INTRODUCTION

India is rich in indigenous herbal resources permitting the growth of more than 20,000 plant species of which, about 2,500 are of medicinal value (Choudhari and Maheswari, 2009). The medicinal value of these plants lies in some active chemical substances called phytochemicals that produce a definite physiological action of human body. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatment (Arunkumar and Muthuselvam, 2009). Alkaloids, flavonoids, phenolic compounds and tannins are the major phytochemicals present in this plants (Edeoza et al., 2005). Coleus amboinicus, Karpooravalli in Tamil is a country borage plant. It is also referred as Plectranthus amboinicus. It belongs to the family lamiaceae (Labiatae) and is also called as country borage in English (Kirtikar and Basu, 1999). It is a large succulent aromatic perennial herb found throughout India, Moluccas and Ceylon (Warrier and Nambier, 1996, Nadkarni, 2002). It is a medicinal plant, widely used in the Indian system of medicine. Many pharmacological properties have been reported including urolithiasis, antiepileptic, antitumorogenic, antimutagenic, radioprotective effect, antiviral, antifungal and neuropharmacological properties (Patel et al., 2010). Afaf Mohammed Weli et al. (2011) found out twenty six constituents present in the essential oil of Coleus aromaticus growing in Oman. The major constituent being M-thymol (63.4%) followed by terpinene (11.8%), P-cymene (7.7%), Caryophyllene (7.1%) and beta – seline (2.7%). The oil showed potent antibacterial activity against all tested micro organisms but it did not show any antifungal activity.

Synthetic antioxidants and antimicrobials in use have been shown to have harmful side effects (Gao et al., 1999; Williams et al., 1999 and Osawa and Namiki, 1981); therefore there is a need for more effective, less toxic and cost effective antioxidants and antimicrobials from natural sources. Several medicinal plants with ethno – botanical uses have been used traditionally in the treatment of disease and have been exploited for these desired traits (Patel et al., 2010, Okoro et al., 2010; Lagnika et al., 2011). Consequently there has been a growing interest to identify natural antioxidants and antimicrobials from these plants (Chanda and Dave 2009; Rice, 2004). Khory and Katrak (1999) reported that the decoction of Coleus amboinicus leaves is used in cases of chronic cough and asthma, and also as an antispasmodic, stomachic, and for the treatment of headache, fever, epilepsy and dyspepsia (Morton, 1992). It is also used in the treatment of skin ulcerations and urinary diseases, as well as to alleviate inflammation, kidney

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF COLEUS AMBOINICUS ON SIX BACTERIAL STRAINS

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ABSTRACT

Coleus amboinicus is a commonly available medicinal herb in India. Ethanolic extract of Coleus amboinicus leaves were used for study of antimicrobial activity. The antimicrobial activity was evaluated against six bacterial strains with four different concentrations by detecting minimum inhibitory concentration and zone of inhibition. The highest antibacterial activity was observed in Salmonella typhi at a concentration of 1000µg/ml. The lowest antibacterial activity was observed in Staphylococcus aureus at a concentration of 250µg/ml of ethanolic extract of Coleus amboinicus leaf extract.

Key words:
Coleus amboinicus,
Antimicrobial activity,
Ethanolic extract,
Minimum inhibitory concentration.

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troubles, nervous disorders and in conditions of congestive heart failure (Franca et al., 1996; Jain and Lat, 1996). Lukhoba et al. (2006) recently reviewed the ethanobotanical uses of *P. amboinicus*.

Malini et al. (2013) studied antimicrobial activity of Ethanolic and Aqueous extracts of *Aloe vera* and *Coleus aromaticus* against waste water pathogens like *E. coli, Pseudomonas sp, Klebsiella sp, Bacillus sp* and *Staphylococcus sp*. They found out minimum antibacterial activity of 6.66 ± 0.667 mm zone of inhibition in aqueous extract and maximum antibacterial activity of 20.50± 0.500 mm zone of inhibition in ethanolic extract. They further confirmed the ethanolic extracts of *Aloe vera sp* showed the maximum activity 22.46± 0.2990 mm zone of inhibition against waste water pathogen. Nivya Mariam Paul et al. (2014) studied phytochemical screening and antimicrobial activity of *Coleus amboinicus* against twelve different bacterial strains. Phytochemical analysis of ethanolic extract of fresh leaves showed the presence of flavonoids, terpenoids, phenolic compounds tannins and cardiac glycoside. Ethanolic extracts of shade dried and oven dried leaves showed the presence of terpenoids, phenolic compounds, tannins and cardiac glycoside. Aqueous extract of fresh leaves showed the presence of Alkaloids, flavonoids, phenolic compounds and tannins and gum. Aqueous extract of shade dried leaves showed the presence of alkaloids, phenolic compounds, tannins and gum. Aqueous extract of oven dried leaves showed the presence of phenolic compounds, tannins, morphine alkaloids and gum. This work was supported by the work of Roshen Patel et al. (2010) they detected the presence of alkaloids, carbohydrates, glycosides, proteins aminoacides, terpenoids, quinine, tannins and flavanoids in *Coleus amboinicus* leaves.

They further found out the ethanolic leaf extract of *coleus amboinicus* showed more antibacterial activity against all the bacterial strains than the aqueous leaf extract of *Coleus amboinicus*. Melva Silitonga et al. (2014) found out Bangun bangun (*Plectranthus amboinicus* Lour) increased the productivity of breast milk in black tribe woman who consumed *Plectranthus amboinicus* just gave birth in North Sumatra Indonesia. This plant is known to have a high content of nutrients, especially iron carotene. Also known to have many benefits among other as an antipyretic, analgesic, wound medicine, cough medicine and thrush, antioxidant, antitumour, anticancer and hypotensive.

**MATERIALS AND METHODS**

**Plant Materials**

Fresh leaves of *Coleus amboinicus* collected from Kanchipuram District, Tamil Nadu. It was authenticated by Dr. P. Jayaraj Director, Plant Anatomy Research Center Thambaram Chennai. Retired Professor of Botany, Presidency College, Chennai, Tamil Nadu. (Reg.No. PARC/2013/2081) The plants were freshly collected and the leaves were separated from the stem, washed with running tap and rinsed in distilled water. The leaves were shade dried after complete dryness they were ground well to fine powder and then transferred into airtight containers.

**Extraction**

The leaves were chopped into small pieces and were shade dried for 2 weeks. The dried leaves were powdered, using mechanical grinder. 20g of the plant powder sample was ground and soaked with ethanol in a 250 ml conical flask. The flask was plugged with cotton wool and aluminium foil to prevent the solvent from escaping. The flask was placed in a shaker for 24 hrs. The filtrate was concentrated in a rotary evaporator to get the crude plant extracts.

**Antimicrobial screening**

Bacteria: *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherchia coli, Klebsiella pneumoniae* and *Proteus species*. The bacterial cultures were maintained in Nutrient Agar (NA) slants at 4°C.

**Bacterial inoculums preparation**

Bacterial cultures used in this study were obtained from Microbial Type Culture Collection (MTCC). Lab Chandigarh, India. Bacterial cultures included in this study were *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Escherchia coli, Klebsiella pneumoniae* and *Proteus species*. All the cultures were grown in Muller – Hinton broth medium. The inoculums was used for antibacterial assay. Stock cultures were maintained at 4°C on nutrient agar slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated at 37°C for 24 hrs.

**Antibacterial assay**

The media and the test bacterial cultures were poured into dishes (Muller – Hinton agar media). The test strain (0.2 ml) was inoculated into the media to inoculums size (108 cells / ml) when the temperature reached 40–42°C care was taken to ensure proper homogenization. The plant extracts was tested for antibacterial activity in the agar well diffusion assay against *Staphylococcus aureus, Escherchia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae* and *Proteus spp*.

**Agar well Diffusion Method**

The inoculums of microorganism was prepared from pure culture according to the method of Parihar and Bohra, (2006). The antibacterial activity of plant extract was determined by Disc Diffusion Method of Muller Hinton Agar (MHA) medium (Bauer et al., 1966). The Muller Hinton Agar medium is poured into the pertiplate, after solidification the inoculums, were spread on the solid plates with sterile swab moistened with the bacterial suspension. The crude extracts were dissolved in Dimethyl sulphoxide (DMSO) and extracts were loaded on the 6 mm diameter, sterile disc (Himedia, Bombay) with the different concentration.

The disc were placed in MHA plate. Different concentration of leaf extract (1000µg/ml, 750µg/ml, 500µg/ml and 250µg/ml)
were added to the bacterial suspension. The samples were placed in the disc. The plates were incubated for 24 hrs at 37°C. DMSO (20µg/disc) was used as control. Ampicillin (100 µg) was used as standard. The inhibition zone around the disc (diameter) was measured and recorded. The antimicrobial activity was determined by measuring the diameter of zone of inhibition.

RESULTS

Antibacterial activity of Coleus amboinicus was analyzed against Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, E.coli, Klebsiella pneumoniae and Proteus species. The Staphylococcus aureus showed 8 mm zone of inhibition in 1000µg/ml, 7 mm zone of inhibition in 750µg/ml, 4 mm zone of inhibition in 500µg/ml and 3 mm zone of inhibition in 250µg/ml of leaf extract of Coleus amboinicus. The E.coli showed 7 mm zone of inhibition in 1000 µg/ml, 6 mm zone of inhibition in 750µg/ml, 4 mm zone of inhibition in 500µg/ml and 4 mm zone of inhibition in 250µg/ml of leaf extract. Pseudomonas aeruginosa showed 4 mm zone of inhibition in 1000µg/ml, 4 mm zone of inhibition in 750µg/ml. No inhibition were observed in the concentration of 500µg/ml and 250µg/ml of leaf extract against Pseudomonas aeruginosa.

On the other hand Salmonella typhi showed 11 mm zone of inhibition in 1000µg/ml, 6 mm zone of inhibition in 750µg/ml, 6 mm zone of inhibition in 500µg/ml and 5 mm zone of inhibition in 250µg/ml of leaf extract. Klebsiella pneumoniae showed 7 mm zone of inhibition in 1000µg/ml, 6 mm zone of inhibition 750µg/ml, 5 mm zone of inhibition in 500µg/ml and 4 mm zone of inhibition in 250µg/ml of plant extract. Proteus species showed 5 mm zone of inhibition in 1000µg/ml, 750µg/ml and 500µg/ml and 4 mm zone of inhibition was found in 250µg/ml of Coleus amboinicus leaf extract. The highest antimicrobial activity was observed in Salmonella typhi (11 mm zone of inhibition) at 1000µg/ml of leaf extract, whereas lowest antimicrobial activity was observed in Staphylococcus aureus (3 mm zone of inhibition) at 250µg/ml of concentration of leaf extract. But in Pseudomonas aeruginosa showed no antibacterial activity at 500µg/ml and 250µg/ml of leaf extract. In standard treated with Amphicillin (100 µg) showed 10mm zone of inhibition in Staphylococcus aureus, E.coli, Pseudomonas aeruginosa and Proteus spp. 20mm zone of inhibition in Salmonella typhi and 13mm zone of inhibition in Klebsiella pneumoniae. The results are presented in Table -1, Bar graph-1 and Figure 1 – 6.

Table 1. Screening of antibacterial activity of Coleus amboinicus leaves extracts against different bacterial strains

<table>
<thead>
<tr>
<th>S.No</th>
<th>Micro Organisms</th>
<th>Concentration (µg/ml)</th>
<th>Std</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>750</td>
<td>500</td>
</tr>
<tr>
<td>1.</td>
<td>Staphylococcus aureus</td>
<td>8</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>E.coli</td>
<td>7</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>Pseudomonas aeruginosa</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Salmonella typhi</td>
<td>11</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>5.</td>
<td>Klebsiella pneumonia</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>6.</td>
<td>Proteus spp</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Bar graph-1. Screening of antibacterial activity of Coleus amboinicus leaves extracts against different bacterial strains
Fig. 1. Antibacterial activity of *Coleus amboinicus* leaves extracts against *Staphylococcus aureus*

Fig. 2. Antibacterial activity of *Coleus amboinicus* leaves extracts against *E. coli*

Fig. 3. Antibacterial activity of *Coleus amboinicus* leaf extracts against *Pseudomonas aeruginosa*

Fig. 4. Antibacterial activity of *Coleus amboinicus* leaf extracts against *Salmonella typhi*

Fig. 5. Antibacterial activity of *Coleus amboinicus* leaf extracts against *Klebsiella pneumoniae*

Fig. 6. Antibacterial activity of *Coleus amboinicus* leaf extracts against *Proteus spp*
Numers in the agar plate indicates the concentration of the leaf extract.

1. Coleus amboinicus : 1000 µg
2. Coleus amboinicus : 750 µg
3. Coleus amboinicus : 500 µg
4. Coleus amboinicus : 250 µg
5. Standard – Ampicillin : 100 µg
6. DiMethyl Sulphoxide (DEMSO): 20 µg

**DISCUSSION**

Plants are important source of potentially useful structure for the development of new chemotherapeutic agents (Tona et al., 1998). The ethanolic crude extract of Coleus amboinicus leaf showed the highest antimicrobial activity against Salmonella typhi 11 mm and *Staphylococcus aureus* 8 mm zone of inhibitions at 1000 µg/ml concentration. The results are in agreement with those of Shiny ramya et al. (2012). They concluded that alcoholic crude extract of Coleus aromaticus and Luecas aspera leaves were exhibited antibacterial activity against Shigella sp, Salmonella typhi and *E. coli*, this is due to the presence of antimicrobial substances in the leaf extract like tannis, alkaloids and glycosides. Roa et al. (1991) reported that oil isolated from the leaves of Coleus amboinicus exhibited varying degree of antimicrobial activity against a number of pathogenic and non-pathogenic Fungi and Bacteria. Prudent et al. (1995) also analyzed the essential oil of Coleus aromaticus for its bacteriostatic and fungistic properties. The results are further supported by the work of Subhas Chandrappa et al. (2010). They found out ethanol extract of Coleus aromaticus showed strongest antibacterial activity with the MIC value of 25-39µg/ml against 3 Gram positive and 2 Gram negative human pathogenic bacteria. Ethanol extract of Coleus aromaticus (ECA) showed more potential of antibacterial effect than Hot water Coleus aromaticus extract (HWCA). This may be because the alcohol extract is rich in polyphenol and other bioactive compounds, which are responsible for its antibacterial activities.

The work of Pritima and Pandian (2008) is of great interest. They found the Coleus amboinicus species growing in India have more effective in antibacterial and antifungal activities. They conducted an experiment, the Coleus amboinicus plant was tested on micro-organism causing reproductive tract infections (RTI) in females. When tested against *Candida Krusei* there was the highest 20 mm zone of inhibition, followed by *C. albicans*, *Proteus mirabilis*, *E.coli*, *S.aureus*, *E.faecalis*, Kieshiella pneumoniae and the least inhabitation was observed on *Neisseria gonarreae*. In the present study the Lamiaceae (Labiatae) leaf extract from Kanchipuram District, Tamilnadu had greater on the diameter of the inhibition zone for *S. aureus* than *E. Coli*, indicating that the gram positive bacteria for *S. aureus* is more sensitive than the gram negative strain. The leaf extract from Tamilnadu having inhibitory effect on the gram positive bacteria (*Staphylococcus aureus*) compared to the gram negative bacteria (*Escherichia coli* and *Pseudomonas aruginosa*). Generally gram negative bacteria are more resistance to plants extract compared to gram positive bacteria (Basri and Fan, 2005). This may be due to the permeability barrier provided by the cell wall. The reason is that the density of the lipopolysaccharide layer in the outer surface of bacterial cell wall, which is greater in the gram negative bacteria as compared to the gram positive bacteria (Burn, 1988). It has been concluded that the antibacterial properties is of due to nucleotide damage with increase in spatial division and condensation of genetic material (Chakruborty et al., 2007).

The results proves that the use of the leaves of Coleus amboinicus to cure several illness, especially those caused by microbes, is valid.

**REFERENCES**


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