PHYTOCHEMICAL SCREENING FROM LEAF EXTRACTS OF THE PLANT COLEUS FORSKOHLII (BRIG) COLLECTED FROM THE ANANTHAGIRI FOREST AREA, RANGAREDDY DISTRICT, ANDHRA PRADESH, INDIA.

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ABSTRACT

The medicinally important plants of Coleus forskohlii were collected from the forest areas of Ananthagiri, Rangareddy District. This plant belongs to family Lamiaceae. A total of fourteen (14) phytochemicals were screened from the leaf extracts of the above plant. The analysis was studied in different solvents like methanol, acetone, petroleum ether and chloroform including the aqueous extracts. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, Xanthoproteins, glycosides, steroids, phenols, resins, carboxylic acid group in varying concentrations. The present study provides an evidence that solvent extract of Coleus forskohlii contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases and ailments.

Keywords: Coleus forskohlii, Ananthagiri forest, medicinal plant, phytochemical screening,

INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. The use of medicinal plants as traditional medicine is well known in rural areas of the many developing countries. [1] Herbs are mine of medicinal agents and a large number of medicinal herbs are found to be efficacious, cheap and safe in preventing various diseases.
Moreover, use of herbal medicines for the treatment of different ailments is very important in developing countries where the cost of conventional medicines is a burden to the population. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. Herbs being easily available to human beings, have been explored to the maximum for their medicinal properties. Various parts of the plants like roots, leaves, bark, exudates etc. are used as per medicinal properties. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils. Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives. Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defense mechanisms against predation by microorganisms, insects and herbivores.

MATERIALS AND METHODS

Collection and authentication of plant materials: The leaves of the plant species were collected wildly from the forest areas of Ananthagiri, Rangareddy District, with the help of local people. The collected plants were identified using available published literature at the Department of Botany, Osmania University, India.

Preparation of extracts: To prepare the Methanolic, Acetonic, Chloroformic and Petroleum ether extracts, 150 g of each of the ten plant material were collected, dried in the oven at 70°C for 4 h and reduced to powder. It was separately macerated with the above solvents and allowed to stand for 72 hrs and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at 5°C in the refrigerator, until when required for use. For the aqueous extraction, 50 g of the plant powder was weighed into 50 ml Eylen-Mayer flask and to this was added 400 ml of distilled water. This was heated to boil using hot plate. The mixture was stirred at regular intervals (3-5 min) for one hour after which it was filtered with No. 1 Whatman filter paper (W AND R BALSON LTD, ENGLAND). The filtrate was then filtered sterilized using a membrane filter of pore size 0.45 cm diameter (miillipores corp, England). The extracts were concentrated in a hot water bath at 80°C for 5 h during which 0.5 g charcoal was added to decolorize it. Sterile decolorized filtered extract was then refrigerated at 50°C until required for use.
Phytochemical analysis: Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts as well as powder specimens using the standard procedures as described by [9, 10, 11, 12] [14, 15, 16, 17].

Qualitative analysis: Preparation of reagents: Preparation of Maeyer’s reagent: 0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0 g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water. Preparation of Dragendorff’s reagent: Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in 1:1 ratio.

Phytochemical screening for different compounds
Test for Flavonoids: 0.5 g of various extract was shaken with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following test: 3 ml of the filtrate was mixed with 4 ml of 1% aluminium chloride in methanol in a test tube and the colour was observed. Formation of yellow colour indicated the presence of flavonols, flavones and chalcones.

Test for Alkaloids: 0.5 to 0.6 g of various extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer’s and Dragendorff’s), after which it was observed whether the alkaloids were present or absent in the turbidity or precipitate formation.

Test for Glycosides: Five ml each of various extract were hydrolysed separately with 5 ml each of conc. HCl and boiled for few hours on a water bath and hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

Test for steroids: 0.5 g of the various solvent extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids.
Test for Phenols: To 1ml of various solvent extracts of sample, 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

Test for Terpenoids (Salkowski test): 5 ml of various solvent extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H2SO4). A layer of the reddish brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Test for Saponins: 0.5 g of various solvent extract was dissolved in boiling water in a test tube. Test cooling aqueous extracts were mixed vigorously to froth and the height of the froth was measured to determine the saponin contents in the sample. 2.0 g of the powdered plant material was boiled in distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponins.

Test for Resins: One ml of various solvent extract were treated with few drops of acetic anhydride solution followed by one ml of conc. H2SO4. Resins give colouration ranging from orange to yellow.

Test for Tannins 0.25 g of various solvent extract was dissolved in 10 ml distilled water and filtered. 1% aqueous Iron chloride (FeCl3) solution was added to the filtrate. The appearance of intense green, purple, blue or black colour indicated the presence of tannins in the test samples.

Test for Cardiac glycosides (Keller-Killani test) 5 ml of various solvent extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride (FeCl3) solution, followed by the addition of 1 ml concentrated sulphuric acid. Brown ring was formed at the interface which indicated the presence of deoxy-sugar of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the layer.

Test for Carboxylic acid: One ml of the various extracts was separately treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of carbon dioxide) indicates the presence of carboxylic acid.
Test for Coumarins: 0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Test for Quinones One ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

Test for Xanthoproteins One ml each of the various extracts were treated separately with few drops of conc.

RESULTS AND DISCUSSIONS
The results of the phytochemical analysis of the leaf extracts in various solvents has shown a remarkable variation in the presence the above studied phytochemical compounds in the studied taxa. The detailed investigations of phytochemicals in various solvents are shown in TABLE 1. The study revealed that the leaf extracts of *Coleus forskohlii* is showing maximum presence of Flavonoids and alkaloids in aqueous and acetonic extracts, whereas it is minimum in methanolic, petroleum ether and Chloroformic extracts. The glycosides are adequately present in all the solvents except in methanol and chloroform extracts they are absent. Steroids are maximum in aqueous extracts, adequately present in methanolic and petroleum ether extracts but they are absent in acetic and chloroformic extracts. Phenols are maximum in acetonic extract and adequately present in all other four studied extracts. While terpenoids are completely absent in petroleum ether and chloroformic solvents but are adequately present in other three extraction. Saponins are adequately present in all the solvent extracts i.e., methanolic, acetic, petroleum ether and chloroformic including the aqueous extractions. In contrast to the above all resins are completely absent in the studied taxa in all the solvents. Tannins are maximum in acetonic extracts but adequately present in other extracts. Whereas cardiac glycosides are maximum in aqueous extractions and adequately present in other four solvent extracts. Similarly the carboxylic acids, coumarins, quinones and Xanthoproteins are observed maximum in acetonic extractions but are adequately seen in methanol, petroleum ether and Chloroform solvents including aqueous extractions. The present study regarding the qualitative analysis of the selected medicinal plants is in agreement with the previous findings of the various researchers.
Table -1 Phytochemical screening in leaf powder extracts of Coleus forskohlii

<table>
<thead>
<tr>
<th>S.No</th>
<th>PHYTOCHEMICAL CONSTITUENTS</th>
<th>DIST. WAT</th>
<th>METH</th>
<th>ACE</th>
<th>P.ETH</th>
<th>CHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
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<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
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<td>+</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
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<td>Phenols</td>
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<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>Terpenoids</td>
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<td>+</td>
<td>++</td>
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</tr>
<tr>
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<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>10</td>
<td>Cardiac Glycosides</td>
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</tr>
<tr>
<td>11</td>
<td>Carboxylic acid</td>
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<td>++</td>
<td>++</td>
<td>++</td>
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</tr>
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<td>+</td>
</tr>
<tr>
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<td>Xanthopretins</td>
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CONCLUSION

Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs. Plants have successfully passed the tests of commercial screenings. Thus, from the present study the plant leaf extracts of Coleus forskohlii shown an abundant production of Phytochemicals as secondary metabolites and they can be used in the pharmaceutical industries for producing a potent drug against various diseases and disorders. The results of the study gives a basis of its use in traditional medicine to manage ailments and disorders. It also contains some biologically active constituents worthy of further investigations.

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REFERENCES


