International Journal of Advanced Research in Chemical Science (IJARCS)
Volume 4, Issue 7, 2017, PP 32-37
ISSN No. (Online) 2349-0403
DOI: http://dx.doi.org/10.20431/2349-0403.0407005
www.arcjournals.org

The Chemical Constituents and Bioactivity of Ethyl Acetate (Leaf) Extract of *Palicourea croceiodes*

Udegbunam, I.S.[1*], V.I.E., Ajiwe[2], Omovo, A.Marvis[3], Ghibbono, Sani[3]

[1]*Department of Chemical sciences, Federal University Wukari, Taraba State*

[2]*Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Anambra*

[3]*Central Laboratory, Federal University Wukari, Taraba State*

Corresponding Author:** ***Udegbunam, I.S, *Department of Chemical sciences, Federal University Wukari, Taraba State*

Abstract: *Palicourea croceiodes (Rubiaceae) is a plant claimed in traditional medicine in the eastern part of Nigeria to cure various ailments such as urinary tract infections, vaginal infections which cause infertility in women,, sexually transmitted diseases and other related ailments. The GC-MS analysis of the ethyl acetate leaf extract revealed nineteen compound with prevailing compounds as 11, 14,-Eicosadienoic acid methyl ester,Hexadecenoic acid, 2,6,10,15,19,23,-Hexamethyl-2,6,10,14,18,22-tetracosahexaene (squalene) and 10-octadecenoic acid methyl ester. Antimicrobial assay of the extract against some pathogenic bacteria and fungi including Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Klebsiella aerogenes, E.coli, Proteus mirabilis, Enterobacter aerogenes, Candida albican and Aspergillus niger using the agar well diffusion method. The extract was also found to exhibit selective activities against some of the organisms with Minimum Inhibitory Concentration (MIC) of between 0.25mg/ml and 0.0625mg and a Minimum Fungicidal Concentration of (MFC) of 0.125mg/ml and 0.0625mg/ml. The study justified the use of the plant as an antibiotic in ethno-medical applications.*

Keywords: *phytochemical, Palicourea croceiodes, ethyl acetate, anti-microbial, ethno-medical.*

1. INTRODUCTION

Medicinal plants have become important elements in indigenous medicinal systems (Heinrich *et al.*, 2001), and these plants contain various secondary metabolites which can be used against many diseases in the world today. Many plants also have been shown by studies as sources of various nutrients and non-nutrient molecules. These medicinal plants are known to produce certain bioactive molecules which are responsible for their antimicrobial properties (Rios and Recio, 2005., Sonibare *et al*., 2009., Kuete, 2010). A lot of them are responsible for plant flavours (e.g. the terpenoid capsaicin from chilli peppers) while others give plants their characteristic odour. Yet, others are responsible for the kind of pigments seen in plants (Cowan, 1999).

Currently, plants are still being employed by numerous developing countries as sources of therapeutic agents because they accept that medicinal plants are readily obtainable, reachable, inexpensive, and potent and with relatively lower occurrences of antagonistic reactions compared to modern conventional drugs (Adomi, 2008). In addition to their medicinal uses, herbs are of great importance in the maintenance of general health and well-being. They are valuable sources of vitamins, minerals and other nutrients. Some are delicious to taste, example mint and betelwhile others are not, and still, they are full of beneficial properties in their diverse ways. Juices are often consumed for their perceived health benefits for example; orange juice is rich in vitamin C, folic acid and potassium. It is an excellent source of bioavailable antioxidant phytochemicals (Pascal *et al.*, 2006).

According to the world Health Organization (WHO, 2001), 75% of people still depend on plant-centered traditional medicine for primary health care globally. It seems likely that up to 80% of the world's populace rely primarily on so-called "traditional" medicine for primary health care; in many developing countries, the mainstream of the population depend on traditional therapies. This is to some extent due to poverty; but also because traditional systems are more culturally conventional and are able to meet psychological needs in a way western medicine does not (Prescott-Allen and Prescott-Allen, 1982).

Nigeria is rich in varieties of medicinal plants among the 50,000 species of medicinal plants recognized all over the world (Schippman *et al*., 2002).

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

The plant materials were collected in December 2014, from Urum, Awka North Local Government Area of Anambra State and was identified and authenticated in the Biological Sciences Department of Federal University Wukari, Taraba State, Nigeria

2.2. Extraction of Plant Material

The collected plant material was air-dried at room temperature for two weeks then pulverised into powder using electric blender, kept in a sterile container and labelled. About 500g of the material was extracted using 1 litre of ethyl acetate using the cold maceration method; the homogenous solution was filtered using Whatman No. 1 filter paper. The filtrate was concentrated with rotatory evaporator at 40^0C. The extract was placed in container and kept refrigerated for further analysis.

2.3. Gas-Chromatography Mass Spectrometry Analysis

The ethyl acetate leaf extract of *Palicourea croceiodes* were investigated on a Shimadzu QP2010 PLUS, GC-MS, interfaced with a BG mode analytical spectrometer was used. Helium was used as a carrier gas. Initial column temperature was 120^0C held for 5minutes and increased at 5^oC/minutes to 230^oC and held for 5minutes and helium was used as carrier gas at 1ml/min. For the MS, electrons impact ionization was carried out at 70eV. Identification of the constituent compounds was done with the chem-office software along with the MS library.

2.4. Phytochemical and Antimicrobial Screening

The ethyl acetate extract was screened for the presence of secondary metabolites using standard methods, (Silva *et al.,* 1998). The antimicrobial activity of the extract was screened using agar well diffusion method. Both sensitivity and minimum inhibitory concentration (MIC) were determined. The agar well diffusion method was used (Nostro *et al.,* 2002). Ethyl acetate leaf extract of *Palicourea croceiodes* were diluted with Dimethyl Sulphoxide (DMSO). Bacteria from 24 hours slants were suspended in saline solution (0.9% w/v Nacl) individually till the turbidity matches with that of McFarland 0.5 solution (mixture of 9.9ml of 1% w/v solution of $BaCl_2$).

The standardized innocula of the isolates were uniformly streaked unto freshly prepared Mueller Hinton Agar plates with the aid of a sterile swab stick. The prepared plates were allowed to dry by keeping them half open and face downwards for 30minutes. With the aid of sterile cork borer (6mm in diameter), four appropriately labeled wells were punched into each agar, 0.05ml of each extract was used. DMSO was used as negative control while Oxfloxacin and Augmentin were used as positive control. The plates were kept for 30minutes in the bench for the diffusion of the crude extracts to take place before incubation. After, these plates containing bacterial cultures were lifted gently and placed in the incubator at 37^oC for 24hrs.

After incubation, the plates were examined and the inhibition zones were measured in mm a ruler. The observed inhibition zones were recorded appropriately in mm for the various diameter zone of inhibition. (Bryant, 1972 and Cruckshank *et al.,* 1975). Triplicate plates were prepared for each extract and controls.

While for the fungi, sabouraud dextrose agar was used and the incubation period was 48hours. Clotrimazole was used as positive control. The zones of inhibition of the antifungal activities were also determined

2.5. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of the extracts were determined using the tube dilution method as outlined by Onyeagba 2004.

3. RESULTS AND DISCUSSION

The GC-MS results of the leaf crude extracts of *Palicourea croceiodes* showed that the extract contained nineteen (19) compounds (Table 1). The identified compounds arephenyl-2(-1-piperidinyl)-

ethanone, 3,5-Dimethyl peperidine, 4,5-Dimethyl-4-hexen-3-one, α-D-glucopyranoside, β-D-fructofuranosyl, 2-Nonen-1-ol, 2,6,10,14,-Tetramethylhexadecane, Hexadecanoic acid methyl ester, Octadecanoic acid, 11, 14,-Eicosadienoic acid methyl ester, 10-octadecenoic acid methyl ester, Docosanoic acid methyl ester, Hexadecenoic acid, 2-Dimethylamino ethyl acrylate, Cyclohexyl-(5-methyl-3-isoxazolyl) sulfamide, 2-(Hydroxylethyl) octanamide, Propyl hexedrine, 2,3-Dihdroxypropyl palmitate,13-Docosenoic acid and 2,6,10,15,19,23,-Hexamethyl-2,6,10,14,18,22-tetracosahexaene.

The bioactivity of many of the compounds identified have been reported. It was reported that 2,6,10,15,19,23,-Hexamethyl-2,6,10,14,18,22-tetracosahexaene (Squalene),is an imidazole derivative and it is associated with many therapeutic fields. It has activity against broad range of microorganisms specifically bacteria and fungi and it is employed in the treatment of Candidiasis, superficial dermatomycoses and other vaginal infections (Mallesshappa *et.al.*, 2014).

Squalene is also a natural detoxifier, protecting the body from the detrimental effects of chemicals and toxins, it also blocks the cancer-causing effects of certain industrial chemicals and environmental toxins. Results of animal studies indicate that squalene surpress the growth of tumour cells, partially prevent the development of chemically induced cancer and cause the regression of already existing tumours. (Malleshappa *et.al.*, 2014). `

Hexadecanoic acid methyl ester has been reported to have anti proliferative properties on tumour cells. This is supported by Fa-rong *et.al.*, (2005) who isolated methyl ester derivatives such as Hexadecanoic acid methyl ester from *Euphorbia kansui* and reported that the methyl ester extract effectively inhibited the proliferation of SGC-7901 tumour cells by initiation growth inhibition and induce apoptosis in the tumour cells. Also, anti-inflammatory, antioxidant, hypocholesterolemic, and antibacterial activities have been reported for hexadecanoic acid.(Aparna *et al.*,2012, Kumar et al., 2010 and Rahuman et al., 2000)

The leaf extract of *Palicourea croceoides* was also investigated for their antibacterial and anti-fungal activities and the results are presented in table 2 and 3. The sensitivity test on extract showed that *Pseudomonas aeruginosa* (28.00mm), *E.coli* (24.00mm) and Staphylococcus aureus (26.00mm) were the most inhibited organisms and were higher than that of commercial antibiotics like Oxfloxacin and Augmentin. The high diameter zone of inhibition exhibited by these test organisms corroborated to a good antimicrobial agent and could be an alternative to the antibiotics in the treatment of infections they cause since most of them have developed resistance against the known antibiotics. (Singleton, 1999). The antifungal assay of the ethyl acetate leaf extract of *Palicourea croceiodes* showed different responses to the test organisms with activity reported for *candida albican* and *Aspergillus niger* with diameter zone of inhibition of 20mm and 18mm respectively. The level of antimicrobial activities exhibited by these extracts from the antibacterial and antifungal screening results with the test bacteria and fungi justified the traditional uses of the plant, and this pathogens cause infection and infertility problems. (Greenwood and Pentherer, 1992).

The result shown in Table 3 revealed that the extracts were sensitive on the fungi tested and is less compared with the bacterial counterpart (Table 2), this is because bacteria are more sensitive than fungi. The reason for this low susceptibility is probably their eukaryotic nature which is responsible for the advance cellular and molecular process when compared to bacteria which are prokaryotic in nature (Momoh *et.al.*, 2012).

The MIC was the lowest concentration of the extract that is required to inhibit visible growth of test organism and is recorded ranging from 0.25 mg/ml – 0.0625mg/ml for the test bacteria, for the fungi, the MIC/MBC was reported in the range of 0.125-0.0625mg/ml. This indicates that the extract is effective even at low concentrations.

4. CONCLUSION

The GC-MS analysis showed the presence of nineteen compounds from the ethyl acetate leaf extract of Palicourea croceiodes. The antimicrobial analysis of the extract also showed excellent activity against the tested pathogens. Therefore, this research is in agreement with the traditional use of the plant for the treatment of urinary tract infection and female related infertility.

ACKNOWLEDGEMENT

The authors are grateful to the National Research Institute for Chemical Technology (NARICT) Zaria and Central Laboratory of Federal University Wukari for providing the equipment for this research.

Table1. *Chemical composition of the Ethyl acetate leaf crude extract*

Peak	Name of compounds	RT	Peak Area	Reported Antimicrobial activities
1.	1-phenyl-2(-1-piperidinyl)-ethanone	4.600	5.33	No activity reported
2.	3,5-Dimethyl peperidine	5.575	2.29	No activity reported
3	4,5-Dimethyl-4-hexen-3-one	7.629	2.82	No activity reported
4.	α-D-glucopyranoside,β-D-fructofuranosyl	11.820	5.76	Anti-cancer, Anti-inflammatory
5.	2-Nonen-1-ol	14.567	1.96	No activity reported.
6.	2,6,10,14,-Tetramethylhexadecane	14.725	2.19	No reported activity
7.	Hexadecanoic acid methyl ester	16.788	4.27	Antioxidant, anti bacterial, antifungal
8.	Octadecanoic acid	17.821	4.89	Anti bacterial. Anti-oxidant
9.	11, 14,-Eicosadienoic acid methyl ester	19.832	6.12	Antibacterial, antifungal.
10.	10-octadecenoic acid methyl ester	19.922	6.87	No reported activity
11.	Docosanoic acid methyl ester	20.274	1.31	No reported activity.
12.	Hexadecenoic acid	20.731	21.25	Antioxidant, antibacterial.
13.	2-Dimethylamino ethyl acrylate	22.253	1.19	No reported activity.
14.	Cyclohexyl-(5-methyl-3-isoxazolyl) sulfamide	22.600	0.70	Antibacterial.
15.	2-(Hydroxylethyl) octanamide	22.662	1.24	No report activity.
16.	Propyl hexedrine	24.056	2.79	No reported activity.
17.	2,3-Dihdroxypropyl palmitate	24.609	7.54	No reported activity.
18.	13-Docosenoic acid	26.350	19.98	Anti inflammatory
19	2,6,10,15,19,23,-Hexamethyl-2,6,10,14,18,22-tetracosahexaene.	27.744	5.33	Anti proliferative, anti inflammatory

Table2. *Results of the Antibacterial Activities of the Crude Extracts of Palicourea croceiodes*

	AVERAGE DIAMETER ZONE OF INHIBITION (mm) ON TEST ORGANISMS												
EXTRACT/SOLVENT	**Vol. used (cm^3)**	***P.a***	***E.a***	***K.a***	***S.a***	***K.p***	***P.m***	***E.c***					
Ethyl Acetate (Leaf)	0.05	28.00	10.00	16.00	26.00	20.00	0.00	24.00					
Oxfloxacin 200mg + distilled water	0.05	20.00	24.00	18.00	25.00	16.00	10.00	18.00					
Augmentine 625mg + distilled water	0.05	18.00	20.00	22.00	22.00	0.00	0.00	4.00					
Control	0.05	–	–	–	–	–	–		0.125	0.0313	0.125	0.0313	0.625
MIC mg/ml		0.0625	0.0625	0.125	0.0313	0.125	0.0313	0.0625	0.25	0.0625	0.25	0.0625	0.125
MBC mg/ml		0.125	0.25	0.25	0.0625	0.25	0.0625	0.125					

*P.a =Pseudomonas aeruginosa, E.a = Enterobacter aerogenes, K.a = Klebsiella aerogenes , S.*a=*Staphylococcus aureus, K.p= Klebsiella pneumonia, P.m =Proteus mirabilis, E.c= E.coli*

Table3. *Results of the Antifungal Activities of the ethyl acetate leaf extract*

		DIAMETER ZONE OF INHIBITION(mm)	
SAMPLE TESTED	**VOLUME USED (cm^3)**	***Candida albican***	***Aspergillus niger***
Ethylacetate Leaf	0.05	20	18
Clotrimazole (100mg)	0.05	14	10
MBC mg/ml		0.0625	0.0313
MFC mg/ml		0.125	0.0625

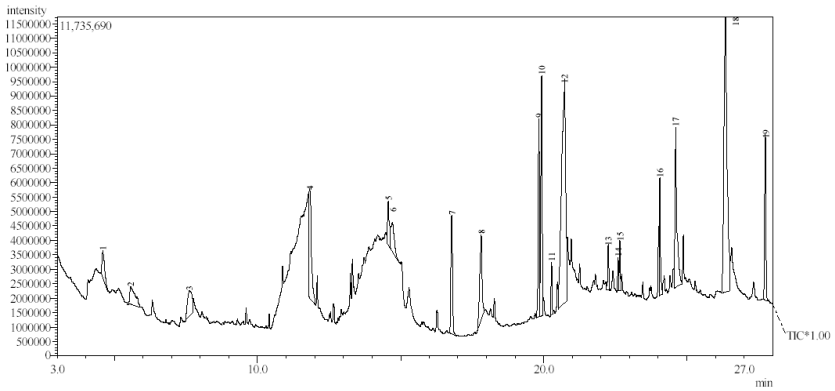

Fig1. *GC_MS Spectrum of the Ethyl acetate leaf extract of Palicourea croceiodes*

REFERENCES

Adomi, P.O., (2008). Screening of the leaves of three Nigerian medicinal plants for antibacterial activity. African Journal of Biotechnology 7, 15:2540-2542.

Bryant, M.C., (1972). Antibiotics and their laboratory control, 2nd edition, Buterworths, London p.85.

Cowan, M.M., (1999). Plants products as anti-microbial agents.Clinical Microbiology Reviews. 12:564-582.

Cruckshank, R., Dagaid, J. P., Morimion, B. P., Swain, R. H. A., (1975). Medical Microbiology Vol. 11& 12th edn. Churchill Living Stone, Edinburgh, London and New York pp. 194-203.

Fa-Rong, Y., Xu-Zhen, L., Hong-Yun, G., McGuire, P.M., Ren-De, L., Rui, W., Fa-Hong, Y., (2005).Isolation and characterization methyl esters and derivatives from *Euphorbia kansui* and their inhibitory effects on the human SGC-7901 cells. Journal of Pharmaceutical Science, 8(3):528-535.

Gill, A. F., (1992). Economic Botany: A textbook of useful plants and plant products, 2nd edition, McGraw Hill book Co. Incorporated U.S.A, pp 242-266.

Greenwood , D.S., Pentherer, C.B.,(1992). Medical Microbiology:A Guide to Microbial Infections, Pathogens, Immunity, Laboratory Diagonosis Control (14th edition). Churchill Livingstone Medical Division of Longman Group, Bungay, U.K. pp. 72-73.

Heinrich, M., Barnes, J., Gibbons S., Williamson, E.M., (2001). Fundamental of Pharmacognosy and Phytotherapy. Churchill Livingstone Press, Edinburgh.Pp15-18.

Kubmarawa ,D., Khan,M., Punah,E., Hassan,A. M.,(2007). Journal of Medicinal Plants Research, 2(12): 5352-5335.

Kuete, V., (2010). Potential of Cameroonian plants and derived products against microbial infections: A review. Planta Medica, 76:1-13.

Malleshappa, N., Nodvi, A.J., Harun, M. P.,(2014).Textbook of Medicinal Chemistry. CBS Publishers and Distributors PVT Limited, India, pp.678-704.

Momoh , A.O., Oladunmoye, M.K., and Adebolu, T.T., (2012). Evaluation of the antimicrobial and phytochemical properties of oil from castor seeds (*Ricinus communis Linn*), Bulletin of Environmental Pharmacology and Life Sciences, 1(10): 21-27.

Nostro, A., Germano, M. P., Diangelo, V., Marino, A., Cannattelli, M. A. (2002). Application Microbiology, 30:379.

Onyeagba, R.A.(2004).Laboratory Guide for Microbiology First Edition, Crystal Publishers, 95-117.

Pascal, M.E., Carretero, M.E., Slowing, K.V., Villar, A.,(2006). Phytochemical investigation of *Altea officinalis l.* Natural Product Research, 124(14):1358-1364.

Prescott-Allen,R., and Prescott-Allen C.,(1982). What's Wildlife Worth? Economic Contributions of Wild Animals and Plants to Developing Countries London.Earthsacan.

Rios, J.L., and Recio, M.C., (2005). Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100:84-94.

Schippman, U., Leaman, D.J., Counningham, A.B., (2002). Impact of cultivation and gathering of medicinal plants on biodiversity: Global trends and issues. Biodiversity and the ecosystem approach in agriculture. Preoceedings of the 9th Session of the Commission on Genetic Resources for Food and Agriculture, FAO, Rome. Pp 125-127.

Silva, G.L., Lee, I., and Douglas, K.A.,(1998).Special problems with extraction of plants. In: Cannel, J. P. R. (ed.). Natural Products Isolation. Humana Press Publishers, New Jersey, (USA). Pp. 356-358.

Singletton, P., (1999). Bacteria in Biology, Biotechnology and Medicine, 4th edition, John Wiley and Sons Limited, New York.P.61.

Sonibare, M.A., Soladoye, M.O., Esan, O.O., Sonibare, O. O.,(2009). Phytochemical and antimicrobial studies on four species of Cola schott and endl.(Sterculiaceae).African Journal of Traditional Complementary and Alternative medicine. 6(4): 518-522.

Sultana, B., Anwar, F., Ashraf, M., (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 14, 2167-2180.

Taylor, Charlotte M. (2008) Palicourea Aubl. (Rubiaceae: Psychotrieae) Retrieved 28th February, 2015

World Health Organization (2001).Legal Status of traditional medicines and complementary/alternative medicine: A world-wide review of World Health Organization, http://apps.who.int/medicine/ dous/en/d/ Jh2943e/. Retrieved 28th February, 2015

World Health Organization.(WHO), (1976). African Traditional Medicine, Technical Report Series, NO.1. Brazzaville.

Citation: *I. Udegbunam et al., "The Chemical Constituents and Bioactivity of Ethyl Acetate (Leaf) Extract of Palicourea croceiodes", International Journal of Advanced Research in Chemical Science (IJARCS), vol. 4, no. 7, pp. 32-37, 2017. http://dx.doi.org/10.20431/2349-0403.0407005*