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STUDY OF ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT OF *OCIMUM AMERICANUM* L. (LAMIACEAE)

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ABSTRACT

Essential oils are one of the most attractive products obtained from the plants for the development of new drugs and may present different pharmacological properties and an excellent antimicrobial activity. Presently researches for new natural antibiotics from essential oils are being attempted and extended to various areas. In last two decades the search for new antimicrobials from natural products has been intensified due to emergence of strains resistant to antibiotics. In present attempt antimicrobial activity of plant extracts including leaves of Ocimum americanum L. belonging to family Lamiaceae which is medicinal herb has been studied using Kirby-Bauer disc diffusion method. Microbial susceptibility assay was carries against test bacterium Escherichia coli (ATCC 14948) and Staphylococcus aureus (ATCC 33591) and dermatophytic fungus Candida albicans (NCIM 3100) and Trichophyton mentagrophyte (MTCC 7687). The results were compared with the standard drugs, tetracycline for bacteria and ketocanazole for fungi respectively. The extract of Ocimum americanum exhibited significant antimicrobial efficacy against test organisms.

Keywords: Ocimum americanum, antimicrobial activity, Escherichia coli, Lamiaceae

Introduction

In the last two decades the search for new antimicrobial compounds from natural products has been intensified around the world mainly due to the emergence of strains resistant to antibiotics. The well known bacteria methicilin resistant *Staphylococcus aureus* – MRSA (Struelens, 2009), vancomycin resistant *Enterococcus* – VRE (Pearson *et al.*, 1992), *Clostridium difficile* and Enterobacteriaceae producing broad-spectrum b-lactamase – ESPL

(Lee *et al.*, 2003), besides the fungi *Candida* spp and *Aspergillus* spp, represent one constant threat and has spread in hospitals and communities.

The antibiotic resistance is inevitable and irreversible. A natural consequence of adaptation of the bacterial cell exposure to antibiotics, mainly due to the indiscriminate use in medicine, agriculture, cosmetics and as food preservatives (Duarte *et al.*, 2012). The current need is to search for new antimicrobials able to reach different targets in the microorganism cells from those in which conventional drugs attack (Ahmad and Beg, 2001).

In the last decade numerous studies involving the assessment of plants for antimicrobial activity, in the form of crude extracts, essential oils, fractions and compounds isolated were performed on several continents and countries, including the flora of South Africa (Kamatou et al., 2008; Van Vuuren, 2008), Lebanon (Formisano et al., 2010), Malaysia (Humeirah et al., 2010), India (Bhakshu and Raju, 2009), Italy (De Martino et al., 2009) and Brazil (Silva et al., 2010; Duarte et al., 2005; Duarte et al., 2007) etc. From a recent survey, it is known that potential antimicrobial activity (Minimal Inhibitory Concentration – MIC until 1.000 $\mu g.ML^{-1}$) was observed only for 10% of the species studies (Duarte et al., 2012). These potentially active species are belonging to 30 botanical families, mainly to the families Apiaceae, Asteraceae, Boraginaceae, Lamiaceae, Lauraceae and Myrtaceae. The information available in the literature can now be used as a source for choosing of plants for advanced studies.

In this context, in present attempt antimicrobial activity of plant extract of *Ocimum americanum* L. belonging to Lamiaceae has been studied.

Ocimum americanum L. is a medicinal and aromatic herb belonging to family Lamiaceae with strong citrus smelled aroma, 15-35 cm high, diffuse; branches arise from woody root stock, 4-angled, pubescent, leaves 1.0–3.5 x 0.6–2 cm, ovate, gland dotted, apex acute, base cuneate, margins entire or finely serrulate, flowers white or pale liliac in 6-flowered whorls in 5-10 cm long racemes; bracts up to 0.1 cm long, hairy; calyx campanulate 0.3 cm long in flower, enlarged in fruits, hairy below outside; corolla 0.6 cm long, lips ovate-oblong, upper lip shorter than lower lip. Nutlets subglobose, ovoid, brown, sub-trigonous. It is distributed throughout India. (Flowers and Fruits: June–October)

The leaves are used for flavoring sauces, soups etc. The seeds are considered diuretic and tonic, and are used in the preparation of cooling drinks. A decoction of the plant is taken for coughs, that of leaves for dysentery, it is also used as a mouthwash for relieving toothache, Singh *et. al.* (1983).

Essential oils are one of the most attractive products obtained from plants for the development of new drugs and may present different pharmacological properties and an excellent antimicrobial activity. For this proposal, current search aiming at new natural antimicrobials from essential oils has been extended to various areas, such as human and veterinary medicine, agriculture, cosmetic, personal care, oral care, food and feed, among others. This can be confirmed through the hundreds of articles published in this area over the last decade, in addition to numerous patents registered in several countries. The growing interest is mainly due to the need to replace antibiotics currently available on the market for new drugs that can control microbial resistance, beyond the need for its replacement as preservatives in food and cosmetics, as growth promoters in animal feed and for disease control in agriculture. Thus, there is vast information available in the literature concerning the antimicrobial activity of essential oils of native species from all continents.

Material and Methods

1. Extract Preparation:

After the proper identification, bark and leaves were cut it into small pieces and shade dried at room temperature and made into coarsely powdered using mechanical grinder and preserved in air tight container. Extract was prepared by soaking 15g powder of plant material in 150ml of petroleum ether. It was kept for 24 hrs. After 24 hrs, the material extracted with ether was evaporated. The residue was extracted with 150ml of mathanol and kept for 24 hrs. after 24 hrs the methanol was evaporated and the residue used as methanol extract.

2. Antimicrobial Activity:

Common human pathogens both bacteria and fungi were used for the assessment of antibacterial activity of plant extract by Kirby-Bauer disc diffusion method, a modification of the agar disk diffusion method of the Clinical Laboratory Standard Institute (CLSI). Log phase inocula (10^6cfu/ml) of the bacterial strains *Escherichia coli* (ATCC 14948) and *Staphylococcus aureus* (ATCC 33591) standardized against MacFarland's standard and were swabbed onto the nutrient agar plates. For antifungal activity against dermatophytic fungus, *Candida albicans* (NCIM 3100) and *Trichophyton mentagrophyte* (MTCC 7687) inocula was maintained and swabbed onto the potato dextrose agar plates. Filter paper disks saturated with plant extract, solvent used for extract preparation and antibiotic discs as a positive control were

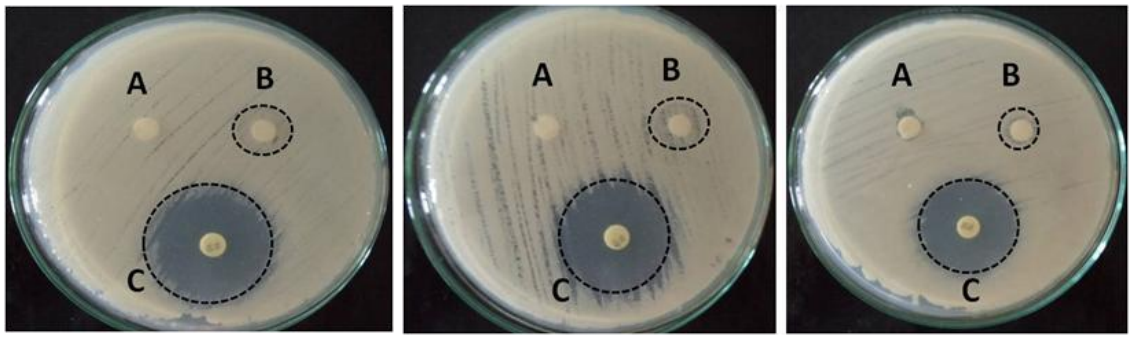
placed onto the surface of the medium with the help of sterile forceps and incubated at 37^0C. After 18 h of incubation, the plates were examined for evidence of zone of inhibition, which appears as a clear area around the disks. All experiments were carried out in triplicates.

Result and Discussion

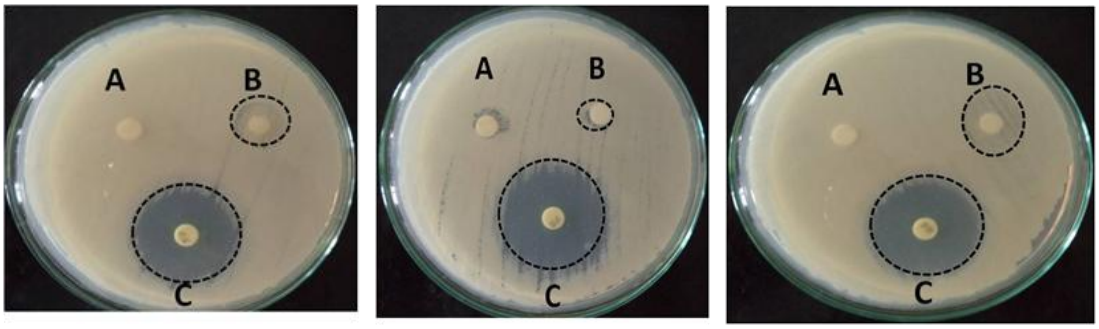
Table 1:

Antimicrobial activity (zone of inhibition, mm) of plant extracts *Ocimum americanum* against pathogens.

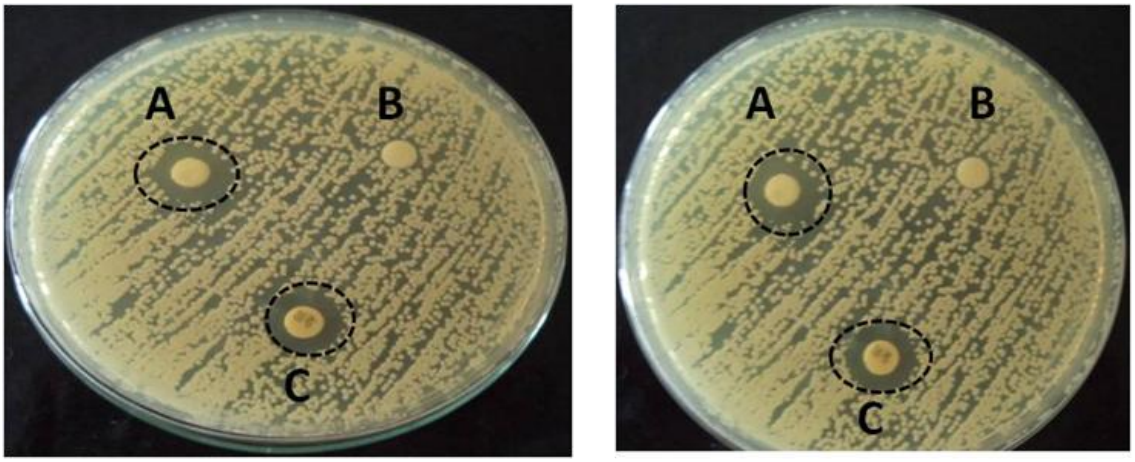
Sr. No.	Name of the Pathogen	Zone of Inhibition (mm)		
		Antibiotic	Methanolic Plant Extract	Solvent
1	***E. Coli*** **(ATCC 14948)**	**28±0.35**	**18±0.43**	**10**
2	***S. aureus*** **(ATCC 33591)**	**28±0.56**	**15±0.82**	**10**
3	***Candida albicans*** **(NCIM 3100)**	**14±0.40**	**11±0.28**	**10**
4	***Trichophyton mentagrophyte*** **(MTCC 7687)**	**10±0.08**	**12±0.31**	**10**



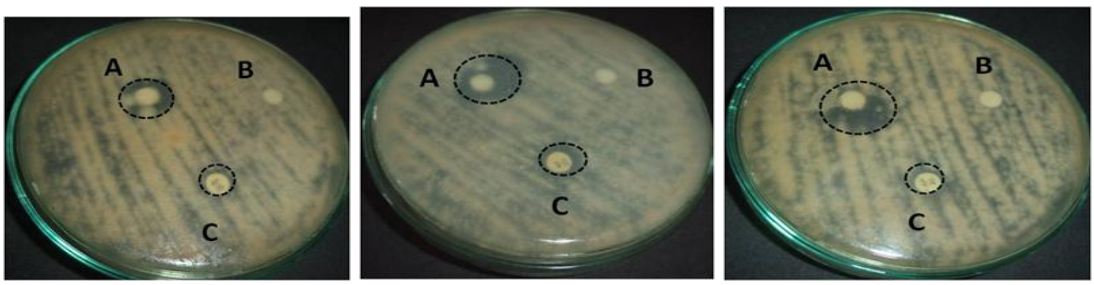
Antimicrobial activity of plant extract against *E. coli* (ATCC 14948), where A – Solvent, B – Plant Extract, C – Antibiotic (Tetracyclin)



Antimicrobial activity of plant extract against *S. aureus* (ATCC 33591), where A – Solvent, B – Plant Extract, C – Antibiotic (Tetracyclin)



Antimicrobial activity of plant extract against *Candida albicans* (NCIM 3100), where A – Plant Extract, B – Solvent, C – Antibiotic (Amphotericin B)



Antimicrobial activity of plant extract against *Trichophyton mentagrophyte* (MTCC 7687), where A – Solvent, B – Plant Extract, C – Antibiotic (Ketocanozol)

In the present investigation, microbial susceptibility assay was carried against test bacterium *Escherichia coli* (ATCC 14948) and *Staphylococcus aureus* (ATCC 33591) and dermatophytic fungus *Candida albicans* (NCIM 3100) and *Trichophyton mentagrophyte* (MTCC 7687) by using Kirby Bauer disc diffusion method. The results were compared with the standard drugs, tetracycline for bacteria and ketocanazole for fungi respectively (Table 1). The extract of *O. americanum* exhibited significant antimicrobial efficacy against test organisms with the inhibition zone ranged from 11-18mm. The solvent methanol did not show any antimicrobial activity. There are several reports supporting significant antimicrobial activity of methanol extracts of several medicinal plant species as higher than that of any other alcoholic solvents [1-4]. Methanol extract shows highest antimicrobial activity against both bacterial and fungal isolates as methanol is good solvent for better extraction of bioactive metabolites. Plant metabolites exert antimicrobial activity through different mechanisms.

Database containing information about the activity of plant species, besides information on the activity of their fractions, identification of its active compounds, oil yield and seasonal activity, should be used for the planning of projects focusing on the control of specific microorganisms, with the goal of replacing antibiotics currently known by essential oils in different areas, such as human and animal health, agriculture, food, feed and cosmetics.

Conclusion

The development of new antimicrobial from natural products such as essential oils poses several challenges. From the present work it has been seen that plant extract of *Ocimum americanum* shows pronounced antimicrobial activity and hence it can be used as a source of novel antimicrobial agent against multiple drug resistant bacterial strains. However, as little is known about the implementation of new antimicrobial drugs in the market, and in order to achieve success in this direction the research should be conducted by a multidisciplinary group.

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