



E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2017; 5(2): 31-34
Received: 08-01-2017
Accepted: 09-02-2017

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Qualitative study of certain phytochemical and pharmacognostic properties of two ethnomedicinal plants used for gastrointestinal disorders: *Centella asiatica* (L.) and *Paederia foetida* (L.) of Darrang district, Assam

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Abstract

Phytochemical properties of two medicinally important plants of Darrang district, Assam, viz. *Centella asiatica* (L.) and *Paederia foetida* (L.), has been studied using six different extract solutions- Methanol, Chloroform, Petroleum ether, Benzene, Hexane and water. All the four plants contain more or less amount of tannin, flavanoids, phenol and alkaloid. Cardiac glycoside and trapezoid are completely absent in *Paederia foetida* whereas present in *C. asiatica*. Anthraquinone, steroid, reducing sugar and resin are absent in both. *P. foetida* shows highest extractive value in Methanol extract while *C. asiatica* in hexane extract.

Keywords: *Centella asiatica* (L.) *Paederia foetida* (L.), phytochemistry, gastrointestinal activity

1. Introduction

From ancient time huge number population is depending on the traditional practitioners, who use locally available medicinal plants to meet their primary health care needs. Though, modern medicines are available in the market, herbal medicine still retained their image. Medicinal properties of plants are due to the active chemical constituents present in different parts of the plant such as carbohydrates, proteins, enzymes, fats, oils, terpenoids, flavonoids, sterols simple phenolic compounds etc. are present in ample amount^[1,2]. Also the content of such chemicals may vary according to the change in their geographic positions. Since from the beginning of this century, there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. In this study a brief comparative analysis of few aspects of two medicinally important plants commonly used for gastrointestinal disorders has been carried out^[3-5].

Paederia foetida (L.) (Rubiaceae) is commonly known as 'Bhedai lata' in Assam. *P. foetida* is known for the strong odour exuded when its leaves or stems are crushed or bruised^[6]. It is used to treat enteromegaly, enteritis, flatulence, gastromegaly, rheumatism, rhinosis, toothache, stomachache and sore in folk medicine^[7]. *P. foetida* is also a popular shrub used as a remedy for diarrhea and dysentery in Assam. Its stems & leaves, after crushing are applied on the broken part of hands or legs for sucking damaged blood as well as quick healing^[8]. Juice of the root is also used for piles, liver and spleen ailments. In Ayurveda, used for asthma, bowel problems, diarrhea, diabetes, rheumatism and seminal weakness^[9].

Centella asiatica (L) (Apiaceae) is a small herb and grows wild in wet places. The herb is used for treatment of bronchitis, asthma, gastric, kidney troubles, leprosy, skin diseases, cough, cold, fever, to improve memory, measles, diarrhoea, dysentery, constipation, leucorrhoea, jaundice, dysmenorrhoea and applied on rheumatism. The plant has a good number of phytochemicals and high antioxidant activity^[10,11].



Fig 1: *Paederia foetida* (L.)



Fig 2: *Centella asiatica* (Linn.),

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2. Material and methodology

2.1 Collection of plant material

Plants were collected from Mangaldai, Darrang district, Assam, India and identified by IBT Hub, Mangaldai College. Collected plants were washed with water and dried in the ventilated shed area in the lab. Grind powder was used for determination of powder microscopy, physiochemical characteristics and phytochemical analysis.

2.2 Powder Microscopic Examination

Glycerol reagent as per WHO guideline was prepared. Small amount of powder was taken on the slide to this mixture was added and covered with cover slip and examined under microscope.

2.3 Determination of Extractive Value

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material.

1. Evaluation of water extractive value: About 3 g of accurately weighed coarsely powdered, air-dried sample was transferred into a glass-stoppered, 30 mL reflux conical flask, followed by the addition of 50 mL of boiled water. The flask was well shaken, and allowed to stand for 10 minutes. It was cooled and filtered. Filtrate was transferred to an evaporating dish; the solvent was evaporated on water bath, allowed to dry for 30 minutes, finally dried in an oven and residue was weighed. Percentage of water-soluble extractives was calculated with reference to the air-dried drug.

$$\text{Extractive value} = \frac{\text{final weight of the extract}}{\text{initial weight of the dry matter}} \times 100$$

2. Alcohol soluble extractive value: 3 g of dried samples of plant material were macerated with 60 mL of alcohol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution to minimize the loss of methanol. Evaporated the filtrate to dryness in a petri dish dried at 105 °C and weighed. Percentage of Alcohol soluble extractive was calculated with reference to the air-dried samples.
3. Petroleum ether extractive value: 1.5 g of dried samples of plant material was macerated with 30 mL of alcohol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution to minimize the loss of Petroleum ether. Evaporated the filtrate to dryness in a petri dish dried at 100 °C and weighed. Percentage of Alcohol soluble extractive was calculated with reference to the air-dried samples.
4. Benzene extractive value: 1.5 g of dried samples of plant material was macerated with 30 mL of alcohol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution to minimize the loss of Benzene. Evaporated the filtrate to dryness in a petri dish and dried at 100 °C and weighed. Percentage of Alcohol soluble extractive was calculated with reference to the air-dried samples.
5. Chloroform extractive value: 1.5 g of dried samples of plant material was macerated with 30 mL of alcohol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter,

it was filtered rapidly taking precaution to minimize the loss of Chloroform. Evaporated the filtrate to dryness in a petri dish dried at 100 °C and weighed. Percentage of Alcohol soluble extractive was calculated with reference to the air-dried samples.

6. Hexane extractive value: 1.5 g of dried samples of plant material was macerated with 30 mL of alcohol in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours separately. Thereafter, it was filtered rapidly taking precaution to minimize the loss of Hexane. Evaporated the filtrate to dryness in a petri dish dried at 100 °C and weighed. Percentage of Alcohol soluble extractive was calculated with reference to the air-dried samples.

2.4 Phytochemical Analysis of Stem and Leaf of the Plant:

Test for tannin: i) To 0.5 ml extract solution added 1ml distilled water and 1-2 drops of ferric chloride solution to it and observed blue black coloration which indicate presence of tannin. ii) 10% lead acetate solution was added to 0.5 ml extract solution and observed for white precipitation which indicates presence of tannin. Test for saponin: i) 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing shows the presence of saponin. Test for flavonoid: 0.2 g of the extract was dissolved in 10% NaOH solution, yellow coloration indicates the presence of flavonoid. Test for phenol: To 2ml of extract solution, added 2ml of alcohol and few drops of ferric chloride solution and observed for coloration. Test for cardiac glycoside: 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of conc. Sulphuric acid. A brown ring at the interface indicated the presence of cardiac glycoside. (A violet ring may appear below the ring while in the acetic acid layer a greenish ring may be).

Test for alkaloid: Hager's test- Drug solution + few drops of Hager's reagent (Saturated solution of Picric acid). The formation of crystalline yellow ppt confirms the presence of alkaloid. Wagner's test- Drug solution + few drops of Wagner's reagent (dilute Iodine solution). The formation of redish-brown precipitate confirms the presence of alkaloid. Test for anthraquinone: To 0.2g of extract, added 5ml of chloroform and 5ml of 10% ammonia solution. The presence of bright pink colour in the aqueous layer indicated the presence of anthraquinone.

Test for trapezoid and steroid: 5ml of extract solution was mixed in 2ml of chloroform, and 3ml of conc. sulphuric acid was added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of trapezoid. Red colour at the lower surface indicates presence of steroid. Test for reducing sugar: To 0.5 ml of extract solution, 1ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitation. Test for resins: HCl test- One gram of the drug was extracted with a few drops of acetone and 3 ml of dilute HCl was added to it. Formation of pink colour after heating the solution on water bath for 30 minutes indicates the presence of resins. FeCl₃ test- Few drops of FeCl₃ solution was added to the alcoholic extract of the drug. Formation of the greenish blue colour indicates presence of resins.

Presence of phytochemicals in the solvents are expressed according to their intensity of presence using '+' signs.

3. Result and Discussion

Powder analysis shows significant micro morphological features present in the tissue of the four plants.

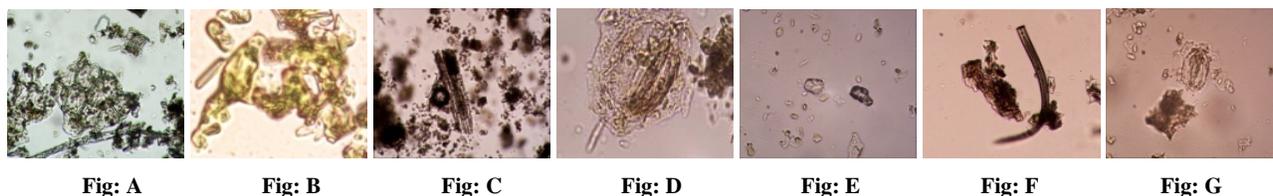


Fig 1: Powder analysis of *Paederia foetida* (A-D): A. Tissue part, 40x, B. Loose chloroplast, 40x, C. Fibre,40x, D. Stomata, 40x, Powder analysis of *Centella asiatica* (E-G): E. Pulp cells and crystals, 40x, F. Trichome, 40x, G. Stomata, Microsphenoidal crystals, 40x.

Both the plants contain several microsphenoidal crystals and druses which indicates the rich secondary metabolite content and the high medicinal potentiality of the plants. Structures

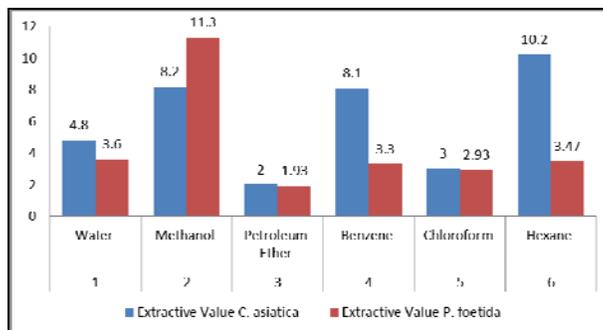
like stomata, trichome and fibres are also distinct in powder analysis. Presence of pulp cells coincides with the good food quality of the leaves.

Table 1: Data showing the presence or absence of various phytochemicals in *P. foetida*

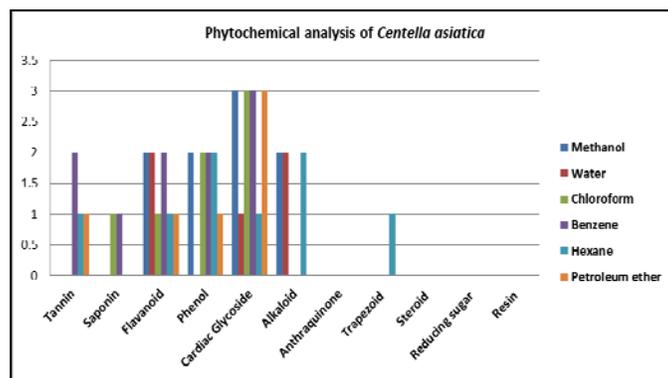
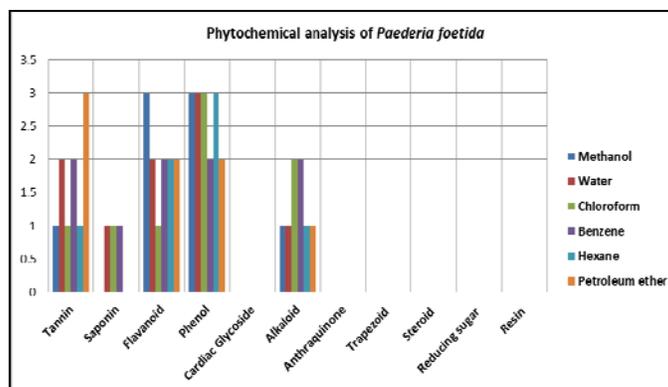
Chemicals	Methanol	Water	Chloroform	Benzene	Hexane	Petroleum
Tannin						
a)FeCl ₃ test	-	+	-	++	-	-
b)PbAc ₃ test	+	++	+	+	+	+++
Saponin	-	-	+	+	-	-
Flavanoid	++	++	+	++	++	++
Phenol	+++	+++	+++	++	+	++
Cardiac glycoside	-	-	-	-	-	-
Alkaloid	+	+	++	++	++	+
Anthraquinone	--	--	--	--	--	--
Trapezoid	--	--	--	--	--	--
Steroid	--	--	--	--	--	--
Reducing sugar	--	--	--	--	--	--
Resin						
a)HCl test	--	--	--	--	--	--
b)FeCl ₃ test	--	--	--	--	--	--

Table 2: Data showing the presence or absence of various phytochemicals in *C. asiatica*

Phytochemicals	Methanol extract	Water extract	Chloroform extract	Benzene extract	Hexane extract	Petroleum extract
Tannin						
1. FeCl ₃ Test	-	-	-	-	-	+
2. (Pb(CH ₃ COO)) ₂	-	-	++	++	+	-
Saponin	-	-	+	+	-	-
Flavonoid						
1. 10% NaOH Test	-	-	+	-	-	+
2. NH ₄ Test	++	++	+	++	+	+
Phenol	++	-	++	++	++	+
Cardiac glycoside	+++	+	+++	+++	+	+++
Alkaloid						
1. Hager's Test	++	++	-	-	++	-
2. Wagner's Test	-	-	-	-	-	-
Anthraquinone	-	-	-	-	-	-
Trapezoid	-	-	-	-	-	-
Steroid	-	-	-	-	-	-
Reducing sugar	-	-	-	-	-	-
Resin	-	-	-	-	-	-



Graph 1: Extractive values in different solvents

Graph 2: Phytochemical analysis of *C. asiatica*Graph 3: Phytochemical analysis of *P. foetida*

For *P. foetida* Methanol is the best extractive solution whereas for *C. asiatica* water and hexane shows high extractive values. Methanol, Benzene and hexane equally serve as good extractive solvent for *C. asiatica*. Petroleum ether and Chloroform shows equally low extractive value for both the plants.

Tannin content is higher in *P. foetida* than *C. asiatica*. Cardiac glycoside is present in high amount in *C. asiatica* indicating potent medicinal value for heart ailment also which coincides with the review by Mota, 2016. But it is totally absent in *P. foetida*. Saponin, flavanoids, phenols and alkaloids are present more or less in equal amount in both the plants. Anthraquinone, steroids, trapezoids, reducing sugar and resins are absent in both the plants.

4. Conclusion

Present study suggests that various medicinally important secondary metabolites are present in both the plants collected from Darrang District, Assam. There is scope of detail studies on chemical composition, isolation of active constituents and pharmacological evaluation of the plants collected from this region.

5. Acknowledgement

Authors are thankful to DBT, Govt. of India for providing necessary funding and the Principal, Mangaldai College, for his encouragement and for the facilities provided in the Institutional Level Biotech Hub, during the preparation of this paper.

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