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Ethno Medicinal, Phyto-Chemical and Physico-chemical Characterization of Selected Endangered Medicinal Plants of Indravati National Park, Bijapur, Chhattisgarh, India

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Abstract: Medicinal plants are beneficial for curing several ailments among the traditional healers, indigenous people, local practitioners and forest dwellers. If harnessed, traditional knowledge of the ethnomedicinal plants can play a pivotal role in drug discovery and development. In many cases a single medicinal plant can be of multiple uses also, different plant parts of the same plant can be utilized for more than one disease condition. The Ethno-botanical, Phytochemical and physico-chemical characterization of five selected medicinal plants viz., Nyctanthes arbor - tristis, Andrographis paniculata, Cissus quadragularis, Plumbago zevlania and Costus specious is an essence and has been carried out to assess bioactive potential and to establish traditional belief in the light of scientific interpretation. Fresh leaves were collected from Indravati National Park, Bijapur, Chhattisgarh, India, dried and powdered for phytochemical and Proximate, ultimate, and compositional analysis. Study revealed that the highest concentration of Ash Content (18.513%), Moisture Content (8.56%), Carbon content (48.77%), Hydrogen Content (24.490%), Nitrogen Content (23.860%) was observed to occur in *Plumbago zeylanica* leaf than other experimental plants. In Cissus quadrangularis, the percentage composition of fat content (0.230%), extractive content (1.05%), Lignin Content (5.53%) was higher than others. Fat content (0.230%), Moisture Content (8.56%), Vit. C content (64.63%) and Oxygen contents (36.655 %) were observed to be higher in Nyctanthes arbortristis leaf than others. The concentrations of Crude Fibre (14.49 %), Moisture Content (8.56%), Protein content (12.16%), Carbon content (75.66 %), Cellulose content (47.63 %) were observed to be highest in Costus speciosus species than others. Carbon content (48.77%) and Hydrogen Content (24.490%) were higher in Andrographis paniculata than in others.

Introduction

Nature has been an immortal source of medicinal substitutes and plays a prime role in the management of human illnesses. The rich diversity of the plant kingdom offers an inexhaustible source of active agents that are invaluable ingredients for medicinal purposes. Use of plant-derived substances has recently garnered great interest throughout the globe for the rich biological diversity of the traditional and ethnomedical system of medicine (Maiti et al., 2010, 2013; Banerjee et al., 2014; Sarkar et al., 2021, 2022, 2024; Ghosh et al., 2022; Sarkar et al., 2023). Medicinal plants have countless abilities to synthesize bioactive compounds like phenols, flavonoids and steroids that can be harnessed in many pharmaceutical applications (Dey-Ray et al., 2024; Rai Sharma, 2024). The bioactive compounds synthesized by the medicinal plants serve as a defence against predators, micro-organisms herbivorous animals. Bioactive compounds protect plants in the form of strong odour (terpenoids), pigments (tannins and quinones) and strong flavor (capsicin) also, these properties enhance the medicinal value of the plants, including antimicrobial properties and antioxidant properties (Dawurung et al., 2021).

The state of Chhattisgarh has been known as a herbal state due to dense greener patches spread across the state.

Chhattisgarh is situated in the Deccan biogeographic area, which abodes rich and unique biodiversity and also serves as a rich native place for numerous endemic plant species of high medicinal importance (Darro and Khan, 2023). Medicinal plants belonging to around 911 genera and 196 families have been documented in the state, of which 90% of the medicinal plant species are naturally growing while 10% are grown by farmers. However, over-exploitation of raw herbs in the forms of roots, leaves, fruits and bark to overcome the high pressure of supply has resulted in destructive harvesting and raised threat to several medicinal plant species, sending them on the threshold of endangered plant species (Darro and Khan, 2023). According to a survey, around 65 medicinal plant species have been enlisted in the endangered plant species taxa that need immediate conservation.

In the present study, we have selected five endangered medicinal plant species of Chhattisgarh observed at the Indravati National Park, Bijapur, Chhattisgarh, including Plumbago zeylanica, Cissus quadrangularis, Nyctanthes arbor-tristis, Costus speciosus and Andrographis paniculata.

Materials and Methods Collection of Plant material

The forest area of the Indravati National Park was visited during the study period of June 2021 to July 2022. The medicinal plants during the study were collected from the study site 19.2059°N, 81.0313°E from their natural habitat and identified with the help of the tribals and the forest resource persons (Khanna et al., 2004; Singh, 2016). The medicinal plants collected during the study period included including *Plumbago zeylanica*, *Cissus quadrangularis*, *Nyctanthes arbor-tristis*, *Costus speciosus* and *Andrographis paniculata*. After collection, the plant material was washed thoroughly with distilled water, and the cleaned plant parts were kept for shade drying.

Determination of Ethnomedicinal importance

Frequent field visits were conducted during the study period to determine the ethnomedicinal importance of the collected medicinal plants. Semi-structured interviews were designed and conducted with the local informants, forest dwellers, and persons belonging to the tribal communities after the aim of the study was explained to them. The informants for the semi-structured interviews and group discussions were selected based on age, knowledge of the local area and the floral diversity and experience of using locally available medicinal plants, local Vaidya and guniya were also selected for the study.

The selected informants were addressed with questions regarding the traditional knowledge of medicinal plants, local names of plants, diseases cured, plant parts used and mode of administration. The collected plant samples were further categorized into threatened, endangered, rare, vulnerable and least concern category based on the IUCN red data list for medicinal plants.

Preparation of Extract

The plant parts (leaves) of the selected medicinal (including Plumbago zevlanica, Cissus quadrangularis, **Nvctanthes** arbor-tristis. Costus speciosus, Andrographis paniculata) were collected and shade dried for a time period of 15days. The dried plant material was powdered using a grinder to obtain a fine powder for extraction. The powdered plant samples were stored in sealed bags to prevent moisture. The dried plant parts were used for Soxhlet extraction using distilled water and methanol as extraction solvents. Several solvents, including both polar and non-polar groups, can be used to extract phytochemicals.

In the present study, two solvents including distilled water and methanol were selected for extraction. The dried plant powder (25g) was extracted sequentially using organic solvents separately through Soxhlet extractor (Redfern et al., 2014; Zhang et al., 2018). The extraction was performed for a period of 24-48 hours accompanied by slight shaking. The crude extract was filtered using a Whatman filter paper no.1 and concentrated using a drier at 40°C. Later the concentrated extracts were dried aseptically at room temperature and further stored in sterile bottles at 4°C for further investigation.

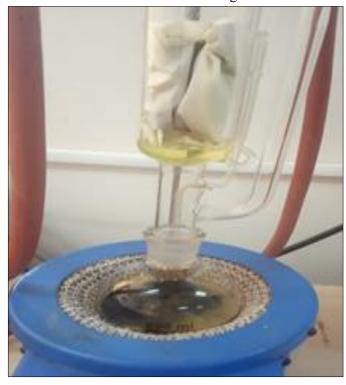




Figure 1. Soxhlet extraction using Distilled water and Methanol.

Phytochemical analysis of plant extracts

The phytochemical study of the selected tuber extracts was performed based on the standard protocols. Phytochemical tests for screening the extracts obtained using Soxhlet extraction were evaluated to identify the biochemical constituents (Harborne, 1973; Trease and Evans, 1989). The phytochemical tests were performed to detect alkaloids, flavonoids, phenols, terpenoids, tannins, saponins and coumarins.

Test for Alkaloids:

Hager's test: A small amount of extract is taken to it and a few drops of picric acid (1%) are added and observed for the formation of yellow precipitate, indicating the presence of alkaloids.

Wagner's test: A small amount of extract and a few drops of potassium Iodide (KI) solution are added to it, and the emergence of brown /red precipitate indicates the presence of alkaloids.

Mayer's test: A small amount of extract is taken and a few drops of potassium mercuric iodide solution are added. The formation of yellow precipitate depicts the presence of alkaloids.

Test for flavonoids:

Three methods are performed for the determination of flavonoids in the plant sample (Harbrone, 1973). For the study, 5 ml of dilute ammonia solution was added to aqueous filtrate, followed by the addition of concentrated sulphuric acid. After addition yellow colouration appears, indicating the presence of flavonoids; however, the yellow colour disappears on standing.

To the aqueous filtrate, few drops of aluminium solution (1%) were added and a yellow colouration appeared, indicating the presence of flavonoids.

Test for tannins:

0.5 g of the dried cladodes powder was boiled in 20 ml of water in a test tube and filtered further. A few drops of 0.1% ferric chloride were added and observed for brownish-green color or a blue-black colouration.

Test for saponin:

Around 2g of dried cladodes powder was boiled in 20 ml of distilled water using a water bath and filtered. 10 ml of the so-obtained filtrate was further mixed with 5 ml of distilled water and shaken vigorously to obtain a stable, persistent froth. The frothing was later mixed with 3 drops of olive oil, shaken vigorously, and observed for emulsion formation.

Test for terpenoids (Salkowski test):

For the study, 5 ml of plant extract is mixed in chloroform (2 ml) and concentrated sulphuric acid (3 ml) carefully to form a layer. The occurrence of reddish-brown colouration at the interface indicates positive results for the presence of terpenoids.

Test for phenols:

Ferric chloride test: A small amount of extract is taken and a few drops of ferric chloride (FeCl3) solution are added. Formation of a bluish-black colour indicates the presence of phenols.

Test for coumarins

For the test, around 3ml of 10% NaOH solution was added to the plant extracts; the yellow color indicated the presence of coumarins.

Physical analysis

Proximate Analysis

Proximate composition analysis has been achieved through a standard test method for ash in biomass (ASTM E1755). The obtained biomass sample's moisture content (%) was ascertained using the convection oven dry method. The method for determining crude fiber in leaves involves a series of chemical treatments to remove all soluble and easily digestible components from the sample. Proteins, starches, and sugars are normally eliminated while leaving the fiber component behind by acid and alkali treatments. By drying the samples at 105±2°C until a consistent weight was achieved, the moisture content for proximate composition was ascertained using a hot air oven (Acharya et al., 2021b). The ash content was ascertained after 20 hours of combustion at 550°C. The total amount of carbohydrates was calculated by deducting the total of moisture, ash, protein, and fat from 100 (Acharya et al., 2021b). The protein content was ascertained by converting the nitrogen content discovered using Kjeldahl's technique (Acharya et al., 2022). Fat and crude fiber were measured using procedures outlined in (Acharya et al., 2022). The

titration method was used to determine the level of vitamin C (Acharya et al., 2021b).

The aluminium weighing plates were dried in an oven set to $(105 \pm 3)^{0}$ C for at least 4 hours. After the dishes cooled in a desiccator, the weight was recorded. Each gr. of powdered biomass sample was weighed on an predried aluminium dish. It was then baked for approximately four hours at $(105 \pm 3)^{0}$ C in a convection oven. The plate and sample were removed and placed in a desiccator to cool. The oven-dried sample weighed nearly 0.1 mg in addition to the meal (Acharya et al., 2022).

The sample was reprocessed using the same convection oven at the same temperature in order to obtain a dry and consistent weight. The following formulas are utilised to determine the total solid content and moisture content (Acharya et al., 2022).

Moisture (%) =
$$[1 - \frac{A-B}{C}] \times 100$$

Here,

A = Weight of dried sample along with dish

B = weight of dish

C = Weight of collected sample

Total solid (%) = [100 - Moisture (%)]

To determine the volatile chemicals present in the biomass samples, 1 g of each oven-dried sample is placed in a crucible and heated to (925 10) 0 C for 7 minutes with the lid closed in a muffle furnace. After heating up, the crucible is removed and placed in desiccators to cool. The following formula is used to determine the samples' volatile content:

$$C (\%) = \left[\frac{A-B}{A}\right] X 100$$

Here,

A = weight of sample before heating

B = weight of the sample after heating

C = weight loss

Volatile matter in sample (%) =(C-D)

Here, D = moisture (%)

The ash content of the samples is measured in a muffle furnace. One gr of oven-dried biomass sample was placed in a crucible and heated to $575 \pm 10^{\circ}\text{C}$ for a minimum of three hours. After it has reached the appropriate temperature, the crucible is taken out and put into a desiccator. The ash, which weighs roughly 0.1 mg, is heated in the crucible until no weight loss is apparent. The following formula is used to compute the fixed carbon content (FC): FC is equal to [100 - (moisture % + volatiles percentage + ash percentage).

Bulk density and Swelling index

 The bulk density, also known as the specific gravity of soil solids, provides information about the density of soil particles. It is used to calculate various soil properties, such as void ratio and degree of saturation.

 The swelling index (EI) indicates the swelling potential of compacted soil when inundated with distilled water.

Bulk density was analyzed through Standard Test Methods for Specific Gravity of Solids (ASTM D 854-92) and the swelling index was calculated through ASTM D4829 – 11 (Acharya et al., 2022).

Compositional analysis

Compositional analysis was achieved through ASTM: American Society for testing and materials (ASTM International) standards, 2015, Van Soest et al., 1991.

The polysaccharide content of Leaf samples was determined using the Pelican fibra plus automatic fibre estimate device. In a crucible at room temperature, 0.5–1 gm of the sample in powdered form was mixed with 100 ml of neutral detergent solution and 0.5 g of sodium sulphate to determine the neutral detergent fibre (NDF). The crucible was heated to boiling point at 4000 C and refluxed for 60 minutes thereafter. After filtering, the mixer was meticulously cleaned using acetone and water. After the crucible was dried at 105°C for about 8 hours, its weight was noted. This is how the NDF was determined:

NDF %=
$$\frac{[(weight\ of\ crucible+NDF)-weight\ of\ crucible]}{weight\ of\ sample}\ X\ 100$$

Similar methods of estimating NDF are used to assess acid detergent fibre (ADF). Acid detergent solution is used as a substitute for neutral detergent solution. The reduced weight is calculated as follows:

ADF %=
$$\frac{[(weight\ of\ crucible + ADF) - weight\ of\ crucible]}{weight\ of\ sample}\ X\ 100$$

By generating ADF in a crucible, adding 72% concentrated H2SO4 and letting it mix for three hours while continuously stirring, filtering, and twice washing with water, the amount of ADL (acid detergent lignin) was ascertained. The weight loss was calculated following about eight hours of air drying at 100 0C in the hot air oven. After the crucible has gained the necessary amount of ash, it is placed in a muffle furnace that is set to 500°C for at least 7 minutes. The weight loss is then calculated:

Hemicellulose (%) = NDF(%) – ADF(%)
Cellulose (%) =
$$\left(Y - \frac{L}{W}\right)$$
 X100

Lignin (%) =
$$\left(L - \frac{A}{W}\right) \times 100$$

Here,

Y = weight of ADF + crucible L = weight of crucible+ lignin

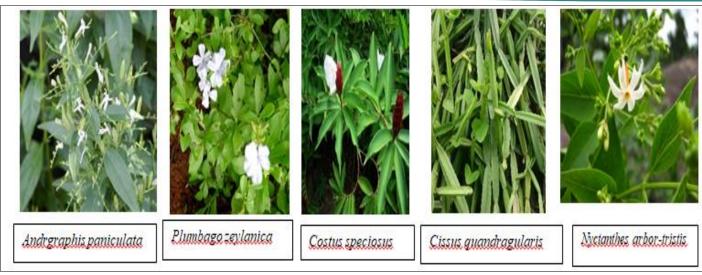


Figure 2. Experimental Plants.

A = weight of crucible + ash W = weight of a sample

Ultimate analysis

The plant species was analyzed by the method- Solid biofuels - Determination of total carbon, hydrogen, and oxygen content is covered in CEN/TS 15104:2005.

The final analysis of the biomass that has been gathered is done using the C, H, N, S, and O elemental analyzer (Eurovector EA3000). 5 tin capsules containing a 5L cysteine test are used to calibrate the analyzer. A 0.1 mg tin pill containing the dusted biomass sample was consumed. To generate heat of 980 degrees Celsius, helium- and oxygen-enriched gas was continually supplied. Callidus® software was utilised to analyse the biomass's constituent elements.

Results and Discussion

In the present study, five different endangered medicinal plants belonging to five different plant families including Acanthaceae, Costaceae, Oleacea, Plumbagoniaceae and Vitaceae collected from the Indravati National Park, Bijapur district, Chhattisgarh were selected for the study. The medicinal plants chosen for the study offer numerous ethnomedicinal benefits and have been used by the locals, ayurvedacharya and pharmaceutical companies for the preparation of different medicinal formulations. The high demand for these medicinal plants for healing purposes has resulted in the overexploitation and destruction of the natural habitat of these plants, and hence, they are facing the threat of extinction. The medicinal plants Plumbago zeylanica and Costus speciosus have been categorized as endangered

plants, *Nyctanthes arbor-tristis* has been categorized as critically endangered, while *Cissus quadrangularis* and *Andrographis paniculata* have been categorized in the least concern category.

Following the status of these endangered medicinal plants, the selected medicinal plants were grown in the home garden using the potting method for the present study. The plant material obtained was used as the primary material for determining the bioactive compounds and physico-chemical analysis. The detailed ethnomedicinal uses of the selected plant species have been documented here.

The dried plant material of the selected medicinal plants was used for isolation of aqueous and methanolic extracts using the Soxhlet apparatus. The finally obtained aqueous and methanolic extracts were subjected for phytochemical analysis for the determination of the presence of alkaloids, terpenoids, tannin, phenols, flavonoids, coumarin and saponins. The study's results depicted that the methanolic extract was much more active than the aqueous extract. the methanolic extract showed a strong presence of alkaloids, phenols, flavonoids, saponins, terpenoids, tannins, and coumarins. The methanolic extract of *A. paniculata* showed the highest number of bioactive compounds, followed by *N. arbor-tristis*, *C. speciosus* and *P. zeylanica*.

Further, the dried plant material was used for the physico-chemical analysis; during this study, bulk density, swelling index, proximate analysis, ultimate analysis and compositional analysis were checked, the results of which have been given in the tables.

Table 1. Ethnomedicinal uses of selected medicinal plants.

Medicinal Plant	Common Name	Family	Status	Ethnomedicinal Uses	Plant Parts Used
Plumbago zeylanica	Chitra, Chitrak	Plumbagoniaceae	Endangered	Skin disease, Hysteria, Cancer	Root, bark, leaves
Cissus quadrangularis	Hadjora, Asthishrinkala	Vitaceae	Least Concern	Hemorrhoids, upset stomach, asthma, allergies	Roots, stem, leaves
Nyctanthes arbor- tristis	Parijaat, Shefali	Oleaceae	Critically Endangered	Dandruff, arthritis, hair growth, skin problems, malarial fever	Flowers, leaves, seeds, bark
Costus speciosus	Kemuka, Kewkand	Costaceae	Endangered	Pneumonia, Rashes, cough, fever, asthma, bronchitis, constipation	Roots, stem, leaves, shoots
Andrographis paniculata	Kalpanth, Kalpu	Acanthaceae	Least Concern	Flu, common cold, fever, malaria, bronchitis, intestinal worms	Leaves, flowers, stem

Table 2. Determination of bioactive compounds.

		Andrographis paniculata		Plumbago zeylanica		Costus specious		Cissuss quadrangularis		Nyctanthes arbor-tristis	
S.no	Phytochemical test	D. W. extract	Meth extract	D. W. extract	Meth extract	D. W. extract	Meth extract	D. W. extract	Meth extract	D. W. extract	Meth extract
1.	Alkaloid	++	+++	-	+	++	++	+	+	++	+
2.	Terpenoid	-	+	++	-	+	+++	-	-	+	++
3.	Tannin	++	+	-	++	+++	++	-	++	+	+
4.	Phenol	++	++	++	+++	1	+	+	+	1	++
5.	Flavanoid	+++	+++	+	+	+	+	++	+	+	+++
6.	Coumarin	-	++	++	++	+	-	-	++	+	++
7.	Saponin	++	+++	+	++	++	+	+	-	+	++

Physio-chemical Analysis

Table 3. Bulk Density.

Sample	BD 1 (gm/cm3)	BD 2 (gm/cm3)	BD 3 (gm/cm3)	Mean (gm/cm3)	SD (gm/cm3)
Plumbago zeylanica	0.102	0.133	0.145	0.126	0.018
Cissus quadrangularis	0.200	0.272	0.291	0.254	0.039
Nyctanthes arbor-tristis	0.102	0.178	0.182	0.154	0.035
Costus speciosus	0.098	0.088	0.099	0.095	0.004
Andrographis paniculata	0.104	0.198	0.196	0.166	0.043

Table 4. Swelling Index.

Sample	SW-1 (%)	SW-2(%)	SW-3(%)	Mean (%)	SD (%)
Plumbago zeylanica	0.235	0.21	0.211	0.21	0.011
Cissus quadrangularis	0.421	0.422	0.400	0.414	0.010
Nyctanthes arbor-tristis	0.985	0.999	0.952	0.979	0.019
Costus speciosus	1.322	1.355	1.213	1.296	0.06
Andrographis paniculata	0.888	0.848	0.846	0.86	0.019

Table 5. Proximate Analysis.

Sample	Crude	Crude Fibre Ash Co		ontent Fat Content		Protein Content		Moisture Content		Carbon Content		Vitamin C		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plumbago zeylanica	12.480	0.160	18.513	0.118	0.123	0.015	10.460	0.235	8.56	0.24	64.63	0.18	58.5	0.17
Cissus quadrangularis	12.550	0.090	18.460	0.274	0.230	0.000	11.530	0.278	8.22	0.08	65.3	0.05	50.55	0.15
Nyctanthes arbor-tristis	11.42	0.07	15.55	0.17	0.230	0.004	11.088	0.101	8.56	0.24	60.07	0.13	64.63	0.18
Costus speciosus	14.49	0.2	15.69	0.04	0.123	0.015	12.16	0.04	8.56	0.24	75.66	0.42	60.07	0.13
Andrographis paniculata	12.33	0.124	15.55	0.14	0.14	0.05	10.27	0.06	8.22	0.08	64.63	0.18	59.5	0.08

Table 6. Ultimate Analysis.

Sample	Carbon Content		Hydrog	en Content	Oxygen	Content	Nitrogen Content		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Plumbago zeylanica	48.77	0.21	24.490	34.337	19.679	17.565	23.860	9.135	
Cissus quadrangularis	48.65	0.14	24.395	34.302	19.612	0.108	18.007	17.153	
Nyctanthes arbor- tristis	42.6	0.21	3.564	0.375	36.655	0.609	1.718	0.365	
Costus speciosus	42.6	0.21	21.405	29.974	17.196	15.322	2.268	0.103	
Andrographis paniculata	48.77	0.21	24.490	34.337	32.800	17.565	1.831	0.445	

Table 7. Compositional Analysis.

Sample	Cellulose content		Hemicellulose Content		Lignin Content		Extractive Content	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plumbago zeylanica	42.22	0.09	17.32	0.004	5.232	0.012	0.749	0.064
Cissus quadrangularis	47.39	0.28	19.37	0.35	5.53	0.099	1.05	0.088
Nyctanthes arbor- tristis	42.453	0.183	20.01	0.57	5.42	0.106	0.97	0.006
Costus speciosus	47.63	0.3	17.26	0.045	4.278	0.033	0.865	0.009
Andrographis paniculata	44.302	0.063	19.785	0.54	5.14	0.07	0.852	0.004

The detailed study of ethnomedicinally important plants is of utmost importance as they form the backbone of folk medicine and an integral part of the traditional medicinal system of Ayurveda. Also, the overexploitation of medicinal plants has led to the degeneration of valuable medicinal assets. The present study emphasizes on the conservation of the endangered medicinal plant species while also performing research studies on the plants. In this manner, the natural flora of the plant is also not destroyed and there is no hindrance in the research work also.

Natural products derived from plants are of great significance due to their unique therapeutic properties, medicinal efficacy and minimal adverse effects. Bioactive compounds observed in the plants consist of alkaloids, flavonoids, terpenoids, nitro and sulfur-containing compounds, phenols, coumarins and saponins. These bioactive compounds contribute to the plant extracts' antimicrobial, anti-inflammatory, anti-oxidant, anti-cancer, and immunostimulatory activity (Stephane et al., 2022). Study revealed that the highest concentration of Ash Content (18.513%), Moisture Content (8.56%), Carbon content (48.77%), Hydrogen Content (24.490%), Nitrogen Content (23.860%) was observed to occur in Plumbago zeylanica leaf than other experimental plants. In Cissus quadrangularis, the percentage composition of fat content (0.230%), extractive content (1.05%), Lignin Content (5.53%) was higher than others. Fat content (0.230%), Moisture Content (8.56%), Vit. C content (64.63%) and Oxygen contents (36.655 %) were observed to be higher in Nyctanthes arbor-tristis leaf than others. The concentrations of Crude Fibre (14.49 %),

Moisture Content (8.56%), Protein content (12.16%), Carbon content (75.66 %), Cellulose content (47.63 %) were observed to be highest in *Costus speciosus* species than others. Carbon content (48.77%) and Hydrogen Content (24.490%) were higher in *Andrographis paniculata* than in others.

Conclusion

The present study details the study of five selected endangered medicinal plants for the determination of their ethnomedicinal importance, presence of bioactive compounds and physic-chemical analysis. The observations noted from the above study depict promising results for the ethnomedicinal applications of the plants. The presence of a high amount of bioactive compounds accounts for their therapeutic applications and provides insight into the conservation of endangered medicinal plants.

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Conflict of interest

Nil

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