European Journal of Applied Sciences – Vol. 10, No. 2
Publication Date: April 25, 2022
DOI:10.14738/aivp.102.11978.
Joseph, O. V., Agbagwa, O. E., Frank-Peterside, N. (2022). Antioxidant Potential of Ethanolic and Aqueous Extracts of Selected Medicicinal Plants on Fungal Species. European Journal of Applied Sciences, 10(2). 84-98.

Antioxidant Potential of Ethanolic and Aqueous Extracts of Selected Medicicinal Plants on Fungal Species

Joseph, O. V.
Department of Science Laboratory Technology
Heritage Polytechnic Ikot Udoata Eket, Nigeria

Agbagwa, O. E.
Department of Microbiology
University of Port Harcourt, P.M.B. 5323
East-West Road Choba Rivers State, Nigeria

Frank-Peterside, N.
Department of Microbiology
University of Port Harcourt, P.M.B. 5323
East-West Road Choba Rivers State, Nigeria

ABSTRACT

Medicinal plants are plants in which at least one of its parts possesses therapeutic properties that are useful for the treatment of aliments. The study was carried out to determine the antioxidant potential of *Borreria verticillata, cassia alata, Carica papaya, Diodia Sarmentosa,* and *Ocimum gratissimum* on *Rhizopus oryzae*, *Aspergillus tamarii*, *Tricholoma matsutake*, *Kodamaea ohmeri*, *Aspergillus awamori* , *Aspergillus fumigatus*, *Aspergillus nomius*, *Aspergillus awamori* and *Aspergillus nomius*. The five medicinal plants were collected from University of Uyo botanical garden, while the nine fungal isolates were stock cultures from previous studies. The leaves of the five plants were extracted by ethanolic and aqueous methods, and subjected to phytochemical screening. The extracts were also subjected to antifungal assay by the agar diffusion methods. Results obtained on the phytochemical screening of leaf extracts indicated the presence of alkaloids, tannins, saponins, terpenes, flavonoids, salwoski, Keller-killian, liberman, gyanogenetic glycosides, phlobactanin, and free and combined anthraquinone in varying quantities. The antifungal assay of the aqueous and ethanolic leaf extract for all the plants at five different concentration (31.25mg/ml,62.5mg/ml,125mg/ml,250mg/ml and 500mg/ml), showed that that *Cassia alata*(14mm ethanol,10.67mm aqueous) had the highest inhibition zone, which was followed by *Ocimum gratissimum* (12.89mm ethanol,and 10.56 aqueous), *Diodia sarmentosa* (12.87mm ethanol and 8.22mm) and *Borreria verticillata* (12.44mm ethanol and 7.78mm aqueous). The lowest inhibition was observed in *Carica papaya* (11.78mm ethanol and 6.56mm aqueous) at concentration 500mg/ml. Minimum inhibitory concentration obtained ranged between 31.25-125mg/ml. Generally, ethanolic extract were more effective than aqueous extract, although all the plant had inhibitory effect in both solvents. The outcome of the work justifies the use of these plants in ethno-medicine.

Keywords: Antioxidant; Aqueous Ethanolic; Fungi; Bioactive; Phytochemical

INTRODUCTION

Medicinal plants have been found to be effective in the treatment of diseases caused by fungi and bacteria for more than three decades due to their antioxidant activity [1]. Antioxidant constituents of the plants have the ability to act as a radical scavengers thus converting free radicals to less reactive species. Studies have shown that most natural antioxidants associated with plants are flavonoids, vitamins, phenols, carotenoids and dietary glutathione [2] Medicinal plants are still recognized as the preferred primary healthcare system in many communities, with over 60% of the world's population and about 80% in developing countries depending directly on medicinal plant for their medical purposes [3]. Modern medicine today utilizes active compounds isolated from plants and about 80% of these active ingredients indicate positive correlation between their modern therapeutic use and traditional use [4].The effectiveness and antioxidant properties of these plants on fungi and diseases are due to the presence of some phytochemical constituents and bioactive compounds which can be determined in the extracts [5]. The search for use of drugs and dietary supplements obtained from plants has increased in recent years. Scientists have explored plants for phytochemicals present in them in order to develop into medicines for various disease treatments. *Carica papaya* is an eminent medicinal plant that in use in most parts of the world to treat diseases such as malaria, dengue, inflammation, and skin infections and to neutralize free radicals. Others use the flowers of *C. papaya* as a fresh vegetable to supplement the diet of our society and support higher levels for the growth of the individuals. Studies have shown that the flowers of *C. papaya* to prevent cancer, increase digestion and appetite, and delineate heart problems [6]. *Diodia sarmentosa* is mostly found in the tropics it is a used traditionally for the treatment of ulcer, diabetes, eczema, and , injury oedema and other ailments in some parts of Nigeria. It is been used because of its antioxidant and anti-inflammatory properties [7, 8]. *B. verticillata* occurs in agriculture areas, grass lands, rural and urban areas commonly found in tropical Africa including Nigeria. The flower is used as antipyretic and analgesic [9, 10]. The roots of *B. verticillata* act as anti diarrhoea and for treatment of erysipelas and haemorrhoids. In some countries its used for diabetes, amenorrhoea and dysmenorrhea [11, 12]. *B. verticillata* is used to treat bacteria skin infections and leprosy in Senegal [13]. The juice of fresh aerial part is used in Nigeria for the treatment of skin eczema. [14]. A study on the phytochemical screening and antimicrobial activity of plant extracts such as *Carica papaya*, *Citrus paradise*, *Citruse sinensis* and *Vernonia amygdalina* revealed the presence of Alkaloid, Tannin,Phenol and Flavonoid [15] Tannin, Plobatannin, Flavonoid, Saponnin and Anthraquinone were identified in *Ocimum gratissimum* leaf extract following a phytochemical screening to determine its mineral composition [16]. Flavonoid, Saponnin and Tannin were found to be present in *Cassia alata* following a study on its screening and bioactivity [17]. Studies carried out on the on the phytochemical screening of *Borreria verticillata* leaves revealed arthraquinone, saponins, tannins, and flavonoid [18,]. *Dioda sarmentosa swartz* leaves have shown to contain alkaloid, tannins, flavonoid and terpenoid [19]. These medicinal plants if used in the right mix and concentration can inhibit the growth of some microbial activities. This study therefore aims at determining the bioactive components present in five medicinal plants namely *Cassia alata*, *Diodia sarmentosa*, *Borreria verticillata* , *Carica papaya* and *Ocimum gratissimum on fungal isolates* and to ascertain their antioxidant potentials on nine fungal isolates namely: *Rhizopus*

oryzae, *Aspergillus tamarii*, *Tricholoma matsutake*, *Kodamaea ohmeri*, *Aspergillus awamori*, *Aspergillus fumigatus*, *Aspergillus nomius*, *Aspergillus awamori*, *Aspergillus nomius*.

MATERIALS AND METHODS

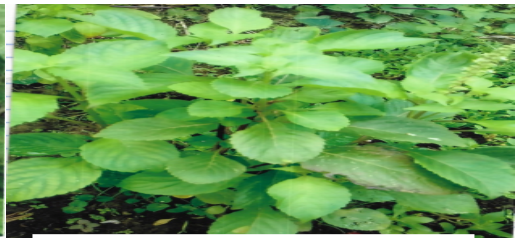
Collection and Preparation of the five Medicinal Plants

Leaves of *Cassia alata*, *Diodia sarmentosa*, *Borreria verticillata*, *Carica papaya* and *Ocimum gratissimum* were collected in separate sterile polythene bags from the University of Uyo Teaching Hospital Botanical Garden, Uyo, Akwa Ibom State Nigeria. The leaves were subject to proper identification and authentication in the Department of Microbiology, Faculty of Science in the same University. The leaves samples were washed with distilled water chopped into pieces and air-dried for two weeks after which they were grounded into powder with mortar and pestle for extraction.

Five Medicinal Plants Sampled



Scientific Name: *Cassia alata*
Family: Caesalpiniaceae
Common Name: Candle bush, Ringworm bush, Craw craw plant
Local Names: Okorojo, AdayaOkon (Ibibio)



Scientific Name: *Ocimum gratissimum*
Common Name: Scent leave
Local Names: Ntong (Ibibio)
Part used: Leaves



Scientific Name: *Borreria verticillat*
Common Name: buttom wood
Local Names:Abiaikara (Ibibio)
Part used: Leaves



Scientific Name: *Carica Papaya*
Family: Caricaceae
Common Name: Paw-paw
Local Names: Akpot (Ibibio)
Part used: Leaves



Scientific Name: *Diodia sarmentosa*
Family: Rubiaceae
Common Name: Turtle's shell, Diodia
Local Names: EDEM Ikid (Ibibio)

Fungal Isolates

Fungal isolates used in this study were identified in our previous study, they include *Rhizopus oryzae* MN525245 (J1), *Aspergillus tamari* MN653149 *(*J2), *Tricholoma matsutake* MF038193 (J3), *Kodamaea ohmeri* LC 317627 (J4), *Aspergillus awamori* MG 827310 (J5), *Aspergillus fumigatus* LC 171332 (J6), *Aspergillus nomius* AF 104447 (J7), *Aspergillus awamori* MG 827310 (J8) and *Aspergillus nomius* AF 104447 (J9)

Extraction of the Sample

Aqueous Extraction: A mixture of 20grams of each powdered leaf and 200ml of distilled water was thoroughly prepared and kept for 3days (72 hours). After 3 days, the plant extracts were filtered through a membrane filter (Whiteman No. 1 paper) into a sterile beaker and placed in a water bath for sterilization in order to obtain the crude extract. The sterile extract obtained was then transferred into a sterile capped MacCartney bottles and refrigerated at 4^{o}C until further use [20].

Ethanolic Extraction: A mixture of 20grams of each powdered leaf and 200ml of 70% ethanol was thoroughly prepared and kept for 3days (72 hours). After 3 days, the plant extracts were filtered through a membrane filter (Whiteman No. 1 paper) into a sterile beaker and placed in a water bath for sterilization in order to obtain the crude extract. The sterile extract obtained was then transferred into a sterile capped MacCartney bottles and refrigerated at 4^{o}C until further use [20].

Preparation of Extract Concentrations from Various Extracts: The concentrations of the crude extracts obtained from the respective solvents were prepared using appropriate volume of distilled water to obtain concentration of 31.25, 61.5, 125,250 and 500 mg/ml for each extract.

Phytochemical Studies

Qualitative studies were carried out to identify the presence of some phytochemical constituents in the plant extracts using standard methods [21]. The presence of Saponin was identified using Frothing test and Bicarbonate test; Ferric chloride test and Bromine water test were used to identify the presence of Tannin; Borhtrager's test was used to identify both free hydroxy Anthraquinone and Anthraquinone derivatives; Alkaloid was identified by treating a 1ml solution that contains 5ml 1% hydrochloric acid and 0.5grams of the plant extract with Mayer's reagent and Dragendoeff reagents respectively. A yellow precipitate was taken as an indication of the presence of alkaloids [22]. Flavonoid was determined by using the Shinoda reduction test. Other test carried out include Hydrochloric Acid Test to identify Phlobatannin, Lieberma's test to identify Terpenes, Salkowski test to identify Cardiac Glycosides and Keller-killani test to identify Deoxy sugar.

RESULTS

Phytochemistry and Assay of the Plant Leaves for Antimycotic Properties

Phytochemical analysis conducted on the five medicinal plants (*Cassia alata, Ocimum gratissimum, Carica papaya, Diodia sarmentosa* and *Borreria verticillata*) showed that flavonoids, tannins, Salkwoski and Liberman were extracted from all the five plants. Keller-Killian was extracted from *C. alata*, *O. gratissimum*, *C. papaya*, and *Diodia sarmentosa*. Alkaloids

were only extracted from C. papaya and *Diodia sarmentosa*. Saponins were extracted from *C. alata*, *O. gratissimum* and *B. verticillata*. Phlobacternin was extracted from *O. gratissimum*, *C. papaya* and *B. verticillata*. Free Anthraquinone and combined Anthraquinone were only found in *C. alata* and *B. verticillata*, whereas Terpenes and cyanogenetic glycosides were not extracted from any of the five plants leaf. The result is presented in Table 1. The percentage occurrence of alkaloids, tannins, sapnnons and flavonoids are presented in Figure 1.

Table 1: Phytochemistry and Assay of the Plant leaves for Antimycotic Properties

PLANT NAME	Alkaline	Tannis	Saponins	Terpenes	Falvonoids	Salkwoski	Keller-killiani	Liberman	Combined Anthraquinone	Cyanogenetic glycosides	Anthraquinone	Phlobactanins
Cassia alata	-	+	+	-	+	+	+ +	+ +	+	-	++ +	-
Ocimum gratissimum	-	+ +	+	-	+ +	+	+	+	-	-	-	+
Carica papaya	+	++	-	-	+++	+	+	+	-	-	-	+
Diodia sarmentosa	+	+	-	-	+	+	+	+	-	-	-	-
Borreria verticillate	-	+	++ +	-	+ +	+	-	+	+	-	+	+

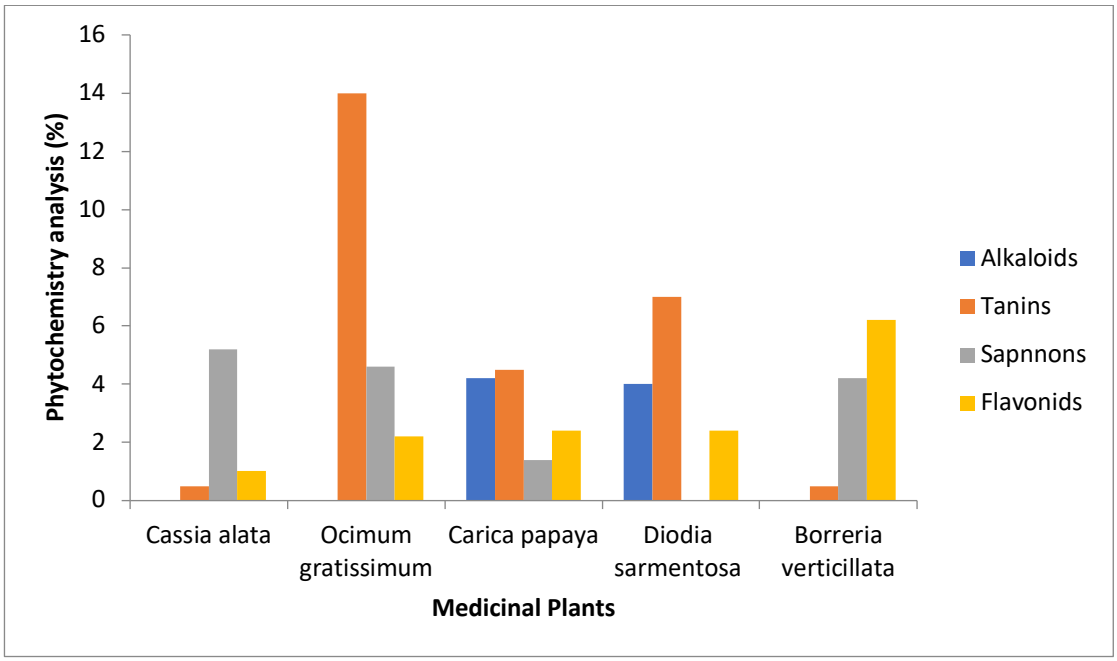
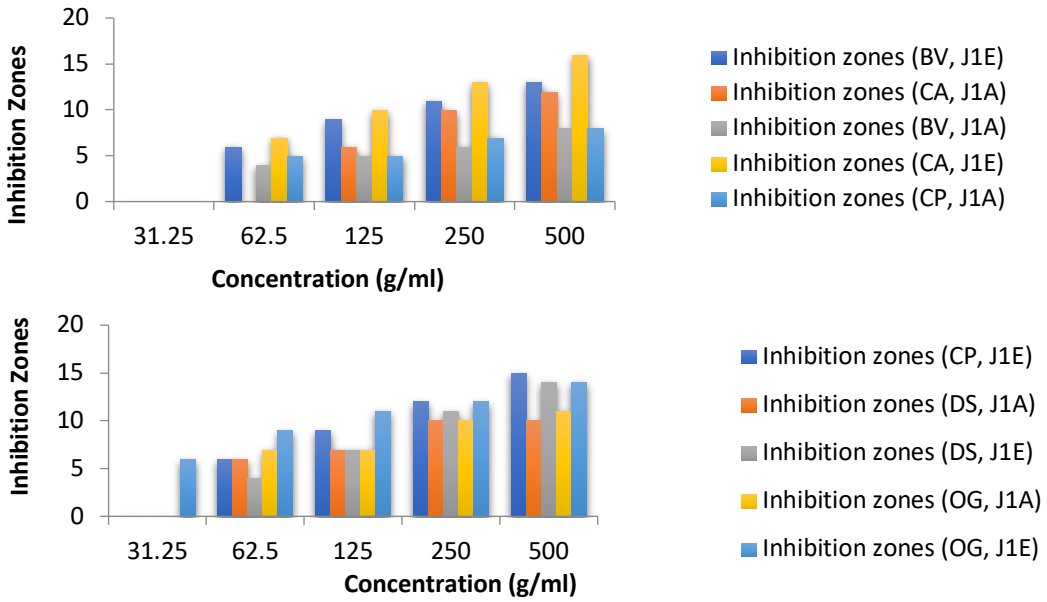


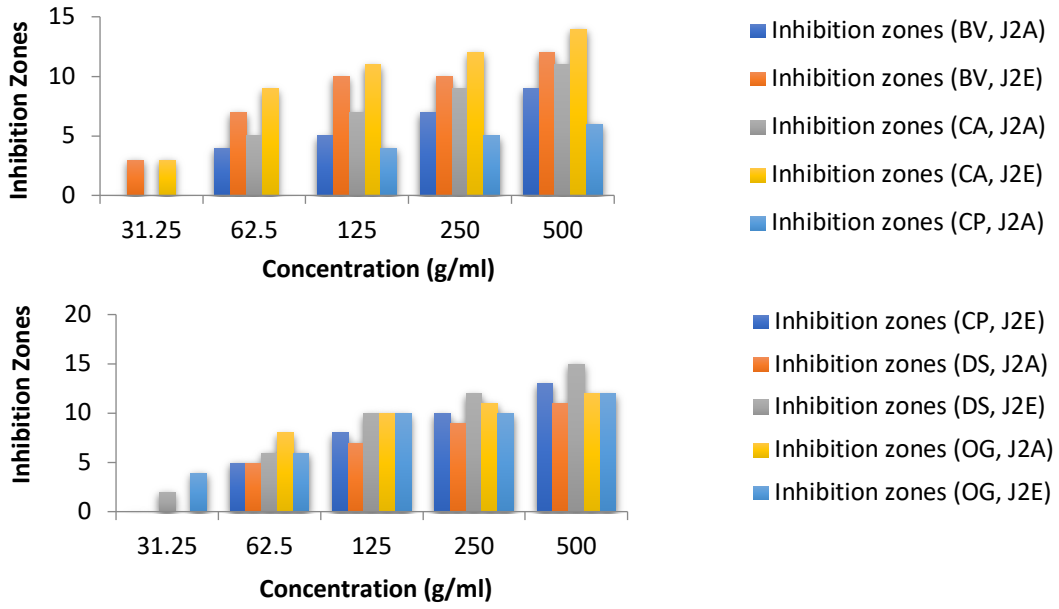
Figure 1. Percentage occurrence of alkaloids, tannins, sapnnons and flavonoids on plant extract

Antioxidant Potential of Plant Extracts

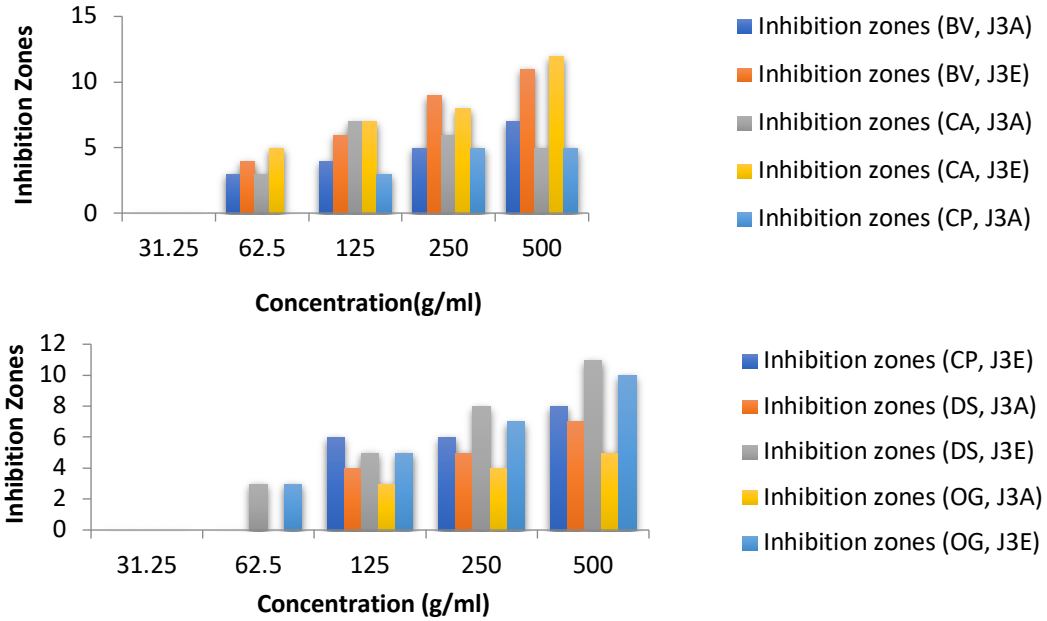
The ability of the five aqueous and ethanolic leaf extracts of the plants to inhibit nine fungal isolates was determined at different concentrations. Results obtaine dshowed that efficacy was higher at high concentration. J1(*Rhizopus oryzae*) and J2 (*Aspergillus tamarii*) similart inhibition on the average for both ethanolic extract (15mm) and aqeuous extracts(12mm). This shows that *Cassia alata* extracts at a high concentration is suggestive for the treatment of *Rhizopus oryzae*. For the second higher concentration (250mg/ml), *Cassia alata* showed the highest inhibition for the ethanolic extracts (13mm) while *Cassia alata*, *Ocimum gratissimum* and *Diodia sarmentosa* showed the highest inhibition (10mm) for the aqueous extracts. The effect of *Borreria verticillata, Cassia alata, Carica papaya, Occimum gratissimum, Diodia sarmentosa* and *Ocimum gratissimum* on J3(*Tricholoma matsutake*, J4 (*Kodamaea ohmeri*) and J5(*Aspergillus awamori*) showed that *Cassia alata* ethanolic extract had reduced inhibition at the highest concentration for J1 (12mm) while *Borreria verticillata* showed the highest inhibition (9mm) for the aqueous extract at the same concentration (500mg/ml). At concentrations 250mg/ml and 62.5mg/ml, *Cassia alata* had the highest antifungal activity for the aqueous extract while *Borreria verticillata* showed the highest inhibition for the ethanolic exract. No inhibition was observed however at the lowest concentration (31.25mg/ml). For isolate J4(*Kodamaea ohmeri*), *Cassia alata* and *Diodia sarmentosa* showed the highest inhibition zones(13mm) for the ethanolic plant extract at 500mg/ml while *Cassia alata*, *Diodia sarmentosa, Borreria verticillata* and *Ocimum gratissimum* showed the highest inhibition(8mm) at the same concentration(500mg/ml) for the aqueous extract. For concentrations 62.5mg/ml, 125mg/ml and 250mg/ml, *Cassia alata* showed the highest antigungal activity for the ethanolic extract while *Diodia sarmentosa* also showed the highest inhibition zones for these concentrations. *Borreria verticillata* however showed very low inhibition at 31.25mg/ml (2mm). For the antifungal activity on *Kodamaea ohmeri*, *Cassia alata* ethanolic extract and *Diodia sarmentosa* aqueous extract dominated on the average. For isolate J6, the highest antifungal activities were observed for both *Cassia alata* and *Ocimum gratissimum.* At 500mg/ml, *Ocimum gratissimum* showed the highest inhibition zone (15mm) for the etholic extract, while *Cassia alata* showed the highest inhibition (12mm) zone for the aqueous extract. For concentrations 62.5mg/ml, 125mg/ml and 250mg/ml respectively, *Cassia alata* dominated for the ethanolic extract in their antifungal activity while *Ocimum gratissimum* dominated in their antifungal activity for the aqueous extract. Only *Cassia alata* showed inhibition zones at 31.25mg/ml for both plant extracts. Fungal isolates J5 and J8 being the same fungal strands, were susceptible to both the ethanolic and aqueous plant extracts in the same manner. *Cassia alata* showed the highest antifungal activity on *Aspergillus awamori* for concentrations 31.25mg/ml, 62.5mg/ml, 125mg/ml, 250mg/ml and 500mg/ml respectively. Similarly, *Ocimum gratissimum* showed the inhibition zones for concentrations 62.5mg/ml, 125mg/ml, 250mg/ml and 500mg/ml respectively. Fungi J7 and J9 (*Aspergillus nomius*) were susceptible to both the ethanolic and aqueous plant extracts in the similar manner. For all the concentrations, *Cassia alata* showed the highest inhibition zones for both extracts on the average. It showed the highest inhibition at various concentrations of ethanolic and aqueous plant extracts. Detailed results of the antioxidant potential of the plant extracts are shown in figures 2 -10.



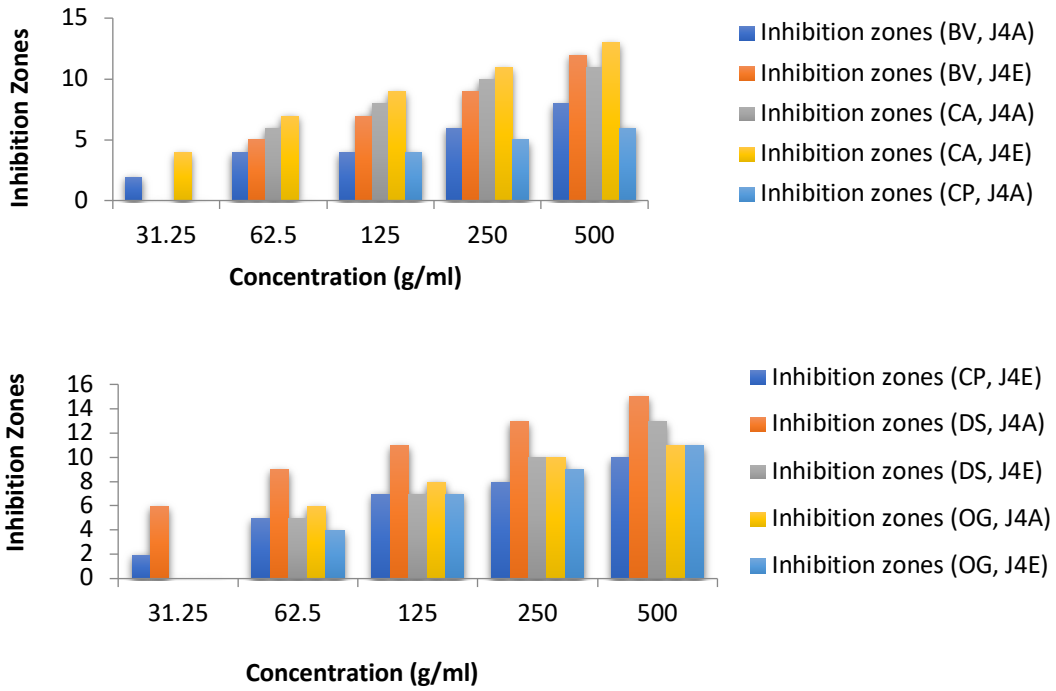
Figures 2a and 2b: Antifungal potential of aqueous and ethanolic plant extracts on isolate J1 at various concentrations



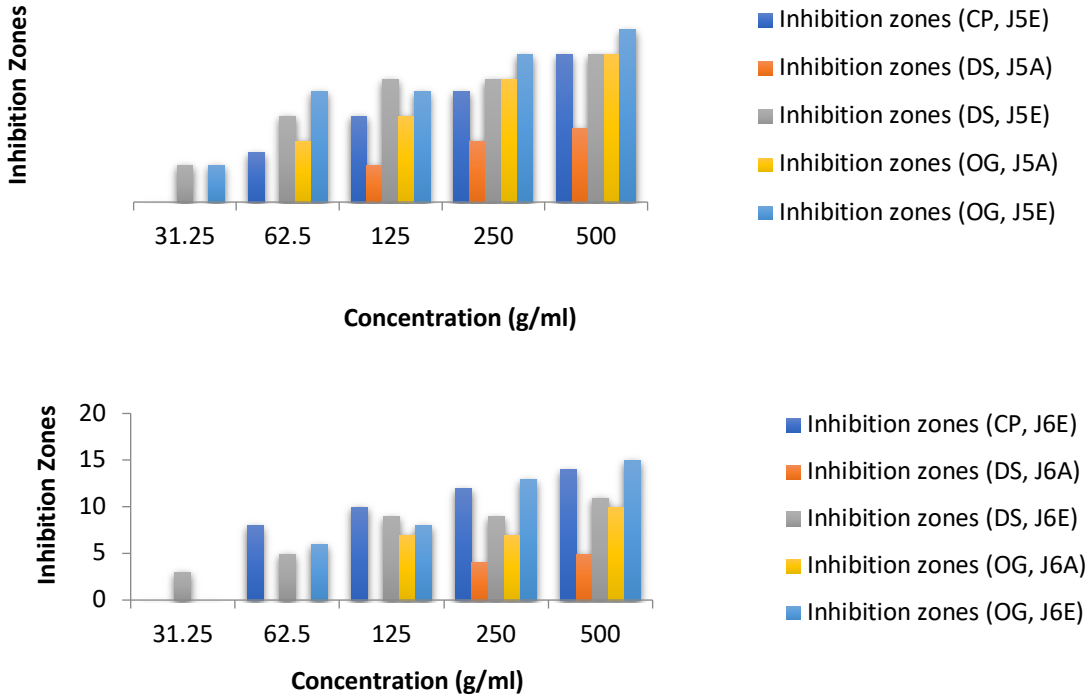
Figures 3a and 3b: Antifungal potential of aqueous and ethanolic plant extracts on isolate J2 at various concentrations



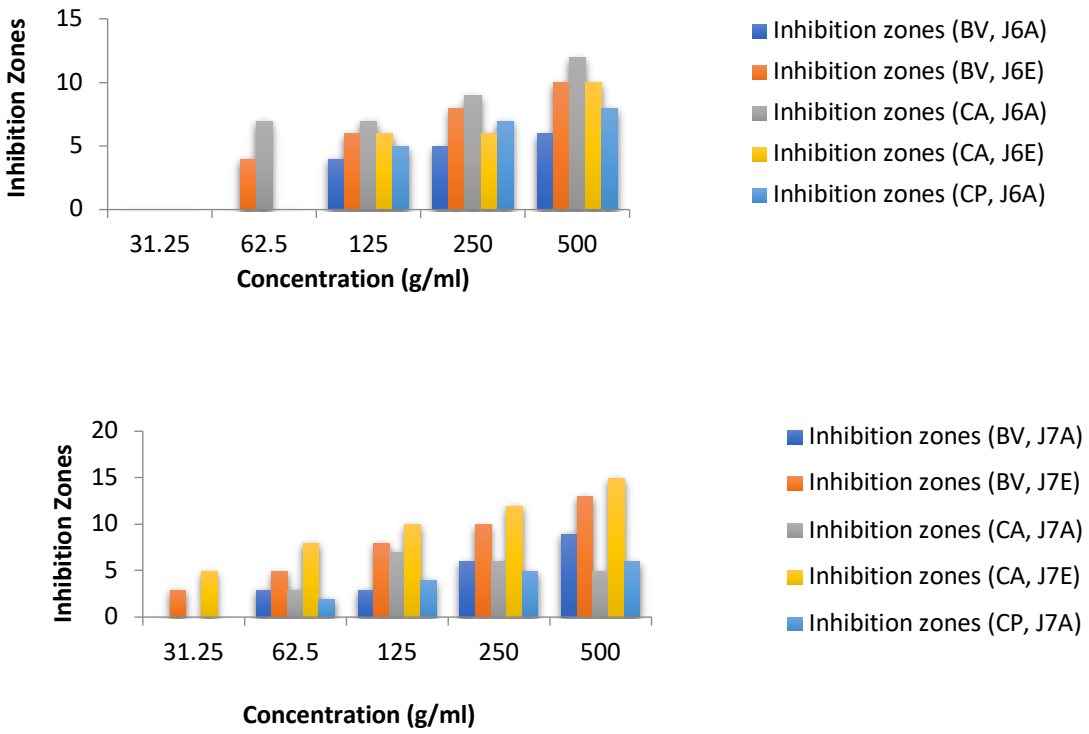
Figures 4a and 4b: Antifungal potential of aqueous and ethanolic plant extracts on isolate J3 at various concentrations



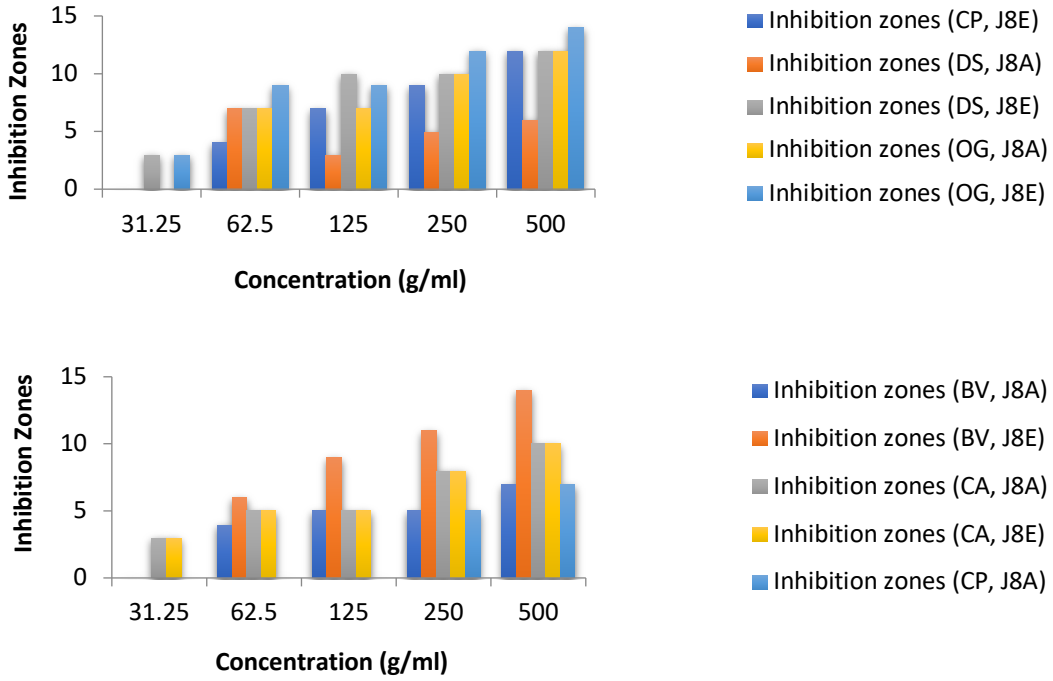
Figures 5a and 5b: Antifungal potential of aqueous and ethanolic plant extracts on isolate J4 at various concentrations



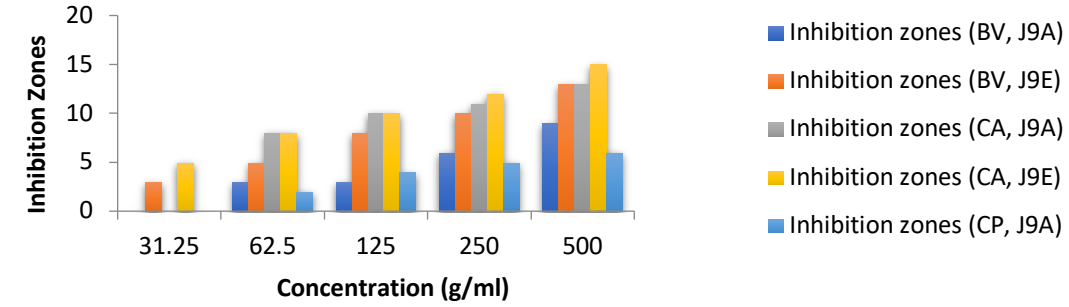
Figures 6a and 6b: Antifungal potential of aqueous and ethanolic plant extracts on isolate J5 at various concentrations

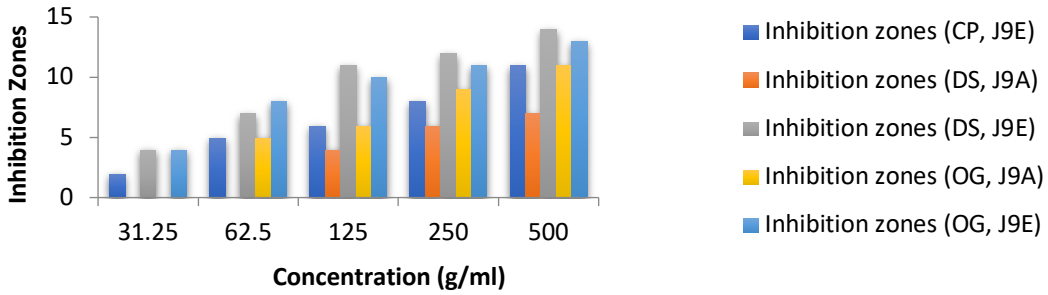


Figures 7a and 7b: Antifungal potential of aqueous and ethanolic plant extracts on isolate J6 at various concentrations

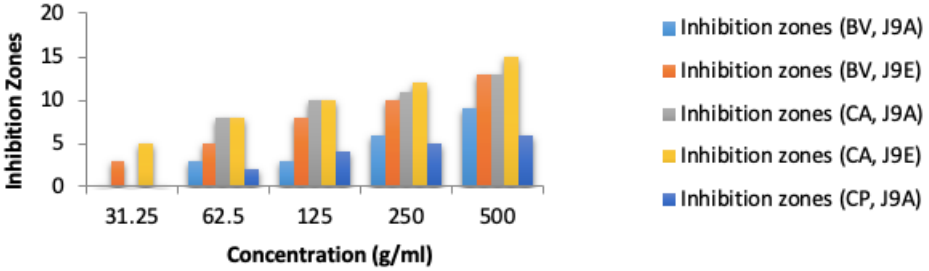


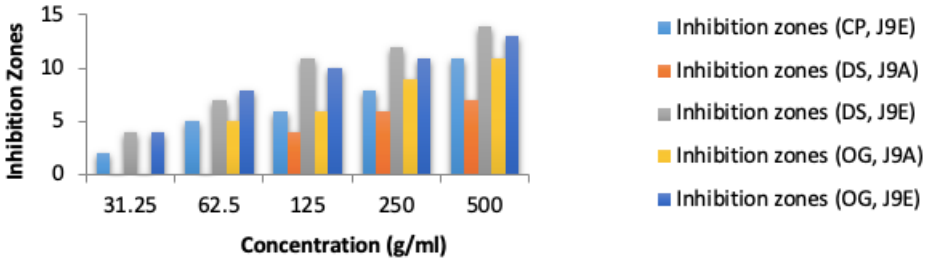
Figures 8a and 8b: Antifungal potential of aqueous and ethanolic plant extracts on isolate J7 at various concentrations





Figures 9a and 9b: Antifungal potential of aqueous and ethanolic plant extracts on isolate J8 at various concentrations





Figures 10a and 10b: Antifungal potential of aqueous and ethanolic plant extracts on isolate J9 at various concentrations

Minimum inhibitory concentration (MIC) is the lowest concentration of the crude extract to have an inhibitory effect on the isolates (J1-J9).Concentration of both plant extracts (ethanol and aqueous) were varied between 31.25 – 250mg/ml as shown in Table 2.

Table 2: Minimum Inhibitory Concentration of Aqueous and Ethanolic Extract of Fungal Isolates

Plant Extracts	J1	J2	J3	J4	J5	J6	J7	J8	J9
Borreria verticillata aqueous (mg/ml)	62.5	62.5	62.5	31.25	62.5	125	62.5	62.5	62.5
Borreria verticillata ethanol(mg/ml)	62.5	31.25	62.5	62.5	62.5	62.5	31.25	62.5	31.25
Carica papaya aqueous (mg/ml)	62.5	125	125	125	250	125	62.5	250	62.5
Carica papaya ethanol (mg/ml)	62.5	62.5	125	31.25	62.5	31.25	31.25	62.5	31.25
Cassiaalata aqueous(mg/ml)	62.5	31.25	62.5	31.25	31.25	125	31.25	31.25	31.25
*Cassia alata*ethanol(mg/ml)	125	62.5	62.5	62.5	31.25	62.5	62.5	31.25	62.5
Diodia sarmentosa aqueous(mg/ml)	62.5	62.5	125	31.25	125	250	125	125	125
Diodia sarmentosa ethanol(mg/ml)	62.5	31.25	62.5	62.5	31.25	62.5	31.25	31.25	31.25
Ocimum gratissimum aqueous (mg/ml)	62.5	62.5	125	62.5	62.5	125	62.5	62.5	62.5
Ocimum gratissimum ethanol(mg/ml)	31.25	31.25	62.5	62.5	31.25	62.5	31.25	31.25	31.25

DISCUSSION

From the phytochemical analysis of the five medicinal plants (*Borreria verticillata, Cassia alata, Carica papaya, Occimum gratissimum, Diodia sarmentosa* and *Ocimum gratissimum*), the result obtained showed that the five plants extracts contained Flavonoids, Tannins, Salkwoski and Liberman. Keller-Killian was extracted from *C. alata*, *O. gratissimum*, *C. papaya*, and *Diodiasarmentosa*. Alkaloids were only extracted from C. papaya and *Diodia sarmentosa*. Saponins were extracted from *C. alata*, *O. gratissimum* and *B. verticillata*. Phlobacternin was extracted from *O. gratissimum*, *C. papaya* and *B. verticillata*. Free Anthraquinone and combined Anthraquinone were only found in *C. alata* and *B.verticillata*, whereas Terpenes and cyanogenetic glycosides were not extracted from any of the five plants leaf. The presence of these bioactive compounds accounts for the antimicrobial actions of the extracts on the fungal isolates. This is in conformity with that of [15] who reviewed alkaloid, tannin, flavonoid and phenols in *Carica papaya*. This also is in agreement with the findings of other researchers that

detected the presence of these bioactive components in varying proportion. In their studies flavonoid, saponin and tannin were detected in *Cassia alata, Ocimum gratissimum, Borreria verticillata and Diodia sarmentosa*. While anthraquinonein was not detected in some of the plant extract. The yield of bioactive metabolites in plant extracts varies considerably with the solvent of extraction. Stusies have shown that organic extracts are more active than aqueous extracts based on better solubility of the active components in organic solvents [23 24]. The variability observed in the bioactive components can also be attributed to location of different plant types which can be affected by the soil and weather conditions and the section used for the extraction [16, 17, 18, 25]. The five medicinal plants studied showed varying efficacy on the fungal isolates which was observed to be dose-dependent. Findings showed that a synergy of *Cassia alata*, *Ocimum gratissimum* and *Diodia sarmentosa* can be used to treat fungal infection at a concentration of 250mg/ml. This suggests that *Ocimum gratissimum* can prove to be more effective on the average for the treatment of *Rhizopus oryzae* at concentrations between 31.25mg/ml to 125mg/ml. From the results of the study obtained, ekthanolic extracts of the five plants were generally more effective compared to the aqueous plant extracts for all concentrations. This is supported in the previous studies documented by other researchers [26, 27] which showed that ethanolic leaf extracts possesses more antimicrobial activities than aqueous extracts. This corresponds with other studies where it was stated that some medicinal plants have more abundant and highly active compounds than others [28]. Minimum inhibitory concentration (MIC) is the gold standard for determining the susceptibility of organisms to antimicrobial agent, and therefore used to judge the performance of all other methods of susceptibility testing [29, 30]. Generally MIC differed within the five medicinal plants on the nine isolates. The present work has revealed has re-emphasized the need to explore plant, as well as justifying its ethno-medicinal use.

CONCLUSIONS

The study concludes that both ethanolic and aqueous extracts showed remarkable antifungal activity on the isolates wih *C. alata* activity being the most effective followed by O*. gratissimum, Diodia sarmentosa, C. papaya* and *B. verticillata*. Ethanolic leaf extract had the highest zones of inhibition compared to aqueous extract. Concentration 500mg/ml had the highest zones of inhibition, although resistance was encountered at concentration 31.25mg/ml in some of the isolate. The antifungal activity of the plants could be as a result of the presence of certain bioactive compounds which are responsible for the antioxidant potential of these plants. The bioactive components of these medicinal plants can be extracted and composed into to drugs for effective therapy of fungal infections. A synergy of these plants is recommended so as to produce higher efficacy in fungal treatment. Further studies should be carried out to establish appropriate doses of the plant extract.

LIMITATIONS OF THE STUDY

Paucity of recent studies using aqueous and ethanolic leaf extracts for the treatment of fungal infections was a challenge in the present study. The study was limited to five medical plants of which more plants can be studied. Toxicity testing was not carried out in the present study.

References

[1] Adetunji, C., Olaniyi, O. O. and Ogunkunle, A. T. J. (2013). Bacterial activity of crude extracts of *Vernonia Amygdalina* on Clinical Isolates. Journal of Microbiology and Antimicrobials 5:60-64

[2] Manach C., Morand C., Crespy V., Demigne C., Texier O., Regerat F., Remesy, C. Quercitin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. 1998; FEBS Lett. 426: 331-336.

[3] Etukudo I. Ethnobotany: Conventional and traditional use of plants. (1st ed.). Uyo: Verdict Press Ltd; 2003. 24-35 p. 1

[4] Asghar, N., Naqvi, S.A.R., Hussain, Z. *et al.* Compositional difference in antioxidant and antibacterial activity of all parts of the *Carica papaya* using different solvents. *Chemistry Central Journal*. 2016; **10,** 5

[5] Ajuru M. G., Williams L.F., Ajuru G. Qualitative and quantitative phytochemical screening of some slants used in ethnomedicine in the Niger Delta Region of Nigeria. J Food Nutr Sci. 2017; 5(5):198-205.

[6] Yogiraj, Vijay, Goyal P.K., Chauhan, C.S. Carica papaya Linn: an overview. 2014. Int J Herbal Med 2(5): 1–8.

[7] zejiofor, N. T. I., Chinedumije, K. S., Chukwudi, P. Free Radical Scavenging and Antioxidant Potential of Ethanolic Leaf Extract of Diodia sarmentosa on High Fat Fed Wistar Rats. Journal of Advances in Biology & Biotechnology, 2020; *23*(10), 54-63.

[8] zejiofor, N. T. I., Chinedumije, K. S., & Chukwudi, P.. Free Radical Scavenging and Antioxidant Potential of Ethanolic Leaf Extract of Diodia sarmentosa on High Fat Fed Wistar Rats. *Journal of Advances in Biology & Biotechnology*, 2020; *23*(10), 54-63.

[9] Elechi NA, Okezie-Okoye C. Abo KA. Antidiabetic Potentials of *Diodia sarmentosa* SW (Rubiaceae) Leaves on Alloxan-Induced Diabetic Rats. Saudi J Med Pharm Sci. 2020; 6(9):622-626

[10] Ekpe. E.L and Ijomone, O.R. Investigation of anti-diabetic potential of *Diodia sarmentosa* in alloxan-induced diabetic albino rats. J Appl Pharm Res. 2018; 6(1):26-32.

[11] Lorenzi, H. and Matos, F. J. A. Plantas medicinais no Basil: Nativas e exoticas cultivadas, Instituto Plantarum, Nova Odessa. 2002.

[12] van Andel, T., de Boer, H., Barnes, J., Vandebroek, I. Medicinal plants used for menstrual disorders in Latin America, the Caribbean, sub-Saharan Africa, South and Southeast Asia and their uterine properties: A review. Journal of Ethnopharmacology. 2014; 155 (2): 992- 1000.

[13] Sofowora, A. Medicinal Plants and Traditional Medicine in Africa. 3rd edition Ibadan: Spectrum Books; 2008.

[14] Ajibesin, K.K., Ekpo, B.A., Bala, D.N., Essien, E.E., Adesanya, S. A. Ethnobotanical survey of Akwa Ibom State of Nigeria. Journal of Ethnopharmacology. 2008; 115:387 -408.

[15] Nduche, M.U., Nkaa, F.A., Onyebinime, A. "Phytochemical Screening and ntimicrobial Activity of *Carica papaya,Citrus paradise,Citrus sinensis* and *Vernonia amygdalina*"Journal of Dairy and Veterinary Sciences. 2019; 9(4):555-768.

[16] Alexander, P."Phytochemical Screening and Mineral Composition of the Leaves of *Ocimum gratissimum*" Int Journal of Applied Sciences and Biotechnology. 2016; 4(2):161-165.

[17] Raji, P., Screenidhi, J., Sugithra, M., Renugadevi, K., Anthony,V.Samrot, V. Phytochemical *Screening and Bioactivity Study of Cassia alata* leaves" 2015; Biotechnology Research 12(2)291-296.

[18] Ushie, O. A. and Adamu, H. M. Phytochemistry Screening of *Borreria verticillata* Leaves Journal of Agriculture,Biotechnology and Ecology. 2019; 3(1):108-117.

[19] Okoroafor, H.C., Awayi, F. E., Azeke, E. A. Phytochemical and Antioxidant Properties of *Dioda srmentosa* swarts Leaves"Mongolian Journal Chemistry. 2020; 21(47); 27-32.

[20] Newton, S.M., Lau C., Gurcha, S. S., Bersra, G.S. Wright C.W. The evaluation of forty-three plant species for invitro antimycobacterial activities., isolation of active constituents from Psoralea coryliforia and Sanguinaria Canadensis" Journal of ethno-pharmacology. 2002; 9:57-67.

[21] Trease, C. E. and Evans, W.C. Phytochemicals of Plants. *A textbook of Pharmacognosy.* 1989; 13:200-235.

[22] ofowara, E. A. Phytochemicals of Plants. Medicinal Plants and Traditional Medicine in Africa. 1993; 4:150-28.

[23] Boer, H.J., Kool, A.., Broberg, A.., Mziray, W. R., Hedberg, I., Levenfors, J.J. Antifungal and antibacterial activity of some herbal remedies from Tanzania. Journal of Ethnopharmacology 2005; 96:461-469.

[24] Doughari, J.H., Elmahmood, A. M. Manzara, S. Studies on the antibacterial activity of root extracts of *Carica papaya*. 2007

[25] Nićiforović, N., Mihailović, V., Mašković, P., Solujić, S., Stojković, A., Muratspahić, D.P. Antioxidant activity of selected plant species; potential new sources of natural antioxidants. Food Chem. Toxicol*.* 2010, *48*, 3125–3130.

[26] Ibekwe V. I., Nnanyere, N. F., Akujobi C.O. Studies on Antibacterial activity and phytochemical qualities of extracts of orange peels. International Journal of Environmental Human Health. 2001*;* 2:4-46.

[27] Adetutu, A., Morgan, W.A., Corcoran, O. Ethnopharmcological survey and invitro evaluation of wound – healing plants used in southwestern Nigeria. 2011; Journal of Ethnopharmacology 137:50-56.

[28] Ramatosobane, M. M. and Anthony, J. A. Comparative Phytochemical Constituents and Antioxidant Activity of Wild and Cultivated Alepidea amatymbica Eckl and Zeyh"BioMed Research International 2020; 13:5808624.

[29] Jennifer, M.A. Determination of Minimum Inhibitory Concentrations. .Journal of Antimicrobial Chemotherapy. 2001; 48:5-16.

[30] Prajna, L. Organism, Minimum Inhibitory Concentration, and Outcome in a Fungal Corneal Ulcer Clinical TriaL. National Library of Medicine. 2012; 31(6):662-7