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PHYTOCHEMICAL EVALUATION AND QUANTIFICATION OF SELECTIVE PRIMARY METABOLITES FROM *CYPERUS ROTUNDUS* LINN.

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ABSTRACT

The present investigation was to develop a protocol for the production of some selective important primary metabolites viz. carbohydrate, proteins, lipids, phenols from a medicinally important plant of family Cyperaceae namely *Cyperus rotundus* Linn. It is an important medicinal plant widely used in traditional medicine around the world to treat stomach aliments, wounds, boils and blisters.. The present investigation quantify that *C. rotundus* contains many primary metabolites like carbohydrates, proteins, phenols, lipids, etc. in relatively high amounts. The plant parts (leaves and rhizomes) and *in vitro* callus cultures of *C. rotundus* varied in composition of primary metabolites studied.

KEYWORDS: *Cyperus rotundus*, Callus cultures, Primary metabolites.

INTRODUCTION

Cyperaceae is a large family of plants possessing medicinal properties. *Cyperus rotundus is an* important plant of this family commonly known as "Nagarmotha" and is originated in India as a weed and it is now widely distributed in the tropics and subtropics,[1] The phytochemical investigation of *Cyperus rotundus* rhizome have revealed the presence of polyphenol, flavonol glycoside, alkaloid, saponins, sesquiterpenoids[2] and essential oil[3] The callus *in vitro* cultures of many grasses and other monocotyledons have been grown successfully in tissue culture.[4] Smith (1968) and Mohan Ram and Batra (1970)[5,6] grew young inflorescence meristems of *Carex* and *Cyperus* in tissue culture, respectively but did not subculture or induce callus. The growth of nutsedge *in vitro* should provide a powerful

tool in fundamental studies of growth and development of the species. *C. rotundus* is a multipurpose plant, widely used in traditional medicine around the world to treat stomach aliments, wounds, boils and blisters.[7]

Primary metabolites are directly involved in growth and development of plants. Primary metabolites *viz*, protein, lipid, carbohydrates and so forth play a key role in metabolic processes such as photosynthesis, respiration and nutrient assimilation. They are used as raw material and food additives. Primary metabolites viz, chlorophyll, amino acid, nucleotides and carbohydrates have a key role in metabolic process such as photosynthesis, respiration and nutrient assimilation. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in pharmaceutical compounds. Plant synthesizes primary metabolites (lipid, protein, starch, sugar, phenol etc.) for the normal growth and development of itself. They are also used as raw material and food additives. The present study was conducted to investigate phytochemical estimation of primary metabolites viz, total soluble sugar, starch, lipids, proteins and phenols in *Cyperus rotundus.*

MATERIAL AND METHODS

In the present investigation, *in vivo* plant parts (leaves and rhizomes) and *in vitro* raised callus cultures[8] of *Cyperus rotundus* were analyzed for their biochemical estimation of primary metabolites viz, total soluble sugar, starch, lipid, protein and phenol. The plant material was collected from nursery of University of Rajasthan campus, Jaipur. The plant was identified and voucher specimen was deposited to the Herbarium, Department of Botany, University of Rajasthan, Jaipur. The callus culture of the plant was raised on Murashige and Skoog's (1962) medium[9] supplemented with growth hormones, using rhizomes as explants. The various plant parts (leaves and rhizomes) as well as calli (6 weeks old) of *Cyperus rotundus* were separately dried, powdered and extracted for various primary metabolites viz. carbohydrate, proteins, lipids, phenols using various standard techniques of extraction.

Estimation of primary metabolites

Carbohydrates

Total Soluble Sugars: The dried plant materials leaf, rhizome and callus (50 gm each) were homogenized separately in a mortar and pestle with 20 ml of 80 % ethanol and left overnight. Each of the sample was centrifuged at 1200 rpm for 15 min, the supernatants were collected separately and concentrated on a water bath. Distilled water was added to make up the volume up to 50 ml and processed further for quantitative analysis.

Starch: The residual mass obtained after extraction of total soluble sugrs of each of the test samples was suspended in 5.0ml of 52% perchloric acid. Then, 6.5 ml of water was added to each sample and the mixture was shaken vigorously for 5 min.

Quantitative Estimation

1 ml aliquot of each sample was used for the estimation of carbohydrates using the phenol-sulphuric acid. A Standard regression curve of standard sugar (glucose) was prepared. A stock solution of glucose 100 µg/ml was prepared in distilled water. From this solution, 0.1 to 0.8 ml was pipette out into eight separate test tubes and volume was made up to 1 ml with distilled water. These tubes were kept on ice; 1 ml of 5% phenol was added in each tube and shaken gently. 5 ml of concentrated Sulphuric acid was rapidly poured so that the steam hits the liquid and tubes were gently shaken during the addition of the acid. Finally the mixture was allowed to stand on a water bath at 26-30°C for 20 min. The characteristic yellow orange colour was developed. The optical density was measured at 490 nm using spectrometer (Carlzeiss, Jena DDR, VSU 2 P) after setting for 100% transmission against a blank (distilled water). Standard regression curve was computed between the known concentration of glucose and their respective optical density, which followed Beer's law.

All samples were analyzed in the same way as described above and contents of the total soluble sugar and starch were calculated by computing density of each of the sample with Standard curve.

Proteins: The test samples (50 mg each) were separately homogenized in 10ml of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4°C for 24 hrs. These mixtures were centrifuged separately and supernatant were discarded each of the residues was again suspended in 10ml of 5% TCA and heated at 80°C on a water bath for 30 min. The samples were cooled, centrifuged and supernatant discarded. The residue was then washed with distilled water, dissolved in 1N NaOH, and left overnight at room temperature.[10]

Quantitative estimation

Each of the above samples (1ml) was taken and the total protein content was estimated using the spectrophotometer and method of.[11] A regression curve of the standard protein (Bowin serum albumin, BSA) was prepared. A stock solution of BSA (Sigmachem.co, St. Louis USA) was prepared in 1N NaOH (1 mg/ml). Eight concentrations (ranging from 0.1-0.8mg/ml) were separately measured in test tubes and the volume of each was made up to 1

ml by adding distilled water to each, 65 ml of freshly prepared alkaline solution (prepared by mixing 2% Na_2CO_3) in 0.1N NaOH and 1ml 0.5% $CuSO_4.5H_2O$ in 1% Sodium Potassium Tartarate) was added and kept at room temperature for 10 min. In each sample 0.5 ml of Folin-ciocaltau reagent (commercially available reagent was diluted with equal volume of distilled water just before use) was added rapidly with immediate mixing and optical density of each sample was measured after 3min at 750 nm using spectrophotometer against the blank. Five replicates of each concentration were taken and the average value was plotted against their respective concentration to compute a regression curve.

All sample were processed in the same manner and the concentration of total protein content in each sample was calculated by referring the optical density of each sample with standard curve. Five replicate samples were taken in each case and mean value was calculated.

Lipids: The test samples were dried, powdered and 100 mg of each was macerated with 10 ml distilled water, transferred to a conical flask containing 30ml of Chloroform and Methanol (2/1; V/V:).[12] The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Late, 20ml of Chloroform was mixed and 2ml of water was added and centrifuged. 2 layers separated, the lower layer of Chloroform which contained all the lipids, was carefully collected in the pre-weighed glass vials and the colored aqueous layer of Methanol which contained all the water soluble substances and thick pasty interface layer were discarded in each test ample. The Chloroform layers were evaporated to dryness and weighed. Each treatment was replicated thrice and their mean values calculated.

Phenol: The test samples dried and powdered and 200 mg was macerated with 80% ethanol (10ml) for 2hr and left overnight at room temperature. It was centrifuged, the supernatant was collected individually and volume of each was raised to 40ml with 80% ethanol. A stock solution (40%) of galic acid was prepared in 80% ethanol, from which 0.1 to 0.9 ml was transferred into test tubes separately and the volume in each case was raised to 1 ml with 80% ethanol. To each of these tubes 1 ml of folin-Ciocalteau reagent (dilute the reagent 1:2 with distilled water) and 2 ml of 20% of Na_2CO_3 solution was added. The mixture was shaken vigorously. Each of the tubes was boiled on water bath and cooled at room temperature and diuted to 25 ml with distilled water. The OD was taken at 750 nm using spectrometer against blank. The experiment performed in triplicate and average OD was plotted against respective concentration to compute in similar manner, OD's were measured and the total level of phenol was calculated from the mean value by referring and the total level of phenol was

calculated from the mean value by referring the OD of the test sample with regression curve of the standard.

RESULTS AND DISCUSSION

Primary metabolites are the basic substances involved in the biosynthesis of complex substances in the cellular machinery. Biochemical investigation of plant parts is a necessary prerequisite to understand all metabolic and physiological processes in the plant kingdom. Carbohydrate, protein, lipids, amino acids, phenols, chlorophylls, vitamins, hormones etc. are the soul of plant without which the processes, such as glycolysis, respiration, or photosynthesis are hampered. In the present investigation, the plant of family Cyperaceae *viz. C. rotundus* was evalualted quantitatively *in vivo* and *in vitro* for the analysis of total soluble sugar, starch, lipid, protein and phenol (Table1).The plant parts (leaves and rhizome) and six weeks old callus cultures of *C. rotundus* were dried and powdered for the analysis of various primary metabolites. The results obtained in the present investigation are as follows.

Carbohydrates

Total soluble sugars

In the present investigation, maximum content of soluble sugar level was observed in rhizome of *C. rotundus* (54.0±1.38 mg/g.dw) and minimum in calli (37.8±0.53 mg/g.dw) (Table 1). Plant suartificial sweetener and they can even help in diabetes by supporting the body in its rebuilding (Freeze, 1998).

Starch: The maximum content of starch was observed in leaves of *C. rotundus* (45.8±0.28mg/g.dw) and lowest content in calli (28.2±1.97mg/g.dw) (Table 1). The major source of starch are wheat, potato and cassava mostly used as food.[13] Although, starch is also used in cosmetic formulation like face powder and in dusting preparations that use aerosol dispensing systems. Starch can also be used as a substitute for petroleum based plastics.[14]

Protein: The maximum content of proteins was observed in the leaves of *C. rotundus* (47.6±1.81mg/gdw) while minimum was in the calli (21.7±0.15mg/g.dw) (Table 1). The presence of higher protein level in the plant parts towards their possible increase in food value or that a protein based bioactive compounds can also be utilized in future for enhancing food quality.

Lipid: The observed mean value for lipid was higher in rhizome part of *C. rotundus* (51.1±1.74 mg/g.dw) while minimum amount of lipid was observed in the calli (19.4±0.61 mg/g.dw) (Table 1). Lipids are reserve plant material, involved as an integral part of biological cell membrane serving as module to hormone and vitamins such as fats, essential oils, waxes, terpenoids and oleoresin. With a strong foundation in research and development, plant lipid have developed products that work with diverse requirements, be it culinary, medicinal or cosmetic.

Phenols: The highest amount of phenol was found in the rhizome part of *C. rotundu* (57.04±1.92 mg/g.dw) while minimum amount was observed in the calli (24.1±0.3 mg/g.dw (Table 1). Plant phenols are getting more attention as safe antioxidants and they have enormous contribution to combat chronic diseases. Nutraceuticals like polyphenols are in high demand against the dreadful diseases including cancer, cardiovascular, infectious disease and disorders of immune system. Indeed, these compounds may have beneficial effects on human health.[15] The higher amount of phenols is important in the regulation of plant growth development and diseases resistance.

Present study concludes that *C. rotundus* is a rich source of primary metabolites *viz*. sugar, starch, protein, lipid, and phenols.

Table1: Yield content (mg/g dw) of primary metabolites of *C. rotundus.*

Plant parts	Primary metabolites (mg/g. dw)				
	Sugar	Starch	Proteins	Lipids	Phenols
Leaf	41.3± 0.26	45.8± 0.28	47.6± 1.81	33.6±0.36	42.04± 1.92
Rhizome	54.0± 1.38	32.1± 0.45	38.3± 0.42	51.1± 1.74	57.04± 1.92
Callus	37.8± 0.53	28.2± 1.97	21.7± 0.15	19.4± 0.61	24.1±0.3

Abbreviations: mg/gdw = milli gram dry weight

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