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Phytochemical studies of some plants used by the Paite tribe of Manipur, North-East India.

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Abstract- *The present study deals to find out the total content of some phytoconstituents like phenols, tannins, flavonoids of some plants used by the Paite tribe of Manipur. The Paite occurred mainly in Churachandpur district of Manipur. The study results of the presence of secondary metabolites. The plant parts may be employed as an alternative source for flavonoids, phenols and tannins for primitive traditional remedies.*

Keywords- Manipur, metabolites, Paite, Phytoconstituents

1. INTRODUCTION

Nature has endowed us with a variety of plants with immense medicinal values. India is one of the 12 megadiversity regions of the world. India harbors two major hotspots, namely the Eastern Himalayas and the Western Ghats. From the earlier societies till date, people depend on plants and plant products to meet the initial and day today requirements. Higher plants possess active compounds among 80% shows a positive interconnection between the modern therapeutic use and the traditional use of the plants from their derivatives. The knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Farnsworth, 1994).

Wendakoon *et al.*, (2011) have evaluated medicinal plants with selected ethanol concentrations. Khan *et al.*, (2011) have been carried out phytochemical screening and reported the presence of secondary metabolites in 20 medicinal plants of Pakistan. Hidayathulla *et al.*, (2011) revealed the presence of secondary metabolites with antibacterial activity.

Material and methods: The fully matured plants were collected from Churachandpur district. The materials were washed with water thoroughly and shade dried. The plant extracts were taken out using Soxhlet apparatus and the amount of the phytoconstituents were found out by using UV-VIS spectrophotometer.

Extraction of plant material:

200 gm of the fine powdered sample of each of the plant with 250 ml of methanol was placed in the Soxhlet extractor for 48 hours and filtered with Whatman filter paper no.1. After filtration the solvent collected from the extractor was evaporated. The concentrated residue obtained which contains the plant extract were kept in respective airtight bottles was stored for further use.

Qualitative Preliminary Screening:

Phytochemical analysis of all the samples was determined as follows:

1. Test for Flavonoids**:** 250 mg of the alcoholic sample was dissolved in 3.5 ml of ethanol, slightly warmed and then filtered. A few pieces of magnesium chips were added to the filtrate followed with few drops of conc. HCl. A pink, orange or red to purple coloration was taken as a confirmation for the presence of flavonoids (Trease and Evans, 2002).
2. Test for Phenols: To 5ml of the alcoholic sample, 2 ml of distilled water was mixed with a few drops of 10% of aqueous $FeCl_3$ was added. The colour changed into blue-greenish yellow indicated the presence of phenols. (Harborne, J.B, 1873).
3. Test for Tannins**:** 500mg of the extract was mixed with 10 ml of distilled water and filtered. The filtrate was treated again with the addition of few drops of 1% of ferric chloride

solution. Development of a blue-black, green or blue-green precipitate indicated the presence of tannins (Trease and Evans, 2002).

Quantitative Determination of Phenolic compounds and Antioxidants

1. Estimation of Total Flavonoid Content:

20 g of powdered dried sample was weighed and extracted using 200ml of 80% methanol in Soxhlet apparatus for 24 hrs and filtered through Whatman paper no. 1 filter paper. Crude methanolic extract of the sample was obtained by evaporating the extract to dryness. 100 µl of the extract was taken and 100µl of aluminium chloride (10%), 0.1ml of potassium acetate (1M) and 2.7ml of distilled water to make the volume to 3ml. The reaction mixture was kept at room temperature for 30 mins. The absorbance was measured at 415nm using spectrophotometer. The calibration curve was prepared using different concentrations of quercetin expressed in mg/gm dry weight.

2. Estimation of Total Phenol Content:

20 g of powdered dried sample was weighed and extracted using 200ml of 80% methanol in Soxhlet apparatus for 24 hrs and filtered through Whatman paper no. 1 filter paper. Crude methanolic extract was obtained by evaporating the extract to dryness. 100µl of the sample was taken and its volume was made upto 3ml with distilled water. In the test tube containing the sample , 0.5ml of Folin-ciocalteu reagent was added . Then after 2 mins. 2ml of 20% Na_2CO_3 was added and mixed thoroughly. The contents were kept in a boiling water bath for 1 minute. Then the test tubes were cooled in running tap water and absorbance of the blue coloured complex were taken against blank at 650nm with the help of spectrophotometer. The total phenol content was calculated and expressed in mg/g using a standard curve prepared from catechol. Total phenol content was estimated using Folin- Ciocalteu's reagent (FCR) (Thimmaiah, 1999).

3. Estimation of Tannin:

20g of the dried parts were weighed and kept in magnetic stirrer for 3hr after adding 200ml of 80% ethanol (Thimmaiah, 1999). The extracts were centrifuged for 15 min at 10,000 rpm. The supernatants were collected and stored it for further analysis. From the collected supernatants, 0.1 ml of Folin Denis Reagent (FDR) followed by 1 ml of 35% Na_2CO_3. The final volume is made up to 10 ml with distil water. The blue colour appeared is measured at 700 nm by using UV-VIS Spectrophotometer. The calibration curve was prepared using tannic acid in mg/gm dry weight. Total phenol content, flavonoids content and tannin content were expressed as the mean + Standard Deviation.

RESULTS AND DISCUSSION

A total of 5 plant species was taken for phytochemical study. The qualitative preliminary screening of the plants revealed the presence of phenolic compounds in trace amount or abundant. From the qualitative phytochemical analysis, it showed the presence of phenolic compounds viz. flavonoids, phenols, and tannins in all the selected plant species either in trace amount or abundantly. Moreover, to obtain an accurate amount of the present phenolic compounds, quantitative screening was also carried out. Quantitative estimation of phenols was observed as the highest in *Oreocnide frustences* with 55.0 ±0.4750 and lowest in *Artabotyrs hexapetalus* with 0.5 ± 0.0568. Quantitative estimation (in %) of tannins was found to be the highest in *Oereocnide frutescens* Miq. with 11.1± 1.5637 and the lowest in *Portulaca oleracea* L. with 0.6±0.5510. Quantitative estimation of flavonoids (mg/g) in the plants yielded a high content of 47.6±2.89 in *Oreocnide frustences* Miq. while the lowest occurred in *Mallotus philippensis* (Lam.) Mull.Arg.with a value of 2.06±0.1625. It was summarized in Table 2.

Table 1: Preliminary screening of flavonoids, phenols and tannins.

Name of the plant	**Parts used**	**Flavonoids**	**Phenols**	**Tannins**
Artabotrys hexapetalus (L.f.) Bhandari	Leaves	+	+	++
Mallotus philippensi	Leaves	++	+	++

s (Lam.) Mull.Arg.				
Oreocnide frutescens (Thunb.) Miq.	Leaves	+	++	+
Parkia timoriana Merr.	Kernels of pods	+	++	+
Portulaca oleracea L.	Whole aerial part	+	+	+

Key: +=Present (trace amount); ++= abundant

Table 2: Total content of secondary metabolites (mg/g) (*n=3)*

Name of the plant	**Flavonoids**	**Phenols**	**Tannins**
Artabotrys hexapetalus (L.f.) Bhandari	2.3425 ±1.2402	0.5 ± 0.0568	1.1575± 2.3556
Mallotus phillipensis (Lam.) Mull.Arg.	12.15±0.91526	2.0625 ±.1552	1.2638±3.7756
Oreocnide frutescens (Thunb.) Miq.	2.06 ±0.1625	55.0 ±0.4750	11.1± 1.5637
Parkia timoriana Merr.	47.6 ±2.8926	28.25 ±0.2558	8.93 ± 2.1094
Portulaca oleracea L.	3.537 ± 0.7351	2.2325 ±.3798	0.6±0.5510

Conclusion: The plants that we have studied here can be taken as a potential source of useful drugs. It has also been a proof that the folklore medicinal uses of these plants as curative agent and therefore it can also be suggested for further isolation, purification, identification, characterization and elucidation of the structure of the bioactive compounds of the plants that would be obtained with a view to get useful chemotherapeutic agents.

Flavonoids become an integral part of a useful component in pharmaceutical, medicinal and cosmetic applications Presence of flavonoids indicates of having anti-oxidative, anti-inflammatory properties (Panche *et al.*, 2016). Tannins are polyphenolic biomolecule that binds to and precipitates proteins and other organic compounds which are widely found in food plants. They act as natural defender against antimicrobial activity (Chung *et al.*, 1998). Presence of phenols indicates it can act as antioxidant which in turn protecting the human body (Kokate *et al.*,1998). The presence of phenolic compounds validate the claims of the Paite tribe in curing various ailments by using these plants then and now.

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