

Antibacterial and antioxidant activities of methanolic leaf extract of *Maerua crassifolia*

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ABSTRACT

The objective of this study was to investigate the antibacterial and antioxidant activities of the methanol leaf extract of *Maerua crassifolia*, an important medicinal plant used in Nigeria. The antibacterial properties of the methanol extract were studied against clinically important bacteria viz; *Staphylococcus aureus*, *Shigella spp.*, *Salmonella typhi*, *Bacillus subtilis* and *Escherichia coli* by disc diffusion method. The free radical scavenging potential of the extract was assessed by measuring its capability for scavenging 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The methanol leaf extract of *Maerua crassifolia* at the concentration of 12.5-150 µg/ml, showed significant activity against all the tested organisms. The observed antioxidant activity of the leaf extract suggests that the extract is a potential source of natural antioxidant and could be useful in the treatment of diseases.

INTRODUCTION

The use of medicinal plants as traditional health remedies is the most popular for over 80% of the world population, and it has been reported to possess minimal side effect (Kumar *et al.*, 2012). The importance of medicinal plants remains even of greater relevance with the global shift to obtain drugs from plants sources, and as a result of which attention has been given to the medicinal value of herbal remedies for safety and efficacy (Abubakar *et al.*, 2009). These plants are used in herbal medicine in different countries, and are a source of numerous potent and effective drugs (Mahesh and Satish, 2008). There is an urgent need to investigate the medicinal plants used in traditional medicine with the aim of establishing their potential antimicrobial and antioxidant activities, and identifying the constituents responsible for these properties (Akuodor *et al.*, 2013; Gupta and Mazumder, 2007; Lata and Ahuja, 2003).

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Maerua crassifolia which belong to the family Capparaceae is mainly found in the Saharan Africa. In Nigeria, the plant is mainly found in Sokoto and some parts of Zamfara and Katsina States. The leaf of this plant has long being used for the treatment of malaria (Akuodor *et al.*, 2014), gastric acid, tooth ache and intestinal diseases (Rahman *et al.*, 2004; Idris-Usman *et al.*, 2010). However, there has not been any scientific information on antioxidant and antimicrobial activity of *Maerua crassifolia* leaf extract on some clinical isolates that can help ascertain some folkloric claims. This study is therefore, aimed at examining the antibacterial and antioxidant potentials of *Maerua crassifolia* leaf extract.

MATERIALS AND METHODS

Plant Collection and identification

The leaves of *M. crassifolia* were collected in March 2009 and were identified by Dr. (Mrs) Jemilat A. Ibrahim of the herbarium unit, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja with voucher specimen number NIPRD/H/6406 for future reference. The international plant number index of *M. crassifolia* is Fl. Aegypt.-Arab. P. Cxiii. 1775.

Plant extraction

500 g of pulverized coarse powdered leaves was macerated overnight in methanol (1.5 L). The mixture was filtered and dried on a water bath at reduced temperature, and was stored in refrigerator for subsequent use.

Phytochemical screening

The methanol leaf extract was tested to determine the secondary constituents using standard procedures (Mukherjee, 2006; Parekh *et al.*, 2006).

Test organisms: Disc diffusion test

The antibacterial activity was carried out by disc diffusion method as described by Mbaveng *et al.* (2008) with slight modifications. Fresh inoculum was prepared by suspending the colonies in physiological saline water (0.9% Normal saline). Using McFarland turbidity standards (0.5), the bacteria suspension were adjusted to 1×10^6 CFU/ml and were aseptically inoculated by swapping the surfaces of the Muller-Hinton agar plates. Whatman (No. 1) filter paper discs of 6 mm in diameter were made by punching the paper, and the blank discs were sterilized in the hot air oven at 120 °C for 90 min. The discs were however saturated with 12.5, 25, 50, 100 and 150 µg/ml of the extract solution. With the aid of a sterile forceps, the dry impregnated discs were carefully placed on the agar plates. The positive and negative control were incorporated in each plate and incubated at 4 °C for 2 h for the extract to diffuse into the media. The experiments were carried out in duplicate. Thereafter, they were incubated at 37 °C for 24 h. Antimicrobial activity of the extract was determined by measuring the size of the inhibition zone in mm.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined (Mothana *et al.*, 2008).

Determination of the Minimum Inhibitory Concentration

The method as described by Andrew (2001) was adopted to determine the Minimum Inhibitory Concentration (MIC). Six tubes were properly arranged in a rack and 0.5 ml of nutrient broth was put into each test tube. The extract (6.25, 12.5, 25, 50, 100 and 125 µg/ml) were differently prepared. 0.5 ml of the test organism was transferred into each of the test tube and incubated at 37°C for 24 h. The MIC was considered as the least concentration of the extract which completely inhibited the growth of the test organism.

Minimum Bactericidal Concentration (MIC and MBC)

The Minimum Bactericidal Concentration was carried out following National Committee for Clinical Laboratory Standard (1993) method. A loopful of the broth taken from the MIC tubes without growth and streaked on a nutrient agar. The plates were incubated at 37 °C for 24 h. The lowest concentration of the methanol leaf extract without visible growth after incubation was considered as Minimum Bactericidal Concentration.

Antioxidant activity: 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH scavenging activity of the extract was measured by the spectrophotometric method of Aweh *et al.*, (2010) with slight modifications. To a methanol solution of DPPH, 0.05 ml of the extract dissolved in ethanol was added at different concentrations (100-500 µg/ml) Control was prepared as above but without the sample extract and methanol was used for the baseline correction. The changes in the absorbance of the plant sample were measured at 517 nm and the percentage inhibition calculated by using the formula:

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Test} \times 100}{\text{Control}}$$

Statistical analysis

The mean of the reading measured for each zone in the sensitivity assay was taken as the zone of inhibition of the clinical isolates. One way ANOVA was used to compare mean and the differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemical studies

Phytochemical screening of methanol leaf extract of *Maerua crassifolia* revealed the presence of alkaloids, saponins, tannins, terpenoids, flavonoids, steroids phenol and cardiac glycosides. However, phlobatannins and anthraquinones were absent. These secondary metabolites have been reported to possess different biological activities (Panda and Kar, 2007).

Table 1: Antibacterial effect of the methanol leaf extract of *M. crassifolia*

Organisms	Extract (µg/ml) zone of inhibition (mm).				
	12.5	25	50	100	150
<i>S. aureus</i>	12.00±2.00	14.33±1.20	15.67±2.10	20.75±2.15	29.60±0.00
<i>S. typhi</i>	10.67±0.81	13.00±1.64	16.33±1.35	19.00±2.00	24.10±1.67
<i>B. subtilis</i>	9.33±1.04	12.00±2.11	14.55±2.11	18.67±1.33	22.00±1.11
<i>Shigella spp.</i>	14.60±2.10	16.30±2.04	20.49±2.70	22.80±3.40	27.50±2.05
<i>E. coli</i>	7.79±3.00	10.00±2.30	12.00±2.00	16.70±1.40	20.30±1.45

Values are expressed as mean ± S.E.M

Table 2: Antibacterial effects (MIC/MBC in µg/ml) of the methanolic leaf extract of *M. crassifolia*

Test organism	Methanolic leaf extract	
	MIC	MBC
<i>S. aureus</i>	10	20
<i>S. typhi</i>	7	14
<i>B. subtilis</i>	9	18
<i>Shigella spp.</i>	9	18
<i>E. coli</i>	12	24

Table 3: DPPH scavenging effect of methanol root extract of *M. crassifolia* at different concentrations (µg/ml) and IC₅₀ values.

Conc. µg/ml	MEMC	Standard (Ascorbic acid)
100	58.7±2.3	92.4±2.2
200	67.8±3.6	94.3±1.5
300	78.5±2.7	95.5±1.3
400	86.9±2.5	96.3±0.7
500	91.2±1.5	96.2±0.9
IC ₅₀	58.9±3.7	4.8±0.9

Results are expressed as mean ± SEM, n= 3 replicate.

M = methanol, E = extract, M = *Maerua*, C = *crassifolia*.

The preliminary phytochemical screening of the methanol leaf extract showed the presence of flavonoids, alkaloids, tannins, saponins. Plant secondary constituents show different biochemical and pharmacological actions in animals and bacteria when ingested (Trease and Evans, 1989). Alkaloids, tannins and flavonoids have shown to be responsible for the antibacterial and antioxidant activities of different medicinal plants (Gandhare *et al.*, 2010; Nwaogu *et al.*, 2008; Nweze *et al.*, 2004). Thus, the observed antibacterial and antioxidant effects of the leaf extract may be due to the presence of the secondary metabolites. Medicinal plants are essential source of potentially structures for the development of new agents. These observations have helped in identifying the active principle responsible for such activities in developing new drugs for therapeutic use in humans.

The demonstration of activity against test organisms indicates scientific reasons for the local use of this potential medicinally important plant in the treatment of different ailments. The fact that the leaf extract was active against the organisms tested (gram-negative and gram-positive) may suggest a broad spectrum of activity. The observed effect is very important because of the possibility of producing therapeutic agents that will be against drug-resistant organisms. The low minimum inhibitory concentration value observed for *Salmonella typhi*, is a good indication of high activity against this bacterium. This result is outstanding considering the fact that typhoid fever is on the increase, and also becoming recalcitrant to first-line antibiotics treatment of the organism in developing countries. Apart from antimicrobial properties, the plant extract is also exploited for therapeutic purpose to cure other diseases. The methanol leaf extract of *Maerua crassifolia* was found to exhibit Antimalarial, anti-diarrhoeal, analgesic, anti-inflammatory, antipyretic and gastrointestinal activities (Akuodor *et al.*, 2015; Akuodor *et al.*, 2014; Akuodor *et al.*, 2014; Idris Usman *et al.*, 2010). Free radicals have been implicated in a variety of diseases including bacterial infection and neurological diseases (Rafikali and Nair, 2001).

Although there are medications to manage free radical damage and to protect the body against oxidative stress, the drugs available and in use are known to have severe side effects. The need for natural antioxidants to replace the synthesized ones cannot be overemphasised. The leaf extract exhibited strong antioxidant activity. The addition of methanol leaf extract of *Maerua crassifolia* to the DPPH solution caused a rapid decrease in the optical density at 517 nm, indicating good scavenging activity of the extract. The extract exhibited substantial antioxidant effect in a dose dependent manner similar to ascorbic acid which was used as a control standard antioxidant. The findings of this study supports the traditional application of the leaf extract and suggests that it possess constituents with antimicrobial and antioxidant properties.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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