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**PHYTOCHEMICAL SCREENING ON THREE TRADITIONAL
MEDICINAL PLANTS AGAINST PILES**

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ABSTRACT

*The aim of the present study was to determine phytochemical analysis and the total phenol contents of the leaves of *Paederia foetida*, *Clerodendrum siphonanthos* and *Blumeopsis flava*. Leave extract were prepared with methanol by Soxhlet apparatus. Total phenolic contents were determined by Folin-Ciocalteus reagent method. Alkaloid, phenol, flavonoid, terpenoid, glycoside, saponin and steroid were detected in *Paederia foetida* but high concentration in terpenoid and steroid. Saponin was absent in *Blumeopsis flava*. Steroid was also not found in *Blumeopsis flava* and *Clerodendrum siphonanthos*. The total phenolic contents of the methanol extract of *P. foetida*, *C. siphonanthos* and *B. flava* in terms of Gallic acid equivalent were 138.33 ± 6.41 , 131.67 ± 5.77 and 71.25 ± 7.60 mg/g of extract respectively. *Paederia foetida* exhibited highest total phenolic contents then followed by *C. siphonanthos* and *B. flava*. It is evident that *Paederia foetida* has highest therapeutic efficacy. The present study indicates that these plants are of therapeutic potential due to the presence of various phytochemicals.*

Keywords:

Phytochemical, Phenol, Methanol extract, Soxhlet, Gallic acid.

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1. INTRODUCTION

Plants synthesize numerous complex phytochemicals for nutrition, defense against stress, to fight unwanted predators and facilitate pollination. The different phytochemicals synthesised by plants are very useful for human healthcare as well as for curing of diseases [1]. A number of plants contain secondary phytochemicals such as alkaloid, steroid, tannin, glycoside, phenol, flavonoid, volatile oil, essential oil etc[2,3]. Alkaloids, saponin, tannin, flavonoids are known to have activity against pathogens and therefore aid the antimicrobial activities of medicinal plants

[4]. Phytochemicals are known to possess antioxidant [5], antibacterial [6], antidiabetic and anti-inflammatory[7]. The importance of *Paederia foetida*, *Clerodendrum siphonanthos* and *Blumeopsis flava* are well known as folk medicine.

Paederia foetida of family Rubiaceae is an annual semi woody climber with foetid smell and bitter taste, whole plant has medicinal value. It is used in gout, diarrhoea, dysentery, piles, inflammation of the liver[8]. In Manipur, leaves are used as curry for treatment of Piles and bleeding due to piles. It is used by diabetic patients as helpful in lowering of glucose level in the blood. *Paederia foetida* leaves are useful for the treatment of piles, pain in chest and inflammation of spleen and liver[9].

Clerodendrum siphonanthos is a shrub of family Verbenaceae. This is being used in Indian, Chinese, Thai, Korean, Japanese systems of medicine for the treatment of various diseases such as typhoid, cancer, jaundice and hypertension and also as medicine for applying wounds. However in Manipur, leaves are used as a good medicine for treatment of Piles, tonsillitis, dysentery, cough and fever.

Blumeopsis flava is a herb belongs to family Compositae. Leaves decoction is useful in bronchial congestion, Cold, skin diseases in Mizoram[10]. Its leaf extract is given in bronchial congestion, cold, cough, effective in skin diseases and applied as bandage against back-ache[11]. However, decoction of whole plant is used externally for treatment of Piles in Manipur.

A piles is an illness that gives excruciating pain to the patients because of a swollen rectum. Haemorrhoids are the most common Piles and painful to patients. Haemorrhoid (Piles) are swellings that develop from the lining of the anus and lower rectum. Sometimes Piles patients face severe bleeding with pain. However, there are no sufficient reports about the uses of plants in treatment of piles in India. Therefore the present study is under taken the quantitative and qualitative analysis of these three plants.

2. MATERIALS AND METHODS

Plant Material Collection: The present study included three wild plant species which were *Paederia foetida*(Oinam), *Clerodendrum siphonanthos*(Charoi -utong), and *Blumeopsis flava*(Haochak). Fresh leaves were collected from different places of Sadar Hills, Senapati District of Manipur. The collected plant species were used for phytochemical analysis.

Preparation of Plant Extract: The leaves of collected three wild medicinal plants were washed under running tap water to remove dust particles and rinsed again with distilled water. The plant samples were shaded; air dried for one week and then evaporated water molecules under hot air oven at a temperature of 35°C- 40°C for 2 days. The dried leaves were ground into powder form by using grinder and stored in polythene bags for chemical analysis.

Solvent extraction: Crude plant extract was prepared by Soxhlet extraction method. For methanolic extraction, each 20gm of powdered plant material was uniformly packed into thimble and extracted with 250 ml of methanol separately at 65°C. The process of extraction continues

till the solvent in siphon tube of an extractor become colourless. The extracted aliquotes were cooled down and taken in colour amber bottles and kept in refrigerator for further analysis.

Phytochemical screening: All the plant extracts were quantitatively tested by using standard method for the presence of chemical constituents such as alkaloids, phenols, flavonoids, saponin, glycosides, steroids and terpenoids (12-16) (Table 1).

3. DETERMINATION OF TOTAL PHENOLIC CONTENT

The phenolic contents in the methanolic extract was estimated by Folin-Ciocalteu method with little modification as previously described (17-19). 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na₂CO₃ (2%W/V) were added to 0.5 ml of the sample (3 replicates) of each plant extract solution (1 mg /ml). The mixture was allowed to stand at 20°C for 30 minutes and the absorbance of the developed colour was measured at 760 nm using UV-visible Spectrophotometer. Gallic acid was used as standard (1 mg/ml) to calculate total phenolic content. All the tests were performed in triplicates. Total phenolic content in the plant extract was expressed as Gallic acid equivalent (mg of Gallic acid equivalent /g of dry weight sample) and was calculated by the formula [20].

$$T = CXV/M$$

Where, T = Total content of phenolic compound, mg/g of plant extract, in GAE;

C = Concentration of Gallic acid established from the Calibration curve, µg/ml;

V = Volume of extract; M = Weight of methanolic plant extract, g.

4. STATISTICAL ANALYSIS

The result of phenolic content in the plants extract were expressed as Mean ± Standard Deviation (SD) by using Microsoft excel 2007.

5. RESULT AND DISCUSSIONS

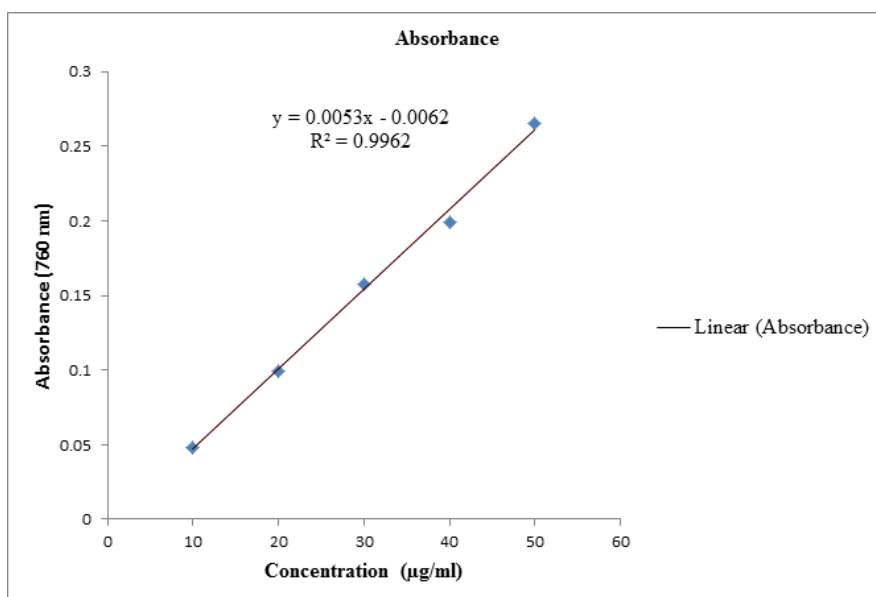
Table 1: Comparative Analysis of Phytochemical Constituents

Chemical Constituents	Chemical tests	<i>Paederia foetida</i>	<i>Clerodendrum siphonanthos</i>	<i>Blumeopsis flava</i>
Alkaloid	Mayer's test	+	+	+
Phenols	Potassium dichromate	+	+	+
Flavonoid	Shinoda test	+	+	+
Terpenoid	Salkowski's test	++	+	+
Saponins	Form test	+	++	-
Steroid	Salkowski's test	++	-	-
Glycosides	Keller-Kiliani test	+	+	+

- indicates absent
+ indicates present
++ indicates high concentration

Table 2: Total Phenolic content in the methanolic extracts

Sample	Mg of gallic acid/g of extract (Mean $\pm$ Standard Deviation)
<i>Paederia foetida</i>	138.33 $\pm$ 6.415
<i>Clerodendrum siphonanthos</i>	131.67 $\pm$ 5.770
<i>Blumeopsis flava</i>	71.25 $\pm$ 7.603

**Graph 1:** Gallic acid Standard Curve

The phytochemical analysis of three selected plants have shown the presence of alkaloids, phenols, glycosides, flavonoids and tenpenoids (Table 1). They also contained saponins (except *Blumeopsis flava*), and steroids (except *Clerodendrum siphonanthos* and *Blumeopsis flava*).

However, Terpenoid and steroid concentration were highly present in *Paederia foetida*. High concentration of saponin was shown in *Clerodendrum siphonanthos*. Terpenoids are known to posses antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic and antiinflammatory properties [21,22]. Steroid help in regulating the immune response [23]. Flavonoid are present in all three selected plants. It is a water soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [24,25]. Some workers also reported that *Paederia foetida* has antioxidant activity [26].) and antioxidant and antimicrobial activity [27] in Assam. Antibacterial efficacy of *Paederia foetida* against multidrug-resistant enteric pathogenic bacteria have been reported [28]. They also detected the presence of phenol, saponin, tannin, terpenoid, alkaloid, reducing sugar, flavonoids in *Paederia foetida*. Some workers studied phytochemical analysis in different *Clerodendrum species* [29,30] and *Blumeopsis flava* [10] and revealed the presence of alkaloids, steroids, phenol, flavonoid, terpenoids in methanolic extract.

The total phenol contents of three crude extracts determined by Folin- Ciocalteu method were reported as Gallic acid equivalent. The standard graph for Gallic acid is represented in Graph . ($Y=0.0053x-0.0062$; $R^2=0.9962$). Where, Y is absorbance at 760 nm and X is concentration. The total phenolic contents of the methanolic extract of *Paederia foetida*, *Clerodendrum siphonanthos* and *Blumeopsis flava* were 138.33 ± 6.415 , 131.67 ± 5.770 and 71.25 ± 7.603 mg/g of Gallic acid equivalent per gram of extract respectively (Table 2). *Paederia foetida* extract contained the highest phenolic content then followed by *Clerodendrum siphonanthos* and *Blumeopsis flava*. In Assam, the total phenolic content in *P. foetida* were recorded as 143, 266 and 384.5 mg/g [10] . The present study shows that phenolic content of *Paederia foetida* (138.33 ± 6.415 mg/g) is near the above recorded data.

6. CONCLUSION

In the present study, phytochemical screening revealed that the presence of alkaloid, phenol, flavonoid, saponin, glycosides, high concentration of terpenoid and steroid in *Paederia foetida* . However, in *Clerodendrum siphonanthos* steroid was not found but saponin was recorded in high concentration. In *Blumeopsis flava* saponin and steroid were absent. Reports from different literature have shown that secondary metabolites are of great importance in the field of drug research for the production of new drugs for curing of various diseases. *Paederia foetida* shows maximum total phenolic contents then followed by *Clerodendrum siphonanthos* and *Blumeopsis flava*. It proves that *P.foetida* has the maximum therapeutic potential than others. This suggest that the investigated plants may be possess antiinflammatory, antimicrobial, antioxidant, antitumour, antiviral and insecticidal activities. Therefore these plants may be used to cure piles and other diseases as traditional herbal medicine.

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