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ANTIMICROBIAL PROPERTIES OF ESSENTIAL OILS FROM SOME MEDICINAL PLANTS

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ABSTRACT

This research was focus on the extraction and antimicrobial screening of essential oil obtained from some medicinal plants. The antimicrobial screening of the various essential oils were carried out using micro broth dilution technique The result of the antimicrobial activities ofmethanolic extract of the stem-bark/leaves of the plants revealed that Bosweilliadalzielii, Ocimumamericanus, Hyptisspicigera, Hyptissuaveolens, Eucalyptus cammaldulensis, vossiacuspidata, Lavandulaofficinalis, Cinnamondedronecubenes, *D. microcarpun, D. Mespilisformis, Isoberliniadoka, L. korstringi, and K. sengalensis* have actitivtiesagains microorganisms.. The result shows that the essential oil of *Ocimumamericanus* has wide spectrum antimicrobial activity whereas the other essential oils exhibited selective antimicrobial activities.

Keyword: Antimicrobial Activity, Extract, Essential Oils, Methanol, *Ocimumamericanus*

INTRODUCTION

Our earliest human ancestors found plants to heal wound, cure diseases and ease troubled minds. People have long used indigenous medicinal plants for treatment of various ailments dating back to prehistory. Knowledge about the healing properties or poisonous effects of plants started from these earliest times in order to provide health and predates all other

medical treatment. Medicinal plant is defined as any plant which in one or more of its organ contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora,1982). This definition makes it possible to distinguish medicinal plants whose therapeutic constituent have been established scientifically from plants that are regarded as medicinal but which have not yet been subjected to a thorough scientific study. However, through trial and error, humans and some other species have discovered that under certain circumstances, physiological effects of some secondary metabolites can have medicinal benefits (Kubmarawa *et al.*, 2013). Essential oils contain highly volatile substances that are isolated by a physical method or process from plants of a single botanical species. These volatile substances are complex mixtures, constituted by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. They originate from the plant secondary metabolism and are responsible for their characteristic aroma. The oils normally bear the name of the plant species from which they are derived. (Abdurahman *et al.*, 2013). A large number of herbal materials contain essential oils with extensive bioactivities. These oil-plants and the culinary herbs include a broad range of plant species that are used for their aromatic value as flavorings in foods and beverages and as fragrances in pharmaceutical and industrial products (Baser, 2010).

Oils are used in the embalming process, in medicine and in purification rituals. Research has confirmed centuries of practical use of essential oils, and we now know that the 'fragrant pharmacy' contains compounds with an extremely broad range of biochemical effects. There are about three hundred essential oils in general use today by

professional practitioners (Hamid *et al.*, 2011). Continual contamination in our body which occurs as a result of bombaredment by virae, bacteriae, parasites and fungi, as such proper utilization of essential oils can be of a great benefit to help protect our bodies and homes from this onslaught of pathogens. Our immune system also needs support and these essential oils can give the required endorsement (Baser, 2010). The various applications of essential oils account for the great interest in their study. Such applications may be found in the cosmetic industry, as ingredients of fragrances, decorative cosmetic, and flavouring, in the food industry, as aromas and flavours, in the pharmaceutical industry, as active components of medicines and as antibacterial/antimicrobial, and in aromatherapy. At present, there are many studies in which they are used as intermediaries in certain chemistry reactions, among other applications (Baser *et al,* 2010).The aim of this research is to extract essential oils from some plants using modified steam distillation method and study their antimicrobial activities.

Plants Collection and Preparation

The collection site of the plants material was Girei Local government Area of Adamawa State. The list of plants and their parts used in this research are given in Table 1 below:

Table 1 List of Plants and their Parts

Plants	Plant part used
*Anogeissusleiocarpus*Guill and Perr	Bark

*Boswelliadalzielii*Hutch	Bark
*Cinnamondedronecubenes*Roxb	Leaves
*ComiphoraKerstungii*Engl	Bark
*Deteriummicrocarpum*Guill and Per	Leaves
*DiospyrosMespiliformes*Hochstex.A.Dc	Leaves
*Eucalyptus camaldulensis*Dehnn	Leaves
*FicusSyconmorus*Linn	Leaves
*Hiptisspicigera*Murubio	Leaves
*Hyptissuaveolens*Poit.	Bark
*Isoberliniadoka*Craib and Sapt	Leaves
*KhayaSenegalensis*A. Juss	Bark
*LannaeKerstingii*Engl and K	Leaves
*Lavandulaofficinalis*Buscal and Muchl	Leaves
*Ocimumamericanus*Sims.	Leaves
*Parkiaclapertonia*Keay	Leaves
*Vitexdoniana*SWEET	Leaves
Vossia cuspidate Griff.	Leaves

Preparation of Plants Extracts

200 g of the plant materials each were shade dried at room temperature, powdered using a grinder and soaked in 200ml of 70% methanol for 24 hours. At the end of the extraction each extract was then filtered using whatman filter paper. The filtrates further concentrated in vacuum at 30°C and stored at 4°C for further use.

These plants were then subjected to a modified steam distillation technique for possible extraction of essential oils, out of which 8 plants yielded oil in significant percentage

Antimicrobial Investigation for Essential Oils

Test Organisms

The bacteria and fungal strains that were used for the investigation includes: *Escherichia coli, Staphylococcus aureus, S. perfrinens, Pseudomonas aerugunosa, Candida albicans and Micobacterium tuberculosis.*

Determination of Antimicrobial Activity

The antimicrobial activity of the extract was determined using Disc Diffusion Method (Ravi *et al.*, 2010). Baur *et al.*, 1966, Mitscher *et al.*, 1972). Petri plates containing 10ml of Mueller Hinton agar medium was seeded with 24 hrs old cultured selected bacteria strain. Sterile filter paper disc (9mm in diameter) containing 1000-5000 ppm of the essential oil dissolved in dimethyl sulphuroxide (DMSO) placed on the medium, then another DMSO and water alone which served as negative controls. A standard disc containing chloramphenicol antibiotic drug (30 µg/disc) was used as a positive control. Incubation was done for 24 hrs at 37°C. The assessment of antimicrobial activity was base on the measurement of diameter of incubation zone formed around the disc. (diameter of inhibition zone minus diameter of the disc).

An average zone of inhibition was calculated for three replicates. An inhibition zone of 8 mm or greater was considered as a good antimicrobial activity (Ali *et al.*, 2001). According to Ogunwande, (2001) a cleared zone > 10 mm will be interpreted as sensitive while < 9 mm will be interpreted as resistance.Essential oils that showed positive activity in the preliminary screening were serially diluted (two-fold) and loaded on the filter paper disc. These serially diluted concentrations of the extracts, assayed in triplicate as described above to determine the Minimum Inhibitory Concentration (MIC) i.e. the minimum concentration per disc to inhibit growth of the test microorganism (Habsah*et al.*, 2000).

Microbroth Dilution Technique

Dilution of the oils: One 0.1 ml of the oil was dissolved in 0.1 ml of 10% dimethylsulphoxide (DMSO) to give a concentration of 50% oil.

Organism preparation: Five hundred microliter of test organism *mycobacteriumtuberculosis* (BCG) freshly thawed stock was inoculated into 50 mI of sterile Middlebrook 7H9/ADC broth medium and incubated at 30^0C for 5-7 days. The optical density (OD) of resulting culture determination at 650 nm was approximately 0.2 which equal to cfu/mI. Sample screening for antituberculosis activity: into each well of 96 microwell plate was transferred 50µ of sterile 7H9 broth starting from well 2 to 12. To each of the first well was added 100µl of 10% DMSO, 100 µI of 25 µg/mI solution of rifampicin (control drug) and 100 µI of each diluted oil sample. Using a multichannel pipettor 50µI was carefully removed from well 1 to 2, mixed thoroughly and the process continued to well 11 from which 50µI was withdrawn and discarded. The well were inoculated with 50 µL of diluted BCG culture and

incubated at 30^0C for 7 days. The results were confirmed by adding tetrazolium dye after the incubating period. The wells where there was no colour change were regarded as activity of test samples indicating inhibition of test organism. The last well where there was no growth is regarded as the minimum inhibitory concentration (MIC) of the sample.

RESULTS AND DISCUSSION

Percentage Yield of Essential Oil of the Various Plants

1 kg of each plant material was subjected to steam distillation for extraction of essential oils. The results obtained show that *Ocimumamericanus* leaves has the highest percentage yield of 0.16 %, followed by *Eucalyptus camaldulensis* and *Bosweilliadalzielii* each with percentage yield of 0.12 %. Other percentage yields include; *Cinnamondedronecubenes* (0.1 %), the two lamiceae family, *Hyptisspicigera* and *Hyptissuaveolens* (0.08 %) each, *Lavandulaofficinalis* (0.06 %) and *Vossiacuspidata* (0.05 %). The yield of essential oils varies with factors like site of collection, time of collection, part and form of plant used and the extraction method employed among others (Baser *et al.*, 2010). Different percentage yield have been reported by other researchers for *Hyptisspicigera* to be 0.2 % obtained from Benin republic, Mali (0.3 %), Cameroon (0.12 %), and Togo (1.2 %) (Kini*et al.,* 1993, Sidibe *et al.*, 2001 Tchoumbougnong *et al*, 2005, Koba *et al.*, 2007, and, Bognonou *et al.*, 2013). On the other hand, the percentage yield of the essential oils of leaves, stem bark and flowers of *Eucalyptus camaldulensis* obtained from Malaysia were 1.4, 0.5 and 0.46 % respectively (Elanaiem*et al.*, 2015). Similar report shows that the percentage yield of *Ocimumgratissimum* a mint plant like *Ocimumamericanus* were 0.97 and 0.83 % as reported by Owokotomo*et al.,* 2012 which is higher than 0.16 yield for *Ocimumamericanus* obtained from this work. Hydrodistillation of *Boweilliadalzielii obtained* from

Nigeria was reported to have yielded 1.25 % essential oil (Kubmarawa*et al.,* 2011) higher than what was obtained in our work. The variation observe in percentage yields as reported by other authors and that of the present work can be attributed to the geographical characteristics of the ecological zone, vegetative state of the plant species as well as the plant part and the method of extraction of the essential oil (Bognonou*et al.*, 2013).

Table 2 Percentage Yield of Essential oils of the Various Plants

Plants	Plants Part and Form	Volume (ml)	Appearance	% yield (v/w)
B. dalzielli	Dried stem bark	1.2	Colorless	0.12
C. cubenes	Fresh leaves	1.0	Clear, yellow	0.1
E. camaldulensis	Fresh leaves	1.2	Clear yellow	0.12
H. spicigera	Fresh leaves	0.8	Colorless viscous	0.08
H. suaveolens	Fresh leaves	0.8	Light-yellow viscous	0.08
L. officinalis	Dried leaves	0.6	Yellow viscous	0.06
O. americanus	Fresh leaves	1.6	Light-yellow	0.16
V. cuspidata	Fresh leaves	0.5	Yellow viscous	0.05

Antimicrobial Activities of the Essential oils of Various Plant

Table 3 Antimicrobial Activities of the Isolated essential oils.

	Microorganisms/Zone of Inhibition (mm)				
Plants Essential Oils	*E.coli*	*C.perfringens*	*C.albicans* 22018	*P.aeruginosa* 27853	*S.aureus* 2592
B.dalzielliі	25	-	-	-	25
E. camaldulensis	-	-	25	25	12.5
C. cubenes	25	-	-	-	-
H. spicigera	25	6.25	-	6.25	6.25
H.suaveolens	-	-	1.56	25	-
L. officinalis	25	-	25	50	-
O. americanum	3.125	6.25	0.39	3.125	3.125
V. cuspidata	-	-	-	-	-

The screening for the antimicrobial activities of the essential oils from the various plant parts was carried out using micro broth dilution technique. The result shows that the essential oil of *O. americanus* inhibit all microorganisms assayed (bacteria and fungi) (Table 3). This result also shows that all the microorganism were resistant to the essential oils of *V. cuspidata*, on the other hand *S. aureus* and *E.coli* are the only microorganism that present some degree of sensitivity to the essential oils of *B. dalziellii*. *Hiptisspicigeria* has appreciable degree of inhibition on all the microorganisms except *C.albicam*. The inhibitory effect of the essential oil of *Hyptissuaveolens* is only seeing in *C. albicam*, whereas the essential oil of *C. cubenes* inhibits only the growth of *E.coli.* Three microorganisms were sensitive each to *E.comandulensis* and *L. officinalis*, these includes; *C.albicans*, *P.auruginosa* and*S. aureus* to *E. comondulensis*, whereas *E. coli*, *C. albican* and *P. auerugnosa* were sensitive to *L. officinalis* essential oil. The Broad spectrum antibacteria and antifungal activities of the essential oil of *O. americanus* could be related to the presence of monoterpenesand sesquiterpene present in the oil. These compounds were equaly reported to have wide spectrum anti bacteria and antifungal activity as revealed in the works of Carson *et al.,* (2006), Solis *et al.,* (2004) and Piscopo*et al.,* (1991). The inhibition of *H. spicigera* essential oil on the microorganism could be traced to the appreciable percentage concentration of α-pinene, β-pinieneand Eucalyptol, eventhough the oil has no anti-fungal activity. In a related study by Sandra *et al,* (2007) and Fereshteh *et al.,* (2005) reveal that fraction of essential oil which contain high percentage of sabinene has

highest antifungal activity compare to the rest of the fractions. It is therefore possible to relate the inhibition of *C.albicans* by the essential oil of *Hpytissuaveolens* to the presence of sabinene. On the other hand the essential oil of *C. cubenes* inhibits the growth of *E.coli* due to the presence of p-mentha-2, 5-diene-7-ol trans, Elemol and p-mentha,2. *L. officinalis* contain β-myrcene, Germacrene A and Elemene. β-myrcene has been reported by Gallu*ci et al.,* (2010) to have no antimicrobial activity althought the work carried by Sinan*et al.,* (2009) showed high inhibition for *C.albicans*. Devandra *et al.,* (2011) shows the inhibition of both gram positive and gram negative bacteria by Germacrene A, while β-Elemene exhibit broad range antimicrobial as revealed by Murtala*et al.*, (2014). This has somewhat agreed with the result obtained in this test for essential oil of *L. officinalis*. M.cymene is the major compound of the essential oil of *E. camaldulensis* followed by a-phellandrene and Eulalyptol. Therefore the antifungal activity of this oil could be related to the presence of M.cymene this compound has been reported in the work of Carson &Riky (1995) to have no antibacterial activity rather has anti fungal activity. Consequently the inhibition observed in *P.auruginosa* and *S. aureus* by the essential oil of *E.camaldulensis* will be credited to the presence of a-phellandreneand Eucalytol. This is also aligned with the work of Zoran *et al,*(2000) and Fereshteh *et al,*(2005).

CONCLUSION

From the results obtained it can be generally concluded that only eight (8) plants namely; *Bosweilliadalziellia, cimumamericanun, Hyptis Spicigera, Hyptis Suaveolens, Vossia Cuspidata, Eucalyptus camaldulensis, Lavandulaofficinalis*, and *cinnamondedronecubines*having strong

odouringcharecterestics, and as such yielded essential oils when subjected to steam distillation process. Therefore, the result on the yield of essential oil by plant shown in this work is in line with the definition that essential oils are obtained from odoriferous plants (Baser *et al,* 2010). The result of antimicrobial analysis in this work reveals that each essential oil exhibited various degree of growth inhibition on different microorganism except the oil of *Vossiacuspidata* which does not possess antimicrobial activity on the tested bacteria. On the other hand *Ocimumamericanum* was shown to possesed a broad spectrum antimicrobial activity compare to the essential oils of the rest of the plants when tested on *E. coli, S. perfringens C. albicans, P. avengunosa*and *S. aureus.*

REFERENCES

Abdurahman, H.N., Ranitha, M., Azhari H.N., (2013); Extraction and Charecterization of essential oils from Ginger (*zingiberofficinale roscoe*) and Lemongrass (*cymbopogancitratus*) by microwave assisted hydrodistilation. *International journal of Chemical and Environmental Engineering* (4)4: 221-226

Ali, N. A. A; Julich, W.D. Kusnick C. and Lindequist U. (2001); Screening of Yemeni medicinal plants for Antibacterial and cytotoxic Activities. *Journal of Ethnophamacology* 74: 173-179.

Baser, K.H. (2010). Hand Book of Essential Oils: Science, Technology, and Application. CRC Press, London. 17-19

Baur, A.W., Kerby W.M.M; Sherris J.C. and Turck M. (1966); Antibiotic susceptibility testing by a standardized single disc method. America Journal of Clincal Pathology 45: 493-496

Bognonou A.G.S., Yedomonhan H., Avlessi F., Sohounhloue D. Chalard P. Chalchat J.C Delort L., Billard H., Caldefie-chezet F. and Figueredo G. (2013); Volatile oil Composition and Antiploriferative Activity of *Hyptisspicigera* Lam Against Human Breast Adenocarcinoma cells MCF-7. *Research Journal of Chemical Science*. 3(1): 27-31

Carston C.F Hammer K.A and Riley T.V (2006); *Melalencaalternifilia* (Tea tree) oil a Review of antimicrobial and other medicinal properties.*Journal of Applied Bacteriology* 106(4): 64-69

Carson C.F. and Riley T.V. (1995); Antimicrobial Activity of a major component of the essential oil of *Melaleucaalternifolia.Journal of Applied Bacteriology* 78(3): 264-269

Devandra, M., Shwani, J., Sangeeta, P.S., Abhimanyu, D. and Ganga, B. (2011); Chemical Composition and Antimicrobial Activity of the Eseential oils of Seneciorufinervis D.C. (Asteraceae).*Indian Journal of Natural Product and Resources.* 2(1): 44-47

Elnaiem, E.M., Land, Z. A., Ibrahim, F.A.A., Abdul, B.A.A. and Rosna, M.T. (2015); Essential oil Compositions and Cytotoxicity from various Organs of *Eucalyptus camaldulensis.International Journal of Agriculture and Biology*. 17: 320-326

Fereshteh E., Mortiza Y., Dina A., Ali S. and peyman S. (2005); Essential oil composition and antimicrobial Activity of Diplotacniadamavardica Z.

nature fosch. 60: 821-825.

Galluci N., Oliva M., carezzano E., Zygado J. and Damo M. (2010);Terpeness antimicrobial activity against shim producing and non producing staphylococci., molecular medicinal chemistry vol 21: 132-136.\

Habsah, M., Amram M., Mackeen M.M., Lajis N.H., Kikuzaki H., Nakatani N., Rahama A,A. and Ali A.M (2000); Screening of zingiberaceae extracts for antioxidant activities. *Journal of Ethnopharmacology* 72: 403-410.

Hamid, A.A., Aiyelagba, O.O., Usman, L.A. (2011); Essential oils: its medicinal and pharmacological uses. *International journal of current research* (3)2: 86

Kini F., Kam B., Aycard J.P., Gaydon E.M. and Bombarda I. (1993); Chemical Composition of the Essential oil of *Hyptisspicigera* Lam. From Burkinafaso.*J. essenti. Oil Res* 5(2): 219-221

Koba K., Raymoud C., Millet J., Chaumout J.P. and Sanda K. (2007); Chemical Composition of *Hyptispectinata* L.H. *lanceolata*poit H. *Suaveolens* L and H. *spicigera* Lam, Essential oil from Togo *Journal of Essential oil Bearing Plant* 10(5): 357-364

Kubmarawa, D., Akiniyi J.A. and Okorie D.A.(2013); Ethnomedicinal survey of traditional medicine of Lala people of Nigeria. *International Journal of Medicinal plant and Alternative Medicine*, 1(3): 39-57.

Kubmarawa D., Oguwande I.O., Okorie D.A., Olawone N.O., and Adaleke K. (2011); Constituent of essential oil of *Bosweilliadazielin* Hutch from Nigeria.*Journal of*

Essential oil research. 10: 119-120.

Mitscher, L.A., Leu R.P,.Bathala M.S., Wu W.N., Beal J.I. and white R. (1972); Antimicrobial agents from higher plants.Introduction, rationale and methodology *Lloydia*. 35: 175-166

Murtala B.I., Mohammed A.I., Aminu M. and Abubakar B.A. (2014); Antitrypanosomal activity of African Medicinal Plants; A Review Update.*Journal of Ethnopharmacology.* (1): 4-30

Ogunwande, I.A. (2001). Composition pattern of essential oils of the leaves of eucalyptus thuja, callitris and melaleuca specie growing in Nigeria University of Ibadan pp: 2-3.

Owokotomo I.A., Ekundayo O. and Dina O. (2012); *OcimumGratissumum* the Brine hrumps lethality of a Newchemotype Grown in South Western Nigeria. *Global Journal of Science frontier Research chemistry.*12(6): 56-49

Piscopo E., Diumo M.V., Mazzomi O., Ciron O.G and Bilognes A. (1991); synthesis and antimicrobial activity of hydroxyl –isophthadehyde acid derivative farmaco 46 (5): 669-76

Pierce A. (1999); The American pharmaceutical Association practical guide to natural medicine.

Ravi, K.U., Pratibha, D. Shoeb, A. (2010); Screening of Antibacterial Activity of six Plant Essential Oils against Pathogenic Bacterial Strains.*Asian Journal of Medicinal Science.* 2(3): 152-158

Sandra B.G Sue tomir Z.M., Suzan I.D., Alek Sandra M.O and Dejamu S. (2007); Antimicrobial activity of essential oil and different fractions of Jumiperciscommunis L.

and a comparison with some commercial antibiotics *J. Serb. Chem. Soc*. 72(4): 311-320

Sidibe L. Chachat J.C. Garry R.P and Harama M. (2001); Aromatic Plants of Mali (part III): Chemical Composition of Essential oil of 2 *Hyptis species*, *Hyptissuaveolens* (L)poit and *H. spicigera* Lam. *J. Essenti. Oil Res*. 13(1): 55-57

Sofowora, A. (1982); Medicinal Plants and Traditional Medicine in Africa.Wiley, New-York.Pp: 10-13

Solis S. C., Bacerra J., Florens C., Robied J. and Silva M. (2004); Anti bacterial and anti fungalTerpenes from pilgerodendronuniferum (D.Don) flonri.*J. chil. Chem. Soc* 49 (2): 157-161

Sinman K. D., Ahmet D.D., Hakki A. M. and metrin D. (2009);Anti microbial activity of the essential oils of rosini from cones of Abies Cilicia subsp.*African Journal of Biotecnology*. 19(8): 5021-5024

Tchoumbougnang F., Zollo P.H.A., Boyon F.F., Nyegue M.A., Bessiere J.M and Menut C. (2005); Aromatic Plants of Tropical Central Africa. XLVIII, Comparative Study of the Essential oils of four *Hyptis* species from Cameroon: *H.Lanceolata*poit*;* *H.pectinata* (L) poit, *H. spicigera* Lam. And *H. suaveolens*poit.*Flavor Frag. J*. 20(3): 340-343

Zoran K., Ivica A., Ivana M., Goranka P., Sonja P. and Tenta M. (2000); Antibacterial activity of Halothane Eucalyptol and orange oil.*Actastomatcroat* 34(3): 307-309

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