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ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF THREE MEDICINAL PLANTS USED IN SOUTH-WEST NIGERIAN FOLK MEDICINE ON SOME FOOD BORNE BACTERIAL PATHOGENS.

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Abstract

Three medicinal plants *Trema guineensis*, *Phyllanthus discoideus*, and *Acalypha wilkesiana* traditionally used in South-West Nigerian communities for the treatment of gastroenteritis were investigated for antibacterial activity against strains of three food borne pathogens that resisted conventional orthodox antimicrobials. The extracts were screened against *Salmonella enteritidis*, *Escherichia coli* and *Staphylococcus aureus* by standard methods. The results of antimicrobial activity showed that water and ethanol crude extracts were active on all the strains of pathogens tested at different concentrations, with ethanol extracts exerting more activity. Using the disc containing 20 mcg of the extracts, the average diameter zone of inhibitions observed against these organisms ranged from 10.6 to 13.1 mm. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 32.5 & 35.3 and 31.5 & 33.0 mcg/ml were obtained for water and ethanol extracts of *T. guineensis* against *S. enteritidis* respectively, similar values were recorded against *E. coli* in this study.. On the other hand, MIC & MBC values of 28.5 & 39.2 and 28.5 & 39.3 as well as 26.4 & 35.6 and 27.0 & 36.2 mcg/ml were recorded for water and ethanol extracts of *A. wilkesiana* and *P. discoideus* against *S. aureus* respectively, higher values were obtained against the strains of *S. enteritidis* and *E. coli*. Although, both water and ethanol extracts of the three medicinal plants were considered active on all the strains of the three food borne pathogens in this study thus justifying their traditional use as antimicrobials, the extracts of *T. guineensis* seems to exert more activity on gram negative bacteria. All the plants extracts exhibited positive reactions to alkaloids, tannins, saponins, flavonoids and anthraquinones but in variable degrees. None contained cardiac glycosides and cyanogenic glycosides.

Key words: Medicinal plants, Antibacterial activity, Foodborne pathogens, Minimum Inhibitory Concentrations, , *Acalypha wilkesiana*, *Phyllanthus discoideus* and *Trema guineensis*.

Introduction

Food-borne diseases result from eating food contaminated with bacteria (or their toxins) or pathogens such as parasites or viruses. The illnesses range from stomach upset to more serious symptoms such as diarrhoea, fever, vomiting abdominal cramps and dehydrations (Slutsker *et al.*.,1998 ; Adak *et al.*, 2002). Outbreaks of food-borne diseases have been reported for decades and have been associated with the consumption of foods like soft cheese, processed meat, beef, poultry or eggs and other related foods . Food borne diseases associated with pathogens such as *Salmonella enteritidis*, *Staphylococcus aureus*, *Campylobacter spp*, *Escherichia coli* have been reported in many part of the world particularly in Australia, Canada, Japan, United States, European countries and in South Africa (Adak *et al.*,2002; Slutsker *et al.*.,1998; Moterjemi and Kaferstein,1997; Bäumlner *et al.*, 2000) and in Nigeria the situation is the same (Akinyemi *et al.*, 1998). Acute food borne infections and intoxications are much more of a concern to government and the food industry today than a few decades ago. Some of the factors that have led to that include, the identification of new aetiological agents, increasing number of large outbreaks being reported, the impact of food-borne diseases on children, the aging population and the immunocompromised individuals (Moterjemi and Kaferstein,1997; Bäumlner *et al.*, 2000).The epidemiology of food borne diseases is changing and reports from different parts of the world indicate that strains of resistant food borne pathogens have emerged as public health problem. Over the last two decades for examples, bacterial infections caused by *Salmonella enteritidis*, *Staphylococcus aureus*, , *Escherichia coli* and newer food borne pathogens have become increasingly resistant to empirical antimicrobial agents (Slutsker *et al* 1998, 2001). The search for new antimicrobial natural products from plant materials is essential in order to curb the menace of multiple antibiotics resistant pathogens.

Medicinal plants constitute an effective source of antimicrobial natural products. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into Africa continent (Haslam *et al.*,1989).Plants have been used in traditional medicine for many centuries as abortifacients, contraceptives, for menstrual regulation, fertility control, as well for the treatment of ailments of both microbial and non-microbial origins (Gill and Akinwunmi,1986). The Nigeria flora is rich in medicinal plants which are usually exploited by herbal doctors otherwise called ‘‘native doctor’’. The indigenous population in Southwest, Nigeria for example has developed a vast knowledge on the use of plants as traditional remedies (Ekundayo,1986). Some of the plants collections are used against a variety of diseases such as typhoid fever gastroenteritis, dysentery, malaria and others which are typical diseases of tropical countries (Sofowora,1993; Nick *et al.*, 1995).

Acalypha wilkesiana is a shrub with heart-shaped leaves available in varying mottled combinations of colours. The plant is available in tropical forest of western, Nigeria and often used traditionally for the treatment of malaria, healing of wounds and dermatological as well as gastrointestinal disorders (Akinde and Odeyemi,1987). *Phyllanthus discoideus* is a small tree widely used in tropical West-Africa. In South-west part of Nigeria, the bark extract is used locally to cure stomachache and lumbago. It is also useful in the treatment of helminthes infections. *Trema guineensis* is a tree abundant in the tropical forest of South-west, Nigeria. The leaf and the back extracts are used for the treatment of fever , bronchitis, pneumonia and gastrointestinal disorders. Several workers have proved and disproved scientific efficacies of some

Afr. J. Trad. CAM (2006) 3 (4): 13 - 22

medicinal plants claimed by traditional practitioners to cure some ailments of microbial origins (Akinyemi *et al.*, 2005 ; Ajaiyeoba,2002; Ilori *et al.*, 1996; Akinsinde and Olukoya ,1995).

In recent times , persistent claimed of efficacy of the crude extracts of *A. wilkesiana*, *P. discoideus*, *T. guineensis* when used separately in the treatment of ailments associated with gastrointestinal disorders has been on increased among traditional medical practitioners. The reputed efficacy of the plants had been experienced and passed on from one generation to the other. It is an established fact that some medicinal plants contain compounds that have lethal effect on some microorganisms. We therefore embark on this study with a view to establishing the effect of these three medicinal plants on some food borne bacterial pathogens in order to substantiate the therapeutic indications claimed by traditional medical practitioner on gastroenteritis..

Materials and Methods.

Fresh plant materials were collected from users of these plants in Epe, Ikorodu, and Imota in Lagos State, Nigeria. Their botanical identities were determined and authenticated in Botany department, Lagos State University, Ojo, Lagos. Samples have been deposited in the herbarium of the department.

Extraction

The extraction method used in this study was a modification of those used by Akinsinde and Olukoya (1995) and Akinyemi *et al.* (2000) Shredded plant materials were put in sterile bottles containing either distilled water or 40% ethanol, in line with the conditions similar to those used by traditional Medical practitioners for their preparation.

Water Extract

Leaves of *T. guineensis* (A) and *A. wilkesiana* (B) and the bark of *P. discoideus* (C) were oven-dried at a temperature of 60°C for 6 days. They were subsequently ground into fine powder in 25 ml of sterile distilled water, maintained at 60°C for 3 hr. The resulting suspensions were filtered and evaporated to dryness at 60°C in vacuo. The resulting extracts were weighed to produce 0.036, 0.037 g and 0.041g of A,B and C respectively. They were further labeled as aqueous extracts and designated as AW, BW and CW respectively.

Ethanol Extract

Six grams of the powdered plant material was extracted in a soxhlet extractor using 25ml of 40% ethanol. The extract was filtered using What man filter paper no.1 and the filtrates were then evaporated and dried at 60°C in vacuo. The resulting extracts were subsequently weighted to produce 0.034, 0.035 and 0.039g of A B and C respectively. They were labeled as ethanol extracts and designed AE, BE, and CE respectively. The extracts were tested against some food borne pathogens

Bacterial Culture

The following food borne pathogens *Escherichia coli*, *Salmonella enteritidis* and *Staphylococcus aureus* were used for the antibacterial screening in this study. They were isolated from patients presenting with symptoms of food poisoning and/or gastrointestinal disorders. The isolates were identified by standard methods (Cowan and Steel,1993). All the isolates used were resistant to conventional antibiotics chloramphenicol, ampicillin and cotrimoxazole. Resistance was determined by reference microdilution methods using the established Standards (NCCLS,1993). The organisms were maintained on agar slope at 4°C and sub-cultured for 24hr before use.

Bacterial Susceptibility Testing

A standardized inoculum of $1-2 \times 10^7$ cfu/ml with 0.5 McFarland standard was introduced onto the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of inoculum. A sterile paper disc previously soaked in known concentration of extracts (20 mcg/ml per disc) was carefully placed at the centre of the seeded labeled agar. The plates were incubated aerobically at 37°C and examined for zone of inhibition after 24 hrs. Each zone of inhibition was measured with a ruler and compared with the control (disc containing only physiological saline) (Sardari *et al.*,1998).

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

The MIC of the extracts were determined by diluting the various concentrations (0.0-36, 0.0-41, and 0.0-37 mcg/ml) of A,B, and C respectively. Equal volume of the extracts and nutrient broth were mixed in the test tube. Specifically 0.1ml of standardized inoculum of 1 to 2×10^7 cfu/ml was added to each tube. The tubes were incubated aerobically at 37°C for 18-24hrs. Two control tubes were maintained for each test batch. This is as follows: tube containing extracts and the growth medium without inoculum (antibiotic control) and the tube containing the growth medium, physiological saline and the inoculum (organism control). MIC was determined as the lowest concentration of the extracts permitting no visible growth (no turbidity) when compared with the control tubes. The MBC was determined by subculturing the test dilution on fresh solid medium and further incubated at 37°C for 18-24hrs. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC.

Phytochemical screening Methods

The screening procedures used were adapted from the work of Wall *et al.*(1952) and Sofowora (1993). All the plant parts were extracted on the day of collection. An extraction of each plant part was carried out by macerating a known weight of the fresh plant material in a blender with redistilled methylated spirit. Each plant extract was taken, suction-filtered and the process repeated until all soluble compounds had been extracted, as judged by loss of color of the filtrate. The plant extract in each case was evaporated to dryness in vacuo at about 45°C and further dried to a constant weight

Afr. J. Trad. CAM (2006) 3 (4): 13 - 22

at the same temperature in a hot-air oven. The yield of residue was noted and a portion of it was used to test for plant constituents.

The test for alkaloids was carried out by subjecting 0.5g aqueous extract in 5ml 1% HCl, boiled, filtered and Mayer's reagent added (Harborne, 1973 and Trease & Evans, 1989). Cyanogenic glycosides were identified by subjecting 0.5g extract in 10 ml sterile water, filtering and adding sodium picrate paper to the filtrate and heated to boil. The presence of flavonoids was determined using 1% aluminum chloride solution in methanol. Concentrated HCl and magnesium turnins, and potassium hydroxide solution (Kapoor *et al.*, 1969) For cardiac glycosides, legal test and the Killer-Kiliani test were adopted (0.5g of extract was added to 2ml acetic anhydride plus H₂SO₄) (Trease & Evans, 1989). The extract was also tested for carbohydrates using resorcinol solution as described by Wall *et al* (1952). The extract was subjected to frothing test for the identification of saponin, also hemolytic test was further performed on the frothed extracts in water to remove false positive results. For reducing sugar, Fehling's solution was added to the extract and heated. The extract was also tested for free glycoside bound anthraquinones (Wall *et al.*, 1952; Sofowora, 1993). Five grams of extract was added to 10ml benzene, filtered and ammonia solution added. The test for tannins was carried out by subjecting 3g of each plant extract in 6ml of distilled water, filtered and ferric chloride reagents added to the filtrate (Trease and Evans, 1989).

Results

The profile of the medicinal plants used in this study is shown in Table 1. The results of antibacterial activity of both water and ethanol crude extracts of *T. guineensis*, *P. discoideus*, and *A. wilkesiana* showed good activity on all the strains of *S. enteritidis*, *E. coli* and *S. aureus* tested at different concentrations, with ethanol extracts exerting slightly higher activity than water extracts as revealed by mean diameter of zone of inhibitions, minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) (Tables 2 and 3). This study showed that, ciprofloxacin commercial antibiotic was more active than both water and ethanol crude extracts of these three medicinal plants at the same concentrations (20mcg) as revealed by the mean diameter zone of inhibitions (Table 2).

Table 1: Profile of medicinal plants used.

Botanical name	Family name	Local name	Part	Vouchernumber
<i>Trema guineensis</i> Schum.& Thonn.	Ulmnceae	Ayinyin	Leaf	LSH 1030
<i>Acalypha wilkesiana</i> Muel-Arg	Euphorbiaceae	Ewe eela	Leaf	LSH 2100
<i>Phyllanthus discoideus</i> (Baill.) Muel-Arg	Euphorbiaceae	Asin	Bark	LSH 2111

Afr. J. Trad. CAM (2006) 3 (4): 13 - 22

However, MIC and MBC values of 32.5 & 35.3 mcg/ml and 31.5 & 33.0 were recorded for both water and ethanol extracts of *T. guineensis* respectively against *S. enteritidis*. While similar values were obtained against *E. coli*, whereas higher values of MBC of 45.0 and 37.3mcg/ml of water and ethanol extracts of the same plant were recorded against *S. aureus* in this study (Table 3). Also, it was observed that water and ethanol extracts of *A. wilkesiana* and *P. discoideus* exerted more activity on *S. aureus* than *E. coli* and *S. enteritidis*. For example 28.5 & 39.2 and 28.5 & 39.3 as well as 26.4 & 35.6 and 27.0 & 36.2 mcg/ml were the MIC & MBC values obtained for water and ethanol extracts of *A. wilkesiana* and *P. discoideus* against *S. aureus* respectively, higher values of MIC and MBC were recorded against the strains of two other pathogens tested (Table 3). The result of photochemical screening showed that the three plants exhibited positive reactions to alkaloids, tannins, saponins, flavonoids and anthraquinones but in variable degrees.

Table 2: Antibacterial activity of the plant extracts

Plant used	<i>Salmonella enteritidis</i> (n =7)		<i>Escherichia coli</i> (n = 8)		<i>Staphylococcus aureus</i> (n = 7).	
	Water extract	Ethanol extract	Water extract	Ethanol extract	Water extract	Ethanol extract
<i>T. guineensis</i>	12.2	13.1	12.1	12.7	12.3	13.1
<i>A. wilkesiana</i>	10.8	11.6	10.6	11.4	10.7	11.5
<i>P. discoideus</i>	11.3	12.1	12.0	12.0	11.2	11.9
<i>Ciprofloxacin</i> (20 mcg)	27.0		28		27.3	

Each value indicates mean diameter zone of inhibitions.

Ciprofloxacin = Commercial antibiotic, mcg = micrograms

None contained cardiac glycosides and cyanogenic glycosides. Reducing and non-reducing carbohydrates were only found in *P. discoideus* (Table 4).

Discussion.

Medicinal plants are widely used in African communities to treat different types of bacterial diseases (Sofowora, 1993). We investigated three medicinal plants *T. guineensis*, *A. wilkesiana* and *P. discoideus* commonly used by traditional medical practitioners in South western communities of Nigeria to treat bacterial gastroenteritis. Since the strains of bacteria used resisted the conventional antibiotics such as chloramphenicol, ampicillin and cotromoxazole, we decided to use a ciprofloxacin (fluoroquinolone) a broad spectrum antibiotic for both gram positive and gram

Table 3: Minimum inhibitory and bactericidal concentrations of the crude extracts.

Plant used	<i>S. enteritidis</i>				<i>E. coli</i>				<i>S. aureus</i>			
	Water extract		Ethanol extract		Water extract		Ethanol extract		Water extract		Ethanol extract	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>T. guineensis</i>	32.5	35.3	31.5	33.0	31.5	33.0	31.5	33.0	33.5	45.0	31.5	37.3
<i>A. wilkesiana</i>	47.5	50.4	45.0	45.3	40.4	50.5	42.0	51.0	28.5	39.2	28.5	39.3
<i>P. discoideus</i>	49.0	51.0	47.5	43.0	39.7	50.0	37.8	49.4	26.4	35.6	27.0	36.2

negative bacteria as control. Discs containing 20mcg of extract were used in the sensitivity test because this amount was contained in the control antibiotic. The result of discs antibacterial susceptibility testing showed that all the strains of the three pathogens vis-a-vis *S. enteritidis*, *E. coli* and *S. aureus* were highly susceptible to ciprofloxacin with average diameter zone of inhibitions of 27, 28.5 and 27.3 mm respectively. The

Table 4 : Phytochemical analysis of crude extract of the screened plants.

Constituents	Screened plants		
	<i>T. guineensis</i> (leaf)	<i>A. wilkesiana</i> (leaf)	<i>P. discoideus</i> (bark)
Alkaloids	+++	++	+++
Tannins	+++	++	+++
Saponins	+++	++	++
Anthraquinone	+	+	+
Flavonoids	+	+	+
Cardiac glycosides	-	-	-
Cyanogenic glycosides	-	-	-
Reducing and non-reducing carbohydrates	-	-	+++

Parenthesis = plant part.

+++ = Appreciable amount

++ = Moderate amount

+ = Trace amount

- = Completely absence.

acceptable standard diameter zone of inhibition for sensitive organism for this antibiotic is >21mm (NCCLS, 1993). However, for the plant extracts, the average zone of inhibitions observed against these pathogens ranged from 10.6 to 13.1 mm. These values fall within the range of resistant and/or intermediate sensitive when compared with control antibiotic. Although, the low values recorded for the plant extracts may be attributed to the fact that the extracts being in crude form, contain very small amounts of bioactive compounds. At the same time, several workers have reported bioactivity of crude extracts of medicinal plants within such range of diameter zone of inhibitions (Olukoya *et al.*, 1993; Ilori *et al.*, 1996; Ogbeche *et al.*, 1997; Akinyemi *et al.*, 2005). Our present study conformed with these previous findings. In order to further substantiate the activity of these plants extracts, we determined the MIC and MBC of the crude extracts using a standard method. The results showed that both water and ethanol extracts of the three plants exerted good antibacterial activity on all the strains of *S. enteritidis*, *E. coli* and *S. aureus* at different concentrations, with ethanol extracts exerting more activity (Tables 2 and 3). It is worthy of note that MBC values obtained for the extracts against the pathogens are higher than MIC, indicating that the extracts are bacteriostatics at lower concentrations and bactericidal at higher concentrations. This suggests that these plant extracts, when used traditionally as antimicrobials inhibit bacteria growth without necessarily killing the bacteria and since most of the traditional preparations lack specific concentrations, this may thus account for the use of large quantity of the extracts by traditional medical practitioners for the treatment of their patients. We obtained MIC & MBC values of 32.5 & 35.3 mcg/ml and 31.5 & 33.0 mcg/ml for both water and ethanol extracts of *T. guineensis* respectively against *S. enteritidis*, and similar values were recorded against *E. coli*, whereas higher values of MBC of 45.0 and 37.3 mcg/ml of water and ethanol extracts of the same plant were recorded against *S. aureus* in this study respectively. On the other hand, water and ethanol extracts of *A. wilkesiana* and *P. discoideus* exerted more activity on *S. aureus* than other two pathogens with MIC & MBC values of 28.5 & 39.2 and 28.5 & 39.3 as well as 26.4 & 35.6 and 27.0 & 36.2 mcg/ml for water and ethanol extracts of *A. wilkesiana* and *P. discoideus* against *S. aureus* respectively, higher values of MIC and MBC were recorded against the strains *S. enteritidis* and *E. coli* tested (Table 3). Therefore, our results tend to suggest that both water and ethanol leaf extracts of *T. guineensis* may be more active on gram negative bacteria than gram positive, and those of *A. wilkesiana* and *P. discoideus* may probably be more active on gram positive bacteria. A more detailed study of the activity of these plants extracts on many gram positive and gram negative bacteria of medical importance is underway to substantiate this assertion. However, our previous studies had proved the efficacy of *T. guineensis* on Typhoid and paratyphoid bacilli (Akinyemi *et al.*, 2000), as well as efficacy of water and ethanol extracts of *A. wilkesiana* and *P. discoideus* on methicillin resistant strains of *S. aureus* (Akinyemi *et al.*, 2005). This study offers scientific rationale for traditional use of these three medicinal plants particularly ethanol extracts, for the treatment bacterial gastroenteritis. The traditional preference of ethanol extracts could be due to two reasons. Firstly, the bioactive constituents such as saponins, tannins, alkaloids and anthraquinones in these plant extracts may be enhanced in the presence of ethanol. Secondly, the stronger extraction capacity of ethanol may be responsible, such that more active ingredients may be present in the ethanol extracts. The results of phytochemical analysis indicated that all the plant extracts contained tannins,

Afr. J. Trad. CAM (2006) 3 (4): 13 - 22

saponins, alkanoids flavonoids and antraquinones in variable degrees. None of the extracts contained cardiac glycosides, and cyanogenic glycosides, only *P. discoideus* contained reducing and non-reducing carbohydrates. It had long been documented that saponins, tannins and alkaloids are plants metabolites known for antimicrobial activity (Tschesche,1970).Some of the detected compounds in these plants extracts may be responsible for the antibacterial activity observed and thus justifying their traditional use as medicinal plants for the treatment of bacterial gastroenteritis. It is essential to carry out the bioautography of the extracts in order to determine the exact antibacterial compound(s) , unfortunately we were unable to perform this due to limited facilities.

Conclusion

The activities of both water and ethanol extracts of *T. guineensis*, *A. wilkesiana* and *P. discoideus* on food borne bacterial pathogens as revealed in this study support the local uses of these plants in traditional therapy for gastroenteritis.

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Afr. J. Trad. CAM (2006) 3 (4): 13 - 22

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