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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL AND ANTIBACTERIAL STUDIES OF BARK EXTRACT OF SOYMIDAFEBRIFUGA (ROXB.) JUSS

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ABSTRACT

Soymida febrifuga (Roxb.)A. Juss. of the family Meliaceae traditionally used for the treatment of various diseases. The present study focuses on the screening of the preliminary Phytoconstituents and antimicrobial activity of barks of S. febrifuga. The screening of the phytoconstituent was carried out by dilution extract of 1gm/ml respectively and antimicrobial activity was performed by using disc diffusion methods. The preliminary phytoconstituents showed the presence of Alkaloids, Carbohydrate, Flavonoids, Glycoside, Phenol, Saponins, Steroids, Tannins and Terpinoids. The results shows that the antibacterial activity of aqueous extract acetone and methnolic bark extract have maximum inhibition zone against most of the pathogenic bacteria. While chloroform, ethanol and petroleum ether showed moderate activity against some of the pathogenic bacterial strains. The streptomycin was used as control.

INTRODUCTION

Soymida febrifuga is an indigenous and lofty deciduous medicinal tree found frequently on dry stony hills. Leaves are paripinnately clustered at the end of twigs, petioles swollen at base, leaflets opposite, coriaceous, and ovate to lanceolate with entire margin. [1] Flower is greenish white in colour, in large terminal or axillary divertically branched panicles often equating the leaves. Fruits are 2.5 to 6 cm long, black, woody in colour, obovoid in shape with 5 celled and 5 valved with winged seeds. Heartwood is dark blood red to reddish brown in colour. Bark occurs in the form of half quills of red brown colour and has astringent and anti-periodic properties. [2, 3]
Moreover nature has rich deposits of botanical affluence and a large number of varied types of plants grown in different parts of the country. Traditional medicine has been enhanced in developing countries as an unconventional solution to health problems and costs of pharmaceutical products. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to the host cells are considered for developing new antimicrobial drugs [4]. Secondary metabolites such as flavonoids, alkaloids, tannins and phenolic compounds have been established as the bioactive compounds of plants [5]. The aim of the present study is to screen in vitro antimicrobial activity and phytochemical analysis of bark extracts of *Soymida febrifuga*.

Ethno medicinal Values

The decoction of the bark containing bitter resin is used in rheumatic pains and stomach pains. It is also used for the treatment of wounds, dental diseases, uterine bleeding, and haemorrhage and as anticancer agent [6].The bark is crushed and mixed with water and administered in cough [7]. In addition to remove blood impurities, various parts of the plant are claimed to be good for ulcers, leprosy, and dysentery and have an anti-inflammatory activity. It is considered to be as good as the cinchona bark for the treatment of malaria. In Unani Medicine, decoction of the bark is used in fevers, and is astringent to bowels and a good substitute for oak-bark used for vaginal infections, preparation of gargles, and enemas. Literature surveys showed that methyl angolensate and steryl glycoside [8] were isolated from bark. Quercetin-3-O rhamnoside and Quercetin-3-Orutinoside [9] were isolated from leaves. The phytomedicines which can be derived from any part of the plant like bark, leaves, flowers, fruits, seeds, etc. i.e., any part may contains active components[10].

MATERIAL AND METHODS

Collection and Authentication

The plant was collected from Balrampur district, Chhattisgarh, India. The taxonomic identification of the plant was carried out by Dr.S.John Britto,Director and Head, The Rapinat Herbarium and Centre for Molecular Systematics St. Joseph's College (*Autonomous*) Tiruchirappalli, India. The voucher specimen was deposited at the centre (RHT 67112).

Extraction Procedure

The collected barks of plant were dried at room temperature,

powdered, and was then stored in air tight container till use. It was weighed in a selected quantity and was subjected to Rotary shaker extraction using solvents such as Acetone, Aqueous, Chloroform, Ethanol, Methanol and Petroleum ether respectively. The solvent was then evaporated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of phytochemical and antibacterial studies.

Preliminary Phytochemical Screening

The extracts of the powdered barks of *S.febrifuga* was analysed for the presence of various phytoconstituents like Alkaloids, Carbohydrate, Flavonoids, Glycosides, Phenol, Saponins, Steroids, Tannins and Terpenoids compounds were identified using standard phytochemical procedure.

Test's for Alkaloids

Hager'sTest

To 2 ml extract, add few drops of Hager's reagent (Saturated solution of picric acid). Formation of yellow colour precipitate signifies positive result.

Mayer's Test

To 2mlextract, add few drops of Mayer's reagent. Formation of cream precipitate indicates the presence of alkaloids.

Wagers Test

To 2ml extract, with few drops of Wagers reagent. Formation of reddish brown precipitate indicates the presence of alkaloids [11].

Test's for Carbohydrate

Benedict's Test

Extract was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test (For reducing sugars)

Extract was hydrolysed with dil. HCL, neutralized with alkali and heated with Fehling's A&B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Molisch's Test

2ml extract + 10ml H_2O + 2 drops Ethanolic α-naphthol (20%) + 2ml conc.H_2SO_4. Reddish violet ring at the junction.

Test's for Flavonoids

Alkaline Test

To 2-3 ml of extract, few drops of 5% NaOH solution were added in a test tube. Formation of intense yellow colour which turns colourless on addition of few drops of dilute HCL indicated the presence of flavonoids [12].

Lead acetate Test

1ml extract was treated with 1ml 10% lead acetate ($pb(OA_C)_4$) solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Pews Test

To 2-3ml extract, add zinc powder in a test tube, followed by drop wise addition of conc. HCL. Formation of purple red or cherry colour indicates the presence of flavonoids [13].

Shinoda Test

To 2-3ml extract, few fragments of magnesium metal were added in a test tube, followed by drop wise addition of concentrate HCL. Formation of red or crimson red colour indicated the presence of flavonoids [11].

Test's for Glycosides

Glycosides Test

To small amount of extract, add 1ml water and shake well. Then aqueous solution of NaOH was added. Yellow colour appeared that indicates the presence of glycosides. [14]

Keller-Kiliani Test (Test for cardiac glycoside)

To 2ml extract, add 1ml glacial acetic acid, one drop 5% $FeCl_3$ and 1ml conc.H_2SO_4. A brown ring of the interface indicates the presence of deoxysugar characteristics of cardenolides, cardiac glycosides [11, 12]

Molisch's Test

To 1ml of extract, 2drops of Molisch's reagent was added in a test tube and 2ml of concentrate H_2SO_4 was added carefully keeping the test tube slightly curved**.** Formation of violet ring at the junction indicated the presence of glycosides [11].

Test's for Phenol

FeCl3

To 2ml of extract add 2-3 drops of 5% ferric chloride solution. Formation of bluish-black colour shows presence of phenol and black colour shows tannins.

K2Cr2O7

To the extract add 5% potassium dichromate solution. Positive result is confirmed by a formation of brown precipitate.

Test's for Saponins

Foam Test

2ml extract was diluted with 10ml of distilled water and warmed gently. It was shaken for 15 minutes. Persistent froth indicated the presence of saponins [11].

$NaHCO_3$ Test

To extract a drop of sodium bicarbonate was added. The

mixture was shaken vigorously and kept for 3 min. A honey comb like froth was formed and it shows the presence of saponins.

Test's for Starch

Iodine Test: 2ml extract was treated with 5 drops of Iodine solution, gives blue colour indicates the presence of starch.

Table 1 Phytochemical screening of *Soymida febrifuga* bark

S.No.	Phytoconstituents	Name of Tests	Solvents used					
			Acetone	Aqueous	Chloroform	Ethanol	Methanol	Petroleum ether
1.	Alkaloids	Hagers	_	+	_	+	++	_
		Mayers	_	_	_	_	_	_
		Wagers	+	+++	_	++	+++	_
2.	Carbohydrate	Benedict's	++	+++	_	++	+++	_
		Fehling's	_	+++	_	++	++	_
		Molisch's	_	_	_	+	+	_
3.	Flavonoids	Alkaline	+++	++	+	++	+++	_
		Lead acetate	+++	+++	++	+	++	_
		Pews	+++	_	_	+	+++	_
		Shinoda	+++	_	++	+	++	_
4.	Glycosides	Glycosides	+	_	_	_	+	_
		Keller-Kiliani	+++	++	+	++	+++	_
		Molisch's	+	++	_	+	++	_
5.	Phenol	$FeCl_3$	++	++	_	++	++	_
		$K_2Cr_2O_7$	++	+	_	++	+++	_
6.	Saponins	Foam	_	++	+	_	_	_
		$NaHCO_3$	_	_	_	+	++	_
7.	Starch	Iodine	_	_	_	_	_	_
8.	Steroids	Salkowski's	++	_	_	_	_	_
		Braymer's	_	++	_	_	_	_
9.	Tannins	$FeCl_3$	+	+++	_	+	++	_
		Lead acetate	+++	++	_	++	++	_
10.	Terpinoids	Salkowski's	+	++	_	++	++	_

- Absent, + Present, ++ good, +++ very good.

Table 2 Antibacterial assays of *Soymida febrifuga* bark

S.No.	Test microorganisms	Inhibition zones (mm)					
		Acetone	Aqueous	Chloroform	Ethanol	Methanol	Petroleum ether
1.	*Bacillus cereus*	0.0	0.0	0.0	0.0	0.0	0.0
2.	*Bacillus subtilis*	0.0	0.0	0.0	0.0	0.0	0.0
3.	*Entero bacteraerogenes*	10±0	7.33±0.33	0.0	0.0	7.33±0.33	0.0
4.	*Escherichia coli*	7.33±0.33	7.67±0.33	7.33±0.33	8±0.58	7.67±0.67	7.33±0.33
5.	*Klebsiella pneumoniae*	7.33±0.33	12.33±1.45	7.67±0.33	9±0.58	10±0.58	0.0
6.	*Proteus mirabilis*	0.0	7.65±0.33	8±0.58	0.0	8.33±0.88	0.0
7.	*Proteus vulgaris*	7±0.33	10±0.58	8±0.58	0.0	0.0	0.0
8.	*Pseudomonas aeruginosa*	0.0	0.0	0.0	0.0	0.0	0.0
9.	*Salmonella paratyphi*	0.0	0.0	0.0	0.0	0.0	0.0
10.	*Serrati amarcescens*	0.0	0.0	0.0	0.0	0.0	0.0
11.	*Staphylococusaureus*	7.33±0.33	10.33±0.88	0.0	8.33±0.88	9.67±1.20	4.67±2.33
12.	*Streptococouspneumoniae*	8.67±0.67	15.33±1.45	7.67±0.33	8±0.58	10.67±1.20	0.0
13.	*Vibrio cholerae*	8±1	13.67±0.88	7.67±0.33	9±0.58	11±0.58	7.33±0.33

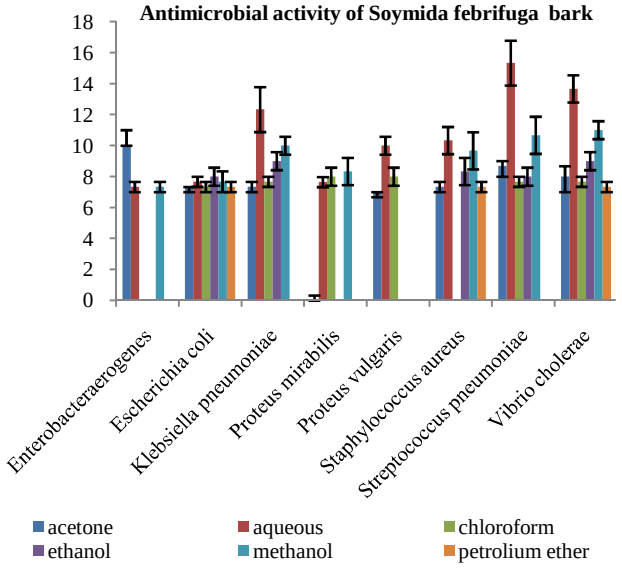

Figure 1 Antibacterial Inhibition Zones

Test's for Steroids

Salkowski's Test

To 2ml of extract, add 2ml chloroform and 2ml conc.H_2SO_4 from the side of the test tube. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols [11].

Test's for Tannins

Braymer's Test

2ml extract + 2ml H_2O + 2-3 drops $FeCl_3$ (5%). Green precipitate shows the presence of tannins.

$FeCl_3$ Test

To 2ml of extract add 2-3 drops of 5% ferric chloride solution.

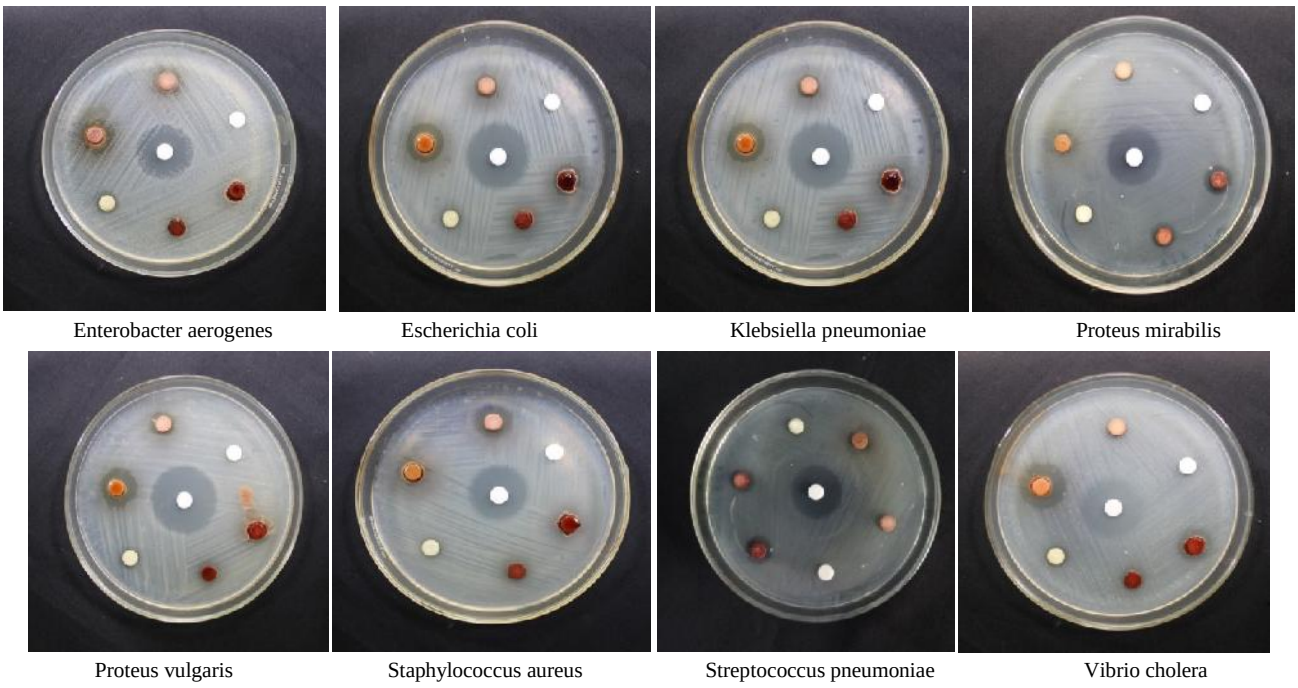

Figure 2 Antibacterial Inhibition zone in Bark Extract of *Soymida febrifuga*

Formation of black colour shows the presence of tannins.

Lead acetate Test

Few drops of 10% lead acetate solution were added into 5ml of extract. Formation of yellow or red precipitate indicates the presence of tannins [14].

Test's for Terpenoids

Salkowski's Test

2 ml of chloroform and 1ml of conc.H_2SO_4 was added to 1ml of extract and observed for reddish brown colour that indicates the presence of Terpenoids.

Test Micro-organisms

13 bacterial strains were used in study namely Staphylococcus aureus (MTCC # 3163), Escherichia coli (MTCC# 199), Klebsiella pneumoniae (MTCC # 3040), Pseudomonas aeragenosa (MTCC # 2474), Salmonella paratyphi (MTCC # 734), Vibrio cholera (ATCC # 14104), Enterobacter aerogenes (MTCC # 2990), Streptococcus pneumoniae (ATCC # 7066), Bacillus subtilis (MTCC # 441), Bacillus cereus (ATCC # 4342), Proteus vulgaris (MTCC # 1771), Proteus mirabilis (MTCC # 1429) and Serratia marcescens (MTCC # 2645). These pathogenic micro-organisms were obtained from Rapinat Herbarium and centre for molecular systematic, St. Joseph's College Tiruchirappalli, Tamilnadu. All the test bacterial strains were maintained on nutrient agar media at 4 °C.

Preparation of Disc

6 mm discs were prepared and sterilized in autoclave. These discs were soaked in different extracts like Acetone, Distilled water, chloroform, Ethanol, Methanol and Petroleum ether. The standard drug streptomycin was used as control.

Determination of Antibacterial Activity

Antibacterial activities of the *S.febrifuga* bark extract was determined by disc diffusion method [15]. Nutrient agar was prepared for the study. Each plate of Nutrient agar was swabbed with each bacterial strain by using sterile cotton swab. The soaked dried discs were placed on the surface of each inoculated plate. The plates were allowed for diffusion for half an hour and then transferred to incubator at 370c for 24 hours. Standard disc of Streptomycin was also placed as positive control. The antibacterial activity of bark extracts was determined by measuring the diameter of zone of inhibition in mm.

RESULT AND OBSERVATION

Phytochemical screening

The phytochemical analysis of *S.febrifuga* bark revealed the presence of Alkaloids, Carbohydrate, Flavonoids, Glycoside, Phenol, Saponins, Steroids, Tannins and Terpenoids. The highest phytoconstituents were observed in acetone, ethanol and methanolic extracts followed by aqueous and chloroform where as the Petroleum ether was observed nil (Table 1).

The antibacterial activity of *S.febrifuga* bark extracts against all test bacterial strains exhibits that the aqueous extract showed effect on 8 different bacterial strains followed by methanolic and Acetone extract. Chloroform showed antibacterial effect on 6 bacterial strains while ethanol 5 and petroleum ether showed effect on 3 bacterial strains. (Table2, Figure 2).

CONCLUSION

The preliminary phytochemical and antibacterial studies of S.febrifuga bark reveals the presence of secondary metabolites. The plant extracts were also found to have positive effective against most of the test micro-organism. Hence the plant can

be used as alternative drug to cure disease caused by pathogenic bacteria. Further studies are required to isolate, characterize and elucidate the structural of the bioactive compounds for better therapeutic values.

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