



## ORIGINAL RESEARCH ARTICLE

SCREENING OF ANTIMICROBIAL ACTIVITY OF *HELICTERES ISORA*, *CICHORY INTYBUS* AND *LITSEA CHINENSIS*Asha Pandey<sup>\*1</sup>, Anshulika Upadhyaya<sup>2</sup> and Kamal K Pande<sup>2</sup><sup>1</sup>Department of Biotechnology & Allied Science, M.B. Govt. P.G. College, Haldwani Nainital, Uttarakhand, India<sup>2</sup>Department of Chemistry and Biotechnology & Allied Science, M.B. Govt. P.G. College, Haldwani Nainital, Uttarakhand, India

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**Abstract:** *Helicteres isora*, *Cichory intybus* and *Litsea chinensis* species samples of leaf and stem were collected from Haldwani Distt (Nainital). Samples were chopped, shed dried and grinded in electronic grinder. After grinding crude extracts were extracted using different solvents viz., Methanol, Chloroform and Acetone. The solvents were evaporated after filtering with whatman filter paper. Antimicrobial activity was evaluated against bacterial strains (*E. coli*, *Pseudomonas*, *S. aureus* and *Bacillus subtilis*) antifungal strains (*Aspergillus fumigatus*, *Aspergillus awamori*, *Rhizopus oryzae*, *Trichoderma viridae* and *Culmularia oryzae*) by using disc diffusion method.

**Key words:** *Helicteres isora*; *Cichory intybus*; *Litsea chinensis*; Stems; Leaves; Solvent Extracts; Antimicrobial Activities.

## INTRODUCTION

About three quarters of world's population is estimated to be dependent mainly on plants and plant extracts for the care of their health (Jamil et al., 2007). Medicinal plants possess potent medicinal value owing to the presence of a variety of phytochemical constituents in the plant tissues which cast a definite physiological action on the human body. A very few of these chemicals are toxic too (Sheeba; 2010). There are a few reports on the use of plants in traditional healing by either tribal people or indigenous community (Sandhy et al., 2006; Ayyanar and Ignacimuthu, 2005; Rajan et al., 2002; Natarajan et al., 1999; Ignacimuthu et al., 1998). The activities have been selected because of their great medicinal relevance with the recent years. Infections have increased to a great extent and their resistance against antibiotics becomes an ever increasing therapeutic problem (Austin et al., 1999). Natural products of higher plants may give a new source of antimicrobial agents. There are research groups that are now engaged in medicinal plants research (Samy et al., 1998; Hamil et al., 2003; Motsei et al., 2003).

*Helicteres isora* belongs to family sterculiaceae and commonly known as Maror phalli. It is a small tree with grey coloured bark, simple leaves, serrate margin distributed widely in forests throughout India and commonly known as East Indian screw tree. The plant is a medicinally important sub-deciduous shrub or a small tree (Varsha et al., 2010). Traditionally, the root, juice and bark of *H. isora* were used against emphysema and diabetes. It is also used as expectorant, astringent, antagalactagogue, to reduce gripping and a cure for snakebite (Kirtikar and Basu; 1993, Singh et al., 1984). *Helicteres isora* fruits are used as astringent, stomachic, vermifuge, and useful in bowel gripes (Chopra et al., 1956). Cell cycle inhibitory

activity of *H. isora* against FT210 cell line was reported by (Quinchun et al., 2005). Fried pods of *H. isora* are given to children to kill intestinal worms ([www.flowerofindia.net](http://www.flowerofindia.net)).

*Cichorium intybus* commonly known as Chicory, Kasani, blue daisy, blue dandelion, blue weed bunk. *Cichorium* is a genus of flowering plants in the family Asteraceae. The species are commonly known as chicory, there are two cultivated species and four to six wild species. It grows as a wild plant on roadsides ([www.wikipedia.org](http://www.wikipedia.org)).

According to the folklore, the juice is said to be a remedy for cancer of the uterus and for tumors. It is well known as a coffee substitute but is also widely used medicinally to treat various ailments ranging from wounds to diabetes. A widespread weed, leaves, stems, and roots are made into a tea for Jaundice and chicory syrup is used as tonic and purifying medicine for infants (Ranee et al., 1987).

*Litsea chinensis* or Lam commonly known as "Meda, Medalaki, Medasakah". Lam belongs to a family Lauraceae. It is a perennial aromatic erect tree or shrub, growing in evergreen forest area at height with a large and well developed trunk up to 1-2ft in diameter and mean sea level of 5000-11000ft of north east states of India (Balakrishnan; 1996).

The family comprises over 3000 species of flowering plants in over 50 genera worldwide. The family are among the top 5 families in terms of the no. of species ([www.wikipedia.org](http://www.wikipedia.org)).

Its bark is good in stomach problems, antiseptic to cure inflammation, wound, and bleeding

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diarrhea. Aromatic leaves are useful in the treatment of spontaneous and excessive flow of semen in young boys. The wood is used in structural timber and excellent for fuel wood. Fruits are edible and yield a fat that is used in soap industry (Lohita, 2010).

### MATERIAL AND METHOD

Preparation of the extract: The aerial parts of *Helicteres isora*, *Cichcory intybus* and *Litsea chinensis* including leaves and stems were collected from Haldwani. It is situated on piedmont grade called Bhabhar where the Mountain Rivers go underground to re-emerge in the Indo gangetic plane, it has an average elevation of 424 meters and is located at 29.22 N and 79.52 E. The plant materials were washed thoroughly and dipped in 70% ethanol for sterilization and dried. The dried samples were then chopped in fine pieces and shade-dried at an ambient temperature (31°C) and were powdered using an electronic blender. The powdered mixture was then soaked in different solvents (acetone, methanol and chloroform) for 72 hrs. After filtering the contents using Whatman No 1 filter paper, the filtrate was left at room temperature for 48 hrs to evaporate partially. The remaining crude extracts thus obtained were used for further analysis. Colours of the extracts were recorded (Table 1)

**Table 1:** Colour of the different plant extracts in solvents

Plant	Solvent	Colour
<i>Helicteres isora</i>	stem+ acetone	Parrot green
	Stem+chloroform	Pale yellow
	Leaf + acetone	Dark green colour
	Leaf + Chloroform	Dark green colour
	Leaf + methanol	Dark green colour
<i>Cichcorium intybus</i>	stem+ acetone	Lemon
	Stem+chloroform	Pale yellow
	Leaf + acetone	Dark green colour
	Leaf + Chloroform	Dark green colour
	Leaf + methanol	Dark green colour
<i>Litsea chinensis</i>	stem+ acetone	Pale yellow
	Stem+chloroform	Lemon
	Leaf + acetone	Dark green colour
	Leaf + Chloroform	Dark green colour
	Leaf + methanol	Dark green colour

**Media Composition and Preparation:** For bacterial culture the media used for checking the antibacterial activity is 'nutrient agar'. Nutrient agar is a microbiological growth medium commonly used for the routine cultivation of bacteria. However, for fungal culture the growth supplement needed is different and therefore Sabouraud dextrose agar is used. Sabouraud dextrose agar is a microbiological growth medium commonly used for the routine cultivation of fungus. The Test Microorganisms used for the screening were the following:

### Bacterial strains

*E. coli* (gram -ve), *Staphylococcus aureus* (gram +ve), *Pseudomonas* (gram -ve) and *Basillus subtilis* (gm +ve).

### Fungal strains

*Aspergillus fumigatus*, *Aspergillus awamori*, *Rhizopus oryzae*, *Tricoderma viridae* and *Culmuralia oryzae*. These strains were maintained in Microbiology lab, Dept. of Biotechnology and Allied Sciences of M.B Govt. PG. College Haldwani.

### Preparation of Culture

To prepare the media firstly, luke warm the autoclaved bacterial and fungal media under running tap water. Secondly, all the activities are to be performed in laminar air flow near the Bunsen burner flame. The bacterial media and the fungal media prepared were inoculated with the bacterial strains and fungal strain. Thirdly, fully prepared media were poured into different Petri-plates. Finally, leave the Petri-plates for 40 minutes providing them low temperature to solidify.

### Determination of Antibacterial and Antifungal Activity

The antibacterial and antifungal activity of all the solvent extracts of *Helicteres isora*, *Cichcory intybus* and *Litsea chinensis* (stems and leaves) was evaluated by disc-diffusion method (Maruzzella & Henry, 1958). When media is solidified or set, the disc (6 mm) of whatman no 1 filter paper was soaked in crude solvent viz. methanol, acetone and chloroform and placed carefully in the centre of Petri-plates containing the solidified media. To compare the antimicrobial activity same concentration of the solvent using disc is placed in plate which acts as control to our crude solvents. Same procedure is applied for the remaining Petri plates (for different solvents). The plates were incubated at 37°C for 24 hrs for bacterial culture and at 28°C for 48 hrs for fungal culture. The antimicrobial activities were assayed by measuring the resultant 'zone of inhibition' with the help of ruler.

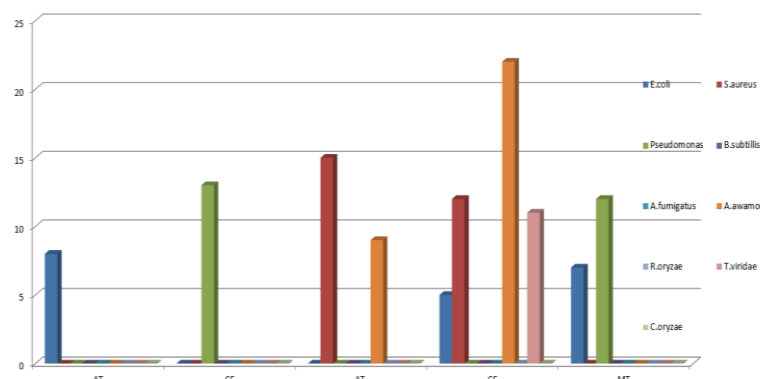
### RESULT AND DISCUSSION

The results of the antimicrobial activity tests of crude extracts are shown in table 2. It was found that the crude extracts of *Helicteres isora*, *Cichcory intybus* and *Litsea chinensis* at 100mg/mL concentration exhibited various antibacterial and antifungal activity.

**Table 2:** Antimicrobial activity tests of *H. isora* plant crude extracts

Plant name	Crude Solvents	Leaf extracts			Stem extracts	
		AT	CF	MT	AT	CF
<i>H. isora</i>	<b>Pathogenic Microorganisms</b>					
	Gram positive Bacteria					
	<i>B.subtillis</i>	0	0	0	0	0
	<i>S.aureus</i>	15	12	0	0	0
	Gram negative Bacteria					
	<i>Pseudomonas</i>	0	0	12	0	13
	<i>E.coli</i>	0	5	7	8	0
	<b>FUNGI</b>					
	<i>A.fumigatus</i>	0	0	0	0	0
	<i>A.awamori</i>	9	22	0	0	0
	<i>R.oryzae</i>	0	0	0	0	0
<i>T.viridae</i>	0	11	0	0	0	
<i>C.oryzae</i>	0	0	0	0	0	

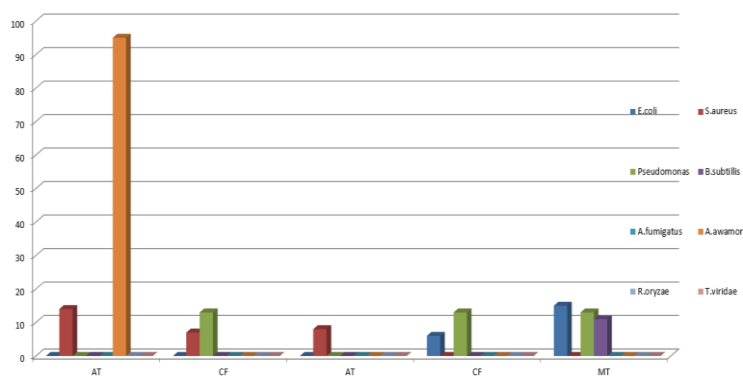
MT- Methanol, AT-Acetone, CF- Chloroform  
Zone of inhibition in mm



**Table 3:** Antimicrobial activity of *C. intybus* plant crude extracts

Plant name	Crude Solvents	Leaf extracts			Stem extracts	
		AT	CF	MT	AT	CF
<i>C. intybus</i>	<b>Pathogenic Microorganisms</b>					
	Gram positive Bacteria					
	<i>B.subtillis</i>	0	0	11	0	0
	<i>S.aureus</i>	8	0	0	14	7
	Gram negative Bacteria					
	<i>Pseudomonas</i>	0	13	13	0	13
	<i>E.coli</i>	0	6	15	0	0
	<b>FUNGI</b>					
	<i>A.fumigatus</i>	0	0	0	0	0
	<i>A.awamori</i>	0	0	0	95	0
	<i>R.oryzae</i>	0	0	0	0	0
<i>T.viridae</i>	0	0	0	0	0	
<i>C.oryzae</i>	0	0	0	0	0	

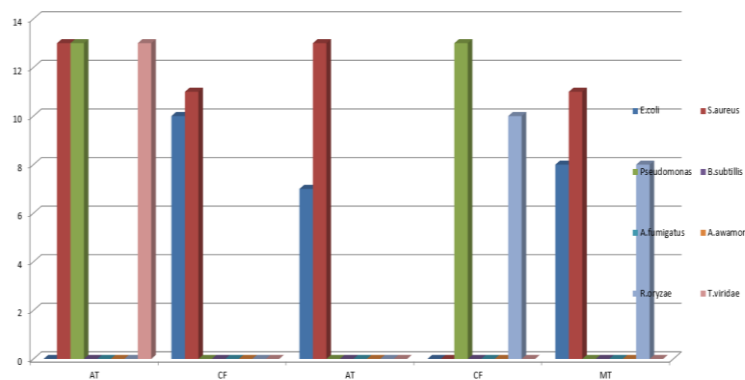
MT- Methanol, AT-Acetone, CF- Chloroform  
Zone of inhibition in mm



**Table 3:** Antimicrobial activity tests of *L. chinensis* plant crude extracts

Plant name	Crude Solvents	Leaf extracts			Stem extracts	
		AT	CF	MT	AT	CF
<i>L. chinensis</i>	<b>Pathogenic Microorganisms</b>					
	Gram positive Bacteria					
	<i>B.subtillis</i>	0	0	0	0	0
	<i>S.aureus</i>	13	0	11	13	11
	Gram negative Bacteria					
	<i>Pseudomonas</i>	0	13	0	13	0
	<i>E.coli</i>	7	0	8	0	10
	<b>FUNGI</b>					
	<i>A.fumigatus</i>	0	0	0	0	0
	<i>A.awamori</i>	0	0	0	0	0
	<i>R.oryzae</i>	0	10	8	0	0
<i>T.viridae</i>	0	0	0	13	0	
<i>C.oryzae</i>	0	0	0	0	0	

MT- Methanol, AT-Acetone, CF- Chloroform  
Zone of inhibition in mm



**Antifungal Activity**

In *Helicteres isora*, against *A. fumigates*, *R. oryzae*, *C. oryzae*, no activity was shown by any crude extract of leaf. Chloroform extract of *H. isora* showed highest activity against *A. awamori*. Then comes the acetone extract, while methanol extract showed zero activity. In the chloroform and acetone stem extract no activity is shown against any fungal strains.

In *Cichorium intybus* all crude extracts of stem samples showed no activity against *A. fumigates*, *T. viridae*, *R. oryzae* and *C. oryzae*. Acetone showed highest zone of inhibition while chloroform showed no

activity against *A. awamori*. While the crude extracts of leaf showed no activity against any fungal strains.

In *Litsea chinensis* the crude extract of stem in acetone showed activity against *T. viridae* fungal strain while the extract of stem in chloroform showed no activity. While in leaf sample, against *A. fumigates*, *A. awamori*, *T. viridae* and *C. oryzae*, no activity was shown by any crude extracts. However, in case of *R. oryzae* only chloroform and methanol show highest activity while acetone showed no activity.

### CONCLUSION

From the present investigation it is clear that leaves and stem of *Helicteres isora*, *Cichorium intybus* and *Litsea chinensis* are potent antimicrobial agents, which can be further used for the preparation of herbal drugs.

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