4. ANTIMICROBIAL ACTIVITY OF PHYSALIS MINIMA L.

4.1. Introduction

Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Diallo et al., 1999). The phytochemical research based on ethnopharmacological information has been generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Kloucek et al., 2005). The plant extracts had been used by humans for their antifungal, antimicrobial, insecticidal, cytostatic and therapeutic activities (Franzios et al., 1997; Pruthi, 1980). The development of drug resistance in human pathogens against commonly used antibiotics had necessitated a search for new antimicrobial substances from other sources including plants. Screening of medicinal plants for antimicrobial activities and phytochemicals was important for finding potential new compounds for therapeutic use (Erdogrul, 2002). It is known that most of the antimicrobial properties are attributed to the essential oils they contain as products of their secondary metabolism (Adam et al., 1998).

Higher plants produced hundreds to thousands of diverse chemical compounds with different biological activities (Hamburger and Hostettmann, 1991).

Most of the plants used for medicinal purpose have been identified, and their uses were well documented and described by different authors (Nadkarni, 1876; Dastur, 1985;
Saradamma, 1990; Daferera et al., 2003), but the efficacy of many of these plants is yet to be verified. Attention has turned to natural antimicrobial agents in recent years. There had been many investigations on the antifungal, antibacterial, particularly enteric pathogenic organisms (Elgayyar et al., 2001; Alanis et al., 2005), by preparations and individual compounds isolated from natural sources.

In the present study, an investigation was carried out to test the antimicrobial activity of different parts; leaves, root and fruit of *Physalis minima* L.

### 4.2. Materials and Methods

#### 4.2.1. Collection of Plant Material:

Fresh leaves, root and fruit of *Physalis minima* L. were collected in and around Tiruchirapalli, Tamil nadu India, shade-dried and powdered.

#### 4.2.2. Solvent Extraction:

25 g of shade-dried powder was filled in the thimble and extracted successively with ethanol solvent in Soxhlet extractor for 48h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

#### 4.2.3. Growth and Maintenance of Test Microorganism for Antimicrobial Studies:

Bacterial cultures of *Lactobacillus* sp., *Escherichia coli* (*E. coli*) and fungal cultures of *Aspergillus niger*, *Candida albicans* were obtained from Department of Clinical Microbiology, K.A.P.Visvanatham Government Medical College, Tiruchirappalli. The bacteria were maintained on
nutrient broth (NB) at 37°C and fungus was maintained on Potato dextrose agar (PDA) at 28°C.

4.2.4. Preparation of Inoculum: The bacteria (Lactobacillus sp., Escherichia coli) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (at 610nm). The fungal inoculum (Aspergillus niger, Candida albicans) was prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (at 595nm) to obtain a final concentration of approximately $10^5$ spores/ml.

4.2.5. Anti-bacterial Activity: The ethanol extract of leaf, root and seed of Physalis minima L. were tested by the disc diffusion method [Anonymous, 1996]. The test microorganisms were seeded into respective medium by spread plate method 10 µl ($10^6$ cells/ml) with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Lactobacillus sp. and Escherichia coli were used for antibacterial test. Streptomycin sulphate (10 µg ml$^{-1}$) used as positive control and ethanol solvent (100 µg ml$^{-1}$) used as negative control the antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.
4.2.6. **Antifungal Activity**: The antifungal activity was tested by disc diffusion method [Taylor et al., 1995]. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µg ml\(^{-1}\) concentrations of the extracts were placed on test organism-seeded plates. Ethanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent ethanol followed by drying off was used as negative control and Nystatin (10 µg disc\(^{-1}\)) used as positive control. The activity was determined after 72 hr of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

4.3. **RESULTS**

Results obtained in the present study revealed that the tested plants extracts possessed potential antibacterial activity against *Lactobacillus sp.* and *Escherichia coli* (Table 4.1) and antifungal activity against *Aspergillus niger, Candida albicans* (Table 4.2). These results were similar to the previous studies (Samy and Ignacimuthu, 2000; Parekh and Chand., 2006). The zone of inhibition above 7 mm in diameter was taken as positive result.

Saini et al. (2006) tested the ethanol and water extracts (5 mg/ml) from leaves of *Melia dubia L.* for their activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*. The extracts exhibited moderate to good antibacterial and antifungal activity.
Comparatively, the ethanol extract was more effective against all test microbes than the water extract. In the present study, ethanol extracts of leaves, root and fruit of *Physalis minima* L. were tested for the antimicrobial activity.

When tested by the disc diffusion method, the ethanol extract of leaves of *Physalis minima* L. showed significant activity against the four micro-organisms. Similar results were observed by Mahesh and Satish(2008). Leaf and root samples of *Withania somnifera* L. were used to examine their antimicrobial potential against some human pathogenic bacteria (Escherichia coli, Bacillus and Shigella) fungi (Aspergillus niger and Trichophyton rubrum) growth inhibition was observed in different volumetric concentrations of this extract. Leaf sample showed higher antimicrobial activity than the root sample. (Senthil Kumar and VinothKumar, 2011).

The results confirm the antimicrobial property of *Physalis minima* L. leaf, root and fruit and support the traditional use of the plant in therapeutic use against microbial infections. Phytochemical analysis showed the presence of Glycerin, 2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1, 8-diol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Undecanal, 2-methyl-, Phytol, 2-Propenamide, N-[2-(dimethylamino)ethyl]-, Stigmasta-5,24(28)-dien-3-ol,(3á,24Z)-, Campesterol, Stigmasterol, Cholest-5-en-3-ol, 24-propyldene-, (3á)- as a major constituent of the plant leaf and roots which are reported to have antimicrobial activity(Dr.Duke’s Phytochemical and Ethnobotanical Databases).
The highest antifungal activity of 23 mm in *Aspergillus niger* was recorded by ethanol extract of *Physalis minima* L. root and least activity recorded in *Lactobacillus* sp. measured 8mm. by ethanol extract of *Physalis minima* L. root.

The anti-bacterial activity of ethanol extract of *Physalis minima* L. root was negligible (8 mm against *Lactobacillus* sp. and 9 mm against *Escherichia coli*). Afolayan et al. 2002 observed that water, methanol and acetone extracts did not have activity on *Strepto myces marcescens*, a gram-negative bacterium.

The anti-fungal activity was more when compared with anti-bacterial activity.

Apart from antimicrobial activities, these plant extracts are also exploited for therapeutic purpose to cure several disorders of man. The results of present investigation clearly indicate that the antibacterial and antifungal activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants which could be of considerable interest to the development of new drugs (*Vanila et al.*, 2005).