Rubiaceae in African Traditional Medicine: Comparative Assessment of Antibacteria Activity of the Leave Extracts of *Cephalanthus Occidentalis.*

***O. Adeyanju., A. Lazarus., P. Ikwuemesi., I.I. Ayodabo., S. Tanko**

Department of Chemistry, Faculty of Natural Sciences, University of Jos, Nigeria.

***Corresponding Author**

DOI: https://doi.org/10.51584/IJRIAS.2024.907035

Received: 24 June 2024; Accepted: 06 July 2024; Published: 12 August 2024

ABSTRACT

The Phytochemical screening, antimicrobial activity and minimum inhibitory concentrations (MIC) of the leave extracts of Cephalanthus occidentallis were studied. Preliminary phytochemical investigation revealed the presence of alkaloids, flavonoids, anthraquinoes, saponins, glycosides, tannins, carbohydrates and terpenes. Antimicrobial studies showed that the ethanol extract had considerable activities and significant inhibition against streptococcus pyogen, salmonella typhii, shigella dysentery and escherichia coli, Ethanol extract had higher zone of inhibition (32mm) against salmonella typhi at 0.5µg/ml to $5x10^4$ µg/ml. The MIC of ethanol extract against S. pyogene, E. coli, S. dysentery and S. typhii were 12.5mg/ml, 25mg/ml, 25mg/ml and 12.5mg/ml respectively.

Keywords: Cephalanthus occidentallis, Medicinal plant, phytochemistry, antimicrobial.

INTRODUCTION

Of the 300,000 plant species acclaimed worldwide only about 5% have been investigated scientifically for their medicinal purposes (Adeyanju *et al.*, 2011a). Researcher has reported that developing countries rely mainly on plants for the treatment of their prevailing ailments especially in rural areas where hospital are not accessible (Adeyanju *et al.,* 2011b). The presences of phytochemicals in plants are actually responsible for their antibacterial and antioxidant properties Plant derived bioactive substances are considered a very good and cheap source of medical disease (Deepika, *et al.,* 2020). Plant have great medical relevance because infections caused by drug resistant microorganisms have become a major therapeutic problem nowadays (Deepika *et al*, 2020). Therefore, thousands of researchers have focused their interest on investigating phytochemical constituents of plant for human health. The bioactive constituents of plants such at tannis, flavonoids, saponins, terpenoids and alkaloids have great antimicrobial activity (Adeyanju, *et al*., 2011a; Deepika *et al.,* 2020). The plant *Cephalathus occidentallis belong* to the family *Rubiaceae.* Native Americans used the bark decoctions as wash for sore eyes, antidiarrheal agents, anti-inflammation and rheumatism medication, headache and fever relievers and venereal disease remedies.

The bark is also chewed to relieve toothaches (Deepika, *et al.*, 2020). Traditional herbal medicine practitioners have put it forward that the leave of *Cephalanthus occidentalis* is used to cure some prevailing ailments. There are no scientific proof to justify these claims, therefore this study was conducted with the aim to investigate phytochemicals present in the leave of *C. occidentalis* and relate this to its potential antimicrobial activity.

MATERIALS AND METHODS

Phytochemical Screening

Plant extracts obtained with ethanol and water were evaluated for the presence of alkaloids, saponins, cardiac glycosides, tannins, steroids and flavonoids (Adeyanju *et al*., 2011a).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VII July 2024

Saponins:

Frothing test: 2 cm^3 of the extracts in a test tube was vigorously shaken for two mins. Frothing observed in the three extracts tested indicated the presence of saponins.

Emulsion test: 5 drops of olive oil were added to 3ml of the extracts in a test tube and the mixture was vigorously shaken. A stable emulsion formed in each extract tested indicated the presence of saponins.

Tannins:

1 ml of freshly prepared 10% KOH was added to 1 ml of the extracts. A dirty white precipitate observed in each extract showed the presence of tannins.

2 drops of 5% $FeCl_3$ were added to 1 ml of the extracts. A greenish precipitate indicated the presence of tannins in the three extracts.

Glycosides:

10ml of 50% $\mathbf{H_2SO_4}$ was added to 10 ml of the extracts in a test tube. The mixture was heated in boiling water for 15 mins. 10 ml of Fehling's solution was added and the mixture was boiled. A brick-red precipitate was observed in the methanol and water extracts, showing the presence of glycosides.

Alkaloids:

2 drops of Mayer's reagent were added to 1ml of the extract. **A** creamy precipitate observed indicates the presence of alkaloids in each extract.

2 drops of Hager's reagent were added to 1ml of each extract. **A** reddish-brown precipitate observed indicates the presence of alkaloids in each extract.

Flavonoids

1 ml of 10% NaOH was added to 3 ml of the extract. A yellow colouration showed the presence of flavonoids in each extract.

Carbohydrate:

Few drops of molisch's reagent were added to 2 ml of the extract. 1 ml of concentrated sulphuric acid was allowed to run down the inclined tube to form a lower layer. The interface was observed for a purple colour. Showing the presence of carbohydrate.

Anthraquinone

0.5 g of powdered plant was boiled with 10 ml of ferric chloride (10%) and 5 ml of dilute HC1 for 5 mins. The mixture was filtered while hot, cooled and the filtrate was shaken with equal volume of chloroform. The layers were allowed to separate in a separating funnel, the chloroform layer was transferred into another test tube containing 5 ml of 10% ammonia solution and the upper aqueous layer was observed for a bright-pink colour showing the presence of anthraquinones.

Terpenes

To 1 ml of the extract was added 1 ml acetic anhydride followed by the addition of 1 ml concentrated sulphuric acid down the wall of the test tube to form a layer underneath. The test tube was observed for red colouration showing the presence of tri-terpenes.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VII July 2024

ANTIMICROBIAL SCREENING

Preparation of Agar Medium

Nutrient agar 2.5g and 2.6 g of nutrient broth were added to 100 ml of distilled water in a 500 ml sterilized conical flask. The suspension was heated to dissolve the nutrient agar and broth. After complete dissolution of the media, the mouth of the conical flask was closed tightly with aluminium foil. The media was then sterilized using autoclave at 121 °C, and 15 mmHg for fifteen (15) mins.

Preparation of Agar Plates

Plates were sterilized in a hot air oven at 160 °C for 2 h. The plates were allowed to cool. 20 ml of the sterilized nutrient agar was poured into each sterilized plate and the medium was allowed to gel. The agar plates were then wrapped with aluminium foil and transferred into a refrigerator until use.

Test Organisms

Cultures of *streptococcus, pyogen, Eschericia coli, salmonella, typhii and shigella dysentery* were obtained from University of Jos Teaching Hospital, Plateau State. All microorganisms were propagated and stored in nutrient agar at 4 °C before use.

Preparation of Stock Solution of Extracts

Stock solutions of extracts were prepared by dissolving 0.2 g of each of the crude extracts in 1 ml of the diluents to give a concentration of 200 mg/ml and were kept in sterile cocked container until use. Concentrations of 300 mg/ml, 400 mg/ml and 500 mg/ml were also prepared together with 250 mg/ml of gentamicin which was aseptically prepared in sterile distilled water and used fresh as the standard antibiotic.

In Vitro Antimicrobial Sensitivity Test

The paper disc diffusion method was used to determine the antimicrobial activity of the test extracts using a standard procedure (Bauer *et al.*, 1996). The solutions of test extracts of varying concentrations, ranging from 200mg/ml to 500mg/ml were prepared**.** Nutrient agar was prepared, sterilized and used as the growth medium for the microorganisms. The sterilized media (20 ml) were poured into each sterilized petri-dish, covered and allow to gel. The nutrient agar were then inoculated with the test microorganisms and left for about 30 mins to dry. The sterilized paper discs were soaked in the prepared solutions of the extract with varying concentration and dried at 50 °C. The dried paper discs were then planted on the nutrient agar seeded with the test microorganism. The plates were incubated at 37 °C for 24 h, after which they were inspected for the zones of inhibitions using a transparent meter rule. The zones of inhibition were measured and recorded in millimeters (mm).

Minimum Inhibitory Concentration (MIC)

This was determined using the broth dilution technique using a standard method (Krivoshan *et al,* 1989). Solution with a concentration of 200 mg/ml was serially diluted (two fold) to varying concentration ranging from 200 mg/ml to 6.25mg/ml using nutrient broth and later inoculated with 0.2ml suspension of the test organism (Usman *et al.* 2007). The inoculated tubes were then incubated at 37 °C for 24 hours and were inspected for non-turbidity. The least concentration of the extract which prevented visible growth (did not show turbidity) was noted and recorded as the Minimum Inhibitory Concentration (MIC).

RESULTS

Table 1: Phytochemical analysis of leave extracts of *Cephalanthus occidentialis*

Phytochemical constituents	water extract	ethanol extract
Anthraquinone	-	-

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VII July 2024

Tannins	+	++
Carbohydrate	++	++
Alkaloid	+	+++
Glycoside	-	++
Flavonoids	+	+++
Terpenes	+	+
Saponins	-	+

+++ = High concentration; ++ = Moderate concentration + = Low concentration; - = Absent

Table 2: Inhibition zone of *C. occidentalis* (Water extract/drug) against the tested microorganism

Zone of Inhibition (mm)				
Extract/drug (mg/ml)	*Streptococcus Pyogene*	*E. coli*	*Shigella Dysentery*	*Salmonella typhi*
200	4	4	0	12
300	6	5	0	14
400	9	8	6	18
500	13	14	10	24
250 GTC	24	23	20	30

GTC = Gentamicin

Table 3: Inhibition zones of *C. occidentalis* (ethanolic extract / drug) against tested microorganisms

Zone of Inhibition (mm)				
Extract/drug (mg/ml)	*Streptococcus Pyogene*	*E. coli*	*Shigella Dysentery*	*Salmonella typhi*
200	20	17	15	23
300	24	20	17	25
400	27	22	21	28
500	29	25	23	30
250 GTC	32	30	25	35

GTC = Gentamicin

Table 4: Minimum inhibitory concentration (MIC) of *C. occidentalis* water extract against the tested microorganisms

Extract/drug	***Concentration mg/ml***				
	100	50	25	12.5	6.25
Streptococcus pyogene	+	+	0+	-	-
E. Coli	+	+	0+	-	-

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume IX Issue VII July 2024

Shigella dysentery	+	0+	-	-	-
Salmonella typhi	+	+	0+	-	-

+ = inhibition, 0+ = minimum inhibition, - = no inhibition (Turbidity)

Table 5: Minimum Inhibitory Concentration (MIC) of *C. occidentalis* ethanolic extract

Extract/drug	***Concentration mg/ml***				
	100	50	25	12.5	6.25
Streptococcus pyogene	+	+	+	0+	-
E. Coli	+	+	0+	-	-
Shigella dysentery	+	+	0+	-	-
Salmonella typhi	+	+	+	0+	-

+ = inhibition, 0+ = minimum inhibition, - = no inhibition (Turbidity)

DISCUSSION

In this research, phytochemical screening, antimicrobial activity and minimum iinhibitory cconcentration of water and ethanol extracts of *C. Occidentialis* were carried out.

The phytochemical screening (Table I) revealed the presence of alkaloids, carbohydrates, tannins, saponins, flavonoids, cardiac glycosides, and terpenes. The ethanol extract of the plant leaves had the most metabolites. This may be as a result of the solubility of the plant extracts in ethanol solvent than the aqueous solvent. Anthraquinone was not detected in both extract. These chemical constituents present in the extracts have many therapeutic values. Tannins and saponins are plant metabolites well known for their antimicrobial properties (Adeyanju *et al.,* 2011a). Flavonoids have both antifungal and antibacterial activity. They posses anti-inflammatory property (Ogundaini, 2005; Iwu, 1984). It was reported that cardiac glycosides have specific action on the cardiac muscles and they are useful for the treatment of congestive heart failure (Sofowora, 1982). Saponins, flavonoids, terpenes and steroids are known to have antimicrobial and curative properties against several pathogens (Usman *et al,* 2007; Hassan *et al,* 2004). In the anti-microbial studies (Table 2-5) there was a variation in the degree of the antimicrobial activity of the two plant extracts. The variation in the degree could be due to the different active compounds present in the plants. Majority of the organisms were more sensitive to the ethanol extract of *C. occidentialis*, particularly the gram positive bacteria, this may indicate that the gram positive organisms are more susceptible to the effect of the active compounds in the plants. The larger zones of inhibition exhibited by the ethanolic extract of *C. occidentialis* may be due to the presence of variety of active compounds in the plants such as tannins, alkaloids, flavonoids and saponins as described by (Abo *et al.,* 2000). It is not unlikely that one or a combination of the chemical constituents identified through phytochemical screening could be responsible for observed antimicrobial properties of the extracts. This is more so since tannins and saponins are plant metabolites well known for their antimicrobial properties (Adeyanju et al., 2011a). Saponins have been used in the treatment of inflammation of the respiratory tract (Trease and Evans, 1989). From the results obtained, the leaf extract of *C. occidentalis* showed more antimicrobial activity even at low concentration. This suggests that the leaf contains more of the active compounds and has high potency. In, Table 5, the ethanol extract of *C. occidentalis* was active against the entire microorganism. *Strptococcus pyogens, E. coli, Shigella dysentery and Salmonella thypii*. It has MIC of 25mg/ml against *Shigella dysentery, E. coli, and,* 12.5mg/ml against *Streptococcus pyogene and Salmonella typhii.* The standard drug (gentamicin) inhibited the growth of the microorganisms tested in this study. These findings were consistent with those of Singh and Agrawal *et al* (2000), who observed that medicinal plant containing alkaloid, flavonoids and reducing sugar showed considerable antimicrobial activity against gram positive microorganisms. They also agreed with the findings of (Abo *et al.,* 1999) that the extract from the leaves and pods of some medicinal plants showed significant antimicrobial activity. (Abo *et al.*,2000) also

found that the ethanol extract of the leaves of *Cassia singueana Del* exhibited significant antimicrobial activity against *P. aeruginosa, S. aureas* and *proteus mirabitis.*

CONCLUSION

The results of the experiment showed that the leaf of *C. occidentialis* may have some valuable anti-microbial activities against gram positive and gram negative microorganisms. This property tends to support the traditional medicinal stage in the treatment of bacterial infections. The result of the study justified the use of the plant extracts in the treatment of diseases of microbial origin in herbal medicine.

Finally, it is apparent from our study that effective drugs could be produced from *Rubiaceae* family of plants used in traditional medicine. This could lead to development of local pharmaceutical industries, thereby enhanced self-reliance and reduced drug importation.

ACKNOWLEDGEMENT

The authors are grateful to the laboratory technologist in the Department of Pharmaceutical Chemistry and Microbiology, University of Jos, Nigeria for their technical assistance.

REFERENCES

1. Abegaz, B.M, Alemajalu G. Kebede T., Mahayan D, Nindia, M., M, (1996). Cevenones and other phenolic compound from marketed African plant. In chemistry Biological and pharmacological properties of African medicinal plants. Wolfender J. L (EDS) university of Zimbawe publication; Pp 63-169
2. Abo, K.A, Adeyemi, A.A, Jegede, I.A (2000) Spectrophtochemical estimation of anthraquinone content and antimicrobial potential of extracts of some cassia species used in herbal medicine in Ibadan, Nigeria Sci. forum 3 (2): 57 - 63.
3. Abo, K.A, Lasaki, S.W Adeyemi, A.A (1999). Laxative and antimicrobial properties of cassia species growing in Ibadan, Nigeria of natural product and medicine 8:47 - 50.
4. Adeyanju, O., Olajide, Olutayo, O. Afolayan, Michael and Arifalo, K. M. (2011a). Preliminary phytochemical and antimicrobial screening of the leaf extracts of Pilostigma reticulatum (DC) Hochst. African Journal of Pure and Applied Chemistry. 5(3): 43-46.
5. Adeyanju, O., Olajide Olutayo O, and Afolayan Michael and Khan, I. Z. (2011b). Preliminary phytochemical and antimicrobial screening of the leaf extract of cassia Singueana Del. African Journal of Pure and Applied Chemistry.5(4): 65-68.
6. Agrawal Ahmad, A. B (2006) Phytochemical screening of cassia general for quinines and
7. Akah P. A, Orisakwe O.E, Gramaniel K.S Shittu, A. (1998). Evaluation of Nigeria traditional medicine:
8. Akinniyi, J.A and Telia, A (1989/1990) Rural resources and National Development. A case study for recognition of traditional medicine in Nigeria. Animals of Borno pp 279- 292, 617.
9. Anthraquinine (emodia) isolated from the; layer of cassi nigracans vah. African J of biotechnology 6(ii) pp 1276-1279.
10. Anthraquinonses, unpubished B. Sc chemistry dissertation University of Maidiguri.
11. Ayo, R.G and Amupitan J.O (2007): cytotorcity and antimicrobial studies of 1,6,8- tryhydroxy-3-cassi
12. Bauer, A.W, Kily, N.M, Sherris J.C and Turck, M (1996) Antibiotics susceptibility testing by a standardize single paper disc, Am. J. Clin. Pathol 45:473 - 496.
13. Benjamin, T.V (1980) Invsetigation of cassia alata plant used in Nigeria in the treatment of skin diseases J. Afri. Med. Plants 3:135-136.
14. Bonja, C and Farrokhi, H (2004), The bacterial activities of amoxicillin with other antimicrobial agent. J. antimicrobi. chemother (3) 273.
15. Burkill, H, M (1995) The useful plants of west African 2nd Edn, vol.3, royal kew Botanical Garden, Kew, London pp 144 - 150.
16. Dalziel, J.M (1956) useful plants of west Tropical Africa. Crown agents for overseas Government; London.

17. Deepika T., R Eena, A and Sourabb. J (2020). Antimicrobia, antioxidant and phytochemical investigation of thuja occidentalis (Arbor Vitae) leave extract, GSC Biologicla and pharmaceutical sciences, 12 (03): 108 – 116.
18. Effect of some Nigerian folk Remedies on peptic ulcer J. Ethanopharmacol. 62 (2): 123-127.
19. Elujoba, A.A, Abere, A.T and Adelusi, S.A (1999) Laxative activities of cassia pod sources from Nigeria. Nigerian, J. of Natural products and medicine 3:51-53.
20. Fadeyi M (1983) A handbook on traditional medicine practice in Nigeria. Longman Nigeria p.35.
21. Harbone, J. B (1973) Phytochemical Methods A. Guide to Modern Techniques of Plant analysis Chapman and Hill, London, pp 182-201.
22. Hassan, M.A, Oyewale; A.O Amupitan, J.O., Abdullahi, M.S. and Okonwo; E.M. (2004). Preliminary phytochemical and antimicrobial investigation of crude extract of root bark of Deterium microcarpum. J. chm. Soc 29:36-49
23. Irvine, F.R (1961). Woody plants of Ghana (with special reference to their uses). Oxford University press, London pp 285 - 286.
24. Iwu, M.M Angela, R.D. and Chris, O. (1999). New microbials of plant origin in J. janick (ed) perspective on crops and their uses. ASHS press Mexandrria pp 457 - 462.
25. Jalalpure s.s, Pall M.B, Aruna P, Shah B.N, (2004) Antidiabetic acitivity of cassia auriculate seed in alloxan induced diabetic rats. J. Nat. prod. Med. 8:22-23.
26. Khan, I. Z (1996) The role of Chemistry in health disease and ageing. A seminar presentation in the Department of chemistry, University of Maiduguri, Nigeria.
27. Krivoshien, Y.S (1989) Handbook on microbiology Russia publisher p. 36.
28. Lambo, J.O (1979) The Healing Power of herbs with special reference to obstetric and Gyncology in African medicinal (2nd ed). Ife press Nigeria pp. 24 - 27.
29. Nwafor, P.A Pkwuasaba, F, (2001). Effect of methanolic extract of cassia Nigerians leaves on rat gastrointestinal track. Fitoterapia 72:206. 214.
30. Odebiyi A. and Sofowora A.E (1990) phytochemical screening of Nigeria.
31. Ogarawu, V. (1992) studies in natural product chemistry, the antimicrobial activity of Senegalensis, M. sc. dissertion present to the department of chemistry, University of Maidiguri.
32. Ogugbuaja, V.O, Akinniyi, J. A., Abdulrahman, F.I., Ogarawu, V. C., (1997) Elemental Contents of Medicinal plants. A monograph. Department of chemistry faculty of science, University of Maiduguri, Maiduguri, Nigeria.
33. Ogundani A.O (2005) from greens into medicine taking a lead from nature" inaugural lecture series, 176, O.A.U press Ltd, lie - Ife Nigeria pp. 1-10.
34. phytochemical and antimicrobial investigation of crude extract of root bark of Deteriummi crocarpum. J. chem. Sci. Nigeria, 29:36-49.
35. Sanusi, S.S. and Rabo, E.T(2004) An inventory of Medicinal plants of the Nigeria Savannah Leviathan book Lagos pp. 21-24.
36. Singh, A.K, Agrawal, P.K (1982) Isopropylideno 3-oxo-phyllcladone, a diterpenoid from Ca/licarpa macrophylla phytochemistry. 38(6): 1560-1563.
37. Sofowora A (1982) Medicinal plants and Traditional Medicine in Africa, spectrum Books, limited, Ibadan Nigeria. Pp 6, 154.