Research Journal of Recent Sciences _____ ISSN 2277-2502
Vol. **3(9),** 52-55, September **(2014)** *Res.J.Recent Sci.*

Terpenes and Antimicrobial Activity from Lantana Camara Leaves

Mariajancyrani[1*], Chandramohan[1] and Ravikumar[2]
[1]Department of chemistry, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur-613 503, Tamil Nadu, INDIA
[2]School of Chemical and Biotechnology, Department of Chemistry, SASTRA University, Thanjavur, Tamil Nadu, INDIA

Available online at: www.isca.in, www.isca.me
Received 19th November 2013, revised 25th February 2014, accepted 26th March 2014

Abstract

The plant leaves contain a number of medicinally important compounds. The present study was carried out to identify the saturated terpenoid alkane, triterpenoid and evaluate antimicrobial activity of hexane extract of Lantana camara leaves. The terpenoids were separated from hexane crude extract by using column chromatography and Thin layer chromatography. Column chromatography was eluted with hexane, chloroform solvent mixtures of increasing polarity. The structures of this isolated compound were identified on the basis of spectral analysis. i.e. FT-IR, H'NMR, ^{13}CNMR, EI/MS. Terpene characterization using various spectroscopic analysis the final identified terpene as Calarane and 3-hydroxy-10,19-en-urs-28-oic acid. The antimicrobial activity was determined by well diffusion method and tested against different pathogens of three bacteria and two funguses. Antimicrobial activity of 50, 100 and 150% Hexane extract of Lantana camara leaves has been evaluated against streptococcus pneumoniae, Aspergillus niger, Salmonella typhi, klebsiella pneumoniae, Trichophyton Violaceum. In 150% concentration of hexane extract showed the highest antibacterial zone against streptococcus pneumoniae. In 100% and 150% concentration the extract showed the highest antimicrobial zone against Aspergillus niger. This is the first report of the isolation and identification of above compound from hexane extract of Lantana camara leaves

Keywords: Calarane, 3-hydroxy-10,19-en-urs-28-oic acid, Salmonella typhi, Trichophyton violaceum.

Introduction

Plants have been used in traditional medicine for several thousand years[1]. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine[2]. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. Documenting the indigenous knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources. According to the Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary health care needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases[3]. Due to less communication means, poverty, ignorance and unavailability of modern health facilities, most people are still forced to practice traditional medicines for their common day ailments[4]. Most of these people from the poorest link in the trade of medicinal plants. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants in still of great importance[5].

Plants contain pharmaceutically active compounds and that the type and concentration of these compounds determine the activity of a plant extract. The full moon is not important, but the concentration of active compounds in a plant nevertheless depends on the time of day. So it can be useful to pick the plants at a certain time or weather, as in the early morning or when the sun is shining, because the metabolic activity and therefore the concentration of the different natural products depends on parameters like temperature and action of light. Not only the methods of treatment but also the techniques of isolation of pure active compounds have been changed very much[6]. Lantana camara are useful as honey plants and spanish flag, sometimes planted lantana camara for this purpose and in butterfly gardening[7]. Lantana camara having important source in pentacyclic triterpenoids that cause hepatotoxicity and photosensitivity when ingested by grazing animals such as bovines, sheep, goats, and horses. This has led to widespread livestock loss in the India, Mexico, United States, South Africa and Australia[8].

The crude extracts of lantana camara used for protection of cabbage against the aphid Lipaphis erysimi. The Lantana camara leaves having a lots of medicinal activity such as anti-inflammatory, analgesic, anti-tumor, antibacterial, sedative, fungicide and antimicrobial[9]. Terpenes are more active in warmer weather released from trees to form a natural cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature[10]. With this background, the present study was carried out to isolate, identify the terpenoids and evaluate the antimicrobial activity of hexane extract of lantana camara leaves.

Material and Methods

Collection of plant material: The leaves of the plant Lantana camara collected from Thanjavur District in the month of July, 2010 and authenticated by Dr. John Britto, Rapinet Herbarium, ST.Joseph's College, Tiruchirappalli.

4.4 kg of leaves were cleansed with running tap water and dried in shadow . The shade dried leaves were crushed into uniform powder using pulverizer. The powdered sample was stored in closed container or air tight polythene bags until use.

Extraction of plant material: The leaf powder was extracted with 95% ethanol by using cold method extraction in room temperature for 1week. The 95% ethanol extract was filtered through whatmann -1 filter paper, distilled and concentrated to obtain the solid greenish residue. The final extract weight was noted and stored in a refriegerator. The 95% extract was further fractionated successively with petroleum ether, n-hexane, chloroform, ethyl acetate, ethanol, n-butanol and methanol. The solvents were recovered under reduced pressure.

Isolation of terpenoids: 7.3g of n-hexane soluble part was subjected to column chromatography using 60-120 silica gel mesh, that was packed using wet packing method in hexane with increasing polarity of hexane to chloroform (100, 75:25, 50:50, 25;75, 100). From that 50% hexane fraction was rechromatographed with thin layer chromatography. Thin layer chromatography (TLC) was carried out using silica gel G. The thickness of the chromatographic plate coated with silica gel was 0.2 mm. All chromatograms were developed in a glass chamber (20 X $10cm^2$) at room temperature. Among different solvent systems tested by micro-TLC, hexane: chloroform (9:1) was found to be suitable for good separation and, therefore, this solvent system was chosen for preparative TLC. The non-polar constituents are moving on the top of the TLC, then the top layer was scraped and separated out. The separated portion was recrystallized with methanol to obtained white color solid amorphous powder Compound-1(38 mg), which was analyzed by spectroscopic studies.

25% eluted hexane fraction was rechromatographed with thin layer chromatography. Hexane: chloroform (9:1) was also found to be suitable for good separation and, therefore, this solvent system was chosen for preparative TLC. The upper middle layer was scraped and separated out. The separated portion was recrystallized with methanol to obtained white color solid amorphous powder. The compound showed single spot on TLC, afforded the compound-2 (19 mg). This was identified by spectral studies.

General experimental procedures: FT-IR (Fourier Transform-Infra red) spectra were obtained using Perkin Elmer FT-IR 450-4000 in KBr disc and absorption peaks in terms of wave numbers (cm^{-1}). EI-MS (electron impact mass spectrum) were recorded on Jeol instrument. NMR (Nuclear magnetic resonance) was acquired on Brucker at 400 MHZ (^1H) and 100 MHZ (^{13}C). Chemical shifts were recorded as δ i.e. (ppm), chloroform as an inert solvent.

Screening test for terpenoids: Compounds 1, 2 were subjected to micro thin layer chromatography with suitable solvent system[11]. The TLC plates were placed in iodine chamber; brown color appeared in iodine vapor indicating the presence of terpenoids. Vanilin sulphuric acid was sprayed by TLC plates and put in an Oven at 110^0C. After minutes, a dark brown color appeared. Presence of terpenoids.

Antimicrobial activity: Microorganisms: Trichophyton violaceum, Aspergillus Niger, Salmonella Typhi, Streptococcus pneumoniae, Klebsiella were the pathogenic micro organisms included in the study. All the cultures were obtained in pure form the culture collection of Institute of Microbial Technology (IMTECH), Chaningarh, India.

Media preparation: Bacterial media: 36gm of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 151b pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plats were bored with 5mm dia cork bored. The plate with wells were used for the antibacterial studies.

Fungal media: 200gm of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20gm of dextrose was mixed with potato infusion. 20gm of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm dia cork borer.

Well diffusion method: Antibacterial and anti-fungal activity of the plant extract was tested using well diffusion method[12]. The prepared culture plates were inoculated with different selected strains of bacteria and fungi using streak plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37 ºC ± 2 ºC for 24 hours for bacterial and 25 ºC ± 2 ºC for 48 hours for fungal activity. The plates were observed for the zone formation around the wells was measured in mm (millimeter). For each treatment three replicates were maintained. The diameter of inhibition zones was measured in mm and the results were recorded. Inhibition zones with diameter less than 12mm were considered as having no antimicrobial activity. Diameters between 12 and 16mm were considered moderately active and these with >16mm were considered highly active.

Results and Discussion

Compound 1: The compound 1 is a white amorphous powder with melting point 20-23 ^0C. The IR spectra shows absorption band at 3097.54, 2896, 1404.54 and 1268 cm^{-1} due to presence of CH_3 and CH_2 groups.

^{1}HNMR- The signals appeared at δppm- 0.856, 0.880, 1.254 and 1.676 indicating the presence of CH_3 and CH_2 Protons.

^{13}CNMR- 13.13, 21.62, 28.29, 28.62, 30.85, 38.46, 38.67, 38.88, 39.09, 39.30, 39.51, 39.71. Correspond to presence of only CH_3, CH_2 groups and indicating the absence of OH, C=C and C-O groups.

The EI-MS spectroscopy of the present compound exhibited a molecular ion peak at m/z value 205 and fragmented peaks at 97.85, 149.05, 137.107 and 167. In accordance with above data the compound may be deduced as calarane.

Based on the above results and by comparing with other similar compounds[11-14], the identified structure of the isolated terpene hydrocarbon is given in figure-1.

Compound 2: The compound 2 is a white amorphous powder with melting point 303 ^{0}C. The IR spectra shows absorption band at 3398 cm^{-1} assigned to the presence of hydroxyl groups in the compound. Apart from this, presence of other vibration frequencies at 2938 and 1659 cm $^{-1}$ were assignments to presence of saturated C-H_3 group and carboxyl group, respectively.

^{1}HNMR- gave the signals appeared at δppm- 0.66, 0.78, 0.82, 0.89, 0.91 and 1.0 indicating the presence of CH_3 protons. The assignments of the various signals are: δppm 3.5 (H-1, C-3 proton) 5.3 (1H-anomeric proton).

The EI-MS spectroscopy of the present compound exhibited a molecular ion peak at m/z 454 and fragmented peaks at 412, 394, 203, 220 and 192. In accordance with above data the compound may be deduced as 3-hydroxy-110,19-en-urs-28-oic acid.

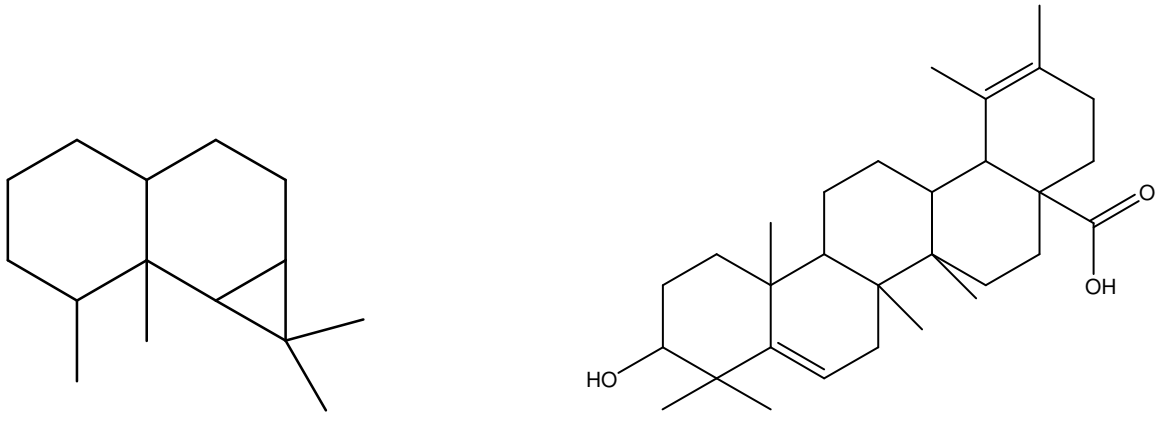
Based on the above deliberations and by comparing with other similar compounds[11-15], the proposed structure of the isolated terpenoid is given in figure-1.

Table-1
Antibacterial activity of different concentrations of hexane extract of Lantana camara leaves

Name of the pathogens	Inhibition in mm				
	Control	**Antibiotic**	**50%**	**100%**	**150%**
Salmonella Typhi	0	20	Nil	Nil	Nil
Streptococcus Pneumoniae	0	22	10	11	20
Klebsiella Pneumoniae	0	44	Nil	Nil	Nil

Table-2
Anti fungal activity of hexane extract of Lantana camara leaves

Name of the species	Inhibition in mm				
	Control	**Antibiotic**	**50%**	**100%**	**150%**
Trichophyton Violaceum	0	31	Nil	Nil	Nil
Aspergilleus Niger	0	32	13	15	20



Calarane 3-hydroxy-10,19-en-urs-28-oic acid

Figure-1
Structure of isolated compounds

Antimicrobial activity: As a result, the comparing of hexane extract of Lantana camara against bacteria and Fungai, In 150% concentration, antimicrobial activity of hexane extract of Lantana camara showed the highest 20mm zone against streptococcus pneumoniae. In 100% and 150% concentration, Lantana camara showed the highest 15mm, 20mm antimicrobial zone respectively against Aspergillus niger. In 50%, 100% and 150% concentration, antimicrobial activity of hexane extract of Lantana camara in salmonella Typhi, klebsiella pneumoniae, trichophyton violaceum not showed. Use of plants as a source of medicine has been inherited and is an important component of the health care system. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests[12-16] . The systematic screening of plant extracts for antibacterial activity is a continuous effort to find new antibacterial compounds. Considering the rich diversity of plants in India, it is necessary to screen plants for their antibacterial activity.

Conclusion

This is the first time, to report the above compounds present in the hexane extract of lantana camara leaves. The pharmacological studies of above compounds were work-in-progress. In future, the analysis by using various solvent system should congregate different other compounds present in the plant. On the basis of our results, Lantana camara appears to have potential for treatment of antimicrobial diseases. It should, however, be explored as a functional medicinal plant for isolating the active ingredients along with animal studies in vivo.

Acknowledgements

The authors thank to IISC-Bangalore, IIT-Chennai and Annamali University for providing spectral facilities to carry out this work.

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