

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 ethanol extract of both the plants were 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

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy, 2001; Erdoorul, 2002; Atef and Erdoorul, 2003). Much work has been done on ethnomedicinal plants in India (Maheshwari, 1986; Rai, 1989; Negi, 1993). Interest

in a large number of traditional natural products has increased (Taylor, 1996).

In the present study deliberates 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. There are four known kaempferol glycosides, mauritianin, clitorin, nicotiflorin and biorobin, have been isolated from the flowers and leaves of *Acalypha indica* (Nahrstedt, 2006). The presence of these flavonoids could be responsible for the wide range of antimicrobial activities. *O. basilicum* is belongs to Lamiales, distributed throughout the southern part of Tamil nadu. It is used for various applications as poultice or salve for the insect bites, acne and ringworms, as a gargle or mouth for 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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Table 1. Antibacterial activity (in mm) of acetone and ethanol extracts of *Acalypha indica* and *Ocimum basilicum* against selected bacterial strains.

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

A: Acetone extract; B: Ethanol extract.

MATERIALS AND METHODS

Plant materials

Whole plants of *A. indica* and *O. basilicum* were collected from green house attached to the college campus, Lady Doak College, Madurai and authenticated by Botanical Survey of India, Coimbatore. 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) HgCl₂ with two drops of Tween 80 for 2 min (Jain et al., 1970), followed by rinsing thrice with sterile distilled water until all traces of sterility are 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 the plants were performed on Nutrient agar plate with discs enriched with various concentrations (20, 40, 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 the extracts in 5ml 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 thiocyanate 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 a 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 was 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 thiocyanate was added. After the addition of 0.1 mL of 2 x 10⁻² M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red color developed was measured in 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 (Yildirim et al., 2001). The inhibition of lipid peroxidation in percentage was calculated by following equation:

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

Where; A1 was the absorbance of the test sample and A2 was the absorbance 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 compare to acetone extract, the ethanol extract of the plant recorded significant zone of inhibition activities against all the tested bacterial strains. The ethanolic extract of *O. basilicum* showed very effective against *E. coli* (19mm), *Klebsiella* (12 mm), *Proteus* (10 mm) where as *A. indica* is 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 20µg/0.1ml concentration. Similarly the ethanol extract of *O. basilicum* showed MIC at 20 µg/0.1ml, 40µg/0.1ml for *E. coli* and *Klebsiella* respectively. Where as, the acetone extracts of *A. indica* and *O. basilicum* showed MIC for *Proteus* at 20µg/0.1ml and 60µg/0.1ml respectively (Table 2). The antioxidant activity

Table 2. Minimum Inhibitory Concentration of acetone and ethanol extracts of *Acalypha indica* and *Ocimum basilicum* against selected bacterial strains.

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

Table 3. Antioxidant activity of Ethanol extracts of *Acalypha indica* and *Ocimum basilicum*.

Extract	% of 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*

of the acetone and ethanol extracts of *A. indica*, and *O. basilicum* were determined by ferric thiocyanate (FTC) and the values are presented in Table 3. FTC method was used to determine the amount of peroxide formed and that react 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 the plants at various concentrations (50, 100, 250 and 500 in µg/mL) showed antioxidant activities in a concentration dependent manner. However ethanol extract of *Ocimum basilicum* at the concentration of 500 µg/mL showed 75.87%, 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 poly-phenols or flavanone or flavanoids may play important roles in the activities.

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

Conclusion

Healing power of the plant is an ancient idea and the 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 the plant extracts have significant antioxidant properties, which reveal that the compounds responsible for this 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 this antibacterial activity of those plants.

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