PHYTOCHEMICAL ANALYSIS OF CAYARTIA PEDATA, ENICOSTEMMA AXILLARE AND TERMINALIA CHEBULA

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ABSTRACT

South-eastern India has been known for its rich biological diversity. For this study, three medicinal plants such as Cayartia pedata, Enicostemma axillare and Terminalia chebula were selected. The aim of the present study was to investigate the presence of phytochemicals and to determine the bioactive components of the selected medicinal plants. Soxhlet apparatus was used for the organic solvent extraction. Solvent used were ethanol. Total phenolic contents of the ethanol extracts of the plants were determined by Ferric chloride test method whereas total flavonoid contents of the ethanol extracts were determined by the Shinoda test. Proteins, carbohydrates, phenols, tannins, flavonoids, saponins, were detected in all of the plants tested.

Our findings provided evidence that ethanol extracts of tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases.

KEYWORDS: Ethanol, Antiulcer, Antibacterial, Cayartia pedata, Enicostemma axillare, Terminalia chebula.

INTRODUCTION

Cayratia pedata (Tamil: Pannikkodi, Kattupirdai, Sanskrit:suuaha, Gobhapadi, Malayalam, Velutta sori, Tripadi) is an indigenous herb belonging to the family Vitaceae, In south region of Tamilnadu it is easily found in kolli hills. It is a woody climber with cylindrical stem and grown mostly in semi evergreen to evergreen forest. Traditionally, the leaves of this plant were used in the treatment of ulcer and diarrhea. The decoction of the leaves was used to check uterine and othersfluxes.¹ The plant has also found to possesses anti-inflammatory,²
and antinociceptive activities. The plant extract has been reported to possess antibacterial, antiviral, anticancer and diuretic activities.

*Enicostemma axillare* (Lam) family (Gentianaceae), Locally called as Choto Chirayita in Marathi and Mamajaka in Sanskrit and Vellerugu has been used traditionally for many diseases.\(^3\) According to Ayurvedic Literature survey. The fresh juice of leaves has been used as a bitter tonic, to control arthritis, in typhoid fever and as cooling agent. It is used as stomachic and laxative, blood purifier in dropsy, rheumatism, abdominal ulcer, hernia, swellings, itches and insect poisoning. The plant paste is applied on boils. The leaves are fed to cattle to increase appetite. Plant extracts were reported for the biological activities such as anti diabetic, anti-inflammatory, stimulant, astringent and diuretic, and useful in skin diseases. The plant possesses stimulant, astringent, diuretic and anthelmintic properties.\(^4\) It also acts as ethnomedicine for snakebite.

*Terminalia chebula* belongs to the family “Combretaceae”, commonly known as black myrobalan and usually called Kadukai in Tamil. *T. chebula* is a medium- to large-sized tree distributed throughout tropical and sub-tropical Asia, including China and Tibet. This tree is found in the forests of northern India, Uttar Pradesh and Bengal, and is common in Tamil Nadu, Karnataka and southern Maharashtra. The traditional Indian systems of Ayurveda and Siddha medicines support the importance of medicinal plants to treat diseases,\(^5\) *T. chebula* is routinely used as traditional medicine by tribals of Tamil Nadu to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections.\(^6\) Plant fruits appear to have evolved complex antibiotic compounds to cure various diseases like cancer, cardiovascular, digestive and pathogenic bacteria.

Nature has been a source of medicinal agents for thousands of years and since beginning of man.

Presently in the developing countries, synthetic drugs are not only expensive and in adequate for the treatment of diseases but are also often with alterations and side effects, therefore the need to search for plants of medicinal value. The plants used in the present study are *Cayartia pedata*, *Enicostemma axillare* and *Terminalia chebula* which are used traditionally for the treatment of ulcer, wounds and others diseases. The main objective of this study is to examine the comparison of bioactive Compounds in ethanolic extract of *Cayartia pedata*,...
Enicostemma axillare and Terminalia chebula using phytochemical analysis by carry out its pharmacognostic evaluations.

MATERIALS AND METHODS
Collection of plant materials and authentication
Cayartia pedata, Enicostemma axillare and Terminalia chebula were collected at the kolli hills of Salem district, Tamil nadu south India. The plants were authenticated at the Herbarium Department of Botany, Annamalai university, Annamalai nagar, Tamil nadu. The voucher number is BSI/SC/5/23/08- 09/Tech.952. The fresh whole plants materials was washed under the running tap water, dipped on saline overnight, air dried and placed in polythene bags.

Drying of plant materials
The leaves were shade dried at room temperature in a clean environment over a period of two weeks and powdered in a domestic grinder and stored in an airtight container in cool and dark place to present the deterioration by evaluated temperature light and moisture.

Preparation of crude ethanol plants extract
The coarse power of plants material was successively extracted with ethanol using hot continuous extraction-soxheletion. All the plants extracts were concentrated to dry using rotary evaporation and presented in refrigerator.

Preliminary phytochemical Screening
Phytochemical screening of the plants extract were carried out to determine the presence and above phyto constituents of as per the method and test screen by.[7]

1. CARBOHYRATE (Molisch’s Test)
Mix the Crude extract with 2ml of Molisch’s reagent and shake the mixture properly. After that, add 2ml of concentrated H$_2$SO$_4$ carefully along the side of the test tube. Appearance of a violet ring at the interphase indicates the presence of carbohydrate.

2. PROTEIN (Millon’s Test)
Mix the crude extract with 2ml of Millon’s reagent, white precipitate appeared which turned red upon gentle heating confirms presence of protein.
3. ALKALOID
a. Wagner’s Test
Treat the extract with few drops of Wagner’s reagent. Formation of reddish brown precipitate indicates presence of alkaloids.

b. Mayer’s Test (Potassium Mercuric Iodine Solution)
Treat the acid layer with few drops of Mayer’s reagent. Formation of creamy white precipitate indicates presence of alkaloids.

4. FLAVONOIDS
a. Shinoda Test
To the extract, add a few fragments of magnesium ribbon and concentrated hydrochloric acid. After a few minutes appearance of red to pink colour presence of flavonoids.

b. Ferric Chloride Test
Add a few drops of neutral ferric chloride solution to the extract, formation of blackish green colour indicates the presence of phenolic content.

5. TERPENOIDS (Liebermann-Burchard Test)
To 1ml of chloroform, add 2ml of concentrated H_2SO_4 and 1ml of acetic anhydride to the sample, formation of deep red colour (or) reddish violet indicates presence of triterpenoids.

6. PHENOL /TANNIN
a. Ferric chloride test
Mix the crude extract with 2ml of 2% solution of FeCl_3. Blue-green (or) black coloration indicates presence of phenols and tannins.

c. Nitric acid test
The extracts were treated with dilute nitric acid separately. Formation of reddish to yellowish colour indicated the presence of tannins and phenolic compounds.

7. ANTHRAQUINONE
   Borntrager’s Test (Free Anthroquinone)
   i. Isolate the extract with chloroform by boiling on water bath. To this extract 2ml add 1ml of dilute 10% ammonia and mixture shake. Record the colour change. A pink-red colour in the ammonial (lower) layer shows anthracene derivatives.
ii. Mix 0.5g sample with 10 ml chloroform for 5 minutes and filter. To the filtrate, add 10% potassium hydroxide solution. Pink-red colour in upper aqueous layer indicates presence of Anthraquinones.

iii. To the extract, sodium hydroxide was added. Blue green or red colour indicates the presence of Quinone.

8. GLYCOSIDE (Salkowski Test)
To the extract, add 2ml of CHCl₃ and Conc. H₂SO₄ and shake gently. Reddish brown colour indicates presence of glycoside.

9. STEROID
a. To the 1ml extract, add 10ml chloroform and concentrated H₂SO₄. Upper layer turns red and sulphuric acid layer show yellow with green fluorescence indicates presence of steroids.

b. To the 1ml extract, add 2ml chloroform and concentrated H₂SO₄ then acetic acid. Formation of greenish coloration indicates the presence of steroids.

c. To the 1ml extract, add 5ml of distilled water shake vigorously. Formation of foam indicates presence of steroid.

9. SAPONIN (Froth Test)
To the 5ml of extract, add few drops of sodium bicarbonate solution. Mix vigorously and left for 3 minutes. Honey comb like froth is formed.

10. PHLOBATANNIN
1 ml of extract was boiled with 1% hydrochloric acid. Formation of red precipitate indicates the presence of phlobatannin.

11. VOLATILE OIL
Mix 0.5g of powdered sample with 1ml of 0.1M NaOH solution and 1% aqueous HCl. Formation of white precipitate indicates presence of volatile oil.

13. RESINS
a. Dissolve the extract in acetone and pour the solution in distilled water. Turbidity indicates presence of resins.

b. Extract 0.5g of pulverized plant with 15ml petroleum ether and filter. Dispense 5ml of filtrate into a test tube and shake vigorously with equal volume of copper acetate solution.
Allow the mixture to stand for a few minutes. Formation of a green colour solution indicates presence of resins.

RESULTS

The Phytochemical active compounds of Cayartia pedata, Enicostemma axillare and Terminalia chebula were qualitatively analyzed and the tested were summarized in Table 1. The results revealed the presence of medically active compounds in the three plants studied. From the table, which could be seem that the ethanolic extracts of three plants showed the presence of Phytochemical active compounds such as carbohydrates alkaloids, Terpenoids, glycoside, steroids, saponin, Volatile oil, Resins, protein, flavanoid, and phenol. Tannin were absent from the leaves of Cayartia pedata. Phlobatannin were absent only in the leaves of Enicostemma axillare and also anthraquinones were absent in the leaves of the plants extracts of Cayratia pedata and Enicostema axillare .while Terminalia chebula plant extract showed the presence of all Phytochemical active compounds mention in the table.

Table -1 Results of Phytochemical Screening

<table>
<thead>
<tr>
<th>S. No.</th>
<th>TEST</th>
<th>PLANT SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cayratia pedata</td>
</tr>
<tr>
<td>1.</td>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Protein</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Flavanoid</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Terpenoid</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Phenol</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Tannin</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>Phlobatannin</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td>Volatile oil</td>
<td>+</td>
</tr>
<tr>
<td>14.</td>
<td>Resin</td>
<td>+</td>
</tr>
</tbody>
</table>

Positive (+) and Negative (-).

DISCUSSION

Medicinal plants contain some organic compounds which provide definite physiological activities on the human body and the active substances include tannins, alkaloids, carbohydrates, terpenoids, steroid and flavonoids.\(^{[8,9]}\) Phytochemical analysis studied on the plant extract revealed the presence of constituents to exhibits medicinal as well as
physiological activities.\cite{10} These bioactive compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compound are widely used in the human health, veterinary, agriculture, scientific research and countless other areas.\cite{11} Phytochemical constituents belonging to large number of chemical classes have been shown to inhibitory effects on microorganisms invitro.\cite{12}

Phytomedicines can be derived from leaves, flowers, roots, fruits and seed of the plant part.\cite{13} Synthesis of complex chemical substance.\cite{14,15}

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites.\cite{16} They process biological properties such as anti apoptosis, antiaging, anti carcinogen, anti inflammations, antiatherosclerosis, antiulcer and cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.\cite{17} The antioxidant of medicinal plants which are rich in the form of phenolic compounds such as flavonoid, phenolic acid, tocopherols etc.\cite{18} Tannins are effective antioxidant and strong anticancer activities.\cite{19,20,21}

The plant extracts were constituted saponins which are known to produce inhibitory effect on inflammation. Saponin has the property of precipitating and coagulating red blood cells and characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity cholesterol binding properties and bitterness.\cite{22} Steroids have been reported to have antibacterial properties and also relationship with compound such as sexhormones.\cite{23}

Alkaloids have been associated with their common biological properties of cytotoxicity, analgesic, antispasmodic and antibacterial,\cite{24,25,26,27} properties of alkaloids. Glycosids are known to lower the blood pressure according to many studies.\cite{28}

This result showed in this study suggested that the Phytochemical compounds have bioactive constitutes and these plants proved to be an increasingly antioxidant properties of bioactive compounds of substantial medicinal uses.

**CONCLUSION**

This study showed the presence of bioactive constituents in the ethanolic plant extracts of *Cayratia pedata, Enicostemma axillare* and *Terminalia chebula*. Several authors confirmed that the phytochemicals in the plants contribute medicinal as well as physiological properties.
Hence, extracts from the plants have been used as drugs and further more to elucidate the plant extracts.

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