



## Phytochemical Analysis and In Vitro Antibacterial Activity of *Soymida febrifuga* (Roxb.) Juss. and *Hemidesmus indicus* (L.)

### KEYWORDS

Antibacterial, Phytochemical, *Soymida febrifuga* and *Hemidesmus indicus* (L.).

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**ABSTRACT** Natural plant products are the source of most active ingredients of the medicine. The extract of many plants used in traditional medicine contain a wide range of curative agents that are used in many modern medicines. The present investigation is on phytochemical analysis and in vitro antibacterial activities of the n-butanolic extracts of *Soymida febrifuga* and *Hemidesmus indicus* (L.) having ethnomedicinal uses collected from the Kadapa district were tested. The phytochemical screening of the leaf extracts revealed the presence of carbohydrates, tannins, alkaloids, flavonoids, steroids, glycosides in the two plants. In vitro antibacterial activity of the extracts was evaluated for selected major human pathogenic bacterial strains like *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, and *Staphylococcus aureus* by agar well diffusion method. The results of antibacterial activity revealed that the extracts showed excellent inhibitory activity against all the tested pathogens and the *Soymida* extract showed comparatively better activity than the other *H. indicus* extract

### INTRODUCTION

Nature has very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Traditional medicine has been improved in developing countries as an alternative 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 (Ahmad and Beg, 2001). Secondary metabolites such as flavonoids, alkaloids, tannins and phenolic compounds have been established as the bioactive compounds of plants (Zakir Ullah et al., 2013). The aim of the present study is to screen *in vitro* antimicrobial activity and phytochemical analysis of leaf extracts of selected medicinal plants of *Soymida febrifuga* and *Hemidesmus indicus* (L.).

*Soymida febrifuga* (Roxb.) Juss. is an indigenous lofty deciduous medicinal tree endemic to India (Table: 1). The decoction of the bark has bitter resin used in vaginal infections, rheumatic pains and stomach pains. It is also used for the treatment of wounds, dental diseases, uterine bleeding, haemorrhage and as anticancer agent (Ambaye, 1971, Kirtikar and Basu, 2003). The bark is crushed and mixed with water and administered in cough (Murthy et al., 2001). 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 angelensate and steryl glycoside (Adesogan et al., 1972) were isolated from bark. Quercetin-3-O rhamnoside and Quercetin-3-Orutinoside (Nair and Subramanian, 1975) were isolated from leaves.

*Hemidesmus indicus* is commonly known as anantmoor or Sugandi pala or sariva belongs to the family Asclepiadaceae and it is well known during the Ayurvedic system of medicine (Shute and Bodhankar, 2010). Sugandi is a perennial, fast-growing thin creeper vine; that sends tendrils out at every node to cling to the surrounding vegetation for stability and support. The roots are known to be very aromatic, emitting a sweet scent reminiscent of a combination of vanilla, cinnamon and almonds. This is a common medicinal plant widely used in Indian and also an official drug in Indian pharmacopoeia and British pharmacopoeia (Anoop, 2008). *Hemidesmus indicus* roots are used as antipyretic, anti-diarrhoeal, astringent and tonic (Gayathri and kannabiran, 2009). Roots are also useful in blood diseases (Verma et al., 2005) biliousness (Kavitha et al., 2006). Furthermore, Das and Devaraj (2006) reported the antibacterial activity of the chloroform and methanol extracts of *Hemidesmus indicus* root.

**TABLE: 1 PLANT SPECIES USED FOR SCREENING OF ANTIBACTERIAL ACTIVITY.**

S.No.	Name of the plant	Kingdom	Family	Genus	Species	Local name
1.	<i>Soymida febrifuga</i>	Plantae	Meliaceae	<i>Soymida</i>	<i>febrifuga</i>	redwood, rohun tree, rohuna, somi
2.	<i>Hemidesmus indicus</i>	Plantae	Asclepiadaceae	<i>Hemidesmus</i>	<i>indicus</i>	Anantamul, Milkweed, sugandhi pala

### MATERIALS AND METHODS

#### Collection of plant material

We have selected the healthy, disease free and mature plants of *Soymida febrifuga* and *Hemidesmus indicus* (L.) from Kadapa district.

#### Sampling of plant material

The fresh and healthy leaves of medicinal plants were washed with running tap water, then with distilled water (three times) and air dried under shade, dried mass was grounded to a fine powder. The powder obtained was kept in small plastic bags with proper labeling.

### Preparation of plant leaf extract

Dried powdered plant material was extracted in soxhlet's extractor for 12 hrs and all extracts were concentrated using rotary evaporator and dry residue was preserved at 4°C in air tight bottles until further use.

### Bacterial strains

*In vitro* antimicrobial activity was examined for n-butanol extracts of plants. Bacterial strains used were, *Bacillus subtilis* (G+ve), *Escherichia coli* (G-ve), *Klebsiella pneumonia* (G-ve), *Proteus vulgaris* (G-ve), and *Staphylococcus aureus* (G+ve).

### Media preparation and antibacterial activity (Agar well diffusion method)

The antibacterial activities of the leaves were tested against the selected bacterial strains. Sterile agar medium was poured into each sterile Petri plate and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile spreader. The antibacterial assay was performed by Agar well diffusion method for solvent extracts. The well of 0.5 cm was made by using a sterile tip. 100 µl of n-butanol plant extracts were added into two wells and 100 µl of tetracycline (antibiotic) was added into one well for control. After these plates were incubated at 37°C for 24 hours. After incubation period the results were observed and antibacterial activities were measured by measuring the diameter of the zones of inhibition around the each well and were compared with the zone of inhibition of standard drug (Tetracyclin).

### Phytochemical screening of the extracts

Plant extracts collected were characterized biochemically by qualitative analysis.

### Detection of alkaloids

About 2 ml each of the extracts were stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath for 10 minutes. 1 ml of the extract was treated with a few drops of Mayer's reagent, precipitation with these reagents was seen as evidence for the presence of alkaloids (Sofowora, 1993).

### Detection of carbohydrates

Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates (Pardhasardhi and Sindhu, 1972).

### Detection of glycosides

Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides (Pardhasardhi and Sindhu, 1972).

### Detection of phenols:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols (Pardhasardhi and Sindhu, 1972).

### Detection of tannins:

Two milliliters each of the methanolic extracts were separately boiled for ten minutes in 10 ml of water in a test tube. A few drops of 0.1% ferric chloride were added to each test tube and observed for 10 minutes for a brownish green or a blue black coloration (Okwu et al., 2005)

### Detection of Flavonoids

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids (Pardhasardhi and Sindhu, 1972).

### Detection of proteins and amino acids

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid (Pardhasardhi and Sindhu, 1972).

### Detection of diterpenes

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Pardhasardhi and Sindhu, 1972).

### Detection of steroids

1 ml each of the extracts was dissolved in 2 ml of chloroform. A few drops of concentrated sulphuric acid were carefully added to form a lower layer. A reddish colour formed at the interphase indicates the presence of a steroid ring (Sofowora, 1993).

## RESULTS AND DISCUSSIONS

In the present investigation the antibacterial properties of n-butanol extracts of three medicinal plants such as *Soymida febrifuga* and *Hemidesmus indicus* (L.) were tested against five human pathogenic bacteria.

**Antibacterial activity of *Soymida febrifuga*:** The n-butanol leaf extracts of *Soymida febrifuga* showed maximum zone of inhibition against *Bacillus* (17 mm), *E. coli* (19 mm), *Klebsiella* (19 mm), *Proteus* (20 mm), *staphylococci* (19 mm) (Table: 2).

**Antibacterial activity of *Hemidesmus indicus*:** The n-butanol leaf extracts of *Hemidesmus indicus* showed maximum zone of inhibition against *Bacillus* (17 mm), *E. coli* (17 mm), *Klebsiella* (17 mm), *Proteus* (16 mm), *staphylococci* (19 mm) (Table: 2).

**TABLE: 2 ANTIBACTERIAL ACTIVITY OF n-BUTANOLIC LEAF EXTRACTS OF MEDICINAL PLANTS AGAINST BACTERIA**

S. No	Name of the organism	Zone of inhibition (mm)		
		Tetracycline	<i>Soymida febrifuga</i>	<i>Hemidesmus indicus</i>
1.	<i>Bacillus subtilis</i>	30 ± 0.35	17 ± 0.51	17 ± 0.15
2.	<i>Escherichia coli</i>	15 ± 0.51	19 ± 0.32	17 ± 0.34
3.	<i>Klebsiella pneumonia</i>	16 ± 0.4	19 ± 0.30	17 ± 0.23
4.	<i>Proteus vulgaris</i>	29 ± 0.3	20 ± 0.25	16 ± 0.31
5.	<i>Staphylococcus aureus</i>	27 ± 0.15	19 ± 0.52	19 ± 0.18

The development of microbial resistance to the antibiotics led to the investigation of antimicrobial drugs from plant extracts. Plant based antibacterial activity have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antibacterials (Lutterodt et al., 1999). The potential of developing antibacterial activity from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes. *Soymida febrifuga* and *Hemidesmus indicus* exhibited good inhibiting activity against all the tested microorganisms. Among the extracts *Soymida febrifuga* exhibited maximum antibacterial activity against all the tested strains. It showed highest activity against *Proteus* and lowest activity against *Bacillus*, (Table: 2). The *Hemidesmus indicus* showed highest activity against *Staphylococci* and lowest activity against *Proteus* (Table: 2).

Phytochemical compounds such as alkaloids, flavanoids, glycosides and several other aromatic constituents are secondary metabolites in plants that alleviate the pathogenic and environmental stress (Marjorie, 1999; Edreva et al., 2008). The results of preliminary qualitative phytochemical study of the plants showed the presence of alkaloids, tannins, cardiac glycosides, steroids, Flavonoids, proteins and diterpenes (Table: 3).

TABLE: 3 PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACTS OF MEDICINAL PLANTS

S. No	Name of the compound	Name of the test	Name of the plants	
			Soymida febrifuga	Terminalia arjuna
1.	Alkaloids	Mayer's test	+	++
2.	Flavonoids	Alkaline Reagent Test	++	++
3.	Carbohydrates	Molisch's Test	+	++
4.	Phenols	Ferric Chloride Test	++	++
5.	Tannins	5% Ferric chloride	++	++
6.	Steroids	Chloroform + Acetic acid + H <sub>2</sub> SO <sub>4</sub>	+	++
7.	Glycosides	Legal's Test	++	++
8.	Proteins	Ninhydrin Test	+	++
9.	Diterpenes	Copper acetate Test	+	+

- = absent

+ = present

++ = more quality

## CONCLUSION

In conclusion both the plant extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. Thus, the plants studied here can be seen as a potential source of new useful drugs and open the possibility of finding new clinically effective antibacterial compounds. The preliminary qualitative phytochemical analysis of the two plants showed the presence of alkaloids, tannins, cardiac glycosides, steroids, flavonoids, proteins and diterpenes. Further, phytochemical characterization of the extracts, the identification and purification of responsible bioactive component and the probable antimicrobial mode of action are necessary for future studies.

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