

## The Effect of Methanolic Extracts of *Anogeissus Leiocarpus* and *Terminalia Avicennioides* on the Growth of Some Food-borne Microorganisms

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**Abstract:** *Anogeissus leiocarpus* and *Terminalia avicennioides* are extensively used in Nupe traditional and folklore medicines to cure various human ailments. The preliminary phytochemical screening of the stem bark and root bark respectively revealed the presence of alkaloids, flavonoids, saponins, tannins and phenolic compounds, cardiac glycoside and terpenoids. *In vitro* antibacterial studies of the methanol extracts of both plants were carried out on six medically important bacterial strains namely: *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus* spp, *Lactobacillus* spp and *Pseudomonas aeruginosa* using agar plug techniques. The results of our antibacterial assay revealed that the extracts showed good inhibitory activity against all the tested pathogens compared with standard antibiotics like streptomycin. The extracts of the both plants showed antibacterial activity, justifying their continued use in treatment of bacterial infections. Further studies are required to isolate and characterise the active phytoconstituents in these plants as well as toxicity studies should also be done to determine their safety.

**Key words:** Medicinal plants, *Anogeissus leiocarpus*, *Terminalia avicennioides*, Antimicrobial activity, Phytoconstituents, Food-borne microorganisms.

### INTRODUCTION

Infectious diseases with increasing trends of drug resistant microorganisms have been common global problem posing enormous public health concerns (Iwu *et al.*, 1999). Antibiotics and the chemotherapeutic agents have been of value in controlling many infections but they depend on judicious use to minimize the incidence of resistance (Coates *et al.*, 2002; Levy and Marshall, 2004). The global emergence of antimicrobial resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections (Hancock, 2005). In many poor countries, the available drugs are costly and beyond the reach of the common man (WHO, 2002). Strategies to improve the current situation include research in finding new and innovative antimicrobials (Freeman, 1997), this include the need to develop new antimicrobial compounds from indigenous plant materials. In most cultures from ancient times to the present day, plants were used as a source of medicines by man to control diseases in humans and animals (Schultes and Raffauf, 1992). These plant-based systems continue to play an essential role in health care, and it has been estimated by the World Health Organization that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care (Arvigo and Balick, 1993; Farnsworth *et al.*, 1985). World Health Organization described plant with one or more organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (WHO, 1992).

Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries. The Nigerian environment is probably the least explored in terms of available untapped resources. Nigeria flora has over seven thousand, three hundred and forty-nine species of higher plants that had made serious impact on the health and wealth of Nigerians and could be an enormous source of foreign exchange for the country (Mann *et al.*, 2003).

Extensive and in-exhaustive lists of plants have been screened for their various chemical constituents that served both economical and medicinal purposes (Faraz *et al.*, 2003). Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. Antibacterial active principles isolated from higher plants used in traditional medicine were developed in to drugs. A substantial number of drugs currently being used are discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine (Grifo *et al.*, 1997). Plant medicine was commonly used

for traditional treatment of some infectious diseases and a lot of plants have been reported to show significant antimicrobial activity (Cowan, 1999).

The present report is to ascertain the claims by trado-medical practitioners on the potency of *Anogeissus leiocarpus* and *Terminalia avicennioides* in the treatment of various infectious diseases like tuberculosis and related cough, fever, nausea and body pains used in the Nupeland of North Central Nigeria. However, several studies of these plants demonstrated antimicrobial activity against common bacteria infections (Mann *et al.*, 2007; 2008a, b,c,d; 2009a, b,c,d; 2010). However, none had been reported against food-borne microorganisms and probably the present findings might be a useful tool in the treatment of such diseases. Therefore, the purpose of this study was to investigate the antimicrobial properties of the extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* against food-borne microorganisms as well as the phytochemical constituents responsible for the activity.

## MATERIAL AND METHODS

### **Plant materials:**

*Anogeissus leiocarpus* (Stem bark) and *Terminalia avicennioides* (Root bark) used in this study were collected in the Federal Polytechnic Bida, Niger State, Nigeria. Voucher specimens were deposited in the Herbarium at the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, and National Institute for Pharmaceutical Research and Development (NIPRD) with the Herbarium numbers ABUHH 167 and NIPRDH 5735 respectively.

### **Preparation of plant extract:**

The plants' parts collected were washed with tap water and air-dried at ambient temperature for 10 days and made into a fine powder of 40 mesh size using the laboratory mill and then packed into polythene bags until when needed. Hundred grams (100g) of the pulverized root bark of *Terminalia avicennioides* and the stem bark of *Anogeissus leiocarpus* were macerated in 100ml of 70% methanol for 72 h with intermittent stirring. The extract was filtered through Whatman no.1 filter paper to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The extracts were filtered through a Whatman No 1 filter paper and the filtrates were concentrated to dryness using rotary evaporator under reduced pressure. The extracts were prepared into 500mg/ml, 1000mg/ml, 1500mg/ml and 2000mg/ml concentrations required for the bioassay. To ensure the purity of each prepared extract, the extract solutions were plated by streaking on a prepared nutrient agar medium for 24 hours and observed for growth.

### **Test organisms:**

The extracts were tested against the following clinical isolates: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Lactobacillus spp* and *Bacillus spp*. which were collected from the Pathology Department of Federal Medical Centre, Bida, Niger State and were maintained on nutrient agar slants at the Department of Science Laboratory Technology, Federal Polytechnic, Bida in refrigerator until needed.

### **Phytochemical screening:**

Phytochemical analysis of the extract was conducted with slight modifications by Trease and Evans, 1989 and Harborne (1998). By this analysis, the presence of several phytochemicals like sugar, protein, alkaloids, flavonoids, saponins, tannins, cardiac glycoside, terpenoids and lipids were tested.

### **Antimicrobial assay:**

The extracts were tested for antimicrobial activity using agar disc diffusion assay according to the method of Bauer *et al.* (1966).

The bacteria were inoculated on the fresh media (Nutrient agar slants), incubated at 37°C for 24 h and were referred to as seeded broth. 0.3ml portion of the new culture was aseptically transferred into petric dishes containing melted soft medium, gently agitated and poured as over lay on assay plate containing 15ml base medium. The preparation was left to gel and dry under hood, spots where extracts were to be introduced into the plates were carefully marked by boring wells equidistant to each other using a sterile cork borer (5mm diameter). A drop of melted agar was introduced into the wells to seal the bottom before introducing 0.1ml of each extract of different concentration (i.e. 500, 1000, 1500 and 2000mg/ml in different wells. The patented antibiotic (streptomycin i.e. 200, 300, 400 and 500mg/ml) was used as standard and a control experiment was

set up by using drops of sterile water in place of different extract concentrations. The plates containing the bacteria, various concentration of extract and the antibiotic used as standard as well as control plate were allowed to stand for an hour at room temperature to allow the growth of the organism commenced.

The plates were observed for zones of inhibition, which was measured in mm, after 24 hours incubation at 37°C in triplicate determination.

## RESULTS AND DISCUSSION

The preliminary phytochemical analysis of the extracts revealed the presence of alkaloid, glycosides, steroids, phenol, tannins, saponins, flavonoids, anthraquinone and terpenes as presented in Table 1. The results obtained from the disc diffusion assay showed that there has been an increasing effect on bacterial growth inhibition with increasing concentration of the extract. And the extract showed good inhibitory activity on almost all the bacteria tested. It has been found that among all the tested organisms, the bacterial strain, *Lactobacillus spp* was found to be more susceptible to the plant extract by showing inhibition zone ranging from 1.8-4.0 mm. The antibacterial activity in terms of zone of inhibition was presented in Table 2. The observed activity may be due to the presence of potent phytoconstituents in the extracts.

**Table 1:** Result of preliminary qualitative analysis of phytochemical present in *A. leiocarpus* and *T. avicennioides*

Plant part	A	St	G	T	F	S	P	An
T.A(root bark)	0	0	0	0	-	0	0	-
A.L(stem bark)	0	0	0	0	-	0	0	-

Key: T. A = *Terminalia avicennioides*, A. L = *Anogeissus leiocarpus*, A = Alkaloid, ST = steroid, G = Glycoside, T = tannin, F = Flavonoid, S = Saponin, P = Phenol, An = Anthraquinone, + = Implies the presence of active compound in plant organ, - = Implies the absence of active compound in plant organ

**Table 2:** Zone of inhibition (mm) of extracts of plant part against the test organisms

Test organism	Plant extracts							
	<i>T. avicennioides</i> (root bark) mg/ml				<i>A. leiocarpus</i> (stem/ bark) mg/ml			
	500	1000	1500	2000	500	1000	1500	2000
<i>Lactobacillus spp</i>	1.8	2.0	2.4	2.8	2.2	2.4	3.0	4.0
<i>St. aureus</i>	1.6	1.6	1.8	2.0	1.6	2.0	2.1	2.4
<i>P. aeruginosa</i>	0.4	0.4	0.4	0.5	0.2	0.4	0.4	0.4
<i>E. coli</i>	0.2	0.2	0.0	0.4	0.0	0.0	0.4	0.6
<i>Bacillus spp</i>	1.8	2.0	2.2	2.6	1.8	2.4	2.8	3.0
<i>S. typhii</i>	1.9	2.0	2.2	2.4	2.0	2.2	2.6	2.8

**Table 3:** Zone of inhibition (mm) of antibiotic (streptomycin) against test organism

Test organism	Control			
	200mg/ml	300mg/ml	400mg/ml	500mg/ml
<i>Lactobacillus spp</i>	3.0	3.0	4.0	4.2
<i>S. aureus</i>	2.6	2.6	4.0	4.8
<i>P. aeruginosa</i>	2.0	2.8	3.8	4.0
<i>E. coli</i>	2.6	4.0	4.4	4.6
<i>Bacillus spp</i>	2.8	2.8	3.8	4.8
<i>S. typhii</i>	2.4	3.8	4.2	4.4

### Discussion:

Susceptibility test of the methanolic extracts of the tested plant parts showed that the parts exhibit antimicrobial activity against *Staphylococcus aureus*, *Lactobacillus spp*, *Bacillus spp* and *Salmonella typhii*. According to World Health report on infectious diseases 2000, overcoming antibiotic resistance is the major issue of the WHO for the next millennium. Hence the last decade witnessed an increase in the investigation of plants as a source of human disease management. The present study has shown that increase in the antimicrobial activities of the extracts was enhanced by the increase in the concentration of the extracts (Table2).

This finding agrees with the report of Banso *et al.* (1999), that higher concentration of antimicrobial substances showed appreciable growth of inhibition. The zones of inhibition of the test organism and their susceptibility to the plant extracts, varied from one organism to another and from one plant extract to another. According to Prescott *et al.* (2002), effect of an agent varies with target species. *S. aureus*, *Lactobacillus spp*,

*S. typhii* and *Bacillus spp* are more susceptible at low concentration of the antimicrobial agent used than *E. coli* or *P. aeruginosa*. Hugo and Russel (1998) also said that the position of the zone edge (diameter of zone of inhibition) is determined by the initial population density of the organism, their growth rate and the rate of diffusion of the antimicrobial agent. These explain the differences in the zones of inhibition observed in the experiments. Since the study has shown that the extracts from the root, stem, barks and a combination of the parts, had antimicrobial activities, it therefore, justifies their traditional usage as medicinal plants.

These might be due to the presence of the active principles in the plants. Plants generally produce many secondary metabolites which constitute an important source of microbiocides, pesticides and many pharmaceutical drugs (Ibrahim *et al*, 1997 and Kolapo *et al.*, 2007). The medicinal value of plants lies in some metabolites such as alkaloids, flavonoids, tannins and phenolic compounds that produce a definite physiological action on the human body (Duraipandiyan *et al.*, 2006).

The combination of some of these chemical substances found in herbal preparations synergistically forms a natural mixture with antimicrobial spectrum. The plant parts used showed significant differences in their bioactivities and in correlation with the standard used had shown that the extract from the root, stem barks as well as the combination of the two parts used could compare well in their bioactivities against the tested pathogenic organisms. Methanol extract of the plant parts used in this study showed activity against disease causing organism. These observations suggest that the constituent of the plant parts could be useful in chemotherapy. It is imperative that more work has to be done using different solvent from the one used in this study for extraction of active ingredients.

In conclusion, efforts could be made to isolate and characterize the active principles with clinical relevance.

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