Evaluation of Antibacterial and Antifungal activity of medicinal plant Solanum erianthum

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ABSTRACT
The antibacterial and antifungal activities of extracts (10, 20, 30 and 40 μg/ml) of Solanum erianthum were tested against five bacterial and five fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. Zone of inhibition of extracts were compared with that of standards like ampicillin and chloramphenicol for antibacterial activity and griseofulvin for antifungal activity. The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: Solanum erianthum, in vitro antibacterial activity, antifungal activity

1. INTRODUCTION
Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds (Edeoga et al., 2005).
Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem (Mahesh and Satish, 2008). Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action (Ahmad and Aqil, 2007; Barbour et al., 2004). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. Systematic screening of them may result in the discovery of novel active compounds (Tomoko et al., 2002).

Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century. However, with the ‘antibiotic era’ barely five decades old, mankind is now faced with the global problem of emerging resistance in virtually all pathogens (Peterson and Dalhoff, 2004). During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. Traditional medicines are used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (Lanfranco, 1999). The herbal medicines serve the health needs of about 80% of the world’s population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality (WHO, 2001).

In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal. In view of this, the searches for new anti-microbial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of bacterial origin are not only rampant, but the causative agents are also developing an increasing resistance against many of the commonly used antibiotics (Abebe et al., 2004). Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in folklore medicine is further justified. It is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as sterols, alkaloids, glycosides, saponins, flavonoids, tannins, and carbohydrates are generally superior in their anti-microbial activities (Cowan, 1999). Leaves and flower of experimental plants have been used for treating many diseases in traditional medicines.

The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world (Vuuren & Naidoo, 2010, Bhengraj et al., 2008). As a result, antifungal therapy is playing a greater role in health care and the screening of traditional plants in search of novel antifungals is now more frequently performed (Motsei et al., 2003). The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (Kusumoto et al., 1995). Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (CSIR, 1998). Human infections, particularly those involving the skin and mucosal surfaces, constitute a serious problem, especially in tropical and subtropical developing countries (Portillo et al., 2001). In humans, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in recent years. The treatment of mycoses has lagged behind bacterial chemotherapy and fewer antifungal than antibacterial substances are available. Therefore, a search for new antifungal drugs is extremely necessary (Fortes et al., 2008).

The purpose of this study was to carry out preclinical evaluation of some popular medicinal plant species, i.e., biological and phytochemical screening with particular emphasis on those that seems to have very little scientific information in the areas intended for the investigation. This study facilitated the selection of plants with relatively high level of potency and wide range of biological activities suggesting that the strength of biological activities of a natural product is dependent on the diversity and quantity of such constituents. Therefore, simultaneous determination of the compounds those are possibly responsible for any biological activity would facilitate decision-making process as in the selection of the plants for in-depth future investigation.

2. MATERIALS AND METHODS
Collection of Plant Materials
The fresh plant leaves of Solanum erianthum were collected randomly from the Yercaud, Salem District, Tamil Nadu. Plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in refrigerator.

Preparation of extract
Crude plant extract was prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml hydroalcohol extract. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C till future use.
Antimicrobial procedure:
Screening of antibacterial activity
Bacteria tested
Bacterial strains and fungal strains were used throughout investigation. All the bacterial cultures and fungal cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Preparation of inoculums
Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37°C and 25°C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0 \times 10^6 colony forming units (CFU/ml) for bacteria.

Antimicrobial susceptibility test
The disc diffusion method (Bauer et al., 1966) was used to screen the antimicrobial activity. Invitro antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Culture Media
The media used for antifungal test was Sabouraud’s dextrose agar/broth of Hi media Pvt. Bombay, India.

### Table 1
Antimicrobial activity

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organism Name</th>
<th>C</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C.bacterium</td>
<td>20</td>
<td>8</td>
<td>17</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>S.sonnie</td>
<td>23</td>
<td>16</td>
<td>21</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>S. metigene</td>
<td>18</td>
<td>14</td>
<td>16</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>E.facilus</td>
<td>20</td>
<td>16</td>
<td>18</td>
<td>19.5</td>
<td>24</td>
</tr>
</tbody>
</table>

### Table 2
Antifungal activity

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organism Name</th>
<th>C</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>T.rubrum</td>
<td>22</td>
<td>6</td>
<td>9</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>T.simii</td>
<td>19</td>
<td>14</td>
<td>22</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>C.lunata</td>
<td>20</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>C. albicans</td>
<td>19</td>
<td>6</td>
<td>8</td>
<td>16</td>
<td>24</td>
</tr>
</tbody>
</table>
Inoculum
The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 h and the suspensions were checked to provide approximately 10^5 CFU/ml.

Determination of antifungal activity
Theagar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 h. The diameters of zone of inhibition observed were measured.

3. RESULTS & DISCUSSION
The antibacterial activity was conducted on the organisms C.bacterium, S.connie, S.metigene and E.facilus. The highest activity is seen in the organism C.bacterium with the concentration 40 and the performance is much higher than the control. For the other organisms, S. sonnie, S. metigene and E. facilus, the activity is highest in 40 concentration and is higher than the control (Figure 1 & Table 1). The antifungal activity was conducted with the organisms T.rubrum, T.simii, C.lunata and C.albicans. The highest activity is seen in T.simii for the concentration 40 and the performance is higher than the control. For the other organisms also the activity is seen in 40 concentrations than the control (Figure 2 & Table 2). Previous studies demonstrated that ascorbic acid, phenolic acids and flavonoids isolated from the fruits of Solanum erianthum (pepino) exhibited anti-oxidative, anti-inflammatory and anti-glycative protection in diabetic mice (Hsu et al., 2011). The antioxidant activity of the ripe pepino fruit was reported to be largely due to polyphenols (Sudha et al., 2011). The anti-tumor effect of pepino fruits has been reported by Ren & Tang (Ren and Tang, 1999). In the Solomon Islands, leaf juice is used as a rinse for sores in the mouth. S. erianthum is considered poisonous to livestock. The root bark is poisonous and can be used as an antiphlogistic and against arthritis. The fruits can be eaten when cooked. The velvety leaves are used to remove grease from dishes in Nigeria and Philippines (Burkill HM, 2000; Blomqvist MM and Nguyen TB, 1999).

4. CONCLUSION
From the study, it is understood that the highest antibacterial activity is seen in C.bacterium and antifungal activity is seen in T.simii with the 40 concentration and the performance is higher than the control. The results of this study
corroborate the usage of these plants in traditional medicine. Further studies will be carried out to isolate and identify the active compounds.

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