Phytochemical screening, Free Radical Scavenging, Antioxidant and Antimicrobial Activities of in Aqueous and Methanolic Extracts of Root of *Maerua Oblongifolia (Forsk)*

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Abstract : This study has been undertaken to investigate the determinants of *Maerua oblongifolia* is one of the botanical sources of the ayurvedic medicine. The root drug is used to treat diseases like reproductive disfunction, anemia, stomach and urinary disorders. In the present investigation of Free Radical Scavenging, Antioxidant and Antimicrobial Activities of In aqueous and Methanolic Extracts of *Maerua Oblongifolia.* The Preparation of aqueous and methnolic extracts of *M. oblongfolia* roots Acute toxicity studies the animals were divided into eight groups of six animals each. The first group served as control group. *M. oblongifolia* was administered orally to different groups at the dose levels of 100, 250, 500, 1000, 2000, 4000, 5000 mg/kg/ body weight. All the animals were observed for toxic symptoms and mortality for 72 h. Phytochemical screening of aqueous and methanolic extracts of *M.oblongigolia*, The *in vitro* free radical scavenging activity in aqueous and methonolic extract of *M.oblongifolia such as* DPPH Free Radical Scavenging Activity, Nitric Oxide scavenging activity (NO^-), Superoxide Anion Scavenging Activity and Assay of DNA Sugar Damage, The antioxidant studies TBA and FTC method, The Antimicrobial (Antibacterial and Antifungal) activity were studied. Preparation of aqueous and methnolic extracts of *M. oblongfolia* roots Acute toxicity studies the absence of any adverse effects and mortality on administration of acute dose of 5000 mg of the aqueous extract of *M. oblongifolia*/ kg body weight indicates the non-toxic nature of the plant extract. Toxicologists agree that any substance that is not lethal when administered acutely at a concentration of 5000mg/kg body weight is non-toxic. Therefore a concentration of 200mg/kg body weight was selected for this present investigation. The preliminary phytochemical analysis of aqueous and methanolic extracts of *Maerua oblongifolia* roots present in the more components. The primary investigations on in vitro free radical scavenging activity were increased in different concentration levels decreased when compared to the vitamin C. The antioxidant activities of *M.oblongifolia* the % of inhibition were decreased in Ist, IInd, IIIrd and IVth days when compared to the Vitamin E. The Antimicrobial activity of *M.oblongifolia* Ciprofloxacin 5µg/disc was used as the standard for gram positive and gram negative bacteria. Ketoconazole d 50µg/disc was used as a standard for fungus. The phyto-chemicals, Free Radical Scavenging, Antioxidant and Antimicrobial Activities of In present in *Maerua oblongifolia* may be responsible for the biological activity to enhance the overall reproductive potential and many more diseases toxicity treated adult rats.

IndexTerms - Maerua oblongifolia, Phytochemical, Freeradicals, Antioxidant, DPPH, Nitric Oxide, DNA Sugar Damage, Antimicrobial.

I. INTRODUCTION

India has a rich heritage of traditional system of medicine, which is proved to be safe and effective. Also, these systems of medicine have the possibility of providing health security to rural people in primary health care. The Phytochemicals isolated from medicinal plants can be used as agents or starting materials in the synthesis of drugs. The use of medicinal plants in the management of various illnesses is due to their phytochemical constituents and dates back to antiquity Yakubu *et al.,* 2007. The use of herbs is very common in developing countries, particularly in rural areas. However, during the last decade, an increase in the use of plants has been observed in developed countries Harnack *et al.,* 2001. Number of researchers has studied the protective effect of medicinal plants against various diseases. The World Health Organization (WHO) estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in ayurvedic, homeopathic, naturopathic and traditional medicine. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. In the last few decades there has been an exponential growth in the field of herbal medicine. Plants that were once considered of no value are now being investigated, evaluated and developed into drugs with little or no side effects Oluyemi Kayode., 2007.

Tropical and Subtropical countries have a number of medicinal plants, which have some potential effect against pathological conditions in reproductive disorder Prakash and Gupta, 2000. Due to their cost effectiveness it provides motivation to look for plant based new therapies Sivaloganathan *et al.,* 2004. Oxidative stress due to reactive oxygen species (ROS) can potentially damage cellular proteins, lipids and DNA. They have also been implicated in various diseases like atherosclerosis, cancer, arthritis, neurodegenerative diseases, pulmonary fibrosis, acute chronic inflammation and infertility. Many effective free radical quenching active principles have been reported from plant sources. The traditional systems of medicine have a vast repository of plant based therapies to combat many chronic degenerative diseases including reproductive disorders (Henkel., 2005) *M.oblongifolia* is a commonly used medicinal plant in Indian systems of medicine. In this investigation the antioxidant properties

of *M.oblongifolia* were studied against various types of free radicals. The induction of reversible male infertility in experimental animals and humans resulting from treatment with medicinal plants and their products had drawn the attention of researchers over the years Toyin, *et al.*, 2008.

Plant

Maerua oblongifolia is a shrub; it belongs to the family *Capparaceae* (caper family). It is widely distributed in India, Pakistan and Ceylon. In India, the plant is distributed in Punjab, upper gangetic plains, Madhya Pradesh, Rajasthan, Gujarat, Deccan and Carnatic districts from Godavari southwards. The part used viz: the earth sugar root of the Tamils has been known in South India for centuries. The plant is used as an alternative, tonic and stimulant. It is also used to treat rigidity in lower limbs, fever, poisoning, tubercular ulcer etc. Taxonomic classification Domain-Eukaryota, Kingdom-Plantae, Subkingdom-Viridaeplantae, Phylum-Tracheophyta, Subphylum-Spermatophytina, Infraphylum-Angiospermae, Class-Magnoliopsida, Subclass-Dilleniidae, Superorder-Violanae, Order-Capparales, Suborder-Capparineae, Family-Capparaceae, Genus-*Maerua*, Specific epithet-*oblongifolia*. Classical and common names English-Earth Sugar root, Hindi-Hemkand, Gujarati-Vika, Telugu-Morinika, Tamil-Bhumichakkarai, Synonyms-Maerua arenaria.

Morphological feautures

The plant is grows up to 3 m long, commonly found growing wild in scrubland. The bark is smooth and pale. Leaves are simple and quite entire. Elliptic –oblong, obtuse mucronate, glaucous, glabrous, petioles stout Flowers corymbose. Greenish white in colour, Sepals 4 lobed calyx, lobes valvate, Petals 4, smaller than the calyx lobes inserted on the edge of the cup shaped disc. Stamens many, inserted on the torus; Filaments free at the base. Ovary seated on the long gynophores, 1-2 celled, placenta 2-4, parietal or cohering in the middle, ovules many, stigma sub-sessile. Fruits are berry fleshy, elongate one or more seeded. Seeds large, cotyledons fleshy, convolute (Plate 1)

Plate 1 Morphological Characters of *Maerua Oblongifolia (Forsk)*

a) Plant b) Root

Phyto-chemical constituents and biological activites

The plant root possesses stimulant properties. Phytochemically the plant contains betaines and other quaternary ammonium compounds such as Prolinebetaine and 3-hydroxyprolinebetaine (William McLean *et al* 1996), lupane triterpenoids (Abdel Mogib, 1999).

Betaines(N, N, N-trimethylglycine) are an important class of naturally occurring quaternary amine compounds and it shows the characteristics of a dipolar zwitterion resulting in osmoprotective properties that function as compatible solutes or osmoprotectants (Wood *et al* 2002). Betaine helps to maintain normal cell volume (Olthof and Verhoef, 2005). Betaine can also be synthesized endogenously through oxidation of excess choline. Promoting effects on the intestinal tract against osmotic stress occurring during diarrhoea or coccidiosis have been reported following betaine supplementation in pigs and poultry. There is also some evidence that dietary betaine may improve the digestibility of specific nutrients. Betaine as a methyl donor provides its labile methyl groups for the synthesis of several metabolically active substances such as creatine and carnitine. They protect some the key regulatory enzymes in mitochondria such isocitrate dehydrogenase, malate dehydrogenase and fumarase (Nash *et al.*,2001*).* Betaine as an antioxidant, helps in the breakdown of fats by the liver, in other words, betaine is a lipotropic substance. It is also a component of lipoproteins and plays a role in the structural integrity of cell membranes. Betaine has been linked to a number of health benefits, particularly heart, liver and kidney health. It can lower blood levels of homocysteine, Therefore lower levels of homocysteine can reduce the risk of heart disease.

Lupane Triterpenoids have been tested for potential anti-inflammatory activity (Carolina *et al.*, 2006), potent anti-tumor initiator and antiviral activity (Yuko *et al.*, 2004). It is reported that *Mearua* species possesses antimicrobial activities. Extracts of this plant have been proved to be effective against Gram-positive bacteria (*Bacillus cereus*, *Streptococcus mutans*, *Lactobacillus acidophilus* and *Staphylococcus aureus*), two Gram-negative bacteria, (*Escherichia coli* and *Klebsiella pneumoniae*) and two yeasts (*Candida albicans* and *Cryptococcus neoformans*). Also it do possess Anti helminthic activity (against intestinal nematode *Heligmosomoides polygyrus***)** (Gakuya *et al*., 2005). Hence it is of interest to evaluate the Phytochemical, Free Radical Scavenging and Antioxidant Antimicrobial Activities of in Aqueous and Methanolic Extracts of *Maerua Oblongifolia* the main objectives of this study.

II. MATERIAL AND METHODS

The roots of *Maerua oblongifolia* was purchased from the local herbal drug supplier in Chennai, Tamilnadu, India in the month of August 2008. The plant roots were authenticated by the chief botanist Prof. S. Jayaraman, Plant Anatomy and Research centre (PARC), Chennai, India.

Preparation of aqueous and methnolic extracts of *M. oblongfolia* roots:

The aqueous extracts dried root plant material was coarsely powdered and 1kg was soaked in double distilled water for 12h at room temperature. Then it was filtered three times using whatman filter paper and the filtrate was poured on petridishes and concentrated at 60°C to obtain the semisolid residue. The weight was measured and the yield of the extract was calculated as 12.4% w/w. It was then stored in refrigerator for further studies. The methnolic extracts dried root plant material was coarsely powdered. 1kg was soaked in methanol for 12h and kept at room temperature. Then it was filtered and the process was repeated for three time and concentrated at 55° C to obtain a semi solid residue,. The weight was measured and the yield of the extract was calculated as 13.2% w/w. It was then stored for further studies.

Acute toxicity studies and dose fixation

Herbal medicines are gaining importance in primary health care because of their efficacy and lesser side effects it is an essential requirement to fulfill the guidelines formulated by the World Health organization (WHO report, 1991) to establish the safety of herbal preparations. In the present investigation the aqueous extract was subjected to the acute toxicity evaluation. The animals were divided into eight groups of six animals each. The first group served as control group. *M. oblongifolia* was administered orally to different groups at the dose levels of 100, 250, 500, 1000, 2000, 4000, 5000 mg/kg/ body weight. All the animals were observed for toxic symptoms and mortality for 72 h. The absence of any adverse effects and mortality on administration of acute dose of 5000 mg of the aqueous extract of *M. oblongifolia*/ kg body weight indicates the non-toxic nature of the plant extract. Toxicologists agree that any substance that is not lethal when administered acutely at a concentration of 5000mg/kg body weight is non-toxic (OECD 1981). Therefore a concentration of 200mg/kg body weight was selected for this present investigation.

Preliminary phytochemical screening

1 g of *M. oblongifolia* extract was dissolved in 100 ml of methanol. The filtrate was subjected to qualitative tests separately for identification of various phytochemical constituents present in the extracts (Wagner *et al.,* 1979;).

Test for alkaloids

50 mg of the aqueous extract of *M.oblongifolia* was dissolved in 10 ml of diluted HCl separately and filtered. The filtrate of *M.oblongifolia* was tested for the presence of alkaloids. The Mayer's test 1.358 g of mercuric chloride was dissolved in 60 ml of distilled water, and 5 g of potassium iodide was dissolved in 10 ml of distilled water, were mixed and made up to 100 ml with distilled water. 0.5 ml of Mayer's reagent (Potassium mercuric iodide) was added to the filtrate of *M.oblongifolia*. The presence of alkaloids was confirmed by the formation of cream precipitate. Dragendorff's test 5 ml of solution 0.85 g of basic bismuth nitrate was dissolved in 10 ml of acetic acid and 40 ml of distilled water and 5 ml of solution 8 g of potassium iodide was dissolved in 20 ml of distilled water were mixed with 20ml of acetic acid and made upto100 ml with distilled water before use. 0.5 ml of Dragendorff's reagent (Potassium bismuth iodide) was added to 1 ml of *M.oblongifolia* filtrate. The presence of alkaloids was confirmed by the formation of orange precipitate. Wagner's test 1.27 g of iodine and 2 g of potassium iodide were dissolved in 5 ml of water and made up to 100 ml with distilled water.0.5 ml of Wagner's reagent (aqueous solution of iodine and potassium iodide) was added to 1 ml of *M.oblongifolia* filtrate. The presence of alkaloids was confirmed by the formation of reddish brown precipitate. Hager's test 1 ml of filtrate of *M.oblongifolia*, 0.5 ml of saturated aqueous solution of picric acid was added. The presence of alkaloids was confirmed by the formation of yellow precipitate.

Test for glycosides

A portion of *M.oblongifolia* was hydrolyzed by boiling with diluted HCl in a water bath for 30 min, cooled and filtered. The hydrolysates were subjected to Legal's; Borntrager's and modified anthraquinone tests. The Legal's Test to each 1 ml of the hydrolysates of *M.oblongifolia*, 1 ml of pyridine and a few drops of sodium nitroprusside solution were added and then it was made alkaline with NaOH solution. Appearance of pink colour shows the presence of glycosides. Borntrager's test 1 ml each of hydrolysate of *M.oblongifolia* was shaken gently with equal volume of chloroform and then the chloroform layer was separated. To this, equal quantity of diluted ammonium solution was added. If ammonia layer acquires pink colour it shows the presence of O-anthraquinone glycosides. Modified anthraquinone test to 1ml of the hydrolysate of *M.oblongifolia*, equal volume of 5% $FeCl_3$ solution and diluted HCl were added and heated on boiling water bath for 5 min, cooled and shaken gently with benzene. Benzene layer was separated and equal volume of ammonia was added to it. Formation of pink colour indicates the presence of C-anthraquinone glycosides.

Test for phenol

To the *M.oblongifolia* extract (1ml), two drops of ferric chloride solution was added. Formation of blue colour indicates the presence of phenol.

Test for flavonoids

To the filtrate of *M.oblongifolia* extract (1ml), a pinch of magnesium and 2 drops of concentrated hydrochloric acid were added and gently heated. Formation of pink colour indicates the presence of flavonoids.

Test for triterpenoids

To 1 ml of *M.oblongifolia* extract, a pinch of tin and 2 drops of thionyl chloride were added. Formation of pink colour indicates the presence of triterpenoids.

Test for coumarins

To 1 ml filtrate of the *M.oblongifolia* extract (1ml), 1 ml of 2N NaOH was added. Formation of yellow colour indicates the presence of coumarins. Further 1 ml of 5N HCl was added. If colourless solution formed at the upper layer, it shows the presence of coumarins.

Test for saponins

Foam test the *M.oblongifolia* extract was diluted with 1 ml of water separately and shaken well. Stable froth formation indicates the presence of saponins.

Test for tannins

To the *M.oblongifolia* filtrate of extract (1 ml), 0.5 ml of 10% lead acetate was added. Appearance of white precipitate indicates the presence of tannins.

Test for Carbohydrates

To the filtrate of the *M.oblongifolia* extract (1 ml), 2 drops of concentrated H_2SO_4 was added slowly on the sides of the test tube and then 0.5 ml of α-naphthol solution was added. Formation of purple ring at the junction of two liquids shows the presence of carbohydrates.

Test for reducing sugars

To the aliquot of *M.oblongifolia* extract (1 ml), 2 ml of diluted HCl was added and boiled for 10 min and it was neutralized with diluted NaOH then cooled for 5 min. After cooling, equal volume of Fehling's A and B solutions were added and boiled in a water bath. Formation of reddish brown precipitate indicates the presence of reducing sugar.

Test for phytosterol

The *M.oblongifolia* extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted with distilled water and extracted with ether. The ether layer was evaporated and the residue was tested for phytosterols by Libermann Burchard test. The Libermann Burchard test the *M.oblongifolia* extract (10 mg) was dissolved in 3 ml of acetic anhydride. To this solution, two drops of concentrated sulphuric acid were added slowly along the sides of the test tube. Appearance of bluish green colour shows the presence of phytosterol. Salkowski test 1 ml each of *M.oblongifolia* filtrate was mixed with 1 ml of chloroform separately. To these solutions, 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of red colour indicates the presence of sterols.

Test for proteins

Biuret test to 1 ml of filtrate of *M.oblongifolia* extract, 1 ml of 5% copper sulphate and 1% NaOH solution were added. Deep blue colour indicates the presence of protein.

Test for amino acids

Ninhydrin solution 100 mg of ninhydrin was dissolved in 100 ml of n-butanol. To 1 ml aliquot of the *M.oblongifolia* extract, 2 ml of ninhydrin solution was added. Formation of violet colour indicates the presence of amino acids.

Test for gums and mucilage

Extract of *M.oblongifolia* was dissolved in water and filtered. To the filtrate a few drops of alcohol was added. The precipitate was examined for swelling property. If the precipitate swells, it indicates presence of gums and mucilage.

***In vitro* antioxidant studies**

DPPH Free Radical Scavenging Activity

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was assayed by the method of Ursini *et al.*, (1994). Various concentrations of *M.oblongifolia* (5, 10, 15, 20, 25 µg/ml) in methanol were prepared separately. Extract (1 ml) was mixed with equal volume of DPPH in methanol (0.1 mM) and shaken vigorously. The mixture was kept at room temperature for 50 min and the absorbance was measured at 517 nm. Ascorbic acid was used as standard. The percent free radical inhibition was calculated as IC_{50}. It denotes the concentration of the sample required to scavenge 50% DPPH free radical.

Nitric Oxide scavenging activity (NO^-)

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of *Griess Illosvoy* reaction (Garrat, 1964). In this experiment, *Griess Illosvoy* reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). Scavengers of nitric oxide compete with oxygen leading to decreased production of nitric oxide (Marcocci *et al.*, 1994). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and various concentrations of *M.oblongifolia* (50,100,150,200,250 µg/ml) in methanol (0.5 ml) were incubated at 25°C for two and a half hours. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for complete diazotization. 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine. The absorbance was read at 540 nm. The results were expressed as % of scavenging.

Superoxide Anion Scavenging Activity

Measurement of Superoxide anion scavenging activity of *M.oblongifolia* was based on the method described by Liu *et al.,* (1997). Superoxide anions were chemically generated in a mixture of Phenazine Methosulphate (PMS) and NADH. The reaction was quantified by coupling superoxide generation to the reduction of Nitroblue Tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3 ml of Tris–HCl buffer (16 mM, pH 8.0) containing 1 ml of NBT (50 µM) , 1 ml of NADH (78 µM) and 1ml of various concentrations of *M.oblongifolia* (100,200,300,400,500 µg/ml) were mixed. The reaction was started by adding 1 ml of PMS (10 µM) to the mixture. The reaction mixture was incubated at 25˚C for 5 min and the absorbance was measured at 560nm in a spectrophotometer. Results were expressed as % of inhibition.

Assay of DNA Sugar Damage

The protective role of *M.oblongifolia* on the inhibition of free radical mediated DNA-sugar damage was assayed by the method of Halliwell and Gutteridge (1981). The reaction mixture in a total volume of 1.24 ml contained 0.5 ml of calf thymus DNA (1 mg/ml of 0.15 M NaCl), 0.5 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of ascorbic acid (1 mM) and 0.04 ml of ferric chloride (100 µM). To the reaction mixture various concentrations of *M.oblongifolia* (5, 10,15,20,25 µg/ml) *was* added. The reaction mixture was incubated for 1 h at 37˚C in a water bath. After the incubation 1ml of 0.67% TBA was added to the reaction mixture and then it was kept in boiling water bath for 15 min. The TBA reacting species that are generated forms an addict and shows a characteristic absorption at 535 nm.

Antimicrobial Activity

In the present study, *in vitro* antibacterial and antifungal activities of *M. oblongifolia* were studied. The Antibacterial Activity aqueous and methanolic extracts of *M. oblongfolia* were subjected for antimicrobial activity by disc diffusion method against both gram-positive and gram-negative organisms, by the method of Gillespie (1994). Gram Positive Organisms *Staphylococcus epidermidis* (ATCC 155), *Staphylococcus aureus* (ATCC 9144), *Micrococcus luteus* (ATCC 4698)**,** *Bacillus cereus* (ATCC 11778), *Bacillus subtilis (ATCC11774)* and *Streptococci mutans (ATCC35668).* Gram Negative Organisms *Escherichia coli* (ATCC 25922) and *Klebsiella pneumonia* (ATCC 11298), *Pseudomonas aeruginosa* (ATCC 1688).

The antibacterial activity of Nutrient agar medium (Pharmacopoeia of India, 1985) 10g of Beef extract, 10g Peptone, 5g of Sodium chloride, 1.2% Agar dissolved in 1L of water. All the ingredients were dissolved in distilled water and gently heated. PH of the medium was adjusted to 8.0 to 8.4 with 5 M sodium hydroxide and boiled for 10 min then filtered. The filtrate was adjusted to pH 7.2 and sterilized by maintaining at 115 º C for 30 min. The sterilized (autoclaved at 120ºC for 30 min) medium (40ºC -50ºC) was inoculated with the suspension of the various microorganisms and poured into petridishes to give a depth of 3-4 mm. various (250, 500, 750 µg/ml) concentration of *M. oblongifolia* are prepared in methanol separately. Sterile discs (made from whatmann filter paper pre sterilized in UV lamp) dipped in specified concentration of the extracts and standard (Ciprofloxacin, 100 µg/ml). The impregnated discs are allowed to dry. The dried discs were placed on the surface of agar plates. A disc dipped in methanol and dried was used as control. The plates were left for 1 h at room temperature and incubated at 37º C for 18 h. The diameter of zone of inhibition of extracts and standard were measured.

Antifungal activity aqueous and methanolic extracts of *M. oblongifolia* was screened for antifungal activity by disc diffusion method against following organisms as described by Gillesbie (1994). *Aspergillus niger* (ATCC 9029), *Aspergillus fumigatus* (ATCC 46645), *Candida albicans* (ATCC 10231). Sabouraud dextrose agar medium was used for the study. Sabouraud dextrose agar media 10 g Mycological peptone and 40 g Dextrose**,** 15 g Agar dissolved in1L distilled water. Suspension of microorganisms were added to sterile sabouraud dextrose agar medium at 45°C and the mixture was transferred to sterile petridishes and allowed to solidify. Sterile discs dipped in various concentrations (50, 100, 250 µg/ml) of the aqueous extract of *M. oblongifolia* was placed on the surface of agar plates. A dried disc was used as a control. The plates were used for 1h at room temperature and incubated at 37°C for 18 hours. The diameter of zone of inhibition of extract was measured.

III. RESULTS AND DISCUSSION

In the present investigation, the preliminary phytochemical analysis the aqueous and methanolic extracts of *Maerua oblongifolia* roots were studied. Preliminary phytochemical analysis of the aqueous and methanolic extracts of *M. oblongifolia* roots Table 1. In the present investigation, preliminary phytochemical analysis of the roots of *M.oblongifolia* was performed on both aqueous and methanolic extracts. It revealed mainly the presence of alkaloids, glycosides, flavonoids, triterpenoids, tannins and phytosterols. The phenolic compounds present in the plant extracts are potential antioxidants. They play a significant role in scavenging the free radicals; also they function as chelating agents, and modifiers of various enzymatic and biologic reactions Lupane triterpenoids (Mogib, 1999). It is reported that flavonoids have many functions (Koshy and Vijayalakshmi.,2001). Betaines(N, N, N-trimethylglycine) are an important class of naturally occurring quaternary amine compounds and it shows the characteristics of a dipolar zwitterion resulting in osmoprotective properties that function as compatible solutes or osmoprotectants (Woollen *et al*., 1961). Betaine helps to maintain normal cell volume Tannins has well-described antimutagenic and antioxidant activities (Lopes., 1998). The steroidal constituents in the plant increase the steroidogenesis and elevate androgen levels which results in observed effect. (Khanduja., 1999). The phyto-chemicals present in *M.oblongifolia* may be responsible for the biological activity to enhance the overall reproductive potential.

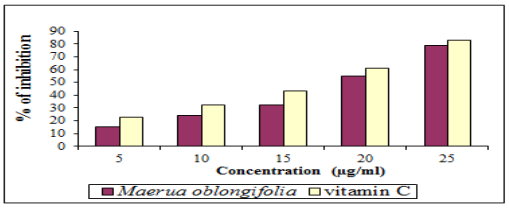
Table 1 Preliminary phytochemical analysis of the aqueous and methanolic extracts of *M. oblongifolia* roots

S.No	Tests	Aqueous	Methanol
1	Alkaloids	++	--
2	Glycosides	++	+++
3	Phenols	++	+++
4	Flavanoids	+++	+++
5	Triterpenoids	++	+++
6	Coumarins	--	--
7	Saponins	++	++
8	Tannis	++	+++
9	Phytosterols	---	++
10	Carbohydrades	+++	+++
11	Proteins	++	++
12	Amino acids	++	+++
13	Gums and mucilage	--	--
14	Volatile oil	---	--

In-Vitro* Antioxidant Studies on *Maerua Oblongifolia (Forsk)

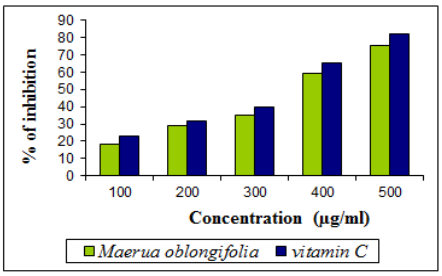
The primary investigation on *in vitro* free radical scavenging activity of aqueous extract of *M.oblongifolia* by positive DPPH test (Fig.1) shows the free radical scavenging at various concentrations from 5-25 µg/ml increased % of the inhibition. At all the concentrations it showed antioxidant capacity near to that of the standard Vitamin C. The DPPH is generally used as a substrate to evaluate the antioxidative activity of antioxidants (Banerjee *et al.*, 2005). The method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non - radical form, DPPH by the reaction. The extract of *M.oblongifolia* showed a concentration dependant antioxidant activity by reducing stable radical DPPH to the yellow coloured diphenyl picryl hydrazine derivative. Positive DPPH test suggests that the plant extract is a free radical scavenger (Sanocka., 2004). The aqueous extract of *M.oblongifolia* showed strong antioxidant activity compared with the standard Vitamin C. Depletion of DPPH by the extract establishes the free radical scavenging potential of *M.oblongifolia.*

FIG.1 Free radical Scavenging acitivity of *Maerua oblongifolia* by DPPH method



The Nitric oxide radicals by the aqueous extract of *M. oblongifolia (forsk)* Fig.2 plant extract is a free radical scavenging at various concentrations from 100-500 µg/ml increased the % of inhibition. The aqueous extract showed strong free radical inhibiting activity when compared with the standard, Vitamin C. Thus by depleting the DPPH and NO^{-}, it proves that the extract might be a potent peroxide inhibitor. The role of free radicals in infertility is well established. (Weinberg *et al.,* 1995). In a previous study of infertile cases, it was shown that increased lipid peroxidation was related to seminal plasma nitric oxide (NO) levels (Romeo., 2003). Therefore, increased lipid peroxidation causes cytotoxic effects, increased NO causes impaired sperm motility. It is well known that nitric oxide is an important second messenger in a number of physiological pathways, Studies reports that in the presence of oxidative stress, nitric oxide can be converted into reactive nitrogen species that contribute to cellular injury and death. (Knowles., 1994) For instance, in the presence of superoxide anion, nitric oxide can be transformed into peroxynitrite ($ONOO^{-}$), a strong oxidant and nitrating species (Lu and Wang, 1998). Proteins, lipids and DNA are all targets of peroxynitrite. Over production of nitric oxide is known to be an important mediator of inflammatory state (Bowen *et al.,* 2001) and cancer. In the present investigation, the aqueous extract of *M.oblongifolia* suppressed the nitric oxide formation comparable to that of the standard. The effectiveness of the plant may be due to its nitric oxide (free radicals) scavenging activity.

FIG.2 Nitric oxide scavenging activity o f *Maerua oblongifolia*



The free radical damage preventing activity of the aqueous extract of *M. oblongifolia (forsk)* against the super oxide radicals shows Fig. 3. The super oxide radicals scavenging activity at various concentrations from 100-500 µg/ml were increased the % of inhibition. The aqueous extract showed strong super oxide anion scavenging activity when compared with the standard Vitamin C. Several phytochemical compounds are able to efficiently scavenge superoxide radicals (Valentao *et al*., 2002). These compounds may react with the superoxide radical via one-electron transfer mechanism or by a hydrogen abstraction mechanism to form the corresponding semiquinone (Wang *et al*., 1996). The present study suggests *M.oblongifolia* has anion scavenging activity and this activity may be attributed to the presence of phytochemical compounds present in it. Superoxide is a well-known free radical of all oxygen derived species (Fridovich, 1978). Therefore it is the first intermediate in the sequential univalent reduction of oxygen that leads to formation of hydrogen peroxide (Harris, 1992). Superoxide radical is unique in that it can lead to the formation of many other reactive species, including hydroxyl free radical, hydrogen peroxide and perhydroxyl radicals (Pryor, 1984). It induces oxidative damage in lipids, proteins and DNA (Pietta, 2000). The most important source of superoxide is oxidative enzymes, among which xanthine oxidase and NADPH/ NADH oxidase are the most effective sources (Cross and Jones, 1991). The activity of SOD is likely to be a result of futile cycling of P450, caused by NAPQI which utilized reducing equivalent of NADPH with concomitant reduction of molecular superoxide anion radical (O^{-}), therefore there will be a reduction in super oxide dismutase activity (Bessems and Vermeulen, 2001).On the other hand, it is also produced in the extra cellular fluid by macrophages during phagocytosis in the inflammatory phase of diseased conditions. Monboisse *et al*., (1983) are of the opinion that superoxide breaks collagen fibrils into relatively small peptides. It is a known fact that superoxide can also alter the normal biochemistry of the major macromolecular constituents like collagen that is need for the repair of damaged tissues (Greenwald *et al*, 1976). Superoxide radical have a direct derivative action on hyaluronic acid. It also plays a crucial role in repairing tissue injury (McCord, 1974).

FIG.3 Superoxide anion scavenging activity of *Maerua oblongifolia*

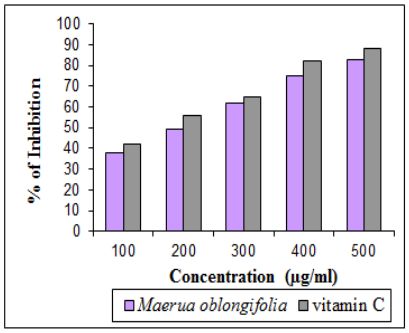
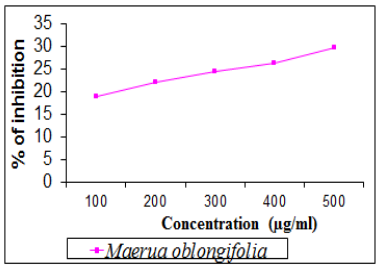


Fig 4 shows the dose dependent DNA sugar, ribose damage inhibition by the aqueous extract of *M oblongifolia (forsk)*. The DNA sugar damage at various concentrations from 100-500 µg/ml was simultaneously increased the % of inhibition. In the present investigation the extract of *M.oblongifolia* prevented the DNA damage in a concentration dependent manner. Oxidative DNA damage has been implicated to be involved in infertility and various degenerative diseases including Alzheimer's disease, Parkinson's disease, Hodgkin's disease, Blooms syndrome (Halliwell and Gutteridge, 1981). The dose dependent decrease in degradation of DNA by the *M.oblongifolia* extract implies that the extract have phytochemical compounds which might combat free radical mediated degradation of deoxyribose sugar moiety of DNA (Soong., 2004).

FIG.4 Effect of *Maerua oblongifolia* on DNA sugar damage



The total antioxidant activity of aqueous extracts of *M. oblongifolia* was assessed by FTC and TBA methods and compared with vitamin-E. From this it showed that (Fig. 5 and Fig. 6) the level of different concentration at the absorbance values of extract when compared with the standard Vitamin-E, which clearly indicates its high level antioxidant activity. Maximum rate of inhibition was observed on day1 and day 2 and further it showed a decrease in the next consecutive days. On the 5th day the extract showed a lower absorbance value which indicates its higher antioxidant activity. The effect of *M. oblongifolia* on linolic acid was examined by FTC and TBA method. Cell membrane is a rich source of unsaturated fatty acids and hydrocloric acids that are most susceptible to oxidative process. During the oxidation process, peroxide is gradually decomposed to lower molecular compounds that are measured by FTC and TBA methods. FTC method is used to measure the amount of peroxide at the primary stage of linolenic acid peroxidation, whereas TBA method measures at the second stage (Kikuzaki and Nakatani, 1993). In this method the concentration of peroxide decreases as the antioxidant activity increases. The results obtained by FTC method revealed that the vitamin E showed least increase in absorbance value, higher the antioxidants activity of the sample. In the TBA method, formation of MDA (malondialdehyde) is the basis for evaluating the extant of lipid peroxidation. In the present investigation the *M. oblongifolia* exhibited antioxidant activity by inhibiting the oxidation of linolic acid in both FTC and TBA method. The antioxidant activity of *M. oblongifolia* was found to be near with the standard. This might be due to the active phytoconstituents present in the extract.

FIG.5 *In vitro* antioxidant activity of *Maerua oblongifolia* by FTC method

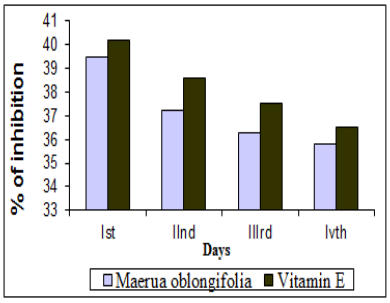
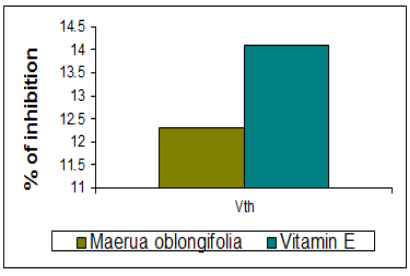


FIG.6 *In vitro* antioxidant activity of *Maerua oblongifolia* by TBA method



Antimicrobial activities of *M.oblongifolia*

The antimicrobial activity of aqueous and methanolic extracts of the plant was studied. Ciprofloxacin 5µg/disc was used as the standard for gram positive and gram negative bacteria. Ketoconazole d 50µg/disc was used as a standard for fungus (Table 2 and Plate 2). Reproductive tract infections are being increasingly recognized as a serious global health problem with impact on individual women and men. Infections of the male genitourinary tract may contribute to infertility by adversely affecting sperm function, causing anatomical obstruction or initiating a leukocyte response. Inflammation and fibrosis could obstruct the sperm passage. The sperm could be directly damaged and immobilized. The chemical composition of the seminal plasma could be adversely affected. .In this context, urethritis, prostatitis, orchitis, and epididymitis have been considered as male accessory gland infections (MAGIs) as reported by the WHO. It is reported that presence of some bacteria in semen is may lead to decreased sperm count and percentage of motility Rehewy *et al.*, 1979, obtained positive bacterial cultures from the semen of 73% of

asymptomatic infertile men. The most common aerobic organisms grown were *Staphylococcus aureus. Staphylococcus epidermidis, Escherichia coli, and Klebsiella pneumouia*e.

Fungal infection such as epididymoorchitis can cause infertility because of gonadal destruction and resultant azoospermia. Fungal infection will produce mycotoxins. These mycotoxins can induce anti-aperm effects and contribute to infertility. *Candida albicans* are the most common infections and they *are* able to inhibit sperm viability and motility in vitro. (Tuttle., 1997)**.**

In the present investigation, the dose levels in the range of 50, 100, 150 µgs of *M.oblongifolia* extracts were analyzed for the antimicrobial activity. It is found that the extracts of M.oblongifolia inhibited the growth of following microorganisms in culture. The present investigation reports the antibacterial activity of *M.oblongifolia* against gram positive bacteria such as *Staphylococcus epidermis, Staphylococcus aureus, Micrococcus aureus, Bacillus cerus, bacillus subtilis, Streptococci mutans and gram negative bacteria such Escherichia coli, Klebsiella pneumoniae and pseudomonas aeruginosa.* Extracts of the plant also showed the antfungal activities against *Aspergillus niger, Aspergillus fumigates, Candida albicans.* Thus *M.oblongifolia* can be considered as an antimicrobial agent to treat male accessory gland infection. (Iwu *et al*.,1999).

Table 2 Antimicrobial activities of *M.oblongifolia*

Microorganisms	**50** µg	**100** µg	**150** µg	**Standard** µg
Gram positive bacteria				
Staphylococcus epidermis				
Aqueous	17	22	26	30
Methanol	19	24	28	31
Staphylococcus aureus				
Aqueous	15	19	25	28
Methanol	16	18	22	28
Micrococcus luteus				
Aqueous	17	20	26	31
Methanol	18	22	25	30
Bacillus cereus				
Aqueous	18	25	30	30
Methanol	19	22	25	32
Bacillus subtilis				
Aqueous	19	24	29	31
Methanol	19	22	26	31
Streptococci mutans				
Aqueous	16	20	27	32
Methanol	19	22	28	30
Gram negative bacteria				
E.Coli Aqueous	16	23	29	30
Methanol	19	21	28	30
Klebsiella pneumoniae				
Aqueous	16	21	25	30
Methanol	17	20	26	30
Pseudomonas aeruginosa				
Aqueous	16	19	23	31
Methanol	17	19	24	30
Fungus				
Aspergillus niger				
Aqueous	14	16	19	28
Methanol	15	17	21	27
Aspergillus fumigatus				
Aqueous	13	16	20	28
Methanol	16	19	24	29
Candida albicans				
Aqueous	17	21	27	29
Methanol	17	23	28	32

IV. SUMMARY

The present study was assigned to evaluate the Free Radical Scavenging and Antioxidant Antimicrobial Activities of in Aqueous and Methanolic Extracts of *Maerua Oblongifolia***,** Preliminary phytochemical analysis on the aqueous and methanolic extracts of the roots of *M.oblongifolia* revealed mainly the presence of alkaloids, glycosides, flavonoids, triterpenoids, tannins and

phytosterols. These phytochemicals play a significant role in scavenging the free radicals; also they function as chelating agents, and modifiers of various enzymatic and biologic reactions. The phytochemicals present in *M.oblongifolia* may be responsible for the biological activity to enhance the overall Free Radical Scavenging and activities of in Aqueous and Methanolic Extracts of *Maerua Oblongifolia.* The *in vitro* study of DPPH, NO, and DNA sugar damage showed the free radical scavenging activities of *M.oblongifolia.* The aqueous and methanolic extracts of *M.oblongifolia* showed antimicrobial activities. The plant root extract inhibited the growth of several gram positive, gram negative bacteria and fungus species in culture.

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