ANTIMICROBIAL ACTIVITY OF *LANTANA CAMARA* LINN.

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Abstract

The *in vitro* antimicrobial activity of ether soluble fraction of upper ground parts of *Lantana camara* Linn. and its petroleum ether insoluble and soluble portions and sub fractions were deliberated against various Gram positive and Gram negative bacteria and fungi. They are found to be active against some Gram positive and Gram negative bacteria while inactive against fungi tested.

Introduction

Lantana camara Linn. generally famous as lantana, bunchberry and natural sage belonging to the family Verbenaceae is a terrestrial, evergreen aromatic and hairy shrub. It is cultivated throughout the world as a decorative or shrubbery plant (Sastri, 1962; Parajapati, 2003). It is used in many parts of the world in conventional medication for the cure of different human diseases. Various pharmacological activities have been reported such as analgesic, antimalarial, antifungal, antibacterial, enzyme inhibition, hepatotoxic, insecticidal and nematicidal activities (Ross, 2003; Ghisalberti, 2003; Patel, 2011; Sousa and Costa, 2012). It is a rich source of terpenoids, steroids and alkaloids (Begum *et al.,* 2015).

For a long era, plants have been a valued source of natural products for sustaining human healthiness, exclusively in the last decade, with more exhaustive studies for natural treatments. Antimicrobial confrontation has turn into a main risk to public and medical health. It undertaken throughout investigations for effectual prevention and action of the globally escalating range of diseases caused by bacteria and fungi. With the persistent manifestation of opposing pathogenic microorganisms, there is an emergent inquisitiveness in the discovery of new antimicrobial agents. The use of plant extracts can be great significance in therapeutic treatments with known antimicrobial properties. In the last few years, a number of studies have been accompanied in different countries to corroborate such effectiveness. Due to their antimicrobial qualities a number of plants have been used that are because of secondary metabolism of the plant for compounds synthesis (Gislene *et al.,* 2001).

The antimicrobial properties of plant have been examined by a numerous researchers worldwide (Mostafa *et al.,* 2011). Because of the therapeutic potential accredited to *L. camara*, the current studies were carried out on the ether soluble fractions and sub fractions of the upper ground parts of the plant.

Experimental

Materials and Methods

General**:** Silica gel PF_{254} (Merck) was employed for Vacuum liquid chromatography (VLC) (Coll and B. F. Bowden, 1986) and FCC (Flash column chromatography, Model Eyela EF-10) on silica gel 9385 (Merck) (Still *et al*., 1978). TLC (Thin layer chromatography) was taken out using precoated aluminium cards (Kieselgel 60 F_{254}, 0.2 mm thickness, Merck) and spots were visualized under UV light at 254/365 nm and through heating silica gel plates by sprayed with 5% H_2SO_4 in MeOH.

Plant Material**:** Upper ground parts of *Lantana camara* were unruffled from the University of Karachi campus. The species was recognized by Mr. Abdul Ghafoor a Senior Taxonomist from University of Karachi (Department of Botany). In the Herbarium a voucher specimen No. 63482 KUH was placed.

Extraction and Isolation**:** The upper ground parts (air dried) of *L. camara* was crushed and repetitively extracted at room temperature with MeOH. The solvent was evaporated under reduced pressure, leaving an extract (**LC**). This extract was partitioned in ethyl acetate phase and aqueous phase. The 4% aqueous solution of Na_2CO_3 was used to treat EtOAc phase to take apart the neutral from the acidic fraction. The EtOAc layer comprising the neutral fraction was splashed with water, dried (Na_2SO_4) and passed over activated charcoal. The

charcoal bed was consecutively washed with EtOAc and MeOH-C_6H_6 (1:1), after that these fractions were combined on the basis of TLC. The residue (LC-EAR) achieved on removal of the solvent from the charcoal filtrates and washings was alienated into petroleum ether-insoluble (LC-PEI) and petroleum ether-soluble (LC-PES) portions. The petroleum ether-insoluble fraction was again divided into ether insoluble (LC-EI) and ether soluble (LC-ES) fractions.

The ether soluble fraction (LC-ES) was further distributed into petroleum ether insoluble fractions (LCE-PEI) and petroleum ether soluble (LCE-PES). Petroleum ether insoluble portion was chromatographed on vacuum liquid chromatography (VLC) (petroleum ether; petroleum ether-EtOAc in order of rising polarity), which yielded 11 fractions LCE-I to LCE-XI. All these fractions were subjected to antimicrobial testing.

Biological assay

Table1. Antibacterial activity of ether soluble fraction (LC-ES) of methanolic extract (LC) and its sub-fractions (zone of inhibition in mm).

BACTERIA TESTED	LC-ES	LCE-PES	LCE-PEI	LCE-I	LCE-II	LCE-III	LCE-IV	LCE-V	LCE-VI	LCE-VII	LCE-VIII	LCE-IX	LCE-X	LCE-XI
GRAM POSITIVE														
Bacillus cereus	11	7	12	0	0	0	10	12	14	12	11	8	0	0
Bacillus subtillus	8	12	13	12	11	0	11	11	14	12	10	0	0	0
Bacillus thuringiensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynebacterium diptheriae	8	8	10	7	7	7	0	11	7	11	7	10	10	7
Corynebacterium hoffmanii	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynebacterium xerosis	8	10	8	0	0	0	0	0	10	7	9	0	0	0
Micrococcus luteus	0	9	10	8	7	11	0	11	0	0	7	0	0	0
Staphylococcus aureus	9	0	0	0	0	0	0	0	9	0	0	0	0	0
Staphylococcus aureus AB, 188	0	8	10	0	0	0	11	11	12	7	9	10	0	0
Staphylococcus epidermidis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylococcus faecalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylococcus saprophyticus	7	7	10	0	0	0	7	7	13	7	0	0	0	0
GRAM NEGATIVE														
Escherichia coli	9	8	10	0	0	0	10	0	10	9	10	9	7	0
Escherichia coli ATCC 8739	11	13	12	11	10	10	12	12	13	12	13	11	9	8
Escherichia coli Multi drug resistant	13	13	13	0	0	7	9	9	13	0	0	0	0	0
Klebsiella pneumonia	0	11	10	11	8	11	10	7	8	7	0	7	8	7
Proteus mirabilis	9	0	7	9	7	0	0	0	7	7	7	7	7	7
Pseudomonas aeroginosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeroginosa ATCC 9027	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salmonella paratyphi A	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salmonella paratyphi B	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salmonella typhi	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shigella dysenteriae	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Conc.: 500 μg/disc

Table 2. Antifungal activity of ether soluble fraction (LC-ES) of methanolic extract (LC) and its sub-fractions (zone of inhibition in mm).

FUNGI TESTED	LC-ES	LCE-PES	LCE-PEI	LCE-I	LCE-II	LCE-III	LCE-IV	LCE-V	LCE-VI	LCE-VII	LCE-VIII	LCE-IX	LCE-X	LCE-XI
FILAMENTOUS														
Aspergillus flavus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus terreus	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhizopus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DERMATOPHYTES														
Trichophyton rubrum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichophyton mentagrophytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichophyton tonsurans	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Microsporum canis	0	0	0	0	0	0	0	0	7	0	0	0	0	0
Microsporum gypseum	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fusarium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTHER FUNGI														
Candida albicans	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Candida albicans ATCC 0383	0	0	7	0	7	7	7	0	0	9	0	7	7	7
Saccharomyces cerevisiae	9	9	11	9	10	10	7	12	11	9	11	10	10	9
Helimanthosporum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Conc.: 500 μg/disc

Screening of Antibacterial Activity: The disc diffusion method (Baur *et al.*, 1966) was used to determine the antibacterial activity of the samples. 100 mg/ml (crude extract), 50 mg/ml (fractions) and 10 mg/ml (pure compounds) of stock solution in DMSO of each sample was prepared. Sterile filter discs containing 10 µl of stock solution were used for screening. The Iso sensitest agar (Oxoid) plates were seeded with 24 hours old culture (containing approximately 1-2 x 10^8 CFU/ml) grown-up in Mueller Hinton broth (Oxoid). The prepared discs were positioned on to the agar surfaces at different points and plates were nurtured at 37 °C for 24 hours. Results were confirmed by measuring the zone of inhibitions in mm (Table 1). DMSO was used as negative control.

Antibacterial activity of ether soluble fraction (LC-ES) of upper ground parts of *L. camara* and its sub-fractions was studied against twelve Gram-positive and eleven Gram-negative bacteria.

Screening of Antifungal Activity: Antifungal activity was also determined by disc diffusion method (Baur *et al.*, 1966) as described above. Briefly, a small amount of culture was transfered to 2-3 ml distilled water or normal saline in a screw capped tube with few glass beads (1 mm in diameter) and vortexes for 5-10 minutes to make a homogeneous suspension of fungal culture. Sabouraud dextrose agar (SDA) plates were seeded with this suspension holding about 1-2 x 10^8 CFU/ml. Sterile filter discs (containing concentration of 1000 µg/disc of extract / 500 µg/disc of fractions and 100 µg/disc of pure compounds) were placed on to the surfaces at different positions. Plates were incubated at room temperature for 1 week. Results were recorded by measuring the zone of inhibitions in mm (Table 2).

Results and Discussion

Antibacterial Activity: The fraction (**LC-ES**) was found effective against 1 out of 12 Gram-positive and 2 out of 11 Gram-negative bacteria tested at 500 μg/disc concentration. Further separation of this fraction through classical method yielded petroleum ether soluble (LCE-PES) and petroleum ether insoluble fractions (LCE-PEI). These fractions were also tested against these bacteria. LCE-PEI was found to be more active. It showed significant zones against 6 Gram positive and 4 Gram negative bacteria at 500 µg/disc concentration. The fraction LCE-PEI was subjected to vacuum liquid chromatography (VLC) which afforded eleven fractions (LCE-I to LCE-XI). Most of the sub-fractions showed activity against various gram positive and gram negative bacteria. Sub-fraction (LCE-VI) was the most active showing activity against 3 Gram-negative bacteria and 5 Gram-positive tested (Table 1).

Antifungal Activity: The *in vitro* antifungal activity of **LC-ES** and its sub fractionswas also determined against four filamentous fungi (*Aspergillus flavus, Aspergillus terreus*, *Rhizopus* sp., *Penicillium* sp.), six dermatophytes, (*T. mentagrophytes, Trichophyton rubrum, T. tonsurans, Microsporum gypseum, M. canis, Fusarium* sp.) and four other fungi (*Candida albicans, Candida albicans* ATCC 0383, *Saccharomyces cerevisiae*, *Helimanthosporum* sp.). All the fungi tested showed resistance against these samples except *Saccharomyces cerevisiae* which was inhibited by almost all fractions (Table 2).

Conclusions

The results of the present examination designate that the antibacterial and antifungal activities contrast with the fraction to fraction used. Therefore the study establishes the significance of plants used in Ayurvedic medicine, which might be substantial attention to the development of innovative drugs.

References

Baur, A. W., Kirby, W. M., Sherris, J. C. and Tink, M (1966). *Am. J. Clin. Pathol*., 45:493-496.

Coll, J. C. and Bowden, B. F (1986). The Application of Vacuum Liquid Chromatography to the Separation of Terpene Mixtures. *J. Nat. Prod.*, 49:934-936.

Ghisalberti, E. L. (2001). *Lantana camara* L. (Verbenaceae), *Fitoterapia*, 71:467-486.

Mostafa, A. A., Al-Rahmah, A. N. and Abdel-Megeed (2011), A. Evaluation of some plant extracts for their antifungal and antiaflatoxigenic activities. *J. Med. Plants Res., 5:4231-4238.*

Parajapati, N. D., Purohit, S. S., Sharma A. K. and Kumar T. (2003). *A Handbook of Medicinal Plants, A Complete Source Book.* Agrobios, India, pp. 306-307.

Patel, S., (2011). A weed with multiple utility: *Lantana camara. Rev. Environ. Sci. Biotechnol*., 10:341-351.

Ross. I. A. (2003). *Medicinal Plants of the World, Chemical Constituents, Traditional and Modern Medicinal Uses.* Vol. 1, 2nd Edition, Humana Press, Inc. Totowa, New Jersey, pp. 289-303.

Sastri,B.N. (1962). *The Wealth of India.* Council of Scientific and Industrial Research, New Delhi, Vol. *VI*, India, pp 31.

Sousa, E. O. and Costa. J. G. M (2012). Genus Lantana: chemical aspects and 22(5): 1155-1180, Sep./Oct. 2012 biological activities. *Braz. J. Pharmacogn*., 22:1155-1180.

Still, W. C., Kahn. M. and Mitra, A. (1978). Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.*, 43:2923-2925.