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Antibacterial, antioxidant and phytochemical properties of the ethanolic extract of *Ocimum obovatum* E.Mey. ex Benth.

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This study evaluated the potential antibacterial, antioxidant and phytochemical properties of the ethanolic extract of the leaves of *Ocimum obovatum* E.Mey. ex Benth. The ethanolic extract of *O. obovatum* was tested *in vitro* against Gram-positive (*Staphylococcus aureus*, *Klebsiella* sp, and *Escherichia coli*) and Gram-negative (*Proteus* sp. and *Pseudomonas* sp.) strains of bacteria by the disc diffusion method. The maximum inhibition was against *Staphylococcus aureus* with an inhibition zone of 18 mm. The minimum inhibitory concentration of the extract against the bacteria was also determined. A standard antibiotic disc with *Chloramphenicol* was used as the positive control. The extract was further tested for antioxidant activity using the 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) method which was then compared with standard butylated hydroxyanisole (BHA). Antioxidant activity was IC_{50}220±0.05µg/ml. Phytochemical screening of the leaves showed the presence of carbohydrates, phenols, flavonoids, tannins, saponins, fixed oils and fats, glycosides and terpenoids.

Keywords: *Ocimum obovatum* E.Mey. ex Benth., Antibacterial, Antioxidant, Phytochemical, South Africa

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A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor for the synthesis of useful drugs (Sofowora, 1982, Medicinal plant and Traditional Medicine in Africa). Traditional medicine continues to provide health coverage for over 80% of the world's population, especially in the developing world. The medicinal values of plants lie in their phytochemicals which produce definite physiological actions on the human body. These phytochemicals can be used as food and medicine to offer protection against illness and to maintain human health[1]. Phytochemicals have antioxidant or hormone-like effects which help in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues[2]. Extracts of *Ocimum* are believed to decrease lipid peroxidation and increase the activity of superoxide dismutase[3]. The constituents of *Ocimum* species have antibacterial, antifungal, antioxidant and radio-protective activity[4-5]. Studies show that many *Ocimum* species are useful for the treatment of disorders of the central nervous system (CNS) and may also have antidepressant activity[6-7]. Oxidative stress has been associated with an increased risk of a great number of pathological disturbances such as atherosclerosis, brain dysfunction, cardiovascular disease, cancer and other chronic diseases which may have a substantial impact on human health[8]. The current interest is towards natural antioxidants, especially plant polyphenolics[9-10]. Tea and herbal sources of antioxidant phenolic compounds are already widely represented in our diet[11].

Among the benefits conferred by the medicinal plants from the *Ocimum* genera with regard to anti-ulcer action, the reduction of the incidence of gastric ulcer induced by aspirin was already observed with the ethanolic extract from *O. sanctum*[12]. The genus *Ocimum* is an important group of aromatic and medicinal plants which yield many essential oils and aromatic chemicals and are widely used in the perfume and cosmetic industries as well as in indigenous systems of medicine. *Ocimum sanctum, Ocimum kilimandscharicum, Ocimum americanum* and *Ocimum micranthum* are important species which exhibit medicinal properties[13].

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Ocimum obovatum subsp. *obovatum* var. *obovatum* (Lamiaceae) is widely used in traditional African medicine, especially in southern African countries where it is believed to have great healing properties. The plant is commonly known as 'Cat's whiskers' in English or '*Umathanjane*' in isiZulu and is used in South Africa and West Africa to treat ailments ranging from skin and chest infections, gastrointestinal illnesses and hair loss[14-15].

Materials and methods

Plant materials

The leaves of *O. obovatum* were collected from Sherwood, Durban, South Africa in 2011. The plants were identified and a voucher specimen has been deposited in the Herbarium, School of Life Sciences, University of KwaZulu-Natal.

Extraction of plant material

Leaves of *O. obovatum* were air dried at room temperature and then powdered using a mill. The leaf powder (250 gm) was extracted using a soxhlet apparatus with ethanol as a solvent. The resultant extract was filtered, concentrated to dryness under reduced pressure in a rotary evaporator and stored at 4 °C for further use.

Determination of antibacterial activity

Test organisms

The extract of *O. obovatum* were tested against the bacteria *Escherichia coli* (MTCC40), *Staphylococcus aureus* (MTCC 3160), *Klebsiella pneumoniae* (MTCC3384), *Pseudomonas aeruginosa* (MTCC741), and *Proteus mirabilis*, (MTCC425). These microorganisms which belong to the Microbial Type Culture Collection (MTCC) were supplied by the Institute of Microbial Technology, Chandigarh, India.

Determination of antibacterial activity

Antibacterial activity assays were undertaken by modified disc diffusion[16]. Two concentrations of extract were prepared by dissolving 1mg/ml and 2mg/ml in 10 % DMSO. The bacterial suspension (100µl), adjusted to contain $1x10^6$ CFU/ml of bacteria, was spread by a sterile glass rod on Nutrient Agar (NA) medium. Filter paper discs (What man No.1, 5 mm in diameter) were impregnated with 4µl of the extract, placed in the inoculated plates, incubated at 27±2°C for 24 hrs and the inhibition zones were measured. Antibiotic discs containing 30µg of *Chloramphenicol* (Himedia, India) were used as the positive control and DMSO as the negative control. There were three replications per assay.

Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) of the extract was determined by a modified broth dilution technique[17]. The extract was diluted to give five different concentrations (100-500 µg/ml) in the nutrient broth. Using a standard micropipette, 0.05 ml of the 18 hrs old bacterial broth (10^6 CFU/ml) culture was introduced into each of the test tubes with different concentrations of extract. A set of tubes containing only growth medium plus each of the test bacteria was set up separately to serve as controls. All tubes were incubated at 27±2°C for 30 hrs. The minimum inhibitory concentration was the lowest concentration of extract that prevented bacterial growth. The same test was repeated with the antibiotic *Chloramphenicol* to serve as a positive control.

Determination of antioxidant activity

The free radical scavenging activity of the crude extracts was determined by the DPPH assay described by Blois[18]. In its radical form, DPPH absorbs maximally at 517nm but upon reduction by an antioxidant or a radical species its absorption wavelength decreases. A 0.1mM solution of DPPH in methanol was prepared and 4mL of this solution was added to 1mL of sample solutions in methanol at different concentrations. After 30 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All the assays were carried out in triplicate. BHA was used as a standard antioxidant. Percentage DPPH scavenging activity was determined as follows:

$$\text{DPPH Scavenging Effect (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

Results and discussion

The antibacterial activity of the *O. obovatum is* presented in Table 1. The extract was evaluated for antibacterial activity against gram-positive and gram-negative bacterial strains. The extract was active against *Escherichia coli, Staphylococcus aureus, Pseudomonas* sp, *Proteus* sp but not against *Klebsiella* sp. The maximum inhibition was against the gram-positive bacterium *Staphylococcus aureus,* with an inhibition zone of 18 mm. The MIC values confirmed the antimicrobial activity against the tested

Table 1—The diameter of the antimicrobial activity zone (mm) of the ethanol extract of *O. obovatum*

Test organisms	Ethanol extract		Positive control	MIC µg/ml
	1mg/ml	2mg/ml		
Escherichia coli	8	14	28	176
Staphylococcus aureus	12	18	32	125
Klebsiella sp	-	-	24	-
Pseudomonas sp	9	16	30	180
Proteus sp	7	13	25	320

Values (mean of three replicates) are diameter of zone of inhibition (mm)

Positive control= each antibiotic disc contained 30µg of *chloramphenicol*

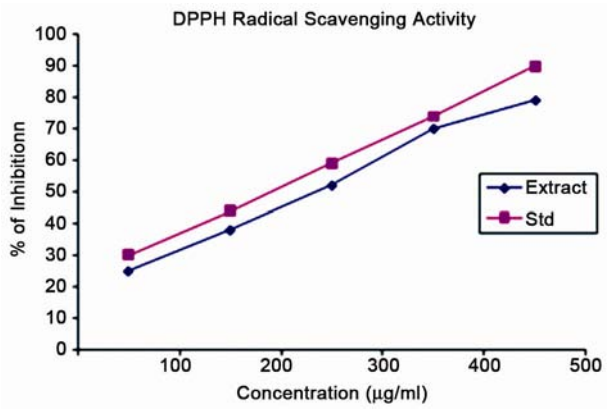

Fig: 1—DPPH free radical scavenging activity (IC_{50} µg/ml) of ethanolic extract of *O.obovatum* leaves and BHA standard

bacteria. The MIC values of the extract ranged from 150 to 500µg/ml (Table 1).

Escherichia coli and *Staphylococcus aureus* test cultures are known to be the leading pathogens in nosocomial infections[19-20]. Ethanol, methanol, and hexane extracts from *O. basilicum* L. were investigated for their *in vitro* antimicrobial properties by Adiguzel and colleagues[21]. The antibacterial activity of different extracts from the leaves of *O. gratissimum*, another species of *Ocimum*, was tested against pathogenic bacteria that cause diarrhea and effective results on all the four bacteria studied were obtained. The antioxidant activity of the *O. obovatum is* presented in Fig. 1. The extract tested for antioxidant activity by the 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) method and then compared with standard butylated hydroxyanisole (BHA). Antioxidant activity is very important in counteracting the deleterious roe of free radicals in food and biological system[22]. The DPPH alcohol

Table 2—Phytochemical screening of ethanolic extract of *O. obovatum*

Tested for	Presence/Absence
1. Carbohydrates	+
2. Phenols	+
3. Flavonoids	+
4. Tannins	+
5.Steroids/ Terpenoids	+
6. Alkaloids	-
7. Glycosides	+
8. Saponins	+
9. Anthraquinones	-
10. Amino acids	-
11. Fixed oils	+

solution is a deep purple color with an absorption peak at 517 nm which disappears in the presence of radical scavengers in the reactive system. Antioxidant activity of *O. obovatum* was IC_{50} 220±0.05µg/ml. The scavenging capacity of biological reagents on DPPH free radicals can be expressed as its antioxidant capability. On accepting an electron or hydrogen atom, it becomes a stable diamagnetic molecule[22]. Evidences gathered in recent years suggest the involvement of free radicals and other oxidants as the major cause of oxidative stress that leads to a variety of diseases and disorders. Strong restrictions have been placed on the use of synthetic antioxidants such as BHT, BHA, gallates due to their carcinogenic potential[23]. This led to an increasing interest in natural products having antioxidant properties. Plants have been considered as richer sources of antioxidants. Flavonoids are reported to exhibit antioxidant activity and are effective scavengers of superoxide anions[24]. Phytochemical screening of the leaves showed the presence of carbohydrates, phenols, flavonoids, tannins, saponins, fixed oils and fats, glycosides and terpenoids (Table 2). The present findings indicate that *O. obovatum* possesses compounds with antioxidant and antimicrobial properties against pathogenic microorganisms.

Traditional significance of study to the farmers/society/researchers

The highly aromatic essential oils found in leaves of *Ocimum* species are produced as secretions by specialized glands on the leaf surface. Significant levels of terpenes detected in leaf glandular trichomes of *Ocimum obovatum* may account for the medicinal properties exhibited by this species, and may also serve a role in plant defense against insects and

pathogens[25]. *Ocimum obovatum* often grows in areas prone to burning or seasonal flooding, and occur at altitudes ranging between 100 and 2100 m. The plants are perennial and have a swollen woody rootstock that produce erect branched or unbranched flowering shoots between 0.15 and 0.60 m high. Inflorescences consist of pink or lilac-white flowers with very long stamens[26]. Naidoo and colleagues[27] showed that *O. obovatum* leaf oil possesses antimicrobial potential against several microorganisms. The essential oil of *O. obovatum*, i.e., phytol (21.46%); 2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene (8.2%); n-hexadecanoic acid (7.4%); 6,10,14-trimethyl 2-pentadecanone (5.2%); 2-ethylhexyl undecy l ester phthalic acid (5.2%);dibutyl phthalate (4.5%); 2,6-lutidine-N-oxide(3.4%) and 2-(1,1-dimethylethyl)-1,4-dimethoxy- benzene (3.1%), has been previously reported.

Conclusion

Extracts of *O. obovatum* possess antimicrobial activity against *Staphylococcus aureus, Pseudomonas sp and Escherichia coli* which may be useful in medicinal and food storage applications. The strong antioxidant potential of *O. obovatum* may be advantageous to the food industry as a food additive, and highlights the broad-spectrum activity of bioactive compounds derived from this species. Furthermore, compounds derived from plant extracts are a safer alternative to synthetic chemical analogues and can be obtained through various purification and isolation methods. Further studies are required, and will be on-going, to test the antimicrobial and antioxidant activity of *O. obovatum* against a wider range of microorganisms.

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