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

Ololade Z.S.[1*], Fakankun O.A.[1], Alao, F.O.[2], Udi O.U.[1]

[1]Department of Chemical Sciences, Bells University of Technology, P.M.B. 1015, Ota, Nigeria
[2]Department of Biological Sciences, Bells University of Technology, P.M.B. 1015, Ota, Nigeria
zacchsnatpdt@gmail.com; suntolgroup@yahoo.com

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 98.9% of the total percentage composition. The essential oil was made up predominantly of monoterpenoids (62.2%). The most abundant components was 1R-*α*-pinene (15.2%) followed by *α*-pinene (9.6%), 1S-*α*-pinene (5.6%), cyclofenchene (5.6%), (+)-3-carene (4.5%), 6,6-dimethyl-2-methylenebicyclo [3.1.1] heptane (4.5%) and *trans*-*β*-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 IC_{50} 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 agar-well 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.

[Ololade Z.S., Fakankun O.A., Alao, F.O., Udi O.U. **Phytochemical and Therapeutic Studies of the Fruit Essential Oil of *Thuja orientalis* from Nigeria.** *J Am Sci* 2018;14(6):96-100]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 11. doi:10.7537/marsjas140618.11.

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

1. Introduction

Essential 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 biodegradation and have no residual effect. The impacts of essential oils and aroma-chemicals on the nervous system, gastrointestinal system, immune system, respiratory system, antimicrobial and antifungal activities have in recent years been the area of interest for researches. 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 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.

2. Material and Methods

Identification, Collection of plant material and Isolation of the Essential Oil

Fresh fruit of the plant was 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^{o}C in a refrigerator (European Pharmacopoeia, 2004).

GC and GC-MS Analyses

Analysis of the fruit essential oil of *T. orientalis* was performed using multi-dimensional gas 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 µl 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^{o}C and held isothermally for 6 minutes. Ion source temperature for these analyses was set at 200^{o}C, while the interface temperature was set at 250^{o}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.

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. Different concentrations (1000, 100 and 10 µgml^{-1}) of the essential oil in methanolic solution of DPPH. After 30 minutes of incubation at room temperature in the dark, the absorbance at 517nm was measured spectrophotometrically. Ascorbic acid was used as reference compound. The assay was carried out in triplicate. The percentage inhibition (I%) 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 IC_{50} value for the essential oil and the standard were calculated (Formagio *et al*., 2011).

Antimicrobial Activity

Antibacterial activities of the fruit essential oil of *T. orientalis* were measured against Gram-positive bacteria (*Streptococcus agalactiae*, *Streptococcus viridans* and *Staphylococcus aureus*), 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 were recorded. Gentamycin (GEN) and augmentin (AUG) were used as control (Pimentel *et al*., 2013).

3. Results and Discussion

Essential Oil Composition

Hydrodistillation of the fruit 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 making up 98.9% of the percentage total composition (Table 1). The most abundant components is 1R-*α*-pinene (15.2%) followed by *α*-pinene (9.6%), 1S-*α*-pinene (5.6%), cyclofenchene (5.6%), (+)-3-carene (4.5%), 6,6-dimethyl-2-methylenebicyclo [3.1.1] heptane (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%, respectively), beyerene (8.54% 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 (*β*-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.

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

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
α-pinene	9.6	938
1S-*α*-pinene	5.6	941
DL-pinene	4.5	943
(+)-2-carene	1.0	948
camphene	0.4	952
β-mycene	1.5	958
1S-camphene	0.4	964
β-pinene	1.5	970
trans-*β*-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
β-*cis*-caryophyllene	1.0	1477
β-*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
epi-globulol	1.0	1582
globulol	1.0	1588
13-tetradecenal	2.8	1591
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

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 2.5µgml^{-1}, the percentage inhibition of free radical was 68% at 10µgml^{-1}, 69% at 100µgml^{-1} and 70% at 1000µgml^{-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 IC_{50} values of 202.45 µgml^{-1} and percentage radical scavenging values of 73% at 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 µgml^{-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 *T. orientalis*

Essential Oil and Reference Compound	DPPH IC_{50} µgml^{-1}
T. orientalis	2.5
Ascorbic acid	9.0

Data are presented as triplicate of the mean

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 obtained antibacterial activities were categorized as follows: (a) - = no inhibition, (b) 6-9 mm = low inhibition, (c) 10-15 mm = moderate inhibition and (d) ≥ 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 excellent 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 the Antimicrobial Properties of the Fruit Essential oil of *T. orientalis*

	Fruit Essential Oil			GEN	AUG
Organism \ Conc.	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. viridans	30	30	30	-	-

Key note: --- = Resistant, 6-9 mm = low inhibition, 10-14 mm = moderate inhibition and ≥ 15 mm = high inhibition.

Conclusion

This study demonstrated that the fruit essential oil *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.

***Corresponding Author:**
Ololade Z.S.
Department of Chemical Sciences,
Bells University of Technology,
P.M.B. 1015, Ota, Nigeria
Email: zacchsnatpdt@gmail.com;
suntolgroup@yahoo.com
Tel: +2348034836546

References

1. Angienda PO, Onyango DM, Hill DJ. Potential application of plant essential oils at sub-lethal concentrations under extrinsic conditions that enhance their antimicrobial effectiveness against pathogenic bacteria, *African Journal of Microbiology Research*. 2010; 4 (16): 1678-1684.
2. Buchbauer G. The detailed analysis of essential leads to the understanding of their properties, *Perfumer flavorist*. 2000; 25: 64-67.
3. Das S, Rani R. Antioxidant and gastroprotective properties of the fruits of *Thuja occidentalis* Linn, *Asian Journal of Biochemical and Pharmaceutical Research*. 2013; 3(3): 80-87.
4. Dubey SK, Antioxidant Activities of *Thuja Occidentalis* Linn., *Asian Journal of Pharmaceutical and Clinical Research*. 2009; 2(1): 73-76.
5. Baharoglu Z, Garriss G, Mazel D. Multiple Pathways of Genome Plasticity Leading to Development of Antibiotic Resistance. *Antibiotics*. 22013; 2: 288-315.
6. European Pharmacopoeia Commission. *European Pharmacopoeia 5th Ed.* Council of Europe: Strasbourg Cedex, France. 2004.
7. Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A. Antioxidants: Its medicinal and pharmacological applications, *African Journal of Pure and Applied Chemistry*. 2010; 4(8): 142-151.
8. Ibrahim HA, Imam IA, Bello AM, Umar U, Muhammad S, Abdullahi SA. The Potential of Nigerian Medicinal Plants as Antimalarial Agent: A Review, *International Journal of Science and Technology*. 2012; 2(8): 600-605.
9. Jirovetz L, Buchbauer G, Denkova Z, Slavchev A. Stoyanova A, Schmidt E. Chemical composition, antimicrobial activities and odour descriptions of various *Salvia* sp. and *Thuja* sp. essential oils, Ernahrung/Nutrition. 2006; 30: 4.
10. Pimentel RBQ, Costa CA, Albuquerque PM, Junior SD. Antimicrobial activity and rutin identification of honey produced by the stingless bee *Melipona compressipes manaosensis* and commercial honey. BMC Complementary and Alternative Medicine. 2013; 13(151): 1-14.
11. Pham-Huy LA, He H, Pham-Huy C. Free Radicals, Antioxidants in Disease and Health, *Int. J. Biomed. Sci*. 2008; 4(2): 89-96.
12. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in food and dietary supplements. *J. Agric. Food Chem*. 2005; 53: 4290-4302.
13. Savithramma N, Rao ML, Suhrulatha D. Screening of Medicinal Plants for Secondary Metabolites, *Middle-East Journal of Scientific Research*, 2011; 8 (3): 579-584.
14. Shah SMM, Sadiq A, Shah SMH, Ullah F. Antioxidant, total phenolic contents and antinociceptive potential of *Teucrium stocksianum* methanolic extract in different animal models, *BMC Complementary and Alternative Medicine*. 2014; 14(181): 1-14.
15. Tsiri D, Graikou K, Pobłocka-Olech L, Krauze-Baranowska M, Spyropoulos C, Chinou I. Chemosystematic Value of the Essential Oil Composition of *Thuja* species Cultivated in Poland-Antimicrobial Activity, *Molecules*. 2009; 14: 4707-4715.
16. Weli AM, Al-Hinai SRK, Hossain MM, Al-Sabahi JN. Composition of essential oil of Omani *Juniperus excelsa* fruit and antimicrobial activity against foodborne pathogenic bacteria, *Journal of Taibah University for Science*. 2014; 8(3): 225-230.
17. Zhang XW, Choe YH, Park YJ, Kim BS. Effect of Korean arbor vitae (*Thuja koraiensis*) extract on antimicrobial and antiviral activity, *Afr. J. Pharm. Pharmacol*. 2014; 8(10): 274-277.
18. Zhu K. Information transparency of business-to-business electronic markets: A game-theoretic analysis. 2004; 50(5): 670-685.

6/25/2018