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Antibacterial activity and preliminary phytochemical screening of the extracts of the bark of *Saraca asoca* (Roxb.) Wild. (Caesalpiniaceae)

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Abstract

Ethyl acetate, acetone, methanol and aqueous extracts of the bark *Saraca asoca* (Roxb.) Wild. were screened against the enteric pathogenic isolates, namely, *Escherichia coli, Shigella sonnei* and *Salmonella enteritis*. All the extracts other than aqueous extract showed antimicrobial activity with the methanol extract having the highest percentage of activity. Preliminary phytochemical screening revealed the presence of flavones and tannins in the methanol extract.

Keywords: *Saraca asoca*, bark, antibacterial activity, flavones, tannins

INTRODUCTION

Herbal medicines contribute significantly in the primary health care of mankind. Diarrhoea is a second killer disease of children in the developing world. Many of the diarrhoeal pathogens have developed resistance to most of the modern drugs. To overcome this serious health hazard researchers resort to herbal medicines. WHO has recognized herbal medicine as an alternate therapy for various human ailments. The bark of *Saraca asoca* (Roxb.) Wild. is used in Indian traditional medicines as an antidiarrhoeal drug. It is a small tree belonged to the family Caesalpiniaceae. The bark of this tree has been frequently used in the Indian traditional systems of medicine. It is used as an astringent, to treat hemorrhagic dysentery, diarrhoea, diabetes, syphilis, and as a uterine tonic as it has a stimulating effect on the endometrial and ovarian tissue (Yoganarasimhan, 2000). The bark is also used in treating gynaecological problems, especially decoction of bark in mixture with milk and water used to prevent leucorrhoea and menorrhoea as stated in Indian folklore medicine. It has cosmetic application also, such as removing black spots on face (Kurian, 1995). Chemicals from several plant species have been reported to be effctive in controlling various human pathogens such as *Escherichia coli, Salmonella enteritidis* and *Shigella sonnei* (Kishore, 1991). In this paper, antimicrobial activity of the extract of the bark of *S. asoca* has been evaluated.

MATERIALS AND METHODS

Selection of medicinal plants

Bark of *S. asoca* was collected from the trees in and around Ponmalai, Tiruchirappalli, India. Shade dried bark was coarsely powdered using mechanical grinder and passed through 40 mesh sieve. This coarsely powdered bark was extracted with various solvents in increasing polarity. i.e., 500 g of powdered plant was successively extracted with ethylacetate, methanol, water, alcohol and acetone. The extracts so collected were evaporated on a waterbath at atmospheric pressure and the solvents were completely removed *in vacuo*.

Phytochemical analysis

All the extracts were subjected to preliminary phytochemical screening as per the standard methods (Harborne, 1984).

Isolation of enteric pathogens

Stool samples were collected from acute gastroenteritis cases of Child Jesus hospital, Tiruchirappalli, India. All the samples were processed for the recovery of enteric pathogens. Selective and differential media such as Hektoen enteric agar and XLD agar were used for the primary recovery of the pathogens and identified by biotyping method (Elmer *et al.*, 1994).

Test organisms

Escherichia coli, Salmonella enteritis and *Shigella sonnei* were the predominant pathogens isolated from the diarrhoeal cases and were subjected to antimicrobial screening. All the isolates used in this study are multiple drug resistant pathogens.

Antibacterial assay

Petri plates containing 20 ml of Mueller Hinton agar medium were seeded with a 24 hours old culture of the bacterial strains individually. The extracts and fractions were dissolved in Dimethyl Sulfoxide (DMSO) and sterilized by using Sortorious syringe filter of pore size 0.22 µm. Various concentrations of the extracts, 100 µg, 200 µg, 400 µg and 800 µg were impregnated into the sterile 6 mm diameter discs. Discs were dried and dispensed on to the solidified Mueller Hinton agar, previously inoculated with test organisms. Antibiotic discs and DMSO discs were used as positive and negative controls. Incubation was made at 37^0C for 24 hours. The assessment of antibacterial activity was based

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on the measurement of diameter of the inhibition zone formed around the disc (Anonymous, 1993).

RESULTS AND DISCUSSIONS

The preliminary phytochemical screening of the extracts showed the presence of triterpenoids, alkaloids, tannins, phenolic compounds, saponins and carbohydrates (Table 1). It is already reported that secondary metabolites such as alkaloids, tannins, phenolic compounds and saponins show antimicrobial activity (Tonalutte *et al.,* 1999).

All the stool specimens were subjected to culturing by using selective and differential procedure and analyzed for prevalence rate of microbial etiology. It was observed that 51% of the infection was due to *E.coli* followed by *Shigella* sp. (26%) and *Salmonella* sp. (23%). Similar type of incidences have been reported by different authors from different countries (Bonfiglio *et al.,* 2002; Banajeh *et al*., 2001). *Escherichia coli*, *Salmonella* sp. and *Shigella* sp. were reported to be the most common isolates associated with diarrhoea is tropical areas (Bonfiglio *et al.,* 2002), whereas *Escherichia coli* (58.4%) *Salmonella* sp. (20%) and *Shigella* sp. (20%) and *Campylobacter* sp. (1.6%) were found to be extremely uncommon agents of childhood diarrhoea making only 1.6 per cent of the positive cultures in Yemen (Banajeh *et al*., 2001). Incidence of *E. coli* was 59% followed by *Salmonella* sp. (16%), *Campylobacter* sp. (3%), *Giardia* sp. (3%) and *Shigella* sp. (3%) in Kolkata, India (Kahali *et al.,* 2004). Diarrhoeal infection caused by *Escherichia coli* is common in India with occasional outbreaks (Kahali *et al*., 2004).

Ethyl acetate, methanol and acetone extracts of barks of *S. asoca* showed the potential antimicrobial activity. Methanol extract showed highest activity against all

Table 1. Preliminary phytochemical screening of extracts of bark of *Saraca asoca*

S.No	Test	Ethyl acetate fraction	Acetone extract fraction	Methanol fraction	Ethanol extract fraction
1	Steroid	-	_	-	-
2	Triterpenoids	+	+	+	-
3	Reducing sugar	_	_	_	_
4	Carbohydrate	+	+	+	+
5	Alkaloids	+	_	+	-
6	Phenolic compounds	+	+	+	-
7	Saponins	+	+	+	-
8	Xanthoprotein	+	+	+	-
9	Tannins	+	+	+	-
10	Flavanoids	+	+	+	-

+ - Positive - - Negative

Table 2. Antibacterial activity of extracts of bark of *Saraca asoca* Roxb. Wild. against enteric isolates of bacteria

Test organism	Zone of inhibition in mm												Standard
	Ethyl acetate extract (µg/disc)				Methanol extract (µg/disc)				Acetone extract (µg/disc)				
	100	200	400	800	100	200	400	800	100	200	400	800	
Escherichia coli	--	09	11	13	08	12	15	18	08	10	13	17	15(C)
Salmonella enteritis	--	--	10	13	--	10	14	17	--	--	09	14	20(Ac)
Shigella sonnei	--	09	13	16	--	09	15	19	--	10	11	15	23(OT)

C - Chloramphenicol (30µg/disc) ; OT - Oxy tetracycline (30µg/disc); Ac - Amoxyclave ; - Negative

the pathogens tested (Table 2). Phytochemical screening of bark also revealed the presence of flavone and tannins (Table 1) in methanol extract, which showed the highest antimicrobial activity. Antimicrobial activity of the bark of *S. asoca* (Roxb.) Wild. was also evaluated by Parotta (2002). But, he used some non-pathogenic strains of bacterium while in the present study enteric pathogenic isolates were used against which also its methanol extracts are highly effective. The aqueous and ethanol extracts of bark didn't show any antibacterial activity even at higher concentrations, indicating the compounds in different extracts have different potentials.

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