Phytochemicals analysis and antimicrobial activity of *Ruellia patula* L. against pathogenic microorganisms

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Abstract
The present work aimed to estimate the phytochemical profile and antimicrobial activity of medicinal plant *Ruellia patula* (L.) against human pathogenic bacteria. Medicinal plants are the effective source for the development of drug against several diseases. Nowadays, medicinal plants were used to treat most diseases among humans because of its medicinal value. In Ayurveda and Siddha, many medicinal plants have been recommended for the management of common diseases. The extraction was done by using different solvent such as ethanol, methanol and acetone by using standard procedures. The antibacterial assay was carried by using agar well method with different organisms and also antifungal activity against *Aspergillus niger* using disc diffusion method. The ethanolic extract of *R. patula* L. (0.4 mg/mL) showed higher antibacterial activity against Gram positive bacteria and Gram negative bacteria. In the antifungal activity also ethanolic extract shows highest activity compare to other extract. From the present work, we conclude that the ethanolic extract of plant *R. patula* L. have potential of antibacterial activity and antifungal activity because of its secondary metabolites in the plant which responsible for biological activities. Due to the presence of phyto-constituent in the plant extract may control the bacterial growth either in high concentration/long durations and it may have the ability to control the human pathogenic organisms.

Keywords: Phytochemical analysis, *Ruellia patula* L., Disc diffusion, Antimicrobial activity

1. Introduction
Plants have been an important source of medicine for thousands of years. The World Health Organization estimates that around 80% of the world population in developing countries relies on traditional plant medicines for primary healthcare needs, of which a major proportion corresponds to plant extracts or their active principle (Salatino et al., 2007). They are also the source of many modern medicines. It is estimated that approximately one fourth (1/4⁰) of the prescribed drugs contain plants extracts or active ingredients obtained from the plant substances.
Herbs are more important in human’s life and fulfill his everyday’s needs. Herbal products are the effective source of both traditional and modern medicines which are used extensively to treat several medical problems. In ancient times, plants have provided a source of idea for the production of new drug and medicines derived from different plants have made to improve the human health and well being (Igbinosa et al., 2009). India is one of the biodiversity nations, which embrace the indigenous knowledge of traditional healers. In India, throughout its long history, has accumulated a rich body of empirical knowledge of utilizing the medicinal plants for the treatment of various diseases of veterinary and also to improve the health problem of people (Mohana et al., 2008). They are food flavor, cosmetics, ornamentals, fungiants, insect deterrents and medicines.

The higher plants are used to treat a number of infectious diseases in almost all parts of the world. In many parts of India, herbal medicine is used for treating various diseases. India possesses a vast number of medicinal plants (Srinivasan et al., 2001). Even though medicinal plants are used and distributed and throughout the world, they are more abundantly present in tropical countries like India. Greater demand for these plants especially for the purpose of food and medicine is one of the causes for their rapid deletion. Medicinal plants contain large varieties of chemical substances which possess important therapeutic properties that can be utilized in the treatment of human diseases. Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro-organisms against these drugs. Thus, there is need to search for new and more potent antimicrobial compounds of natural origin to combat the activities of these pathogens which is the basis for this study (Akinpelu et al., 2008). Commercial drug has some side effects and the resistance that pathogenic micro-organisms build against antibiotics, recently much attention has been paid to extract and biologically active compounds isolated from the plant species used in herbal medicine. Antimicrobials of plant origin have enormous therapeutic potential Antonisamy et al., 2015; Balamurugan 2015; Barathi and Agastian 2015; Nandhini and Bai 2015; Rathi et al., 2015; Narendran et al., 2016; Puthur 2016; Noorudheen and Chandrasekharan 2016; Santhosh et al., 2016; Greeshma 2016; Sreeshma et al., 2016; Nair et al., 2016). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Several screening has been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (Dulger et al., 2002) Opportunistic fungal infections represent a significant cause of morbidity and mortality in immuno compromised patients, including those with AIDS, cancer and organ transplants (Fisher-Hoch and Hutwagner 1995). Despite the increase in fungal infections, therapeutic options are very limited and are often unsatisfactory because of elevated toxicity and an inability to eradicate infections (Sheehan et al., 1999). In the last few years the incidence of fungal infections in the immuno compromised host has increased greatly (Masoko and Eloff 2005). In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. Medicinal plants contain a number of substances such as phytochemicals, which serve as drug for several diseases caused by microorganisms, herbivores and insects. Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections and it also for the antifungal diseases.

The medicinal plant, Ruellia patula (L.) (Acanthaceae) is commonly known as ‘kiranthinayagam’ or ‘kayappachilai’. It is a medicinal plant traditionally used in the treatment of wounds. In folklore remedies, the leaves are used to treat cuts, wounds, cure eye sore, gonorrhea, syphilis and renal infections and poisonous bites (Akhtar et al., 1992). The plant is commonly distributed on Africa, Arabia and South West India wastelands. The leaves are used
for treating itches, insect bites, sores, eye diseases, tumors, skin diseases, rheumatic complaints, renal affections, dental problems and insect bites. Pharmacological studies indicated its cardio tonic; wound healing, antiulcer and antioxidant activities. Aim of the present work, is to analysis the antimicrobial and antifungal activity of the medicinal plant R. patula L. against different organisms.

2. Materials and methods
2.1. Collection of plant material
The plant leaves of Ruellia patula (L.) were collected from different places of Thanjavur district. The leaves were washed thoroughly 2-3 times with running water and then air dried under shade.

2.2. Collection of plant material
The collected leaves were cleaned and shade dried. The dried plants were pulverized by an electrical blender and passed through the 20 mesh sieve. A powdered of dried leaves (20 g) was extracted successfully with ethanol, acetone and methanol by using Soxhlet apparatus. The extraction was carried out for 24 h at room temperature with mild shaking. The extract were filtered and concentrated at room temperature.

2.3. Extraction of the components
The medicinal plant R. patula (L.) were collected and dried under shade for few days. The dried plant materials were ground to fine powder by using mixer. The powdered material (50 g) was transferred into a Soxhlet apparatus containing 200 mL solvents such as ethanol, acetone and methanol for 48 h. All the extracts were concentrated using rotary vacuum evaporator. After the completion of solvent evaporation, each of these solvent extract were weighed and preserved at 5 °C in airtight bottles until further use.

2.4. Preliminary phytochemical screening of plant extract
The presence of alkaloids, steroids, glycosides, phenols, flavonoids, tannins and terpenoid, in the plant Ruellia patula (L.) were analysed by the standard methods of Harborne (1973).

2.5. Antibacterial study
2.5.1. Microorganisms
For this study, both Gram Positive [Bacillus Subtilis (MTCC 441)] and Gram negative [Escherichia coli (MTCC 433)] bacteria were used to determine the antibacterial activity of different extracts of plant R. patula (L.).

2.5.2. Preparation of inoculum
Bacterial broth was prepared by dissolving 1.3 g of nutrient broth (NB) in 100 mL of distilled water. Then, took a loopful of bacterial culture from the slant and inoculate bacteria in the broth medium. Then, incubate the culture broth for 18 - 24 h at 37 °C.

2.5.3. Agar-well diffusion method
Antibacterial activity of plant R. patula (L.) extracts were carried out by a modified well in agar method (Ahmad et al., 1998). Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 24 h old broth culture of respective bacteria. consequently, using sterile borer, well of 0.5 cm diameter was made into the each agar plate and then 20, 30 and 40 µL containing 500 µg/mL concentration of each extract (ethanol, methanol, and acetone) in aseptic condition filled into the
well. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 h at 37 °C. The results were recorded by measuring the diameter of inhibitory zone using a transparent meter rule at the end of the 24 – 48 h. Amoxillin and gentamycin were the standard drugs for antibacterial activities, respectively.

### 2.6. Antifungal study

#### 2.6.1. Microorganisms

For this study, fungal strain [Aspergillus niger (MTCC 1344)] fungi was used to determine the antifungal activity of different extracts of plant R. patula (L.).

#### 2.6.2. Microorganisms

Potato dextrose broth was prepared by dissolving 3.9 g of PDB in 100 mL of distilled water. Then, took a loopful of fungal culture from the slant and inoculate fungi in the broth medium. Then, incubate the culture broth for 48 h at 37°C. After adding all the ingredients into the distilled water it was boiled to dissolve completely and was sterilized by autoclaving at 121°C for 20 min. Then it was cooled and poured into petriplates and allowed to solidify. And the fungal suspension was spreaded in the potato dextrose agar plate.

#### 2.6.3. Disc diffusion method

Disc diffusion method was used to determine the zone of inhibition against chosen fungi by the R. patula (L.) plant extract. Sterile Whatmann no. 01 paper disc (6.0 mm in diameter) was used. Discs were stored at 5°C prior to use. Plant extract of concentration ranging between 0.1 mg – 0.8 mg/mL was applied in each plate. The diluted fungal cultures were spread over potato dextrose agar plates using sterile glass L rod. Various concentration of the extract was applied in filter paper (0.1mg – 0.8 mg/mL) and allowed to dry before being placed on the top layer of the agar plate. The plates were incubated at 37°C for 48 h and growth of inhibition zone was measured.

### 3. Results

The present the study was carried out on antimicrobial, antifungal and phytochemical screening of ethanolic extracts, methanolic extract and acetone extract of R. patula (L.).

#### 3.1. Phytochemical analysis

The phytochemical analyses of leaves extract was carried out and were shown in (Table 1). The ethanolic dried leaves extract of R. patula L. contains alkaloids, steroids, glucosides, phenols, flavonoids, tannins, terpenoids compare to other extracts.

#### 3.2. Antibacterial activity

The bacteria culture of B. subtilis, E. coli in petriplates were incubated along with test were checked for growth inhibitions zone of organisms after 24 h, the anti bacterial activity of ethanolic extract, methanolic extract acetone extract of the plants R. patula was studied and listed (Table 2). Antibacterial activity of the dried leaves extract and their efficiency were quantitatively assessed using agar well diffusion methods. The present studies indicate that the ethanolic extract of R. patula L. at different concentrations (0.1 mg/mL, 0.2 mg/mL and 0.4 mg/mL) significantly suppressed the growth of the human pathogenic bacteria. The ethanolic extract of R. patula L. was most active against the micro organism’s B. subtilis, E.coli. The maximum inhibition zone was found in B. subtilis in 0.4mg/mL concentration and it was (19 mm). The minimum inhibition zone was acetonic extract found in B. subtilis in 0.1mg/mL concentration and it was (7 mm). The maximum inhibition zone was found in E.coli in 0.1 mg/mL concentration and it was (29 mm).
The minimum inhibition zone was acetonic extract found in *B. subtilis* in 0.1mg/mL concentration and it was (10 mm) when compared to the ethanolic, methanolic extract and acetonic extract, ethanolic extract showed the highest zone of inhibition among the organisms.

**Table 1.** Preliminary phytochemical analysis of *R. patula* L.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Compound</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Amino acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) present; (-) absent

**Table 2.** Antibacterial activity of different extracts of *R. patula* (L.).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Microorganism</th>
<th>Diameter of the zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td><em>Bacillus subtilis</em></td>
<td>18 19 19</td>
</tr>
<tr>
<td></td>
<td><em>E.coli</em></td>
<td>18 18 18</td>
</tr>
<tr>
<td>Methanol</td>
<td><em>Bacillus subtilis</em></td>
<td>17 17 17</td>
</tr>
<tr>
<td></td>
<td><em>E.coli</em></td>
<td>17 17 16</td>
</tr>
<tr>
<td>Acetone</td>
<td><em>Bacillus subtilis</em></td>
<td>8 7 8</td>
</tr>
<tr>
<td></td>
<td><em>E.coli</em></td>
<td>7 8 9</td>
</tr>
</tbody>
</table>

**3.4. Antibacterial activity**

The fungal culture of *Aspergillus niger* in petriplates was along with test were checked for growth inhibitions zone of organisms after 48 h, the fungal activity ethanolic extract , methanolic extract and acetone extract of the plants *R. patula* (L.) was studied and listed (Table 3). The ethanolic extract of *R. patula* (L.) showed maximum zone of inhibition *A. niger* at higher concentrations 0.8 mg/mL and it was 33 mm. The acetone extract showed minimum zone of inhibition *A. niger* at higher concentrations 0.1 mg/mL and it was 6 mm. When compared to the ethanolic, methanolic extract and acetonic extract, ethanolic extract showed the highest zone of inhibition the organism.

**Table 3.** Antifungal activity of different extracts of *R. patula* (L.).

<table>
<thead>
<tr>
<th>Micro organism</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Concentration/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>16</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>0.2</td>
<td>28</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>0.4</td>
<td>31</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>0.8</td>
<td>33</td>
<td>20</td>
<td>7</td>
</tr>
</tbody>
</table>
4. Discussion

The phytochemical screening and antimicrobial potentiality of *Phyllanthus amarus* against multidrug resistant pathogens were investigated using standard microbiological techniques. The extracts were tested by agar well diffusion method for activity against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Klebsiella* sp. isolated from clinical samples. The results revealed that the extracts did not inhibit the growth of *E. coli*, *Pseudomonas* sp. and *Klebsiella* sp. at 10 mg/mL but the largest zones of growth inhibition for the ethanolic extract was recorded with *S. aureus*, *E. coli* and *Klebsiella* sp. with a mean zone diameter of 20 mm concentrations. However, the isolates were subjected to antibiotics susceptibility testing and found to be resistant to Gram negative and Gram positive antibiotics with little variations in sensitivity pattern. The observed antibacterial effects were believed to be due to the presence of alkaloids, tannins, and flavonoids identified in the extracts. The results apparently justified their use in the treatment of infections (Adegoke et al., 2010).

Phytochemical analysis of methanol extract of *Origanum vulgare* and *Althea officinalis* revealed that antibacterial activity is due to the presence of phenolic and acidic fractions respectively. The results suggest that *O. vulgare* and *A. officinalis* are potential candidate plants for the management of phytopathogenic *Xanthomonas* which are known to cause diseases on many crop plants (Babu et al., 2007). The leaves extracts of *Urena lobata* L were examined for their antioxidant, antibacterial and antifungal activities. Preliminary evaluation of both the crude and the solvent fractions showed a broad spectrum of activity since the extracts inhibit the growth of both Gram positive and Gram negative bacterial isolates. The ethyl acetate and n-butanol fractions had a fast antioxidant reaction with DPPH solution, while the n-hexane and dichloromethane fractions gave no reaction. Three compounds were isolated from the ethyl acetate fraction and their structures determined, on the basis of spectroscopic data, tobe kaempferol 1, quercetin 2, and 3-O-β-D-(6″-O-trans-p-coumaroyl)-α-L-glucopyranosyl kaempferol3 (tiliroside). The compounds showed strong antimicrobial activity against *E. coli*, *B. subtilis*, *K. pneumoniae*, *B. polymyxa* and *Candida albicans*. The compounds also showed moderate to fast radical scavenging properties against DPPH radical. It was concluded that the isolated flavonoids may be part of the compounds responsible for the biological activity of *U. lobata* leaf extract. This study therefore supports the traditional uses of the plant in the treatment of infectious diseases (Adeloye et al., 2007). The phytochemical analysis of leaves extract was carried out and was shown in (Table 1).

The microorganisms used in these study such as *E.coli* which act as agent for urinary tract infection (Bichler et al., 2002) and it also cause diarrhea, sepsis and meningitis (Adegoke et al., 2010). *Staphylococcus aureus* is the major human pathogen which causes food poisoning and infections such as septicaemia, skin infection (Adegoke et al., 2008) and urinary tract infection (Bichler et al., 2002). Antibacterial activity of the dried leaves extract and their efficiency were quantitatively assessed using agar well diffusion methods by measuring the diameter of growth inhibition zone. The present studies indicate that the ethanolic extract of *R. patula* L at different concentrations (0.1 mg/mL, 0.2 mg/mL, and 0.4mg/mL) significantly suppressed the growth of the human pathogenic bacteria. The ethanolic extract of *R. patula* L. was most active against the microorganism’s *B. subtilis*, *E.coli*. The maximum inhibition zone was found in *B. subtilis* in 0.4 mg/mL concentration and it was (19 mm). The minimum inhibition zone was aceton extract found in *B. subtilis* in 0.1mg/mL concentration and it was (7 mm). The maximum inhibition zone was found in *E.coli* in 0.1mg/mL concentration and it was (29 mm). The minimum inhibition zone was aceton extract found in *B. subtilis* in 0.1mg/mL concentration and it was (10 mm). When compared to the ethanolic, methanolic extract and aceton extract, ethanolic extract showed the highest zone of inhibition among the organisms.
The fungal culture of *A. niger* in petriplates was along with test were checked for growth inhibitions zone of organisms after 48 h, the fungal activity ethanolic extract, methanolic extract and acetone extract of the plants *R. patula* (L.) was studied and listed (Table 3). The ethanolic extract of *R. patula* (L.) showed maximum zone of inhibition *A. niger* at higher concentrations 0.8 mg/mL and it was 33 mm. The acetic extract of *R. patula* showed minimum zone of inhibition *A. niger* at higher concentrations 0.1 mg/mL and it was 6 mm. When compared to the ethanolic, methanolic extract and acetone extract, ethanolic extract showed the highest zone of inhibition the organism.

5. Conclusion
*Ruellia patula* is commonly known as kayapachilai an important medicinal plant. Among three different extracts, (ethanol, methanol and acetone) ethanolic extract of leaves of *R. patula* L. exhibited maximum zone of inhibition effect than methanolic extract and acetone extract of leaves of *R. patula*. Ethanol extract of leaves was highly affected the activity of *B. subtilis, E. coli* and *A. niger* when compared to the other extract.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgement
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