Bioprospecting medicinal plant *Aerva lanata* Juss. ex Schult. flowers for potential antimicrobial activity against clinical and fish-borne pathogens

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Multidrug resistant microorganisms are increasing these days from medical and aquaculture backgrounds. In present investigation chloroform extract of the flowers of the medicinal plant *Aerva lanata* Juss. ex Schult. was evaluated for antimicrobial activity against both the clinical and fish-borne microorganisms. The antimicrobial activity was found to be maximum against *Aeromonas hydrophila*, *Vibrio alginolyticus* and also against *E. coli* with the inhibition zone of about (23.33±0.33, 23.33±0.33 & 25.67±0.33), respectively for the above pathogens. The minimum level of antimicrobial activity was found against *Klebsiella pneumoniae*, *Plesiomonas shigelloides*, *Vibrio cholerae* and also against *Salmonella paratyphi* with the inhibition zone of about 14.33±0.33, 15.33±0.33, 16.33±0.33 and 14.00±0.58, respectively for the above pathogens when assayed with 100 µL of chloroform extract of *A. lanata* flower (100 µL/mL w/v). Moderate antimicrobial activity was found against the fish-borne pathogens *Vibrio mimicus* and *Vibrio harveyi*. The results of the phytochemical analysis revealed the presence of phytochemicals such as alkaloids, steroids, flavonoids and xantho proteins.

Keywords: Medicinal plants, Bioactive compounds, Pathogenic microbes, Antimicrobials, Phytomedicine, *Aerva lanata*.

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Introduction

A large portion of the world population especially in developing countries depend on the traditional system of medicine for a variety of diseases. Several hundreds of plant genera are used medicinally as herbal preparations in the indigenous system of medicine in different countries and are sources of potent and powerful drugs. Hence, it is essential to study the medicinal plants in order to promote their proper use and also to determine their potential as the primary source for the preparation of new drugs. The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs for their primary health care needs. The chemical substances that are present in the medicinal plants were responsible for their physiological action on the human body. The most significant of these bioactive compounds of the plants includes alkaloids, flavonoids, tannins and phenol compounds. Thus, the phytochemical researchers mainly focus on the ethno-pharmacological information which is generally considered to be an effective approach in the part of discovering new anti-infective agents from the higher plants. Medicinal plants are distributed worldwide but India is not only a varietal emporium of medicinal plants but is one of the richest countries in the world with regard to genetic source of medicinal plants.

The medicinal plant *Aerva lanata* Juss. ex Schult. of family Amaranthaceae is one of the important medicinal plants that grows throughout the plains of India. It is an erect or the prostrate herbaceous weed, common throughout the hotter parts of India especially all over the plains that extends up to an altitude of 3,000 m. It is also found in Sri Lanka, Arabia, Egypt, Tropical Africa, Java and Philippines. In India, it spreads especially in Tamil Nadu, Andhra Pradesh and Karnataka states. *A. lanata* commonly known as ‘Sunny-khur’ is widely used in Indian folk medicine for the treatment of *Diabetes mellitus*. It is also known as, *Pashana Beda*. Leaves are woolly, tomentose throughout and smaller in flowering branches. Flowers are very small, sessile, bisexual, greenish or dull white, often clustered with spikes. Seeds are kidney shaped and shining black in colour. The root has camphor like aroma and possesses high

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medicinal value. The antioxidant activity of A. lanata showed protective effects against diseases without reducing their therapeutic efficacy. This plant has been reported to possess hypoglycaemic and anti-hyperlipidemic activities. It contains phytochemicals of immense medicinal and pharmaceutical importance such as O-acyl glycosides, β-sitosterol, daucosterol, cyringic acid, vanillic acid, feruloyl tyramine, feruloyl homovanillylamine, narcisin and aervitrine which are effective as nephroprotective and used in jaundice. Hence, considering its medicinal value an attempt was made to present and overview of phytochemical and pharmacological activity of this plant. The alcoholic extract of the aerial parts showed significant diuretic, anti-inflammatory, antimicrobial and cytotoxic properties. The alcoholic extract of leaves, stem and roots possess potential diuretic activity. In present investigation A. lanata was evaluated for its antimicrobial activity against the clinical and the fish-borne pathogens and also screened for the presence of potential phytochemicals.

Materials and Methods

Plant collection
The plant was collected from Alwarkurichi region (Elevation-102 m, latitude 8.7792°N, longitude 77.4031° E), Tirunelveli District, Tamil Nadu during the month of February 2012 in the morning time and was brought to the laboratory as early as possible without the exposure of the plant material to scorching sun. In the laboratory the plant flowers were allowed to dry for 10 days under shade condition.

Crude extract preparation
The extraction of the plant flowers was carried out by Soxhlet method. Soxhlet apparatus was set in a suitable protective place. The water flow was checked regularly and the solvent chloroform was taken in a Soxhlet flask. The powdered flowers, about 200 g, were teased in the Soxhlet extraction unit and the process was allowed to run until getting the fine extract of A. lanata (32 cycle). The crude extract of about 6 g was obtained.

Antibacterial activity
The standard bacterial cultures were obtained from Muthayammal College of Arts and Sciences, Raspuram, Namakkal district, Tamil Nadu. The bacterial cultures, viz. Vibrio mimicus, V. alginolyticus, V. cholerae, V. harveyi, Klebsiella pneumoniae, Aeromonas hydrophila, Plesiomonas shigelloides, E. coli and Salmonella paratyphi were used as test microorganisms.

Agar disc diffusion method
Muller-Hinton Agar of about 180 mL was prepared and sterilized. After sterilization was over, the media was allowed to hand bearable temperature and immediately poured into the petriplates and kept for solidification. After solidification, the appropriate bacterial cultures were swabbed over the media. After swabbing, the discs impregnated with different volumes of (25 µL, 50 µL, 75 µL, 100 µL) crude extracts of A. lanata was placed over the media and kept for incubation at 37°C for 24 h and in the next day fresh cultures of respective bacterial strains were inoculated into the respective test tubes marked with different names of the culture. The bacterial cultures, viz. Vibrio mimicus, V. alginolyticus, V. cholerae, V. harveyi, Klebsiella pneumoniae, Aeromonas hydrophila, Plesiomonas shigelloides, E. coli and Salmonella paratyphi were used as test microorganisms.

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Antifungal activity
The fungal cultures were obtained from Muthayammal College of Arts and Sciences, Raspuram, Namakkal district, Tamil Nadu. The fungal cultures, viz. Cladosporium sp., Tricoderma sp., Aspergillus sp., Candida albicans, Trycophyton rubrum were maintained in potato dextrose agar slants and stored in refrigerator for further usage. The fungal broths were prepared and the appropriate fungal cultures were inoculated and incubated at 37°C for 24 h for the preparation of seed culture.

Agar disc diffusion method
Muller-Hinton Agar of about 100 mL was prepared and sterilized. After sterilization, the media was allowed to hand bearable temperature and immediately poured into the petriplates and kept for solidification. After solidification, the appropriate bacterial cultures were seeded over the media. After swabbing, different volumes (25 µL, 50 µL, 75 µL, 100 µL) of crude extract of A. lanata impregnated discs were placed over the media and kept for incubation at 37°C for 48 h. After the incubation time was over, the zone of inhibition was measured using a zone measurement scale. Fluconozole disc was used as positive control (25 mcg/disc).
Phytochemical analysis
Phytochemical tests were performed based on the proposed methods to find out the phytoconstituents of the crude chloroform extract of *A. lanata* flower.\(^{13}\)

**Test for reducing sugars (Fehling’s test)**
The chloroform extract (0.3g in 3 mL of water) was added with Fehling’s solution (A and B) in a test tube and heated. The solution was observed for a colour reaction.

**Test for anthraquinones**
Chloroform extract of about 0.5 g was boiled with 10 mL of sulphuric acid (H\(_2\)SO\(_4\)) and filtered. The filtrate was collected and shaken with 5 mL of chloroform. The chloroform layer was collected in another test tube and added with 1 mL of dilute ammonia solution. The reaction mixture was then observed for the formation of colour changes.

**Test for terpenoids (Salkowski test)**
The chloroform extract of about 0.5 mL was mixed with 2 mL of chloroform. Concentrated H\(_2\)SO\(_4\) (3 mL) was added along the sides of the test tube. Formation of reddish brown colour indicates the presence of terpenoids.

**Test for flavonoids**
5 mL of dilute ammonia solution was added to a portion of the chloroform extract. Concentrated sulphuric acid (1 mL) was then added along the sides of the test tube. A yellow colouration that disappears on standing indicates the presence of flavanoids.

**Test for saponins**
Chloroform extract of about 0.5 mL was added with 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for the formation of foam.

**Test for tannins**
The extract of about 0.5 g was boiled with 10 mL of water in a test tube and filtered. A few drops of 0.1% ferric chloride solution was added and observed for the formation of brownish green or a blue-black colour.

**Test for alkaloids**
The extract of about 0.5 g was diluted to 10 mL with acid alcohol, boiled and filtered. To 5 mL of the filtrate was added 2 mL of dilute ammonia solution. 5 mL of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 mL of acetic acid and added with Dragendorff’s reagent. The formation of reddish brown precipitate indicates the presence of alkaloids.

**Thin Layer Chromatography Technique**
TLC technique was performed to separate the secondary metabolites present in the crude flower extracts of *A. lanata*. The TLC plates were prepared by mixing the silica gel with distilled water in the ratio 2:4 and were run with different solvent systems to find out the RF value of the different bioactive compounds. The separated spots were observed under the light of UV-transilluminator and the RF values were recorded.

**Statistical Analysis**
Student T-test was performed using the triplicate values of inhibition zone formed against the bacterial and fungal strains by the crude flower extract of *A. lanata*. \(P>0.5\) was considered as significant.

**Results and Discussion**

**Antibacterial activity**
The results of the antimicrobial activity of chloroform extract of the medicinal plant *Aerva lanata* showed potential antimicrobial activity against both the clinical and the fish-borne pathogens. The antimicrobial activity was found to be maximum against *Aeromonas hydrophila*, *Vibrio alginolyticus* and also against *E. coli* with the inhibition zone of about 23, 23 and 26 mm, respectively for the above pathogens. The minimum level of antimicrobial activity was found against *Klebsiella pneumoniae*, *Plesiomonas shigelloides* and *Vibrio cholerae* and also against *Salmonella paratyphi* with the zone of inhibition of about 14, 15 and 16 mm, respectively for the above pathogens when tested with 100 µL volume of chloroform extract of *A. lanata* flower (100 µg/mL w/v). Moderate activity was found against the fish-borne pathogen *Vibrio mimicus* and *V. harveyi*. The alkaloids present in the flower extracts of *A. lanata* is expected to be the major reason for its potential antimicrobial activity against both the clinical and fish-borne pathogens. Many medicinal plants have been reported with potential antimicrobial activity including the parts i.e. flower, bark, stem, leaf, etc.\(^{14}\) The methanol and aqueous extracts of *A. lanata* showed pronounced antimicrobial activity against the fish-borne pathogen *Aeromonas hydrophila*.\(^{15}\) In this research, the chloroform extract of *A. lanata* also shown with potential antimicrobial activity in inhibiting the
growth of pathogenic Aeromonas hydrophila, one of the pathogens which infects fishes in aquaculture. Naylor and Burke (2005) reported that bacterial infections are the major reasons for fish mortality in aquaculture industry. These outbreaks are mainly due to the evolution of multidrug resistant microbes from the aquaculture background. So, we need an alternative drug to save the fishes from the attack of pathogenic microbes which leads to increased mortality in fish farms and in turn affect the economical growth. Treatments of bacterial diseases with various herbs have been safely used widely in organic agriculture, veterinary and human medicine. So, we can utilize the bioactive compounds present in the flower extract of the medicinal plant A. lanata as an effective antibiotic in fish disease management in the days to come. Ramalakshmi et al. (2013) reported that, the flower extracts of the plant Couroupita guanensis showed potential antimicrobial activity against the fish-borne pathogens, viz. Vibrio alginolyticus and Plesiomonas shigelloides and so it can be utilized as an effective antibiotic in aquaculture industry to control the fish disease outbreaks. The results of the present study reiterate the potential antimicrobial activity of the chloroform extract of the medicinal plant A. lanata against the pathogenic microbes. Isolation of the individual compounds from the crude chloroform extract of A. lanata is an essential need as to find out the exact compound responsible for the potential antimicrobial activity displayed against pathogenic clinical and fish-borne pathogens (Table 1 and Plate 1).

### Antifungal activity

The antifungal activity of chloroform extract of A. lanata flowers showed effective antifungal activity.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Bacterial cultures</th>
<th>Different volumes of crude extract of A. lanata flowers extract (100 µg/mL w/v) and the recorded zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>21.67±0.33, 23.67±0.33, 25.67±0.33, 11.67±0.33</td>
</tr>
<tr>
<td>2</td>
<td>Vibrio mimicus</td>
<td>17.67±0.33, 19.67±0.33, 22.33±0.33, 14.33±0.33</td>
</tr>
<tr>
<td>3</td>
<td>Klebsiella pneumoniae</td>
<td>10.67±0.33, 12.67±0.33, 14.33±0.33, 0.00±0.00</td>
</tr>
<tr>
<td>4</td>
<td>Vibrio alginolyticus</td>
<td>17.33±0.33, 21.00±0.00, 23.33±0.33, 12.67±0.33</td>
</tr>
<tr>
<td>5</td>
<td>Plesiomonas shigelloides</td>
<td>10.67±0.33, 12.67±0.33, 15.33±0.33, 18.33±0.33</td>
</tr>
<tr>
<td>6</td>
<td>Vibrio cholerae</td>
<td>11.67±0.33, 13.00±0.00, 16.33±0.33, 0.00±0.00</td>
</tr>
<tr>
<td>7</td>
<td>Aeromonas hydrophila</td>
<td>16.33±0.33, 21.33±0.33, 23.33±0.33, 11.67±0.33</td>
</tr>
<tr>
<td>8</td>
<td>Salmonella paratyphi</td>
<td>10.33±0.33, 11.67±0.33, 14.00±0.58, 0.00±0.00</td>
</tr>
<tr>
<td>9</td>
<td>Vibrio harvey</td>
<td>17.67±0.33, 19.67±0.33, 19.00±3.00, 12.00±0.00</td>
</tr>
</tbody>
</table>

n=3, expressed as M± S.D.
only against the fungal pathogen *Cladosporium sp.* and no inhibitory activity was recorded against *Tricoderma sp.*, *Aspergillus niger*, *Candida albicans* and *Trycophyton rubrum*. The zone of inhibition observed is as follows: 13.66±0.33, 16.33± 0.33 and 19.33 ± 0.33 at 50, 75 and 100 µL, respectively. The control Fluconozole showed 13.00 ± 0.57 zone of inhibition.

**Phytochemical test**

The evaluated phytochemical screening of the chloroform extract of *A. lanata* flower revealed the presence of the phytoconstituents, viz. alkaloids, steroids, flavonoids and xanthoproteins. These bioactive secondary metabolites would have been responsible for the antimicrobial activity displayed by the chloroform extract of *A. lanata* flowers. It is further needed to isolate the above metabolites using the column chromatography technique to find out the exact compound responsible for this potential antimicrobial activity.

**Thin Layer Chromatography**

The thin layer chromatography profile of the chloroform extract of *A. lanata* flowers showed the presence of yellow coloured compound with Rf value 0.30 when it is separated with the solvent system Petroleum ether: Hexane (3:2).

**Conclusion**

The phytochemical screening of the chloroform extract of *A. lanata* flowers showed the presence of phytoconstituents, viz. flavonoids, steroids, xanthoproteins and alkaloids. The methanol extract of stem, leaves, roots and reproductive parts showed the presence of steroids, alkaloids and glycosides and terpenoids. The TLC profile of the chloroform extract of flowers showed the presence of a yellow coloured compound with the Rf value 0.30. Thus, the active compound could be isolated by using column chromatography technique. A novel antibiotic useful in the medical and aquaculture industry to overcome the microbial outbreaks may be developed from flowers of this plant.

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**References**


