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Full Length Research Paper

Isolation of potential antibacterial and antioxidant compounds from *Acalypha indica* and *Ocimum basilicum*

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In recent years multiple drug resistance has been developed in many microbes due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. This paves the way for reconsidering traditional medicine; hence a study was carried out to explore the antimicrobial activities of the acetone and ethanol extract of *Acalypha indica* and *Ocimum basilicum* against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus* sp. The results revealed that the ethanol extract of both the plants was more effective than acetone extract particularly on *Proteus* sp. Hence, both the plants can be vitally used in treating various diseases caused by those pathogens.

Key words: *Acalypha indica*, *Ocimum basilicum*, antibacterial activity, antioxidant activity.

INTRODUCTION

The herbal medicine has recently been used in the United Nations and other developing countries as an alternative to conventional medicine. In many parts of the world, medicinal plants have continued to be an integral part of the health care system and the people's culture. Traditional medical treatments in daily life are now being used with empirical methods (Tanaka et al., 1999).It was also reported by Gupta (2003) that contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infections and diseases. Akhtar et al. (1989) revealed that the prevalence of multi-drug resistant strains of bacteria, the recent appearance of strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectra of untreated bacterial infections and adds urgency to the search for new infections finding strategies.

Since the use of medicinal plants have seconded a wide range in treating diseases, the present study concentrates on commonly available medicinal plants *Acalypha indica* and *Ocimum basilicum* and their effects on human pathogens like, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus* sp.

A. indica belongs to the family Euphorbiaceae. The leaf can be used for the treatment of throat infections and wound healing, and also used as anti-venom and migraine pain relief. *O. basilicum* belongs to Lamiacheae, distributed throughout the Southern part of Tamil Nadu. It is used for various applications as poultice or salve for insect bites, acne and ringworms, as a gargle for mouth or thrush, as a bath herb for increased energy and eye wash for tired eyes. The essential oils of the basil are added to massage for sore muscles. The dried herb used as antiseptic incense and the juice can be applied to fungal infections (Oudhia, 2003; Valsara, 1994).

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Abbreviation: MIC, Microbial inhibition concentration; **FTC,** ferric thiocynate; **MTCC,** microbial type culture collection gene bank.

MATERIALS AND METHODS

Plant materials

Whole plants of *A. indica* and *O. basilicum* were collected from a

Table 1. Antibacterial activity (in mm) of acetone and ethanol extracts of *A. indica* and *O. basilicum* against selected bacterial strains.

Bacterial strain	*Acalypha indica*						*Ocimum basilicum*					
	20 µl		40 µl		60 µl		20 µl		40 µl		60 µl	
	A	B	A	B	A	B	A	B	A	B	A	B
Escherichia coli	3	13	12	12	7	10	3	19	5	16	7	16
Klebsiella pneumonia	1	0	3	0	9	0	2	10	2	12	4	12
Staphylococcus aureus	2	11	12	11	9	16	3	8	7	9	13	10
Proteus sp.	16	16	12	11	8	18	4	13	2	18	12	22
Pseudomonas aeruginosa	2	11	7	17	12	12	2	11	2	16	4	11

A: Acetone extract; B: ethanol extract.

green house attached to the college campus, Lady Doak College, Madurai and authenticated by Botanical Survey of India, Coimbotore. A voucher specimen was deposited in our departmental laboratory. The whole plant was refluxed in running tap water for 1 - 2 h. Leaves were detached and surface sterilized by 0.1% (w/v) $HgCl_2$ with two drops of Tween 80 for 2 min (Jain et al., 1970; Tepe et al., 2005), followed by rinsing thrice with sterile distilled water until all traces of sterilent were removed.

Bacterial cultures

E. coli, *K. pneumonia*, *S. aureus, P. aeruginosa* and *Proteus sp* were obtained from MTCC, Chandigar, India.

Extraction

Surface sterilized leaves were subjected to ethanol and acetone solvent extraction. Samples were extracted with solvent one after another by Soxhlet apparatus for about 24 h (Brantner and Grain, 1994).

Antimicrobial activity

Antibacterial assay of the crude extracts of acetone and ethanol of both plants were performed on nutrient agar plate with discs enriched with various concentrations (20, 40 and 60 µg) of extracts. The antibacterial activity was measured as the zone of inhibition. The disc enriched with sterilized distilled water was used as a control.

For the determination of minimum inhibitory concentration, all the bacterial cultures were co- cultivated with various concentrations of both extracts in 5 ml of nutrient broth medium. After the specified incubation period (24 h at 37°C), 0.1 ml of cultures from all the test tubes were plated on nutrient agar medium to find out the MIC(Cappucino, 1999).

Antioxidant activity

The antioxidant activities of acetone and ethanol extract of leaves of *A. indica* and *O. basilicum* were determined by ferric thiocynate method (Karthikumar et al., 2007). 10 mg of each extract was dissolved separately in 99.5% of ethanol and various concentrations (50, 100, 250, 500 µg/mL) were prepared. A mixture of 2 ml of sample in 99.5% ethanol, 2.052 ml of 2.51% linoleic acid in 99.5% ethanol, 4 ml of 0.05 M phosphate buffer (pH 7.0) and 1.948 ml of water were placed in a vial with a screw cap and placed in an oven at 60°C in the dark. To 0.1 ml of this sample solution 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocynate was added. 0.1 ml of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture and the absorbance of the red color developed was measured after 3 min at 500 nm (Matook and Hashinaga, 2005). The control and standard were subjected to the same procedures as the sample, except that for the control, only solvent was added, and for the standard, sample was replaced with the same amount of α -tocopherol (Ali Yildirim et al., 2001). The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\% \text{ Inhibition} = 1 - (A1/A2) \times 100$$

Where A1 was the absorbance of the test sample and A2 was the absorbance of control reaction.

RESULTS AND DISCUSSION

Table 1 shows the antibacterial activities of acetone and ethanol extracts of *A. indica* and *O. basilicum* against tested organisms. In this present investigation, when the acetone extract was compared to the ethanol extract of the plants, it recorded significant zone of inhibition activities against all the tested bacterial strains. The ethanolic extract of *O. basilicum* showed very effective activity against *E. coli* (19 mm), *Klebsiella* (12 mm), *Proteus* (10 mm) whereas *A. indica* was found to be effective only against *P. aeruginosa* (18 mm) and *S. aureus* (16 mm). The acetone extract of *A. indica* showed the maximum zone of inhibition for *Proteus* (16 mm) and the acetone extract of *O. basilicum* exhibited significant result against *S. aureus* and *Proteus* ranging from 13 and 12 mm, respectively. Broth dilution screening for antibacterial activity showed a promising effect. The ethanol extract of *A. indica* inhibited the growth of *E. coli* at 20 µg/0.1 ml concentration. Similarly, the ethanol extract of *O. basilicum* showed MIC at 20 µg/0.1 ml, 40 µg/0.1 ml for *E. coli* and *Klebsiella,* respectively, whereas, the acetone extracts of *A. indica* and *O. basilicum* showed MIC for *Proteus* at 20 µg/0.1 ml and 60 µg/0.1 ml, respectively (Table 2).

The antioxidant activities of the acetone and ethanol extracts of *A. indica* and *O. basilicum* were determined by ferric thiocynate FTC and the values are presented in

Table 2. Minimum inhibitory concentration of acetone and ethanol extracts of *A. indica* and *O. basilicum* against selected bacterial strains.

Bacterial strain	*Acalypha indica*		*Ocimum basilicum*	
	Acetone extract (µg/0.1 ml)	Ethanol extract (µg/0.1 ml)	Acetone extract (µg/0.1 ml)	Ethanol extract (µg/0.1 ml)
Escherichia coli	40	20	70	20
Klebsiella pneumonia	80	90	100	60
Staphylococcus aureus	40	60	60	60
Proteus sp.	20	60	60	60
Pseudomonas aeruginosa	60	40	90	40

Table 3. Antioxidant activity of ethanol extracts of *A. indica* and *O. basilicum*.

Extract	% Inhibition							
	50 µg/ml		100 µg/ml		250 µg/m		500 µg/ml	
	A	B	A	B	A	B	A	B
Acetone	8.43	15.64	24.69	12.3	35.72	34.92	38.92	36.61
Ethanol	45.21	35.36	47.34	39.36	58.46	56.19	64.38	75.87
α - Tocopherol	59.41	62.9	66.84	75.94	79.82	80.25	82.14	82.36

A: *Acalypha indica*; B: *Ocimum basilicum.*

Table 3. FTC method was used to determine the amount of peroxide formed and that which reacted with ferrous chloride ($FeCl_2$) to form a reddish ferric chloride ($FeCl_3$) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases. Acetone and ethanol extracts of both plants at various concentrations (50, 100, 250 and 500 in µg/ml) showed antioxidant activities in a concentration dependent manner. However, ethanol extract of *O. basilicum* at the concentration of 500 µg/ml showed 75.87% activity, an antioxidant activity very close to that of 500 µg/ml of α-tocopherol (82.14%), the reference compound. It has been observed that the extract exhibited strong activity with the increase in polar solvent, indicating that polyphenols, flavanone, and flavanoids may play important roles in the activities. The present findings are in agreement with the report of Tepe et al. (2005).

When compared to *A. indica*, *O. basilicum* is very effective against *E. coli*, *Klebsiella* and *Proteus.* Furthermore, it possesses very strong antioxidant activity than *A. indica*, whereas, *A. indica* was found to be active against *S. aureus* and *P. aeruginosa.*

Conclusion

The healing power of plants is an ancient idea and traditional medicine is an integral part of rural health care. In many areas, local plants are effectively used to treat ailments. In this study *A. indica* and *O. basilicum* were chosen because they are easily available, economical and have high medicinal values. The study revealed that the ethanol extract of *A. indica* and *O. basilicum* were more effective against the pathogens when compared with the acetone extract. Furthermore, both plant extracts have significant antioxidant properties, which reveal that the compounds responsible for these antibacterial and antioxidant activities might be tannins, flavonol, terpenoid or alkaloids (Oudhia, 2003). Further study can be done on identifying and isolating the active components responsible for these antibacterial activities of these plants.

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