

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS OF *OCIMUM AMERICANUM* L.

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The dried pulverized plant extracts (methanol, hexane and chloroform) from *Ocimum americanum*, were screened for its antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Erwinia carotorola*, *Streptococcus faecalis*, *Klepsiella pyogenes*, *Proteus mirabilis*, *Micrococcus lutes* and *Enterococcus faecalis*. The results clearly indicate that methanolic plant extract was found to have better activity against all the microbial strains tested followed by chloroform and hexane extracts.

Keywords : Antimicrobial activity; Human bacterial pathogens; *Ocimum americanum*; Solvent extracts.

Introduction

The traditional medicinal methods especially the use of plants, play a vital role in the basic health needs in the developing countries and the use of herbal remedies has enhanced in the developed countries¹. In India, large number of medicinal plants are found in their natural habitats. Antimicrobial substances are also found in numerous plants. The active principles of many drugs found in plants are produced as secondary metabolites. Phytochemical and biological analysis of medicinal plants is providing remarkable contribution to the drug industry all over the world².

It is estimated that there are about 500000 sps. of plants on earth. The importances of medicinal plants in India, providing health care against various ailments including infectious diseases are well documented. However, over 50% of all modern clinical drugs are of natural product origin and they play an important role in drug programmes of the pharmaceutical industries³. Considering the rich diversity of these plants, it is expected that screening and scientific evaluation of plant extract for their active principles may provide new antimicrobial substances.

Ocimum americanum belongs to the family Lamiaceae. The aerial parts of *Ocimum americanum* were used in folk medicine for several purposes. Historically the plant material is used in vitiated conditions of vata and kapha, helmenthiasis, anorexia, dyspepsia, flatulence, leprosy, migraine, malaria and fever. Previous studies have shown that the essential oils of the leaves of *Ocimum americanum* possess antibacterial⁴ and insecticidal⁵ properties. Hence, the present research was motivated to

evaluate the activity of *Ocimum americanum* extract against the selected microbial strains.

Material and Methods

Plant Material: The plant material was collected from Botanical garden of Andhra University and its surroundings. The plant material was washed thoroughly under running tap water and dried under shade conditions. After drying, the plant material was powdered and it was stored in a cool and dry place until use.

Preparation of Extracts: The method of extract preparation was followed as per Trease and Evans⁶ with slight modifications. About 30 g of each powdered material was separately extracted with 150 ml of 50% Methanol, Hexane and Chloroform solvents, respectively. Initially plant material powder was soaked in their respective solvents for about 24 hrs and the contents were extracted by using soxhlet apparatus. Then the extract was filtered through Whatman's No.1 filter paper and was reduced to dryness under vaccume. The sticky black substances were obtained and stored in refrigerator and dissolved in DMSO (Dimethyl Sulphoxide) prior to use.

Microorganisms used: Eight human and bacterial pathogens namely *Escherichia coli*, *Bacillus subtilis*, *Erwinia carotorola*, *Streptococcus faecalis*, *Klepsiella pyogenes*, *Proteus mirabilis*, *Micrococcus lutes* and *Enterococcus faecalis* were used in this investigation. All the cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

Media and inoculum preparation: The media used for antimicrobial test were nutrient agar/ Broth and Mueller Hintoagar purchased from Himedia. The test microbial strains were inoculated on nutrient broth and incubated

Table 1. Antimicrobial activity of plant extracts from *Ocimum americanum*

S. No.	Organisms tested	Diameter of inhibition zone in mm* (Mean \pm SD)		
		Solvents used		
		Methanol	Hexane	Chloroform
1	<i>Pseudomonas aeruginosa</i>	7.85 \pm 8.13	6.02 \pm 6.18	6.52 \pm 6.98
2	<i>Enterococcus faecalis</i>	5.73 \pm 6.76	—	—
3	<i>Micrococcus luteus</i>	5.97 \pm 6.34	4.06 \pm 3.76	4.89 \pm 4.41
4	<i>Escherichia coli</i>	7.14 \pm 8.55	6.13 \pm 6.80	5.89 \pm 6.60
5	<i>Bacillus subtilis</i>	4.52 \pm 4.94	3.81 \pm 4.35	5.13 \pm 6.75
6	<i>Klebsiella pyogenes</i>	4.73 \pm 4.86	3.14 \pm 3.91	3.47 \pm 4.05
7	<i>Proteus mirabilis</i>	8.97 \pm 9.61	4.14 \pm 5.02	6.97 \pm 7.99
8	<i>Streptococcus faecalis</i>	5.81 \pm 6.06	—	4.56 \pm 4.26

- = No activity

* - Average value of triplicate

at 37°C for 24 hrs. After the incubation period, the culture tubes were compared with the turbidity (opacity) standard. Antimicrobial activity test: Antimicrobial activity test was carried out by the disc diffusion method⁷ with little modifications. Initially, 500 ml of nutrient agar media was prepared and it was sterilized in an autoclave at 121°C and 15lb pressure for 15 minutes. 10 ml of nutrient agar media at liquid state was transferred into 8 different test tubes. When the temperature of the nutrient agar is around 45°C, 20 μ l of one day old cultures of microbial strains were taken and added to the nutrient agar tubes. Nutrient agar medium was mixed well and then the medium in the 8 test tubes were poured in 8 different Petri plates. After solidification of media, wells were made with cup borer 6 mm/10 mm. With the help of micropipette constant volume of each extract (100 μ l) were added and incubated for 24 hrs at 37°C. The inhibition of bacterial growth was determined by measuring the diameter of the clear zone around each well. The experiment was conducted in triplicate and mean⁸ was calculated.

Results and Discussion

Antimicrobial activity of the plant extracts of *Ocimum americanum*, were presented in Table-1. The results suggested that methanolic extract of *O. americanum* showed an excellent antimicrobial activity against *Proteus mirabilis* followed by *Pseudomonas aeruginosa* and *Escherichia coli*, which is clearly documented. The other tested organisms like *Micrococcus luteus*, *Streptococcus faecalis*, *Enterococcus faecalis*, *Klebsiella pyogenes* and

Bacillus subtilis exhibited moderate activity. However, the hexane extracts reflected good activity against *Escherichia coli* and *Pseudomonas aeruginosa* and exhibited moderate activity towards *Proteus mirabilis* and *Micrococcus luteus*. The same extract shows least activity in case of *Klebsiella pyogenes* and *Bacillus subtilis* and no inhibition was seen in *Streptococcus faecalis* and *Enterococcus faecalis*.

Chloroform extracts showed good to moderate inhibitory activity against the tested microorganisms namely *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Micrococcus luteus*, *Streptococcus faecalis* and *Klebsiella pyogenes*. Both the hexane and chloroform extracts found to be inactive against the growth of *Enterococcus faecalis*.

The results clearly indicate that methanolic plant extract was found to have better activity against all the microbial strains tested followed by chloroform and hexane extracts. This may be due to the presence of chemical constituents, which are soluble in organic solvents and responsible for bioactivity. It can be interpreted that the antimicrobial activity against microorganisms is due to presence of alkaloids⁹ in them. The present findings support *O. americanum* has a long history of use in traditional medicine to treat many diseases like helmenthiasis, anorexia, dyspepsia, flatulence, leprosy, migraine, malaria and fever etc. Further research is necessary to isolate and purify the active compounds in this plant, which allow the scientific community to recommend their utilization as an accessible alternative to

synthetic antibiotics.

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