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COMPARATIVE PHYTOCHEMICAL CHARACTERIZATION OF SELECTED MEDICINAL PLANTS FROM DHEMAJI DISTRICT, ASSAM AND EAST DISTRICT, SIKKIM, INDIA

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Abstract

The aim of the study is to correlate the phytochemical constituents of three potential medicinal plants i.e. *Alstonia scholaris* (L), *Colocasia esculenta* (L), *Nyctanthes arbor-tristis* (L), from Dhemaji district of Assam and East district of Sikkim, India. Methanolic extracts of the plants were used to determine the total quantity of phytochemicals specifically phenols (gallic acid equivalent GAE), flavonoids (Rutin equivalent RT). Bark juice of *A. scholaris* collected from Dhemaji district of Assam contains comparably greater quantity of phenols (51.99 ± 0.020 mg/g) and flavonoids (57.64 ± 0.010 mg/g) than the sample collected from East district of Sikkim (phenol- 47.33 ± 0.019 mg/g and flavonoids- 47.03 ± 0.008 mg/g respectively). The total phenol and flavonoids contents in the leaves of *N. arbor-tristis* collected from Dhemaji district of Assam contains higher quantity (33.40 ± 0.057 mg/g & 86.12 ± 0.015 mg/g) than the sample collected from East district of Sikkim (26.04 ± 0.023 mg/g & 59.71 ± 0.005 mg/g). Though the total quantity of phenols in the rhizome of *C. esculenta* was found to be higher in the plant sample collected from East region of Sikkim (26.96 ± 0.040 mg/g), total quantity of flavonoid was found greater in the sample collected from Dhemaji district of Assam (76.54 ± 0.107 mg/g) than sample collected from East region of Sikkim (31.76 ± 0.007 mg/g). *C. esculenta* and *N. arbor-tristis* collected from Dhemaji district Assam show better free radical scavenging activity than the samples collected from East district of Sikkim. But *A. scholaris* collected from East district of Sikkim showed greater scavenging activity than samples collected from Dhemaji district of Assam.

Keywords: Phytochemical, phenol, flavonoid, scavenging activity

1 Introduction:

There is an inseparable relationship between mankind and plants. People are very much dependent on plants for food, shelter, and medicine etc. people from all over the world uses plants as their primary healthcare support. In India also, mankind are very much dependent on plants for their medicinal values. In India, almost 4, 60,000 people are involved in different kinds of traditional and non-traditional medicine (Mazid *et al.* 2012). This traditional

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knowledge was evolved according to civilization, culture and practices. The medicinal properties of plants lie on certain chemical constituents like alkaloids, phenols, flavonoids etc. These chemical constituents may be different with the variations in geographical areas as well as in meteorological variations. It is therefore, very important to know about the effectiveness of the medicinal plants for the treatment of various diseases by comparing them with the plants found in various parts of the world. In recent years, most of the modern medicines are synthesized from plants. Out of 45,000 plant species found in India, about 15,000-20,000 plants have been found to be of good medicinal value, however, 7500-8000 species are used for their medicinal values by the traditional or tribal communities in the world.]]] They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. 39% of the 520 new permitted drugs between 1983 and 1994 were of natural products or their by-products (Cragg, 2003). The objective of the work is to study the phytochemical constituents along with the antioxidant activity of the plant parts collected from East district of Sikkim and Dhemaji district of Assam and making comparison between them.

2 Materials and methods

2.1. Collection of plants

Different plant parts viz; bark juice of *A. scholaris*, rhizome of *C. esculenta* and leaves of *N. arbor-tristis* were collected from the Dhemaji district of Assam (94° 95 E and 27°05'27"N – 27°57'16" N) and East district of Sikkim (88.36° E 27.19° N), India. The plants were collected on random basis. Standard method regarding collection and preservation of plant specimens was followed (Jain and Rao 1977). The plants were brought to the laboratory to compare their quantitative and qualitative properties.

2.2. Enumeration of studied plants

2.2.1 *Alstonia scholaris*

Belonging to the family Apocynaceae. Also known as “Sotiona” in Assam and “Chattiwan” in Sikkim. These are evergreen plants with flowering season during the month of October. In Dhemaji district of Assam the stem bark juice is used to treat Gastritis. In East district of Sikkim people use the freshly collected stem bark as toothbrush and also used in the treatment of snake bites.

2.2.2 *Colocasia esculenta*

Also known as ‘Kosu’ or ‘Pind-alu’ that belongs to the Araceae family. It is an herbaceous perennial plant; leaves are dark green above and light green beneath, rhizome bearing plant. In Dhemaji district of Assam, the underground part of the plant is used in the treatment of abdominal pain during menstruation cycle. In East district of Sikkim the stem sap is used in the treatment of cuts and wounds.

2.2.3 *Nyctanthes arbor-tristis*

Commonly known as ‘Sewali’ or ‘Parijat’ belonging to the family Verbanaceae. The plants have simple and opposite leaves with an entire margin. The flowers are produced in clusters. They are bisexual and actinomorphic.



Flowering season starts from September to October (Grierson, 1999). The leave juice is used in the treatment of typhoid-pneumonia and common fever.

2.3 Preparation of the plant material

The collected plant parts i.e. stem bark of *A. scholaris*, underground part of *C. esculenta* and leaves of *N. arbor-tristis* were washed properly and separated from undesirable materials. Then the stem bark and the rhizome were sliced into small pieces. The samples were dried at constant temperature (45 °C) for 24 hours and ground into fine powder with the help of grinder. The powdered samples were kept for future analysis in air-tight containers.

2.4 Preparation of Methanolic extract

The powdered plant materials were extracted with methanol by cold maceration. Powdered plant materials (10gm) were macerated with 100ml of methanol separately at room temperature for 2days with frequent shaking. After 2days, the extract was filtered by using Whatman no.1 filter paper. The filtrates were then used for qualitative phytochemical analysis. The filtrates were concentrated and evaporate to dryness. The dried extracts were kept in appendroff tubes inside refrigerator at 4 °C until further use.

2.5 Qualitative phytochemical analysis

Qualitative phytochemical screening of methanolic extract of selected medicinal plants was done according to standard procedures (Sofowara, 1993; Trease and Evans, 1989; Harbone, 1973) to determine the presence or absence of various phytochemical contents.

2.5 Quantitative determination of total phenol contents

The total phenol content in the methanolic extract of the selected plants was estimated by the method of Lin *et al.* 2001. 1.0ml of extract was mixed with 5.0ml of 10% Folin-ciocalteu reagent and 4.0ml of 7.5% sodium carbonate. The mixture was allowed to stand for 90minutes at room temperature. Then the absorbance was measured at 760nm in UV-vis spectrophotometer. The phenol content was determined using the Gallic acid as standard. Results were expressed as µg/g. Measurement of the samples was made in triplicate.

2.6 Quantitative determination of total flavonoids contents

The total flavonoid content was measured by the method of Lin *et al.* (2001). 5ml extract was transferred to the test tube and mixed with 0.3ml of 5% sodium nitrate for 5 minute. Then 0.3ml of 10% aluminium chloride was added after 6 minute, reaction was stopped by addition of 2ml of sodium hydroxide. The mixture was further diluted with distilled water upto 10ml. The absorbance was measured at 510nm. Rutin was used as standard and flavonoid contents were calculated and expressed as rutin equivalent (Rt).

3. Antioxidant analysis

3.1 DPPH free radical scavenging assay

The free radical scavenging activity of the methanolic extract of the selected medicinal plants were determined on

The free radical scavenging activity of the methanolic extract of the selected medicinal plants were determined on the basis of the scavenging activity of the stable DPPH (1,1- diphenyl-2-picrylhydrazyl) as the standard method of Hatano *et al.* (1988). 1mL of each solution of different concentrations (100,200,300,400 and 500µg/ml) of methanol extract was added to 3mL of 0.04% methanolic DPPH free radical solution. After 30 minutes the absorbance of the solution was taken at 517nm by using UV spectrophotometer which was compared with the absorbance of standard ascorbic acid concentrations. Percentage inhibition was measured by using inhibition formula. From the calibration curves obtained from different concentrations of extract, IC₅₀ was determined. IC₅₀ value denotes the concentration of plant sample that inhibit the DPPH free radical by 50%.

The Linear regression equation of standard curve method was performed for the statistical analysis of the data.

4. Results

Qualitative phytochemical screening reveals the presence and absence of various kinds of phytochemicals like alkaloids, glycosides, flavonoids, phenols, tannins etc in the methanolic extracts of selected medicinal plants. Qualitative phytochemical analysis of *A. scholaris*, *C. esculenta* and *N. arbor-tristis* are presented in the tables.

4.1 Quantitative phytochemical estimation for phenols

A comparative study was performed between the same plants from both the regions for the estimation of total phenol content. The total phenol content of all the plants was expressed in gallic acid equivalents (GAE) and calculated from linear regression equation of the standard curve ($y=0.014x+ 0.102$, $R=.991$). During the study the level of phenolics was found to be higher in *A. scholaris* and *Nyctanthes arbor-tristis* collected from Dhemaji district of Assam in compare with the sample collected from East district of Sikkim. In *Colocasia esculenta* the phenol content is higher in Sikkim’s sample than the sample from Dhemaji, Assam (table 4).

Table: 1 Qualitative phytochemical analysis of *Alstonia scholaris*

Name of the species	Sr. No.	Name of Phytoconstituents compounds	East-Sikkim	Dhemaji, Assam
<i>Alstonia scholaris</i>	1	Alkaloids	+	+
	2	Flavonoids	+	+
	3	Phenols	+	+
	4	Tannins	+	+
	5	Steroids	-	-
	6	Saponins	+	+
	7	Phlobatanins	-	-
	8	Carbohydrates	+	+
	9	Glycosides	+	+
	10	Protein	-	-

Table 2: Qualitative phytochemical analysis of *Colocasia esculenta*

Name of the species	Sr. No.	Name of Phytoconstituents compounds	East Sikkim	Dhemaji, Assam
<i>Colocasia esculenta</i>	1	Alkaloids	-	-
	2	Flavonoids	+	+
	3	Phenols	+	+
	4	Tannins	+	+
	5	Steroids	+	+
	6	Saponins	-	-
	7	Phlobatanins	-	-
	8	Carbohydrates	+	+
	9	Glycosides	+	+
	10	Protein	+	+

Table 3: Qualitative phytochemical analysis of *Nyctanthes arbor-tristis*

Name of the species	Sr. No.	Name of Phytoconstituents compounds	East Sikkim	Dhemaji, Assam
<i>Nyctanthes arbor-tristis</i>	1	Alkaloids	+	+
	2	Flavonoids	+	+
	3	Phenols	+	+
	4	Tannins	+	+
	5	Steroids	+	
	6	Saponins	+	+
	7	Phlobatanins	-	-
	8	Carbohydrates	+	+
	9	Glycosides	+	+
	10	Protein	+	+

Table 1-3 shows the results of phytochemical screening analysis and presence (+) and absence (-) of different phytochemicals in the selected medicinal plants.

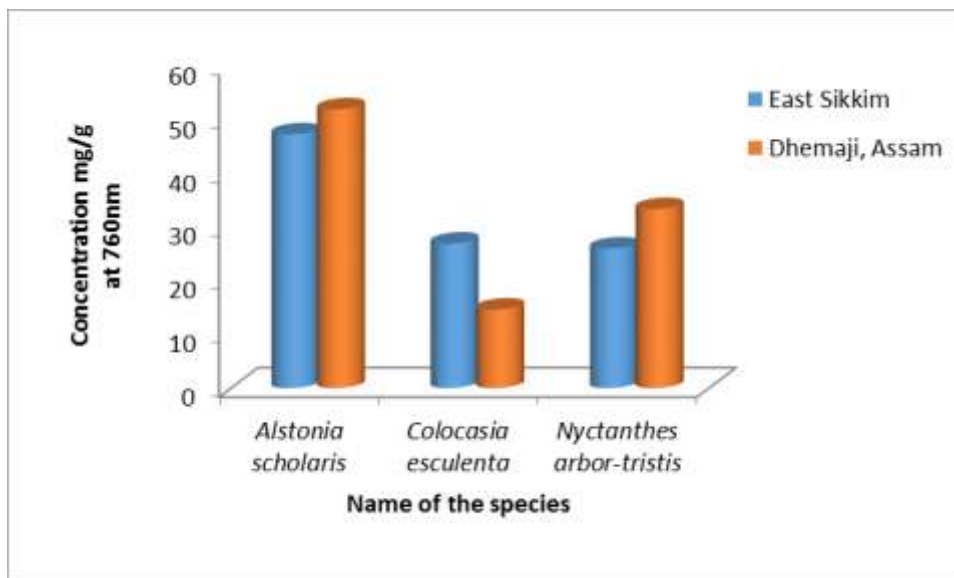


Fig 1: Total phenol content in the selected medicinal plants

The total flavonoids content of the plants was expressed in rutin (Rt) equivalent ($y=0.036x+0.050$, $R=0.948$). Total flavonoid content was found to be of higher in all the plant samples that were collected from Dhemaji district of Assam than the sample collected from East district of Sikkim (table 4).

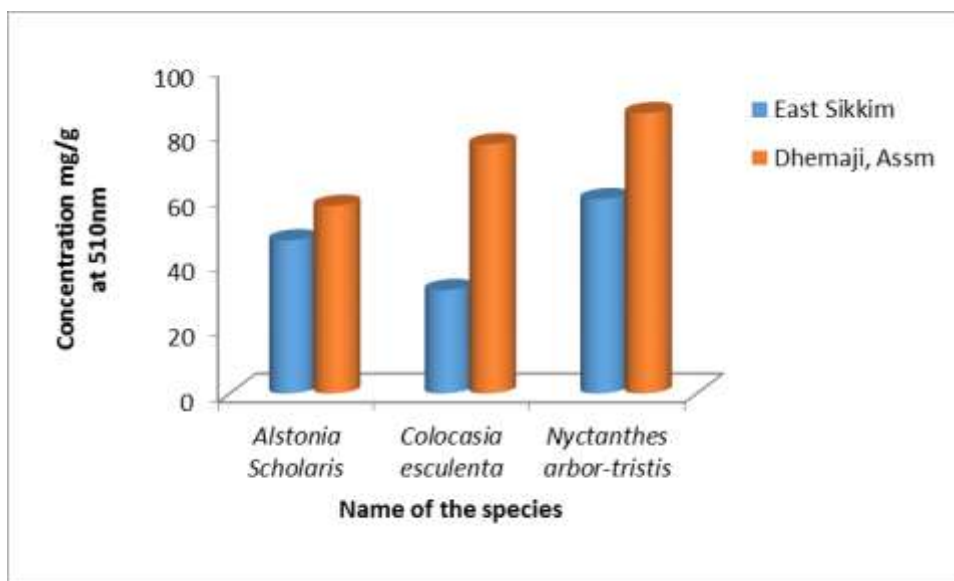


Fig 2: Total flavonoid content in the selected medicinal plants

Table 4: Quantitative phytochemical analysis of the plants

Name of the species	Phytoconstituent (mg/g)	Sikkim region	Assam region
<i>Alstonia scholaris</i>	Phenol	47.33±0.019	51.99±0.02
	Flavonoid	47.03±0.008	57.64±0.010
<i>Colocasia esculenta</i>	Phenol	26.96±0.04	14.66±0.015
	Flavonoid	31.76±0.007	76.54±0.107
<i>Nyctanthes arbor-tristis</i>	Phenol	26.04±0.023	33.40±0.057
	Flavonoid	59.71±0.005	86.12±0.015

DPPH free radical assay was used to investigate the free radical scavenging activity (table 5).

Table 5: DPPH scavenging activity of the selected medicinal plants

Name of the species	East district of Sikkim	Dhemaji district of Assam
	IC 50 µg/ml	IC 50 µg/ml
<i>Alstonia scholaris</i>	4.32	5.44
<i>Colocasia esculenta</i>	3.75	2.11
<i>Nyctanthes arbor-tristis</i>	0.69	0.55

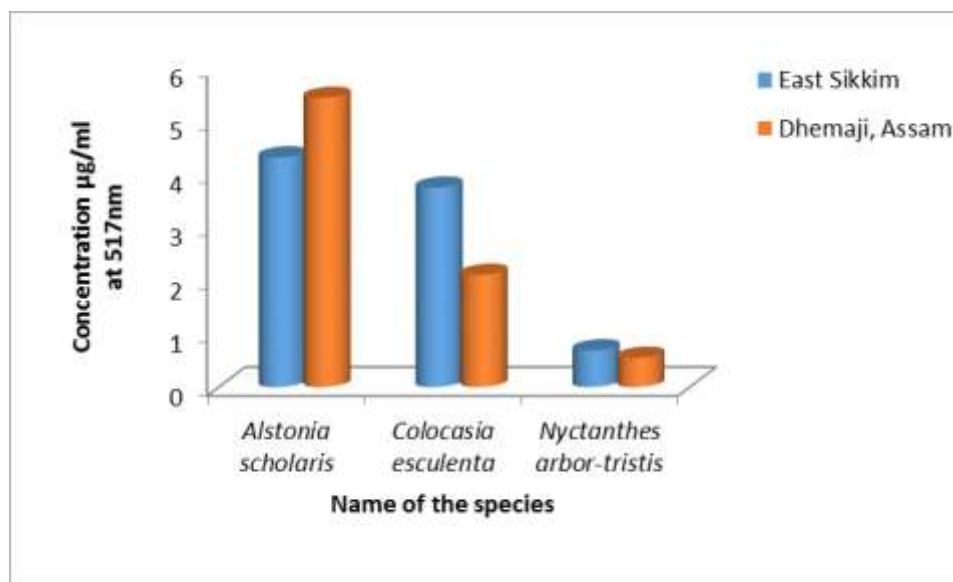


Fig 3: DPPH scavenging activity of the selected plants

The figure 1, 2 and 3 shows the difference in the total content of the phytochemicals and the antioxidant properties.

5. Discussion:

The plant based medicinal compounds have fewer side effects than the synthetic ones. The secondary metabolites present in the plants are important for their medicinal values. Due to the modern day's medicines the dependency of these plants has gradually declining. But still in many of the North-Eastern regions plants are being used as source of medicine for the treatment of various diseases. Phytochemical analysis of the selected medicinal plants from both the region showed the presence of a number of medicinally active compounds like Saponin, flavonoids, phenolic compounds, and alkaloids etc. This main objective of the study is to compare the presence of different secondary metabolites, quantity of the compounds along with their anti-oxidant properties. Phenols are the main and most abundant groups of plants metabolites. Natural antioxidants are generally derived from plants in the form of phenolic compounds (Ali *et al.* 2008). Flavonoids, a group of phenolic compounds are free radical scavengers which prevent oxidative cell damage through their water soluble property and also possess strong anti-cancer property.

The human body is always in a constant attack by free radicals and as a result the chain reaction starts in a cell which can cause damage or even death to the cell in the body. Antioxidants can inhibit or terminate these chain reactions by removing free radicals and other oxidative processes and ultimately prevent the progress of chronic diseases. In this study, DPPH scavenging activity was used to evaluate the anti-oxidant activities in the methanolic extracts of the selected medicinal plants.

6. Conclusion

From the studies of the above selected medicinal plants, it is clear that these plants contain various bioactive compounds. Moreover, these plants have been used by local communities of both East-Sikkim and Dhemaji district of Assam region. From the study it is clear that total flavonoid content of all the three plants viz *A. scholaris*, *C. esculenta* and *N. arbor-tristis* is higher in the sample collected from Dhemaji district of Assam. Thus due the *C. esculenta* and *N. arbor-tristis* collected from Dhemaji, Assam are found to be more effective than the sample collected from East district of Sikkim. And *A. scholaris* from East district of Sikkim is found to be more effective than the plant sample from Dhemaji district of Assam for the treatment of various kinds of diseases.

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