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Full Length Research Paper

Phytochemical screening and antimicrobial activity of three plants used in traditional medicine in Northern Nigeria

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***Trichilia roka* (Forsk) Choiv, *Tridax procumbens* Linn and *Maerua angolensis* DC are medicinal plants commonly used in traditional medicine to treat various diseases. The aims of this study were to screen the extracts of the leaves of the three medicinal plants for phytochemicals, and to determine the antimicrobial activity of the extracts against nine common strains of bacteria and one yeast strain. The extraction of the leaves was performed using methanol and petroleum ether. The phytochemical screening and the antimicrobial activity of the extracts were carried out using standard laboratory methods. The results of the phytochemical screening showed that the methanol extracts contained tannins, saponins, flavonoids, cardiac glycosides and alkaloids. The minimum inhibitory concentrations (MICs) of the methanol extracts of *M. angolensis* and *T. roka* ranged between 6.25 and 25 mg/ml for all the test microorganisms, while those of petroleum ether extracts of *M. angolensis* and *T. procumbens* obtained for *Bacillus subtilis* were 6.25 and 12.5 mg/ml, respectively. The petroleum ether extract of *T. roka* was active against *Streptococcus pyogenes*, *Escherichia coli* and *Neisseria gonorrhoeae,* and the MIC was between 6.25 and 12.5 mg/ml. The methanol extracts of the plants were demonstrated to possess greater inhibitory activity against the pathogenic microorganisms than the petroleum ether extracts. In conclusion, the results of the study support the use of the plants in traditional phytomedicine for the treatment of diseases caused by the microorganisms.**

Key words: *Trichilia roka, Tridax procumbens, Maerua angolensis,* secondary metabolites, antimicrobial activity.

INTRODUCTION

Medicinal plants are the most productive source of new compounds and drugs of natural origin. Most of the natural products isolated from medicinal plants are the secondary metabolites, which include alkaloids, tannins, flavonoids, steroids, terpenoids, phenylpropanoids (Harvey, 2008) and anthraquinones (Ayo, 2010). Some of the products have nutritive value (Prasad and Bisht, 2011), and antifungal and antibacterial activities. However, some possess cytotoxicity (Wang et al., 2009). *Trichilia roka* (Forsk) Choiv, (Family: Meliaceae), *Tridax procumbens* Linn (Family: Asteraceae) and *Maerua angolensis* DC (Family: Capparidaceae) are common medicinal plants found growing in the wild in Zaria, located in the Northern Guinea Savannah zone of Nigeria. Species of *Trichilia*, including *Trichilia connaroides,* have been shown to exert protective effects against hypercholesterolaemia-induced oxidative stress in Wistar rats (Prasanna and Purnima, 2011), and *in vitro* antiplasmodium activity (Kumar et al., 2011), while *Trichilia priedreana* leaves have been shown to possess antimicrobial activity (Arotupin et al., 2008). *T. roka* (Forsk) Choiv (syn - *Trichilia emetica* Vahl) is a tree 8 to 10 m high. It is widely distributed, especially in the semi-deciduous forest and Savannah zones of Tropical Africa

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(Ghazanfar, 1989; Mann et al., 2003). The decoction of *T. roka* is used for the treatment of many diseases. It is used as a purgative, anti-epileptic, antipyretic (Watt and Breyer-Brandwijk, 1962; Iwu,1993), and antimalarial agent (Traore-Keita et al., 2000), and in the treatment of hepatic disorders (Germano et al., 2001, 2005). It also possesses antioxidant (Lindsey et al., 2002; Germano et al., 2006), anticancer, antimutagenic (Verschaeve and van Staden, 2008), antidiabetic and hepatoprotective activities (Germano et al., 2001; Lindsey et al., 2002). *T. procumbens* Linn. (Asteraceae) is a common annual herb and hairy plant found in Asia, Australia, India and Nigeria. It is used traditionally in the treatment of jaundice (Wagh and Shinde, 2010), eye diseases and typhoid fever (Mann et al., 2003). It also possesses hypotensive (Salahdeen et al., 2004; Ikewuchi et al., 2010), antidiabetic, antioxidant (Bhagwat et al., 2008; Habila et al., 2010), hepatoprotective (Wagh and Shinde, 2010), insecticidal, parasiticidal (Wani et al., 2010), wound healing (Diwan et al., 1982; Wagh and Shinde, 2010) and immunomodulatory properties (Oladunmoye, 2006). Rabbits fed with concentrated diet supplemented with *T. procumbens* forage (containing 14% crude protein) *ad libitum* had high feed utilisation efficiency, optimum weight gain and least cost of production (Mmereole et al., 2011). *T. procumbens* is used extensively in Indian traditional medicine as an anticoagulant (Ali et al., 2001).

M. angolensis DC (Capparidaceae) is a medium to big self-planted tree of up to 10 m high, growing in bush and rocky areas. It is widespread in the Savannah zones of Tropical Africa (Burkill, 1997). The leaves of the plant are used as vegetables and they are rich in crude protein (Tairo et al., 2011). The anti-inflammatory (Adamu et al., 2007), anticonvulsant and antidiarrhoeal (Magaji et al., 2008) activities of the aqueous methanolic extract of the stem bark of the plant have been reported (Magaji et al., 2009). The leaves of the plant are used in the treatment of convulsion (Stafford et al., 2008), dysentery, epilepsy (Adamu et al., 2007), stomach ulcer, skin rashes (Burkill, 1997) and diabetes mellitus (Mohammed et al., 2007). There is paucity of information in the available literature on the phytochemical screening and antimicrobial activities of petroleum ether and methanol extracts of the leaves of the three plants, used for the treatment and prevention of various infectious diseases.

The aims of this study were to determine the phytochemical constituents and antimicrobial activity of *T. roka, T. procumbens* and *M. angolensis* leaf extracts. The screening of these plants was based on the claims of their effective use in traditional medicine for the treatment of many diseases in the zone. Preliminary phytochemical screening and the testing of antimicrobial activity of the extracts were carried out against nine bacterial strains and one yeast strain: *Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Bacillus subtilis, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Neisseria gonorrhoeae and Candida albicans.*

MATERIALS AND METHODS

Plants

The leaves of *T. roka, T. procumbens* and *M. angolensis* were collected in July, 2009 from Ja'ama village near the dam of the Ahmadu Bello University, Zaria (11° 04′N, 07° 42′E), Nigeria. The plants were confirmed and authenticated by a botanist, Mr U. S. Gallah at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The voucher specimen numbers for *T. roka, T. procumbens* and *M. angolensis,* deposited at the Herbarium are Nos. 7232, 260 and 900119, respectively. The leaves were air-dried, powdered and kept in polythene bags before use.

Extractions

The powdered leaves (250 g) of each plant were extracted exhaustively with 1 L of petroleum ether (60 to 80°C) and methanol, respectively using Soxhlet extractor. Each extract was concentrated *in vacuo* at 40°C using rotary evaporator.

Phytochemical screening

The phytochemical screening of the extracts of the leaves of *T. roka, T. procumbens and M. angolensis* was carried out using standard procedures (Sofowora, 1993; Evans, 1996).

Test microorganisms

Nine bacterial strains and one yeast strain were used in this study: *S. aureus* (ATCC 13704), *S. pyogenes* (Local strain), *C. ulcerans* (Local strain), *B. subtilis* (NCTC 8230), *E. coli* (NCTC 10418), *S. typhi* (ATCC 9184), *K. pneumoniae* (ATCC 10031), *P. aeruginosa* (NCTC 6750), *N. gonorrhoeae* (NCTC 10341) and *C. albicans (*ATCC 10231). The pathogenic microorganisms were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Antimicrobial screening test

The antimicrobial activity of the leaves of crude extracts was determined by paper disc diffusion method (Ericsson et al., 1960; Bauer et al., 1966). Briefly, 0.1 g of each extract was dissolved in 2 ml of dimethyl sulphoxide (DMSO) to obtain a concentration of 50 mg/ml. This concentration was used to determine the activities of the extract on the microorganisms. Nutrient agar was prepared, sterilised and used as the growth medium for the microorganisms. Twenty millilitres of the sterilised medium was poured into each sterilised Petri dish, covered and allowed to solidify. The Müeller-Hinton sensitivity agar (oxoid) plate was then seeded with the test microorganisms by the spread plate techniques, and left for about 30 min to dry. The sterilised paper discs of 6 mm diameter were soaked in the prepared solutions of the extracts and dried at 50°C. The dried paper discs were then planted on the nutrient agar seeded with the test microorganisms. Sparfloxacin and fluconazole, prepared using the same solvent as for the tested extracts, were used as positive standards at the concentration of 30 µg/ml each. The bacterial culture plates were incubated at 37°C for 24 h, while the fungal culture plates were incubated at 27°C for 48 h. Thereafter, all the plates were examined for zones of inhibition of

Table 1. Phytochemical constituents of extracts of the leaves of *T. roka, T. procumbens* and *M. angolensis*.

Secondary metabolites	*Trichilia roka*		*Tridax procumbens*		*Maerua angolensis*	
	TRPE	TRME	TPPE	TPME	MAPE	MAME
Carbohydrates	-	+	-	+	-	+
Reducing sugars	-	+	-	+	-	+
Alkaloids	+	+	-	+	+	+
Cardiac glycosides	+	+	+	+	+	+
Saponins	-	+	-	+	+	+
Flavonoids	+	+	+	+	-	+
Tannins	+	+	-	+	-	+

TRPE = petroleum ether extract of *Trichilia roka*, TRME = methanol extract of *Trichilia roka*, TPPE = petroleum ether extract of *Tridax procumbens*, TPME = methanol extract of *Tridax procumbens*, MAPE = petroleum ether extract of *Maerua angolensis*, MAME = methanol extract of *Maerua angolensis*, + = positive, - = negative.

growth. The diameters of zone of inhibition of growth were measured and recorded. A negative control experiment was also set up using pure DMSO for each of the test microorganisms.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was carried out on the leaf extracts that showed sensitivity against the growth of the test microorganisms using Kirby and Bauer diffusion method (Bauer et al., 1966). Briefly, the concentration of the extracts were prepared by dissolving 0.1 g of each extract in 2 ml DMSO to obtain a concentration of 50 mg/ml. From the stock solution of 50 mg/ml, concentrations of 25, 12.5, 6.25 and 3.125 mg/ml were equally prepared by serial dilution for each of the six extracts. Each standard control was dissolved in DMSO and diluted to obtain concentrations ranging from 1.875 to 30 µg/ml. The test microorganisms were then seeded on prepared blood agar plates, left to dry for 30 min. Filter paper discs (6 mm diameter) were cut and sterilised. The paper discs were soaked into different concentrations of the extracts and dried at 50°C. The dried discs were then planted on the medium, seeded with test microorganisms. The bacterial culture plates were incubated at 37°C for 24 h, while the fungal culture plates were incubated at 27°C for 48 h, after which all the plates were examined for zone of inhibition of growth. The plate with the lowest concentration of the plant extract that had inhibitory effect was taken as the MIC of that extract against such microorganism.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the petroleum ether and methanol leaf extracts of *T. roka*, *T. procumbens* and *M. angolensis* are shown in Table 1. The results showed that the plants contained carbohydrates, reducing sugars, alkaloids, cardiac glycosides, saponins, flavonoids and tannins. The antimicrobial activity exhibited by the extracts of the leaves of the three plants may be due to the presence of the secondary metabolites revealed in their phytochemical screening. The secondary metabolites possess pharmacological activities, responsible for the use of plants in traditional phytomedicine to treat diseases caused by pathogenic microorganisms (Akinjogunola et al., 2009; Wang et al., 2009; Olajuyigbe and Afolayan, 2012). The finding of this study agrees with the result obtained by Olajuyigbe and Afolayan (2012) that phenolic compounds, including the flavonoids, and alkaloids and tannins exhibit a wide range of antimicrobial, including antibacterial and antifungal, activities.

The results of antimicrobial activities carried out on the three medicinal plants are summarized in Tables 2 to 5. The antimicrobial activity, determined based on the inhibition zone, demonstrated that *S. typhi* had the highest inhibition zone of 27 mm, while most of the microorganisms had inhibition zones greater than 15 mm at the highest concentration of 50 mg/ml (Table 2). Petroleum ether extract of *T. roka* exhibited activity against three microorganisms, while those of *T. procumbens* and *M. angolensis* showed activity each against only one microorganism. Methanol extract of *T. roka* was active against eight microorganisms, while that of *T. procumbens* showed activity against nine microorganisms. Methanol extract of *M. angolensis* was active against all the test microorganisms (Table 2). The results showed that methanol extracts possessed more potent antimicrobial activity than petroleum ether extracts of the plants, and that methanol extract of *M. angolensis* is the most potential source of antimicrobial agents. In this study, the antimicrobial activities were compared with standard antimicrobial agents, sparfloxacin and fluconazole, which were used as positive controls. The diameters of zone of inhibition of the crude extracts ranged from 10 to 27 mm and were lower than those of the standard antibiotics, which ranged from 30 to 49 mm. The crude extracts were less potent than the standard antibiotics, to which the bacteria and fungi were highly susceptible at 30 µg/ml.

The results of the MIC of *T. roka*, *T. procumbens* and *M. angolensis* extracts are shown in Tables 3 to 5. The MIC of petroleum ether extract of *T. roka* was found to be 6.25 mg/ml for *S. pyogenes* and *E. coli*, but the methanol extract of *T. roka* had MIC of 6.25 mg/ml for *S. aureus, S. pyogenes, E. coli* and *S. typhi* (Table 3). Petroleum ether extract of *T. procumbens* was active against only *B.*

Table 2. Susceptibility patterns of the leaf extracts of *T. roka, T. procumbens and M. angolensis* to the test microorganisms.

Test microorganism	Diameter of zone of inhibition (mm)							
	TRPE	TRME	TPPE	TPME	MAPE	MAME	SFX	FNZ
S. aureus	R	22	R	16	R	20	45	R
S. pyogenes	14	20	R	14	R	19	40	R
C. albicans	R	19	R	10	R	17	R	37
P. aeruginosa	R	R	R	19	R	21	R	R
C. ulcerans	R	R	R	10	R	22	30	R
B. subtilis	R	21	10	12	11	20	49	R
E. coli	15	20	R	11	R	21	31	R
S. typhi	R	27	R	12	R	10	30	R
K. pneumoniae	R	19	R	12	R	10	42	R
N. gonorrhoeae	14	17	R	R	R	10	32	R

TRPE = petroleum ether extract of *Trichilia roka*, TRME = methanol extract of *Trichilia roka*, TPPE = petroleum ether extract of *Tridax procumbens*, TPME = methanol extract of *Tridax procumbens*, MAPE = petroleum ether extract of *Maerua angolensis*, MAME = methanol extract of *Maerua angolensis,* R = resistant to test microorganism, SFX = Sparfloxacin, FNZ = Fluconazole.

Table 3. Minimum inhibitory concentration of leaf extracts of *T. roka*.

Test microorganism	Minimum inhibitory concentration (mg/ml)									
	TRPE					TRME				
	3.125	6.25	12.5	25	50	3.125	6.25	12.5	25	50
S. aureus	ND	ND	ND	ND	ND	-	0+	+	+	+
S. pyogenes	-	0+	+	+	+	-	0+	+	+	+
C. albicans	ND	ND	ND	ND	ND	-	-	0+	+	+
P. aeruginosa	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C. ulcerans	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B. subtilis	ND	ND	ND	ND	ND	-	-	-	0+	+
E. coli	-	0+	+	+	+	-	0+	+	+	+
S. typhi	ND	ND	ND	ND	ND	-	0+	+	+	+
K. pneumoniae	ND	ND	ND	ND	ND	-	-	0+	+	+
N. gonorrhoeae	-	-	0+	+	+	-	-	0+	+	+

TRPE = petroleum ether extract of *Trichilia roka*, TRME - methanol extract of *Trichilia roka*. + = inhibition; 0+ = minimum inhibition; - = no inhibition; ND = not determined.

subtilis and the MIC was 12.5 mg/ml. The methanol extract of *T. procumbens* was active against all the test microorganisms, except *N. gonorrhoeae*. The MIC was 6.25 mg/ml for *S. aureus, S. pyogenes, C. albicans* and *P. aeruginosa*. The rest microorganisms had MIC of 12.5 mg/ml (Table 4). The MIC recorded for the petroleum ether extract of *M. angolensis* was 6.25 mg/ml against *B. subtilis*. For methanol extract of *M. angolensis*, the MIC of 6.25 mg/ml was obtained for all the pathogens; exceptions were *S. pyogenes* and *C. albicans* which had 12.5 mg/ml, while that of *B. subtilis* and *N. gonorrhoeae* was 25 mg/ml (Table 5).

The MICs recorded for the standard antibiotics, sparfloxacin and fluconazole are shown in Table 6. The microorganisms were highly susceptible to both the standard antibacterial agent, sparfloxacin (with MIC values of 3.5 to 7.5 µg/ml) and the standard antifungal agent, fluconazole (with MIC value of 15 µg/ml for *C. albicans*); thus, the extracts were less potent as compared to the antibiotics. The difference between the degrees of potency of the extracts and those of the antibiotics may be mainly due to the mixture of bioactive constituents contained in the unrefined extracts, unlike the standard antibiotics which contained the pure active compounds.

The results showed that the methanol extract exhibited considerable inhibitory activities against the test microorganisms as demonstrated by its highest diameters of zones of inhibition. The methanol extracts of the leaves of the three plants are potential sources of new antimicrobial agents for the treatment of diseases caused by the bacterial strains and *C. albicans.* The petroleum extracts did not show any antifungal activity. The results of the present study suggest that the

Table 4. Minimum inhibitory concentration of leaf extracts of *Tridax procumbens*.

Test microorganism	Minimum inhibitory concentration (mg/ml)									
	TPPE					TPME				
	3.125	6.25	12.5	25	50	3.125	6.25	12.5	25	50
S. aureus	ND	ND	ND	ND	ND	-	0+	+	+	+
S. pyogenes	ND	ND	ND	ND	ND	-	0+	+	+	+
C. albicans	ND	ND	ND	ND	ND	-	0+	+	+	+
P. aeruginosa	ND	ND	ND	ND	ND	-	0+	+	+	+
C. ulcerans	ND	ND	ND	ND	ND	-	-	0+	+	+
B. subtilis	-	-	0+	+	+	-	-	0+	+	+
E. coli	ND	ND	ND	ND	ND	-	-	0+	+	+
S. typhi	ND	ND	ND	ND	ND	-	-	0+	+	+
K. pneumoniae	ND	ND	ND	ND	ND	-	-	0+	+	+
N. gonorrhoeae	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

TPPE = petroleum ether extract of *Tridax procumbens*; TPME = methanol extract of *Tridax procumbens*; + = inhibition; 0+ = minimum inhibition; - = no inhibition; ND = not determined.

Table 5. Minimum inhibitory concentration of leaf extracts of *Maerua angolensis*.

Test microorganism	Minimum inhibitory concentration (mg/ml)									
	MAPE					MAME				
	3.125	6.25	12.5	25	50	3.125	6.25	12.5	25	50
S. aureus	ND	ND	ND	ND	ND	-	0+	+	+	+
S. pyogenes	ND	ND	ND	ND	ND	-	-	0+	+	+
C. albicans	ND	ND	ND	ND	ND	-	-	0+	+	+
P. aeruginosa	ND	ND	ND	ND	ND	-	0+	+	+	+
C. ulcerans	ND	ND	ND	ND	ND	-	0+	+	+	+
B. subtilis	-	0+	+	+	+	-	-	-	0+	+
E. coli	ND	ND	ND	ND	ND	-	0+	+	+	+
S. typhi	ND	ND	ND	ND	ND	-	0+	+	+	+
K. pneumoniae	ND	ND	ND	ND	ND	-	0+	+	+	+
N. gonorrhoeae	ND	ND	ND	ND	ND	-	-	-	0+	+

MAPE = petroleum ether extract of *Maerua angolensis*, MAME = methanol extract of *Maerua angolensis.* + = inhibition; 0+ = minimum inhibition; - = no inhibition; ND = not determined.

Table 6. Minimum inhibitory concentration of sparfloxacin and fluconazole.

Test microorganism	Minimum inhibitory concentration (µg/ml)									
	Sparfloxacin					Fluconazole				
	1.875	3.75	7.5	15	30	1.875	3.75	7.5	15	30
S. aureus	-	0+	+	+	+	ND	ND	ND	ND	ND
S. pyogenes	-	0+	+	+	+	ND	ND	ND	ND	ND
C. albicans	ND	ND	ND	ND	ND	-	-	-	0+	+
P. aeruginosa	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C. ulcerans	-	-	0+	+	+	ND	ND	ND	ND	ND
B. subtilis	-	0+	+	+	+	ND	ND	ND	ND	ND
E. coli	-	-	0+	+	+	ND	ND	ND	ND	ND
S. typhi	-	0+	+	+	+	ND	ND	ND	ND	ND
K. pneumoniae	-	-	-	-	-	ND	ND	ND	ND	ND
N. gonorrhoeae	-	-	0+	+	+	ND	ND	ND	ND	ND

+ = inhibition; 0+ = minimum inhibition; - = no inhibition; ND = not determined.

methanol, and not the petroleum ether, extracts are more of potential sources of antibacterial agents, rather than antifungal agents. Of the three plants investigated, methanol extract of *T. procumbens*, with the lowest MIC, is the most potential source of antifungal agents. This requires further study. Further investigation is also required on the bio-assay guided isolation and characterisation of the active components, contained in the methanol extracts of the leaves of the three plants. The result of the antimicrobial activity of the leaves of *T. roka* agrees with the previous finding on *T. emetica* (Germano et al., 2005; Geyid et al., 2005) and *T. priedreana* (Arotupin et al., 2008) that plants belonging to the genus *Trichilia* possess antimicrobial activity. The present results obtained for *T. procumbens* are also in agreement with the earlier report of Sharma and Kumar (2009) that the leaves of the plant exhibited antimicrobial activity against *C. albicans*.

Conclusions

The demonstration of the antimicrobial activity of the leaf extracts of the plants, *T. roka, T. procumbens* and *M. angolensis* against pathogenic microorganisms is evidence that the extracts are potential sources of antibiotics with a broad spectrum of activity. Results of this study validate the use of the plants in traditional phytomedicine to treat diseases caused by the pathogenic microorganisms.

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