Antimicrobial activity of various root extracts of *Coleus forskohlii*

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Abstract

The antimicrobial activity and phytochemical properties were screened using ethanol, methanol, chloroform, ethyl acetate, petroleum ether, hexane, hot water, and acetone extracts of *Coleus forskohlii*, Lamiaceae. Root extracts of *Coleus forskohlii* were evaluated by agar well diffusion method against six bacterial species and five fungal species. Preliminary phytochemical analysis of the root extract revealed the presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, tannins, phenolic compounds and terpenoids in the different root extracts was established. The ethanol extract was more effective against *Bacillus cereus* and *Micrococcus luteus* and *Klebsiella pneumoniae* and *Staphylococcus aureus*, whereas acetone extract was more effective against *Micrococcus luteus*, *Bacillus cereus* and *Klebsiella pneumoniae*. The different extracts were also found to be effective against the test fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Candida tropicalis*, and *Cryptococcus neoformans*. The results of the present study suggest that *Coleus forskohlii* roots can be used in treating diseases caused by the tested organisms.

Key words: *Coleus forskohlii*, antibacterial activity, root extract, medicinal plants, phytochemicals

Introduction

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine. The ancient indigenous practice of combining and concentrating several plants as decoction (extracting together in boiling water) to treat the whole person and focused different organ system along with the presenting complaint (Scott, 1998).
of antimicrobials have been key contributors to ineffective management of infectious diseases in many developing countries (Kapila, 2005; Runyoro et al., 2006). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Runyoro et al., 2006; Shahidi et al., 2004). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Reddy, 2001). Much work has been done on ethnomedicinal plants in India (Maheshwari et al., 1986). It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents. 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, 1995).

In an effort to expand the spectrum of antibacterial agents from natural resources, Coleus forskohlii belonging to Lamiaceae family (Mint family) has been selected. The leaves of the green type of country borage are often eaten raw with bread and butter. The chopped leaves are also used as substitute for sage (Salvia officinalis L.) in stuffing. Coleus forskohlii is used for seasoning meat dishes and in food products, while a decoction of its leaves is administered in cases of chronic cough and asthma (Kusumoto et al., 1995). It is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia. It is used to treat conditions such as indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis (Warrier et al., 1995). It is also known to be a very powerful painkiller, stimulates flow of bile aiding digestion. Mast cell stability property of Coleus forskohlii leaves was checked in rat peritoneal mast cells (Kumar et al., 2007). Freeze-dried aqueous extract of Coleus forskohlii extract clearly established antioxidant potency (Kumaran and Karunakaran, 2006). It has been reported to exhibit antioxidant, leishmanic, uroliothiasis (Baskar et al., 1992; Jose et al., 2005) antiepileptic (Buznego and Perez-Saad 1999), antitumor and antimutagenic (Annapurani and Priya 1999), neuropharmacological (Pirez Saad et al., 2003), radioprotective effect (Rao et al., 2006), antimicrobial (Rao et al., 1991; Deena et al., 2002), antibacterial, antifungal properties (Prudent et al., 1995). Certain active principles of this plant are being effective in relieving the intraocular pressure of glaucoma, lowering blood pressure in patients with heart disease, also found to stabilize the cells that release histamine and other inflammatory compounds (Andre et al., 1995). The plant also finds prominent importance in modern medicine. Coleus forskohlii has been used historically for menorrhagia in Trinidad (Lam, 2007). In the present study, antibacterial activity of ethanol and water extract of Coleus forskohlii leaves was determined.

Materials and methods

Plant materials

The Coleus forskohlii roots collected during June-July of 2010 in and around Arani, Tamilnadu were authenticated by Department of Botany. The voucher specimens were kept in the Department of Botany in C. Abdul Hakeem College, Melvisharam, Vellore, Tamil Nadu, India.

Extraction procedure

All the laboratory works are done in Microlabs, Institute of Research and Technology, Arcot, Tamil Nadu, India. The plants washed with fresh water and dried under shade at room temperature, cut into small pieces and powdered in a mixer grinder. The roots were powdered and stored in sterile containers for further use. Then these powdered samples (100 g / 100 ml) were mixed in solvents such as hot water, ethanol, methanol, chloroform, Ethyl acetate, Petroleum ether, hexane and acetone extracts for overnight at room temperature. Soxhlet apparatus was used for this extraction (Grouch et al., 1992; Matanjun et al., 2008). The extract from three consecutive soaking were pooled and evaporated under pressure. The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, protein, tannins, phenols.
Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites.

Antibacterial activity

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic Ciprofloxacin (100 µg/mL) in-vitro by well diffusion method (Perez, 1999; Bagamboula et al., 2004). Lawn culture was prepared using the test organism on Muller Hinton Agar (MHA). The inoculated plates were kept aside for a few minutes. Using well cutter, four wells were made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. Using sterilized micropipettes 30µl of different solvents with selected Coleus forskohlii root extract was added in to the well. The plates were incubated at 37°C for overnight. The activity of the root extract was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents without root extracts were used.

Results

The presence of various phytochemicals is shown in Table 1. The results of antibacterial activity are given in the Table 2, which clearly shows that all the extracts have shown antibacterial activity almost equivalent to that of standard against the entire tested organisms. Ethanol, methanol, Ethyl acetate, acetone, chloroform, Petroleum ether, hexane, and hot water extracts have shown better activity than the standard against all the six test bacteria. Ethanol extract was more effective against Bacillus cereus and Micrococcus luteus. Methanol extract was more effective against

and furanoids using the method of Harborne (Harborne 1973).

Phytochemical test

The extracted samples were stirred with dil Hcl and filtered. This filtrate was tested carefully and used for compound analysis. In this alkaloids (Mayer’s test), carbohydrates and glycosides (Molish test), Saponins (Chloroform and H2SO4 test), protein and amino acid (Millon’s Test), Phytosterols (Libermann- Burchard’s test), Phenolic compound (Ferric chloride test) and Tannin (Lead acetate test) were followed (Okigbo and Omodamiro, 2006; Ogueke et al., 2007).

Test organisms

The bacterial spp. used for the test were Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus), Micrococcus luteus,(M.luteus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumonia(K.pneumoniae) The fungus spp. used for the test were Aspergillus niger (A.niger), Aspergillus flavus(A.flavus), Candida albicans (C.albicans), Candida tropicalis(C. tropicalis), and Cryptococcus neoformans. All the stock cultures were obtained from Microlabs, Institute of Research and Technology, Vellore, Tamilnadu, India.

Culture media and inoculums preparation

Nutrient agar / broth (Himedia, India.) were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth and incubated at 37°C for 72 hrs and Potato dextrose agar and potato dextrose broth (Himedia, India) were used as the media for the culturing of fungal strains. Loops full of all the fungus cultures were inoculated in the potato dextrose broth (PDA) and incubated at room temperature for 72 hrs.

Preliminary phytochemical screening

All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites.
**B. cereus** and **S. aureus.** Ethyl acetate extract was more effective against **S. aureus** and **M. luteus.** Acetone extract was more effective against **M. luteus, B. cereus** and **K. pneumoniae.** Chloroform extract was more effective against **S. aureus** and **E. coli.** Petroleum ether extract was more effective against **B. cereus** and **K. pneumoniae** hexane extract was more effective against **S. aureus** and **E. coli.** Hot water extract was more effective against **B. cereus** and **K. pneumoniae.** The results of antifungus activity are given in the Table II, which clearly show that all the extracts have shown antifungal activity against all the five microorganisms. Ethanol extract was more effective against **C. albicans** and **A. flavus.** Methanol extract was more effective against **A. niger** and **C. neoformans.** Ethyl acetate extract was more effective against **A. niger** and **C. neoformans.** Acetone extract was more effective against **Candida tropicalis, C. neoformans and C. albicans.** Chloroform extract was more effective against **A. niger** and **C. neoformans.** Petroleum ether extract was more effective against **A. niger.** Hexane extract was more effective against **A. flavus** and **C. albicans.** Hot water extract was more effective against **A. flavus** and **C. neoformans.**

**Table 1.** Preliminary phytochemical analysis of **Coleus forskohlii** root

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Test performed</th>
<th>E</th>
<th>M</th>
<th>C</th>
<th>Et</th>
<th>P</th>
<th>H</th>
<th>Aq</th>
<th>Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molish test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Chloroform and H$_2$SO$_4$ test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Molish test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins&amp; aminoacids</td>
<td>Millon’s Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterol</td>
<td>Libermann-Burchard’s Test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>Ferre chloride test and Lead acetate test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>Shinoda test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpinoids</td>
<td>Noller’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Neutral FeCl$_3$</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

E – Ethanol; M- Methanol; C- Chloroform; Et – Ethylacetate; P – Petroleum ether; H – Hexane; Aq – Aqueous; Ac – Acetone extracts; (+) Positive (-) Negative

**Discussion**

The therapeutic value of medicinal plants lies in the various chemical constituents in them. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane (Mohamed Sham et al., 2010). Flavonoids are a major group of phenolic compounds reported for their antiviral (Mehrangiz et al., 2011), antimicrobial and spasmylytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties. The antibacterial activity of the root extracts of **Coleus forskohlii** as recorded in present study may therefore be attributed to the presence of above phytochemicals i.e Alkaloids, Carbohydrates, Glycosides, Proteins and aminoacids, Phenolic, Flavonoids, Terpinoids, Tannins in Ethanol extracts and Alkaloids, Carbohydrates, Glycosides, Proteins, Phenolic, Flavonoids, Terpinoids, Tannins in Methanol extract and Saponins, Phytosterol in Chloroform extract and alkaloids, Carbohydrates, Saponins, Glycosides, Phenolic, Flavonoids, Tannins in ethyl acetate extract and Saponins, Phytosterol in Petroleum ether extracts and Saponins and Saponins, Phytosterol in Hexane extracts and Alkaloids, Carbohydrates, Glycosides,
Protins & aminoacids, Phenolic compounds, Flavonoids, Terpinoids, Tannins in aqueous extracts and Alkaloids, Carbohydrates, Glycosides, Proteins and aminoacids, Phenolic compounds, Flavonoids, Terpinoids, Tannins in acetone extracts.

It is concluded that the plant extract possess antimicrobial activity against tested organisms. The zone of inhibition varied suggesting the varying degree of efficacy and different phyto constituents of herb on the target organism. The antimicrobial activity of the plants may be due to the presence of various active principles in their roots. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs. Based on the results of the present study it is concluded that the Coleus forskohlii plants have potent antimicrobial activity against various bacteria and fungi which might be due to the phytochemicals present in the plants. Also, there is further scope to study the identification and purification of active compound(s) involved in this antimicrobial activity of Coleus forskohlii.

Acknowledgments
The authors thank the Microlabs, Institute of Research and Technology, Vellore and Arcot, Tamil Nadu, India for channelizing required facilities.

Table 2. Antibacterial activity of different root extracts of Coleus forskohlii against different organisms (Mean±SEM) (mm)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>E.coli</th>
<th>Micrococcus luteus</th>
<th>Pseudomonas aeruginosa</th>
<th>Bacillus cereus</th>
<th>Klebsiella pneumoniae</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>15.23±0.25</td>
<td>31.23±0.25</td>
<td>15.23±0.25</td>
<td>32.23±0.25</td>
<td>30.30±0.17</td>
<td>29.23±0.25</td>
</tr>
<tr>
<td>Methanol</td>
<td>12.03±0.25</td>
<td>23.23±0.25</td>
<td>12.23±0.25</td>
<td>25.27±0.25</td>
<td>21.17±0.15</td>
<td>25.27±0.30</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>14.13±0.32</td>
<td>14.20±0.26</td>
<td>10.17±0.15</td>
<td>17.23±0.25</td>
<td>15.23±0.25</td>
<td>22.27±0.25</td>
</tr>
<tr>
<td>Acetone</td>
<td>13.10±0.10</td>
<td>40.23±0.26</td>
<td>8.27±0.25</td>
<td>30.23±0.25</td>
<td>28.23±0.25</td>
<td>19.23±0.25</td>
</tr>
<tr>
<td>Chloroform</td>
<td>15.90±0.10</td>
<td>15.23±0.25</td>
<td>6.27±0.25</td>
<td>13.23±0.25</td>
<td>11.27±0.25</td>
<td>20.27±0.25</td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>14.03±0.15</td>
<td>12.23±0.25</td>
<td>9.37±0.32</td>
<td>15.20±0.20</td>
<td>14.20±0.26</td>
<td>13.10±0.10</td>
</tr>
<tr>
<td>Hexane</td>
<td>13.23±0.25</td>
<td>12.27±0.25</td>
<td>8.23±0.25</td>
<td>12.37±0.32</td>
<td>10.17±0.15</td>
<td>19.23±0.25</td>
</tr>
<tr>
<td>AQUEOUS EXTRACT</td>
<td>6.23±0.25</td>
<td>6.23±0.25</td>
<td>6.17±0.15</td>
<td>8.10±0.10</td>
<td>7.17±0.15</td>
<td>7.10±0.10</td>
</tr>
<tr>
<td>CIPROFLOXACIN(10)</td>
<td>19.20±0.26</td>
<td>16.90±0.36</td>
<td>15.37±0.32</td>
<td>13.23±0.25</td>
<td>16.23±0.25</td>
<td>17.37±0.32</td>
</tr>
</tbody>
</table>

Table 3. Antifungal activity of different extracts of Coleus forskohlii root of against different organisms (Mean±SEM) (mm)

<table>
<thead>
<tr>
<th>EXTRACTS</th>
<th>Aspergillus niger</th>
<th>Aspergillus flavus</th>
<th>Candida albicans</th>
<th>Candida tropicalis</th>
<th>Cryptococcus neoformans</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETHANOL</td>
<td>10.07±0.11</td>
<td>12.03±0.06</td>
<td>15.07±0.11</td>
<td>10.07±0.11</td>
<td>10.03±0.06</td>
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<tr>
<td>METHANOL</td>
<td>17.10±0.10</td>
<td>10.17±0.15</td>
<td>8.07±0.06</td>
<td>8.07±0.11</td>
<td>11.07±0.11</td>
</tr>
<tr>
<td>ETHYL ACETATE</td>
<td>10.10±0.17</td>
<td>-</td>
<td>5.10±0.10</td>
<td>9.10±0.10</td>
<td>10.10±0.10</td>
</tr>
<tr>
<td>ACETONE</td>
<td>6.03±0.06</td>
<td>9.03±0.06</td>
<td>10.03±0.06</td>
<td>19.03±0.06</td>
<td>19.07±0.11</td>
</tr>
<tr>
<td>CHLOROFORM</td>
<td>11.03±0.06</td>
<td>6.03±0.06</td>
<td>8.03±0.06</td>
<td>7.03±0.06</td>
<td>10.03±0.06</td>
</tr>
<tr>
<td>PETROLEUM ETH</td>
<td>8.06±0.11</td>
<td>5.0±0.00</td>
<td>6.07±0.11</td>
<td>-</td>
<td>8.03±0.06</td>
</tr>
<tr>
<td>HEXANE</td>
<td>-</td>
<td>6.03±0.06</td>
<td>6.03±0.06</td>
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<td>5.03±0.06</td>
</tr>
<tr>
<td>AQUEOUS EXTRACT</td>
<td>5.07±0.11</td>
<td>6.03±0.06</td>
<td>-</td>
<td>-</td>
<td>6.03±0.06</td>
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<tr>
<td>KETOCONAZOLE</td>
<td>10.17±0.15</td>
<td>9.17±0.29</td>
<td>19.70±0.60</td>
<td>28.0±0.50</td>
<td>19.73±0.64</td>
</tr>
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</table>

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