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Phytochemical screening and comparative study of bioactives in *Phyllanthus niruri* L. from different geographical locations

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ABSTRACT

Medicinal plants are resources of new drugs. Phyllanthus niruri L.(Euphorbiaceae) is a widespread tropical herb. The phytochemical screening and quantification of primary metabolites in the plants collected from three geographical locations. Maximum extractive value (3.951%) was found in water extract among test solvents. All metabolites were qualitatively present in water and ethanolic extracts.

Keywords: *Phyllanthus niruri*, Phytochemicals, Proteins, Carbohydrates, Lipids, Flavonoids

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INTRODUCTION

India is endowed with a rich wealth of medicinal plants, and a large number of plant species are recognized which have medicinal values and are main sources for the bioactive compounds like primary metabolites. Prehistorically these plants were acknowledged with curative properties against various diseases and aliments. Plants have formed the basis of traditional medicine systems as Ayurvedic, Unani, and Chinese. Several drugs have their origin in these systems and are successfully used [1, 2]. Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds [3].

Phyllanthus niruri L. is an annual herb of the family Euphorbiaceae occur in most tropical countries. It is known for a hepatoprotective action [4], lipid lowering [5] and antifungal action [6], antidiabetic action [7], etc. It holds a reputed position in both Ayurvedic and Unani systems of medicine.

Scientists estimate that there are as many as 10,000 different phytochemicals which have the potential to treat diseases like cancer, stroke or metabolic syndrome. Primary metabolites are essentially required for the growth of plants. Many primary metabolites are precursors or pharmacologically active metabolites in pharmaceutical compounds such as antipsychotic drugs [8, 9].

EXPERIMENTAL SECTION

The plants *Phyllanthus niruri* were collected from three different geographical areas : rocky areas of Jaipur, Rajasthan University Campus and swampy areas in Jaipur .

The collected plants from different locations were subjected to separation into its parts roots, stems and leaves. All the separated plant parts and callus raised from intermodal and nodal part of plant was collected, dried in shade and

subjected for successive extraction with petroleum ether, benzene, chloroform, ethanol and water with soxhlet apparatus. The extracts were then concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in desiccators. Then they were subjected to preliminary phytochemical screening for the detection of various constituents. Each extract was processed further to evaluate the presence of carbohydrates, proteins, flavonoids and alkaloids following the established protocols16 for root, stem, leaf and callus. A comparative study was done quantitatively to estimate the total levels of soluble sugars, starch, proteins, lipids and phenols following the established methods for the sugars, starch [10], lipid [11], protein [12] and phenol [13] of experimental plants from different geographical loci showing the effect of diverse climatic conditions on metabolism. All experiments were repeated five times for precision and values were expressed in mean ± standard deviation in terms of air dried material.

RESULTS

Phytochemical screening

Plant material dried under shade was subjected to sequential extraction in petroleum ether, benzene, chloroform, ethanol and water. The maximum extraction value was found in aqueous extract as shown (Table 1).

Table 1 Successive solvent extraction of air dried *Phyllanthus niruri*

S. No.	Solvent	Color and Consistency	Extractive value (%w/w)
1	Petroleum ether	Yellowish green viscous	0.459
2	Benzene	Yellowish green sticky	0.885
3	Chloroform	Yellowish green viscous	0.774
4	Ethanol	Yellowish green sticky	2.625
5	Aqueous	Brownish powder	3.951

Preliminary phytochemical investigation of the whole experimental plant revealed that petroleum ether extract of swampy area shows the presence of proteins, carbohydrates and tanins, which was comparable to campus area, benzene extract of rocky area as well as swampy areas contains proteins and flavonoids, chloroform extract of campus area contains proteins, carbohydrates and flavonoids which was equally present in all the three geographical locations ,ethanol extract of three areas contains the highest amount of flavonoids, alkaloids and tannins, flavonoids, carbohydrates and proteins, which was comparable to aqueous extract content.

Table 2: Physico-chemical evaluation for different extracts of *Phyllanthus niruri*

S. No	Test	PE			BZ			CHL			ETH			AQS		
		C	SW	RA	C	SW	RA	C	SW	RA	C	SW	RA	C	SW	RA
1	**Proteins**	+++	+++	++	+	++	+	++	++	+	+++	+++	++	+++	+++	++
2	**Carbohydrate**	+	++	+	_	_	_	+++	++	+	+++	+++	++	++	++	+
3	**Flavonoids**	+	+	+	+++	+++	++	+++	+++	++	+++	+++	+	+++	+++	++
4	**Alkaloids**	_	_	_	_	_	_	_	_	_	++	++	+	++	++	+
5	**Tannins**	+	+	-	_	_	_	_	_	_	+	++	+	++	++	+

C: campus, RA: rocky areas, SW: swampy areas; PE: pet ether, BZ: benzene, CHL: chloroform, ETH: ethyl alcohol, AQS: aqueous
- absent; + trace amount; ++ moderate amount; +++significant amount

Primary metabolite quantification

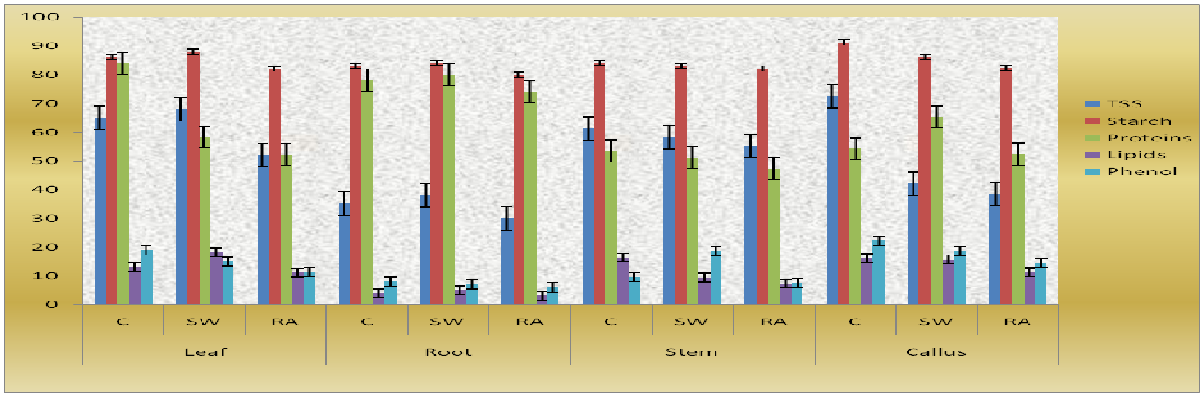
Primary metabolites quantified from root, stem, leaves and callus are shown in table 3. Quantitative estimation shows that sugar content was higher in leaves of this plant collected from swampy area(68.02±0.47mg/gdw),callus showed the highest amount of sugar i.e 72.66±0.51 mg/gdw, and minimum in roots of rocky areas 30.12±0.53 mg/gdw. Maximum amount of starch was observed in stem 91.10±0.87 mg/gdw (campus area) and leaf part (swampy area) 88.01±0.86 mg/gdw and minimum in roots (rocky area) 80.04±0.55 mg/gdw. Maximum amount of protein was observed in leaf part (campus area) 84.04±0.76 mg/gdw and minimum in stem (swampy area) 51.18±0.58 mg/gdw. Total levels of lipids were found to be higher in leaf part (swampy area) 18.28±0.58 mg/gdw and minimum in root part (campus area**)** 4.06±0.44 mg/gdw. The maximum amount of phenols was found in callus (campus area)

22.18±0.98 mg/gdw which was at par with leaves of plant of campus area **19.10±0.55** mg/gdw and minimum in roots (rocky areas) 6.05±0.96 mg/gdw.

Table 3: Total level of primary metabolites (mg/gdw) in various plant parts and callus culture of *Phyllanthus niruri.*

S. No.	Test	Plant Parts											
		Leaf			Root			Stem			Callus		
		C	SW	RA	C	SW	RA	C	SW	RA	C	SW	RA
1	Sugar	65.14± 0.58	**68.02± 0.47**	52.06± 0.59	35.16± 0.87	38.15± 0.45	30.12± 0.53	61.24± 0.66	58.26± 0.50	55.28± 0.52	**72.66± 0.51**	42.18± 0.56	38.54± 0.66
2	Starch	86.26± 0.76	**88.01± 0.86**	82.09± 0.57	83.02± 0.56	84.08± 0.56	80.04± 0.55	**91.10± 0.87**	83.06± 0.77	82.20± 0.51	84.16± 0.59	86.28± 0.56	82.32± 0.59
3	Protein	**84.04± 0.76**	58.31± 0.87	52.23± 0.86	78.18± 0.67	80.15± 0.89	74.13± 0.49	53.55± 0.53	**51.18± 0.58**	47.22± 0.57	54.29± 0.34	65.34± 0.59	52.42± 0.68
4	Lipids	13.24± 0.52	**18.28± 0.58**	11.16± 0.55	**4.06± 0.44**	5.09± 0.53	3.02± 0.75	16.44± 0.54	9.44± 0.96	7.35± 0.88	16.27± 0.83	15.78± 0.79	11.32± 0.53
5	Phenolcontent	**19.10± 0.55**	15.12± 0.53	11.43± 0.86	8.08± 0.78	7.10± 0.98	**6.05± 0.96**	9.62± 0.86	18.60± 0.66	7.54± 0.87	**22.18± 0.98**	18.62± 0.58	14.55± 0.76

mg/ gdw- milligram per gram dry weight ; C: campus, RA: rocky areas, SW: swampy areas; Data are presented as mean ±S.E.M



Concentration of primary metabolites in Phyllanthus niruri (mg/gdw)

DISCUSSION

Preliminary phytochemical screening of plant is very useful for determination of the active constituents in different solvents and their yields. Most of the active principles are found in alcoholic and aqueous extracts. Our results were in agreement of previous reported results. Most of the pharmacologically active metabolites in pharmaceuticals have their origin from primary metabolites which serves as precursors to synthesize them. Plant synthesizes primary metabolites (lipid, protein, starch, sugars, phenol etc.) for the normal growth, survival and development of itself. A comparative analysis reveals the effect of various biotic and abiotic factors in their formation as well as serves as mode to collect them from their rich source. Many polysaccharides purified from Chinese medicinal herbs and phenols are bioactive and possess immuno- modulating, anti-tumor and antibacterial activities [14, 15].These results are suggestive of primary bioactive compound of commercial importance and may result in great interest in plants pharmaceuticals. Primary metabolites analysis is necessary for knowing the nutritional potential of plants and them also from the precursors for the synthesis of secondary metabolites.

CONCLUSION

In recent investigation maximum extractive value was (3.951%) found in aqueous extract among test solvents. All the metabolites were qualitatively present in water and ethanolic extracts. Total levels of soluble sugars (68.02±0.47mg/gdw) in leaves of swampy area, starch (91.25±0.59mg/gdw) in stem, protein (84.04±0.76mg/gdw) in leaves of campus, lipid (18.28±0.58mg/gdw) in swampy area leaves and maximum phenolic content (22.18±0.98mg/gdw) was found in callus as compared to its root and stem. Callus also showed significant amount of all above metabolites. These primary metabolites further used for biosynthesis of secondary metabolites or bioactive compounds.

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