In Vitro Antibacterial Activity and Preliminary Phytochemical Screening of Four Plants on Selected Clinical Pathogens

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Abstract- Twelve preparation of the ethanol and water extracts of four medicinal plants,*Anogeissus leiocarpus(*root and bark*), Terminalia glaucescens(*root and bark*), Adansonia digitata(bark)* and *Lennea welwitschii(bark) were screened for their inhibitory effect on Escherichia coli, Salmonella typhii, Staphylococcus aureus* and *Shigella dysentriae* using the agar-diffusion method. The efficacy of the extracts was assessed by measuring the diameter of zone of inhibition around the colonies on nutrient agar medium. The result obtained showed that the ethanol extracts (root) from *T. glaucenscens* and *A. leiocarpus* were active against all the test bacteria. The ethanol and aqueous extracts of *A. digitata* did not exert any inhibitory effect on test organisms, while the other eight extracts exhibited variable antibacterial activities. The minimum inhibitory concentration (MIC) of the potent extracts ranged from 0.625 mg/ml to 5.0mg/ml while a minimum bactericidal concentration (MBC) was between 1.25mg/ml - 10.0mg/ml. Photochemical screening of the extracts revealed that they contain saponin, tannin, alkaloid and glycoside .Statistical analysis (T-test) showed that there was no significant difference between the ethanolic extract ofPurification of these active components in the extracts could enhance their antibacterial activity.

Index Terms- Antibacterial, clinical origin, phytochemical constituents, and Plant extracts.

I. INTRODUCTION

Most developing countries including Nigeria are endowed with vast resources of medicinal and aromatic plants and these plants have been used over the millennia for human welfare (Rukangira, 2001). Throughout history, plant have been the principal source of drug used in preventing and curing of disease and in the production of some drugs currently used in modern medicine. The use of higher plants and their extracts to treat infection is an age – old practice in African medicine and it is an effective practice in many third world countries (Olorundare *et al.*, 1992). Medicinal plants represent a rich source from which antimicrobial agents may be obtained. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The active components of many drugs found in plants are secondary metabolites. (Sofowora, 1982).

Traditional medicine practices in Nigeria have continued to provide remedies for various diseases (Adelakun *et al.*, 1999) and most rural dweller depends on it for their health care needs (Ekpa and Ebana, 1992). This is because of the variety of herbal preparations that can be made from plants to treat different kinds of ailment including microbial infections (Akinyemi *et al.*, 2000). The pharmaceutical potential of medicinal plants is immense and various publications have reported the antimicrobial activities of some plants extracts. Black and green tea extracts have been shown to inhibit the growth of a variety of enteric bacteria (Toda *et al.*, 1989). Alade and Irobi, (1993), indicated that the extract *of Alcalypha wilkesiana* leaves was active against bacteria and fungi such as *Staphylococcus aureus, Candida albicans* and *Aspergillus flavus*. *Pseudomonas aeruginosa* and *Enterococcus faecalis* are inhibited by the sawdust extract of *Mansonia altissima* (Ejechi, 1996) also *Occium gratismum* has been shown to be antidiarrhoel (Ilori *et al.*, 1996).

In the recent past, attention has been directed towards medicinal plant research to substantiate the claims of cure made by traditional healers and thus providing a scientific basis for their efficacy (Sofowora, 1984).The lack of health care system in rural area has led to self-medication either by buying high cost medicine or by using medicinal plants (Rukangira, 2001).

Lannea welwitschii belongs to the family *Anacardiaceae.* The trunk bark is used for the treatment of diarrhoea, anaemia and haemorrhoids (Adjanohoum *et al.*, 1991).*Anogeissus leiocarpus* belongs to the family *Combretaceae*. The plant is a deciduous tree 15-20m high with bole sometimes fluted. The trunk bark and the roots are effective against haemorrhoid. (Adjanohoum *et al.*, 1999).*Terminalia glaucescens* belongs to the family *Combretaceae*. The tree is 8m high,black gray bark with deeper cracks. The plant is spread all over tropical Africa. The bark of the trunk is anti-diarrhoeal and is used to treat haemorrhoid, the roots are used for the treatment of haemorrhoid also. (Adjanohoum *et al.*, 1991).The bark has been shown to be effective against *vibrio cholerae* (Akinsinde and Olukoya, 1995) and also against *salmonella typhi* (Akinyemi *et al.*, 2000). *Adansonia digitata* belongs to the family Malyaceae. The main stem of larger baobab trees may reach enormous proportions of up to 28m in girth.

With the current trend in biotechnology of plant tissue and with the emergence of antimicrobial resistance, it would appear that man might soon have to depend on herbs as sources of a number of antimicrobial agents. (Babalola, 1988). The aim of this research work was to find out possible antibacterial potential in the extracts of these plants against some clinical isolates.

II. MATERIALS AND METHODS

Collection and Identification of plant sample.

The root and bark of the plant species *Anogeiossus leiocarpus* and *Terminalia glaucescens* and the bark of *Adansonia digitata* and *Lannea welwitschii* were sourced with the help of traditional herb sellers from their farms across western Nigeria.

The plants were identified and authenticated at the Botany department, University of Lagos, Nigeria.

Preparation of plant extract: The four plants were pulverized into fine powder. Their ethanol and aqueous extracts were prepared using the cold maceration and soxhlet extraction techniques. The cold extracts were dried under vacuum using the freeze drier while the hot extracts were concentrated to dryness at 50^oC in the vacuum oven. 1g portion of each dried extract was reconstituted in 100ml sterile distilled and sterilized using a 0.2µm membrane filter. The resulting sterile filtrate was transferred aseptically into a labeled sterile bottle and stored in the refrigerator at 4^0C till needed for use.

Source of clinical pathogens: Clinical bacterial isolates were obtained from the Molecular Biology and Biotechnology Division, Nigerian Institute of Medical Research, Lagos, Nigeria.The organisms included *Escherichia coli*, *Shigella dysenteriae Salmonella typhi* and *Staphylococcus aureus*. These were subcultured on Mac Conkey Agar at 37^0c for 24 hr. Bacterial colonies on Mac Conkey Agar plates for each organism was emulsified in 3mls sterile saline and adjusted to obtain a concentration of 1.5×10^6 cells/ ml.

Sensitivity test: Agar well diffusion method was used to determine the antibacterial activity of the extracts. Wells of 7mm diameter were made into previously seeded Nutrient agar plates. Each well was filled with 0.1ml of each plant extract. The control experiment was setup using sterile distilled water and 75% ethanol. The plates in duplicated were incubated at 37^0C for 24 hrs. The diameter of zones of inhibition was measured and expressed in millimeter (mm).The transparently cleared zones showed bactericidal activity while the cleared zones containing micro colonies showed bacteriostatic activity.

Determination of Minimum Inhibitory Concentration (MIC) AND Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration of the active extracts was determined by diluting the extracts in nutrient both to give concentrations of 10.0, 5.0, 2.5, 1.25, 0.625 and 0.313. While 2ml of sterilize extract was added to the first tube containing 2ml of nutrient broth. The tube was shaken and 2ml transferred aseptically to the next tube containing the same quantity of broth. This was done until serial dilution was achieved in the last tube i.e. the sixth tube. An aliquot of 1ml of bacterial suspension ($1.5x10^6$) was inoculated into each tube .The control tubes were inoculated with same quantity of sterile distilled water and 75% ethanol respectively .All tubes were incubated at 37^0C for 24 hours. The minimum inhibitory concentration was regarded as the lowest concentration of the extract that did not permit any visible growth when compared with the control tube. The minimum bactericidal concentration was determined by culturing the content of the tubes, which had no visible growth on MacConkey Agar, the plates were incubated at 37^0C for 24 hours. The lowest concentration of the extract, which did not produce any bacterial colony, was regarded to be the minimum bactericidal concentration (Alade and Irobi, 1993).

Phytochemical screening of plant extracts:The reconstituted extracts were evaluated for the presence of alkaloid, tannin, glycoside, flavonoid and saponin using the procedure described by Okerulu and Chinwe,2001.

III. RESULTS

SENSITIVITY TEST

Two extracts were able to produce clear zones against all the test organisms indicating bactericidal activity. These are the ethanol extracts of *A. leiocarpus* (Fig 1) and *T. glaucescens* (Fig 1). Two extracts were not inhibitory to all the test organisms, i.e. the ethanol and water extract of *Adansonia digitata*, however, eight of the extracts exhibited bacteriostatic properties.

Determination of MIC and MBC

The minimum inhibitory concentration of the extracts varied between 0.625 mg/ml - 5.0 mg/ml while a range between 1.25 mg/ml - 10.0 mg/ml was recorded for the minimum bactericidal concentration (Table1).

Phytochemical properties of plants extracts: The screening revealed that flavonoid was not detected in any sample while Some of the extracts showed the presence of glycoside, alkaloid, saponin and tannin (Table2).

TABLE1: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) expressed in mg/ml

Sample	Ec		Sa		St		Sd	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
F	0.625	1.25	0.625	-	*		1.25	2.5
D	0.625	1.25	1.25	2.5	2.50	5.0	5.0	10.0
H	1.25	2.5	1.25	-	0.625	1.25	0.625	2.5
L	2.5	-	*		2.50	5.0	-	-

Ec = *Escherichia coli*; Sa = *Staphylococcus aureus;* St = *Salmonella typhi*
Sd = *Shigella dysenteriae;* - = No MIC/ MBC; *= Not tested

TABLE 2: Result of Phytochemical properties of the plant extracts

Extract	Tannin	Flavonoid	Alkaloid	Saponin		Glycoside
	$FeCl_3$			Frothing	Emulsion	
A	-	-	-	+	+	+
B	-	-	+	-	-	+
C	-	-	-	+	+	+
D	-	-	+	+	+	+
E	+	-	-	+	+	-
F	+	-	+	+	+	-
G	+	-	-	+	+	-
H	+	-	-	+	+	-
I	-	-	-	+	+	-
J	-	-	+	+	+	+
K	-	-	-	+	+	+
L	-	-	+	+	+	+

+ = present; - = absent

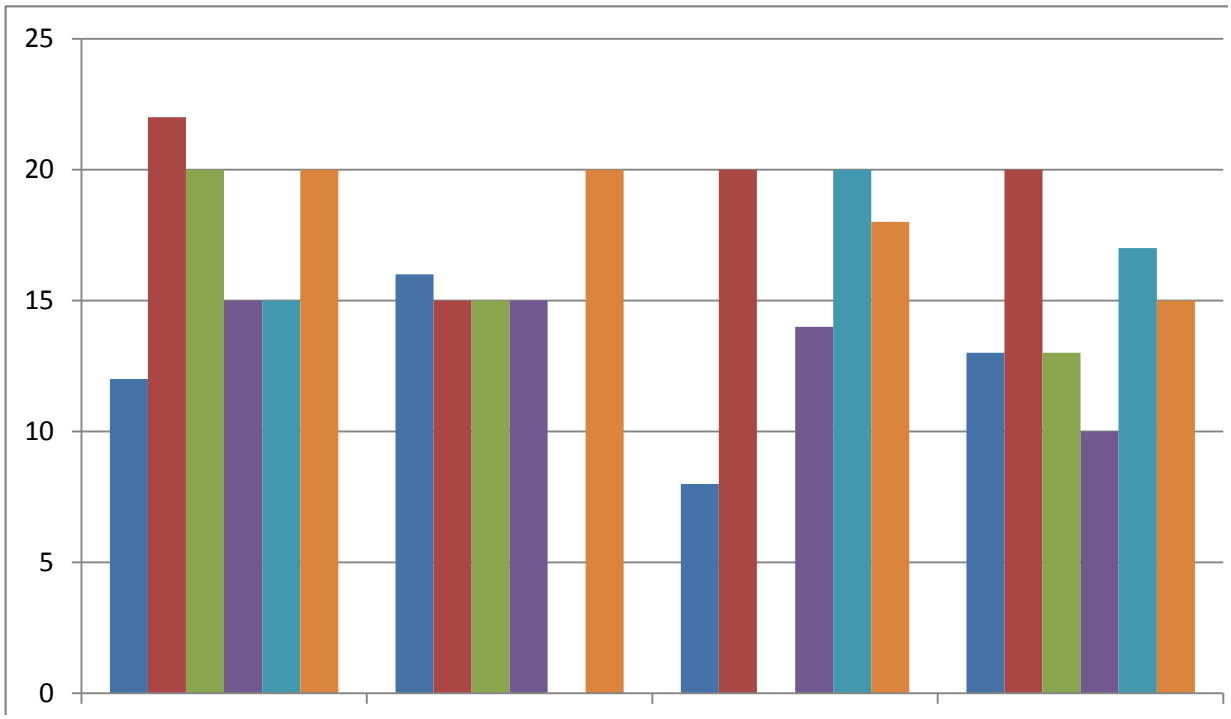

Figure 1: Antibacterial Activity of the Medicinal Plants Used Against the Test Organisms

B =*Anogeissus leiocarpus* (Bark) Ethanol extract; D = *Anogeissus leiocarpus* (Root) Ethanol extract; F = *Terminalia glaucessens* (Bark) Ethanol extract; G = *Terminalia glaucessens* (Root) Water extract; H = *Terminalia glaucessens* (Root) Ethanol extract; L = *Lannea welwitschii* (Bark) Ethanol extract

IV. DISCUSSION

Results obtained in this study showed that ethanol extracts of *A. leiocarpus* and *T. glaucescens* clearly showed that the extracts were inhibitory to all the test isolates in varying degrees. It was shown in this study that the ethanolic extract is more potent than aqueous extract. The relatively high potency of the ethanol extract may be attributed to the dissolving power of alcohols over water. (Majorie, 1999). This can also be deduced to the ability of the ethanol to extract more secondary plant metabolites which are believed to exert antibacterial activity on the test isolates (Nwinyi *et al.*, 2009).It had earlier been reported by several investigators that these plants contain antimicrobial substances (Adjanohoum *et al.*, 1999, Ilori *et al.*, 1996, Akinyemi *et al.*,2000, and Okujaga, 2005). Eight of the extracts were weakly inhibitory to almost all the test isolates, while two extracts were not inhibitory to all the test isolates used i.e. the ethanol and aqueous extracts of *A. digitata*. This conforms to the report of Kubmarawa *et al.*, 2007.Failure of these extracts to exert antibacterial effect on the test isolates is not enough to conclude that the plant does not contain substances that can exert antibacterial activity against the test organisms because the potency of the extract depends on the method used to obtain the

extract(Unaeze *et al*.,1986).Sofowora,1982 reported that the age of plant and the season of harvest determine the amount of active constituents and this varies in quality and quantity from season to season. The active components usually interfere with growth and metabolism of microorganisms in a negative manner and are quantified by determining the minimum inhibitory concentration and minimum bactericidal activity. These values are used as guide for treatment of most infections. Result obtained showed that the minimum inhibitory concentration values of the four extracts that showed the most sensitivity where lower than the minimum bactericidal concentration suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration. Phytochemical screening of the plant extracts showed the presence of saponin, alkaloids, glycoside and tannin, but flavonoids were not detected at all. Several phenolic compounds like tannin present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes used by plant pathogens. Other preformed compounds like saponins also have antifungal properties.(Aboaba *et al*.,2006).

The results obtained indicated the presence of antibacterial compounds in these plants and some also showed a good correlation between the claims of the traditional healers and the use of these plants in traditional medicine against clinical isolates.

Purification, identification, quantification, stability and toxicological studies should be carried out on the active components of these plants. These plants whose antibacterial activities have been demonstrated in this study could be used as raw materials in pharmaceutical industries.

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