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Full Length Research Paper

In Vitro Antimicrobial Activity and Cytotoxicity of *Maerua oblongifolia*

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Abstract

The research was carried out to search new antimicrobial agents mainly from plant extracts with the goal to discover new chemical structures by investigate *in-vitro* antibacterial and antifungal activity of *Maerua oblongifolia*. Eighty percent Methanol and chloroform extracts of leaves and stems were screened for their antimicrobial activity against different pathogenic bacteria and fungi. These were *Staphylococcus aureus, Bacillus subtilus, Escherichia coli, Salmonella typhi*, and *Aspergillus niger* and *Candida albicans* using the cup plate agar diffusion method. All extracts exhibited inhibitory effects against most of the tested organisms with zones of inhibition ranging from 13-20 mm. The largest inhibition zones were obtained from methanol stem extract of *Maerua oblongifolia* against *Aspergillus niger* and chloroform stem extract against *Escherichia coli*. In vitro cytotoxicity assays were performed using MTT assay and used 3T3 NIH mouse embryo fibroblast cell line, and CC-1, a rat Wistar hepatocyte cell line. The results obtained indicate that this plant has nontoxic effect on 3T3 with less than 20% of growth inhibition and has nutritional value on CC-1. Therefore, this finding indicated that these extracts of such plant promising antimicrobial agents.

Keywords: Antimicrobial activity, Medicinal plants, *Maerua oblongifolia,* Cytotoxicity, Sudan.

INTRODUCTION

The researchers all over the world are working in drug discovery research from medicinal plants which are representing a rich source of antimicrobial agents. However, many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann *et al.,* 2008). Indeed, Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997).

Maerua oblongifolia (Forssk.) A. Rich. is one of the Sudanese medicinal plants named Surreih in Capparaceae family, has been traditionally used to cure various diseases. (Madhava Chetty *et al*., 2008). Ethanomedical survey reveals that Murva (*Maerua oblongifolia*) is used to cure various diseases such as fever, stomach ache, skin infections, urinary calculii, diabetes mellitus, epilepsy, pruritis, rigidity in lower limbs, and abdominal colic .Murva is an important controversial drug used in diseases like anaemia; fever;

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diabetes; stomach disorders; typhoid; urinary infection and cough (Alice and Asha, 2007). It has botanical description as, a low woody bushy under-shrub sometimes scandent to 2-3 meters high, with a thick root stock and thick leaves, and strongly scented flowers. The root of this plant, which tastes like coconut pulp, is edible and is eaten with sugar (Boulos, 1999). This plant is widely distributed in grassland with scattered trees (Acacia), deciduous bushland and semi-desert scrub in dry, stony and sandy places. Occurring in savanna woodland from Senegal to Nigeria and in Sudan to the Red Sea and Arabia. The plant survives annual burning by throwing up shoots from its thick rootstock (Boulos, 1999). Arulanandraj *et al.* (2011) were establishing the antifungal activity of aqueous and alcoholic extracts of *Maerua oblongifolia*. It was carried out by agar diffusion method. Serial dilution was done to find out Minimum inhibitory Concentration (MIC) and disc diffusion method to carry out the zone of inhibition (ZI). In Sudan there is great number of traditional medicinal plants uses daily by traditional healer, the biological and toxicity information for these plants are restricted. Therefore, for first time in Sudan this study aims to screen the antibacterial and antifungal activity of *Maerua oblongifolia* using two solvents, and also study cytotoxicity using 3T3 AND CC-1 cell lines and MTT assay.

METHODS

Collection and Identification of plant specimens

Plant specimens were collected from February to May 2012 from Al-Gazira state. Identification was done in Plant Taxonomy, Herbarium Curator, Medicinal and Aromatic Plants Research Institute, National Research Center, Khartoum, Sudan, and identified as *Maerua oblongifolia* (Forssk).A. Rich.

The leaves and stems were dried in the shade to prevent cells from sun light which destroy the cell for 1 week until a constant weight were obtained, and ground to powder using mortar and pestle.

Extracts Preparation

Samples were ground into fine powder then extraction was done using maceration procedures. In accordance with such method fifty gram of the powdered leaves were macerated successively in chloroform and 80% methanol and kept for 5 days at room temperature with occasional shaking. Each mixture was then filtered and the filtrate was evaporated to dryness in an evaporating dish on a steam bath at a temperature of 70ºC. The process was repeated four times with intervals of 5 days (Hagerman, 1987). These extracts were stored in screw-capped bottles and kept in the laboratory refrigerator.

Antimicrobial activity of plant extracts

A test stock concentration of 10mg/ml for methanol/H_2O (80:20) extracts were prepared by dissolving 0.1g of each extract in 10 mls of methanol in separate test tubes and chloroform extracts were dissolved in petroleum ether: methanol (1:2).The antimicrobial activities of each of the methanol/H_2O (80:20) and chloroform were tested against standard Gram positive bacteria (*Staphylococcus aureus* American Type Culture Collection ATCC 25923 and *Bacillus subtilus* National Culture Type Collection NCTC 8236), Gram negatie bacteria (*Escherichia coli* ATCC 25922, and *Salmonella typhi* NCTC 0650) and fungi (*Aspergillus niger* ATCC 9763 and *Candida albicans* ATCC 7596) using agar well diffusion method (NCCLS, 2000) and the resultant inhibition zones were measured and tabulated as means. The zones were measured with a transparent ruler and the result recorded in millimeters. The screening was done in triplicates. Negative controls involving the addition methanol instead of the extracts were included.

Minimum inhibitory concentration (MIC)

MICs were carried out according to the method described by Hirasawa *et al.* (1999). Different concentrations (2.5, 5, 10 and 20 mg\mL) were prepared using sterile distilled water as the diluents. Again, the agar well diffusion method was used. The test was carried out in duplicate and the mean recorded.

Cytotoxicity assays using 3T3 and CC-1 cell-lines by MTT assay

The antiproliferative activity of plant extracts was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Lau *et al.,* 2004). The assay detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product, which reflects the normal function of mitochondria and cell viability.

In vitro cytotoxicity assays were performed as described by (Lau *et al.,* 2004), using the 3T3 NIH mouse embryo fibroblast cell line and CC-1, a rat Wistar hepatocyte cell line, from European Collection of Cell Cultures, (Salisbury, UK).

The CC-1 cells were cultured in Minimum Essential Media (MEM) supplemented with 10% FBS, 2 mM glutamine, and 20 mM HEPES. The 3T3 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) formulated with 10% FBS. All of these cells are adherent cells and required to be detached from culture flask surfaces by trypsin/EDTA treatment. The media were removed from the cell culture and sterile Phosphate buffer saline (PBS) was added to each flask to wash cells from cell debris. To each flask, 0.25%

Trypsin/EDTA solution was added to the attached cells and incubated for 2-3 min at 37 °C. The flasks were gently tapped and observed under microscope to check for detachment of cells from flask surfaces followed by addition of media containing 10% FBS. Cells were collected in a 15 mL centrifuge tube and centrifuged at 1200 rpm. The pellet was resuspended in a complete media and cells were enumerated using microscope and Neubauer counting chamber.

The MTT assays on the 3T3 and CC-1 cells were performed using 6 $\times 10^3$ cell/well in a 100 µL complete media in a flat-bottomed 96 wells plate. All plates were incubated for 24 hrs at 37 °C in a CO_2 incubator. After attachment of cells, media was replaced by 200 µL of media containing the test extracts at variable concentrations (100, 50, 25 and 12.5 µg/mL) in triplicates and further incubated for 48 hrs at 37 °C in a CO_2 incubator. Following exposure to each test extracts, cell viability was assessed by using 0.5 mg/mL of MTT in complete media for 4 hrs followed by the removal of supernatant and addition of 100 µL of Dimethylsulfoxide (DMSO) to each well to solubilize the formazan complex formed by the action of mitochondrial dehydrogenases. Untreated cells were used as a negative control while, cells treated with Triton were used as a positive control at the following concentrations 0.01 µg/mL. The plates were read at 540 nm after one minute of gentle shaking. The optical density readings were recorded using MS Excel software and the percentage of antiproliferative and / or cytotoxic activity is calculated as (A-B)/A. x 100, where A and B are the OD_{540} of untreated and of treated cells, respectively. The results were expressed as means ± SD of triplicate readings.

RESULTS

The results showed that both solvents were good solvent for extracting antimicrobial substances from the tested plants (Table 1). This finding was based on the number of pathogenic microorganisms inhibited and the diameter of inhibitory zones produced.

It was also observed that, *Bacillus subtilus* and *Escherichia coli* were the most sensitive microorganism inhibited by all extracts followed by *Salmonella typhi* and *Staphylococcus aureus* were inhibited by chloroform extracts only. While *Candida albicans* inhibited by stem extracts and resisted to leave extracts. Futhermore, all of the stem chloroform extracts exhibited inhibitory activity against the entire tested organism with zones of inhibition ranging from 13-20 mm. Moreover, *Aspergillus niger* was superior and sensitive against all of the tested extracts with high inhibition zone of 19 mm. The largest inhibition zone (20 mm) obtained from stem chloroform extracts against *Escherichia coli*.

The Minimum Inhibitory Concentrations (MICs) of the most active extracts were determined against reference organisms (*Staphylococcus aureus, Bacillus subtilus, Escherichia coli, Salmonella typhi, Candida albicans* and *Aspergillus niger*) it was found that MICs a ranging between concentration 2.5-5 mg\mL.

In vitro cytotoxicity assays using the 3T3 NIH mouse embryo fibroblast cell line and CC-1, a rat Wistar hepatocyte cell line measured by MTT indicate that, all extracts of leaves and stems is non toxic for 3T3 And CC-1 cell line with IC_{50} > 100 µg/ml. The least a viability of CC-1 was 112.26%, whereas, the negative control was 100% and the positive control was 32.95%, while the least a viability of 3T3 was 80.6%. (Table 2, Figures 1, 2).

DISCUSSION

From the Table 1, showed that the extracts derived from chloroform extracts were more active than methanol extracts. This may indicate that the non polar active principles are responsible for the antimicrobial activity in *Maerua oblongifolia*. This result agrees with many previous researches that reported the bioactivity of non polar principles in plants like *Achillea santolina*, *Typhonium flagelliforme*, *Schisandra sphenanthera,* and *Scutellaria barbata* (Abu-Dahab and Afifi (2007), Huyke *et al.,* (2007) and Lai *et al.,* (2008)).

Babu and Subhasree, (2009) reported that, due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over the drugs. Medicinal plants produce slow recovery; the therapeutic use of medicinal plant is becoming popular because of their lesser side effects and low resistance in microorganisms (Seyyednejad and Motamedi, 2010).

The results observed in the In-vitro antifungal activity of *M. oblongifolia* were significant and these support the uses of it in African countries as antifungal in traditional medicine and agree with Arulanandraj *et al*., 2011, whom established the antifungal activity of aqueous and alcoholic extracts of *Maerua oblongifolia.* And investigate preliminary phytochemical constituents and showed the presence of triterpenoids and alkaloids could have responsible for the activity. Plants like *Curtisia dentate*, *Glaucium oxylobum* and *Epinetrum villosum* showed the presence of phytoconstituents such as triterpenoids and alkaloids might be responsible for antifungal activity (Shai *et al.,* 2008; Morteza-Semnani *et al.,* 2003).

The inhibitory activities exhibited by the extracts tend to agree with the reports of Jayaveera *et al.* (2010) and Elmahmood *et. al.* (2008) all of whom linked antimicrobial properties of plants to the presence of bioactive secondary metabolites.

Antimicrobial activities shown by plant extracts and some nutritional value in CC-1 normal cell line (Table 1, 2), is could have due to the presence of bioactive substances in these plants, the preliminary phytochemical constituents studies showed the

Table 1. Antimicrobial activity of *Maerua oblongifolia* against certain microorganisms*

Parts used	Solvents used	*Test organisms used MDIZ (MM) *					
		Bacteria				Fungi	
		S.a	***B.s***	***E.c***	***S.t***	***As.n***	***Ca.a***
Leaves	Chloroform	13	13	15	14	14	-
	Methanol 80%	-	15	15	-	15	-
Stems	Chloroform	13	14	20	15	12	16
	Methanol 80%	-	15	15	-	19	13

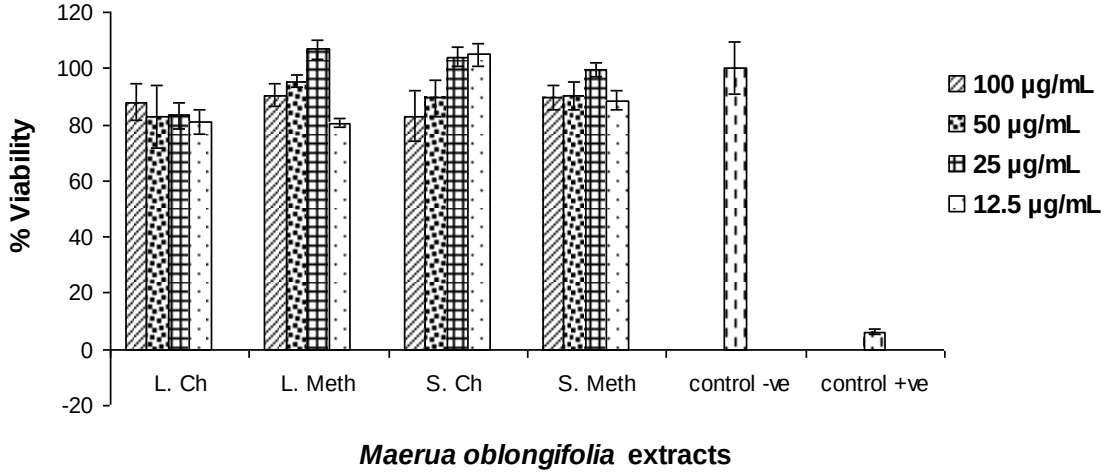
a.S*=*Staphylococcus aureus*, **B.s**= *Bacillus subtilus*, **E.c**=*Escherichia coli*, **S.ty**=*Salmonella typhi*, **Ca.a**= *Candida albicans* and **As.n**= *Aspergillus niger*.

**M.D.I.Z= Mean Diameter of Inhibition Zones (mm)
MDIZ >18 Sensitive; 14-18 Intermediate; <14 = Resistant (-) No activity.

Table 2. Cytotoxicity of *Maerua oblongifolia* extracts on normal cell lines as measured by the MTT assay.

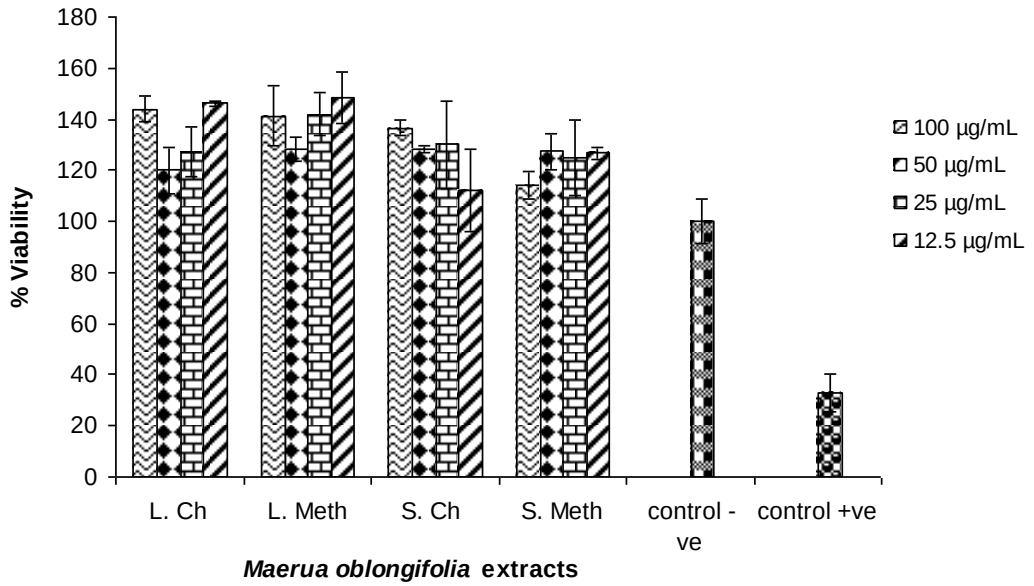
Parts and Solvent used	Conc. µg/mL	% Viability ± STD	
		3T3	CC-1
Leave Choloroform	100	88.0 ± 6.44	143.85 ± 4.94
	50	82.7 ± 11.08	120.07 ± 9.11
	25	83.3 ± 4.49	127.07 ± 9.72
	12.5	81.1 ± 4.47	146.11 ± 0.75
Leave Methanol	100	90.3 ± 4.01	141.16 ± 11.86
	50	95.5 ± 2.10	128.28 ± 4.79
	25	106.7 ± 3.30	141.77 ± 8.39
	12.5	80.6 ± 1.37	148.41 ± 9.96
Stem Chloroform	100	83.1 ± 9.26	136.60 ± 3.20
	50	89.4 ± 6.29	128.38 ± 1.49
	25	104.1 ± 3.43	130.21 ± 17.21
	12.5	105.0 ± 3.93	112.26 ± 16.31
Stem Methanol	100	89.4 ± 4.26	114.29 ± 5.16
	50	90.2 ± 4.78	127.37 ± 6.92
	25	99.5 ± 2.64	124.89 ±14.95
	12.5	88.5 ± 3.42	126.61 ± 2.13
Control – ve	-	100 ± 9.26	100 ± 8.50
Control +ve	0.01	6.27 ± 1.09	32.95 ± 7.17

Key: 3T3 = NIH mouse embryo fibroblast cell line, CC-1 = a rat Wistar hepatocyte cell line



Key: L = Leaves, S = Stems, Ch = Chloroform, Meth = Methanol

Figure 1. Cytotoxicity of *Mearua oblongifolia* extracts on 3T3 NIH mouse embryo fibroblast cell line measured by the MTT assay



Key: L = Leaves, S = Stems, Ch = Chloroform, Meth = Methanol

Figure 2. Cytotoxicity of Mearua oblongifolia extracts on CC-1 a rat Wistar hepatocyte cell line as measured by the MTT assay

presence of triterpenoids and alkaloids (Arulanandraj *et al.,* 2011). Since prehistoric times, people have used natural resources for medicinal purposes.

The result supports the traditional use of *Maerua oblongifolia* for the treatment of various infectious diseases in different regions of the Sudan. The study also showed that these plants may be good as an antibacterial and antifungal recipe and not toxic. More work is needed to isolate the bioactive components of these plants.

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