International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 6 Issue 9, September 2019

ISSN: 2394-4404

Antibacterial Actions Of Anogeissus Leiocarpus And Morinda Lucida Leaves, Stems And Roots Extracts Against Some Enteric Bacteria

Agada, J.O

Gberikon, G.M

Amuta, E.U

Department of Microbiology, Federal University of Agriculture, Makurdi

Abstract: ***Antibacterial actions of Anogeissus leiocarpus and Morinda lucida leaves, stems and root extracts against some enteric bacteria was carried out to ascertain their potency. Samples of Anogeissus leiocarpus and Morinda lucida leaves, stems and roots were collected from otukpo in Benue State. Clinical isolates of enteric bacteria such as E. coli, P. mirabilis, S. typhi, K. pneumonia and S. dysentriae were collected from Veterinary Research Institute, Vom, Plateau State. Phytochemical analysis were carried out on the leaves, stems and roots of both plants to ascertain their bioactive components like phenol, alkaloid, flavonoid, tannin, saponin, steroid, phytosterol and cardiac glycoside. Standard microbiological and biochemical tests were carried out on the isolates for revalidation and confirmation. Susceptibility tests were carried out using agar well diffusion method. Two way analysis of variance (ANOVA) was employed in data analysis. Quantitative phytochemical results showed that both the aqueous and ethanolic extracts of the leaves, stems and roots of these plants contained these phytochemicals assayed but in varied quantities, Phenol was highly present in the aqueous leaf extract of A. leiocarpus (4.032,4.030), aqueous root extract (3.130,3.130), ethanolic leaf extract (3.810,3.812) and ethanolic root extract (3.501,3.500) but moderately present in the aqueous stem extract (2.101,2.100) and ethanolic stem extract (2.500,2.501). Morinda lucida leaf ethanolic extract was the most potent with a zone of inhibition (29.00±0.00) against Proteus mirabilis. Anogeissus leiocarpus leaves aqueous extract was least active against K. pneumoniae with a zone of inhibition of 12.50±0.00 and most active against S. dysentriae with a zone of inhibition of 21.50±0.00. M. lucida leaves aqueous extract was least active against E. coli with zone of inhibition of 13.50±0.00 and most active against P. mirabilis with zone of inhibition of 23.50±0.00. A. leiocarpus root ethanolic extract has the least effect on P. mirabilis with a zone of inhibition of 12.50±0.00 and had the highest inhibition zone on E. coli (24.00±0.00) Anogeissus. leiocarpus root aqueous extract had the least effect on K. pneumoniae and P. mirabilis with zones of inhibition of 12.50±0.00 and its highest effect on S. dysentriae with a zone of inhibition of 17.50±0.00.There was significant difference between the test enteric organisms (P < 0.01) whereas there was no significant difference between the plants' extracts (P > 0.05). Leaves, stem and root extracts of A. leiocarpus and M. lucida are therefore recommended for the treatment of some enteric infections.***

Keywords: ***Anogeissus leiocarpus and Morinda lucida, Antibacteria, Extracts, Enteric pathogens***

I. INTRODUCTION

The use of herbs has been known and accepted by all nations and has been known also as the first art of treatment available to man (Kafaru, 1994). People in all continents of the world have long applied imbibed infusions of hundreds (if not thousands) of indigenous plants dating back to prehistoric period (Nostro *et al*., 2000). The mechanisms of initiation of inflammations as well as cellular damage by microorganisms are pharmacologically well understood and can be

ISSN: 2394-4404

counteracted with the use of phytoconstituents of herbs and plants (Rahal *et al*., 2014). Despite seeming progress made in the development of antimicrobial agents, occurrence of drug resistant microorganisms and the emergence of unknown disease causing microbes, pose enormous public health concern (Ibezim, 2005). Due to high cost of effective antibiotics and the predicament of antibiotic resistance microbial strain worldwide, about 60 – 85% of the population of developing world relies either on plants or indigenous forms of complementary and alternative medicine (CAM) for their various general health related issues and countering several diseases/disorders (Yarney *et al*., 2013). New therapeutic agents are of great demand. Many infectious diseases are known to be treated with herbal medicines throughout human civilization. Even today, plant materials continue to play major role in primary health care and higher plants have been shown to be potential sources for the new antimicrobial agents (Yarney *et al*., 2013). Herbal plants have been used as a source of valuable medication in virtually all cultures worldwide due to the presence of important antimicrobial principles, immunomodulatory activities, and maintenance of general health, precious therapeutic properties and healing potentials; thus ensure prevention and cure for several diseases and disorders of humans and animals (Ibezim, 2005)

II. MATERIALS AND METHODS

SAMPLE COLLECTION

Anogeissus leiocarpus and *Morinda lucida* leaves, stems and roots were obtained from *Anogeissus leiocarpus* tree and *Morinda lucida* tree respectively in Otukpo. The plants leaves were authenticated by a Botanist, Department of Botany, Federal University of Agriculture Makurdi. The various plant leaves, stems and roots were dried at room temperature (30^0C) for two weeks. Samples were pulverised into powdered form, and packed separately into clean polythene bags and were labelled accordingly.

Clinical isolates of enteric bacteria *(Escherichia coli, Salmonella typhi, Shigella dysentria, Klebsiella pneumoniae* and *Proteus mirabilis*) were obtained from Veterinary Research Institute, Vom, Plateau State. Microbiological and biochemical tests were carried out on them for confirmation

EXTRACTION METHOD

The extraction of the blended plant leaves, stems and roots was carried out using ethanol and distilled water as extracting solvents. The cold maceration extraction method of Cowan (1999) was used.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Preliminary quantitative phytochemical analysis like flavonoids, phenols, alkaloids, tannins, ferric chloride, saponins, steroids, phytosterols, and cardiac glycosides were carried out to identify the secondary metabolites present in the various ethanolic and aqueous extracts of leaves stems and roots of the two plants using methods of Soforowa (1993).

PREPARATIONS OF CULTURE MEDIA

All the media used for culturing were prepared according to manufacturer's instructions using methods of Cheesbrough (2006).

DETERMINATION OF ANTIMICROBIAL ACTIVITY

The agar well diffusion method was used to determine the antimicrobial activity of the plant extracts as described by Adegoke and Adebayo-Tayo (2009). Prior to streaking the plates with bacteria, a cork borer was used to make a well of 5mm diameter into the medium .All plates were inoculated with the test bacterium, a sterile cotton swab was dipped into the suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level to remove excess inocula. The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inocula with a final swab around the rim. The plates were allowed to air dry for five minutes. Both plant extracts were not diluted to find the appropriate dilution for its effectiveness because local herbal practitioners do not dilute them before use. Fifty (50) microliter aliquot of each test extract were dispensed into each well after the inoculation of the plates with bacteria. On each plate was a positive control (Tetracycline) while the pure solvent (water / alcohol) was used as negative control. The plates were allowed to stand for one hour for pre-diffusion of extracts to occur and then incubated for 24 hours at 37^oC. The diameter of zone of inhibition was measured to the nearest millimetre (mm) using a meter rule. Each experiment was done in duplicates and the mean value was taken. All plates were incubated and the diameter of the zones of inhibition was measured by calculating the difference between the well (5mm) and the diameters of inhibition as described by Hewitt and Vincent (1989).

DETERMINATION OF ACTIVITY INDEX

The activity index of the extracts were calculated according to Hewitt and Vincent (1989).

$$\text{Activity Index A.I} = \frac{\text{Mean of zone of inhibition of extract}}{\text{Zone of inhibition obtained from standard antibiotic drug}}$$

STATISTICAL ANALYSIS

The results were subjected to two way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20 to determine the level of significance of the various zones of inhibition that were observed.

III. RESULTS AND DISCUSSION

PHYTO CHEMICAL CONSTITUENTS	AQUEOUS LEAVE EXTRACT	AQUEOUS STEM EXTRACT	AQUEOUS ROOT EXTRACT	ETHANOLIC LEAVE EXTRACT	ETHANOLIC STEM EXTRACT	ETHANOLIC ROOT EXTRACT
Phenol	4.031	2.101	3.130	3.811	2.501	3.501
Alkaloid	1.801	0.951	2.301	2.501	1.351	3.012
Flavonoid	2.104	0.981	4.321	5.012	1.496	5.021
Tannin	0.511	1.231	1.821	1.231	2.101	2.350
Saponin	6.021	2.521	3.651	5.021	1.821	2.421
Steroid	_	_	_	0.981	_	_
Phytosterol	_	_	_	0.851	_	_
Cardiac glycoside	0.511	_	0.981	_	_	1.012

Key: (minus) = absent.

Table 1: Quantitative Phytochemical Constituents of Sample (g/100g) (Anogeissus

PHYTO CHEMICAL CONSTITUENTS	AQUEOUS LEAVE EXTRACT	AQUEOUS STEM EXTRACT	AQUEOUS ROOT EXTRACT	ETHANOLIC LEAVE EXTRACT	ETHANOLIC STEM EXTRACT	ETHANOLIC ROOT EXTRACT
Phenol	3.751	_	2.106	4.051	0.501	1.891
Alkaloid	2.166	1.851	3.534	3.233	2.103	3.831
Flavonoid	2.088	2.301	1.851	1.831	2.310	2.051
Tannin	1.218	1.799	2.125	1.201	0.970	2.015
Saponin	1.019	_	1.022	0.951	_	1.022
Steroid	1.999	0.521	0.316	2.120	0.851	0.521
Phytosterol	1.218	3.423	_	0.981	3.550	_
Cardiac glycoside	0.563	_	0.350	1.311	0.521	0.620

Key: (minus) = absent.

Table 2: Quantitative Phytochemical Constituents of Samples (g/100g) (Morinda lucida)

EXTRACTS	*E.coli*	*P. mirabilis*	*S. typhi*	*K. pneumoniae*	*S. dysentriae*	SD MEAN
MLAE	13.50±0.00	23.50±0.00	16.50±0.00	18.00±0.00	17.50±0.00	17.80±3.63
MLEE	16.50±0.00	29.00±0.00	16.50±0.00	22.00±0.00	16.00±0.00	20.00±5.60
ALAE	21.00±0.00	20.50±0.00	21.00±0.00	12.50±0.00	21.50±0.00	19.30±3.82
ALEE	18.50±0.00	18.50±0.00	18.00±0.00	14.50±0.00	19.00±0.00	17.70±1.82
MSAE	13.50±0.00	21.50±0.00	17.50±0.00	18.00±0.00	18.50±0.00	17.80±2.86
MSEE	15.00±0.00	25.00±0.00	17.50±0.00	20.00±0.00	17.50±0.00	19.00±3.79
ASAE	20.50±0.00	22.00±0.00	21.00±0.00	13.00±0.00	20.50±0.00	19.40±3.63
ASEE	20.50±0.00	21.00±0.00	23.50±0.00	15.00±0.00	12.50±0.00	18.50±4.57
MRAE	18.50±0.00	23.50±0.00	19.50±0.00	18.00±0.00	19.00±0.00	19.70±2.20
MREE	14.00±0.00	27.00±0.00	21.00±0.00	16.00±0.00	20.50±0.00	19.70±5.04
ARAE	13.00±0.00	12.50±0.00	16.50±0.00	12.50±0.00	17.50±0.00	14.40±2.41
AREE	24.00±0.00	12.50±0.00	20.00±0.00	14.50±0.00	19.50±0.00	18.10±4.60
TETRACYCLINE	13.27±0.00	27.00±0.00	30.00±0.00	25.00±0.00	26.00±0.00	24.25±6.42
SD MEAN	17.06±2.67[a]	21.81±5.07[b]	19.89±3.75[ab]	16.85±3.82[a]	18.89±3.14[ab]	18.90±4.24

Organisms: F (4, 48) = 4.095, P < 0.01
Extracts: F (12, 48) = 1.741, P > 0.05
Means with the same alphabet are not significantly different; Means with different alphabets are significantly different.
Key: MLAE = M. lucida leaves aqueous extract, MLEE = M. lucida leaves ethanolic extract, ALAE = A. leiocarpus leaves aqueous extract, ALEE = A.leiocarpus leaves ethanolic extract, MSAE = M. lucida stems aqueous extract, MSEE = M. lucida stems ethanolic extract, ASAE = A. leiocarpus stems aqueous extract, ASEE = A. leiocarpus ethanolic extract, MRAE = M. lucida roots aqueous extract, MREE = M. lucida roots ethanolic extract, ARAE = A.leiocarpus roots aqueous extract, AREE = A. leiocarpus roots ethanolic extract.

Table 3: The Diameter of Zones of Inhibition of Plant Extracts on Test Organisms

EXTRACTS	*E.coli*	*P.mirabilis*	*S.typhi*	*K.pneumoniae*	*S.dysentriae*	SD MEAN
MLAE	1.04±0.00	0.84±0.00	0.55±0.00	072±0.00	0.67±0.00	0.76±0.19
MLEE	1.27±0.00	1.02±0.00	0.55±0.00	0.88±0.00	0.62±0.00	0.87±0.29
ALAE	1.61±0.00	0.76±0.00	0.70±0.00	0.50±0.00	0.83±0.00	0.88±0.43
ALEE	1.42±0.00	0.69±0.00	0.60±0.00	0.58±0.00	0.73±0.00	0.80±0.35
MSAE	1.04±0.00	0.80±0.00	0.58±0.00	0.72±0.00	0.71±0.00	0.77±0.17
MSEE	1.15±0.00	0.96±0.00	0.58±0.00	0.80±0.00	0.67±0.00	0.83±0.23
ASAE	1.58±0.00	0.61±0.00	0.70±0.00	0.52±0.00	0.79±0.00	0.84±0.43
ASEE	1.85±0.00	0.78±0.00	0.78±0.00	0.60±0.00	0.87±0.00	0.92±0.38
MRAE	1.42±0.00	0.87±0.00	0.65±0.00	0.72±0.00	0.73±0.00	0.88±0.31
MREE	1.08±0.00	1.00±0.00	0.70±0.00	0.64±0.00	0.79±0.00	0.84±0.43
ARAE	1.00±0.00	0.83±0.00	0.55±0.00	0.50±0.00	0.67±0.00	0.71±0.21
AREE	1.85±0.00	0.83±0.00	0.67±0.00	0.58±0.00	0.75±0.00	0.94±0.52
TETRACYCLINE	13.00±0.00	27.00±0.00	30.00±0.00	25.00±0.00	26.00±0.00	24.20±6.53
SD MEAN	2.23±3.25	2.85±7.26	2.89±8.14	2.52±6.76	2.68±7.01	2.63±6.49

Organisms: F (4, 48) = 0.260, P > 0.05
Extracts: F (12, 48) = 58.528, P < 0.01
Key: MLAE = M. lucida leaves aqueous extract, MLEE = M. lucida leaves ethanolic extract, ALAE = A. leiocarpus leaves aqueous extract, ALEE = A. leiocarpus leaves ethanolic extract, MSAE = M. lucida stems aqueous extract, MSEE = M. lucida stems ethanolic extract, ASAE = A. leiocarpus stems aqueous extract, ASEE = A. leiocarpus stems ethanolic extract, MRAE = M. lucida roots aqueous extract, MREE = M. lucida roots ethanolic extract, ARAE = A. leiocarpus roots aqueous extract, AREE = A. leiocarpus roots ethanolic extract.

Table 4: The Activity Index of the Extracts of Both Plants in Comparison with Standard Antibiotic (Tetracycline)

IV. DISCUSSION

Leaf, stem and root extracts of *Anogeissus leiocarpus* and *Morinda lucida* from aqueous and ethanolic solvent are both potent on enteric pathogens. They contained all the phytochemicals assayed (Phenol, Alkaloid, Flavonoid, Tannin, Saponin, Steroid, Phytosterol and Cardiac glycoside). The phytochemical constituents present in the extracts in various quantities are presented in Tables 1 and 2. Antimicrobial studies indicated that both the aqueous and ethanol extracts of the plants parts inhibited the growth of the microbes but at varied levels and the inhibition was extracts concentration dependent. The inhibition of enteric bacteria strains suggest that the plants possess broad spectrum antibacterial properties which could be used in the treatment of most bacterial infections. Higher quantities of the phytochemicals were extracted by ethanol as the extracting solvent, this conforms to the findings of Parekh and Chanda (2007). The leaf extracts showed higher antimicrobial activity against the enteric bacteria than the stem and root extracts. This according to Hassan *et al.* (2009) could be attributed to the presence of higher bioactive compounds in leaf extracts, furthermore, the sensitivity and susceptibility of the enteric bacteria to the plants varied. Generally, the ethanol extracts were more effective than the aqueous extracts though the reverse was the case at higher concentration. The findings conform to the study of Thabile (2008) who observed higher microbial activity of aqueous extract of lemon grass against human pathogens at higher concentration of plant extracts. Mada *et al*. (2013) reported that antimicrobial activity is solvent dependent with ethanol extract being most potent than aqueous extract. *Morinda lucida* leaf aqueous extract (MLAE) has a zone of inhibition of 13.00±0.00 against *Escherichia coli* while *Anogeissus leiocarpus* leaf aqueous extract (ALAE) has a zone of inhibition of 21.00±0.00 against *E. coli*

This showed that the antibacterial activity of the extracts was enhanced by the increase in the concentration of the extracts. This agrees with the report of Banso *et al.* (1999), that higher concentration of antimicrobial substances showed appreciable growth inhibition. The zones of inhibition

produced by the test organisms indicated their susceptibility to the plant extracts, it was observed that the zones of inhibition varied from one organism to another and from one plant part extract to another. According to Prescott (2002) the effect of an agent varies with target species.

V. CONCLUSION

Findings from this study shows that aqueous and ethanolic extracts of *Anogeissus leiocarpus* and *Morinda lucida* leaves stems and roots contained all the phytochemicals constituents and their potencies varied at different concentrations. It was concluded that extracts of *A. leiocarpus* and *M. lucida* leaves, stems and roots has different antibacterial activities against some enteric pathogens and thus can be recommended in the treatment of some infections were enteric pathogens are implicated.

REFERENCES

[1] Adegoke, A. A. and Adebayo-tayo, B. C. (2009). Antibacterial activity and phytochemical analysis of leaf extracts of *Lasienthera africanum. African Journal of Biotechnology* 8 (1) 77-80

[2] Banso, A., Adeyemo, S.O. and Jeremiah, P. (1999) Antimicrobial Properties of *Vernonia amygdaline* extract. *Journal of Applied Science Management* 3:9-11.

[3] Cheesebrough, M. (2000). District Laboratory Practice in Tropical Countries. 4th edition. Cambridge university press, United Kingdom. Pp. 108-112.

[4] Cowan, M.M. (1999). Plant Products as Antimicrobial Agents.Clinical Microbiology. Rev., 12: 564-582.

[5] Hassan, H. S., Sule, M.I., Usman, M.A. and Ibrahim, A. (2009). Preliminary Phytochemical and Antimicrobial Screening of the Stem Bark Extracts of *Bauhinia rufescence* using some selected pathogens. *Bayero Journal of Pure Applied science. 2:* 53-55.

[6] Hewitt, W. and Vincent, S. (1989). In: Theory and application of microbiological assay. Academic Press, San Diego, p. 39.

[7] Ibezim, E.G. (2005). Microbial resistance to antibiotics. *African Journal of Biotechnology,* 4(13):1606-1611.

[8] Kafaru, E. (1994). *Immense Help from Natives Workshop,* 1st Ed, Elizabeth Kafaru, Lagos, Nigeria, pp. 11-14.

[9] Mada, S.B., Garba, A., Mohamed, H.A., Olagunju, A. and Mohamed, A.B. (2013). Antimicrobial Activity and Phytochemical Screening of Aqueous and Ethanol Extracts of *Mornordica charantia L.* leaves. *Journal of Medicinal Plants Research*, 7: 579-586.

[10] Nostro A, Germane MP, D'Angelo V, Marino A, Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett.Appl. Microbiol. 30(5): 379.

[11] Parekh, J. and Chanda, S. (2007)'. Invitro Antimicrobial Activity of *Trapa natans L.* Fruit Rind Extracted in Different *Solvents. African Journal of Biotechnology., 6:* 766-770.

[12] Prescott, L.M., Harley, J.P., Klein, D. (2002). Microbiology (international edition), fifth edition. Published by McGraw Hill book company pp. 809-819.

[13] Rahal, A., Mahima, A.K., Verma, A., Kumar and Tiwari R. (2014). Phytonutrients and nutraceuticals in vegetables and their multi-dimensional medicinal and health benefits for humans and their companion animals: A review. *Journal of Biological Sciences,* 14:1-19.

[14] Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Limited, Ibadan, Nigerja.

[15] Thabile, P.N. (2008). Antimicrobial and Hormone Mediated Health Benefit of Grain. Crit. Rev. Food Science Nutrition., 34:437-497.

[16] Yarney, J.A., Donkor, JS.Y., Opoku, L., Agyeman-Dual, A.C., Abakah and Asampong, E. (2013). Characteristics of users and implications for the use of complementary and alternative medicine in Ghanian Cancer patients undergoing radiotherapy and chemotherapy: A cross sectional study. BMC. *Complementery Alternative Medicine,* 1310(1186)1472-6882.