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Identification of phytochemical contents and antimicrobial activity of *Saraca asoca* leaves extract

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Abstract

***Saraca asoca* is an important indigenous and considered as sacred tree by Hindus and Buddhists with lots of traditional importance and employed to cure various diseases in Ayurveda. The methanolic, acetone and aqueous leaves extracts of the *Saraca asoca* plant species namely *S. Asoca caesalpiniaceae* and *S. Asoca leguminosae* were analysed phytochemically and antimicrobial activity against bacterial strains *(Bacillus subtilis* MTCC 121 and *Escherichia coli* MTCC 120) responsible for various human infections. The acetone leaves extracts of *Saraca asoca caesalpiniaceae* shows maximum zone of inhibition (36.80 mm at 100 mg/ml) against *B. subtilis* followed by methanol extract (35.90 mm at 100 mg/ml) followed by aqueous extract (20.38 nm at 100 mg/ml). Minimum inhibitory concentration (MIC) of these extract was also investigated in which *S. Asoca caesalpiniaceae* acetone fraction showed significant antimicrobial activity against *E. coli* followed by methanolic extract. The leaf extracts of *S. Asoca caesalpiniaceae* showed better antimicrobial activity against bacterial strains used as compare to *S. Asoca leguminosae.* Preliminary phytochemical studies revealed the presence of tannins, phenolics, saponins, glycosides and flavonoids.**

Keywords: ***Saraca asoca caesalpiniaceae, Saraca asoca leguminosae, antimicrobial action, Bacillus subtilis, Escherichia coli***

Introduction

Plant materials have been used for the treatment of serious diseases throughout the world before the beginning clinical drugs. *Saraca asoca* is one of the traditional medicinal plant having medicinal values. Mainly the bark, leaves, flowers and buds of the plant are used to treating the various infections. It is an evergreen tree which is 9 m in height and occurs throughout India up to an altitude of 750 m in central and eastern Himalayas (Sarojini *et al.* 2011). Leaves of the plant are paripinnate, stipules intra-petioler, united, and leaflet 4-6 pairs, oblong, lanceolate, glabrous. Flowers are Polygamous apetalous, yellowish orange to scarlet, in dense corymbose panicles; Calyx yellowish orange to scarlet, petaloid, cylindric, four lobed. Petals are absent. Pods are tapering at both the ends. Seeds are 4-8, ellipsoid-oblong and compressed. *S. asoca* is highly regarded as a universal panacea in the ayurvedic medicine. The dried flowers of *S. asoca* are used in diabetes and haemorrhagic dysentery and seeds are used for curing bone fractures, strangury and vesical calculi. The leaf juice mixed with cumin seeds and used for treating stomachalagia. The flowers are considered to be uterine tonic and are used in vitiated conditions of pitta, syphilis, cervical adenitis, hyperdipsia, burning sensation, haemorrhoids, dysentery, scabies in children and inflammation (Panchawat *et al.* 2010). Antimicrobial activity of the plant is used in ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms. It prevents the growth of organism which confirms resistance. Therefore, extracts of *S. asoca* plant were tested for their potential activity against microbial pathogens (Dabur *et al.* 2007).

Phytochemical is a natural bioactive compound present in plant foods that works with nutrients and dietary fibre to protect against disease. The antimicrobial activity of different plant extracts reside in a variety of different phytochemicals such as alkaloids, flavonoids, glycosides, saponins, tannins, steroids. Natural phytochemicals are very effective as precursors for the synthesis of novel useful drugs. About 50% drugs are natural products of medicinal plants, which play an important role in

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drug development in pharmaceutical industry. The methanolic extract of leaves of Asoka was more effective against *Staphylococcus aureus*. This is due to the presence of steroids, but absent in the case of ethanolic extract and the ethanolic extract is more effective against *E. coli* due to the presence of alkaloids and tannins. The methanol extract was effective against different strains of bacteria due to the presence of various phytochemical constituents such as flavonoids, glycosides, saponins and steroids (Sarojini *et al.* 2011). Structural features and activities of various phytochemicals present in *S. asoca* are summarized in the Table 1.

Table: 1. Structural features and activities of various phytochemicals present in *S. asoca*

S.No.	Phytochemicals	Structural features	Examples	Activities
1.	Phenols and polyphenols	C_3 side chain,-OH group,phenol ring	Catechol, Epicatechin, Cinnamic acid	Antimicrobial, antidiarrhoeal, antimicrobial
2.	Flavonoids	Phenolic structure, one carboxyl group, hydroxylated phenols, C_6-C_3 unit linked to an aromatic ring	Chrysin, Quercetin, Rutin	Antimicrobial, Antidiarrhoeal
3.	Tannins	Polymeric phenols molecular weight 500-3000	Ellagitannin	Antimicrobial, antidiarrhoeal, Anthelmintic
4.	Alkaloids	Heterocyclic nitrogen compounds	Berberine, Piperine, Palmatine, Tetrahydropalmatine	Antimicrobial, antidiarrhoeal, Anthelmintic
5.	Glycosides	Sugar and non carbohydrate moiety	Amygdalin	Antidiarrhoeal
6.	Saponins	Amphipathic glycosides	Vina-ginenosides-R5 and–R6	Antidiarrhoeal

The present studies were carried out to identify the presence of different phytochemical constituent and its antimicrobial activities against common bacterial pathogens.

Materials and Method

Plant Material: The plant varieties i.e. leaves of *S. asoca caesalpiniaceae* and *S. asoca leguminosae* were collected from H.N.B. Garhwal University campus, Srinagar.

Microorganisms: Bacterial strains (*Bacillus subtilis* MTCC-121, *Escherichia coli* MTCC-120 were obtained from IMTECH, Chandigarh.

Extract preparation: Aqueous, methanolic and acetone extracts of the leaves of the plant was prepared. The plant leaves was washed several time with tap water and then with distilled water and dried in hot air oven and grounded with mortar pestle. The powdered plant bark (20 gm) was extracted for 8 hours with methanol (200 ml) in Soxhlet apparatus. Same procedure was followed for preparation of acetone and aqueous (distilled water) plant extracts.

Antimicrobial activity:

Antimicrobial activities were tested by the agar well diffusion method. Muller Hinton Agar was prepared by mixing it with the distilled water as per calculation and sterilized in autoclave at 15 psi pressure for 15 mins along with the Petriplate, cotton swab and tips which are required for antimicrobial testing. Petriplates containing 20 ml Muller Hinton Agar (HiMedia) medium were seeded with 24 hr culture of bacterial strains (*E. coli* MTCC-120, *B. subtilis* MTCC-121). Wells were cut and 60 µl of the plant extracts prepared by the (namely aqueous, methanol and acetone extracts) were added. The plates were then

incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Tests were performed in triplicates and values of zone of inhibition were expressed as mean value of three replicates.

Minimum inhibitory concentration (MIC) determination: Minimum inhibitory concentration method was applied on extracts that proved their higher efficacy against microorganisms. This method provides information about the minimum standard extract concentration which is required to inhibit the growth of microorganism. Its amount depends upon the type of microorganism against which it is using.Standardized method for determining minimum inhibitory concentrations were used in which serial dilutions of the initial concentration of the extract was used to check the activity against microorganism using agar well diffusion method.

Phytochemical analysis

Qualitative estimation: Freshly prepared extracts were subjected to standard phytochemical analysis to find the presence of phytochemical constituent's. The method described by Odebiyi and Sofowora (1978), were used to test for the presence of flavonoids, tannins, glycosides, phenolics and saponins.

Tannins: To the 2 ml of plant extract, 2 ml of 0.1% ferric chloride was added. Formation of black precipitate indicates the presence of tannins.

Phenols: Plant extracts (2 ml) were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Flavonoids: 2 ml of the plant extracts were treated with 3-4 drops of concentrated HCL and magnesium ribbon was added to it. Formation of red colour indicates the presence of flavonoids.

Glycosides: To the 2 ml of the plant extract, glacial acetic acid, ferric chloride ($FeCl_3$) and concentrated HCL was added drop wise. Formation of reddish brown colour indicates the presence of glycosides.

Saponins: 0.5 gm of the plant extract was shaken with 2 ml of water. Formation of foam and it persists for ten minutes it indicates the presence of saponins.

Quantitative estimation:

Phenolic estimation: Phenols were estimated by the procedure described by (Sadasivam and Manickam, 1997), in which 1g leaf tissue was grounded in 5 ml 80% methanol. The extract was agitated at 70ºC for 15 minutes. Now this methanolic extract was used for estimation of total phenols.To the 1 ml sample 5 ml distilled water was added to make the final volume 6 ml. To this 250 µl Folin's reagent was added and the mixture was incubated for 3 min at room temperature. After incubation, 1 ml 20% sodium carbonate and 1 ml distilled water were added and the solution was incubated for 1 hr at room temperature. Absorbance was recorded at 725 nm. The amount of total phenols was estimated from the standard curve and expressed as µg phenol g^{-1} fresh weight.

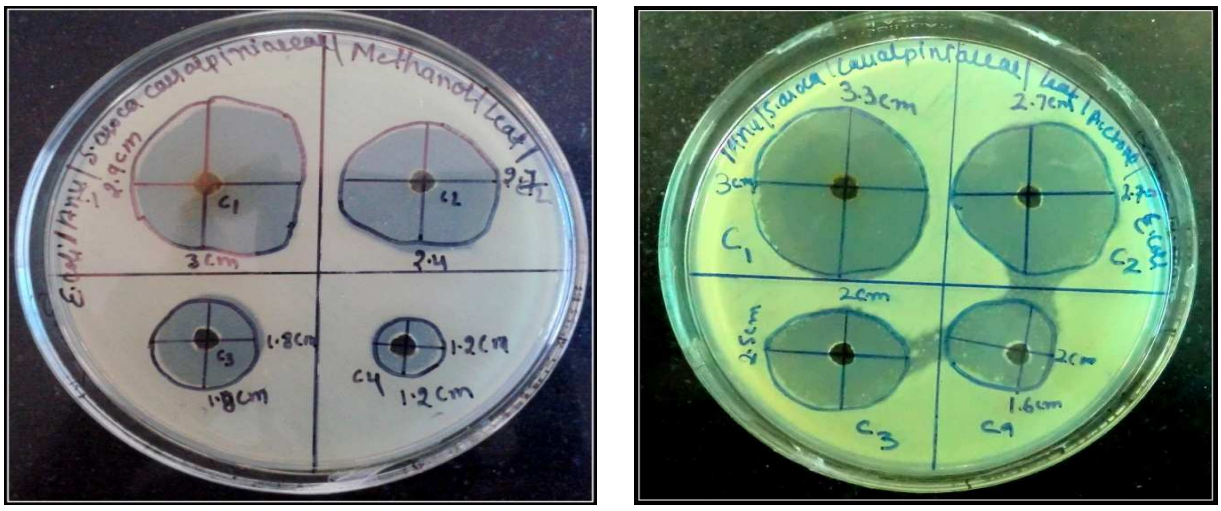
Flavonoids estimation: Flavonoids were estimated by the procedure given by (Boham and Kocipai-Abyazan, 1974), in which 1g leaf tissue was grinded in 5 ml 80% methanol. The extract was agitated at room temperature for 1 hour. Now this methanolic extract was used for estimation of total phenols. 10 gm of the plant sample was extracted repeatedly with 100 ml of 80% methanol at room temperature. Then the whole solution was filtered through filter paper. The filtered solution was then transferred into a flask and evaporated into dryness over hot waterbath and weighted to a constant weight. The remaining content after evaporation was flavonoids and the total amount of flavonoids present in the plant extracts was determined.

Results and Discussion

All the leaves extract of *S. asoca* plant species shows the antimicrobial activity against bacterial strains (Table 2). The methanolic leaf extract of *S. asoca caesalpiniaceae* (100 mg/ml) shows maximum zone of inhibition against *B. subtilis* (27.95 mm) and shows least zone of inhibition against *E. coli* (21.90 mm), the similar result has also been recorded by Preeti *et al.* (2012) that the methanol and water extract of the leaves are valuable against *B. subtilis, Ps. aeruginosa* and *S. typhymurium.* The acetone leaf extract of *S. asoca caesalpiniaceae* (100 mg/ml) shows maximum zone of inhibition when subjected against *B. subtilis* (28.08 mm) but shows least zone of inhibition against *E. coli* (22.16 mm). Aqueous leaf extract of *S. asoca caesalpiniaceae (*100 µg/ml) shows maximum zone of inhibition when used

against *B. subtilis* (20.38mm) but shows no zone of inhibition against *E. coli.*

The methanolic leaf extract of *S. asoca leguminosae* (100 mg/ml) shows significant zone of inhibition against *B. subtilis* (13.00 mm) as compare to the *E. coli* (15.80 mm)**.** Acetone leaf extract of *S. asoca leguminos*ae (100 mg/ml) shows significant zone of inhibition against *E. coli* (17.60 mm) and shows least zone of inhibition in case of *B. subtilis* (11.21 mm). Antimicrobial activity of the plant extracts are also determined by the Minimum inhibitory concentration (Four fold serial dilution) (Table 3).The *S. asoca caesalpiniaceae* methanol and acetone leaves extract show significant antimicrobial activity (Fig.1) against bacterial strains as compare to the *S. asoca*



(a) (b)

Fig.1 Showing antimicrobial activity of *S. Asoca caesalpiniaceae* methanol (a) and acetone (b) leaves extract using MIC against *E. coli* (MTCC120).

Key: C1*: 1st dilution (40 mg/ml), C2*: 2nd dilution (20 mg/ml), C3*: 3rd dilution (10 mg/ml), C4*: 4th dilution (5 mg/ml).

The antimicrobial activity of the plant is due to the presence of several phytochemical constituents like alkaloids, flavonoids, saponins, glycosides, and phenolics. The phytochemical constituents of the plant species investigated are summarized in Table 4**.** Some researchers mentioned that methanol and aqueous extracts of *S. asoca* leaves exhibited antimicrobial activity against *B. subtilis, Ps. aeruginosa,* and *S. typhymurium, Alternania alternata, Colletotrichum gloesporioides* and *Drechlera specifera* (Pradhan *et al.* 2009). Other researchers also found that the methanolic and aqueous leaves extract of *Saraca asoca* showed antimicrobial activity *against B. subtilis, Ps. aeruginosa* and *S. typhymurium* (Seetharam *et al.* 2003). Antibacterial activity of ethyl acetate extract of dried mature leaves of *S. asoca* were tested against *E. coli, S. aureus, Ps. aeruginosa* and *B. cereus*. Ethyl acetate extract shows maximum antibacterial activity against *S. aureus, Ps. aeruginosa* and *B. cereus* as compare to the *E. Coli* (Pradhan *et al.* 2009).The water extract of *S. asoca*, was found to be the most active against bacterial and fungal pathogens. Water soluble fraction of the flowers and bud of *S. asoca* were reported to have significant inhibitory effect against *Sh. boydis* (Narang *et al.* 1962).

Some modern research has explored another useful activity of *S. Asoca* i.e. chemoprevention of skin cancer by the flavonoids fraction of *S. asoca* flower

Identification of phytochemical contents

Table 2: Antimicrobial activity of different fraction of *S. asoca caesalpiniaceae* and *S. asoca leguminosae*

Zone of Inhibition in mm									
Solvent	***S. asoca caesalpiniaceae***				***S. asoca leguminosae***				
	Positive control (Km)*	*E. coli*	Positive Control (Gm)#	*B.subtilis*	Positive control (Km)	*E. coli*	Positive Control (Gm)	*B. subtilis*	Negative control
Aqueous Extract	30.00 ±1.2	0.00	20.00 ±0.2	20.38 ±2.0	30.00 ±1.2	14.75 ±0.4	20.00 ±0.2	10.00 ±1.0	0.00
Acetone extract	30.00 ±0.8	34.12 ±0.5	20.00 ±0.5	36.80 ±1.8	30.00 ±0.8	17.60 ±0.2	20.00 ±0.5	11.21 ±0.6	0.00
Methanol extract	30.00 ±1.2	33.20 ±1.0	20.00 ±0.5	35.90 ±0.5	30.00 ±1.2	15.80 ±0.6	20.00 ±0.5	13.00 ±0.2	0.00

***Kanamycin, # Gentamicin**

Table 3: Minimum inhibitory concentration (MIC) determination of *S. asoca caesalpiniaceae* and *S. asoca leguminosae* leaves extracts by four fold serial dilution method

Extracts	**Microorganism**	**Minimum Inhibitory Concentration (in mg/ml) Zone of inhibition (in mm)**							
		Saraca asoca caesalpiniaceae				***Saraca asoca leguminosae***			
		40mg /ml	20mg/ ml	10mg/ ml	5mg/m l	40mg/ ml	20mg/ ml	10mg/ ml	5mg/ ml
Methanol	*E. coli* (MTCC121)	29.5	25.5	18.0	12.0	14.5	10.5	8.5	8.0
Acetone		31.5	27.0	22.5	18.0	11.6	10.0	9.0	6.5
Methanol	*B. subtilis* (MTCC120)	29.2	19.5	21.5	15.0	10.0	8.5	9.0	9.0
Acetone		28.6	25.5	24.5	15.5	9.5	9.5	8.5	8.0

Table 4. Phytochemical screening of aqueous, acetone and methanol extracts of *S. asoca caesalpiniaceae* and *S. asoca leguminosae* leaves extracts

S.No	**Compound**	***Leaves extracts***					
		S. asoca caesalpiniaceae			***S. asoca leguminosae***		
		Aqueous	Acetone	Methanol	Aqueous	Acetone	Methanol
1.	Tannins	-	+	+	-	+	-
2.	Flavonoids	+	+	+	+	-	+
3.	Phenolics	-	-	-	-	-	+
4.	Glycosides	+	-	+	+	-	+
5.	Saponins (foam test)	+			+		

(+) Positive, (-) Negative

has been found (Cibin *et al.* 2010). Potential anticancer activity of *S. asoca* extracts towards transplantable tumours in mice has also been successfully reported (Varghese *et al.*1992). By the quantitative analysis it has been concluded that the concentration of flavonoids occurs more in *S. asoca caesalpiniaceae* leaves extract (645 µg/ml) as compare to the *S. asoca leguminosae* leaves extract (214 µg/ml) but no phenolic compound present in *S. asoca caesalpiniaceae* leaves extract and in *S. asoca leguminosae* leaves extract phenolic compound present is 0.601 µg/ml. The presence of tannins, proteins, steroids, glycosides, carbohydrates, saponins, flavonoids in *S. asoca* may be responsible for the various pharmacological actions (Saha *et al.* 2011). It has been reported that most active phytochemicals constituent in the flowers are mainly flavonoids, steroids, tannins and glycosides. These phytoconstituents may be responsible for various pharmacological actions of this plant part, like antibacterial, antiulcer, anticancer, larvicidal and chemoprotective activities (Pal *et al.* 1985). The presence of the phytochemical constituents makes the plant parts (bark, leaves, flowers and seeds) pharmacologically important for their usefulness in the treatment of various diseases (Maruthappan *et al.* 2010).

Conclusion

Natural medicinal plants are highly accepted as universal solution in ayurveda medicine. The use of herbal medicine has always been part of human culture, as some plants contain important therapeutic properties, which can be used to cure human diseases. *S.asoca* is also known to possess certain active compounds which can be used as a good antimicrobial agent. In the present investigation the phytochemical screening and anti microbial study of the two species of the *S. asoca* namely *S. asoca caesalpiniaceae* and *S. asoca leguminosae* has been done. *S. asoca caesalpiniaceae* species of the plant show more significant antimicrobial activity towards *E. coli* and *B. subtilis* as compare to the *S. asoca leguminosae* which is due to the presence of phytochemical constituents.The phytochemical screening of the plant shows the presence of phytochemical components (glycosides, phenolics, tannins, saponins and flavonoids). So it has confirmed that the plant extracts could be used for the treatment of various ailments.

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