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Research Article

STUDIES ON ANTIMICROBIAL, ANTIOXIDANT, LARVICIDAL, PESTICIDAL ACTIVITY AND PHYTOCHEMISTRY OF LEAVES OF *ALANGIUM SALVIFOLIUM* (L.f) WANG.

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ABSTRACT

The plant *Alangium salvifolium* is a medium sized tree used in folklore medicine for various ailments. In this study Aqueous, Chloroform, Methanol and Hexane extract of the leaves of the plant were evaluated for their antibacterial, antioxidant, larvicidal and pesticidal potency. Further, the aqueous extract of the plant was subjected to phytochemical analysis to know the phytoconstituents. The different types of the extracts provided inconsistent zone of inhibition against the studied bacteria. There was no antioxidant property recorded (IC_{50}) even at the higher concentration of upto 15mg/ml with any of the extracts. The leaf extract showed significant larvicidal activity against the larvae, *Artemia salina* and the pest, *Sitophilus oryzae.* The aqueous extract of the leaves showed the presence of Tannins, Flavonoids, Terpenoids and Steroids. It is concluded that the leaves of *Alangium salvifolium* can be used as a potential larvicidal and pesticidal agent.

Keywords: *Alangium salvifolium,* Antibacterial, Anti-oxidant, DPPH, Larvicidal, *Artemia salina,* Pesticidal, *Sitophilus oryzae.*

INTRODUCTION

Traditional medicine shares a major part in healthcare system of developing countries[1]. The dependence on traditional medicine is due to its cost effectiveness and more accessible nature[2]. Revival of interest in Herbal medicine is popularly seen in the west and it functions as a primary form of medicine in many countries in the world[3]. This is due to the perception that the incidence of adverse reaction to plant preparation is low when compared to synthetic pharmaceuticals[4]. A major part of total population in developing countries till uses folklore medicine obtained from plant resources[5]. Hamburger and Hostettman[6] stated that plants are the sleeping giants of pharmaceutical industry. India is not remorse in utilizing the plants in its medicinal system. This country is perhaps the largest producer of medicinal herbs and has been in use in one form or another, under indigenous systems of medicine like Ayurveda, Siddha and Unani[7]. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries[8]. The plant, *Alangium salvifolium* (Linn.f) Wang is one among those plants.

The plant genus, *Alangium* is a monogeneric plant of the family Alangiaceae. *Alangium salvifolium* ranges in size from a small shrub to deciduous tree ranging from 3 feet to 12 feet in height. Leaves alternate, unequal, oblong, lanceolate or oval, acute or rounded at base, acuminate and obtuse at apex with 3-6 pairs of oblique veins, glabrous above and pubescent on veins beneath. Flowers white or yellowish with fragrance. Fruit drupe, 1-2 seeded berries, crowned by calyx lobes[9]. It is native to Western Africa, Madagascar, Southern and Eastern Asia (China, Malaysia, Indonesia, India, and Philippines), tropical Australia, the western Pacific Ocean islands and New Caledonia[1]. The *Alangium salvifolium* is also called as Ankola and extensively cultivated in India. Its dried leaves, has traditionally been used to treat various ailments in Asia[10]. It is a popular folk medicine and has been studied for its antifertility[11], anti-inflammatory[12], antimicrobial[13], antioxidant[14], antitumor[15]and anti-ulceric[16] activities. However, the above stated reports were studied using different plant parts of *Alangium* extracted with different solvent system. The present study explores the possibility of the leaf extracts of *Alangium salvifolium* as antibacterial, antioxidant, larvicidal and pesticidal agent using different solvent system like Aqueous, Chloroform, Methanol and Hexane. Further, the aqueous extract was subjected to phytochemical analysis in this present study.

MATERIALS AND METHODS

Plant Source

The leaves of the plant *Alangium salvifolium* was collected near Singaperumal kovil, belonging to Chengalpet district of the state of Tamil Nadu, India. The leaves were chosen such that they are not damaged or diseased and are healthy. The healthy leaves of the collected weeds were cleaned thoroughly in running tap water. They were shade-dried for 4 days. The dried leaves were made powder using electric blender and stored for further use.

Preparation of plant extracts

The plant extracts were prepared using cold-percolation method. To 15g of each dried pulverized sample 150ml of solvent (Aqueous, Chloroform, Hexane and Methanol) was added and stirred in temperature-controlled shaker at 30 ± 2°C. After 48 hours the extract was filtered and concentrated using rotary evaporator. These extracts were used for screening antibacterial, larvicidal, pesticidal and anti-oxidant properties.

Phytochemical analysis

The dried pulverized plant material (15g) was extracted with double distilled water. The aqueous extracts were filtered using Whatman No.1 filter paper and the qualitative phytochemical analysis for the presence of tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and steroids was carried out immediately without storage according to standard procedures[17].

Antibacterial assay

For testing the anti bacterial activity of the plant, bacterial cultures namely *Listeria monocytogenes* (MTCC 657), *Pseudomonas aeruginosa* (MTCC 429), *Staphylococcus aureus* (MTCC 96), *Salmonella typhi* (MTCC 733) and *Vibrio cholerae* (MTCC 3906) were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The cultures for screening were maintained in saline and were suitably diluted prior to use. Disc diffusion method was used to screen the antibacterial activity using Mueller Hinton Agar[18]. Onto the sterile MHA plates 0.1mL of the saline suspension was swabbed uniformly. Different concentrations of the extract (15, 20, 25 µl/disc) that were loaded prior a day on 5 mm sterile discs were placed on the medium along with the control disc Streptomycin. The plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of inhibition zones formed around the discs was measured in millimetre. These studies were performed in duplicates for all the bacterial and solvent samples.

DPPH free radical scavenging assay

The leaf extracts of *Alangium salvifolium* obtained from four different solvents were studied for their Free radical scavenging assay using DPPH (2, 2 diphenyl-1-picryl hydrazyl). To 0.5mL of extract of each solvent and the reference compound in various concentrations (15, 7.5, 3.75, 1.87, 0.93 mg/mL), 0.5mL methanol and 0.5mL of 0.1mM solution of DPPH in methanol was added. After 30 minutes of incubation in dark condition at room temperature, absorbance was measured at 517nm using spectrophotometer. The same solution of DPPH in methanol was used as control, whereas BHA was used as reference.

Percentage inhibition was calculated using the formula

% Inhibition = [(Control absorbance - Test absorbance)/ Control absorbance] x 100

Larvicidal activity

Culture of Larvae

The *Artemia salina* seeds were procured from Philadelphia, USA. The seeds were incubated in marine water for 48 hours for hatching in a small water tank. Aeration was provided with an aerator pump. Required light is provided with Philips 40 Watts lamp for 12 hours cycle. After 48 hours, the larvae were removed and used for the experiments. The hatched seeds were used at the nauplii stage.

Bioassay

Larvae of *Artemia salina* were taken in different test tubes containing extracts of *Alangium salvifolium* at different concentrations. Then four concentrations (0.25, 0.5, 0.75, 1ml) of each extract of different solvents were taken, to which 10ml of sea water and 20 larvae were added. After 24hours and 48hours, the viability of larvae was recorded. The test tubes were maintained in triplicates. The mortality of larvae was observed at 24 hours of challenge[19]. At the end of the experimental period the numbers of mobile and dead larvae in each test tube were checked using hand lens. Nauplii were considered dead when they are immobile and stayed at the bottom of the test tubes.

Pesticidal activity

Weevil cultivation

Sitophilus oryzae adults were collected from naturally infested Rice grains from a local market in Chennai, Tamil Nadu. The insects were reared on clean and un-infested rice grains. Three Hundred adult insects were released in a plastic jar containing sufficient rice grains and were capped with muslin cloth to ensure ventilation. After 48 h, the adults were removed and used for the experiments.

Bioassay

Two ml. of the plant extract constituted using 200 mg of the extract of *Alangium salvifolium* extracted using different solvent system (1:10 w/v) was poured onto a dry and sterile Petridish and was allowed to dry. This plate was exposed to air so that the available solvent in the extract vaporizes. Then a plug of cotton was used to wipe the extract from the plate. The cotton plug on which the extract was adsorbed was placed in a Petridish along with adult *Sitophilus oryzae* (20 numbers) and few grams of rice grains for them to feed on. The observations were recorded in the time interval of 24 and 48 hours. The pesticidal activity against the weevils is provided in percent basis depending upon the mortality of the number of insects.

RESULTS

Antibacterial efficacy

Among the extracts of leaves of *Alangium salvifolium* studied for its antibacterial activity, hexane extract showed maximum zone of inhibition against the bacteria, *Listeria monocytogenes.* For the other bacteria, i.e. *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Salmonella typhi,* chloroform extract of the leaves showed maximum zone of inhibition. It was aqueous extract of the leaf of *Alangium salvifolium* which showed maximum zone of inhibition against the bacteria, *Vibrio cholerae.* The zone of inhibition measured for leaf extracts obtained from different solvent is presented in Table 1.

Antioxidant activity

The leaf extracts of the plant showed no IC_{50} values even at higher concentration. All the solvents used to extract the leaves of *Alangium salvifolium* failed to register IC_{50} value even at higher concentration of 15mg/mL. The values recorded for the assay is presented in Table 2.

Larvicidal Activity

Among the four solvents of leaves of *Alangium salvifolium* tested for its larvicidal activity against *Artemia salina* Chloroform and Methanol extract showed 100 % mortality at the lowest level of concentration, i.e., 0.25ml/10ml v/v. Hexane extract has showed 100 % larvicidal potency at the concentration of 0.5ml/10ml volume. However, very poor activity was recorded for aqueous extract of the leaves of *Alangium salvifolium.* The larvicidal potency recorded for different solvent extracts of the leaves are given in Table 3.

Table 1: Zone of inhibition recorded (in mm) for different solvent extracts of leaves of *Alangium salvifolium*

S. No.	Species	Extract in µl/disc											
		Aqueous			Chloroform			Methanol			Hexane		
		15	20	25	15	20	25	15	20	25	15	20	25
1	*Listeria monocytogenes*	9	10	11	12	13	13	11	11	12	13	13	15
2	*Pseudomonas aeruginosa*	6	6	6	8	10	10	0	9	10	7	7	7
3	*Staphylococcus aureus*	7	7	7	13	14	15	6	6	6	7	7	7
4	*Salmonella typhi*	9	10	11	8	10	12	7	11	11	6	6	6
5	*Vibrio cholerae*	12	15	17	13	14	15	6	6	6	0	0	0

Table 2: Inhibition percentage of the plant extracts and BHA to DPPH and their respective IC_{50} values recorded

Species	15 mg/mL	7.5 mg/mL	3.750 mg/mL	1.875 mg/mL	0.937 mg/mL	IC_{50} values
Aqueous	39.5	20.9	-	-	-	ND
Chloroform	22.15	8.9	-	-	-	ND
Methanol	49	30	-	-	-	ND
Hexane	21.5	7.1	-	-	-	ND
BHA (reference)	35.29	64.70	82.35	88.23	94.11	0.499

ND = Not Detected

Table 3: Larvicidal activity (In %) of leaves of *Alangium salvifolium*

S. No.	Concentration (ml/10ml - v/v)	Aqueous	Chloroform	Methanol	Hexane
1	0.25	20	100	100	75
2	0.5	25	100	100	100
3	0.75	40	100	100	100
4	1	50	100	100	100

Pesticidal Activity

Among the four solvents of leaves of *Alangium salvifolium* tested for its pesticidal activity against the storage pest, *Sitophilus oryzae* Hexane extract showed mortality rate of 80 % and 100 % of mortality at the interval of 24 hours and 48 hours respectively. Aqueous and Chloroform extract showed more than 50 % of mortality after 48 hours of exposure. However, Methanol extract has showed only lower level of mortality rate. The pesticidal potency recorded for the leaves of *Alangium salvifolium* is given in Table 4.

Table 4: Pesticidal activity (In %) of leaves of *Alangium salvifolium*

S. No.	Concentration (ml/10ml - v/v)	24 Hours	48 Hours
1	Aqueous	15	60
2	Chloroform	25	55
3	Methanol	15	45
4	Hexane	80	95

Phytoconstituents

The aqueous extract of the leaves showed the presence of Tannins, Flavonoids, Terpenoids and Steroids. Phlobatannins, Saponins, and Cardiac glycosides were not shown their presence in the leaves of *Alangium salvifolium.*

DISCUSSION

The bio efficacy of different solvent extracts of *Alangium salvifolium* showed significant difference in antibacterial, larvicidal, pesticidal and anti-oxidant properties. Using different solvent system is also recommended to study various bio-efficacies in order to identify the specific solvent system to extract the required compound. Traditional healers use primarily water as the solvent but according to Nair et al[20], the plant extracts in organic solvent provided more consistent antimicrobial activity compared to those extracted in water.

The previous report on antimicrobial activity of *Alangium salvifolium* was on its root[14] and not on leaves of the plant. This present work is carried on the leaves of *Alangium salvifolum* against microbes. The bio efficacy of the plant is attributed to its phytoconstituents[21]and is evident also in this present study. The antimicrobial activity showed by the leaves of *Alangium salvifolium* can be attributed to the presence of flavonoids, terpenoids, and tannins. The bioactivity of the phytochemicals, i.e. Flavonoids[22], terpenoids[23], tannins and phlobatanins[24]are already demonstrated to possess antimicrobial activity. The difference in zone of inhibition recorded for different solvent extracts of the leaves of *Alangium salvifolium* shows that, different phytoconstituents are derived according to the solvents used and the difference in zone of inhibition is recorded.

The present study on antioxidant ability of the leaves of *Alangium salvifolium* showed no satisfactory property. None of the solvent extracts of the leaves studied showed any antioxidant potential (IC_{50} values) even at higher rate of concentration (15mg/ml). Previous report on antioxidant ability of the plant was conducted from root[14]and flower[15,25] reported significant antioxidant property of the plant.

Present study is the first report on the larvicidal activity of leaf extracts of *Alangium salvifolium* against the larvae of *Artemia salina.* In the investigation of the biological activity of plant extracts and natural products, the assay on *Artemia salina* is a valuable tool for establishing toxicity and cytotoxicity parameters. A very positive correlation between the lethality to brine shrimp and cytotoxicity has been established[26]. Thus the lethality toward brine shrimp is recommended as an effective pre-screen for existing in vitro cytotoxicity and antitumor assays[27]. The antihelminthic activity[28], molluscicidal activity[29]of the plant *Alangium salvifolium* and larvicidal activity on *Spodoptera litura*[30] were previously reported.

Similarly, the present study is first of its kind on pesticidal activity of leaf extracts of *Alangium salvifolium* against the storage pest, *Sitophilus oryzae.* An insecticide does not have to cause high mortality on target organisms in order to be acceptable[31]. If this statement is taken into consideration, the extracts of the leaves of *Alangium salvifolium* can also be considered as a potential pesticidal agent for further research. According to Bowers et al[32], the screening of locally available medicinal plants for pest control would generate local employment, reduce dependence on imported products and stimulate local efforts. The present study may provide such basis for using native flora in controlling the larvae of pests. However, the present work is carried out with crude extract and hence, further work is required to isolate the active constituents to test them for their potential cytotoxic, larvicidal and pesticidal activities.

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