%), terpinen-4-ol (4.66% and 3.11%) and sabinene (6.00% and 2.94%) as the most abundant compounds. On the other hand, T. plicata shows higher content of the ketone (fenchone), while T. plicata "gracialis" has high levels of the diterpene (beyerene) (Tsiri et al., 2009), but fruit essential oil of T. orientalis was characterised with high hydrocarbon terpenes rather than ketone. The above results show that the fruit essential oil of T. orientalis grown in Nigeria could serve as good source of these pharmaceutical and industrial useful compounds.

Compounds	% Composition	RI
cyclofenchene	5.6	729
artemesia triene	1.0	896
(+)-sabinene	1.0	897
2-bornene	1.0	932
1R-α-pinene	15.2	937
<i>α</i> -pinene	9.6	938
1S-α-pinene	5.6	941
DL-pinene	4.5	943
(+)-2-carene	1.0	948
camphene	0.4	952
<i>6</i> -mycene	1.5	958
1S-camphene	0.4	964
<i>6</i> -pinene	1.5	970
trans-6-ocimene	4.0	976
(+)-3-carene	4.5	1003
4-methyl-3-(1-methylethylidene)- 1-cyclohexene	1.0	1023
α-terpinolene	1.0	1052
camphene hydrochloride	0.4	1069
L-4-terpineol	1.0	1137
4-terpineol	2.0	1174
2-cyclopropylidene-1,7,7- trimethylbicyclo[2.2.1]heptane	0.2	1251
megastigma-7(E),9,13-triene	0.2	1278
α -terpineol acetate	1.0	1333
aromadendrene	0.5	1386
α-bergamotene	0.8	1430
γ-muurolene	0.2	1435
α-amorphene	0.2	1440
α-farnesene	0.8	1458
6 -cis-caryophyllene	1.0	1477
6-trans-caryophyllene	1.0	1494
α -humulene	1.6	1497
germacrene	0.2	1515
L-globulol	1.0	1530
ledol	2.0	1565
Z,Z,Z-1,5,9,9-tetramethyl-1,4,7- cycloundecatriene	0.4	1579
<i>epi</i> -globulol	1.0	1582
globulol	1.0	1588
13-tetradecenal	2.8	1591

Table 1: Chemical Composition of the Fruit Essential Oil of T. orientalis

9Z-9-tetradecenal	2.8	1609
α-bisabolol	0.4	1625
cis-9-hexadecenal	2.8	1808
dichloroacetic acid, undec-2-enyl ester	2.8	1834
n-pentadecanoic acid	1.6	1869
palmitic acid	3.0	1968
cis-9-octadecenal	2.8	2007
arachidic acid	1.6	2366
1-(+)-ascorbic acid 2,6- dihexadecanoate	1.6	4765
Percentage Total	97.5	

RI = Retention Index

b) Radical Scavenging and Antioxidant Activities

The free radical scavenging and antioxidant properties of the fruit essential oil of T. orientalis were examined using stable DPPH. The essential oil showed high potentials as a natural antioxidant and free radical inhibitor with IC_{50} value 2.5 μ gml⁻¹, the percentage inhibition of free radical was 68% at 10µgml⁻¹, 69% at 100μ gml⁻¹ and 70% at 1000μ gml⁻¹. The results of antioxidant and free radical scavenging activities showed that the fruit essential oil of T. orientalis from Nigeria had better radical scavenging and antioxidant properties than the aerial (twigs) ethanolic extract of Thuja occidentalis (Cupressaceae) with DPPH IC₅₀ values of 202.45µgml⁻¹ and percentage radical scavenging values of 73% at concentration 300µgml⁻¹, 62.7% at 250µgml⁻¹, 50% at 200µgml⁻¹, 43.5% at 150µgml⁻¹, 16.8% at 100µgml⁻¹ (Dubey and Batra, 2009) while the fruit methanolic extract of T. occidentalis had IC_{50} values of 150.98 μ gml⁻¹ (Das and Rani, 2013). Natural antioxidants are essential for helping the body maintain its natural health. They protect cells from free radicals, harmful oxygen molecules thought to damage cells that result in cancer, atherosclerosis and rheumatoid arthritis. Natural antioxidants also have many industrial uses, such as preservatives in food and to prevent the degradation of rubber and gasoline (Hamid et al., 2010). The benefits of antioxidants are very important to good health, because if free radicals are left unchallenged, they can cause a wide range of illnesses and chronic diseases. The human body naturally produces free radicals and the antioxidants to counteract their damaging effects. However, in most cases, free radicals far outnumber the naturally occurring antioxidants. In order to maintain the balance, a continual supply of external sources of antioxidants is necessary in order to obtain the maximum benefits of antioxidants. Therefore, natural antioxidants benefit the body by neutralizing, removing the free radicals from the bloodstream, protects the cells against their toxic effects and contribute to disease prevention (Pham-Huy et al., 2008).

Table 2 : IC ₅₀ of the Antioxidant Properties of the	Fruit
Essential Oil of <i>T. orientalis</i>	

Essential Oil and Reference Compound	DPPHIC ₅₀ µgml ⁻¹
T. orientalis	2.5
Ascorbic acid	9.0

Data are presented as triplicate of the mean

c) Antibacterial Potentials

The fruit essential oil of T. orientalis was screened for the antibacterial activities against clinically isolated Gram-positive and Gram-negative bacteria with the following results as shown in table 3 below. The antibacterial activities obtained were categorized as follows: (a) - = no inhibition, (b) 6-9 mm = low inhibition, (c) 10-15 mm = moderate inhibition and (d) \geq 15 mm = high inhibition. The antimicrobial properties of the fruit essential oil showed different selectivity for each organism. The results revealed that the fruit essential oil have high activities against all the tested organisms with the inhibition zones ranging from 10-30mm. The difference in susceptibility of the bacteria to the essential oil is thought to arise as a result of the differences in their cell membrane structure (Angienda et al., 2010). The fruit essential oil of T. orientalis gave a comparable result with other related species in the Cupressaceae family such as Thuja occidentalis: S. aureus (17mm), E. faecalis (11mm), E. coli (11mm), P. vulgaris (-), P. aeruginosa (9mm), Salmonella sp. (11mm), K. pneumoniae (13mm). Moreover, Thuja plicata shows the following activities with the following organisms: S. aureus (30mm), E. faecalis (10mm), E. coli (11mm), P. vulgaris (7mm), P. aeruginosa (10mm), Salmonella sp. (11mm), K. pneumoniae (-) (Jirovetz et al., 2006), Thuja koraiensis extract gave the following zones of inhibition with S. aureus (17 mm), B. subtilis (13 mm), E. coli (15 mm) and S. typhimurium (12 mm) (Zhang et al., 2014) while the fruit essential oil of Juniperus excelas (Cupressaceae) has no activity

against S. aureus, E. coli and P. aeruginosa (Weli et al., 2014).

Table 3 : Zones of Inhibition (mm) showing theAntimicrobial Properties of the Fruit Essential oil of*T. orientalis*

	Fruit Essential Oil			GEN	AUG
Conc. Organism	1000	100	10	10µg	30µg
E. coli	14	-	-	22	-
K. pneumoniae	15	15	15	21	-
P. aeruginosa	12	12	-	20	11
P. mirabilis	10	10	10	20	-
S. agalactiae	15	13	10	-	-
S. aureus	13	12	11	-	-
S. typhimurium	14	14	12	21	-
S. species	30	30	30	-	-

Keynote:--- = Resistant, 6-9 mm = low inhibition, 10-14 mm = moderate inhibition and \geq 15 mm = high inhibition.

IV. CONCLUSION

This study demonstrated that the fruit essential oil of *T. orientalis* produces a pronounced free radical scavenging, antioxidant and antimicrobial activities and could be further investigated for possibility of developing a cheap, acceptable and easy available therapeutic agent. The study therefore not only reveals the plant excellent natural antioxidants to be utilized nutritionally and pharmaceutically, but also provides good scientific justification for increased in traditional use of the plant. *Conflict of interest:* We have no conflict of interest.

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