RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF *HELICTERES ISORA* FRACTIONS

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Abstract: The aim of the present study was to evaluate the antibacterial activity of *Helicteres isora* extracts. We have tested different concentrations of three Ethanol, aquoues and hydroethanol fractions of *H. isora* root on selected pathogenic gram positive (*Staphylococus aureus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Bacillus cereus*) and gram negative bacteria (*Pseudomonas aerogenosa*, *Proteus mirablis*, *Salmonella typhie*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Escherichia coli*) with the well diffusion method in agar. All the tthree extracts exhibited antibacterial activity against seven strains of pathogenic bacteria. Hydroethanol extract showed best antibacterial activity as compared to both other extracts. Ethanol extract showed good antibacterial activity against *K. pneumonia* in comparison to other extracts. However aqueous extract showed minimum antibacterial activity against all the pathogens. This finding showed the good antimicrobial activity of *H. isora*, so it forms the basis for further antibacterial drug isolation from this medicinal plant.

Keywords: *Helicteres isora*, Antibacterial, Hydroethanol, Medicinal plant

INTRODUCTION

In the current scenario, infectious diseases due to the various pathogenic micro organisms are still one of the leading reasons behind the death in the world. Various conventional antibiotic drugs are available for the treatment of infectious diseases, but microbes evolve in the changes of genetic structure and metabolism to acquire resistance against antibiotic drugs (Fred, 2006; Raghunath, 2008). *S. aureus*, *P. aeruginosa*, *Streptococcus* sps. and *Enterobacteriaceae* add *E. coli*, enterococcus etc. are key microbes that showed antibiotic resistant (Bax *et al*., 2000; Bhavnani and Ballow, 2000). These drug resistant microbes are more pathogenic with high mortality rate and become a great challenge in pharmaceutical and healthcare. These commercially available synthetic drugs also have various side effects, carcinogenicity, high cost and have various environmental problems (Gortzi *et al*., 2006), so to overcome all these problems, researcher looking for the invention of alternative and novel drug from natural sources like plants, algae etc.

Helicteres isora (family- Sterculiaceae) commonly known as "Marorphali" in Hindi and "East India Screw Tree" in English is a medium sized tree, abundantly found in hills and forests, well known for its use in traditional medicinal values (Sabale *et al*., 2012). *H. isora* showed antidiabetic (Chakrabarti *et al*., 2002), hypolipidaemic (Kumar and Murugesan, 2008), anti-oxidant (Basniwal *et al*., 2009), anti-nociceptic (Venkatesh *et al*., 2007a), hepatoprotective (Dhevi *et al*., 2008) and cardiotonic (Dama *et al*., 2011) activities. Root juice of *H. isora* was showed to be useful in fever, cough, asthma, stomach infections, intestinal infections (Kirtikar and Basu, 1991) and was also useful for cuts and wounds healing (Nagaraju and Rao, 1990) and scabies. From the roots, betulic acid, daucosterol, sitosterol, isorin (Qu *et al*., 1991), cucurbitacin B and isocucurbitacin B were isolated (Bean *et al*., 1985). Despite the extensive work on *H. isora*, this plant has not been studied for antibacterial potential. Hence keeping this perspective and the above mentioned medicinal properties of *Helicteres isora*, there is a considerable need to investigate the protective and therapeutic effects of this plant against the ten pathogenic bacteria.

MATERIAL AND METHOD

Bacterial strains and growth conditions

Four gram positive bacterial strain *Staphylococus aureus* (MTCC 87), *Bacillus subtilis* (MTCC 441), *Enterococcus faecalis* (MTCC 9845) and *Bacillus cereus* (MTCC 1306) and six gram negative bacterial strains *Pseudomonas aeruginosa* (MTCC 1934), *Proteus mirabilis* (MTCC 425), *Salmonella typhimurium* (MTCC 3224), *Klebsiella pneumonia* (MTCC 3384), *Proteus vulgaris* (MTCC 426) and *Escherichia coli* (MTCC 1695) were procured from Microbial Type Culture Collection (IMTECH), Chandigarh, India. Nitrogen broth medium was used for the maintenance of all bacterial strains at 4 ºC after the preparing mother culture at 37 ºC in rotator incubator for 24 hrs.

Plant extracts preparation

Plant sample (Roots of *Helicteres isora*) was collected from Shiwalik range of Uttar Pradesh and were washed, shade dried and subjected to coarse powder using electric grinder. The powder was extracted with different solvent ranging from non polar to polar (petroleum ether, benzene, chloroform, ethyl acetate, ethanol and distilled water) and 70% hydroethanol in Soxhlet. Ethanol, aqueous and

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hydroethanol extracts was concentrated using rotary evaporator and stored at 4 ºC in airtight containers. Four concentration (10, 12, 15 and 20 mg/ml) of all the three extract (ethanol, aqueous and hydroethanol) were prepared for further activity.

Well diffusion method: (Perez *et al.* 1990)
Nutrient agar medium was prepared, sterilized (121 ºC for 15 min.), poured into a pre sterilized Petri plates and allowed to solidify at room temp. in laminar air flow. Bacterial culture of bacteria (0.3 ml) was placed on the nutrient agar and spread with the help of glass spreader. Four wells (4 mm diameter) were punched on each agar plate using a sterile cork borer. In each well 20 µl of the sample was added and incubated at 37 ºC for 18 to 24 hrs in inverted position in incubator. Zone of inhibition (mm) was observed. Streptomycin (10 mcg/ disc) and tetracycline (30 mcg/ disc) were used as a positive control.

RESULT

Antibacterial potential of three different fraction of *H. isora* with 4 concentrations (10, 12, 15 and 20 mg/well) were determined against 10 pathogenic bacteria with the help of well diffusion method and summarized in table 1 and figure 1. All the three extract showed antibacterial activity, the response for each bacterium tested was different. Diameter of inhibition was ranging from 6.3 to 22 mm indicating that *Bacillus subtilis* was most susceptible bacteria while *E. ficalis*, *S. aureus* and *P. aerogenosa* were resistant bacteria, and there growth was not inhibited by all the three extracts. Almost all bacteria showed resistant against the aqueous extract except *E. coli* and *B. cereus*. Hence aqueous extract showed antibacterial activity against only *E. coli*. *Bacillus subtilis* showed more sensitivity against hydroethanolic extract in comparison to other extract as well as stranded antibiotic. Most prominent antibacterial activity of all the three extract were observed at 20 mg/well concentration in comparison to all other concentrations (10, 12 and 15 mg/ well). Hydroethanol extract showed prominent antibacterial activity against pathogenic bacteria in comparison to other extracts.

Table 1. Means of inhibition growth diameter obtained by using different concentrations of three extracts of *H. isora* roots.

Bacteria	Sample concentration	Hydroethanol	Ethanol	Aqueous	Positive control
K. pneumonia	10	6.3±1.2	11.3±0.6	ND	26
	12	8.0±1.0	12.0±1.0	ND	
	15	10.3±2.1	11.7±2.1	ND	
	20	13.7±1.5	17.3±1.5	ND	
B. subtilis	10	11.7±0.6	ND	ND	21
	12	13.7±1.5	ND	ND	
	15	14.3±1.2	7.7±0.6	ND	
	20	22±0.4	8.7±0.6	ND	
S. typhi	10	9.7±0.6	ND	ND	22
	12	10.3±0.6	ND	ND	
	15	12.0±1.0	11.0±1.0	ND	
	20	20.0±1.0	11.7±1.5	ND	
P. vulgaris	10	11.7±2.5	11.7±0.6	ND	23
	12	12.0±2.0	13.3±2.5	ND	
	15	10.3±0.6	12.0±2.0	ND	
	20	15.0±2.0	11.0±2.6	ND	
E. coli	10	12.0±1.0	15.0±0.00	7.3±0.6	33
	12	13.0±1.0	15.0±0.00	8.±0.00	
	15	14.0±1.0	15.7±0.6	9.±0.00	
	20	16.0±1.0	17.7±1.5	11.7±0.6	
p. mirabilis	10	7.7±0.6	8.0±1.0	ND	17

	12	10.0±0.00	10.3±0.6	ND	
	15	11.7±1.5	10.7±0.6	ND	
	20	14.3±0.6	13.0±1.0	ND	
B. cereus	10	13.0±1.0	13.0±1.0	ND	24
	12	14.3±0.6	14.3±1.2	8.7±1.5	
	15	15.3±0.6	14.7±1.2	10.0±0.00	
	20	18.3±2.1	18.0±1.0	11.0±1.0	

Positive control- streptomycin for all except *E. coli* (Tetracycline). ND not determined

DISCUSSION

Antibiotics are synthetic drugs which are used against the treatment of various pathogenic micro-organisms. These antibiotics also possess many side effects and high costs. In the present era researchers have immense interest in the search for new antimicrobial drug from herbal plant origin. The result of the current study showed that ethanolic and hydroethanolic extract possess antibacterial activity against all pathogenic bacteria except *E. faecalis, S. aureus* and *P. aerogenosa.* Aqueous extract showed antibacterial activity against only *E. coli* and *B. cereus.*Varghese *et al*. (2012) reported that methanol extract and there isolated constituents (alkaloids, flavonoids and phenolic compounds) of *H. isora* fruits possess antimicrobial activity against *E. coli*, *P. aeruginosa*, *Salmonella abony* and *S. aureus*. Aqueous, ethanol, acetone and methanol extract *of H. isora* fruits also showed antibacterial potential against *S. typhimurium, E. coli*, *Staphylococcus epidermidis, P. vulgaris*, *Enterobacter aerogenes, S. aureus* and least antibacterial potential against *P. aeurogenosa.* (Tambekar et al., 2008) *H. isora* fruits aqueous extract also reported antimicrobial potential against ten pathogen strains -*E. coli*, S. *aureus, E. aerogenes*, *P. aeruginosa, S. typhi*, *S. typhimurium, S. paratyphi*, *P. vulgaris*, *K. pneumoniae* and *Shigella Flexneri*. (Tambekar and khante, 2010). Acetone extract of *H. isora* fruit possess antibacterial activity against *E. coli*, *B. cereus* and *E. faecalis* (Shriram et al., 2010) while methanol and aqueous extract of *H. isora* leaf reported antimicrobial activity against *E. coli* and *A. niger* (Kumar et al., 2013) According to the present study, hydroethanol extract showed prominent antibacterial efficacy against pathogenic bacteria in comparison to other extracts (aqueous and ethanol), because hydroethanolic extract possess more secondary metabolite and other compounds in comparison to others, reason behind this is ethanol and aqueous extract were prepared with sequential method (non polar to polar) but hydroethanolic extract was prepared with direct method. So all the beneficiary compounds were distributed in various fractions like petroleum ether, benzene, chloroform, ethyl acetate, ethanol and aqueous. The antimicrobial efficacy of plant is due to the presence of tannins, phenolic compounds, saponins and flavonoids (Aboaba and Efuwape, 2001), because tannins have been reported to prevent the microorganisms development by precipitating protein of microbes and convert it into unavailable form for microbes (Chung *et al*, 1998 and Phate *et al*., 2011), while flavonoids are able to form complexes with extracellular soluble proteins and bacterial cell wall (Tsuchiya *et al*. 1996; Cushine and Lamb 2005), so flavonoids possess efficacy against gram positive and gram negative bacteria, viruses and fungi (Afolayan *et al*., 1997). So, *H. isora* showed antibacterial activity against pathogenic bacteria because it possess various antibacterial compounds like phenols, flavonoids, tannins, saponins and other active components (Sharma *et al*., 2016). Various modes of action are involved in the antibacterial activity of various extracts. Because different extracts of different plant or same plant have variability and different chemical profile, so their antibacterial activity is not due to single mechanism. It is believed that these active compounds have different sites of action at the cellular level. There are 6 possible mechanisms of antimicrobial action that includes- interactions with membrane proteins; coagulation of cell content; inhibition of enzyme synthesis; disintegration of cell membrane; disturbance of the outer membrane of gram negative bacteria and release of lipopolysacherides and destabilizing of the proton motive force with leakage of ions. (Bakkali *et al*., 2008; Burt 2004; Di Pasqua *et al*., 2007; Hammer *et al*., 2008; Amensour *et al*., 2010). All the extracts of *H. isora* roots showed antibacterial activity against both gram negative and gram positive bacteria. The positive results against both gram positive and negative bacteria may indicate the presence of broad spectrum antibiotic components in the plant (Vaghasiya and Chanda**,** 2007).

CONCLUSION

From the result it was obvious that plant extract have different modes of action and exhibited stronger antibacterial activity against both gram positive and negative bacterial strains. The best antibacterial activity was observed against *Bacillus sutilis*. *H. isora* root seems to a promising natural antibacterial agent that have potential applications for controlling the pathogenic bacteria and have potential

applications in pharmaceutical industry should replace the synthetic ones.

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