7. EXPERIMENTAL STUDY ON THE ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANT LANTANA CAMARA ON FISH PATHOGEN, PROVIDENCIA VERMICOLA

7.1. Introduction

The incidence of infectious diseases in aquaculture leads to significant economic losses causing significant problems in the development of the sector (Direkbusarakom et al., 1998). Increased expectation of consumers for fish meat quality, environmental and animal welfare issues are forcing the intensification of aquaculture industry causing serious outbreaks of diseases and leading to the use of antibiotics (FAO, 2008). Hence, an alternative natural medicine should be developed to treat and control bacterial fish diseases.

Pathogenic bacteria often develop drug resistance if exposed to antibacterial drugs in the long term and this makes infections more difficult to treat (Sugita et al., 2002; Sarter et al., 2007). Common uses of antibiotics in food producing animals resulted in antibiotic resistance in intestinal bacteria and also this resistance genes of which can be transferred to disease causing bacteria, resulting in antibiotic resistant infections for humans (Serrano, 2005). The use of antibiotics in aquaculture has been discouraged since their use would lead to development of drug resistance thereby reducing drug efficacy. Moreover, the accumulation of antibiotics both in the environment and in the fish is potentially risky to consumers as well as to non-target species (Aldeman and Hastings, 1998).

To overcome such problems, use of environment friendly natural products has been promoted as an alternate to control bacterial infection in aquaculture. In this regard use of medicinal herbs having antimicrobial activity are recommended against several pathogenic bacteria as traditional medicines for treatment of diseases (Srinivasan et al., 2001; Volleкова et al., 2001). Since the herbal medicines have minimal side effects and are easily biodegradable, inexpensive and locally available, they have been recommended as an alternative tool for bacterial disease management in aquaculture (Bhuvaneswari and Balasundaram, 2006). Plants have been used as traditional medicine since time immemorial to control bacterial, viral, and fungal diseases. Research has been initiated to evaluate the feasibility of using herbal medicines in fish disease management (Abutbul et al., 2005).
Hence, the present attempt to evaluate the antibacterial activity of methanolic extracts of medicinal plant(s) against the fish pathogenic bacterium, Providencia vermicola.

7.2. Materials and methods
7.2.1. Phytochemical screening of five selected indigenous plants, their antibacterial activity on the bacterial infectivity in *L. rohita*

Five species of indigenous Indian traditional plants with previously reported remedial activity against various pathogens and diseases were selected for this study (Parida et al., 2002; Balasubramanian et al., 2007; Ilango and Chitra, 2010). They were Lantana camara, Aegle marmelos, Cynodon dactylon, Azadirachta indica and Limonia acidissima. All these plants were collected from Periyar University-area, Tamil Nadu (India). Herbarium specimens were made for each plant species and the voucher specimens have been kept at the Department of Biotechnology, Periyar University, Tamil Nadu (India).

The terrestrial plants used to determine the antibacterial activity on *P. vermicola* are described below:

1) Aegle marmelos (Linn.) Corr

The plant is a moderate sized, slender, aromatic tree growing wild throughout the deciduous forests of India.

Description: Branches armed with straight, sharp, axillary, nearly 2.5 cm long spines; bark soft, light gray; leaves attenuate, trifoliolate, leaflet ovate or ovate-lanceolate, terminal long petioled; flowers large, greenish white, sweet scented; fruits globose, gray or yellowish; ring woody.

Properties and uses: The leaves are sweet, astringent, bitter and febrifuge and the leaves are astringent, laxative, febrifuge and expectorant. They are useful in diarrhoea, desenery, dyspepsia, stomachalgia, diabetes, inflammations and asthmatic complaints.

2) Azadirachta indica (A. Juss)

In India, this plant occurs throughout the country, to an altitude of 3,000 ft. believed to be an auspicious tree, this is often cultivated in gardens and along roadsides as an avenue tree.
Description: A medium to large sized tree, 15-20 m in height with a clear bole of 7.0 m having greyish to dark grey tubercled bark; leaves alternate, imparipinnate, 7-13 foliolate; leaflets sub-opposite, falcate-lanceolate, oblique at base, coarsely serrate, acuminate, upto 7.5 x 2.5 cms, glabrous green above, pale beneath; flowers greenish white in axillary panicles; calyx 5 fid; petals 5, free shortly ciliate, staminal tube cylindric, 8-10 lobed, lobes slightly toothed at the tip; ovary three-chambered, superior, style slender, elongate, ending in a three lobed cylindric stigma; drupes ellipsoid, fleshy, yellow when mature.

Properties and uses: The leaves are bitter, astringent, acrid, depurative, antiseptic, ophthalmic, anthelmintic, alexeteric, appetiser, insecticidal demulcent and refrigerant. They are useful in vitiated conditions of pitta, burning sensation, leprosy, skin diseases, tuberculosis, malarial and intermittent fevers.

3) Cynodon dactylon (Linn.) Pers.

The plant is a very common weed throughout India and also cultivated for decorative purposes.

Description: Perennial herb; stem slender, creeping, rooting at all nodes; branches erect; leaves narrowly linear, flat, upto 8x0.3 cm; spikes 3-7, umbellate; spikelets sessile, laterally compressed, arranged in two alternating series on the rachis, each spikelet one flowered; glumes 3, 1 and 2 empty, keeled, glume 3 or lemma larger, boat-shaped membraneous, 3- nerved palea 2- nerved, both enclosing a bisexual flower lodicules 2, short, glabrous; stamens 3; ovary glabrous; fruit oblong, laterally compressed grain.

Properties and uses: The plant is astringent, sweet, cooling, haemostatic, depurative, vulnerary, constipating, diuretic and tonic, and it is useful in vitiated conditions of pitta and kapba, hyperdispsia, burning sensation, haemoptysis, haematuria, haemorrhages, wounds haemorrhoids, conjunctivitis, cephalalgia, erysipelas, leprosy, skin diseases, vomiting, diarrhoea, dysentery, strangury and colporrhagia.
4) Lantana camara (Linn.)

A native of tropical America, but now naturalized in many parts of India as a troublesome prickly weed.

Description: A large scrambling evergreen, strong smelling shrub with stout recurved prickles; leaves opposite, often rugose, scabrid on both sides; flowers small, normally orange but often white to dark red, in heads which are prominently capitate; bracts visible, persistent; fruits fleshy drupes, 5 mm in diameter, endocarp hard, green when young and blue or black on ripening.

Properties and uses: The plant is vulnerary, diaphoretic, carminative, antispasmodic and tonic. Leaves are used for cuts, wounds, ulcers and swellings.

5) Limonia acidissima Linn.

A native of the Indo-Malaya eco-zone to Bangladesh, India, Pakistan, Sri Lanka and in Indo-Chinese eco-region east to Java.

Description: Limonia acidissima is a large tree growing to 9 metres (30 ft) tall, with rough, spiny bark. The leaves are pinnate, with 5-7 leaflets, each leaflet 25–35 mm long and 10–20 mm broad, with a citrus-scent when crushed. The fruit is a berry 5–9 cm diameter and may be sweet or sour. It has a very hard rind which can be difficult to crack open and contains sticky brown pulp and small white seeds.

Properties and uses: Its fruit is being used for medicinal purposes in form of a tonic, a powder or a poultice. Leaves, bark and roots can be used to treat snakebites and other venomous wounds. The fruit pulp is also used as a household cleaner (Plate 9).
Plate 9. Medicinal plants used for antibacterial activity on P.vermicola

(A) Lantana camara; (B) Aegle marmelos; (C) Azadirachta indica; (D) Limonia acidissima and (E) Cynodon dactylon
7.2.2. Preparation of different fractions of crude extracts from plants

7.2.2.1. Processing the plant materials

The parts of selected plants (leaves or whole plant) were removed, cut into pieces and washed thoroughly under running tap water and shade dried at room temperature (28 ± 2°C) for 15-20 days. The air-dried materials were powdered separately using electrical blender.

7.2.2.2. Methanolic crude extract from experimental plants

The pulverized plant powder was passed through 20 micron mesh sieve. 100g of powdered plant materials were extracted with methanol (SDFCL). The extraction was carried out in 24 h at room temperature with mild shaking (Chopra et al., 1992). The crude extract of each plant was obtained through distillation at a temperature 5 °C above the boiling point. The plant residue after solvent extraction was completely air-dried and stored at 4 °C (De et al., 1997).

Experimental media preparation

1g of methanol extract (of individual plants) were taken. 200 mg/mL of stock was obtained as a standard concentration. Ethanol extracts were sterilized using 0.22 μm membrane filters and were pasteurized for 15 minutes at temperature 62 °C (Almola, 2010). The obtained stock solution was used for phytochemical screening following the methodology of Kokate (1997); Harborne (1998).

7.2.3. Antibacterial activity of medicinal plants against P. vermicola

7.2.3.1. Agar-well diffusion method

The agar well diffusion method was performed by following the procedure of Perez et al. (1990). A sterile cotton swab was dipped into the P. vermicola broth culture inoculum. The cotton swab was then rotated pressing against the inside wall of the tube, above the fluid level to remove excess inoculum. The agar surface of the plate was inoculated by swabbing three times, turning the plate by 60° angle in between swabblings. 6 mm diameter wells were made with well puncher into the agar on each plate. Different concentrations 50, 75, 100 and 150 μL of the plant extract was added in four wells of a plate, sterile saline was added as a control
another well. The solutions were allowed to diffuse for 30 min. The plates were then incubated at 30°C for 24-48 h. The antibacterial activity was evaluated by measuring the zone of inhibition around the well. Based on the zone of inhibition after triplicate experiments. The plant extract having maximum zone of inhibition was taken into account for further experiments.

7.2.3.2. Minimum Inhibitory Concentration (MIC) of L. camara against P. vermicola

The MIC against P. vermicola was determined by the tube dilution technique. The peptone broth was prepared and autoclaved. The peptone broth was allowed to cool and varying concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 μg/mL were added to the broth before pouring into the test tubes and allowed to cool.

P. vermicola was inoculated into the tubes and tubes were incubated at 27°C for 24 h. After 24 h the tubes were examined for bacterial growth in the broth. The growth of the organism was measured by the method described earlier in section 4.3.1. The minimum concentration of the L. camara, which inhibited bacterial growth, was recorded as MIC (Adiguzel et al., 2005).

7.2.4. DNA fragmentation

Genomic DNA fragmentation was observed in the same bacterial inoculum, which was used in the microdilution method. This bacterial inoculum was incubated with L. camara extract for 3 h. The concentration of plant extract was predetermined from MIC value (20-30 μg/mL) of microdilution method, which was chosen for this DNA fragmentation test. Ethanol was used as a control.

The formed MIC bacterial culture was centrifuged at 5000 rpm for 5 min to get P. vermicola bacterial pellet. Bacterial pellet was resuspended in 500 μL of solution containing 50 mM Tris, 5 mM EDTA and 50 mM NaCl. Lysozyme was added to a final concentration of 1 mg/mL and sample was kept at 60°C for 30 min. After the incubation period 10 μL of proteinase K (10 mg/mL) and 20 μL of 10% SDS were added and then again incubated at 55°C for 10 min or until the solution cleared. The solution was prechilled by ice and the extract with an equal volume of Phenol: Chloroform: Iso amyl alcohol (25:24:1) was added. To this, an equal volume of 3M sodium acetate was added on the supernatant. Then the DNA was precipitated by adding
2 volumes of isopropanol (or Propane-2-ol), and centrifuged for 10 min. After centrifugation, the supernatant was removed and the pellet was washed with 70% ethanol. DNA pellet was dried and dissolved in 100 µL of TE buffer. The sample was then subjected to electrophoresis on 0.8% agarose gel at 100V as constant. Gel was stained with ethidium bromide solution (10 mg/mL) and it was documented (Koher and Emr, 1993).

7.2.5. Qualitative analysis of phytochemical constituents in methanolic extracts of L. camara

Tests for saponin, phenolic compounds, sugar, tannins, alkaloids, steroids, flavonoid, glycoside, protein, amino acids and oils were performed by adopting the methods of Brinda et al. (1981); Kokate (1997); Harborne (1998).

Test for flavonoids: To 1mL of stock solution in a test tube, few drops of dil. NaOH solution was added. Appearance of an intense yellow colour will indicate the presence of flavonoids after adding a few drop of dil. HCl.

Test for alkaloids: 1g powder of L. camara was mixed with 3 mL of ammonia solution in a conical flask and allowed to stand for few minutes to evaluate free alkaloids. To this, 10 mL of Chloroform was added, mixed well and filtered. The chloroform was evaporated from the crude extract by water bath and 3 mL of Mayer’s reagent was added. A cream colour precipitation will indicate the presence of alkaloids.

Test for saponins: 1mL of stock solution was diluted to 20 mL with distilled water and shaked continuously for 15 min. A foam layer on the top of the test tube will indicate the presence of saponins.

Test for tannins: To 3 mL of stock solution in a test tube was diluted with 1 mL of chloroform and 1 mL of acetic anhydride was added and mixed well. To this mixture, 1 mL of sulphuric acid was added carefully by the side of test tube. Formation of green colour will indicate the presence of tannins.

Test for phenol: For phenolic compounds, 50mg of crude methanol extract was dissolved in 5mL of distilled water. To this, few drops of neutral 5% ferric chloride solution was added. A dark green color will indicate the presence of phenolic compounds.
Test for carbohydrates: 100 mg of crude methanolic extract was dissolved in 5mL of water and filtered. 1mL of filtrate was boiled on water bath with 1mL each of Fehling solution A and Fehling solution B. A red color precipitate will indicate the presence of sugar.

Test for glycosides: 10 mg of methanolic crude extract was hydrolyzed by HCl for few hours on a water bath. To this, 1 mL of pyridine and a few drops of sodium nitroprusside solution were added. Further, 1 mL of NaOH solution was added to make the solution alkaline and observed for the change of color from pink to red which will indicate the presence of glycosides.

Fixed oils (spot test): A small quantity of extracts was pressed between two filter paper. Oil stain on the paper will indicate the presence of fixed oils.

Test for amino acids: To 1 mL of stock solution few drops of ninhydrin reagent was added to observe the appearance of purple color which shows the presence of amino acids.

7.2.6. Gas Chromatography—Mass Spectrometry analysis of Lantana camara extracts

GC-MS analysis was performed to identify the bioactive components present in the methanolic L. camara leaf extract (which showed higher antibacterial activity on P. vermicola pathogen tested) was analyzed under GC-MS for further investigations. Identification of compounds was done by injecting 1 μL of sample into a RT x-5 column (30 × 0.25 nm) of GC-MS model, Perkin Elmer, Clarus 680 and helium (1mL/min) was used as a carrier gas. The following temperature gradient program was used: 60°C for 2 min followed by an increase from 60 to 300°C at a rate of 10°C per min and finally 6min at 300°C. The m/z peaks representing mass to charge ratio characteristics of the antimicrobial fractions were compared with those in the mass spectrum library of the corresponding organic compounds (Pandey et al., 2010). The GC-MS analysis was performed in Sophisticated Instrumentation Facility (SIF), VIT University, Vellore, India.
7.3. Results

Antibacterial activity of medicinal plants against *P. vermicola*

Evaluation of various plant extracts activity against *P. vermicola*

Five Indian medicinal plants with known medicinal properties particularly with antimicrobial activity were selected and tested for their efficacy to inhibit the growth of *P. vermicola* to control its infection in *L. rohita*. The results showed that among five medicinal plants selected, the methanolic extract of *L. camara* (a weed) was found to have antibacterial activity. The methanolic extract of *Lantana camara* showed better inhibitory action against the fish pathogen, *P. vermicola* whereas, remaining crude extracts from other medicinal plants showed resistant against the fish pathogens but lesser than *L. camara* (Table-13 & Plate 10). The MIC of *L. camara* is given in (Table-14).

Table 13. Antibacterial activity of methanolic extracts on *P. vermicola*.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Crude methanol extract of study plants</th>
<th>Zone of inhibition (in mm)</th>
<th>50 µL</th>
<th>75 µL</th>
<th>100 µL</th>
<th>150 µL</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Lantana camara</em></td>
<td></td>
<td>09±1.00</td>
<td>13±1.15</td>
<td>16±1.15</td>
<td>19±0.57</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td><em>Aegle marenolos</em></td>
<td></td>
<td>06±0.57</td>
<td>12±1.00</td>
<td>16±0.57</td>
<td>18±1.15</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cynodon dactylon</em></td>
<td></td>
<td>-</td>
<td>10±1.15</td>
<td>10±1.00</td>
<td>14±1.15</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td><em>Azadirachta indica</em></td>
<td></td>
<td>07±1.15</td>
<td>09±0.57</td>
<td>11±1.15</td>
<td>13±1.00</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td><em>Limonia acidissima</em></td>
<td></td>
<td>-</td>
<td>07±1.00</td>
<td>08±1.00</td>
<td>10±0.57</td>
<td>-</td>
</tr>
</tbody>
</table>

Plate 10. Antibacterial activity of methanolic extract of *Lantana camara*
Table 14. Minimum inhibitory concentration of L. camara against P. vermicola by tube dilution method.

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>L. camara</td>
<td>+</td>
</tr>
</tbody>
</table>

‘+’ Growth of P. vermicola; ‘-’ No growth; (+) Poor growth.

DNA fragmentation

Confirmation of the suppressive effect of L.camara on methanolic extract on P. vermicola was observed by genomic fragmentation assay. From the fragmentation analysis, it was confirmed that the L. camara can be used as a potential source for eradication of P. vermicola. Very clearly fragmented DNA band observed in the lane-1 (loaded with plant extract and P. vermicola) and lane-2 (loaded with chloramphenicol and P. vermicola). No fragmentation was observed in lane -3 & 4 (loaded with bacterial culture and methanol, P. vermicola culture, respectively (Plate 11).

Plate 11. DNA fragmentation analysis using Lantana camara extract and Chloramphenicol on P. vermicola (M- Lambda DNA/ Hind III Marker; Lane-1. Lantana camara treated P. vermicola; Lane-2. Chloramphenicol treated P. vermicola; Lane-3. P. vermicola alone; Lane-4. DMSO alone)
Phytochemical analysis of methanolic leaf extract of L. camara

The results of preliminary phytochemical analysis of methanolic leaf extract of L. camara are given in the Table-15. The preliminary phytochemical screening of L. camara methanolic extract showed positive results for flavonoid, saponin, tannin, phenol, carbohydrate and fixed oil.

Table 15. Results of phytochemical analyses of L. camara methanolic extract.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>L.camara</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compound</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>-</td>
</tr>
<tr>
<td>Fixed oils</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
</tr>
</tbody>
</table>

GC-MS chromatogram of the methanolic extract of Lantana camara showed 21 peaks and have been identified after comparison of the mass spectra with WILEY and NIST libraries, indicating the presence of 17 phytocomponents. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (peak area %) were studied clearly. From the results, it was observed that, Pectolinaringenin (14.52%), 2, 6, 10, 14, 18, 22 - tetracosahexane, 2, 6, 10, 15, 19, 23 - hexamethyl- (14.45%), Caryophyllene (10.72%) and cycloisolongifolene (17.72%) were the major components in the methanol extract (Plate 12).
Plate 12. GC-MS analysis of Lantana camara methanolic extract.
7.4. Discussion

The large-scale settings of aquatic animal husbandry have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish and related environment (Smith et al., 1994; Alderman and Hastings, 1998; Petersen et al., 2002; Alcaide et al., 2005; Cabello, 2006). The continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in the aquatic environment. Continuous use of synthetic antibiotics reveals the threats to consumers and non-target organism in the environment (Muniruzzaman and Chowdhury, 2004; Abutbul et al., 2005). Treatment of bacterial diseases with various herbs has been safely employed widely in organic agriculture, veterinary and human medicine (Direkbusarakom, 2004). Since ancient times, medicinal plants have been used for the treatment of common infectious diseases (Rios and Recio, 2005) and treatments with plants having antibacterial activity are potentially beneficial alternative in aquaculture (Abutbul et al., 2005). Medicinal plants as the alternative agents are effective to treat the infectious diseases and ease many of the side effects that are associated with synthetic antimicrobials (Punitha et al., 2008). Many active plant compounds exert potential immunostimulating activity. Alkaloids, terpenoids, quinones and phenolic compounds, whereas polysaccharides, peptides, glycoproteins and nucleotides are in the second class (Wagner and Proksch, 1985). Also, many plants produce antioxidant compounds which work as protective agents that inactivate reactive oxygen species and thus delay or prevent oxidative damage, therefore playing major roles in the prevention of diseases (Hudec et al., 2007).

Certainly, numerous studies have been carried out on using medicinal plants/herbs in fish to treat bacterial diseases, and also to serve as a cheaper source of nutrients for the animals (Sakai, 1999; Siddhuraju et al., 2000; Hossain et al., 2001; Richter et al., 2003; Harikrishnan et al., 2003; Rath, 2000). For example, herbs have been used with some success to treat ulcerative dermatitis in common carp; a disease which was caused by A. hydrophila (Harikrishnan et al., 2003).

Previous work on the antibacterial activities of plants provides evidence of the importance of some plants against fish pathogens. In the present work, among
five medicinal plants tested, L. camara was found to exhibit potent antibacterial activity against P. vermicola. The methanolic extract of the leaf of L. camara was found to be the most effective against P. vermicola and was used to control the infection in L. rohita. The plant has been reported for its antimicrobial activity against some Gram positive, Gram negative organisms and fungi (Barreto et al., 2010; Ashish et al., 2011; Agarwal et al., 2012). The result of present study agrees with the previous reports of the antimicrobial activity of L. camara leaves done by previous workers (Mahdi Pour and Sasidharan, 2011; Agarwal et al., 2012).

The plant based antimicrobial drug have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobial agents. The results revealed that the extract of L. camara effectively produced inhibitory activities against the fish pathogen, P. vermicola. Presence of chemical components of L. camara might have inhibited the bacterial growth.

The frequency of bacteria with DNA fragmentation was established using the Micro-Halomax kit. DNA fragmentation is a vital characteristic in apoptosis. Proteus mirabilis with spontaneously fragmented DNA during exponential and stationary growth and E. coli with DNA damaged after exposure to hydrogen peroxide or antibiotics, such as ciprofloxacin or ampicillin, were clearly detected and then fragmented DNA was detected in Saccharomyces cerevisiae after amphotericin-B treatment (Ferna’ndez et al., 2008). Our experimental study also supported that antibacterial activity of L.camara extract on the bacterial DNA. The given concentration of the plant extract fragmented the DNA with different size of base pairs, which may provide the evidence for denaturing DNA and degrading the bacterial colony or controlling the bacterial (P.vermicola) growth.

Lantana camara (Linn.) belongs to verbenaceae family, commonly known as wild sage, is a flowering shrub native of tropical America and it is being cultivated throughout the world as an ornamental plant (Sharma and Sharma, 1989). However, it is listed as one of the important medicinal plants of the world (Ross, 1999). L. camara whole plant and plant parts viz., leaves, flowers, and essential oils have been thoroughly studied for their chemical compositions, previously and currently (Saleh
1974; Hart et al., 1976; Sharma and Sharma, 1989; Siddiqui et al., 1995; Ghisalberti, 2000). Different parts of the plant are used in folklore remedies and traditional systems of medicine for the treatment of various human ailments. Over the last two and half decades, a large number of plant species have been evaluated for their antibacterial activity. One of the plants known for having many medicinal uses in traditional system of medicine is L. camara (Juliana et al., 2002). The leaves are used in the treatment of itches, cuts, ulcers, swellings, bilious fever, eczema and rheumatism. L. camara has received attention due to its role in economy and ecology. Many pharmacological investigations indicated that extracts of the leaves of L. camara exhibit antibacterial properties. It is suggested that the crude preparations of the leaves of the plant containing both the active and non-active components to have higher efficacy than semi-crude or pure plant substances (Kafaru, 1994).

Bhakta and Ganjewala (2009) reported that the antibacterial properties of the L. camara were due to the presence of phenolics, anthocyanins and proanthocyanidins in their leaves. The antibacterial activity of the bioactive component parthenin isolated from the plant extracts of column cleaned was high in B. subtilis.

In previous, ethanolic extracts of L. camara leaves and roots were reported for its antibacterial activity. The in vitro antibacterial activity was performed by microdilution method. The extracts exhibited antimicrobial activity against S. aureus, P. vulgaris, P. aeruginosa, V. cholareae, E. coli and two multiresistant strains E. coli and S. aureus (Barreto et al., 2010).

Methanolic extracts of different parts of L. camara were screened for antimicrobial activity against 10 bacteria and 5 fungi by disk diffusion method and broth microdilution method. The leaves extract of L. camara showed highest activity against B. cereus and S. typhi (Badakshan et al., 2009).

Antifungal potential of L. camara was screened against Alternaria sp. which causes different plant diseases especially in vegetable plants. The antifungal activity was performed by food poison plate method at three different concentrations of extract viz, 10 mg/mL, 15 mg/mL and 20 mg/mL. At 20mg/mL dose L. camara
exhibited significant antifungal activity against Alternaria sp. (Srivastava et al. 2011).

Antifungal activity of ethanol and hot water extract of L. camara was screened against wood destroying white and brown rot fungi. Both extracts exhibited efficient antifungal activity against white and brown rot fungi, however ethanol extract was highly potential at very low concentration (0.01%) (Tripathi et al., 2009).

Bioactive potential of flavonoids has been reported by several authors (Illic et al., 2004; Cushner and Lamb, 2005). Lantadenes present in all L. camara is believed to be responsible for almost all the biological activities. In addition, other secondary metabolites such as alkaloids, terpenoids and phenolics could be held partially responsible for some biological activities (Barre et al., 1997).

The biochemical composition of plants is the most common parameter used for the characterization of plants. The preliminary phytochemical screening of our plant, L. camara methanolic extract showed positive results for flavonoid, saponin, tannin, phenol, carbohydrate and fixed oil.

Deepak et al., (2009) reported around 7-8 common secondary metabolites in the leaf and flower extracts such as alkaloids, phenolics, and terpenoids and other minor compounds phytosterols, saponins, tannins, phycobatannin and steroids. It was observed that, Pectolinaringenin (14.52%), 2,6,10,14,18,22-tetracosahexane, 2,6,10,15,19,23-hexamethyl- (14.45%), Caryophyllene (10.72%) and cycloisoolongifolene (17.72%) were the major components in the methanol extract.

Barua et al. (1976) isolated a new triterpene lantanilic acid from the leaves of L. camara and the structure was determined as the β, β-dimethylacryloyl ester of lantaninilic acid. A novel flavonol glycoside (camaroside) and a new phenylpropanoid glycoside (lantanoside) were isolated from the leaves of L. camara, which were potential antitumor agents and by spectroscopic methods and chemical transformations they are named as 3, 5-dihydroxy-4, 6-dimethoxyflavonol-7–O-glucopyranoside and 3, 4-dihydroxy-β-phenylethyl-o-alpha-L-rhamnopyranosyl ( 1→3 ) – 4′O- cis – caffeoyl - β-D-glucopyranoside respectively (Shashi Mahato et al., 1994).
3β, 19α dihydroxy ursan-28-oic acid and 21, 22β-epoxy-3β-hydroxy olean-12-en-28 oic acid, the two novel triterpenoids were isolated from the roots of L. camara. Oleanolic acid a hepatoprotective compound isolated from the roots of the L. camara and was converted into 28 → 13β-lactone by a facile photo-oxidation reaction (Misra and Laatsch, 2000).

A new triterpenoid was isolated from the leaves of L. camara and by means of spectral analysis the structure was elucidated as 3, 24-dioxo-urs-12-en-28-oic acid by Yadav and Vyasji (2003).

Five new derivatives of the pentacyclic triterpenoid lantadene A, isolated from the leaves of L. camara, were synthesized, characterized and screened for their cytotoxicity against four human cancer cell lines. Further, the three most potent compounds were studied for their in vivo tumor inhibitory potential upon oral administration in two-stage squamous cell carcinogenesis using induced by 7, 12-dimethylbenz [a] anthracene (DMBA), and promoted by TPA (Sharma et al., 2007).

Two flavonoids, linaroside and lantanoside were isolated from the L. camara and their common acetyl derivatives were examined against Mycobacterium tuberculosis, strain H (37) for their antimycobacterial activity and the acetylated type of flavonoid compound was found to be the most active (Begum et al., 2008).

Sousa et al. (2012) identified 25 identified components, bicyclogermacrone (26.1%), βcaryophyllene (24.4%), germacrone D (19.2%) and valecene (12.0%) were the main constituents. The essential oil volatile constituents inhibited the growth of Staphylococcus aureus and Pseudomonas aeruginosa with MIC of 1 and > 1 mg/L, respectively.

Among the presently identified 4 compounds from the methanolic extract of L.camara-weed viz: Pectolinaringenin (14.52%), 2, 6, 10, 14, 18, 22 - tetracosahexane, 2, 6, 10, 15, 19, 23 - hexamethyl- (14.45%), Caryophyllene (10.72%) and cycloisongifolene (17.72%), there is a scope for the use of the compound, Pectolinaringenin, as it has already been proved to be having potent larvicidal property (Muthu et al., 2012). Hence the study on the immunostimulant effect of methanolic extract of L.camara on Providencia vermicola-infected L. rohita is useful one.