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EVALUATION OF PHYTOCHEMICAL AND ANTIBACTERIAL POTENTIAL OF *HELICTERES ISORA* L. FRUITS AGAINST ENTERIC BACTERIAL PATHOGENS

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Abstract

Antibacterial activities of aqueous, acetone, ethanol and methanol extracts of fruits of *Helicteres isora* (Mororphali) were studied. The fruit aqueous extracts of *H. isora* showed prominent antibacterial activities against *E.coli, Staphylococcus epidermidis*, *Salmonella typhimurium* and *Proteus vulgaris*; moderate activity against *Enterobacter aerogenes, Staphylococcus aureus, Salmonella typhi* and least activity against *Pseudomonas aeruginosa.* The aqueous extract showed maximal, the ethanol and methanol extract moderate and acetone extracts least antibacterial activities. Phytochemical screening revealed the presence of carbohydrates, anthraquinon glycosides, proteins, tannin and phenolic compounds and steroids These antibacterial properties supports its traditional use of fruits of *H. isora* in the treatment of enteric or diarrhoeal infections.

Keywords: Antimicrobial activity, *Helicteres isora*, enteric pathogens, *E.coli, Salmonella typhi, Pseudomonas aeruginosa*)

Introduction

In India, use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times and inherited traditionally. The fruits of *Helicteres isora* Linn (Sterculiaceae) have been used in the indigenous system of medicine in India for the treatment of griping bowels and diarrheal diaseases (Krishnaraju *et al*, 2006). The roots and the bark are expectorant, demulcent, hypoglycemic and useful in colic, scabies, gastropathy, diabetes, diarrhoea and dysentery (Singh et al, 1985, Kirtikar and Basu, 1995, Prajapathi et al, 2003, Kumar 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 barks of *H.isora* showed prominent antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* (Badgujar et al, 2006) and fruits against *Candida albicans* (Bonjar, 2004). The presence of flavones, triterpenoids, cucurbitacin, phytosterols, saponins, sugars and phlobatannins were demonstrated in roots and barks *H.isora* L. (Bean et al, 1985, Satake et al, 1999, Kumar et al. 2007).

Enteric or diarrheal infections are major public health problems in developing countries and contribute to the death of 3.3 to 6.0 million children annually. Enteric bacteria comprised of *Salmonella* spp., *Shigella* spp., *Proteus* spp., *Klebsiella* spp., *E. coli, Pseudomonas* spp., *Vibrio cholerae* and *Staphylococcus aureus* which are major etiologic agents of sporadic and epidemic diarrhea both in children and adults ((WHO, 1985, Ballal, 2005). W.H.O. (1993) reported that 80% populations rely mainly on traditional therapies, involving the use of plant extracts or their active constituents. The use of medicinal plants in India contributes significantly in primary health care and it is interesting to determine whether actual pharmacological effects support the traditional uses or merely based on folklore. The review revealed that the fruits of *H.isora L*. were used in diarrhoeal infection and it is anti-

candidial but so far no information on antibacterial activities of fruits of *H. isora* is available hence, attempt was made to find out phytochemical contents and antibacterial potentials of fruits of *H.isora* against diarrhoeal/enteric bacterial pathogens.

Materials and methods

The fruits of *Helicteres isora* L. were collected in the forest of Melghat in Amravati district, India and authenticated by P.G. Department of Botany, S.G.B. Amravati University Amravati. The dried fruits of *H. isora* were washed with water, $HgCl_2$ (0.5%), sterile distilled water and ground in to fine powder with auto-mix blender. The 20 g fine powder was suspended in 200ml of water or various organic solvents (methanol, ethanol, acetone) and extracted in soxhlet apparatus and vacuum dried. Amount of dry recovered powder extract was recorded (per 20 g of original fruit powder). This dry mass of various extracts *H. isora* L served for experimentations.

The presence of saponins, tannins, anthraquinones, alkaloids, triterpens, flvonoids, glycosides, reduced sugar and phlobatannins were detected by simple qualitative methods (Khandelwal, 2001).

Table 1: Bacterial cultures used in study (IMTECH, Chandigarh, India)

Bacterial Pathogens	MTCC number
Escherichia coli (E.coli)	452
Staphylococcus aureus (S.aureus)	87
Enterobacter aerogenes E.aerogenes)	111
Pseudomonas aeruginosa (P.aeruginosa)	424
Salmonella typhi (S.typhi)	733
Staphylococcus epidermidis (S.epidermidis)	435
Salmonella typhimurium (S.typhimurium)	98
Proteus vulgaris (P. vulgaris)	426

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacteria rejuvenated in Mueller-Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18 hrs and then stocked at 4^0C in Mueller-Hinton Agar. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 mL of sterile nutrient broth and incubated at 37^0 C for 3 hrs. Turbidity of the culture was standardized to 10^5 CFU with the help of SPC and turbidometer.

Agar disc diffusion antibiotic activities: For antibacterial properties, 0.1 ml bacterial suspension of 10^5 CFU ml^{-1} was uniformly spread on Mueller-Hinton Agar plate to form lawn cultures. The aqueous, acetone, ethanol and methanol extracts were prepared in their respective solvents in such a manner that ultimate amount (in dry form) in each disc came to 10mg, 8mg, 6mg, 4mg and 2mg. The blotting paper discs (10mm diameter) were soaked in various diluted extract, dried in oven at 60^0C to remove excess of solvent and tested for their antibacterial activity against bacterial pathogens by disc diffusion technique. After incubation of 24 hr at 37^0C, zone of inhibition of growth was measured in mm. Ampicillin 10mcg (Hi-Media disc) was used as positive control while discs soaked in various organic solvents and dried were placed on lawns as negative control.

Results and Discussion

During the past decades, traditional systems of medicine have become increasingly important in view of their safety. A current estimate suggests that, in many developing countries, a large proportion of population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. The present study was conducted to investigate antibacterial properties of fruits of *H.isora*, which is less studied and used in Indian Folkloric Medicine. Herbal remedies play a fundamental role in traditional medicine in rural areas of India where the therapeutic treatment of choice as antiseptic, anti-inflammatory and in treatment of infectious diseases including diarrhoea. In present study attempt was made to correlate traditional herbal medicinal knowledge held by the Indian native people with modern scientific laboratory-based assay.

Table 2: Average recovery of dry extract from 20g of fruit powder

Type of extract	Amount (g)
Aqueous	12.56
Acetone	10.43
Ethanol	11.26
Methanol	10.15

The amount of dry extracts recovered from various solvents is shown in Table 2. Antibacterial activities of aqueous, acetone, ethanol and methanol extracts of fruits of *H. isora* were studied. The aqueous extracts showed prominent antibacterial activities against *E.coli, S.epidermidis* and

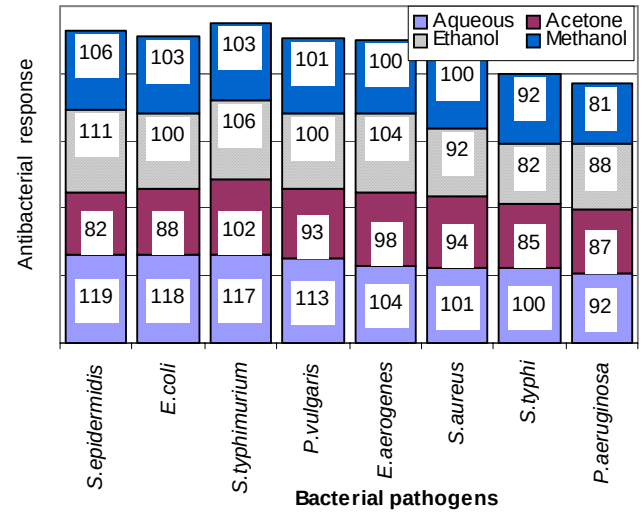
P.vulgaris and *S.typhimurium* whereas it was moderate against *S.aureus, E.aerogenes* and *S. typhi* and least against *P. aeruginosa*. The acetone extract showed maximum activity against *S. typhimurium*, moderate against *S.aureus*, *E.aerogenes* and *P.vulgaris* and least against *E.coli, P. aeruginosa, S. typhi* and *S. epidermidis*. The ethanol extract showed prominent antimicrobial activities against *S.epidermidis, S. typhimurium and E.aerogenes*, moderate against *E.coli, P.vulgaris, S.aureus* and least against *S. aureus*, *P.aeruginosa* and *S.typhi*. The methanol extract showed maximum antibacterial activities against *S. epidermidis, E.coli, S. typhimurium* and *P. vulgaris,* moderate against *S.aureus, E. aerogenes,* and least against *S.typhi and P. aeruginosa* (Table 3). The aqueous extract showed maximal, the ethanol and methanol extract moderate and acetone extracts least antibacterial activities. The aqueous extract showed maximum antibacterial activities against the tested bacterial pathogens, it might be due to higher solubility of antibacterial principles in water as compared to ethanol, methanol and acetone. . Moreover, the fruits are highly fibrous and difficult to ground in fine powder, which may hinder to extract antibacterial component by organic solvents (Figure 1)

Table3: Antibacterial activity of *H. isora* fruit extracts against enteric pathogens(Zone of inhibition of growth in mm, average of 5 readings)

Bacterial pathogens	Aqueous Extract					Acetone extract					Ethanol Extract					Methanol extract					Controls				
	10 mg/disc	8 mg/disc	6 mg/disc	4 mg/disc	2 mg/disc	10 mg/disc	8 mg/disc	6 mg/disc	4 mg/disc	2 mg/disc	10 mg/disc	8 mg/disc	6 mg/disc	4 mg/disc	2 mg/disc	10 mg/disc	8 mg/disc	6 mg/disc	4 mg/disc	2 mg/disc	Ampicillin (10mcg)	DW	Acetone	Ethanol	Methanol
E. coli	30	27	24	20	17	24	19	17	15	13	26	23	20	17	14	27	22	20	19	15	15	-	-	11	11
S. aureus	27	23	20	16	14	24	22	18	16	14	24	20	18	16	14	24	22	21	19	14	17	-	12	12	0
E. aerogenes	26	24	20	19	15	25	22	19	17	15	26	23	21	18	16	24	23	20	18	15	15	-	11	12	11
P.aeruginosa	22	21	18	17	14	21	19	17	15	13	22	20	17	16	13	20	17	16	14	14	16	-	12	0	14
S.typhi	24	21	20	19	16	21	19	17	15	13	22	20	17	15	13	23	21	18	16	14	18	-	13	13	-
S. epidermidis	30	26	24	21	18	24	21	19	16	14	26	25	23	20	17	25	23	22	20	16	18	-	-	13	-
S. typhimurium	28	26	24	21	18	25	23	21	18	15	26	24	21	19	16	25	24	20	18	16	16	-	11	12	11
P. vulgaris	29	25	22	20	17	24	21	19	15	14	25	23	20	17	15	24	22	20	19	16	16	-	11	12	-

The fruits of *H.isora* contain various phytochemical components such as carbohydrates; anthraquinon glycosides, proteins, tannin and phenolic compounds and steroids (Table 4) and combination or joint action of these components in extracts may contribute to the antibacterial properties.

Figure 1: Antibacterial response of bacterial pathogens to various extracts of fruits of *H.isora.*



These fruits are employed in intestinal disturbance such as colic flatulence, diarrhoea, chronic dysentery and stomach-ache (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.* In the present study, the fruit of *H. isora* showed antibacterial activity against all tested enteric pathogens. It authenticates the use of fruits of *H. isora* in the treatment of enteric or diarrhoeal infections and supports the traditional use of the plant.

Table 4: Photochemical analysis of *Helicteres isora* fruits

Phytochemicals	Result
Alkaloid	Absent
Flavonoids	Absent
Carbohydrates	Present
Cardiac Glycosides	Absent
Anthraquinon Glycosides	Present
Saponins	Absent
Proteins	Present
Tannin and Phenolic compounds	Present
Volatile oils	Absent
Steroids	Present

Impact of study: It is clear that *H.isora* L plant's fruits used by people against diarrheal disease showed antibacterial activities. Although the nature and number of active antibacterial principles involved in fruit paste of *H. isora* 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 fruits of *H.isora* L are antibacterial against enteric and diarrhoeal bacterial pathogens. The results of present study may form the basis for further investigation to isolate active compounds, elucidate the structure and evaluate them against wider rage of drug-resistant bacterial strains.

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