

Studies on an Antibacterial Capability of *Helicteres Isora*

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Abstract: Antibiotics played a key role in development of the modern, pharmaceutical industry. Using the commercial antimicrobial drugs soon came up with their limitations, side effects thus making the medical practitioners to think about alternative medical branch. Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity. Present study focuses on potential medicinal properties of *Helicteres isora*, which are used by the people as a folk remedy for various health problems. Fruits of this plant is extracted in four solvents. Antibacterial sensitivity was conducted by well plate method using Sterile Mueller Hinton agar. Antimicrobial activity is measured against Microbial Strains of *E. coli*, *S. aureus*, *P. aeruginosa*, *klebsiella pneumonia*. Zone of inhibition were observed which are measured in mm. Antibacterial capability pattern of all four strains in different solvent extracts were studied.

Keywords: Murudsheng, Common pathogenic bacteria, Organic solvent extraction, Well plate method, Zone of Inhibition

1. Introduction

Bacteria are recognized as the most potent source for various infectious and transmittable diseases. Infectious diseases are the world's leading cause of premature deaths killing almost 50,000 people every day. The use of antimicrobial drugs often considers one of the wonders of modern world. It had achieved dramatic effect on practice of medicine. But antibiotics and its use had a disaster side effect on human body due to Drug-drug interaction, Drug related toxicity Allergies (Hyper sensitivity), Excessive cost, Drug resistance. Some adverse effects of the antibiotics chloramphenicol induce anaemia, Sulphonamide is toxic cause epidermal necrosis. Oxacillin, induce hepatitis in HIV patients. Diseases causing microbes have become resistance to antibiotic therapy. Clinical use of antibiotics has got its own limitations. Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity. Plants have been utilized as medicines for thousands of years.

The *H. isora* (Murul Sheng), a traditional medicine, belongs to family sterculiaceae is a sub – deciduous shrub or small tree of having spreading habit with stem 1-5 inches in diameter, reaching a height of 5 to 15 feet. Species is native to Asia and Australia. It occurs throughout India from Jamuna Eastwards to Nepal, Bihar and West Bengal, and southern India and Andaman Island. It occurs as undergrowth, especially as a secondary growth in forest. The plant is commonly called as “Mrigashringa” in Sanskrit, is an important medicinal plant described in indigenous system of medicine. Its root juice is claimed to be useful in cough, asthma, stomach affections, diabetes and cure scabies when applied topically. Fruits are demulcent, mildly astringent and useful in griping and flatulence. Aqueous ethanol and butanol extracts of *H. isora* root has been reported to possess significant antihyperglycemic activity in both alloxan- and glucose- induced hyperglycaemic rats at a dose of 250 mg/kg. The fruits of it have been use in the indigenous

system of medicine in India for the treatment of griping bowels and diarrheal diseases (Krishnarju et al 2006).

2. Related Work

Antibiotics played a key role in development of the modern, pharmaceutical industry. However, Florey, Ernst chain & their colleagues was paramount in initiating the antibiotic Era (Florey. H. W. 1949). The roots and bark of *Helicteres isora* are expectorant, demulcent, hypoglycaemic and useful in colic, scabies, gastropathy, diabetes, diarrhoea and dysentery (Singh et al, 1985, Kirtikar and Basu, 1995, Prajapati et al 2003, Kumat et al 2006). The fruits are astringents, refrigerant, stomatic, vulnerary and useful in griping of bowels, flatulence of children and antispasmodic (Chopra et al, 1956, Pohocha and Grampurohit, 2001). The literature survey reveals the presence of flavones, triterpenoids, phytosterols, saponins, phlobatannins and sugar (Scarpati M. L. et al, 1958, Kelley C. J. et al, 1975). The fruit extract of *H. isora* possess weak anti HIV activity (WuT-S et al, 1998) The ethanolic root extract possess a cytotoxicity in the human nasopharyngeal carcinoma cell culture assay (Nagai Y. et al, 1995). The fruit also found to possess significant antispasmodic activity S.Venkatesh et al studied on Antimicrobial activity of *Helicteres isora* Root. Y. S. Banginwar et al evaluated antibacterial potential of *helicteres isora* l. fruits against enteric bacterial pathogens. The fruit aqueous extracts of *H. isora* showed prominent antibacterial activities against *E.coli*, *Staphylococcus epidermidis*, *Salmonella typhimurium* and *Proteus vulgaris*; moderate activity against *Enterobacteraerogenes*, *Staphylococcus aureus*, *Salmonella typhi* and least activity against *Pseudomonas aeruginosa*.

Helicteres isora L. has shown medicinal importance. *H. isora* is also known as the Indian screw tree. It demonstrates various health benefits, including anticancer activity (Varghese et.al., Rattanamaneerusee et.al.) antibacterial

and antiplasmid activities. (Shriram *Vet.al.*) And cardiac antioxidant and antiperoxidative potency (Kumar *et al.*) (Prajapati *et al*, 2003). Badgujar *et al*, (2006) had studied antimicrobial activity of Stem bark of *H. isora* and showed antimicrobial activity against *S. aureus*, *Bacillus subtilis*, *P. aeruginosa* and *E. coli*. In the present study, the fruits extracts also showed similar antibacterial activities against these pathogens. Bonjar *et al*, (2004) showed anti-*Candida albicans* activity of fruits of *H. isora*. *Helicteres isora* Linn. has been used for treating various ailments such as diarrhea, sore ear, snake bite, eczema, diabetes, flatulence which reflects its ethanobotanical, pharmacological relevance (Mohan *et al.*, 2015)

Fruit is used in ripping of bowel, flatulence and stomach problems in Santa district of Madhya Pradesh (Divedi *et al.*, 2006). Fruit is also used for gastrointestinal disorder and on a snake bite in the Marundivalmalai, Western Ghats, Tamil Nadu.

3. Experimental Method

1) Preparation of media:

Mueller Hinton Agar was weighted and dispense in D/W & autoclaved at 15 lbs pressure at 121⁰ c for min. For antimicrobial assay well plate technique were used. (John v. Bennett & *et al*)

2) Preparation of materials:

Petriplates, cork borer, micropipette tips, forceps, test tubes, and saline test tubes were autoclaved at 15 lbs pressure at 121c for 15 min.

3) Preparation of Inoculum:

Two or three well isolated colonies of same morphology are selected from overnight grown nutrient agar plate: loopful of growth is transport to test tube containing 5 ml of sterile saline. The correct density of turbidity standard is verified by using colorimeter. The absorbance at 660nm should be 0.1, this corresponds to 10⁶ – 10⁸ cells/ml.

4) Preparation Plant extracts:

Four organic solvents Ethanol, Methanol, acetone, Petroleum ether are used for extract

The 100gm dried fruits of *H. isora* plant are weighted and coarsed into fine powder using mixer and kept in a 500 ml beaker. Methanol is added and kept in a room temperature for 24 hours with shaking the beaker periodically. After 24 hr. Incubation the extract were filtered and kept in water bath at 45°C. till the solvent evaporate. Same procedure repeated for other three solvents.

Sensitivity testing was conducted by well plate method. Solvent extract used as source for testing antibacterial activity. Sterile butts of media are prepared and inoculated with 1 ml suspension of certain microorganism. This butts are poured into sterile plates and allow to solidify. After solidification, with the help of sterile cork borer (8mm) four wells are made in each plate. 50 microliter extract was added in each well. The well filled with solvent was kept as a control. Plates are transferred to incubator at 37°C

4. Results and Discussion

After 24 hours of incubation, zone of inhibition were observed which are measured in mm and are as follows.

Table 1

Microorganisms	Organicsolvent (ZOI inMM)			
	Ethanol	Methanol	PetroleumEther	Acetone
<i>E.coli</i>	9	12	12	16
<i>S. aureus</i>	9	NI	12	13
<i>P. aerogenosa</i>	9	8.5	NI	10
<i>K. pneumoniae</i>	10	8.5	8.5	11

NI = No Inhibition

ZOI= Zone Of Inhibition



Figure 1: Grinded in mixer

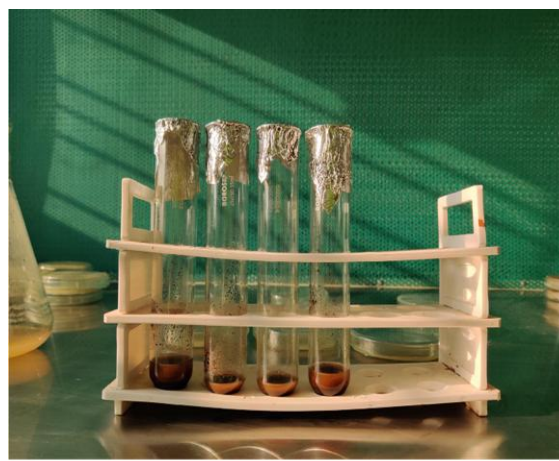


Figure 2: Four solvent extract obtained



Figure 3: 8mm well are made in agar plate

The observation table 1 indicate the antimicrobial sensitivity pattern, in which all four microbial strains include *E. coli*, *S. aureus*, *P. aeruginosa*, *klebsiellapneumoniae*. Shows inhibition of growth against all four extract of *H. isora*. Ethanol extract show antimicrobial activity against all four strains. Methanol extract does not shows any activity against *S. aureus*. Petroleum ether extract does not shows any activity against *P. aerogenosa*. The extract in acetone show maximum zone of inhibition as compared with others.



Figure 4: Antimicrobial activity against *K. pneumoniae*



Figure 5: Antimicrobial activity against *S. aureus*



Figure 6: Antimicrobial activity against *P. aerogenosa*

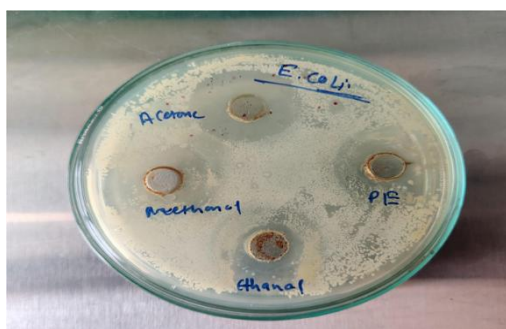


Figure 7: Antimicrobial activity against *E. coli*

5. Conclusion

From present study it can be conclude that, The four solvent extract of *Helicteresisora* have antibacterial potential against *E.coli*, *S. aureus*, *P. aeruginosa* and *K. Pneumoniae*. Acetone extract of *Helicteresisora* showed maximum antibacterial activity when compared with other three solvent extracts i.e. Methanol, Petroleum ether and Ethanol. Acetone is better solvent for extraction of active compound from *Helicteresisora* because, the extract in acetone show maximum zone of inhibition when compare with other solvent used for extraction.

The solvent extract of *Helicteresisora* show least activity against *P. aeruginosa* and *K. Pneumoniae*. It is clear from the results that, antimicrobial activity is due to the presence an active biomolecules present in the plant.

6. Future Perspectives

It is clear that *Helicteresisora* plant's fruits used by people against diarrheal diseases showed antibacterial activities. Although the nature and number of active antibacterial principles involved in fruit paste of *Helicteresisora* are not clear in present research, but the broad spectrum activity of fruit paste especially on enteric pathogens is promising. The present study suggests that fruit of *Helicteresisora* are antibacterial against enteric and diarrheal bacterial pathogens. The result of present study may form the basis of further investigation to isolate bioactive compounds. Separation, identification and purification of active compound by chromatographic technique can be done. Further cytotoxicity testing, lead development and clinical trials can be conducted. The mode of action of drug molecule to be investigated. Formulation of extract for clinical use as well as its antiviral sensitivity pattern can be studied

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