Preliminary phytochemical and anti-bacterial studies on *Physalis minima* Linn.

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Abstract

*Physalis minima* Linn. is widely used in the indigenous system of medicine for the treatment of diuretic, fevers, dropsy etc. In the present study, an attempt was made to evaluate the phytochemical substances and antibacterial activity in the plant parts of *P. minima* (stem, leaf and unripe fruit). Phytochemical constituents like alkaloids, anthraquinones, flavonoids, cardiac glycosides, phenols, quinones, reducing sugars, saponins, steroids, starch, tannin and terpenoids of the stem, leaf and unripe fruit of *P. minima* were examined using the extracts of chloroform, diethyl ether, ethanol, ethyl acetate and methanol. Alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids were present in all the plant parts irrespective of the solvents used. Rich amount of phytochemicals were observed in leaf extracts compared to stem and unripe fruit. The antibacterial activity of *P. minima* was studied using agar well diffusion method. The activity was tested against *Bacillus cereus*, *B. subtilis*, *Citrobacter sp.*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *P. fluorescens* and *Staphylococcus aureus* in its plant parts. Leaf and stem extracts of all solvents invariably showed moderate anti-bacterial activity. *Enterobacter aerogenes* and *Staphylococcus aureus* were found to be more susceptible for the extracts of *P. minima*. *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *P. fluorescens* strains showed resistance against the extracts of *P. minima*. Overall anti-bacterial assay revealed that ethanolic extract was found to be more effective than the other solvents used. It can be concluded that the plant has some antibacterial activity but there is need to do more tests to find new compounds with potential to act against multi resistant pathogenic bacteria.

**Keywords:** phytochemicals, antibacterial activity, *P. minima*

Introduction

Medicinal plants are plants that have at least one of their parts (leaves, stem, barks or roots) used for therapeutic purposes (Bruneton, 1993). The availability and relatively cheaper cost of medicinal plants make them more attractive as therapeutic agents when compared to modern medicine (Agbor et al., 2005). World plant biodiversity is the largest source of herbal medicine and still about 60-80% world population rely on plant based medicines which are being used since the ancient ages as traditional health care system. India is endowed with a rich wealth of medicinal plants, which ranked our country in the list of top producers of herbal medicine. In response to the increased popularity and greater demand for medicinal plants a number of conservation groups are recommending that wild medicinal plants be brought into cultivation (Aqil et al., 2006). It is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. Many medicinal plants were found to possess antibacterial, antifungal and insecticidal properties against wide spectra of organisms. Many active phytochemicals like flavonoids, terpenoids, vitamins, alkaloids etc. were found to be responsible for these activities. With advance in phytochemical techniques several active principle of many medicinal plants have been isolated and introduced as valuable drugs in modern system of medicine (Nirmala et al., 2010). The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with potential to act against multi-resistant pathogenic bacteria and fungi (Shariff, 2006). An anticancer plant selected for the present study is *Physalis*
**minima** Linn. *P. minima*, the wild gooseberry is a pantropical annual herb. The fruit is edible, yellowish and encapsulated in papery cover. The fruit is a good source of vitamin C and is considered to be a diuretic, purgative and used to relieve pain (analgesic action) and cure spleen disorder (Parmar and Kaushal, 1982). The decoction of the whole plant is taken orally to treat cancer and the leaves are used as a poultice for ulcer (Zakaria and Mohamad, 1994). The leaves are crushed and applied over snake bite site (Karthikeyani and Janardhanan, 2003). Based on this background the present study was intened to screen the plant *Physalis minima* for the presence or absence of phytochemical constituents and anti-bacterial activities.

**Materials and methods**

**Collection of plant materials**

*Physalis minima* Linn. plants were collected from disturbed areas in and around Chennai (Fig.1). Whole plants of *P.minima* were uprooted and collected in polythene bags. The collected plant was identified and confirmed by using the Flora of Presidency of Madras (Gamble, 1939). The plants were washed in tap water and air dried. Stem, leaves and unripe fruits were collected in separate paper covers and dried in shade for 20 days.

**Preliminary phytochemical screening of the plant extracts (qualitative analyse)**

The dried plant materials were (stem, leaves and unripe fruits of *P.minima*) made to fine powder using homogenizer. The dried powders (each 250 gms) were extracted separately with continuous shaking for 24 hrs using solvents (chloroform, diethyl ether, ethanol, ethyl acetate, methanol). The extracts were filtered through whatman no.1 filter paper to remove all unextractable matters. The entire extract was concentrated to dryness at room temperature. The dry powder extract obtained was stored at 4°C in air tight containers. The dried condensed extracts of stem, leaves and unripe fruits were dissolved in 20 ml of above mentioned solvents separately and used for preliminary screening of phytochemicals such as alkaloids (Mayer's and Dragendorff’s tests), anthroquinones (Borntrager’s test), flavonoids (NaOH or Alkaline reagent test), cardiac glycosides (Keller-Killani test), phenols (Phenol test), quinines (NaOH test), reducing sugars (Fehling’s test), saponins (Foam test), steroids (Libermann-Burchard test), soluble starch (Iodine test), tannins (gelatin test) and terpenoids (Salkowski test) and were carried out following the methodologies of Harbone (1998), Sofowora (1993), Trease and Evans (2002).

**Test microorganisms taken to study the bioefficacy of P.minima extracts**

**Bacterial strains:** Bacillus cereus, B. subtilis, Citrobacter sp. Enterobacter aerogenes, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, P. fluorescens and Staphylococcus aureus.

**Media Preparation**

Thirty six gram of Muller Hinton medium (HI – media) was mixed with one liter distilled water and then sterilized in an autoclave at 121°C for 15 minutes. The sterilized media were poured into petridishes.

**Broth culture of bacterial strains**

Standardized bacterial suspensions (seeded broth) were prepared separately by picking a colony of respective bacteria using wire loop and suspending it in 10 ml nutrient broth and incubated at 37°C for 16-18 hrs.

**Antibacterial assay**

Antibacterial activities of the plant extracts were tested using agar well diffusion method (Srinivasan et al., 2001). The prepared culture plates were inoculated with different selected strains of bacteria on the surface of sterile Muller Hinten Agar plates by surface spreading using a sterile cotton swab. Nutrient broth cultures of selected bacterial strains were inoculated separately and evenly spread over the entire surface of agar plate to obtain a
uniform inoculum. Agar wells were made on agar surface with 5mm cork borer. The extracts of stem, leaves, and unripe fruits dissolved in respective solvents were poured into the well using sterile syringe. The plates were incubated in an upright position at 37± 2˚C for 4 hrs for bacterial culture. The plates were observed for the zone clearance around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in five different directions in all 5 replicates and the average values were tabulated. The results are statistical presented as mean ± Standard deviation.

### Results and discussion

#### Phytochemical studies

The results of the preliminary phytochemical analyses in the chloroform, diethyl ether, ethanol, ethyl acetate and methanol extracts of stem, leaves and unripe fruits are presented in table 1. Alkaloids, flavonoids, cardiac glycosides, phenols, saponins, steroids, tannins and terpenoids were present in all the plant parts irrespective of the solvents used. Reducing sugars were unable to be separated in all the solvent extracts of *P.minima*. Amount of phenols eluted by the organic solvents was very low in all the plant parts.

#### Table 1. Phytochemical analyses of *P.minima* extracts

<table>
<thead>
<tr>
<th>Organic Solvents</th>
<th>Plant parts of <em>P.minima</em></th>
<th>Alkaloids</th>
<th>Anthraquinones</th>
<th>Cardiac glycoside</th>
<th>Flavonoids</th>
<th>Phenols</th>
<th>Quinones</th>
<th>Reducing Sugars</th>
<th>Saponins</th>
<th>Steroids</th>
<th>Starch</th>
<th>Tannin</th>
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Quinones and starch were present in chloroform, diethyl ether, ethyl acetate extracts. Rich amount of phytochemicals were observed in leaf extracts compared to stem and unripe fruit. Anthraquinones were absent in all the extracts tested. The results of the present study revealed that chloroform, diethyl ether, ethanol, ethyl acetate and methonal extracts were varied in effectiveness in the separation of phytochemicals from stem, leaves and unripe fruits of *P.minima*. It indicates that the strength of active principle depends on the use of suitable solvent besides the type of the plant species to achieve positive results. Occurrence of various phytochemicals in *Physalis minima* indicates the importance of these chemical compounds in the indigenous systems of medicine. According to Ayodele (2003) diverse use of plants in treatment of wide variety of diseases are attributable to the presence of the
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Phytochemicals. Phytochemicals are secondary metabolites produced and used by the plants for protection and repair processes within the natural environment. Phenols, flavonoids and tannins are good antioxidant substances which have been reported to have anti-diarrhoeal activity (Agbor et al., 2004) and prevent or control oxidative stress related disorders (Vinson et al., 1995). Tannins are antiseptic in nature, they have astringent properties and can hasten healing of wounds in a flamed membrane. Tannins can be used to give body resistance against parasites (Tiger, 1980). Flavonoids are potent water soluble anti-oxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Okwv, 2006). Phenolics are employed in adoptive or defense mechanism (Boilley et al., 1998; Giertych et al., 1999). The presence of alkaloids in all the solvent fractions could be well correlated with the antimicrobial activities (Ramkumar et al., 2007). These phytochemicals possesses specific physical, chemical and biological activities that make them useful as drugs.

Some of these biological properties are antimicrobial (Margineau, 1976), antiinflammatory (Singh, 1994), anti-feedent and hemolytic effects (Abeh, 1981). Non observance of alkaloids, flavonoids and tannins in some parts (stem) of P.minima may be due to the differences in the frequency of occurrence could either imply complete absence in the plant part analysed or a differential solubility of the compound in the solvent used. The curative properties of medicinal plants are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids etc. Plants have been components of Phytotherapy because natural constituents of industrial chemicals can be derived from any part of plants like root, stem, bark, leaves, flowers, seeds etc. (e.g) any part of the plant may contain active components (Ara and Nur, 2009). The plant P.minima exhibits strong anticancer, antiviral and several other activities. These properties may be due to the presence of alkaloids, flavonoids, cardiac glycosides steroids, tannins and terpenoids in the crude extracts of P.minima as in several medicinal plants (Vimal kumar, 2009). Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

**Antibacterial activity**

Besides preliminary screening of phytochemicals in P.minima, antibacterial assay of chloroform, diethyl ether, ethanol, ethyl acetate and methanol extracts of stem, leaf and unripe fruits of P.minima against 9 bacterial strains viz. Bacillus cerues, B.subtilis, Citrobacter sp., Enterobacter aerogenes, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, P.fluorescens, Staphylococcus aureus are depicted in table 2.

**Fig.2.** Antibacterial efficacy of leaf extracts of P.minima against Bacillus cerues [A and F], citrobacter sp. [B], Enterobacter aerogenes[C], E. coli [D] and Staphylococcus aureus [E]
antibacterial activity has been screened because of their good medicinal relevance. The extracts of higher plants can be a very good source of antibiotics (Fridous et al., 1990) against various fungal and bacterial pathogens. It has been suggested that the antimicrobial activity is mainly due to the presence of essential oils, flavonoids, triterpenoids and other natural polyphenolic compounds or free hydroxyl groups. Results of the present study are found directly correlated with the observations of previous workers (Wiart et al., 2004; Shariff et al., 2006; Johnson et al., 2008). Pasqua et al. (2005) reported that the oil of *M. officinales* showed low antimicrobial activity against *Lactobacillus, Enterococcus, Pseudomonas* and *Staphylococcus aureus* strains. Chloroformic and absolute alcoholic leaf and callus extracts of *Rauvolfia tetraphylla* and *P.minima* inhibited bacterial and fungal growth (Shariff, et al., 2006).

**Conclusions**

The stem, leaf and unripe fruit extracts gave positive results for the presence of phytochemical substances and antibacterial activity tested. Stem extracts showed negative results for some of the phytochemical substances. The bioefficacy analysis confirms the phytochemical observations. It is concluded that the traditional plants may represent new source of anti microbes with stable, biologically active components that can establish a scientific

<table>
<thead>
<tr>
<th>Phyalsis minima Linn</th>
<th>Bacillus cereus</th>
<th>Bacillus subtilis</th>
<th>Citrobacter spp.</th>
<th>Enterobacter aerogenes</th>
<th>Escherichia coli</th>
<th>Klebsiella pneumoniae</th>
<th>Pseudomonas aeruginosa</th>
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All solvents invariably showed moderate antibacterial activity (Fig. 2). All the solvent extracts of stem, leaf and unripe fruit were positive for antibacterial activity. Bacterial strains *Bacillus cereus, citrobacter sp, Enterobacter aerogenes, E. coli* and *Staphylococcus aureus* showed the zone of inhibition around the wells of chloroform, ethanol, ethyl acetate and methanol extracts of stem, leaf and unripe fruit. *Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *P. fluorescens* strains had no effect with all the extracts of *P.minima*. Unripe fruit extracts were found to be less effective in the inhibition of bacterial growth with all the 9-bacterial strains tested. The antibacterial activity against *Bacillus cereus, Enterobacter aerogenes* and *Staphylococcus aureus* was greater with maximum inhibition zone (10.0 mm ± 0.5) was observed in the ethanol and/or methanol extracts of leaf, stem and unripe fruit. *Staphylococcus aureus* was found to be more susceptible with all the solvent extracts of leaf, stem and unripe fruits with a maximum inhibitory zone of 9.0mm ± 1.0. Least bacterial growth inhibition of 1.0mm ± 0.0 was noticed in *E.coli* in the ethyl acetate leaf extract. Overall antibacterial assay revealed that ethanol extract was found to be more effective than other solvents used. The antibacterial activity has been screened because of their...
base for the use of plants in modern medicine. The Knowledge about the botanical preparation of traditional source of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

References


