

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF LEAVES OF *SOYMIDA FEBRIFUGA* (ROXB.) A.JUSS

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ABSTRACT

Soymida febrifuga (Roxb.) A. Juss belonging to the family *Meliaceae* traditionally used for the treatment of various diseases. The objectives of the present study to evaluate the antibacterial efficacy and phytochemical screening of leaves of *Soymida febrifuga* (Roxb.) A.Juss. The antibacterial efficacy was performed by using disc diffusion method and phytochemical screening was carried out by dilution of extract 1gm/ml respectively. The results shows that the antibacterial activity of acetone and methanol leaves extract shows maximum inhibition against pathogenic bacteria like *Klebsiella pneumoniae* (38mm) and *Pseudomonas aureginosa* (37mm) at its higher concentration. The distilled water extract also shows more

activity against *Pseudomonas aureginosa* (35mm) at 10mg/disc. The amoxicillin was used as standard drug. All the extracts show moderate activity against all the pathogenic bacteria. The preliminary phytochemical screening shows the presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, terpenoids, and amino acids. It can be concluded that the *Soymida febrifuga* leaves may possess excellent antibacterial property because it contains rich phytochemicals.

KEYWORD: Phytochemical analysis, antibacterial assay, MIC, *Soymida febrifuga*.

INTRODUCTION

The history of plants being used for medicinal purpose is probably as old as the history of mankind. India is a nation blessed with rich heritage of traditional systems of medicine and rich biodiversity with 15 agro-climatic zones.^[1] Wild plant species are excellent source of wide range of biochemicals. Indian medicinal plants have been used to treat diseases for

thousands of years. Medicinal plants constitute the main source of new pharmaceuticals and health care products.^[2] The use of medicinal plants in industrial societies is mainly for extraction and to obtain several drugs from these plants. Extraction and characterization of several active phytochemicals from these green factories have produced high activity profile drugs.^[3] It is believed that crude extract in medicinal plants are biologically more active than isolated compounds due to their synthetic effects.^[4] Secondary metabolites of plants having the defense mechanism against predation by many micro organisms, insect and herbivores.^[5] Secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds have been established as the bioactive compounds of plants.^[6] Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available with less side effects. The aim of the present study is to screen the phytochemicals in the leaves and antibacterial activity of the leaves of *Soymida febrifuga* (Roxb) A.Juss.

Soymida febrifuga (Roxb) A.Juss is an indigenous 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.^[7] Flower is greenish white in colour, large terminal or axillary divaricately 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.^[8-10]

Medicinal uses

In Ayurveda the bark is used to remove 'Vata', *tridosha*, fever, cough, asthma, blood impurities, ulcers, leprosy, and dysentery. In *unani* system it is astringent to bowels and useful in fever. It is bitter tonic, antiperiodic, antimalarial and used against rheumatic swellings. The decoction of bark has bitter resin used to cure vaginal infection, rheumatic pains and stomach pains. It is also useful in treatment of dental diseases, uterine bleedings, haemorrhage and as anticancer agent.^[11] Decoction of bark also useful for the treatment of gum infection, tongue sores, and for fixing loose teeth.^[8]

The phytomedicines which can be derived from any part of the plant like bark, leaves, flowers, fruits, seeds, etc. *i.e.*, any part may contain active components.^[12]

MATERIALS AND METHODS

Collection of Plant Material

The fresh leaves of *Soymida febrifuga* were collected from Kandhar tahsil of Nanded district and authenticated by Botanical Survey of India, Pune. The collected sample was washed with tap water to remove soil particles and dirt. The plant material was shade dried and powdered by using electronic grinder.

Preparations of extract

About 30-40 gm powder of leaves was extracted with different solvents by using Soxhlet apparatus until the extractive was clear. The extracts were concentrated with water bath at 50⁰ c then the semi-solid extracts were stored in refrigerator at 4⁰ c for further use.

Phytochemical screening

The extracts were subjected for phytochemical screening by dissolving them in respective solvents (1gm/ml). The extracts show the presence of Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids, Carbohydrates and Amino acids.^{[13][14]}

Test for Alkaloids: To the test solution, add 1% Hcl and any of mayor's reagents, Wagner's reagent or dragendroff's reagent added. A creamish or brownish red or orange precipitate indicates the presence of Alkaloids.

Test for Flavonoids: To the test solution of each extracts added 5 ml of dilute ammonia solution followed by addition of concentrated H₂SO₄. a yellow color in each extract indicates the presence of Flavonoids.

Test for Tannins: Few drops of ferric acid solution were added to the test solution and there is the formation of brownish green or blue black coloration indicates presence of Tannins.

Test for Saponins: Extracts were diluted in 5ml distilled water and shakes vigorously and formation of a stable persistent froth which indicate presence of Saponins.

Test for Terpenoids: To the test solution 2 ml of chloroform and 3 ml of H₂SO₄ was carefully added. Formation of a reddish brown colour at the interface indicates the presence of Terpenoids.

Test for Carbohydrates: The test solution was boiled on water bath and 1ml of fehling's solution A and B were added respectively. A red precipitate indicates presence of Carbohydrates.

Test for Proteins: To the extracts few drops of concentrated nitric acid added. Formation of yellow colour shows the presence of Proteins.

Test Micro-organisms: The microbial strains *E. coli* MTCC (443), *Salmonella typhii* MTCC (734), *Klebsiella pneumoniae* MTCC (2653), *Pseudomonas aureginosa* MTCC (424), *Staphylococcus aureus* MTCC (96), *Shigella flexinari* MTCC (1457), were obtained from Institute of Microbial Technology (IMTECH), Chandigarh.

Preparation of Disc: 10mm discs were prepared and sterilized in autoclave. These discs were soaked in different concentrations of extracts like Petroleum ether, Ethanol, Methanol, Acetone and Distilled water. The standard drug Amoxicillin was used as control.

Determination of Antibacterial Activity

Antibacterial activities of the *Soymida febrifuga* leaves extract were determined by agar 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 glass spreader. 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 Amoxicillin were also placed as positive control and acetone was used as negative control. The antibacterial activity of leaves extracts was determined by measuring the diameter of zone of inhibition in mm.

Minimum Inhibitory Concentration

For determination of MIC, different concentrations of plant extracts prepared ranges from 2 to 10 mg/disc. Each concentration of plant extracts were tested against the different bacterial pathogens and the inoculated plates were incubated for 24 hours. Antibacterial activity was assigned by measuring the zone of inhibition around the disc. Amoxicillin was used as standard.

RESULT AND OBSERVATION

The preliminary phytochemical screening of *Soymida febrifuga* leaves extract shows the presence of phytochemical compounds like Alkaloids, Flavonoids, Saponins, Tannins,

Terpenoids, Carbohydrates and Amino acids in all the extracts. The alkaloids were found only in petroleum ether extract. Flavonoids are not found in the ethanol and acetone extract. Tannins were present in all extracts except petroleum ether extract. Terpenoids were not found in Actone and Distilled water extract (Table-1).

The antibacterial activity of *Soymida febrifuga leaves extracts* with different concentrations against all test bacterial strains exhibits that the Acetone leaves extract shows greatest effect on *Klebsiella pneumoniae* (38 mm) at 10 mg/disc followed by *Staphylococcus aureus* (36 mm) fig-1(1,2), *Shigella flexinari* and *Eschrichia coli* (28mm), *salmonella typhii* (26mm) but *Pseudomonas aureginosa* (16mm) shows moderate activity, Table-2.

The methanol leaves extract also displayed maximum inhibitory growth against *pseudomonas aureginosa* (37 mm) followed by *Klebsiellaa pneumoniae* (33 mm) fig-2(1,2), *Staphylococcus aureus* (30 mm), *Salmonella typhii* and *Escherchia coli* (27 mm) and *Shigella flexinari* (20 mm) at its higher concentrations i.e 10 mg/disc

The distilled water extract also shows marked inhibition at it's higher concentration against the bacterial strains like *pseudomonas aureginosa* (35 mm), *Escherchia coli* (32 mm), *Staphylococcus aureus* and *Shigella flexinari* (30mm) *Klebsiella pneumoniae* (21 mm) and *Salmonella typhii* (20 mm).

The Ethanol and Petroleum ether extract also shows good antibacterial activity against *S.aureus* (32mm) and *E.coli* (31mm) respectively at higher concentrations where as it shows less activity against *S.aureus* (30mm), *K.pheunomonae* (23mm), *P.aureginosa* (22mm), *Sh.flexinari* (21mm). But it shows very less activity against *S.typhi* (18 mm).

Table: 1 Antibacterial assay of *Soymida febrifuga* leaves

Plants	Solvents	Conc. Mg/disc	Pathogens (zone of inhibition in mm)					
			<i>E.coli</i>	<i>S.typhii</i>	<i>K.pneumoniae</i>	<i>P.aeruginosa</i>	<i>S.aureus</i>	<i>Sh.flexinari</i>
<i>Soymida febrifuga</i> (leaves)	Petroleum Ether	2	16	-	-	-	17	-
		4	20	-	-	-	21	-
		6	21	-	15	16	23	16
		8	28	16	19	19	25	19
		10	31	18	23	22	30	21
	Ethanol	2	-	-	-	-	15	-
		4	-	-	-	-	19	-
		6	16	15	-	-	25	-
		8	18	17	16	16	27	17

		10	23	25	20	20	32	20
	Methanol	2	-	14	-	15	18	-
		4	17	22	-	21	20	-
		6	22	23	16	23	22	-
		8	23	25	25	33	27	19
		10	27	27	33	37	30	20
	Acetone	2	-	-	16	-	19	17
		4	16	15	22	-	26	20
		6	19	19	26	-	28	24
		8	22	23	30	14	30	25
		10	28	26	38	16	36	28
	Aqueous	2	-	-	-	17	15	-
		4	14	-	-	18	16	16
		6	20	-	18	20	25	24
		8	23	17	20	30	27	25
		10	32	20	21	35	30	30
	Standards		28	30	30	24	26	24

Table 2: Phytochemical screening of *Soymida febrifuga* leaves

Plant Name	Extracts	Alkaloids	Flavonoids	Saponins	Tanins	Carbo hydrates	Terpe noids	Amino Acids
<i>Pergula daemia</i> leaves	Petroleum Ether	+	+	+	-	+	+	+
	Ethanol	-	-	+	+	+	+	+
	Methanol	-	+	+	+	+	+	+
	Acetone	-	-	+	-	+	-	+
	Aqueous	-	+	+	+	+	-	+

+: Present, - : Absent

1) *Klebsiella pneumoniae*2) *Staphylococcus aureus*Fig-1: *Soymida febrifuga* leaves extract (Acetone)



1) *Pseudomonas aureginosa* 2) *Klebsiella pneumoniae*

Fig-2: *Soymida febrifuga* leaves extract (Methanol)

DISCUSSION

The plant *Soymida febrifuga* is a medicinal plant which has been in use for a long period of time for the treatment of bacterial infections. The present study was undertaken to determine the phytochemical and antibacterial activity of leaves of *Soymida febrifuga*. The Acetone and Methanol extracts shows much inhibition against all test bacterial strains in comparison with the reference antibiotic Amoxicillin. Among the extracts Acetone extract shows significant inhibitory activity against all test organisms (Table-2). Plant based antibacterial activity have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antibacterial.^[16] The results of preliminary phytochemical screening of *Soymida febrifuga* shows the presence of different plant metabolites like Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids, Carbohydrates and Amino acids. Earlier study of Sato *et al*; 1950 and Liu *et al*; 2001 states that the secondary metabolites (phytochemicals) bear antibacterial properties, the phytochemical crude extract along with antibiotics may enhance the inhibitory activity against the micro organisms.^[17,18]

Several studies have been carried out to understand the potential bioactivity of plants, Deba *et al*; 2008 states that antioxidant and antimicrobial properties of various plant extracts are of great interest in research in food industry because of their use as natural additives emerged from growing tendency to replace synthetic antioxidant and antimicrobials with natural ones.^[19]

Baravalia *et al*; 2009 stated that the plants containing high level polyphenols have greater importance as they are the natural antimicrobials.^[20]

The primary benefits of using plant based medicines are comparatively safer than synthetic alternatives, as pure chemicals have non target adverse effects on health, whereas phytochemicals have instances of traditional uses as ethnic medicines.

CONCLUSION

From the overall results it can be concluded that the Acetone and methanol extract of *Soymida febrifuga* leaves have excellent antibacterial activity against *Klebsiella pneumone* and *Pseudomonas aureginosa*. So the plant studied here can be seen as a potential source of new useful drugs. The phytochemical characterisation of the extracts for the identification of effective bioactive compounds and quality standards are necessary for further study.

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