

Antioxidant and Antimicrobial Activities of *Alangium salvifolium* (L.F) Wang Root

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Abstract: The development of bacterial resistance to presently available antibiotics has necessitated the search of new antibacterial agents. Plants and plant products are known to possess excellent antioxidant properties and play a significant role in preventing the conditions due to the excessive free radicals. The present study was aimed to evaluate the antimicrobial and antioxidant activities of the alcoholic and aqueous extracts from the root of *Alangium salvifolium* (L.f) Wang. Aqueous and alcoholic extracts prepared and were tested on Gram positive and Gram negative bacteria. Agar cup plate test was used to determine the sensitivity of the tested samples while the well micro-dilution was used to determine the minimum inhibitory concentration. The DPPH and Nitric oxide radical inhibiting activity were used to detect oxidative activity. The results of antimicrobial assays showed that all tested extract were active against all tested microbial species including gram positive and negative bacteria. The alcoholic extract showed direct antimicrobial activity against all tested microorganism with minimum inhibitory concentration ranging between 0.130 to 0.520 mg/ml, while aqueous extract showed 0.26 to 2.10 mg/ml, respectively. The results of DPPH method showed 50% inhibition rate at the 120.48 µg/ml and 135.14 µg/ml with alcoholic and aqueous extract, respectively. Nitric oxide scavenging inhibition showed 50% inhibition rate at the 308.80 µg/ml and 450.80 µg/ml using alcoholic and aqueous extract, respectively. The overall results of this study indicates that the extract from roots have interesting antimicrobial and potential free radical scavenging activity for treatment of diseases.

Key words: *Alangium salvifolium* (L.f) Wang • Antimicrobial activity • DPPH method • Nitric oxide scavenging activity

INTRODUCTION

In developing countries, infectious diseases remain the main cause of the high mortality rates recorded; the majority of rural people has limited access to formal and adequate health services and thus heavily resources to traditional healers [1]. Indigenous herbal remedies are widely used against many infectious diseases, but only few of them have been studied chemically and biologically in order to identify their active constituents [2]. In modern medical practice, the alarming worldwide incidence of antibiotic resistance causes a need for new compounds that can act either by a direct antimicrobial activity or by inhibiting resistance mechanisms of microorganisms of medical importance. Medicinal plants represent a valuable source for this kind of compounds [3].

Alangium salvifolium (L.f) Wang belongs to family Alangiaceae. Locally it called as Ankolam [4]. Alangiaceae is a monogeneric family of trees and shrubs found in tropical and subtropical region. There are nearly twenty one species of *Alangium* grouped into four sections *Alangium*, *Conostigma*, *Marlea* and *Rhytidendra* [5]. Other two different varieties of this drug namely *Angolam* and *Karaangolam*. They correspond to *Alangium salvifolium*, subspecies *salvifolium* and *hexapetalum* respectively. The plant is distributed in dry regions, plains and lower hills in India, Africa, Srilanka, Indochina and China. Root is used in diarrhea, paralysis, piles and vomiting [6]. They are acrid, astringent, emollient, anthelmintic, thermogenic, diuretic and purgative. Root is useful for external application in acute case of rheumatism, leprosy and inflammation and internal application in cases of bites of

rabbit and dogs [7]. Antibacterial compound was isolated from the flower of *Alangium salvifolium* [8]. Recent phytochemical studies of this plant resulted in the isolation of several flavanoids, phenolic compound, irridoid glycosides and oxyoglucoside.

Methanolic extract of root has been studied for its analgesic and anti-inflammatory activities in animal model [9]. The aerial part of the plant was analyzed and compounds were isolated from chloroform extract employing chromatographic technique [10]. New alkaloid, ankorine was isolated from leaves [11]. Plant is rich in tetrahydroisoquinoline monoterpene glycoside, for e.g., alangiside-1 or ipecoside-2 whose structures are closely related to the ipecac alkaloid [12]. Two sterol alangol (m. p. 296°) and alengol (m. p. 302-307°) were isolated from seed kernels [13].

The plant selected for study was based on its availability and its various therapeutic activities in various ailments mentioned in Ayurveda. In the present work, we have reported for the first time the results of the combined investigations on *in-vitro* antibacterial and antioxidant activities of the extracts of *Alangium salvifolium* (L.f) Wang (ASW).

Experimental

Plant Material: The proposed material of *Alangium salvifolium* (L.f) Wang root was procured from South Gujarat Region, with the help of local tribal and field botanist. The species for the proposed study was identified *Alangium salvifolium* (L.f) Wang root, by Dr. Minoo H. Parabia, Botanist, Department of Bioscience, Veer Narmad South Gujarat University, Surat, Gujarat, India, where the voucher specimen no. was VCJ/02/25032005.

Preparation of Extracts: Preparation of the extract of ASW powdered roots is done using alcohol and distilled water. The shade dried coarse powder of the roots (500 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with 90% alcohol until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. It was dried and kept in a desiccator till experimentation. Similarly, aqueous extract was prepared. Obtained extract was weighed practical yield and percentage yield was calculated in terms of air-dried powdered crude material [14-16].

Antimicrobial Activity

Microbial Strains: The microorganisms used in the antimicrobial tests were Gram-positive (*Staphylococcus aureus* ATCC 25925, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermis* ATCC 12228 and *Micrococcus luteus* ATCC 10240) and Gram-negative (*Enterobacter aerogens* ATCC 13048, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 51812 and *Shigella dysenteriae* ATCC 25931) organisms have been selected. All strains were obtained from C. G. Bhakta institute of Biotechnology, Tarsadi, Bardoli, Gujarat, India.

Culture Media: Nutrient agar (NA) (Himedia Laboratories Ltd., Mumbai, India) medium and Nutrient broth medium were used for the growth of bacteria. The ingredients in 1000 ml distilled water and boiled to dissolve it completely. The pH of media was adjusted to 7.4 ± 0.2 (at 25°C) and sterilized it by autoclaving at 15 lbs pressure (121°C) for 15 min. The solution of the test extracts was prepared at the concentration of 50 mg/ml by dissolving in respective solvent in stopper specific gravity bottle and stored in refrigerator. The solution was removed from the refrigerator one hour prior to use and was allowed to warm up to room temperature.

Chemicals: Gentamycin (Alkem Laboratories Ltd., Mumbai, India) was used as reference antibiotic (RA) against bacteria and dimethylsulphoxide (DMSO) (Himedia, India) was used as solvent for tested samples. Other chemicals were used of analytical grade of S.D. Fine Chemicals, Mumbai, India.

Sensitivity Test

Cup Plate Method (Zone of Inhibition): This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of petri dish, to an extent such that growth of the added micro-organism is prevented entirely in circular area or zone around the cavity containing the extracts. 0.2 ml of each of the seeded broth containing 10^{-6} - 10^{-7} cfu/ml test organism was inoculated on the two plates of solidified agar and spread it uniformly. Sterilize the cup-borer of 10 mm diameter by dipping it in alcohol followed by flaming it and make four wells, one in each quadrant, at equal distance in nutrient agar plate previously seeded with culture. Add 0.2 ml of reference antibiotic Gentamycin (50µg/ml) in to the two wells, in third well kept control solvent-DMSO and in fourth well test extracts solution was added. The plates were kept in refrigerator at 4-5°C for 30 min after addition

to allow diffusion of the solution into the medium and then incubated the plate in upright position at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 hrs. After the incubation period the diameter of the zone of inhibition in mm obtained around the well was measured. Interpret the result of extracts sensitivity as sensitive, moderate sensitive or resistant on the basis of mean zone diameter in mm. The MIC was defined as the lowest concentration of plant extract able to inhibit microorganism growth [17].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbial Concentration (MMC):

The stock solution of different test extracts were prepared in concentrations of 50 mg/ml in respective solvent and used for study. Similarly the reference antibiotic concentration of $50\mu\text{g/ml}$ was prepared. Prepare 8 ml of broth containing 50 mg/ml concentration of extract for the first tube in row. Mix the content of universal bottle, using a pipette and transfer to the first tube in row. Add 4 ml of broth to the remaining 4 ml of the universal bottle, mix and transfer 2 ml to the second tube in row, prepare dilutions up to the 10^{th} tubes. Place 2 ml of extract free broth in the last tube. All tubes were placed in incubator at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 h. Inoculate tube containing 2 ml broth with the organism and keep at 4°C in a refrigerator overnight, to be used as standard for the determination of complete inhibition. The lowest concentration of test extract and reference antibiotic which caused apparently a complete inhibition of growth of organism was taken as minimum inhibitory concentration (MIC). The assay was repeated three times [18-21].

For the determination of MMC, a portion of a liquid (5 μl) from each well that showed minimum zone was again plated on NA media and incubated at 37°C for 24 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MMC.

Antioxidant Study

DPPH Scavenging Activity: The test sample (3ml extract solution in water) was mixed with 1ml (0.1 mM solution of DPPH in methanol) and different concentrations (40-200 $\mu\text{g/ml}$) were prepared. After 30 min at room temperature, the absorbance values were measured at 517nm and converted into percentage of antioxidant activity. Ascorbic acid was used as a standard control. Each assay was repeated three times and the recorded as mean of the triplicate experiments, were graphically illustrated. Capability to scavenge DPPH radical was calculated by using following equation [22-25].

$$\% \text{ Scavenging Effect} = [1 - \text{Abs. (s)} / \text{Abs. (c)}] \times 100 \quad [1]$$

$$\text{Abs. (s)} = \text{Absorbance of sample, Abs. (c)} = \text{Absorbance of control} \quad [2]$$

Nitric Oxide Radical Inhibiting Activity: Nitric oxide scavenging activity was determined using Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide, which interact with oxygen to produce nitrite ions, which can be determined by the use of Griess Illosvoy reaction. Mix 2 ml (10 mM Sodium nitroprusside) in 0.5ml phosphate buffer saline (pH 7.4) with 0.5ml of extract at various concentrations (100-600 $\mu\text{g/ml}$) and was incubated at 25°C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1ml sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1ml naphthylethylenediamine dihydrochloride (NEDA) (0.1%w/v) was mixed and incubated at room temperature for 30 min. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilic acid subsequent coupling with NEDA was read at 540nm. Ascorbic acid was used as a positive control. Experiment was done in triplicate. Capability to scavenge the nitric oxide radical was calculated by using following equation [26-28].

$$\% \text{ Inhibition} = [1 - \text{Absorbance of sample} / \text{Absorbance of control}] \times 100 \quad [3]$$

RESULTS AND DISCUSSION

Antibacterial Activity: The alcohol extract was higher than the aqueous extract in their inhibition zone diameter. These results indicated that most of the active constituents (responsible for exerting antibacterial action) in these plants are expected to be soluble in polar solvent. It authenticates that the entire tested microorganism are susceptible to alcohol extract and degree of susceptibility is given below in the decreasing order *S. dysenteries* > *S. aureus* > *E. aerogenes* > *S. typhi* > *E. coli*. *B. subtilis*. On the other hand, the susceptibility to aqueous extract is in the order: *E. aerogenes* > *S. typhi* > *S. dysenteries*. > *S. aureus*.

Alcohol extract is effective against all tested microorganism except *E. coli*. On the hand, aqueous extract is effective against gram + ive and gram-ive organisms. Alcohol extract is having comparable results with gentamycin against *E. aerogenes* and *S. typhi*. On the other hand, aqueous extract is having comparable against *S. dysenteries*.

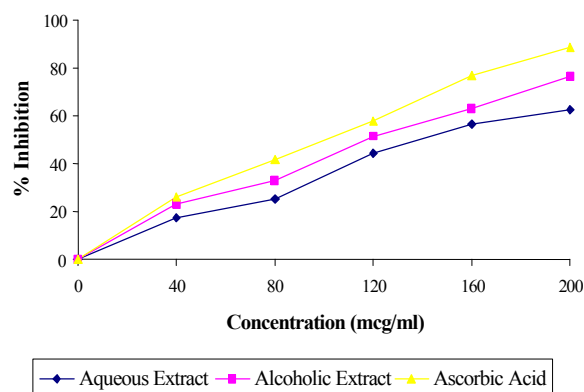


Fig. 1: Graphical representation of *In vitro* Antioxidant activity of ASW root extract by DPPH method

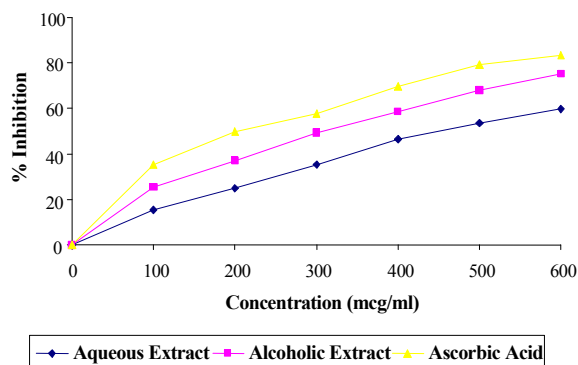


Fig. 2: Graphical representation of *In vitro* Antioxidant activity of ASW root extract by Nitric oxide method

Table 1: Antibacterial activity of the alcoholic and aqueous extracts of the roots of ASW and reference antibiotics determined by the cup plate method

Microorganisms	Aqueous Extract	Alcoholic Extract	RA*
<i>Staphylococcus aureus</i> ATCC 25925	+	+	+
<i>Bacillus subtilis</i> ATCC 6633	+	+	+
<i>Staphylococcus epidermis</i> ATCC 12228	-	+	+
<i>Micrococcus luteus</i> ATCC 10240	-	+	+
<i>Enetrobacter aerogens</i> ATCC 13048	+	+	+
<i>Escherichia coli</i> ATCC 25922	+	+	+
<i>Salmonella typhi</i> ATCC 51812	+	+	+
<i>Shigella dysenterie</i> ATCC 25931	+	+	+

(-): Not active, (+): Active

*RA = Reference antibiotic-Gentamycin (50 µg/ml),

Table 2: Minimum inhibitory concentration (µg/ml) and Minimum microbial concentration (µg/ml) of aqueous and alcoholic extract of the leaves of ASW and reference antibiotic (µg/ml) (Gentamycin)

Microorganisms	Minimum inhibition concentration			Minimum microbial concentration		
	Aqueous extract	Alcoholic extract	RA*	Aqueous extract	Alcoholic extract	RA*
<i>Staphylococcus aureus</i> ATCC 25925	0.520	0.263	9.55	1.05	0.263	9.55
<i>Bacillus subtilis</i> ATCC 6633	2.10	0.130	4.65	4.20	0.263	9.55
<i>Staphylococcus epidermis</i> ATCC 12228	-	0.130	4.66	-	0.263	9.55
<i>Micrococcus luteus</i> ATCC 10240	-	0.263	9.45	-	1.05	9.55
<i>Enetrobacter aerogens</i> ATCC 13048	1.05	0.263	2.45	4.20	0.520	8.34
<i>Escherichia coli</i> ATCC 25922	0.263	0.130	4.91	1.05	0.260	8.34
<i>Salmonella typhi</i> ATCC 51812	1.05	0.520	155.31	2.10	0.520	4.16
<i>Shigella dysenteriae</i> ATCC 25931	0.520	0.263	80.25	1.05	0.520	2.10

(-): not tested because samples were not active by diffusion.

*RA = reference antibiotic (Gentamycin)

The above result shows that the alcohol extract is most active extract with less MIC (0.034-0.263 mg/ml). Aqueous extract has high MIC then alcohol aqueous extract (0.130-0.520 mg/ml). All the strains were more susceptible to alcohol extract, our result indicate the presence of chemical compound in both extract with antibacterial activity against all strains comparable to Gentamycin [29-32].

***In-vitro* Antioxidant Activity:** In today's environment, hyper physiological burden of free radical causes imbalance in homeostatic phenomenon between oxidants and antioxidants in the body. The imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases like arteriosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's and Parkinsonism. Thus free radical

scavenging is very essential for preventing organ injury associated with shock, inflammation and ischemia or reperfusion. Therefore research in recent past have accumulated enormous evidence advocating enrichment of body system with antioxidants to correct vitiated homeostasis and prevent onset as well as treat the disease caused due to free radical and related oxidative stress. Stress, smoking, drugs and diet generates excessive free radicals in human body. Plants and plant products are known to possess excellent antioxidant properties and play a significant role in preventing the conditions due to the excessive free radicals. The results of absorbance and % inhibition showed decrease in the concentration of DPPH radical due to the scavenging ability of extract and standard ascorbic acid, as a reference standard [33-36].

In DPPH method, alcoholic extract of *A. salvifolium* (L. f) Wang root presented more activity than aqueous extract. 200 µg/ml of alcohol, aqueous extracts and ascorbic acid exhibits 76.4, 62.4 and 88.6 % inhibition and the EC₅₀ (µg)-120.48, 135.14 and 96.15 µg/ml, respectively [37-41].

In Nitric oxide method, alcohol extract of ASW roots presented more antioxidant activity than aqueous extract., Alcoholic extracts, Aqueous extract and Ascorbic acid exhibits 74.9%, 59.7% and 83.5%, inhibition and the EC₅₀ (µg)-308.80, 450.8 and 201.32 µg/ml respectively.

The alcoholic extract exhibited more antioxidant activity with low EC₅₀ value in these two methods. The Phytochemical analysis indicated the presence of phenolic compounds and flavanoids in extract. Several such compounds were known to possess antioxidant activity. Hence, the observed activity may be due to the presence of any of these constituents [37-41].

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