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Research Article

Evaluation of Antibacterial Activity of Ocimum

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ABSTRACT
A study was carried out to evaluate the antibacterial activity of Ocimum oil. The oil extracts were prepared using Clevenger-Hydro-Distillation process. Antibacterial activity of oil extracts was tested using Disc diffusion method. Ocimum showed high antibacterial activity. This was compared against antibiotics routinely used as chemotherapeutic agents. Our investigations indicated that Ocimum extracts are equally or sometimes more effective as antibacterial agents in comparison to antibiotics.

Keywords: Antibacterial activity, Ocimum, Antibiotics.

INTRODUCTION

Medicinal plants essentially contain active constituents that are used in the treatment of many human diseases. The essential oils and extracts of plants have been reported to have an antimicrobial activity [3, 7]. Many of these plants which show antimicrobial activity are used in Food preparation. They also serve as food preservatives, examples are spices, turmeric, and basil [8]. The chemical principle of such plants needs to be studied, explored and exploited.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms have increased. Such a fact is cause of concern because the number of patients in the hospitals who have suppressed immunity and new bacterial strains which are multi drug resistant can result in high mortality[8]. This growing problem needs to be addressed by providing effective antimicrobial treatment. Medicinal plants should be investigated to understand their properties, safety and efficacy. Our study aimed at finding out antibacterial activity of various Ocimum varieties grown in experimental fields.

Table 1: Antibiotic sensitivity tested against E.coli wild type and drug resistant strains.

Antibiotic (µg)	Wild strain zone of inhibition (mm)	Resistant strain zone of inhibition (mm)
Streptomycin (25)	22.6	19.5
Kanamycin (20)	21	11
Gentamicin(10)	20.1	15.5
Tetracycline (15)	Resistant	17
Tobramycin (10)	50	22
Chloramphenicol(30)	32.3	19
Amikacin(30)	19	21
Nalidixic acid (30)	Resistant	25
Norfloxacin(300)	29.5	Resistant
Augmentin (30)	40	Resistant
Ampicillin (25)	Resistant	10
Methicillin (30)	Resistant	Resistant
Cephalothin (5)	Resistant	Resistant
Cotrimoxazole (25)	Resistant	Resistant
Cephalexin(30)	Resistant	Resistant
Cephotaxime(30)	40	Resistant

Table 2:

Ocimum ((µg)	Zone of inhibition (mm)
OcimumMG Globe	27.6
OB Tall	28.5
CIM CYU16	28.5
Tirupathi	26.5

In present study effectiveness of Ocimum as antibacterial agent has been evaluated using experimental germplasm of Indian variety. Ocimum oil extracts as antimicrobial agents is reported along with antibiotics commonly used as therapeutics. This evaluation was carried out to know the possibility of using ocimum oils as effective therapy against multidrug resistant nosocomial bacterial agent in combination with antibiotics.

MATERIALS AND METHODS

Essential oil from Ocimum - test sample.

The Ocimum species belongs to the Lamiaceae family includes annual and perennial herbal plants, as well as shrubs from tropical and subtropical zones of Asia, Africa and South America. These plants are widely spread in the world.

The most species of Ocimum genus are usually named common basil or sweet basil, is a herbal, annual and allogamous plant with extraordinary culinary and medicinal properties. Studies have established that compounds have antioxidant, anticancer, antiviral, antimicrobial properties. It is characterized by considerable morphological and biochemical variability. In general, a remarkable intra-specific variation exists in plant morphology and in essential oil composition.

Mostly these essential oils are volatile and aromatic with antimicrobial, antifungal and antioxidant property.

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Table 3: Antibiotic sensitivity tested against Staphylococcus aureus wild type and drug resistant strains.

Antibiotic ((μg)	Wild strain zone of inhibition (mm)	Resistant strain zone of inhibition (mm)
Penicillin (1 unit)	23.5	20
Amoxycillin	Resistant	Resistant
Vancomycin	18	15
Neomycin	27	22
Cloxacillin	Resistant	Resistant
Tetracycline	33	22
Amikacin	28	21.5
Gentamicin	32	25
Erythromycin	28	21
Novobiocin	24	22
Bacitracin	10	08
Clindamycin	42	30
Trimethoprim	Resistant	24
Cotrimexazole	Resistant	24
Fusidic acid	21	21

Leaves and inflorescences, fresh stems of Ocimum species used in this work were harvested from plants at full flowering stage from the experimental field. The extraction of essential oil was done using a Clevenger-hydro-distillation process, with a plant material: water ratio being 1:3, at 60-70° C in 3 hours of extraction time. The anhydrous sodium sulphate was added to remove the moisture. The extracted oil is stored in glass tubes with tight lids and kept under refrigeration.

Antimicrobial testing

Table 4:

Ocimum (μ_g)	Inhibition Zone (mm)
Ocimum Globe	34
Ocimum OGM	51
Ocimum Kushmohak	27
Ocimum Tirupathi	34
MG BD BDL	41

Bacterial strains used:

Two test cultures of bacteria were used namely Escherichia coli and Staphylococcus aureus. Escherichia coli; a Gram negative bacterium is a leading nosocomial pathogen, causes Urinary tract Infections [9].Staphylococcus aureus, a Gram positive bacterium, causes wound and skin infections.

Pure cultures obtained from National Collection of Industrial Microorganisms (NCIM), NCL, Pune were used.

Maintenance of Cultures:

The organisms are maintained on Nutrient agar (HI-Media, India) slopes at 4°C and sub cultured before use.

Determination of antibacterial activity:

1. In vitro antibacterial activity of essential oils of ocimum varieties was studied against two bacterial cultures using Agar diffusion disc method [2].
2. The antibacterial activity of known antibiotics was also tested along with the ocimum essential oils for comparison.
3. Muller – Hinton medium was used for test assay.
4. The extracts i.e. essential oils were diluted to give 200, 400 and 800μg/100 microliter concentration.
5. The overnight broth of the inoculum was seeded on the agar plates (1.5 x 10^8 CFU/ml)
6. The solvent used for preparation of essential oil solution was ethanol. Solvent control well was run for every assay.
7. All the plates were incubated at 37°C for24 hrs.
8. The antimicrobial spectrum of the extract was determined in terms of zone sizes around each well i.e. diameter of inhibition zones. Each result is a mean of three replicates.

RESULTS

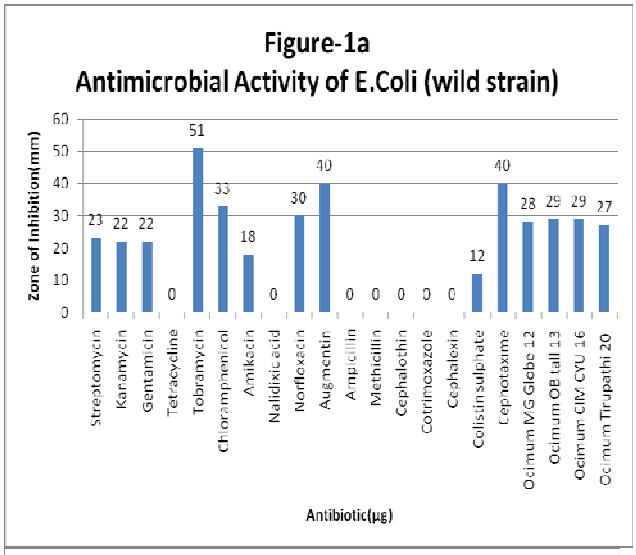
Medically important plant species of Ocimum were cultivated under experimental conditions in Andhra Pradesh, India. Essential oils obtained were tested for their antimicrobial effect. The study was carried out in comparison with the antibiotics used routinely. A Gram negative bacterium Escherichia coli and a Gram positive bacterium Staphylococcus aureus were the test cultures. Both these bacteria are leading nosocomial agents which cause infections like UTI and wound infections [5]. Antibiotics commonly used against them are broad spectrum belonging to protein synthesis inhibitor and cell wall inhibitor groups [5].

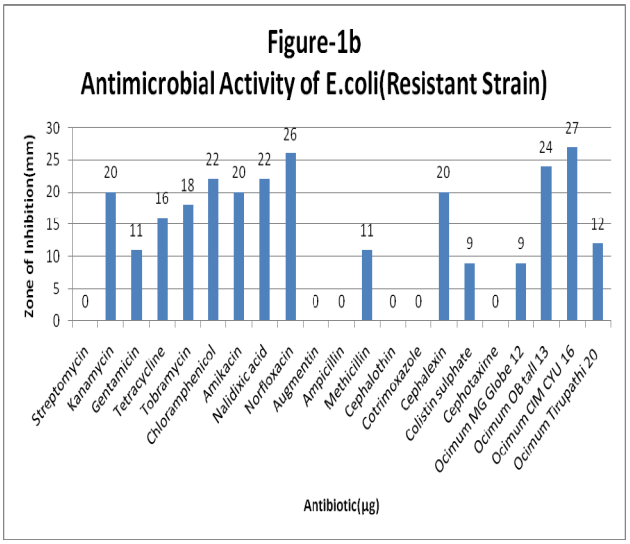
Table 1 shows the results of antibiotic sensitivity invitro of E.Coli, against 16 different antibiotics. E. Coli NCIM strains used were wild and drug resistant variety. Agar diffusion method showed wide zones of inhibition surrounding the antibiotic discs indicating sensitivity to that particular antibiotic. Comparative study of two varieties were carried out using antibiotics like Gentamicin, Amikacin, Tetracycline, Tobramycin as well as antimicrobial agents like Norfloxacin, Nalidixic acid and others as listed in Table. 1.

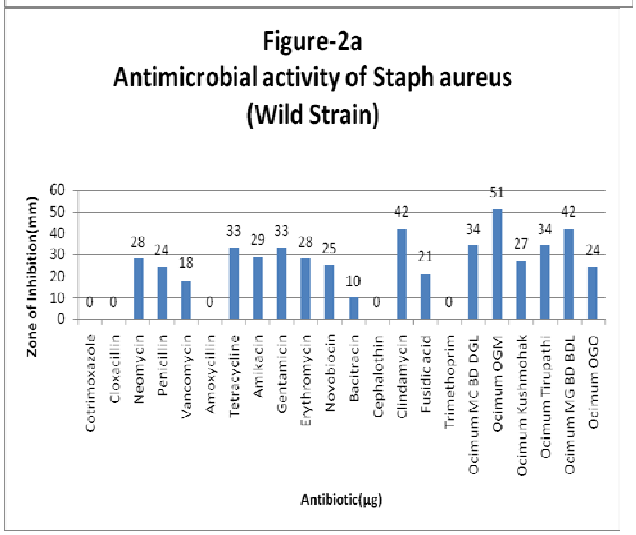
E. coli has been reported to show multidrug resistance. Our results in concurrence showed that wild type responded well to antibiotics than the drug resistant type (Fig.1a).

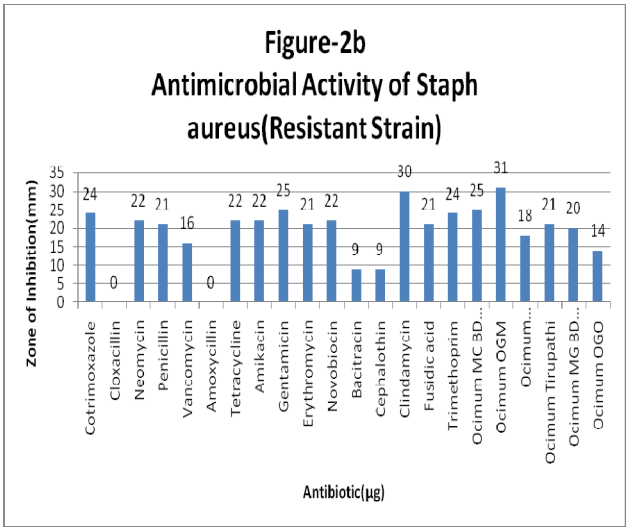
E.Coli wild type was sensitive to antibiotics like Tobramycin with a zone of inhibition with 50mm diameter while Drug Resistant strain has a zone of inhibition of only 17mm.

Table 2 shows results of sensitivity of different Ocimum oil extracts against E.coli wild type and drug resistant strains. E.coli wild type showed highest sensitivity for Ocimum MG Globe variety with inhibition zone of 28mm. (Table 2) OcimumCIM variety could inhibit E.coli drug resistant strain and diameter of zone of inhibition was 27mm which was more than any of the antibiotics tested. This proves effectiveness of Ocimum essential oil used in this study. This is in concurrence









with the reports that Ocimum has extremely good antimicrobial activity [10].

Table 3/Figure 3 and 4 show results of in vitro test of sensitivity of Staphylococcus aureus against commonly used antibiotics. Staphylococcus aureus wild and antibiotic resistant strains were tested against 16 different antibiotics. Staphylococcus being Gram positive the choice of antibiotics for testing included cell wall inhibitors like penicillin and amoxicillin as well as other broad spectrum drugs. Staphylococcus aureus is another leading pathogen in hospital environments. It causes sepsis and skin infections. There are several reports of multidrug resistant Staphylococcus aureus. (MRSA).(9)

Our study indicated that staphylococcus wild and drug resistant strains were sensitive to Clindamycin, Neomycin, Gentamicin and Tetracycline. In contradiction to the reports that Staphylococcus responds to beta lactum antibiotics well (5, 12) our study indicated high resistance to drugs like amoxicillin, Cloxacillin (Fig 3). It confirms emerging trend of MRSA. It is a matter of concern that antibiotics which worked well are of no use today. This can be correlated to the reports of indiscriminate use of antibiotics. Therefore our results of Ocimum oil extracts showing promising inhibitory action against same resistant strains are encouraging. (13) (Fig4).

DISCUSSION

Escherichia coli and Staphylococcus aureus test cultures are known to be the leading pathogens in nosocomial infections [5, 9]. Recent reports indicate that these same pathogens are showing alarming levels of resistance against commonly used antibiotics [14]. In view of this threat our study brings out the following facts.

Firstly our study confirms the high degree of antibacterial activity of Ocimum. Another important finding this study brought out is when E.coli and Staphylococcus cultures namely wild and drug resistant strains were subjected to same antibiotics, wild strains though were more sensitive to same antibiotics showed resistance to others. This confirms emergence of antibiotic resistance as reported by several workers. Gram positive bacteria like test culture Staphylococcus are known to be more sensitive to ß lactam antibiotics like Penicillin group. Development of resistance to these antibiotics in our study is in concurrence with the reports of Multi Drug Resistance of Staphylococcus aureus [14].

Further this study brought out the comparison of Ocimum oil with commonly used antibiotics as therapeutic agents using these test cultures. Findings indicated that Ocimum oil had equal or more antibacterial effect to that of antibiotics used commonly. Drug resistance strains of E.coli and Staphylococcus aureus also were very highly sensitive to Ocimum oil.

Ocimum oil worked better as antibacterial agent against Gram positive culture i.e. Staphylococcus than Gram negative test culture i.e. Escherichia coli. This substantiates the use of Ocimum oil and extracts for septic wounds, skin and wound infections in Traditional Indian therapies like Ayurveda [3, 8, 12].

Our findings are significant in context with present day drug resistance problem as it indicates combined therapy as an answer for reducing drug resistance. This study also forms a good basis for selection or these germplasms of Ocimum for further phytochemical and pharmacological investigations. Effective biomolecules from such medicinal plants have to be identified and extensively tested so that they can be used as sources of new drugs.

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