RESEARCH PAPER

Accepted 21 September 2000
Revised 7 September 2000
Received 12 August 1999
Indian J. Pharm. Sci. 2001, 63(1) 20-23

# Antibacterial Natural Products from Leaves of *Lantana camara* L. with Activity Comparable to some Therapeutically Used Antibiotics

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**Four fractions of petroleum ether extract of the leaves of *Lantana camara* in different solvents showed significant antibacterial activity against some human pathogens under *in vitro* conditions. The MIC of the methanol fraction, containing triterpenoids, active against these pathogens was found to be comparable with those of some therapeutically used antibiotics.**

The genus *Lantana*, a very common weed and popular garden flower, is known to contain sterols[1], alkaloids[1], phenols[2-4], triterpenoids[5-9] and flavoneglucosides[10], the occurrence of which, however, vary with plant variety[11]. Some of these products are toxic to plants[2], animals[11] and insects[12]. *L. camara* with red, pink-red, white, white-pink and orange flowers, reportedly contain the triterpenoids, lantadene A, B and C and icterogenin, which are hepatotoxic[11]. However, the common pink variety is non-toxic and regularly grazed upon[11,13]. Oleanolic acid obtained from the roots of *L. camara* was first to be reported as antibacterial[14,15], whereas, the first record of antibacterial activity of a flavoneglucoside isolated from the leaves was made by Verma *et al.*[10] There is no report of antibacterial activity from the orange-brown flowered variety, though, a triterpenoid lancamarone from this variety, is reported to be poisonous to fish[16]. The present communication reports antibacterial activity of some constituent fractions isolated from the leaves of this plant against some common human pathogens and compares their activity to therapeutically used antibiotics.

## MATERIAL AND METHODS

Powdered shade dried leaves of *L. camara* (20 g) were extracted with petroleum ether (60-80°) for 36 h in a soxhlet. The pale yellow material obtained was vacuum dried and passed through silica gel (60-120 mesh, E Merck) column by elution with distilled solvents, in the order, petroleum ether, benzene, chloroform and methanol and filtered through anhydrous sodium sulphate. The flow rate of the column was 2 ml/min. The four fractions obtained were vacuum dried and a small portion of each was subjected to further analysis for detection of triterpenoids[17], alkaloids and steroids. UV and IR spectra of the fractions were recorded on a Shimadzu UV-160 A and Perkin Elmer 817 spectrophotometer, respectively, to determine the absorption spectra. These different fractions were used for determination of antibacterial activity.

### *In vitro* antibacterial assay:

Twenty four hour cultures of *Escherichia coli* (ATCC10536), *Salmonella typhi* (ATCC686), *Staphylococcus aureus* (ATCC6538) and *Pseudomonas aeruginosa* (ATCC25619) were used in the screening of antibacterial activity. The cultures were obtained from IMTECH, Chandigarh and Central Drug Laboratory, Calcutta. The antibiotics used were chloramphenicol, tetracycline hydrochloride and ampicillin (Sigma). Stock solutions were prepared by dissolving 10 mg of each of the dried fractions and antibiotics separately in 20 ml of sterile polyethylene glycol 600 (PEG). Sterile soyabean casein digest broth (HiMedia) was used to determine minimum

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TABLE 1: CHARACTERISTICS OF FRACTIONS OF *L. CAMARA*

| Fraction | Rf value | Fluorescence | Compound |
|---|---|---|---|
| Petroleum ether | 0.93 | Brown | Steroid |
| Benzene | 0.77 | Yellow-green | Alkaloid |
| Chlcroform | 0.72 | Brick-red | Triterpenoid |
| Methanol | 0.66 | Brick-red | Triterpenoid |

Characteristic Rf values and colour of fluorescence that indicate the type of chemical compound separated from the fractions of *L. camara* by TLC.

inhibitory concentration (MIC). Serial dilutions (5-100 µg/ml) in the sterile broth were done in culture tubes using the stock solutions. In each series of tubes two loopful of bacterial suspension were inoculated. Control with PEG was maintained. All the cultures with bacteria were incubated at 37° for 24 h. Following incubation, the tubes were examined for microbial growth. The concentration without microbial growth was considered to be the MIC. The MIC was determined in triplicate. A comparative study of activity of all treatments was made at 50 µg/ml concentration using the paper disc diffusion method[18]. About 0.01 ml of each of the solutions was placed on sterile 4 mm assay paper discs (Whatman filter paper No. 44) with a micropippette and tried. The discs were then transferred to the surface of soyabean casein digest agar medium (HiMedia, India), previously seeded with 1 ml ($10^8$ cells/ml) of each bacterial suspension. Blanks with PEG and sterile distilled water were maintained. Each treatment was replicated thrice. The plates were incubated at 37° and 25° respectively for 48 h and diameter of the zone of inhibition was recorded.

## RESULTS

The characteristic Rf and colour of fluorescence observed in the thin layer chromatograms indicated the presence of steroids and alkaloids in the petroleum ether and benzene fractions, respectively, whereas, the chloroform and methanol fractions contained triterpenoids (Table 1). This was also indicated by the UV and IR spectral analysis, presented in Table 2.

Bioassay of these fractions against the bacterial pathogens showed different degree of antibacterial activity. The minimum inhibitory concentrations determined were summarised in Table 3. The chloroform and methanol fractions containing triterpenoids showed antibacterial activity against all the human pathogens tested and the MIC of chloramphenicol and tetracycline were comparable with the methanol fraction against *E. coli* (10 µg/ml) and *P. aeruginosa* (15 µg/ml). The petroleum ether fraction inhibited the growth of *P. aeruginosa*, whereas, the benzene fraction showed activity against *S. typhi* only. MIC of methanol fraction against *S. typhi* and *S. aureus* was noteworthy and nearly comparable with the inhibitory activity of chloramphenicol and tetracycline. At 50 µg/ml, the inhibition zones in methanol fraction treatment were almost similar to corresponding zones of inhibition in chloramphenicol and tetracycline against *E. coli* and *P. aeruginosa*.

TABLE 2: MAJOR UV AND IR SPECTRAL FEATURES OF *L. CAMARA* FRACTIONS

| Fraction | UV absorption (nm) | IR spectrum Principal peaks at wave number ($cm^{-1}$)[KBr] |
|---|---|---|
| Petroleum ether | 225.0, 266.4 | 1450, 1550, 3000, 3100 |
| Benzene | 278.2 | 700, 1070, 1570, 1960, 2015, 3100, 3220, 3300 |
| Chloroform | 261.2, 255.0, 249.2, 243.8 | 880, 1355, 1540, 1635, 3190, 3260 |
| Methanol | 214.4 | 790, 970, 1130, 1170, 1490, 1580, 3130, 3750 |

TABLE 3: MIC VALUES OF *L. CAMARA* FRACTIONS AGAINST PATHOGENIC BACTERIA

| Treatments | *E. coli* | *S. typhi* | *P. aeruginosa* | *S. aureus* |
|---|---|---|---|---|
| Petroleum ether fraction | - | - | 50 | - |
| Benzene fraction | - | 35 | - | - |
| Chloroform fraction | 10 | 25 | 30 | 20 |
| Methanol fraction | 10 | 15 | 15 | 20 |
| Chloramphenicol | 10 | 10 | 15 | 15 |
| Tetracycline | 10 | 10 | 15 | 15 |
| Ampicillin | - | 15 | - | 25 |
| Petroleum ether* | - | - | - | - |
| Benzene* | - | - | - | - |
| Chloroform* | - | - | - | - |
| Methanol* | - | - | - | - |
| Sterile water* | - | - | - | - |

Comparison of the MIC values in µg/ml of *L. camara* fractions with those of standard antibiotics against human bacterial pathogen. Asterisk indicates controls and - sign indicates no activity.

## DISCUSSION

The absorption band at 3000 $cm^{-1}$ in the IR spectrum of the petroleum ether fraction indicated the presence of CH, whereas, that of the benzene fraction showing absorption at 3300-2015 and at 1070 $cm^{-1}$ indicated the presence of O-H group and C-OH stretching/deformation, respectively. From the IR spectrum of chloroform fraction, carbonyl absorption is most likely to account for the band at 1635 $cm^{-1}$. In this spectrum, the band at 1540 $cm^{-1}$ could have been caused by NH deformation and when considered with the band at 1635 $cm^{-1}$ could possibly indicate the presence of an amide group. O-H is indicated by the absorption at 3260 $cm^{-1}$. Beckett and Stenlake[19] have reported that the diagnostic regions of aromatic absorption are at 1600, 1500 and 850-700 $cm^{-1}$. In the methanol fraction spectrum, aromatic system is indicated by absorption at 1580, 1490 and 790 $cm^{-1}$ while absorption at 3180 $cm^{-1}$ indicates O-H group.

The present investigation confirms antibacterial activity of natural products from leaves of *L. camara* as

TABLE 4: ACTIVITY *L. CAMARA* FRACTIONS AND ANTIBIOTICS AGAINST SOME COMMON HUMAN PATHOGENS

| Treatments | Average inhibition zone (mm) at 50 µg/ml | | | |
|---|---|---|---|---|
| | *E. coli* | *S. typhi* | *P. aeruginosa* | *S. aureus* |
| Chloramphenicol | 24.4 | 21 | 16.5 | 15 |
| Tetracycline | 25.3 | 22.2 | 15.0 | 19.2 |
| Ampicillin | - | 15.2 | - | 9.2 |
| Petroleum ether fr. | - | - | 5 | - |
| Benzene fr. | - | 16.8 | - | - |
| Chloroform fr. | 24 | 19.5 | 8.2 | 13.7 |
| Methanol fr. | 25.2 | 16.5 | 14.7 | 14.2 |

also reported by Verma *et al.*[8,10] Triterpenoids contained in the methanol fractions were potentially more antibacterial in activity than the triterpenoids, steroids and alkaloids present in the chloroform, petroleum ether and benzene fractions respectively. The former were also found to be significantly comparable in activity to the antibiotics used against the microorganisms tested. From the foregoing discussion it could be concluded that natural products of leaves of *Lantana camara* with orange-brown flowers possess significant antibacterial properties. The active principle needs to be isolated, identified and tested against a wide range of human and plant pathogens.

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