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PHYTOCHEMICAL EVALUATION AND ANTIBACTERIAL ACTIVITY OF *HELICTERES ISORA* FRUITS

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Abstract

Antibacterial activities of methanol, benzene, chloroform, ethyl acetate, petroleum ether and aqueous extracts of fruits of *Helicteres isora* were studied. The fruit aqueous extracts of *Helicteres isora* showed prominent antibacterial activities against the tested bacterial pathogens. The aqueous extract showed maximum activity, the methanol extract moderate activity and other extracts like benzene, chloroform, petroleum ether and ethyl acetate are least antibacterial activities. The aqueous extract showed highest antibacterial activity against *Escherichia coli* with zone of inhibition of 23 mm and lowest activity against *Pseudomonas aeruginosa* with zone of inhibition of 15 mm. Minimum inhibitory concentrations was determined by liquid broth method. Minimum inhibitory concentration of aqueous extract against *E. coli* was found to be 10 µg/ml and that against *P. aeruginosa* found to be 8 µg/ml. The qualitative phytochemical screening revealed the presence of alkaloids, amino acids, proteins, carbohydrates, flavanoids, cardiac glycosides, saponin glycosides, tannins, steroids and terpenoids.

Keywords: *Helicteres isora*, Phytochemical screening, Antibacterial activity, Zone of inhibition, Liquid broth method.

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INTRODUCTION

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 diseases [1]. The roots and the bark are expectorant [2], demulcent [3], hypoglycemic [4] and useful in colic, scabies, gastropathy, diabetes, diarrhea and dysentery [5].The fruits are astringents, refrigerant, stomatic, vulnerary and useful in griping of bowels, flatulence of children and antispasmodic [6].The fruit paste mixed with mustered oil and turmeric is used for massaging in new born baby to cure profound weakness [7]. Approximately 5g fruit powder with salt is to be taken thrice daily with water to cure the gastrointestinal problems [8]. The barks of *H. isora* showed prominent antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*and *Escherichia coli* and fruits against *Candida albicans* [9]. The fruits are useful in the treatment of ophthalmic, flatulence, diarrhoea, dysentery, wounds, ulcers, hemorrhages and diabetes [10]. Due to the uniqueness of fruit property in the treatment of different diseases, this part was selected for the study. The present study was aimed to evaluate phytochemical and antibacterial activity of different fruit extracts of *H. isora*. The presence of flavones, triterpenoids, cucurbitacin, phytosterols, saponins, sugars and phlobatannins were demonstrated in roots and barks *H. isora L* [11]. 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 [12]. The review revealed that the fruits of *H. isora* were used in diarrhoeal infection and it is anti-candidal [13]. To the best of our knowledge no report is available on the antibacterial activities of fruits of *H. isora*. As there is no reference in literature regarding the antibacterial aspects, it was considered to investigate the phytochemical and antibacterial activities of different extracts of fruits of *H. isora*[14].

MATERIALS AND METHODS

Plant material

The fruits of *Helicteres isora* linn were collected from the local market of kodada, Suryapet district during the month of January, 2020 and authenticated by Professor Sk. Mahmood department of botany, Osmania University, Hyderabad T.S, India. The fruits were dried in shade. The dried fruits were powdered using mechanical grinder and sieved to get the powder of uniform size.

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Chemicals

All the chemicals and reagents used for the experiments are of analytical grade.

Preparation of crude extract

The 40g of dried and powdered fruits of *Helicteres isora linn* was subjected to successive solvent extraction using sox let extractor for 48 hours. The fruit powder was extracted with different solvents ranging from non-polar to polar like ethyl acetate, benzene, petroleum ether, chloroform, methanol and water. These extracts were concentrated using rotary evaporator and stored at 5^0 C in airtight containers for experimental studies, and the extractive values of all extracts were calculated in % yield [15].

Antimicrobial activity

Bacterial strains: Antibacterial assay was done using bacteria *Escherichia coli* NCIM 2068; *Pseudomonas aeruginosa* NCIM 2200; *Salmonella typhimurium* NCIM 2501; and *Staphylococcus aureus* NCIM 2079 using nutrient agar medium. All microbial strains were procured from National Collection of Industrial Microorganisms, NCL, Pune, India [16].

Preparation of medium

The media used for antibacterial assay was Muller Hinton agar media. It was prepared by suspending 38g powder in 100 ml water. Heat to boiling to dissolve the media completely. Sterilization was done properly at 15 lbs pressure at 121^0 C for 15 minutes by autoclaving. It was then mixed well before pouring [17].

Cup-plate diffusion method

Antibacterial activities of methanol, benzene, chloroform, ethyl acetate, petroleum ether and aqueous extracts of *H. isora* was carried out using cup-plate agar diffusion method. Prepared 0.9% saline, Muller Hinton agar media and glass wares are sterilized properly at 15 lbs pressure at 121^0C for 15 minutes by autoclaving [18]. Sample organism was inoculated into 0.9% saline and allowed to enrich for 1hr. 300 µl from each standard bacterial stock suspension was mixed thoroughly with 20 ml of sterile Molten Muller Hinton agar (45°C-50°C), poured into sterile petri-dishes and left to solidify. Then, with the help of a sterile cork-borer three cup-shape wells of 10 mm diameter were made in each plate. The agar discs were removed. The wells were filled with test solution, standard and control using sterile adjustable pipettes and allowed for diffusion. The plates were then incubated in upright position for 24 hours at 37°C. After incubation period, the diameter of inhibition zones was measured. Amoxycillin was used as the standard [19].

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Determination of minimum inhibitory concentration by liquid broth method

The Minimum Inhibitory Concentration (MIC) of the aqueous extract of *Helicteres isora* fruits was determined by liquid broth method. The MIC is the concentration of the drug present in the last clear tube, i.e., in the tube having the lowest concentration in which there is no microbial growth [20]. The minimum concentration of plant extract required to inhibit the growth of microorganism was determined. A series of assay tubes were prepared containing uniform volume, 1ml of sterile Sabouraud dextrose broth and the equal volume of known concentration of the plant extract was added. In six tubes, the serial dilutions of test substance were added and seventh tube was left as positive control without test substance. The tubes were inoculated with 0.1 ml of inoculum (1×10^6 CFU per ml). In the experiment, solvent control and sterility controls were maintained. The tubes were incubated at 28°C for 48 hours. The tubes were inspected visually to determine the microbial growth as indicated by turbidity. The tubes having the sufficient concentration of plant extract for inhibiting the microbial growth remain clear. Amoxycillin was used as the standard for tested bacterial pathogens. The lowest concentration of aqueous extract of *Helicteres isora linn* that will inhibit the visible growth of *Staphylococcus aureus* and *Escherichia coli* was found out [21].

RESULTS AND DISCUSSION

The percentage yield of the six extracts (petroleum ether, benzene, chloroform, ethyl acetate, methanol and distilled water) of *H. isora* fruit extracts are summarized in Table 1. The % yield was high in aqueous extract (19.46%) whereas, petroleum ether extract had showed minimum % yield as 0.5%. Primarily water was used as a solvent by traditional practitioners [22].

Table 1: **Percentage yield of various extracts of fruits of *Helicteres isora.***

S. No	Extracts	% Yield (w/w)
1	PEHI	0.5
2	BEHI	0.75
3	CEHI	1.28
4	EAEHI	1.62
5	EEHI	4.88
6	AEHI	19.46

PEHI: Petroleum Ether Extract of *Helicteres isora*; BEHI: Benzene Extract of *HelicteresiIsora*; CEHI: Chloroform Extract of *Helicteres isora*; EAEHI: Ethyl Acetate Extract of *Helicteres isora*; MEHI: Methanolic Extract of *Helicteres isora*; AEHI: Aqueous Extract of *Helicteres isora*.

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Qualitative phytochemical screening

Various phytoconstituents were tested in various extracts of fruits of *H. isora* with standard procedures and results are summarized in Table 2. The qualitative phytochemical screening revealed the presence of alkaloids, amino acids, proteins, carbohydrates, flavanoids, cardiac glycosides, saponin glycosides, tannins, steroids and terpenoids [23].

Table 2: Phytochemical screening of various extracts of *Helicteres isora* fruit.

Compounds	Test name	PEHI	BEHI	CEHI	EAEHI	MEHI	AEHI
Alkaloids	Dragendorff's test	+	-	-	+	+	+
	Mayer's	-	-	-	+	-	+
	Hager's test	-	-	-	-	-	-
	Wagner's test	-	-	+	-	-	-
	Tannic test	-	+	-	+	+	-
Amino acids	Ninhydrin test	+	+	-	-	-	+
Proteins	Biuret reaction	-	-	-	-	-	-
	Xanthroprotective reaction	-	-	-	-	+	+
	Ninhydrin test	-	-	-	-	-	-
Carbohydrates	Molish's test	-	-	-	-	-	-
	Fehling's test	-	-	+	-	-	+
	Benedict test	-	+	-	-	+	+
	Barfoed's test	-	-	-	+	-	+
Flavonoids	Shinoda test	-	-	-	-	-	-
	Lead acetate test	-	-	-	-	-	+
	Zinc HCl test	-	-	-	-	-	+
Fat and fixed oils	Copper sulfate test	-	+	-	+	-	+
Cadiac glycosides	Keller-killianitest	-	-	-	+	-	-
	Baljet test	-	-	-	-	+	+
	Legal'stest	-	-	+	-	-	-
	Borntrager's test						
Saponin glycosides	Froth formation test	-	-	-	-	-	-
Steroids and terpenoids	Salkowtski (steroids)	+	+	+	+	+	+
	Salkowski (terpenoids)	+	+	+	+	-	-
	Liebermann-Burchard reaction	-	-	-	+	+	+
Tannins	FeCl3 test	-	-	-	+	+	+
	Ammonia test	-	-	-	-	-	-
	Lead acetate test	+	+	-	+	+	+

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PEHI: Petroleum Ether Extract of *Helicteres isora*; BEHI: Benzene Extract of *Helicteres isora*; CEHI: Chloroform Extract of *Helicteres isora*; EAEHI: Ethyl Acetate Extract of *Helicteres isora*; MEHI: Methanolic Extract of *Helicteres isora*; AEHI: Aqueous Extract of *Helicteres isora*.

Table 3: Antibacterial activity of *Helicteres isora* fruit extracts.

Organisms	Zone of inhibition (mm)						
	Methanol extract	**Benzene extract**	**Chloroform extract**	**Ethyl acetate extract**	**Pet.ether extract**	**Aqueous extract**	**Amoxicillin standard**
E.coli	16	10	9	-	-	23	23
P.aeruginosa	14	9	8	5	7	15	17
Salmonella typhimurium	12	7	6	-	-	19	15
S.aureus	8	8	7	5	-	16	14

DISCUSSION

Antibacterial activites of Methanol, Benzene, Chloroform, Petroleum ether and aqueous extracts of fruits of *Helicteres isora* were showed in Table 3. The aqueous extract showed maximum activity, the methanol extract moderate and other extract are least antibacterial activities [24]. The aqueous extract showed highest antibacterial activity against *Escherichia coli* with zone of inhibition of 23 mm and lowest activity against *P. aeruginosa* with zone of inhibition of 15 mm. Minimum inhibitory concentration was determined by liquid broth method. Minimum inhibitory concentration of aqueous extract against *Escherichia coli* was found to be 10 µg/ml and that against *P. aeruginosa*found to be 8µg/ml [25].

CONCLUSION

Phytochemical screening and antibacterial activities of methanol, benzene, chloroform, ethyl acetate, petroleum ether and aqueous extracts of fruits of *H. isora* were studied. The qualitative phytochemical screening revealed the presence of alkaloids, amino acids, proteins, carbohydrates, flavonoids, saponin glycosides and cardiac glycosides. In antibacterial activity, the aqueous extract showed maximum activity, the methanol extract showed moderate activity and other extracts like Benzene, chloroform, Ethyl acetate and petroleum ether are least antibacterial activities. Minimum inhibitory concentration of aqueous extract against *Escherichia coli* was found to be 10 µg/ml and that against *P. aeruginosa* found to be 8µg/ml.

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