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A Comparative Study: In-Vitro Anti-Microbial Activity of Various Parts of Lantana Camara Using Ethanolic Extract

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Abstract:
Detecting new sources of antibiotics based on natural products used by traditional practitioners was the main objective to analyse the extracts of root, stem, leaf, flower and fruit of Lantana camara L. (Verbenaceae), a medicinal plant available in India. A group of organisms along with 2 bacteria and 2 fungi were served by L.camara extracts of different parts based on technique. The whole plant extract presented the most active. TheL.camara extract were active against the bacteria Staphylococcus aureus and Pseudomonas aeruginosa (10.25 ± 15.5µg/ml) and fungi Candida albicans and Aspergillus niger (5.25±13.5 and 0 ± 13.25µg/ml). This study may support the conventional use of whole plant extract of L.camara in various disorders, a potential subject to further isolation and identification as a supply of antibacterial substances.

Keywords: Anti-microbial activity, Agra-well diffusion method, Amphotericin B,LantanaCamara, Zone of Inhibition

Introduction:
Plants are effective enzymologist and have been constituents of phytomedicine since time immemorial; man is able to acquire from them a extraordinary mixture of industrial chemicals. Plant based essential constituents can be obtained from any segment of the plant like bark, leaves, flowers, roots, fruits, seeds, etc [1] i.e. any part of the plant may carryintenseelements. The desirable medicinal effects of plant substancesconsistently result from the sequence of secondary products present in the plant. Biologically active compounds are there in the medicinal plants have normally been of prominentengrossment to scientists working in this field. In recent years this interest to appraise plants retaining antibacterial activity for numerous diseases is growing [2]. For instance, in expanding countries 25% of the medicinal drugs are based on plants and their by-products[3]. Even though pharmacological industries have bring about a number of new antibiotics in the last three decades, resistance to these medicaments by pathogens hasmultiplied[4].
Antibiotics are commonly arising or synthetic organic compounds which hinder or pull downselective bacteria, overall at a lower concentrations. Microorganisms have improved resistance to numerous antibiotics and this has produced massive clinical complications in the treatment of infectious diseases [5]. The growth in resistance to microbes due to extensive use of antimicrobial drugs compelled scientists

to look for advanced antimicrobial substances from different sources includes medicinal plants [6]. The antimicrobial potency refer to some plants in treating diseases has been ahead of belief. It is evaluated that localized communities have used about 10% of all the flowering plants on Earth to treat variety of infections, although only 1% have received acceptance by modern scientists [7]. Due to their larger use as remedies for many infectious diseases, exploration for plants containing antimicrobial substances are more often[8].

Lantana camara (Verbenaceae), often known as wild or red sage is the most widespread breed of this genus and considered both as a notorious weed and a well-known ornamental garden plant [9]. However, it is recorded as one of the important medicinal plants of the world [10].

L.camara contains lantadenes, the pentacyclic triterpenes which is noted to contain a number of useful biological activities. Several previous reports have summarize it as antifungal [11],anti proliferative[12]. Moreover, the hydroalcoholic extracts of the leaves have shown anresult on fertility, general reproductive performance, and teratology in rats [13]. Thus, the aim of the ongoing affairs was to screen the antimicrobial activity of Lantana camara against selected clinical isolated strains. Although previous studies have been recorded on the antimicrobial activities of these plants, this work is planned to evaluate the certain antibacterial activity of different extracts of these plants are tested against microorganisms, in order to know the leading extract against a specific microorganism.

Materials and methods:

Plant material

The fresh parts (Root, Stem, Flower, leaves and Fruit) of the plant were collected from a nearby medicinal plant nursery. The collection was under specialist supervision and these plants are commonly known to everyone. These plants were authenticated by a Botanist. The various plant parts were thoroughly washed, sun-dried for 7-10 days and ground into powder using a laboratory mill prior to analysis.

Extraction

Test sample can be fresh (*LC*) or dried. It needs to be crushed, using a pestle and mortar, to provide a greater surface area. The test sample should be sufficient to fill the porous cellulose thimble (in our experiments we use an average of 14 g of thyme in a 25- x 80-mm thimble).All equipment should be too assembled. Build a rig using stands and clamps to support the extraction apparatus. Following this, the ethanol is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an iso-mantle. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor. The side arm is lagged with glass wool. The solvent is heated using the iso-mantle and will begin to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle begins again. The process should run for a total of 4 hours. Once the extraction set up, it can be left to run without direct supervision.

Microorganisms

The clinical isolated cultures of staphylococcus aureus, pseudomonas aeruginosa, candida albicans, aspergillus niger were acquired. The isolates were identified by conventional tests [14,15]. All the strains were maintained on nutrient agar at 4°C and were sub cultured.

Determination Of Antibacterial Activity

The medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium (HiMedia) in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten. Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium (HiMedia) in 100ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15minutes.

Procedure

Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains were adjusted to 0.5 OD value according to McFarland standard, (Staphylococcusaureus-902andPseudomonasaeruginosa- 424)Wells were cut and concentration of sample LC (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA) [16,17].

Determination Of Antifungal Activity

The potato dextrose agar medium was prepared by dissolving 20 gm of potato infusion, 2 gm of dextrose and 1.5 gm of agar in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten.

Procedure

Petri plates containing 20ml potato dextrose agar medium was seeded with 72 hr culture of fungal strain (Candida albicans and Aspergillus niger)wells were cut and different concentration of sample LC (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 28°C for 72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA)[18,19].

Zone of Inhibition

The volume of (50,100,250,500µg/ml) were added to the different petriplatesand were grown within 24hours 100% relative humidity at 37°C. After 24 hours, the plates were taken and observed for their growth. Gentamycin and Amphotericin-B were used for the positive control.

Results

Effect of antibacterial activity

The ethanolic extract of the whole plant of L.camara and its solvent fractions were subjected to antibacterial in agar well diffusion method. The tested samples showed marked antibacterial activity against pseudomonas aeruginosa, staphylococcus aureus. The zone of inhibition were shown in table 1.

Figure 1:effect of LC on S.aureus

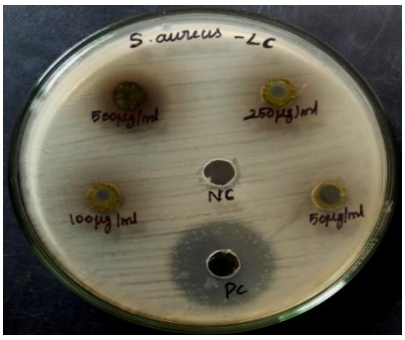

Figure 2: effect onS.aureus

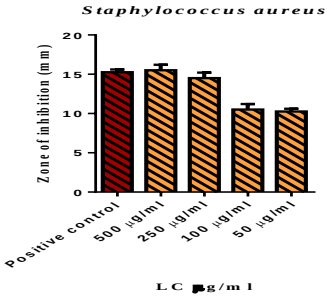

Figure 3: effect of LC on P.aeruginosa

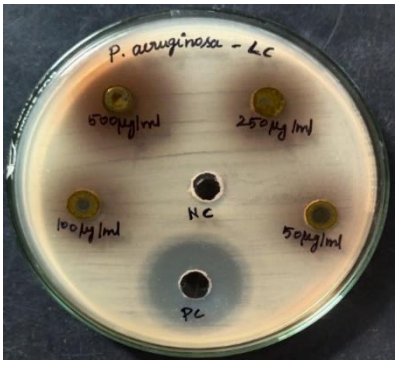

Figure 4: effect onP.aeruginosa

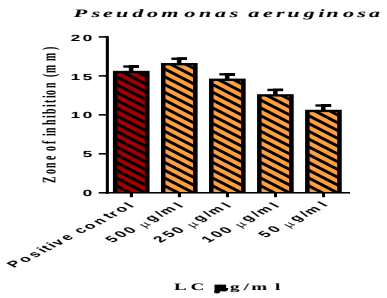

Table 1. SD± Means of zone of inhibition obtained by sample LC against Staphylococcusaureusand Pseudomonasaeruginosa.

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	Staphylococcs aureus	LC	15.5±0.7	14.5±0.7	10.5±0.7	10.25±0.35	15.25±0.35
2.	Pseudomonas aeruginosa		16.5±0.7	14.5±0.7	13.5±0.7	10.5±0.7	15.5±0.7

Effect of antifungal activity

The antifungal activity of the ethanolic extract and subsequent fractions of the plant are presented in table 2. The crude extract are demonstrated activity against Candida albicans and aspergillus niger.

Figure 5: effect of LC on C.albicans

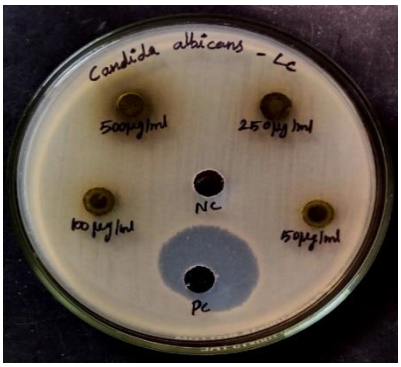

Figure 6:effecton C.albicans

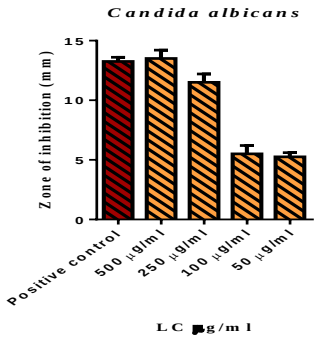

Figure 7: effect of LC on A.niger

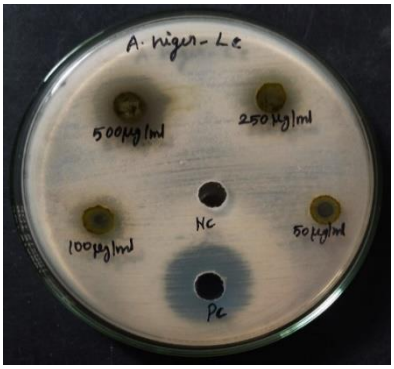

Figure 8: effect on A.niger

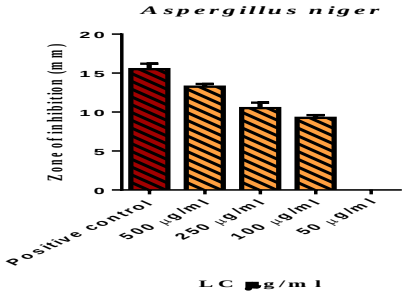

Table 2. SD± Means of zone of inhibition obtained by sample LC againstCandidaalbicansandAspergillusniger.

S.NO	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	Candida albicans	LC	13.5±0.7	11.5±0.7	5.5±0.7	5.25±0.35	13.25±0.35
2.	Aspergillus niger		13.25±0.35	10.5±0.7	9.25±0.35	0	15.5±0.7

Discussions

Crude plant extracts are generally a mixture of active and non-active compounds. A number of medicinal plants are still need to be testify according to the modern parameters to ensure their activity and efficacy.The ethanolic extractts of whole plant of Lantana camara (leaves, stem, root,fruit,flower) were subjected to a preliminary screening for antimicrobial activity against two human pathogenic bacteria staphylococcus aureus and pseudomonas aeruginosa, two pathogenic fungi candida albicans and aspergillus niger.

The antibacterial activity against Gram-positive & Gram-negative bacteria and the ZOI for staphylococcous aureus and pseudomonas aeruginosa were measured from (10.25 ± 15.5µg/ml). the

positive control at (15.5±15.25µg/ml) respectively. Its promising antibacterial activity was shown by the plant fractions tested against gram-positive and gram-negative bacteria. Therefore, it covers a wide range of infections and offers an alternatiive oral therapeutic option for the management of infections caused by these bacteria.

Similarly, the crude extract and fraction of the plant showed sensitivity against tested fungus. The antifungal activity against candida albicans and aspergillus niger, the ZOI were measured from (5.25±13.5 and 0±13.25µg/ml). the positive control at (13.25±15.5µg/ml), hence antifungal activity against candida albicans and aspergillus niger yielded good zone of innhibition, thus the extract was found to be fungistatic in its action.

The results of our study revealed good antimicrobial activity against various pathogens responsible for wide variety of infections.

Authors Biography

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