

ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *Albizia amara*

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ABSTRACT

Albizia amara (Family:Fabaceae) is a plant of Ayurvedic, traditional and ethno medicinal importance as indigenous tribes used different parts of the plant for medicine. The aim of the present study was to evaluate the antibacterial activity of various solvent extracts of *A. amara*. The antibacterial activity of different solvent extracts of *A. amara* were tested against the Gram-positive and Gram-negative bacterial strains by observing the zone of inhibition. The organisms used in the test were *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas auriginosa*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. It was observed that petroleum ether, dichloromethane and methanol extract showed activity against Gram-positive and Gram-negative bacteria. The bark dichloromethane extract of *A. amara* bark showed more activity against Gram-positive and Gram-negative bacteria when compared to other solvent extracts and other different parts of the plant. The results are summarized in the table.

KEYWORDS : *Albizia amara*, Fabaceae, Solvent Extracts, Antibacterial Activity, Phytochemical Tests, Ciprofloxacin, Soxhlet

Infectious diseases remain the main cause of the high mortality rates recorded in the developing nations, the majority of the rural population have limited access to health services provided by the governments, for this the rural population heavily depended on traditional healers. *Albizia amara* (Family:Fabaceae) is one of the 150 species available from the genus *Albizia*. The genus is pan tropical, occurring in Asia, Africa, Madagascar, Central, South, and southern North America and Australia, but mostly in the old world tropics and has been used for various ailments in the traditional as well as folklore system of medicine. *A. amara* is a tree of moderate sized, much branched with smooth, dark green, scaly bark. Leaves; pinnately compound, with 15-24 pairs of small, linear leaflets, on 6-15 pairs of pinnate. The flowers are globose and in clusters with 12-20 globose heads. Fruits are oblong pods, about 10-28 X 2-5 cm, light brown, puberulous, thin, and 6-8 seeded (Orwa et al., 2009).

The seeds of *Albizia amara* used as astringent, treating piles, diarrhea, gonorrhoea, leprosy, leucoderma, erysipelas and abscesses. The leaves of the flowers have been applied to boils, eruptions, swellings, emetic, coughs, ulcer, dandruff and malaria (Woongchon et al., 1991). Phytochemical investigation on *A. amara* revealed the presence of triterpenes, flavonoids, rare amino acids, lipids, steroids and macrocyclic alkaloids (Woongchon et al., 1991, John M. P., 1992, Rajkumar and Sinha, 2010,).

Despite containing budmunchiamins, some of the species of *Albizia* are very much in use in the traditional as well as folklore systems of medicine because of their biological importance. In view of the above finding in the present investigation we tried to explore the antibacterial potential of various parts of the plant *A. amara* using different solvent extracts.

MATERIALS AND METHODS

Collection of Plant Material

Different parts of *A. amara* (Leaf, Flower, Pod and Bark) was collected from Sandur taluk of Bellary district, Karnataka state, India, during the month of April- May, 2010 and authenticated at Department of Botany, Smt. A.S.M. College, Bellary. The freshly collected plant material was washed with water and immediately sprayed with ethanol and dried under shade at room temperature. The dried plant material was cut into small pieces and powdered in a blender. The powdered plant material was stored in sterile containers for further use.

Extraction of Plant Material

The powdered plant material (leaf, flower, pod and bark 100 g each) was subjected for hot continuous extraction by Soxhlet apparatus using solvents of different polarity starting from non-polar to polar (petroleum ether (40-60°C), dichloromethane and methanol). The plant material was extracted with corresponding solvents

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successively for about 48 hours each. After complete extraction the solvents were removed using a buchi type solvent evaporator under reduced pressure and controlled temperature. The petroleum ether extract yielded 1.5, 1.2, 1, and 2g, dichloromethane extract yielded 1.7, 1, 1, and 1.5 g and the methanol extract yielded 4, 3, 3 and 7 g respectively for leaf, flower, pod and the bark. The extracts were stored in freezer of -10°C until further use.

Phytochemical Analysis

The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, anthroquinones, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, tannins, phenols and flavonoids using the method of (Khandelwal, 2007). The extracted samples were stirred with diluted HCl and filtered. This filtrate was tested carefully and used for compound analysis. In this alkaloids (Mayer's test), carbohydrates and glycosides (Molish test), saponins (Chloroform and H₂SO₄ test), protein and amino acid (Millon's test), phytosterols (Liebermann-Burchard's test), phenolic compound and tannin (ferric chloride test and lead acetate test) were carried out.

Antibacterial Activity

The in vitro antibacterial activity of all the extracts of *A. amara* (100 mg/0.1 ml) was carried out against 24 hrs

culture of some selected Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus haemolyticus*) and Gram-negative (*Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) bacterial strains. The anti-microbial activity was performed by cup-plate diffusion method (Saundane et. al., 1998). Nutrient agar was used to culture the bacteria. The size of the cup was 8 mm in diameter. The cups were filled with 100 micro litres of the extract of *A. amara*. For comparison the standard drugs ciprofloxacin and gentamycin (10 micro grams/0.1 ml of distilled water) was used. The petridishes used for anti-bacterial screening were incubated at 37± 0.5° C for 24 hours. The activity was measured in terms of inhibitory zones appearing around the cups.

RESULTS AND DISCUSSION

Phytochemical Investigation

The results of qualitative analysis of phytochemical present in the petroleum ether (40-60°C), dichloromethane and methanol extracts of *A. amara* were presented in [Table 1]. From the results we came to know that the leaf extract of *A. amara* showed positive test for steroids, glycosides, alkaloids, terpenes, saponins and flavonoids. The flower extract showed positive test for steroids, glycosides, alkaloids, terpenes, saponins and

Table 1: Phytochemical Test for Different Solvent Extracts of *Albizia amara*

Sl. No.	Part used/Solvent	Alk	Std	Carb	Tan	Fla	Gly	Sap	Phe	Oils and fats
1	Leaf PE	-	+	-	-	-	-	-	-	+
2	Leaf CHCl ₃	-	+	-	-	-	-	-	-	+
3	Leaf MeOH	+	-	+	+	+	+	-	+	+
4	Flower PE	-	+	-	-	-	-	-	-	+
5	Flower CHCl ₃	+	+	-	-	-	-	-	-	+
6	Flower MeOH	+	-	+	+	+	+	-	+	+
7	Pod PE	-	+	-	-	-	-	-	-	+
8	Pod CHCl ₃	-	+	-	-	-	-	-	-	+
9	Pod MeOH	-	+	+	+	+	+	-	+	+
10	Bark PE	-	+	-	-	-	-	-	-	-
11	Bark CHCl ₃	-	+	-	-	-	-	-	-	-
12	Bark MeOH	+	-	+	+	+	+	-	+	-

- PE-petroleum ether extract, CHCl₃-chloroform extract, MeOH-Methanol extract.
- Alk-Alkaloids, Std-Steroids, Carb-Carbohydrates, Tan-Tannins, Fla-Flavonoids, Gly-Glycosides Sap-Saponins, Phe-Phenolics

Table 2: Antibacterial Activity of Different Solvent Extract of *Albizia amara*

Sl. No.	Extracts	E.c.	S.p.	S.a.	S.t.	B.s.	K.p.
1	Flower pet ether extract	15	16	18	16	18	18
2	Flower dichloromethane extract	15	16	18	16	17	18
3	Flower methanol extract	10	11	-	08	-	08
4	Leaf pet ether extract	15	16	18	16	18	18
5	Leaf dichloromethane extract	15	16	18	16	17	18
6	Leaf methanol extract	12	10	-	10	-	08
7	Pod pet ether extract	15	16	18	16	18	18
8	Pod dichloromethane extract	15	16	18	16	17	18
9	Pod methanol extract	12	11	-	08	-	08
10	Bark pet ether extract	18	20	20	18	18	18
11	Bark dichloromethane extract	20	16	18	18	19	18
12	Bark methanol extract	-	-	-	-	-	-
13	Ciprofloxacin	22	23	22	24	24	22
14	Gentamycin	20	22	20	22	24	20
15	Control	-	-	-	-	-	-

Bore Size: 8 mm, 0.1 ml of test and standard sample

K.p.- *Klebsiella pneumonia*, B.s.-*Bacillus subtilis*, S.t. *Salmonella typhi*, S.a. *Staphylococcus aureus*, S.p.-*Streptococcus pyogenes*, E.c.-*Escherichia coli*

flavonoids. The pod extract showed steroids, glycosides, alkaloids, terpenes, saponins and flavonoids. The bark extract showed the presence of steroids, glycosides, alkaloids, terpenes, saponins and flavonoids.

Antibacterial Testing

The assays were repeated in triplicate and the concurrent values were taken as shown in (table 2), which show the inhibitory activity of the extracts. The activity is expressed as less active, if the zone of inhibition is 9-12 mm, moderate 15-16 mm and high greater than 17 mm. The antibacterial activity of the extract from the table it is evident that, the dichloromethane extract showed high activity than the other two solvents in all the parts of the plant of *Albizia amara*. From the result it is evident that the bark of *Albizia amara* showed greater degree of activity and hence the active compound might be in good concentration in this extract. Further research is in progress to isolate the active metabolite responsible for the activity in the bark extract of *Albizia amara*.

CONCLUSION

Based on the results of the present study it is concluded that the *A. amara* plants have potent antibacterial activity against various bacteria's tested which might be due

to the presence of different alkaloids present in the plants. Also, there is further scope to study the identification and purification of active compound(s) involved in this antimicrobial activity of *A. amara*.

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