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Research Article

Bactericidal activities of different Medicinal plants extracts against Ocular pathogen viz *Corynebacterium macginleyi*

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ABSTRACT

In this contemporary investigation bio-control of ocular pathogen i.e. *Corynebacterium macginleyi* was achieved by methanol, chloroform and hexane extracts of forty different medicinal plants using agar well diffusion method. Among the forty selected plants, methanolic extracts of thirty six plants given remarkable bioactivity than chloroform and hexane extracts against *C. macginleyi*. The methanolic extracts from *Terminalia catappa, Terminalia chebula, Rosa indica, Albizia lebbeck, Butea monosperma* showed maximum activity. *Hibiscus rosa-sinensis, Justicia adhatoda, Physalis minima* and *Pongamia pinnata* did not exhibit antibacterial activity at the condition studied. Among the forty plants studied 90 % of the plants had antibacterial activity while the remaining 10% had no antibacterial activity.

Keywords: Corynebacterium macginleyi*, bactericidal activity, Indian Medicinal plants, Soxhlet extraction, Bactericidal activity, Well diffusion method.*

INTRODUCTION

Although non-diphtherial coryne bacteria are ubiquitous in nature and commonly colonise the skin and mucous membranes of humans, they rarely account for clinical infection. *C. macginleyi* has only recently been reported to be exclusively isolated form ocular surfaces. *C. macginleyi* was recently uniquely isolated from the ocular site and found to cause conjunctivitis and endophthalmitis [1, 2, 3, 4]. Corynebacteria other than *C. diphtheriae* seem to have low virulence against the cornea[5]. Therefore, corynebacteria are considered micro flora if they are isolated in cases of infectious keratitis. However, several studies have found that some strains of corynebacteria cause keratitis [6, 7]. *C. macginleyi* was first identified in 1995 by Riegel *et al*., during investigations of lipophilic corynebacteria[8]. However, it was not clear whether *C. macginleyi* could cause keratitis, and the factors contributing to the virulence of *C. macginleyi* are not well understood. But in the present study, *C. macginleyi* was isolated from sutures are considered causative agent because confluent growth occurred at the site of inoculation on culture plates and the results of the cultures were consistent with direct microscopy findings showing gram-positive pleomorphic rods The isolate, C. macginleyi was resistant to many topical antibiotics commonly used in ophthalmology except Chlor omphenicol, Vancomycin. Problems associated with topical fortified antibiotics such as local toxicity, stability, cost, need for refrigeration and the emergence of antibiotic-resistant organisms have prompted interest in the search for therapeutic alternatives.The increasing prevalence of multi drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infections fighting strategies [9].

The plants that possess therapeutic properties on the animal or plant body are generally designated as medicinal plants. Medicinal plants are rich sources of ecologically developed secondary metabolites, which are potential remedies for different ailments. Extreme interest in plants with microbicial activity has revived as result of current problems such as resistance associated with the use of antibiotics obtained from microorganisms. The main advantage of natural agents that they do not enhance the antibiotic resistance, a phenomenon commonly encountered with the long- term use of synthetic antibiotics. The use of phytochemicals as natural antimicrobial agents commonly called “biocides” is gaining popularity[10]. There is growing interest in correlating phytochemicals constituents of plant with its pharmacological activity[11]. There has been growing interest in the investigation of the natural products from plants for the discovery of new antimicrobial agents as an alternative route for the synthetic chemicals, side effects of which are always in question. It has been reported that the higher plants have shown to be a potential source for the new antimicrobial agents[12]. Accordingly the selected

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medicinal plants organic solvent extracts hexane, chloroform and methanol were evaluated against *C. macginleyi.*

MATERIALS AND METHODS

Plant material and processing

The plant materials of forty plant species (Table.1) were collected from different places at Visakhapatnam. The collected plants were identified and authenticated. The selected parts of different medicinal plants were chopped into small pieces and made into fine powder using blender. The extraction method employed here is a known amount of coarsely powdered plant materials of different plant species were successively extracted with organic solvents like hexane, chloroform and methanol basing on order of their polarity using soxhlet apparatus. The different extracts obtained were subsequently concentrated under reduced pressure to get their corresponding residues. The extracts were screened for antimicrobial activity using the method described under the section.

Table1: List of Investigated Medicinal Plants

Botanical Name	Parts Used
Acacia leucophloea	Bark
Acalypha indica	Aerial parts
Albizia lebbeck	bark
Ammania baccifera	Whole
Boerhaavia diffusa	Whole plant
Bombax ceiba	Bark
Butea monosperma	Bark
Calotropis procera	Flower
Cardiospermum halicacabum	Whole plant
Cassia occidentalis	Whole plant
Catharanthus roseus	Whole plant
Datura stramonium	Whole plant
Cissus Quadrangularis	Whole plant
Cleome viscosa	Whole plant
Clitoria ternatea	Whole
Emblica officinalis	Fruit
Ficus bengalensis	Bark
Ficus religiosa	Bark
Hibiscus rosa sinensis	Leaf
Hildegardia populifolia (Roxb.)	Stem bark
Hyptis suaveolens	Whole
Justicia adhatoda	Whole Plant
Lantana camara	Whole plant
Lawsonia inermis	Leaves
Morus alba	Bark
Solanum khasianum	Whole
Ocimum santum	Leaves, Seeds
Phyllanthus amarus	Whole plant
Physalis minima	Whole plant
Piper betle	Leaves
Plumbago zeylanica	Whole plant
Pongamia pinnata	Bark, Seeds
Rosa indica Linn.	Petal
Sida acuta	Whole plant
Tamarindus indicus	Bark
Terminalia catappa	Bark
Terminalia arjuna	Bark
Terminalia chebula	Bark, Fruit
Tridax procumbens	Whole plant
Vernonia cinerea	Whole plant

Isolation & Identification of Bacterial pathogen used in the present study

The ocular pathogen i.e. *C. macginleyi* was isolated from sutures of post operative keratoplasty patient of L.V. Prasad Eye Institute, Visakhapatnam. The gram positive rods were identified after the 48 hours incubation of sutures on blood agar (Hi-media), and chocolate agar (Hi-media) plates. In order to identify corynebacteria, biochemical testing was performed. The commercial API Coryne system and API Coryne database were used by which the organism was identified as *C. macginleyi* with 99.5% probability.

Antimicrobial Assays

The Hexane, Chloroform and Methanol extracts of forty different plants extracts were screened for antimicrobial activity by agar well diffusion method[13]. The overnight culture grown in nutrient broth was used for inoculation. An aliquot (0.02ml) of inoculums was introduced to molten and cooled at 45º C nutrient agar and placed on petri dish by pour plate technique. After solidification the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05 ml of each extracts of forty different plant extracts were introduced serially after successful completion of one plant analysis. Incubation period of 24 to 48h at 37º C was maintained for observation of antimicrobial activity was evaluated by qualifying zones of inhibition of bacterial growth surrounding the plant extracts. The complete antimicrobial analysis was carried out under strict aseptic conditions and results were tabulated in Table.2.

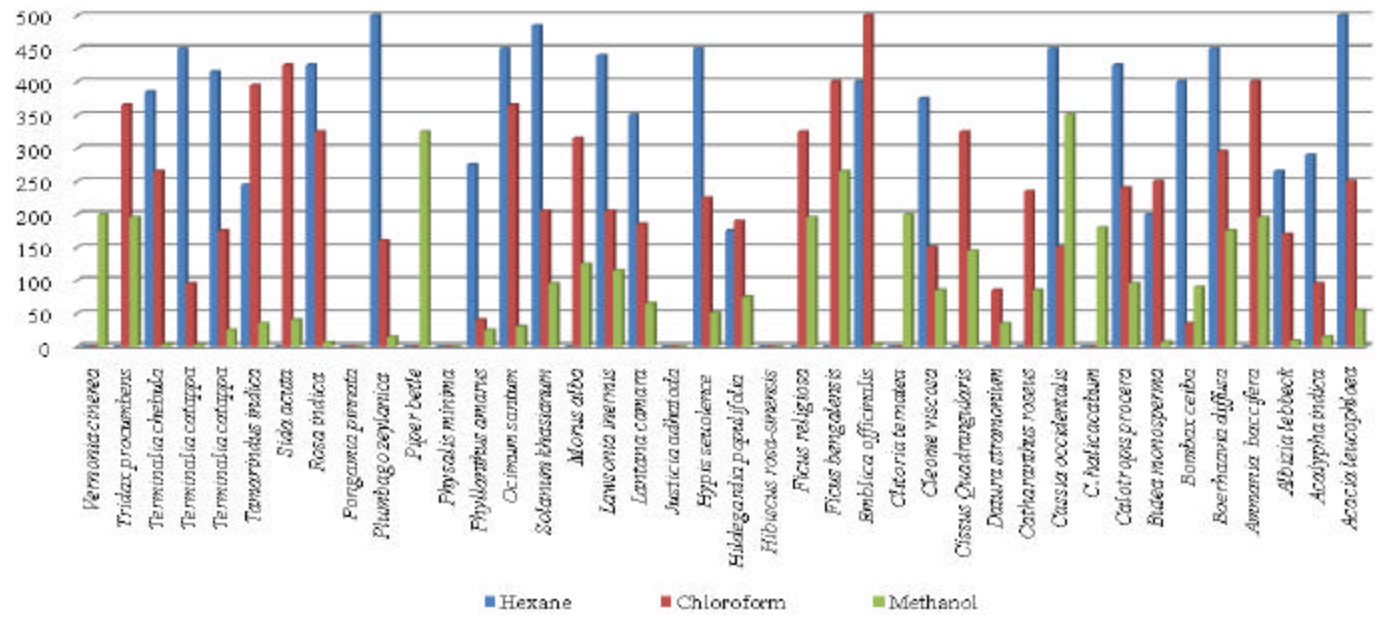
The common tropical antibiotic Chloromphenicol (30mcg) used in treatment to cure this ocular infections, was also used at different concentrations to get MIC (Minimum Inhibitory Concentrations). The zones of inhibition were measured with Antibiotic zone scale in mm duplicates.

RESULTS

The initial examination of bactericidal activity of methanol, chloroform and Hexane extracts forty different plants was reported in table.2. Among the selected forty plants the methanolic extracts of thirty six plants given remarkable bioactivity, chloroform extracts of thirty two plants shown antimicrobial activity and hexane extracts of twenty three plants exhibited antibacterial activity where as four plants such as *A. baccifera, J. adhatoda, P. minimana* and *P. pinnata* were not exhibited antimicrobial activity with any of the three extracts, against *Corynebacterium macginleyi.*

The antimicrobial activity was observed by forty plants with variable concentrations, not with the same concentrations. Based on this criterion plants can be divided as A, B and C and called them as highest activity plants, moderate activity and least activity plants respectively. The plants given activity up to 50 mg/ml, 55-100 mg/ml and 110-500mg/ml concentrations were treated as group A, B and C respectively. Most the methanolic extracts of tested plants were given bioactivity up to 50 mg/ml concentration. Among them the highest activity was exhibited *b T. catappa* and *T. chebula* at 2.5mg/ml and

Table 2: MIC analysis of Methanol, Chloroform and Hexane extracts of forty different plants on *C macginleyi*



MIC Values mg/ml;Borer size used: 6mm, Drug concentration: 0-500 mg/ml

3mg/ml concentrations respectively. Good moderate activity was exhibited by *A. leucophloea* at 55mg/ml. thirteen plants exhibited least bioactivity in which *L. inermis* shown least bioactivity at 115mg/ml. the chloroform extracts of plants *B. ceiba* and *O. santum* were given highest bioactivity at 35mg/ml and 40mg/ml respectively. And good moderate activity also exhibited by *A. indica, C. paleru* and *T. catappa*. Most of the tested plants have reported least bioactivity with chloroform extracts. All hexane extracts except *A. baccifera, J. adhatoda, P. minimana* and *P. pinnata* were shown very least bioactivity. The solvents, without plant extracts were used as negative controls and did not inhibited bacterial growth. Antibiotic-Chloromphenicol was shown the MIC at 5mcg concentration with the isolate.

DISCUSSION

The plants which ecologically synthesis and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, minerals and vitamins, they posses medicinal properties. Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the self of foods, showing inhibition against bacteria, fungi and yeasts [14]. In the present study it was reported that methanol was a better solvent for the consistent extraction of antimicrobial substances from medicinal plants compared to other solvents such as chloroform and hexane. From this present investigation we found some plants which are having very useful antimicrobial properties against *C macginleyi*. But the medicinal plants in current study presented the lowest antimicrobial activity compared to the antibiotic standard. The observed findings suggested the further work on all the selected plants to evaluate their potential for use as antibacterial to treat ocular bacterial diseases in humans.

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