IN VITRO ANALYSIS OF ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF COLEUS AROMATICUS

Amar Deep Shukla*, Tarun Agarwal and Angad Yadav

Amity Institute of Biotechnology, Amity University, Lucknow

Email: mailto.ads.07@gmail.com

ABSTRACT: The Cold Aqueous, Ethanolic, Acetonic and Methanolic extracts of leaves of Coleus aromaticus were evaluated for antibacterial activity against bacterial strains (Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa). The in vitro antimicrobial activity was performed by Agar well diffusion method on Nutrient Agar medium at the final extract concentration of 500mg/ml. The Acetonic extracts of all Coleus aromaticus leaves showed moderate to high activity against all the investigated microbial strains. Acetonic extract of Coleus aromaticus leaves was most effective among all extracts (27mm inhibition zone against S. aureus). Cold Aqueous extracts showed the minimum antimicrobial properties against the selected bacterial strains.

Key words: Antibacterial properties, Coleus aromaticus, Solvent extraction, Agar Well Diffusion

INTRODUCTION

The widespread use of commercially available antimicrobials led to the consequence of emergence of antimicrobial resistant pathogens that ultimately led to the threat to global public health (J. Davis, 2009). Therefore there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious disease from medicinal plant (G.A. Cordell, 2000). According to WHO (1993), 80% of the world’s population of developing countries is dependent on the traditional medicines and a major part of the traditional therapies involve the use of plant extracts or their bioactive components (A. Mathur, et.al., 2011). Research in the field of indigenous plants is a significant aspect of developing a safe antimicrobial principle through isolation, characterization, identification and biological studies (A. Dubey, et.al., 2010). Coleus aromaticus looks as a green, perennial, shrub having heart shaped, and leathery leaves with scalloped edges. The leaves are highly aromatic with a strong flavor of mixed herbs and make an excellent addition in stuffing for meat and poultry. Finely chopped, they can also be used to flavor meat dishes, especially beef and lamb (K.R. Kuebel, A.O. Tucker, 1988). Coleus aromaticus, a plant of medical and food interest is told as “must- have” plant in medicinal herb garden (W. Wong, 2007). It is known to possess antimicrobial (P.S. Murthy, et.al., 2009), antiepileptic, leishmanial, and antioxidant activities (M.T. Buznego, H. Perez-Saad, 1999). The leaves are also used for treatment of cough, throat infection and nasal congestion.

The present paper focuses onto the characterization of the extent of antimicrobial properties present in the leaves of Coleus aromaticus.

MATERIALS AND METHODS

Sample Collection

Fresh and healthy leaves of Coleus aromaticus collected Rajaji Puram area in Lucknow after proper identification. The leaves were washed with tap water followed be distilled water and then dried. Dried sample was ground into fine powder by the help of grinder.

Bacterial strains and culture preparations

Three pathogenic strains namely Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli, available at Amity University, Lucknow were subcultured and used throughout the study.

Preparation of Plant Extract

Antimicrobial metabolites from the dried leaves was extracted in various solvents such as Cold Aqueous, Methanol (80%), Ethanol (70%), and Acetonic (80%). 2 gm of powdered sample was soaked in 20ml of the respective solvents (1:10) and kept in dark for 3-4 days so that secondary metabolites diffuse out into the solvents. It was then filtered in weighed petri plate and dried in hot air oven at 50°C, so that solvents get evaporated. The dried metabolite extract was dissolved in double volume of DMSO (Dimethyl Sulfoxide) thus giving the final concentration of extract to 500 mg/ml.
Antibacterial Susceptibility Assay

Antibacterial susceptibility assay was carried out by well diffusion method (C. Perez, C. Anesini, 1993) wherein sterile Nutrient agar plates were prepared and spreaded with 50μl of the available bacterial cultures against which antibacterial activity was tested. There after 3 wells of 8 mm diameter were dug with the help of sterile borer. Two plates were prepared for each microbial strain.

In the plate 1; the 1st, 2nd and 3rd well was filled with 60μl of standard antibiotic Tetracycline, Methanolic and Ethanolic extract respectively.

In the plate 2; the 1st, 2nd and 3rd well was filled with 60μl of standard antibiotic Tetracycline, Cold Aqueous and Ethyl acetate extract respectively.

Plates were incubated at 37ºC for 24 hours. The antibacterial activity of each extract was expressed in terms of mean of diameter of Zone Of Inhibition (in mm) produced by each extract at the end of incubation period.

RESULTS

Antibiogram analysis:

In order to check the antimicrobial activity of extracted plant samples, agar well diffusion method was used. With the help of this test we can determine whatever the extract being tested has antibacterial property or not.

Table 1 below shows the results of Zone of Inhibitions (ZOI) observed for the antimicrobial properties of Coleus aromaticus leaf extracts and the standard antibiotic tetracycline used throughout the study.

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>EXTRACTS</th>
<th>ZONE OF INHIBITION (ZOI) AGAINST (in mm)</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>By Extract</td>
<td>By Tetracycline</td>
<td>By Extract</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethanol</td>
<td>14.5</td>
<td>16.5</td>
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<tr>
<td>2</td>
<td>Methanol</td>
<td></td>
<td>Methanol</td>
<td>-</td>
<td>16.5</td>
</tr>
<tr>
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<td>Acetone</td>
<td></td>
<td>Acetone</td>
<td>18.5</td>
<td>15.0</td>
</tr>
<tr>
<td>4</td>
<td>Cold Aqueous</td>
<td></td>
<td>Cold Aqueous</td>
<td>-</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Note: Well diameter = 8mm.

Figure 1: Comparative analysis of Coleus aromaticus leaf extracts against chosen pathogens.

DISCUSSION

Researchers have extensively studied the biological properties of Coleus aromaticus and their results showed that this plant is ethno-medically valuable. Some antibiotics have become almost obsolete because of the problem of drug resistance (T.O. Ekpendu, et.al., 1994). Coleus aromaticus leaf extracts were found to be active against used pathogens and this could be the drugs without side effects for the future.
Agar well diffusion method was used here in order to determine the antimicrobial properties of the plant extracts against the pathogens. Acetonic extract showed the maximum Zone of Inhibition (27.0 mm) against *Staphylococcus aureus*, followed by zone of Ethanolic extract of 21.0 mm against the same. Methanolic extracts also showed the zone of 17.5 mm and 16.0 mm respectively, against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. The Cold Aqueous extract showed no zone against E. coli and S. aureus, but shown a inhibitory zone of 12.5 mm against *P. aeruginosa*.

The antibacterial activity of leaves of *Coleus aromaticus* may be indicative of presence of metabolic toxins or broad spectrum antimicrobial compounds that act against gram +ve as well as gram –ve bacteria. Acetonic extracts exhibited higher degree of antibacterial activity as compared to that of other extracts tested against bacterial strains.

**CONCLUSION**

Based on the above research work it can be concluded that the leaves of *Coleus aromaticus* can be a very good source for herbal drugs and specially the solvents like Methanol, Ethanol, Ethyl Acetate and Cold aqueous can be explored further for the extraction of antimicrobial compounds by more sophisticated procedures for extraction in order to increase the yield. The future prospects of present research work includes isolation and purification of the therapeutic antimicrobial agents from the active extract and there further pharmacological evaluation by several method such as – NMR, MS, GC-MS, TLC, HPLC.

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**REFERENCES**