Synergistic Effects of *Anogeissus leiocarpus* and *Morinda lucida* Leaves, Stems and Roots Extracts against Some Enteric Bacteria

***Gberikon, G.M, *Agada, J.O, *Yaji, M.E**

*Department of Microbiology, Federal University of Agriculture, Makurdi
Email Address of Corresponding Author: gracegberikon@yahoo.com. 08034519567

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Author: Synergistic effects of *Anogeissus leiocarpus* and *Morinda lucida* leaves, stems and roots extracts against some enteric bacteria were carried out. 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. Standard microbiological and biochemical tests were carried out on the isolates for confirmation and revalidation. Susceptibility tests were carried out using agar well diffusion methods. The synergistic effects of these plants' extracts against these enteric bacteria were also investigated using different combination ratios by standard methods. Two way analysis of variance (ANOVA) was used in data analysis. Qualitative phytochemical results showed that both the aqueous and ethanolic extracts of the leaves, stems and roots of these plants contained bioactive compounds in varied quantities, Phenol was highly present in the aqueous leaves extract of *A. leiocarpus* (+++), aqueous root extract (+++), ethanolic leaves extract (+++) and ethanolic root extract (+++) but moderately present in the aqueous stem extract (++) and ethanolic stem extract (++). Phenol was highly present in the aqueous leaves extract and ethanolic leaves extract of *M. lucida*, moderately present in the aqueous and ethanolic root extracts, absent in the aqueous stem extract but present in the ethanolic stem extract. Test for synergism showed that *E.coli* was the most susceptible among all the test organisms. Combination of *A. leiocarpus* and *M. lucida* ethanolic root extracts of ratio 50:50% gave highest diameter of zone of inhibition of 25.00±00 against *E.coli*. Similarly, ethanolic leaf extracts of *A. leiocarpus* and *M. lucida* in the ratio of 60:40% gave the highest zone of inhibition of 28.00±00 against *E.coli* as compared to the other test organisms. This also showed that there is greater antibacterial activity in the leaf extracts as compared to the stems and roots. There was no significant difference between the plants' extracts ($P > 0.05$). The various combination ratios of the plants' extracts showed varied levels of inhibition on the test organisms, however, there was no significant difference between the different combination ratios of the extracts used on the test organisms ($P>0.05$). Combination of different parts of plant extracts can be used in the treatment of some bacterial infections.

Key words : Enteric bacteria, Synergistic, *Anogeissus leiocarpus*, *Morinda lucida*,

INTRODUCTION

The search for healing powers in plants is as old as man (Ibezim, 2005). Man used herbs in their raw and cooked forms to keep fit. Till date natural plants of various types are used in traditional African medicine for providing healing to various ailments before and after the spread of modern and scientific medicine. 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). The Yoruba's and south eastern people of Nigeria use *A. leiocarpus* as an antibacterial agent against bacteria infections (Dweek, 1996). The leaves of the plant are used externally as a decoction for the treatment of skin diseases and the itch of psoriasis. The powdered bark is applied to wounds, sores, boils, cysts and diabetic ulcers with good results. The infusion and decoctions are used as cough medicine. The pulped roots are applied to wounds and ulcers. The powdered bark is also rubbed to reduce tooth ache on gums, it is also used as vermifuges and the leaves decoction is used for washing and fumigation (Ibrahim *et al*., 1997). Extract of *A . leiocarpus* is traditionally acclaimed to be effective in treating infectious wounds in man and animals (Dweek, 1996).

Morinda lucida stems, bark, roots and leaves infusion is used as an antimalaria, antidiabetic, jaundice and dysentery treatment (Burkill, 1997). *Morinda lucida* extracts have been reported to have antioxidant and reducing activities (Ogunlana *et al.*, 2008). Extracts of these two plants confers antimicrobial activities on some organisms, and when they are combined their strength cannot be over emphasised, hence the need to assess their synergistic effects on pathogens.

MATERIALS AND METHODS

Sample Collection

Anogeissus leiocarpus and *Morinda lucida* leaves, stems and roots were obtained from Otukpo, Benue State.The plants leaves were authenticated by a Botanist in the 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 and were pulverised into powder. They were packed into sterile containers and labelled appropriately. Clinical isolates of these 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 the various isolates for confirmation.

Extraction Method

The extraction of the powdered form of the plant materials, were carried out using ethanol and distilled water as extracting solvents. The cold maceration extraction method of Cowan (1999) was used. Fifty grams of powdered samples of plant leaves, stems and roots were weighed and dissolved in 1000ml of the extracting solvent inside a 2 litre conical flask and covered with parafilm (Ogunjobi and Nndozie, 2004). The flasks were shaken vigorously at 30 minutes interval and left to stand for 24 hours at room temperature. The resultant mixture was then filtered with Whatman's No. 4 filter paper and cotton wool to remove particles of plant sample. The clear solution obtained was distilled at 65^{o}C under low pressure on a steam bath. The semi solid concentrations of the extracts were then collected in sterile pre-weighed screw capped bottles and labelled accordingly (Ogunjobi *et al.*, 2007). The extracts were stored at 4^{o}C until when needed.

Preliminary Qualitative Phytochemical Analysis

Preliminary qualitative 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^{o}C. The diameter of zones 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. The following combination ratios (80:20, 60:40, 50:50, 20:80 and 40:60) of both plant extracts was used to test the synergistic effect of both plant extracts on the enteric bacteria. All plates were incubated at 37^0C for 24 h 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.

RESULTS AND DISCUSSION

Table 1: Qualitative Phytochemical Screening of *Anogeissus leiocarpus*

PHYTOCHEMICALS	AQUEOUS LEAVE EXTRACT	AQUEOUS STEM EXTRACT	AQUEOUS ROOT EXTRACT	ETHANOLIC LEAVE EXTRACT	ETHANOLIC STEM EXTRACT	ETHANOLIC ROOT EXTRACT
Phenol	+++	++	+++	+++	++	+++
Alkaloid	++	+	++	++	+	+++
Flavonoid	++	+	+++	+++	++	+++
Tannin	+	+	++	+	++	++
Saponin	+++	++	+++	+++	++	++
Steroid	_	_	_	+	_	_
Phytosterol	_	_	_	+	_	_
Cardiac glycoside	+	_	+	_	_	+

Key: + (plus) = present, - (minus) = absent.

Table 2: Qualitative Phytochemical Screening of *Morinda lucida*

PHYTOCHEMICALS	AQUEOUS LEAVE EXTRACT	AQUEOUS STEM EXTRACT	AQUEOUS ROOT EXTRACT	ETHANOLIC LEAVE EXTRACT	ETHANOLIC STEM EXTRACT	ETHANOLIC ROOT EXTRACT
Phenol	+++	_	++	+++	+	++
Alkaloid	++	++	+++	+++	++	+++
Flavonoid	++	++	++	++	++	++
Tannin	+	++	++	+	+	++
Saponin	+	_	+	+	_	+
Steroid	++	+	+	++	+	+
Phytosterol	+	+++	_	+	+++	_
Cardiac glycoside	+	_	+	+	+	+

Key: + (plus) = present, - (minus) = absent.

Table 3: Diameter of Zones of Inhibition of Different Combination Ratios of the Extracts on the Test Organisms (ARAE versus MRAE)

EXTRACTS	***E. coli***	***P. mirabilis***	***S. typhi***	***K. pneumoniae***	***S. dysentriae***	**SD MEAN**
ARAE 80%						
MRAE 20%	19.00±0.00	15.00±0.00	19.00±0.00	21.00±0.00	20.00±0.00	18.80±2.28
ARAE 60%						
MRAE 40%	22.00±0.00	17.00±0.00	20.00±0.00	18.00±0.00	19.00±0.00	19.20±1.92
ARAE 50%						
MRAE 50%	21.00±0.00	19.00±0.00	21.00±0.00	17.00±0.00	22.00±0.00	20.00±2.00
ARAE 20%						
MRAE 80%	24.00±0.00	18.00±0.00	23.00±0.00	17.00±0.00	21.00±0.00	20.60±3.05
ARAE 40%						
MRAE 60%	23.00±0.00	20.00±0.00	22.00±0.00	19.00±0.00	20.00±0.00	20.80±1.64
TETRACYCLINE	13.00±0.00	27.00±0.00	30.00±0.00	25.00±0.00	26.00±0.00	24.60±6.54
SD MEAN	20.33±3.98	19.33±4.13	22.50±3.94	19.50±3.08	21.33±2.50	20.60±3.54

Organisms: $F(4, 20) = 0.925$, $P > 0.05$

Extracts: $F(5, 20) = 1.627$, $P > 0.05$

Key: ARAE = *Anogeissus leiocarpus* roots aqueous extract, MRAE = *Morinda lucida* roots aqueous extract,

Table 4: Diameter of Zones of Inhibition of Different Combination Ratios of the Extracts on the Test Organisms (AREE versus MREE)

EXTRACTS	*E.coli*	*P.mirabilis*	*S.typhi*	*K.pneumoniae*	*S.dysentriae*	SD MEAN
AREE 80%						
MREE 20%	22.00±0.00	20.00±0.00	22.00±0.00	17.00±0.00	21.00±0.00	20.40±2.07
AREE 60%						
MREE 40%	23.00±0.00	19.00±0.00	20.00±0.00	20.00±0.00	22.00±0.00	20.80±1.64
AREE 50%						
MREE 50%	25.00±0.00	18.00±0.00	20.00±0.00	18.00±0.00	19.00±0.00	20.00±2.92
AREE 20%						
MREE 80%	24.00±0.00	16.00±0.00	19.00±0.00	14.00±0.00	20.00±0.00	18.60±3.85
AREE 40%						
MREE 60%	20.00±0.00	15.00±0.00	19.00±0.00	18.00±0.00	21.00±0.00	18.60±2.30
TETRACYCLINE	13.00±0.00	27.00±0.00	30.00±0.00	25.00±0.00	26.00±0.00	24.20±6.54
SD MEAN	21.17±4.36	19.17±4.26	21.67±4.23	18.67±3.67	21.50±2.43	20.43±3.80

Organisms: $F(4, 20) = 0.895$, $P > 0.05$

Extracts: $F(5, 20) = 1.598$, $P > 0.05$

Key:AREE = *Anogeissus leiocarpus* roots ethanolic extract, MREE = *Morinda lucida* roots ethanolic extract.

Table 5: Diameter of Zones of Inhibition of Different combination Ratios on the Test Organisms (ASEE versus MSEE)

EXTRACTS	***E.coli***	***P.mirabilis***	***S.typhi***	***K.pneumoniae***	***S.dysentriae***	SD MEAN
ASEE 80%						
MSEE 20%	21.00±0.00	19.00±0.00	21.00±0.00	18.00±0.00	23.00±0.00	20.40±1.95
ASEE 60%						
MSEE 40%	18.00±0.00	22.00±0.00	20.00±0.00	16.00±0.00	22.00±0.00	19.60±2.61
ASEE 50%						
MSEE 50%	19.00±0.00	19.00±0.00	19.00±0.00	15.00±0.00	20.00±0.00	18.40±1.95
ASEE 20%						
MSEE 80%	20.00±0.00	25.00±0.00	17.00±0.00	20.00±0.00	19.00±0.00	20.20±2.95
ASEE 40%						
MSEE 60%	22.00±0.00	18.00±0.00	19.00±0.00	19.00±0.00	20.00±0.00	19.60±1.51
TETRACYCLINE	13.00±0.00	27.00±0.00	30.00±0.00	25.00±0.00	26.00±0.00	24.20±6.54
SD MEAN	18.83±3.19	21.67±3.67	21.00±4.60	18.83±3.55	21.67±2.58	20.40±3.58

Organisms: $F(4, 20) = 1.148$, $P > 0.05$

Extracts: $F(5, 20) = 1.784$, $P > 0.05$

Key: ASEE = *Anogeissus leiocarpus* stems ethanolic extract, MSEE = *Morinda lucida* stems ethanolic extract.

Table 6: Diameter of Zones of Inhibition of the Different Combination Ratios of the Extracts on the Test Organisms (ALEE versus MLEE)

EXTRACTS	*E. coli*	*P. mirabilis*	*S. typhi*	*K. pneumoniae*	*S. dysentriae*	SD MEAN
ALEE 80%						
MLEE20%	26.00±0.00	16.00±0.00	20.00±0.00	20.00±0.00	21.00±0.00	20.60±3.58
ALEE 60%						
MLEE 40%	28.00±0.00	17.00±0.00	19.00±0.00	18.00±0.00	20.00±0.00	20.40±4.39
ALEE 50%						
MLEE 50%	26.00±0.00	20.00±0.00	22.00±0.00	16.00±0.00	20.00±0.00	20.80±3.63
ALEE 20%						
MLEE 80%	25.00±0.00	17.00±0.00	20.00±0.00	16.00±0.00	21.00±0.00	19.80±3.56
ALEE 40%						
MLEE 60%	25.00±0.00	15.00±0.00	21.00±0.00	12.00±0.00	19.00±0.00	18.40±5.08
TETRACYCLINE	13.00±0.00	27.00±0.00	30.00±0.00	25.00±0.00	26.00±0.00	24.20±6.54
SD MEAN	23.83±5.42	18.67±4.41	22.00±4.05	17.83±4.40	21.17±2.48	20.70±4.54

Organisms: $F(4, 20) = 1.998$, $P > 0.05$

Extracts: $F(5, 20) = 1.021$, $P > 0.05$

Key: ALEE = *Anogeissus leiocarpus* leaves ethanolic extract, MLEE = *Morinda lucida* leaves ethanolic extract

Table 7: Diameter of Zones of Inhibition of Different Combination Ratios of the Extracts on the Test Organisms (ALAE versus MLAE)

EXTRACTS	*E. coli*	*P. mirabilis*	*S. typhi*	*K. pneumoniae*	*S. dysentriae*	SD MEAN
ALAE 80%						
MLAE 20 %	27.00±0.00	18.00±0.00	22.00±0.00	20.00±0.00	21.00±0.00	21.60±3.36
ALAE 60%						
MLAE 40%	27.00±0.00	14.00±0.00	20.00±0.00	15.00±0.00	22.00±0.00	19.60±5.32
ALAE 50%						
MLAE 50%	25.00±0.00	15.00±0.00	21.00±0.00	15.00±0.00	20.00±0.00	19.20±4.27
ALAE 20%						
MLAE 80%	23.00±0.00	18.00±0.00	22.00±0.00	14.00±0.00	21.00±0.00	19.60±3.65
ALAE 40%						
MLAE 60%	27.00±0.00	17.00±0.00	23.00±0.00	18.00±0.00	24.00±0.00	21.00±4.21

TETRACYCLINE	13.00±0.00	27.00±0.00	30.00±0.00	25.00±0.00	26.00±0.00	24.20±6.54
SD MEAN	23.67±5.47	18.17±4.62	23.00±3.58	17.83±4.17	22.33±2.25	21.00±4.62

Organisms: F (4, 20) = 2.728, P < 0.05

Extracts: F (5, 20) = 1.081, P > 0.05

Key: ALAE = *Anogeissus leiocarpus* leaves aqueous extracts, MLAE = *Morinda lucida* leaves aqueous extract.

Table 8: Diameter of Zones of Inhibition of Different Combination Ratios of the Extracts on the Test Organisms (ASAE versus MSAE)

EXTRACTS	***E. coli***	***P. mirabilis***	***S. typhi***	***K. pneumoniae***	***S. dysentriae***	**SD MEAN**
ASAE 80%						
MSAE 20%	19.00±0.00	17.00±0.00	20.00±0.00	15.00±0.00	19.00±0.00	18.00±2.00
ASAE 60%						
MSAE 40%	13.00±0.00	18.00±0.00	20.00±0.00	16.00±0.00	16.00±0.00	16.60±2.61
ASAE 50%						
MSAE 50%	17.00±0.00	18.00±0.00	22.00±0.00	16.00±0.00	20.00±0.00	18.60±2.41
ASAE 20%						
MSAE 80%	15.00±0.00	20.00±0.00	21.00±0.00	19.00±0.00	19.00±0.00	18.80±2.28
ASAE 40%						
MSAE 60%	18.00±0.00	17.00±0.00	20.00±0.00	17.00±0.00	18.00±0.00	18.00±1.23
TETRACYCLINE	13.00±0.00	27.00±0.00	30.00±0.00	25.00±0.00	26.00±0.00	24.20±6.54
SD MEAN	15.83±2.56	19.50±3.83	22.17±3.92	18.00±3.69	19.67±3.39	19.03±3.89

Organisms: F (4, 20) = 4.885, P < 0.01

Extracts: F (5, 20) = 5.241, P < 0.01

Key: ASAE = *Anogeissus leiocarpus* stems aqueous extract, MSAE = *Morinda lucida* stems aqueous extract

DISCUSION

Aqueous and ethanolic, leaf, stem and root extracts of *Anogeissus leiocarpus* and *Morinda lucida* contained bioactive compounds (Phenol, Alkaloid, Flavonoid, Tannin, Saponin, Steroid, Phytosterol and Cardiac glycoside). The phytochemicals were present in the extracts in various quantities (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.

Phenol was highly present in both plants leaves aqueous and ethanolic extracts, this account for the high zones of inhibition exhibited by the extracts of the leaves of both plants on the test enteric bacteria. This is in agreement with the findings of Arnold *et al.* (2011) that natural phenolic compounds have varying antimicrobial activities against enteric pathogens as it contains major antibacterial components and have great potential to be used as natural antimicrobials and food preservatives. *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* but the combination of both extracts in the ratio of 20:80 respectively has a zone of inhibition of 27.00±0.00 against *E.coli*, this agrees with the findings of Hewitt and Vincent (1989) that synergistic effect of plant extract is more effective against some bacteria. These findings are in coherence with the study reported earlier on synergistic activity of six different plants against pathogenic bacteria by Arnold *et al*. (2011). Ogunjobi *et al*. (2007) reported that the ethanolic leave extract of some medicinal plants showed least activity against Methicilin Resistant *Staphylococcus aureus* (MRSA) when used individually. Whereas the combination of these plant extracts exerted higher activity against MRSA.

Conclusion

It was concluded from findings of this study that both aqueous and ethanolic extracts of *Anogeissus leiocarpus* and *Morinda lucida* leaves stems and roots has antimicrobial activities on enteric pathogens. Higher zones of inhibitions were obtained when the extracts were combined. Therefore combination of *Anogeissus leiocarpus* and *Morinda lucida* extracts for synergistic effects is encouraged for effective treatment

REFERENCES

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

Arnold, S.R., Thomka, S.S., Philips, M., Kristie, J.J. and Morgan, O.J. (2011). Emergence of

Klebsiella pneumonia carbagenemase-producing bacteria. Southern medical journal,

104(l):40-4.

Burkill, H. M. (1997). The Useful Plants of West Tropical Africa, 2nd edition,.

Cheesebrough, M. (2000). District Laboratory Practice in Tropical Countries. 4th edition.

Cambridge university press, United Kingdom. Pp. 108-112.

Cowan, M.M. (1999). Antimicrobial Activity. Clinical Microbiology Reviews. 12 (4) 564-

582.

Duke, J.A. and Wain, K.K. (1981). Agriculture Research Service, Beltsville, Maryland.

Dweek A.A. (1996). Plant for Africa, part 2 http://www.dweek data.co.uk/published papers.

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

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

Ibrahim, M.B., Owonubi, M.O., Onaopo, J.A. (1997). Antibacterial Effect of Extract of Leaf, Stem and Root Bark *of Anogeissus leiocarpus* on Some Bacterial Organisms. *Journal of pharmaceutical Research-Development 2* (l):20-23Intracellular bacterial biofilm-like pods in urinary tract infections. *Science* 301 (5629):105-107.

Ogunjobi A. A., Fagade O. E and David O. O. (2007). Antimutagenic and potential

Anticarcinogenic activities of Aloe-vera gel and aqueous Garlic extract in the Bacterial

reverse mutation test (Ames assay) *African Journal of Biomedical Research* 10:275-278.

Prescott, L.M., Harley, J.P., Klein, D. (2002). Microbiology (international edition), fifth edition.

Published by McGraw Hill book company pp. 809-819.

Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa, Spectrum Books

Limited, Ibadan, Nigerja.