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Antibacterial Activity of *Alangium salviifolium* (L. f.) Wangerin

DHARMENDRA KUMAR PARTE[1], ARVINDER SINGH[2], SOUMYA GUPTA[3], SAURAV BISWAS[4], B. L. MANJULA*, SUGIMANI MARNDI[5] AND SANJEET KUMAR[5]

Department of Botany, Sri Jagadguru Renukacharya College of Science, Arts and Commerce, Bengaluru-560 009 (Karnataka), India
**(e-mail: manjulasrivats@gmail.com; Mobile: 99451 81890)*

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ABSTRACT

Alangium salviifolium belonging to the family Alangiaceae is used globally for the treatment of various diseases. All parts of this plant including roots, stem, bark, leaves and fruits are used for the treatment of various diseases. An attempt was made to evaluate its pharmacological value through phytochemical analysis and antibacterial activity of leaves and fruits extract against two gram positive bacteria, namely, *Streptococcus mutans* and *Streptococcus pyogenes* and two gram negative bacteria *Vibrio cholerae* and *Shigella flexneri*. Results revealed that the leaves and fruits extracts were rich in diverse types of bioactive compounds and showed inhibition potential against all selected bacteria.

Key words: Phytochemicals, antibacterial activity, *Alangium salviifolium*

INTRODUCTION

The green leaves and wild edible fruits of plants have played an important role in supplementing the diet of the people for many decades. Most of the wild edible fruits have additional usages. Some of them have medicinal properties, while some others are having antinutritional factors though some of them may be poisonous (Sreekumar *et al.*, 2020). Several ethnobotanical studies have indicated that some fruits are not tasty and desirable like cultivated fruits but they are rich in nutritional content (Saravanan *et al.*, 2020). They contain nutritional food value providing vitamin C, carotenoids, polyphenolic compounds and several vitamins and mineral salts. Research has confirmed that food rich in antioxidants plays a vital role in the prevention of cardiovascular diseases, cancer and neurodegenerative diseases.

Alangium salviifolium (L.f.) Wangerin belongs to the family Alangiaceae . The synonyms of *A. salviifolium* are *A. decapetalum* Lam, *A. lamarckii* Thw, *A. latifolium* Miq.ex C.B. Clarke, *A. mohillae* Tul., *A. salviifolium* subsp. Decapetalum (Lam.) Wangerin, *A. sundanum* var. Miqueliana Kurz., *A. tomentosum* Lam., *Grewia salviifolia* L.f, Karangolummohillae (Tul.) Kuntze and *Karangolum salviifolium* (L. f.) Kuntze (Panara *et al.*, 2016). It is widely distributed in South East Asia, from India to China, Thailand, southern and eastern Asia. *A. salviifolium* is an excellent medicinal plant which holds numerous bioactive phytochemicals. Almost every part of this plant has been used in the Ayurveda, Siddha and various other traditional systems of medicines for the treatment of various diseases (Shravya *et al.*, 2017). The medicinal value of plant parts such as leaves, flower, root, root bark, stem and stem bark contain various biologically active phytochemicals such as alangine, ankorine, tubulosine, alangicine, salsoline, etc (Panara *et al.*, 2016). It is well known for its medicinal properties in India like anti-inflammatory, antimicrobial and antioxidant. It is also antifungal due to the presence of

[1]Government Arts Commerce College, Keolari, Seoni-480 994 (Madhya Pradesh), India.
[2]Department of Botany, Akal University, Talwandi Sabo, Bathinda-151302 (Punjab), India.
[3]School of Bioscience and Technology, Vellore Institute of Technology, Vellore-632 014 (Tamil Nadu), India.
[4]School of Biotechnology, Shoolini University of Biotechnology and Management Sciences, Solan-173 229 (Himachal Pradesh), India.
[5]Biodiversity and Conservation Labs., Ambika Prasad Research Foundation, Cuttack-753 014 (Odisha), India.

active compounds like alkaloids, phenols, tannins, terpenoids, flavonoids and glycosides in aqueous and ethanol extracts (Aphajal *et al.*, 2019). The leaves and fruits are used for diarrhoea and dysentery (Plate 1). Roots are used to expel intestinal worms to treat burning sensation and constipation (Kapoor *et al.*, 2017). The plant has been studied worldwide by the researchers for its medicinal properties (Table 1). The present work was aimed at studying phytochemical analysis and antibacterial activity of leaves and fruits of *A. salviifolium*.

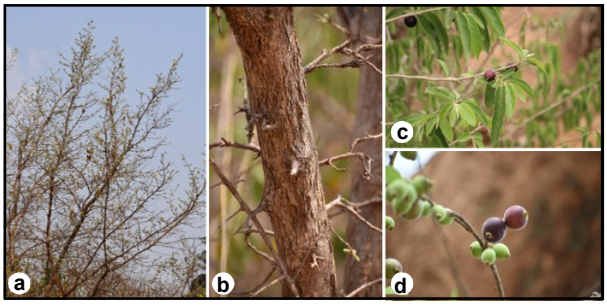

Plate 1. *A. salviifolium* (a) Tree (b) Bark, (c) Leaves with fruits and (d) fruits.

MATERIALS AND METHODS

The leaves and fruits of *A. salviifolium* were collected from different parts of Odisha and kept in the polybags tagged with the botanical name and sorted out as per standard sampling procedure and passport descriptions for taking to laboratory. The collected experimental plant materials were dried at room temperature under shade and were powdered after drying using mechanical devices. The powdered material of the experimental plant was kept in thimble and extraction was carried out using the Soxhlet apparatus. The residue was collected and left for air drying and dried crude extracts were stored in refrigerator for further phytochemical analysis and antibacterial activities.

Phytochemical analysis was carried out using standard procedure to identify the bioactive compounds. Five drops of 10% lead acetate were added to 5 ml of plant extract. Formation of a light-yellow precipitate indicated the presence of tannins. 1 ml of the extract was boiled in 10 ml of distilled water and filtered with Whatman 42 filter paper. Five ml of filtrate was mixed with 2 ml of normal distilled water and shaken vigorously. Occurrence of stable persistent froth indicated the presence of saponins. Five drops of 1% ferric chloride and 1 ml of potassium ferro cyanide were added to 2 ml of plant extract. A bluish green solution showed the presence of phenolic compounds. 0.5 g of plant extract was mixed with 2 ml of chloroform. To this, equal volume of concentrated sulfuric acid was added. Reddish-brown colouration of interface confirmed the presence of terpenoids. Five ml of plant extract was mixed with 3 ml of aqueous HCL on water bath and then filtered. One ml of Dragendorff's reagent was added to the filtrate. The occurrence of orange red precipitate indicated the presence of alkaloids in the sample extract. Few drops of dilute sodium hydroxide were added to 1 ml of the extract. The presence of flavonoids was indicated by the production of an intense yellow colour in the plant extract which became colourless on addition of 2-3 drops of 50% HCL.

The extracts of leaves and fruits were screened for antibacterial activity against two Gram positive bacteria *Streptococcus mutans* (MTCC 497) and *Streptococcus pyogenes* (MTCC 1926); and two Gram negative bacteria *Vibrio cholerae* (MTCC 3906) and *Shigella flexneri* (MTCC 1457). All these four MTCC (Microbial Type Culture Collection) bacterial strains were collected from Institute of Microbial Technology (IMTECH), Chandigarh. Antibacterial activity was done using slight modification of standard methods of agar well diffusion assay, disc diffusion method and broth dilution assay.

Agar well diffusion method was followed to test the antibacterial activity of extracts of experimental plant parts against four bacterial

Table 1. Medicinal uses of different plant parts of *A. salviifolium*

Plant parts	Medicinal uses	References
Whole plant	Boils	Panara *et al.*, 2016
Roots	Jaundice	Phulwaria *et al.*, 2019
Bark	Emetic	Panara *et al.*, 2016
Leaves	Diabetes, Asthma	Zahan *et al.*, 2013
Leaves and fruits	Diarrhoea , Dysentery	Kekuda *et al.*, 2020
Roots and fruits	Rheumatism	Shravya *et al.*, 2017
Fruits	Eye diseases	Panara *et al.*, 2016

strains. Nutrient agar plates were prepared as per instructions of the manufacturer. Hundred µl of nutrient broth cultures of the test microbes prepared a day before were poured over the plates uniformly and a lawn culture was prepared using a sterile spreader in a laminar hood. Six mm wells were made using sterile borer. Stock solutions of samples were prepared in 100% DMSO (Sigma) and two-fold serial dilutions were made in amount of 100 µl per well at concentrations of 100 mg/ml. Hundred µl of samples were added by sterile syringes into the wells and allowed to diffuse at room temperature for 2 h. Plates were incubated at 35±2°C for 18-24 h. Kanamycin served as standard antibiotic control. Triplicates were maintained and the experiment was repeated thrice. For each replicate the readings (diameter of zone of inhibition in mm) were taken and the mean±SD values (diameter of zone of inhibition) were recorded.

Antibacterial activity using disc diffusion assay was done using the 6 mm disc prepared from Whatman filter paper. Each extract was dissolved in DMSO. The sets of dilutions (100 mg/ml) of crude extracts and standard drugs were prepared. Six mm discs were kept in the drug for 12 h before placing on the agar plates. The zones of growth inhibition around the discs were measured after 18 to 24 h of incubation at 37°C for bacteria. The sensitivity of the microbial species to the plant extracts was determined by measuring the size of inhibitory zones (including the diameter of disc) on the agar surface around the disc and values less than 8 mm were considered as not active against microorganisms.

All the extracts of experimental plant were screened for their antibacterial activity. Antibacterial activity was assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method. Selected colonies of aforesaid bacteria were picked off to a fresh isolated plate and inoculated in corresponding tubes containing 5 ml of trypticase soy broth. The broth was incubated for 8±1 h at 35±2°C until there was a visible growth. McFarland No. 5 standard and PBS (Phosphate Buffer Saline) were used to adjust the turbidity to get 105 CFU/ml.

After the incubation, the tubes of respective concentration showing no visible growth after 8-12 h represented MIC value of a respective concentration of extract. Inoculum control showed visible growth due to no antimicrobial agents. However, the broth control showed no growth due to the absence of bacteria. Triplicates were maintained and the experiments were repeated thrice for each replicate.

RESULTS AND DISCUSSION

The secondary metabolites present in most of the aqueous extract of leaves were saponins, phenolic compounds and flavonoids. Secondary metabolites in methanolic extract of leaves were saponins and phenolic compounds. In methanolic extract of fruits, secondary metabolites were tannins, saponins, phenolic compounds and flavonoids. In aqueous extract of fruits, tannins, phenolic compounds and flavonoids were present (Table 2). The plant is widely used for its various phytoconstituents such as flavonoids, terpenoids, phenolics, glycosides, tannins, steroids, β-carboline and quinolizidine alkaloids such as alangine, alangicine, tubulosine, deoxytubulosine, emetine, ankorine and alangimarckine. It has biological activities like antidiabetic, antiulcer, analgesic, anti-inflammatory, antimicrobial, antioxidant, antihelmintic and antifungal. The leaves and fruits are used in the treatment of diarrhoea and dysentery, while roots are used to expel intestinal worms,

Table 2. Phytochemical analysis of leaves and fruits of *A. salviifolium*

Plant extract	Solvent	Tannins	Saponins	Phenolic compounds	Terpenoids	Alkaloids	Flavonoids
Leaf	Aqueous	-	+	+	-	-	+
	Methanol	-	+	+	-	-	-
	Petroleum ether	-	-	-	-	-	-
	n-Hexane	-	-	-	-	-	-
Fruit	Aqueous	+	-	+	-	-	+
	Methanol	+	+	+	-	-	+
	Petroleum ether	-	-	-	+	-	-
	n-Hexane	-	-	-	-	-	-

burning sensation, constipation. Root is useful for external application, rheumatism, leprosy and inflammation. Leaves are useful for curing diabetes and asthma. Fruit juice is applied to cure eye diseases (Phulwaria *et al.*, 2019). The potential of leaf as well as fruit of *A. salviifolium* to exhibit antimicrobial and antioxidant activities justified the traditional medicinal uses of the plant. (Lavanya *et al.,* 2021).

It was observed that aqueous and methanolic extract of leaf and fruit of *A. salviifolium* showed zone of inhibition in Disc diffusion assay (Table 3). It was noted that the aqueous leaf extract showed highest zone of inhibition of 8.5 mm against *S. flexneri* (MTCC 1457). Methanolic extract of fruits showed highest zone of inhibition of 11.5 mm against *S. flexneri*. The aqueous extract of fruits showed zone of inhibition of same readings to all tested bacterial strains. The methanolic extract of leaves and fruits showed highest zone of inhibition 11.5 mm against *S. flexneri*.

It was observed that the aqueous and methanolic extract of leaves and fruits of *A. salviifolium* also showed zone of inhibition in Agar well diffusion assay (Table 3). The aqueous extract of leaves showed maximum zone of inhibition of 9.5 mm against *V. cholerae* and *S. flexneri*. Methanolic extract of leaves showed zone of inhibition of 10.8 mm against *S. flexneri*. Aqueous extract of fruits showed highest zone of inhibition of 10.0 mm against *S. flexneri*. The methanolic extract of fruits showed maximum zone of inhibition of 12.0 mm against *S. flexneri*.

Minimum inhibitory concentration of aqueous extract of leaves was 200 mg/ml against all four bacteria *S. mutans, S. pyogenes, V. cholerae* and *S. flexneri*. However, minimum inhibitory concentration of aqueous extract of fruits was 300 mg/ml against *S. mutans, S. pyogenes, V. cholerae* and *S. flexneri* (Table 4). Minimum inhibitory concentration of methanolic extract of leaves was 100 mg/ml against *S. mutans, S. pyogenes* and *V. cholerae* and *S. flexneri*. Further, minimum inhibitory concentration of methanolic extract of fruits was 200 mg/ml

Table 3. Antimicrobial activity of aqueous and methanolic extract of leaves and fruits of *A. salviifolium* using Disc Diffusion Assay and Agar Well Diffusion Assay

Extract (100 mg/ml)	Disc Diffusion Assay			
	Zone of inhibition (mm)			
	Streptococcus mutans	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous leaf	≤7.0	≤7.0	8.0	8.5
Methanolic leaf	8.0	8.0	10.0	11.5
Aqueous fruit	≤7.0	≤7.0	≤7.0	≤7.0
Methanolic fruit	9.5	8.5	11.0	11.5
	Agar Well Diffusion Assay			
Extract (100 mg/ml)	Zone of inhibition (mm)			
	Streptococcus mutans	*Streptococcus pyogenes*	*Vibrio cholerae*	*Shigella flexneri*
Aqueous leaf	8.0	8.0	9.5	9.5
Methanolic leaf	9.0	9.0	10.6	10.8
Aqueous fruit	8.5	8.0	9.5	10.0
Methanolic fruit	10.5	10.0	11.5	12.0

Table 4. MIC of leaves and fruits extracts of *A. salviifolium* against selected bacterial pathogens (50 to 500 mg/ml)

Extract	*Streptococcus mutans* (mg/ml)	*Streptococcus pyogenes* (mg/ml)	*Vibrio cholerae* (mg/ml)	*Shigella flexneri* (mg/ml)
Aqueous leaf	200	200	200	200
Aqueous fruit	300	300	300	300
Methanolic leaf	100	100	100	100
Methanolic fruit	200	200	200	200
Inoculum	Growth	Growth	Growth	Growth
Broth	No growth	No growth	No growth	No growth

against *S. mutans, S. pyogenes, V. cholerae* and *S. flexneri*.

Alangium salviifolium is rich in numerous bioactive phytochemicals. Almost every part of this plant has been used in various traditional systems of medicines for the treatment of various diseases. Now, the present study concluded that the plant which was used for few decades, could be used to develop antimicrobial agents against studied bacteria.

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