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Antibacterial Activities of Aqueous Extracts of *Terminalia catappa*, *Momordica charantia* and *Acalypha wilkesiana* on *Escherichia coli* Isolated from Pediatrics

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Abstract: Antibacterial activity of aqueous extract of *Terminalia catappa, Momordica charantia* and *Acalypha wilkesiana* was investigated against *Escherichia coli* isolated from pediatrics with the minimum inhibitory concentration (MIC) of 0.5mg/mL by agar dilution technique. The antibacterial potency of the extracts as evaluated by broth dilution technique, showed diameter of inhibition zone of 22.80 mm, 14.20 mm and 21.00 mm at a concentration of 0.5 mg/mL for *T. catappa, M. charantia* and *A. wilkesiana,* respectively. The antibacterial effect of *T. catappa* was found to be more pronounced with its plausible use for the treatment of infections caused by *E. coli.*

Keywords: *Acalypha wilkesiana, Escherichia coli, Momordica charantia, Terminalia catappa,* pediatrics.

Introduction

The use of plants for therapeutic purposes in Yoruba land in Nigeria dates back to centuries where they first applied the use of plant parts in the cure of different ailments (Sofowora, 1993). Presently, use of modern medicines as antimicrobial agents led to the loss of eminence in the use of perceived healing plants of traditional use which still remains dominant in health care of developing countries especially in rural areas. In Nigerian ethno-medicine extract of different parts of one plant such as stems, leaves, barks and roots are still used for the treatment of a variety of diseases.

The healthcare delivery of the larger proportion of the rural communities in Nigeria, and most part of Africa, today hinge to a large extent on medicinal plants based on traditional health care delivery system and there is a need to identify natural products that could give potent therapy at low or no cost at all. Even today, as many as 80% of the world's population depend on traditional medicines for their primary health care needs (WHO, 2002). The role of plants in health care delivery is even more prominent among rural parts of Nigeria (Osho *et al.,* 2007), and with the relevance of plants in health care of humans, various government and nongovernmental organisations are supporting the development of traditional medicines (Briskin, 2000).

Infectious diseases are one of major health problems in Nigeria, which includes common infectious diseases such as diarrhoea caused by *Escherichia coli.* Limited access to modern drugs has driven the rural Nigerian to rely on medicinal plants including the uses of *Terminalia catappa, Acalypha wilkesiana* and *Momordica charantia.*

T. catappa is commonly called tropical almond in Nigeria (Christian and Ukhun, 2006), and leaves, bark and fruit has been traditionally prepared to treat dysentery, rheumatism, cough and asthma. The fruit is also helpful in the treatment of leprosy and headache and the leaves are specifically used in getting rid of intestinal parasites, treatment of eye problems, wounds, and liver problems, and also for treatment of antifungal infections (Irobi and Adedayo, 1999).

A. wilkesiana is locally named as copper leaf or firedragon, and its ointment is used to treat fungal skin diseases. A previous study revealed that this ointment successfully controlled the mycoses in 73.3% of 32 affected patients (Oyelami *et al*., 2003). It was very effective in treating *Pityriasis versicolor, Tinea pedia* and *Candida intetrigo,* with 100% cure and useful in superficial mycoses (Akinyemi *et al*., 2005).

M. charantia (locally named as bitter melon or ejirin) has been used for a variety of ailments in Nigeria, particularly stomach complaints. Bitter melon (*M. charantia*) is generally, consumed either cooked in the green or early yellowish stage. The young shoots and leaves of the bitter melon may also be eaten as greens (Sofowora, 1993). *M. charantia* seeds possess antimicrobial activity (Braca *et al.,* 2008), antispermatogenic

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activity and androgenic activity (Naseem *et al.,* 1998). They are also used in reproductive health as an abortifacient, birth control agent or to treat painful menstruation and to facilitate child birth (Belion *et al.,* 2005).

E. coli is commonly present in the gastro-intestinal flora of most vertebrates, including humans, and mostly nonpathogenic. Most *E. coli* strains fall into 4 main phylogenetic groups, designated A, B_1, B_2 and D (Arpin *et al*., 2007) with extra intestinal infections derived predominantly from group B_2 and, to a lesser extent, group D whereas, group A and B_1 strains are largely devoid of virulence determinants (Johnson *et al*., 2009). Although strains harboring a robust extra-intestinal virulence factors repertoire cluster predominantly in groups B_2 and D, isolates within each phylogenetic group can be further classified as extra-intestinal pathogenic *E. coli* (EXPEC) or non-EXPEC depending on whether specific virulence traits are present (Johnson *et al*., 2009; Calbo *et al*., 2005).

The aim of this study is to determine the antibacterial potency of aqueous extracts of *A. wilkesiana*, *M. charantia* and *T. catappa* against *E. coli*.

Materials and Methods

Collection, identification and processing of plants. Young leaves of *T. catappa, A. wilkesiana* and *M. charantia* were collected from farmlands at Ado Ekiti, Nigeria. The plant samples were identified at the Department of Science Technology, Federal Polytechnic Ado Ekiti, Nigeria and a voucher specimen was kept in the laboratory No: Med Plant 2011/098. The method described by Osho *et al.* (2007) for extraction of plants active components was used. Samples were air-dried at room temperature of (26 °C ± 1 °C) and milled using a Thomas Willey milling machine. 100 g of the milled samples was soaked with 200 mL of distilled water. The aqueous extract was filtered and evaporated to dryness at 20 °C using a rotary evaporator.

Isolation and identification of *E. coli.* Strains of *E. coli* were isolated from stool samples of pediatrics between 9 months and 2 years of age that were referred to the laboratory of the University Teaching Hospital, Ado- Ekiti, Nigeria. The bacteria were identified using conventional methods and were maintained on nutrient agar slants at 4 °C in the refrigerator until required.

Extraction of bioactive components from the plant materials. Extraction method described by Ajibade and Famurewa (2011) was employed. Fifty grams (50 g) of the powdered plant materials *(T. catappa, A. wilkesiana* and *M. charantia)* were poured into different beakers and 500 mL of distilled water was poured into each beaker, respectively and were boiled on electric cooker at 100 °C. The contents were stirred using a sterile glass rod and allowed to stand for 72 h at room temperature (25 °C ± 1). The contents were filtered through a filter paper (Whatman No. 1) and the filtrate concentrated and evaporated using water-bath at the temperature of +95 °C. Extracts were then kept at 20 °C prior use.

Reactivation of organism. The bacterium was re-suspended in 20 test tubes containing nutrient broth and these test tubes were incubated at 37 °C for 18 - 20 h.

Determination of minimum inhibitory concentration (MIC). This was carried out using the agar dilution method previously described by Odelola and Okorosobo (1996). A colony from stock was sub-cultured into 5 mL of nutrient broth and incubated at 37 °C for 18 h. 0.1mL of the overnight broth of each organism was pipetted into 9.9 mL of the broth to yield a 10^1 dilution. The procedure was continued to obtain a final dilution of 10^3 (Smith *et al*., 2000). Streak of bacterial strains A (2 cm) were made on an oven-dried nutrient agar plates containing increasing concentrations (0.5–2.5 mg/mL) of the extracts. The lowest concentration that gave no visible growth after overnight incubation at 37 °C was taken as the minimum inhibitory concentration (MIC) of each extract.

Determination of the degree of antibacterial potency. The disk diffusion method described by Brady and Katz (1990) was employed. Various concentrations of the extracts were prepared in test tubes (2.5 mg/mL – 0.5mg/mL). Disks obtained from Whatman No. 1 filter paper were sterilised in an oven at 160 °C for 30 min. and soaked in the extracts for 24 h. A loopful of the final dilution (10^3) of the test bacterial suspension was spread on an oven-dried nutrient agar. The disks of different concentrations of the extracts were placed at equidistance on the agar and incubated at 37 °C for 24 h. Zones of inhibition were measured in millimeters (mm) with a meter rule. Whatman No. 1 filter paper disks were placed at the centre of each agar plate as a control.

Phytochemical analysis. *Determination of saponins*. Separately, plant extract (0.5 g) was shaken with distilled water (10 mL) in a test tube and frothing which persisted on warming was taken as evidence for the presence of saponins.

Determination of tannins. Plant extract (5 g) was stirred with 100 mL of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black green precipitate indicated the presence of tannins.

Determination of alkaloids. Plant extract (0.5 g) was diluted with acid alcohol (10 mL), boiled and filtered. Diluted ammonia was added (2 mL) to the filtrate (5 mL). Five milliliter of chloroform (5 mL) was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with acetic acid (10 mL). This was divided into two portions. Meryer's reagent (5 mL) was added to one portion and Draggendorff's reagent (5 mL) to the other. The formation of a cream (with Meryer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was taken as positive for the presence of alkaloid.

Determination of steroids. Plant extract (0.1 g) was dissolved in chloroform (2 mL) and sulphuric acid (2 mL) was carefully added to form a lower layer. A reddish brown color at the interphase was indicative of the presence of steroidal ring.

Determination of flavonoids. Powdered sample (2 g) was mixed with acetone (50 mL). The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the sample mixed with acetone. The mixture was filtered while hot. The filtrate was cooled and sodium hydroxide (20% 5 mL) was added to equal volume of the filtrate. A yellow solution indicated the presence of flavonoids.

Determination of terpenoids. This was done using Sakowski test as described by Sofowora (1993). Extract (5 mL) was mixed with chloroform (2 mL) and concentrated H_2SO_4 (3 mL) was added to form a layer. 2:4 formation of a reddish brown colouration at the interphase indicated the presence of terpenoids.

Statistical Analysis. Statistical analysis of P-value was calculated by using Fisher exact test; a test of comparison of 0.50 – 2.50 mg/mL between plant extracts (*T. catappa* and *A. wilkesiana*) was done. Variables with ≥ 1.0 diameter of zones of inhibition and a P-value of ≤ 0.10 in univariate analysis were subsequently analysed in a multivariate model.

Results and Discussion

The susceptibility of different concentrations of the extracts on test microorganism is shown in Table 1. The isolates were susceptible to *T. catappa, M.charantia* and *A. wilkesiana* with *T. catappa* showing the highest potency ranging from the diameter of inhibition of 22.80 ± 0.13 – 27.70 ± 0.20 mm at concentration ranging from 0.5 – 2.50 mg/mL, respectively. Susceptibility was not highly pronounced with *M. charantia* as seen in *T. catappa* and *A. wilkesiana* except at a higher concentration of 2.50 mg/mL. The result showed that *T. catappa* and *A. wilkesiana* have more efficacies in the treatment of both diarrhoea from the diameter of zone of inhibition observed against *E. coli.* This is an indication that their extract could be useful in the therapy of diarrhoea. The potency showed by *T. catappa* is highly significant P ≥ 1.80, 1.20 and 1.30 at the concentration of 0.50 mg/mL, 1.00 mg/mL and 2.00 mg/mL, respectively than other extracts. These showed the relevance of the plant extract (*T. catappa*) compared to other plant extracts. The P-values of only the plant extracts with the highest zones of inhibition were compared, that is, *T. catappa* and *A. wilkesiana*. These plants extracts were more potent against the test organism (*E. coli*).

The qualitative chemical analysis (Table 2) showed that *T. catappa* and *A. wilkesiana* contain saponin and flavonoid while *M. charantia* and *A. wilkesiana*, steroid is also present in *M. charantia*. However, all the plant extracts contained flavonoid. The quantity of the phytochemicals e.g., saponin was higher in *A. wilkesiana* (12.85%); tannin (7.14%) and flavonoid (10.6%) (Table 3).

Minimum inhibitory concentration (MIC) of the extracts on *E. coli* has been presented in Table 4. *T. catappa* having the MIC of 22.80 ± 0.13 mg/mL at the concentration of 0.50 mg/mL; *M. charantia* having the lowest MIC of 16.00 ± 0.92 mg/mL at the concentration of 1.00 mg/mL; and *A. wilkesiana* having the MIC of 21.00 ± 0.01 mg/mL at the concentration of 0.50 g/mL.

The bioactive compounds responsible for the inhibitory effects of the leaf extracts were detected in its phytochemical screening, some of which were reported

Table 1: Antibacterial activity of aqueous extracts of *T. catappa, M. charantia* and *A. wilkesiana* on *E. coli.*

Conc. (mg/mL)	Plant extracts Zones of inhibition (mm)			
	T. catappa	*M. charantia*	*A. wilkesiana*	P-value
0.50	22.80 ± 0.13	14.20 ± 0.58	21.00 ± 0.01	1.80
1.00	24.30 ± 0.30	16.00 ± 0.92	23.10 ± 0.09	1.20
1.50	26.50 ± 0.58	18.10 ± 0.29	25.40 ± 0.40	1.10
2.00	27.30 ± 0.08	19.80 ± 0.14	26.00 ± 0.50	1.30
2.50	27.70 ± 0.20	21.50 ± 0.26	27.60 ± 0.18	0.10

Table 2. Phytochemical (qualitative) analysis of aqueous extracts of *T. catappa, M. charantia* and *A. wilkesiana.*

Bioactive constituent	Plant extracts		
	T. catappa	*M. charantia*	*A. wilkesiana*
Saponin	+	–	+
Tannin	–	+	+
Alkaloid	–	–	+
Steroid	–	+	–
Flavonoid	+	+	+
Terpenoid	–	–	–

+ = present; – = not present

Table 3. Phytochemical (quantitative) analysis of aqueous extracts of *T. catappa, M. charantia* and *A. wilkesiana.*

Bioactive constituent	Plant extracts % composition		
	T. catappa	*M. charantia*	*A. wilkesiana*
Saponin	2.24	–	12.85
Tannin	–	5.6	7.14
Alkaloid	–	–	0.36
Steroid	–	0.47	–
Flavonoid	9.32	7.15	10.6
Terpenoid	–	–	–

– = not present

Table 4. Minimum inhibitory concentration (MIC) of the extracts on *E. coli.*

Plant extracts	Minimum inhibitory concentration (MIC) (mg/mL)
Terminalia catappa	22.80 ± 0.13
Momordica charantia	16.00 ± 0.92
Acalypha wilkesiana	21.00 ± 0.01

in literature as antimicrobial constituents (Oluduro *et al*., 2011). The qualitative and quantitative analysis of the leaves of *T. catappa*, *M. charantia* and *A. wilkesiana* revealed that they contain flavonoid, saponin and tannin in varying proportions with traces of steroid and alkaloid, while terpenoid was absent. The antimicrobial activities observed in this study may be attributed to the presence of these phytochemicals in the leaves (Table 3). Plants such as *Phyllanthus niruri*, *Acalypha hispida*, and *Mormodica charantia* that are rich in a wide variety of secondary metabolites have been found *in vitro* to have antimicrobial properties (Ajibade and Famurewa, 2011; Oluduro *et al*., 2011).

Herbal medicines in developing countries are commonly used for the traditional treatment of health problems (Martinez *et al*., 1996). In recent years multiple drug resistance in human pathogenic microorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Service, 1995). In addition to this problem, antibiotics are sometimes associated with adverse effects on host including hypersensitivity, immune suppression and allergic reactions (Ahmad *et al*., 1998).

Evaluation of these plants in rural areas of Nigeria is more urgent than ever. Thus, ethnobotanical studies of Africa could provide inputs with the isolation of new phytochemicals and their pharmacological studies. Therefore, scientific documentations of plants with effective use against certain microorganism could lead to the sustainable cultivation of plant resources for the small-scale production of raw phytotherapeuticals. and new findings will help to develop alternative antimicrobial medicines for the treatment of infections using plants (Dulger and Gonuz, 2004).

Conclusion

The results of the present study signify the potentiality of *T. catappa* leaf as a source of therapeutic agent which is encouraging in the ongoing search for antimicrobial botanicals. Thus, there is a need for a continuous search for new effective and affordable antimicrobial drugs.

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