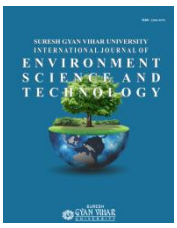

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***Research Article* :**

Evaluation of Primary Metabolites from *'Bunium persicum (Boiss.) B. Fedtsch'*

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Keywords

Biochemical PrimaryMetabolites; *Bunium persicum,* Leaves, Phenols, Lignin

Abstract

Traditional remedies have a long base line in India and and have gained tremendous attention and applicable tools for treating various disorders. The traditional medicinal plants are gaining keen attention in the health care sector. Biochemical studies of the individual plant parts is a necessary prerequisite in order to evaluate their importance in the over all metabolism of the plant as well as the role of phytochemicals that are synthesized as crucial products of metabolism in various physiological phenomena . Plant synthesize a wide range of chemical compounds which are classified based on their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites directly involved in growth and development while secondary metabolites are not involved directly and they have been worked as biocatalysts. In the present study various plant parts of *Bunium persicum* were evaluated, separately for their metabolite content. The maximum content of total soluble sugars was observed in leaves. All primary metabolites were more in leaves as compared to stem. Total phenol content was found to be more

in stem due to presence of lignin than leaves.

Introduction

Biochemical studies of the individual plant parts is an necessary prerequisite in order to evaluate their importance in the over all metabolism of the plant, as well as the role of specific substances that may be produced as direct or indirect products of metabolism in same physiological processes. Metabolism comprises coordinate series of coupled enzymatic conversions in living organisms. Hence, carbohydrates, proteins, amino acids, chlorophyll, vitamins, hormones, phenols etc are basic building blocks of plant without which the plant life is hampered (Dhania et al., 2022)

Carbohydrates comprise the bulk of organic matter of plants having storage and skeletal function, in addition to their involvement in the basic metabolism of all organisms. Comparative distribution of carbohydrates in the plant kingdom has been well studied. Different methods for determining reducing sugars, sucrose and starch have been described in detail (Bravo et al 1998., Walker et al 1975.,Osborne et al 1962.,Utsumi et al 1987).

Proteins are complex nitrogenous biopolymers and areubiquitous components of all living tissues. They have indispensable function in cellular architecture, catalysis metabolic regulation and are an important weapon in the defense arsenal of many higher organisms. Plant proteins have been well reviewed (Utsumi et al., 1987) and a method for estimation of total protein content had been described by different workers (Niranjan et al., 2013,Cronin et al., 1979).

Lipids are molecular organic compounds, composed largely of carbon and hydrogen and are essential for cell growth. They combine with carbohydrates and proteins to form the major component of plant and animal cells. Phenolic compounds comprise a wide range of plant metabolites, which bear at least one hydroxyl group attached to an aromatic ring system.

Various workers described that the phenolic substances are the active products of cellular metabolism and of great importance as they act as analog of growth hormones (Loomis et al., 1937) .Based on their chemical structures more than 10 classes of polyphenols have been identified (Semeniuc et al.,2017).

- ***'Bunium persicum (Boiss.) B. Fedtsch'*** is a endemic of west Asia and has a finite dispersion. It relevant to the family Apiaceae (Odeh et al., 2021). In Kashmir it has been revealed from Dara, Chrarisharief

, Harwan Dara, WasturwanTral, Khrew, Gurez, Baramulla (Muthu et al .,2006) deliberate its therapeutic valuables by appraise the crucial oil and disparate extricate of the seeds of this plant for antioxidant activity by DPPH assay, β-carotene bleaching and ammonium thiocyanate methods and reported its usage as antiobesity and lactogage , carminative and antispasmodic, (Bansal et al., 2021). *B. persicum* seeds have commonly been passed down in analysis of digestive disorders and urinary are certified for their anti-convulsion, anti-nocceptive, anti-dyspnea, antidiarrheal, anti-asthma, anthelmintic and properties in Iranian folk medicine (Sharififar et al., 2010). In Indian folk medicine, leaves of plant are used to cure abdominal pain and flower heads as carminative (Sofi et al., 2009). Fruit decoction in water is used to treat cold, headache, stomachache, joint pain, tuberculosis, fever etc. *B. persicum* seed oil is capable of suppressing the initial stage of an inflammatory process and has been found effective in treatment of gastro-intestinal disorders including diarrhea (Shahsavari et al., 2008) .Antibacterial effects of different extracts against different strains of bacteria showed that essential oil has higher inhibitory effect on gram-positive bacteria than gram-negative bacteria (McCredeay et al., 1950).

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Material and Methods

Plant parts (Leaves and Stem) of ***'Bunium persicum (Boiss.) B. Fedtsch'*** (were separated, shade dried and powdered for evaluation of various primary metabolites.

Carbohydrates

Total Soluble Sugars

Extraction: The dried test materials (50 mg each) were homogenized in a mortar and pestle with 20 ml of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 min; the supernatants were collected separately and concentrated on a water bath using method of Loomis and Shull (1973). Distilled water was added to make up the volume up to 50 ml and processed further for quantitative analysis.

Starch

Extraction: The residual mass obtained after extraction of total soluble sugars of each of the test samples was suspended in 5.0 ml of 52% perchloric acid (Bansal et al., 2021). Later, 6.5 ml of water was added to each sample and the mixture was shaken vigorously for 5 min.

Quantitative Estimation: 1ml aliquot of each sample was used for the estimation of carbohydrates using the phenol-sulphuric as substrate reagents (Bashir et al 2014). 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 kept on ice; 1 ml of 5% phenol was added in each tube and shaken gently. 5 ml of conc. 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^{o}C for 20 min. The characteristic yellow orange colour was developed. The optical density was measured at 490 nm using spectrometer after setting for 100% transmission against a blank (distilled water). Standard regression curve was computed between the known concentrations 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 sugars and starch were calculated by computing optical density of each of the samples with standard curve.

Proteins

Extraction*:* The test samples (50 mg each) were separately homogenized in 10 ml of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4^{o}C for 24 h. These mixtures were centrifuged separately and supernatants were discarded. Each of the residues was again suspended in 10 ml of 5% TCA and heated at 80^{o}C on a water bath for 30 min. The samples were cooled, centrifuged and the supernatants of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH, and left overnight at room temperature (Hassanzad et al .,2018).

Quantitative Estimation: Each of the above samples (1ml) was taken and the total protein content was estimated using the spectrophotometer and method of Lowry *et al* (1951). A regression curve of the standard protein (bovine serum albumin, BSA) was prepared. A stock solution of BSA (Sigma Chem. Co., St. Louis, USA) was prepared in 1N NaOH (1mg/ml). Eight concentrations (ranging from 0.1 to 0.8 mg/ml) were separately measured in test tubes and the volume of each was made up to 1ml by adding distilled water. To each, 5ml of freshly prepared alkaline solution (Prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4$. 5 H_2O in 1% Sodium potassium tartarate) was added and kept at room temperature for 10 min. In each sample 0.5 ml of Folin-Ciocalteau 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 30 min

at 750nm using spectrophotometer against the blank (Khan et al.,2019). Five replicates of each concentration were taken and the average value was plotted against their respective concentrations to compute a regression curve. All samples were processed in the same manner and the concentration of the 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

Extraction and Quantification: The plant material were dried, powdered and 100 mg was crushed in 10 ml distilled water and then whole mixture was transferred to a conical flask containing 20 ml of chloroform and 10 ml of methanol (Khan et al ., 2019). The mixture was thoroughly mixed and left overnight at room temperature in dark for complete extraction. Later, 20 ml of chloroform mixed with 2 ml of water were added and centrifuged. Two layers separated, the lower layer of chloroform, which contained all the lipids, was carefully collected in the pre weighed glass vials and the coloured aqueous layer of methanol which contained all the water soluble substances and thick pasty inter face layer were discarded in each test sample. The chloroform layers were evaporated to dryness and weighed. Each treatment was replicated thrice and their mean values calculated.

Phenols

Extraction: The deproteinized test materials (200 mg each) macerated with 10 ml of 80% ethanol for 2 h, and left overnight at room temperature. The mixtures were centrifuged and the supernatants were collected separately and maintained up to 40 ml by adding 80% ethanol.

Quantitative Estimation: Total phenol content in each sample was estimated by the spectrophotometer method of Bray and Thorpe (1954). It included the preparation of a regression curve of standard phenol (Tannic acid). A stock solution of tannic acid was prepared by mixing 40 mg of standard phenol in 1 ml of 80% ethanol. Eight concentrations ranging from 0.1 to 0.8 ml were prepared in test tubes and volume was raised to 1 ml with 80% ethanol. To each test tube, 1ml of Folin-Ciocalteau reagent (commercially available reagent was diluted by distilled water in 1:2 ratio just before use) and 2ml of 20% sodium carbonate solution was added and the mixture was shaken thoroughly. The samples were placed in boiling water for 1 min and cooled under running water. These reaction mixtures were diluted to 25 ml by adding distilled water and the optical density was read at

Samples	Total Soluble Sugars	tf Starch	Proteins	Lipids	Phenols
Stem	51.36 (±0.18)	24.72 (±1.26)	239 (±0.78)	33.58 (±0.18)	16.13 (±0.44)
Leaves	55.65 (±0.44)	29.82 (±0.81)	418 (±1.69)	37.64 (±0.56)	14.13 (±0.88)

750nm against a blank (Dowd et al., 2011). The optical density of each sample was plotted against the respective concentration of total phenols to compute a regression curve. The concentrations in the test samples were calculated by referring the respective optical density of test samples against standard curve of tannic acid.

Results and Discussion

The maximum content of total soluble sugars was observed in leaves (55.65 mg/gdw) and in stem it was in lower quantity (51.36 mg/gdw). Starch content was more in leaves (29.82mg/gdw) as compared to stem (24.72 mg/gdw). Maximum content of proteins was present in leaves (418 mg/gdw) as compared to stem . Quantitatively total lipids were found to be more in leaves as compared to stem (37.64 mg/gdw). Total phenol content was found to be more in stem due to presence of lignins (16.13 mg/gdw) than leaves (14.13 mg/gdw). Except Phenols all metabolites were found to be more in leaves **(Table 1)**

Table 1 Total levels of various Primary Metabolites (mg/gdw) in *'Bunium persicum (Boiss.) B. Fedtsch'*

Since earlier times traditional practice to cure diseases through medicinal plants has

been followed from mankind. The correlation between man and keen urge for novel drugs in nature has very old history ,agreed by several substantiation mentioned in documents , preserved monuments, and even original floral specimen . Keen attention of use of medicinal plants is outcome of fight for several decades against drastic life threatening diseases which forced scientific communities to urge for new medicines in barks, seeds, fruit bodies, and other plant parts. Primary metabolites are are crucial for essential phenomena of organisms. Since primary metabolites are precursors of other primary metabolites or as building blocks for the synthesis of secondary metabolites, research on biosynthetic routes is crucial for understanding the physiology and for designing new plants with stimulated nutritional importance (Geeta et al.,2014, Baek et al., 2021).

Primary metabolites such as amino acids, organic acids, or nucleosides are among the simplest biotechnological products, and are commonly used as raw materials for other processes such as fermentation or chemical syntheses. While they are commonly used as nutritional supplements or flavoring agents, they are rarely used as therapeutic compounds (Bray et al.,1954**) .** Some primary metabolites such as peptides and lectins also exhibit antimicrobial properties. Their mechanism of action is believed to be through the formation of ion channels in cell membrane. Lectins such as ConA from *Canavalia ensiformis* and Con Br isolated from *Canavalia brasiliensis* have been shown to retard the biofilm development in *C. tropicalis* (Jayaraman et al.,1984). In ***'Bunium persicum (Boiss.) B. Fedtsch'*** Cuminaldehyde (27.8%), γ-terpinene (23%), c-Terpinen-7-al(19.2%), p-Cymene(13.5%), limonene (5.8%) (Iran) Carvone (23.3%), limoneItaly) (18.2%), germacrene D (16.2%), trans-dihydrocarvone (14.0%), carvacrol (6.7%) (Italy) , Trans-anethole (93.9%), limonene (1.05%) and estragole (1.05%), Linalool (0.29%), 3-Carene (0.15%) (India) these are the main component of ***'Bunium persicum*** (Dubois et al .,1956).

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