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Isolation and Evaluation of Antimicrobial Properties of Isolated Phytoconstituents of Fruits of *Helicteres isora* Linn.

Elsa Varghese*, K Leena Pappachen, S Sathia Narayanan

Department of Pharmaceutical Chemistry, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Health Sciences Campus, Kochi-682041, Kerala, India.

ABSTRACT

Helicteres isora linn, an ayurvedic herb, is distributed widely in forest throughout India. Almost its all parts are used traditionally. The fruits are useful in diarrhoea, dysentery, wounds, ulcers, hemorrhages and diabetes. The fruit after collection, authentication and drying, was extracted with petroleum ether, chloroform, methanol and water using soxhlet extractor.The present study was aimed to isolate the constituents and evaluate the antimicrobial properties of isolated constituents of methanolic extract of fruits of *Helicteres isora* linn. Isolation of alkaloids, flavonoids and phenolic compounds was done by using HPTLC technique. The *invitro* antimicrobial activities of methanolic extract, isolated alkaloids, flavonoids and phenolic compounds were investigated against Escherichia coli, Pseudomonas aeruginosa, Salmonella abony and Staphylococcus aureus by cup-plate diffusion method.The methanolic extract showed activity against Escherichia coli, Pseudomonas aeruginosa, Salmonella abony and Staphylococcus aureus., isolated phenolic compounds against Escherichia coli, Pseudomonas aeruginosa and Salmonella abony., isolated flavonoids against Pseudomonas aeruginosa and Staphylococcus aureus., and isolated alkaloids against Escherichia coli and Staphylococcus aureus.Minimum inhibitory concentration was determined by liquid broth method. Minimum inhibitory concentration of methanolic extract against Pseudomonas aeruginosa was found to be 10µg/ml and that against Staphylococcus aureuswas found to be 8µg/ml.

Keywords: *Helicteres isora* linn, Sterculiaceae, Antimicrobial activity, Zone of inhibition, MIC.

Corresponding author

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INTRODUCTION

Infectious diseases are the transmissible diseases caused by infection of microorganisms. They are the major cause of death in the world. Antimicrobial agents are those substances that inhibit the growth of microorganisms or kill them. The emergence of multidrug-resistant pathogens adversely affects the efficacy of many antibiotics [1]. Herbal medicines are used for the treatment of many infectious diseases throughout the history of mankind. The increasing antibiotic resistance exhibited by microorganismshas led to the phytochemical screening of medicinal plants for antimicrobial activity [2, 3]. Many infections can be treated by phytochemicals possessing potent antibacterial efficacy [4, 5].

In India, *Helicteres isora linn* is distributed from Jammu Eastwards to Nepal, Bihar, West Bengal, Central, Western and Southern India and Andaman islands. It is a medicinally important sub-deciduous shrub or small tree and commonly known as East India Screw tree [6]. Almost all parts of the plant are used traditionally for the treatment of various diseases. The roots and stem barks are useful in gastric problems, diabetes, diarrhoea and dysentery. The seeds are used for curing ulcers in ears, otorrhoea etc. The drug is also used in snake bite. The pods are used for killing intestinal worms in children. The fruits are useful in the treatment of ophthalmic, flatulence, diarrhoea, dysentery, wounds, ulcers, hemorrhages and diabetes [7]. Due to the uniqueness of fruit property in the treatment of different diseases, this part was selected for the study.

Escherichia coli, a gram negative bacterium, can cause food poisoning. The diseases caused by E. coli include gastroenteritis, urinary tract infections, neonatal meningitis, septicaemia and pneumonia.Pseudomonas aeruginosa is a gram negative aerobic bacteria. It infects pulmonary tract, urinary tract, outer ear, burns, wounds and blood. The infections caused by Pseudomonas aeruginosa include pneumonia, septic shock, urinary tract infection, gastrointestinal infection, skin and soft tissue infections. Rarely they can cause community acquired pneumonias [8].

Salmonella abony is a gram negative bacterium. The diseases caused by Salmonella abony include septicaemia, meningitis, gastroenteritis and sepsis [9]. Staphylococcus aureus is a facultative anaerobic gram positive coccal bacteria. It is found on the skin as a part of normal skin flora and nasal passages [10].It can cause a range of infections. Infections caused by Staphylococcus aureus include minor skin infections such as pimples, impetigo, boils, cellulitis, folliculitis, scaled skin syndrome and abscesses [11] and life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis. It can cause staphylococcal scaled skin syndrome, a severe disease, in infants [12].

The present research was therefore, undertaken to isolate the active constituents, to examine the possible antibacterial effects of the methanolic extract of fruit of *Helicteres isora linn* and the isolated constituents and to determine the minimum inhibitory concentration of the plant extract.

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MATERIALS AND METHODS

Plant material

The fruits of *Helicteres isora linn* were collected from the local market of Ootacamund, India during the month of June, 2011 and authenticated by Dr. M.E. Kuriakose, Principal, K.G.College, Pampady, Kottayam, Kerala, India. (Reader in Botany and Research Guide, School of Environmental Science, M.G. University, Kottayam.) The fruits were dried in shade. The dried fruits were powdered using mechanical grinder and sieved to get the powder of uniform size.

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

Preparation of Crude Extract: The 50g of dried and powdered fruits of *Helicteres isora* linn was subjected to successive solvent extraction using soxhlet extractor for 72hrs. The solvents used were petroleum ether, chloroform, methanol and water. The dried methanolic extract was used for isolation and evaluation of antimicrobial properties.

Isolation: 16 spots of the methanolic solution of the extract (20µL) were applied on the precoated silica gel 60 F_{254} TLC plates of size 20cm x 10cm and were dried. The plate was developed in the solvent system. After development and drying, the plate was observed under 254nm and 366nm. The developed plate was then scratched, added 25 ml methanol and sonicated. It was then centrifuged, filtered and concentrated. The solvent system used for the isolation of flavonoids was chloroform: ethyl acetate 7.5:2.5. The solvent system used for the isolation of alkaloids was toluene: ethyl acetate: diethyl amine 7:2:1. The solvent system used for the isolation of phenolic compounds was ethyl acetate: 1, 4-dioxane: formic acid: water (5:3:1:1).

Antimicrobial activity

Bacterial strains

Antibacterial assay was done using bacteria Escherichia coli, Pseudomonas aeruginosa, Salmonella abony and Staphylococcus aureus. Microorganisms were obtained from the stock cultures from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India.

Preparation of medium

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

Cup-plate diffusion method

Antibacterial activity of plant extract and isolated constituents was carried out using cup-plate agar diffusion method. Prepared 0.9% saline, Muller Hinton agar media and glass wares are sterilized properly at 15lbs pressure at 121^{0}C for 15mts by autoclaving. Sample organism was inoculated into 0.9% saline and allowed to enrich for 1hr. 300µl from each standard bacterial stock suspension was mixed thoroughly with 20 ml of sterile Molten Muller Hinton agar (45-50°C), poured into sterile petri-dishes and left to solidify. Then, with the help of a sterile cork-borer three cup-shape wells of 10mm 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. Allowed for diffusion. The plates were then incubated in upright position for 24 hr at 37^{0}C. After incubation period, the diameter of inhibition zones was measured. Bacitrim was used as the standard for Salmonella abony. Amoxycillin was used as the standard for Staphylococcus aureus and Escherichia coli. Ciprofloxacin was used as the standard for Pseudomonas aeruginosa [13].

Determination of minimum inhibitory concentration by liquid broth method:

The Minimum Inhibitory Concentration (MIC) of the methanol 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. 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 (1x10^6 CFU per ml). In the experiment, solvent control and sterility controls were maintained. The tubes were incubated at 28^{0}C for 48 hr. 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 Staphylococcus aureus. Ciprofloxacin was used as the standard for Pseudomonas aeruginosa. The lowest concentration of methanolic extract of *Helicteres isora* linn that will inhibit the visible growth of Staphylococcus aureus and Pseudomonas aeruginosa was found out [14].

RESULTS AND DISCUSSION

Results obtained from cup-plate diffusion method showed the antibacterial activity of plant extract and isolated constituents as in Table No.1.

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Table No.1.Zone of inhibition of methanolic extract of *Helicteres isora* linn fruits and isolated constituents

	ZONE OF INHIBITION(mm)			
	Escherichia coli	Pseudomonas aeruginosa	Salmonella abony	Staphylococcus aureus
Methanolic Extract	14	19	14	13
Isolated Phenolics	12	10	8	-
Isolated Flavonoids	-	14	-	15
Isolated Alkaloids	11	-	-	7

The methanolic extract of fruits of *Helicteres isora* linn exhibited potent antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Salmonella abony and Staphylococcus aureus. The methanolic extract showed highest antimicrobial activity against Pseudomonas aeruginosa with zone of inhibition of 19mm and lowest activity against Staphylococcus aureus with zone of inhibition of 13mm.

The isolated phenolic compounds exhibited potent antibacterial activity against Escherichia coli, Pseudomonas aeruginosa and Salmonella abony. The isolated phenolic compounds showed highest antimicrobial activity against Escherichia coli with zone of inhibition of 12mm and lowest activity against Salmonella abony with zone of inhibition of 8mm.

The isolated flavonoids exhibited potent antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus.The isolated flavonoids showed highest antimicrobial activity against Staphylococcus aureus with zone of inhibition of 15mm and lowest activity against Pseudomonas aeruginosa with zone of inhibition of 14mm.

The isolated alkaloids exhibited potent antibacterial activity against Escherichia coli and Staphylococcus aureus.The isolated alkaloids showed highest antimicrobial activity against Escherichia coli with zone of inhibition of 11mm and lowest activity against Staphylococcus aureuswith zone of inhibition of 7mm.

The results obtained from liquid broth method showed minimum inhibitory concentration of methanolic extract of *Helicteres isora* linn fruits against Staphylococcus aureus and Pseudomonas aeruginosa..Minimum inhibitory concentration of methanolic extract against Pseudomonas aeruginosa was found to be 10µg/ml and that against Staphylococcus aureus was found to be 8µg/ml.

CONCLUSION

The present study revealed the first time for the antibacterial activities of isolated constituents of methanolic extract of fruits of *Helicteres isora* linn against Escherichia coli,

Pseudomonas aeruginosa, Salmonella abony and Staphylococcus aureus. The presence of phenolic compounds, flavonoids, and alkaloids are responsible for the potent antibacterial activity of the plant. The phenolic compounds and alkaloids are responsible for the potent antibacterial activity against Escherichia coli. The phenolic compounds and flavonoids are responsible for the potent antibacterial activity against Pseudomonas aeruginosa. The phenolic compounds are responsible for the potent antibacterial activity against Salmonella abony. The flavonoids and alkaloids are responsible for the potent antibacterial activity against Staphylococcus aureus.

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