

BIOCHEMICAL ESTIMATION OF PRIMARY METABOLITES AND ANTIOXIDANT ACTIVITY OF *CORCHORUS OLITORIUS* L. AND *LEPTADENIA PYROTECHNICA* (FORSK) DECNE

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ABSTRACT

In the present investigation, fibre yielding plants *Corchorus olitorius* L. and *Leptadenia pyrotechnica* (Forsk) Decne were carried out for their biochemical estimation of primary metabolites (carbohydrates, lipid, protein and phenol) and antioxidant activity was performed using FRAP, Peroxidase assay, LPO. The highest amount of lipid (140mg/gdw) from shoot and the minimum was phenol (0.22mg/gdw) observed from root of *L.pyrotechnica*. whereas in the case of *Corchorus olitorius* L. maximum quantity of lipid (130mg/gdw) from fruit and the minimum was starch (1.5mg/gdw) observed from stem. Maximum protein was observed in shoot of *C.olitorius* i.e. 16.5mg/100g.d.w. whereas in *L.pyrotechnica* maximum amount of protein were found in roots i.e. 15mg/100g.d.w. *Corchorus olitorius* fruit was found to be better antioxidant when observed by FRAP while shoot was more potent when analyzed by peroxidase and LPO. In *Leptadenia pyrotechnica* shoot had better antioxidant potential than root.

Keywords: Primary metabolites, *Corchorus olitorius*, *Leptadenia pyrotechnica*, FRAP and LPO.

INTRODUCTION

Most of auervedic medicine obtained from plants, like normal laboratories where a huge amount of chemicals are synthesized so plants are unique source of chemical compounds. Herbal drugs are of great importance to human population¹. Plants are worldwide used for the management of various diseases.

Corchorus olitorus locally called jute plant belongs to the family, Tiliaceae and found globally, for the treatment of dysuria and chronic cystitis². The edible plant parts are consumed and safe to eat as potherbs in Africa, Nigeria, Ghana and Cameroon, in West Africa, the shoots and leaves are combined in soups and stews. It has large quantity of calcium, folic acid, fibre, Vitamin A, protein, iron, carotene³.

Leptadenia pyrotechnica (Forsk) Decne belong to Asclepiadaceae, locally called as Khimp in India and Pakistan⁴. It is used usually for anti-inflammatory, anabolic, analgesic, astringent and laxative⁵. diabetes and eczema are also treated by this plant⁶. During photosynthesis primary metabolites are synthesized and these bioactive compounds

are necessary for plant life, growth and development⁷. Many primary metabolites used as pharmacologically active metabolites in pharmaceutical compounds. Many types of metabolic reactions creates diverse types of 'oxidant' and 'antioxidant' species in human bodies. Super oxide anion, hydrogen peroxide, hydroxyl radicals are work as Reactive Oxygen Species (ROS)⁸. When oxidants increase in human body as compared with antioxidant, Oxidative stress is developed. Due to this oxidative stress many diverse diseases and complexities including aging, cataract cancer, autoimmune disorders, arthritis, cardiovascular and neurodegenerative diseases developed in body⁹.

In the present research biochemical estimation of primary metabolites was done by using different protocols and antioxidant potential of *L. pyrotechnica* and *C. olitorius* in both selected plant parts was carried out by FRAP, Peroxidase, LPO methods.

MATERIALS AND METHODS

Collection of plant material

The powdered parts of *Leptadenia pyrotechnica* (shoot and root) was collected from the field of Heerapura (Jaipur) and *Corchorus olitorius* (fruit and shoot) was collected from University of Rajasthan campus, Jaipur. The plant was identified and voucher specimen was deposited to the Herbarium of Department of Botany, University of Rajasthan, Jaipur. The voucher (RUBL*No.211572 for *Leptadenia pyrotechnica* and RUBL*No.211573 for *Corchorus olitorius*)

Evaluation of Primary Metabolites

Plant parts of *L.pyrotechnica* and *C. olitorius* were harvested and cleaned again and again 4-5 times with water and then put the sample in shade for drying. When samples were dried, grinded in powdered form to conduct quantification of primary metabolites.

Carbohydrates

Total Soluble Sugars

The grinded plant parts (50 mg each) were crushed with 20 mL of 80% ethanol and kept for 24 h. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected independently¹⁰. Distilled water was added to rise volume till 50 mL and processed as per described method.

Starch

The remaining mass obtained after centrifugation was suspended in 5 mL of 52% perchloric acid¹¹. Later, 6.5 mL of water was added and shaken dynamically for 5 minutes. The protocol further was done using the phenol sulphuric acid method¹². A standard regression curve of glucose was used as reference compound. A stock solution of glucose (100 μ g mL⁻¹) was prepared. Further, 0.1 to 0.8 mL was pipette out and volume was made up to 1 mL with distilled water. Further, 1 mL of 5% phenol was added and shaken gently. 5 mL of conc. sulphuric was added rapidly. Finally the mixture was stable at 26-30°C for 20 minutes. The characteristics yellow orange colour was developed. The optical density was measured at 490 nm (Carl Zeiss, Jena DDR, VSU 2 P), against a blank (distilled water). Standard regression curve was computed between the known concentration of glucose and their respective optical density, which followed Lambert Beer's Law.

Proteins

The plant material (50mg each) were crushed in 10 mL of cold 10% trichloroacetic acid

(TCA) for 30 min and kept at 4°C for 24 hours. These mixtures were centrifuged and residues was again suspended in 10 mL of 5% TCA and heated at 80°C for 30 minutes. The samples were cooled, centrifuged and supernatants of each were discarded. The residue was then washed with distilled water, dissolved in 10 ml of 1N NaOH, and left overnight¹³.

Established protocol was used for quantification¹⁴. A stock solution of BSA (Sigma Chem. Co., St. Louis, USA) was prepared in 1N NaOH (1mgL⁻¹). Different stock (ranging from 0.1 to 0.8 mgL⁻¹) were used and volume of each sample was made to 1 mL. To each, 5 mL of freshly prepared alkaline solution (Prepared by mixing 50 mL of 2% Na₂CO₃ in 0.1 N NaOH and 1 mL of 0.5 % CuSO₄ .5H₂O in 1% Sodium potassium tartarate) was added and kept at room temperature for 10 minutes. In each sample 0.5mL of Folin-Ciocalteu reagent was added rapidly with immediate mixing and optical density of each sample was measured after 30 minutes at 750 nm. Average value was plotted against their respective concentrations to compute regression curve.

Lipids

The dried plant samples were powdered and 100mg was crushed with 10 mL distilled water then transferred to a flask which containing 30 mL of chloroform and methanol¹⁵. Later, 20 mL of chloroform mixed with 2 mL of water were added and centrifuged. After centrifugation layers were separated, the lower layer of chloroform, which contained all the lipids, was separated in the preweighed glass vials and the upper aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers which contained all the lipids, dried *in vacuo* and weighed. Each procedure was repeated three times and their mean values were calculated.

Phenols

The deproteinized plant samples (200mg each) were homogenized with 10 mL of 80% ethanol for 2 hours, and left overnight. The mixtures were centrifuged and the supernatants were collected separately and maintained up to 40 mL by adding 80% ethanol.

Total amount of phenol was done using established protocol¹⁶. Tannic acid was used as reference. A stock solution of tannic acid was prepared by mixing 40 mg of in 1 mL of 80% ethanol. Different concentrations ranging from 0.1 to 0.8 mL were prepared in the test tube and volume was raised to 1mL by

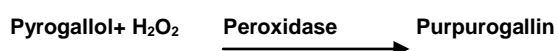
addition of 80% ethanol. To each test tube , 1mL of Folin-Ciocalteu reagent (commercially available reagent was diluted by distilled water in 1:2 ratio just before use) and 2 mL of 20% sodium carbonate solution was added and then mixture was shaken thoroughly. The samples were heated for 1 min and cooled under running water. These reaction mixtures were diluted to 25 mL by adding distilled water and optical density was read at 750 nm against a blank. The optical density of each sample was plotted against the respective concentration of total phenols to compute regression curve.

FRAP Assay (Ferric reducing ability of Plasma)

The FRAP assay depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH. The protocol was followed as per prescribed¹⁷.

Peroxidase Assay (POXA)

The method of assay measures the oxidation of pyrogallol to purpurogallin by peroxidase when catalyzed by peroxidase at 420 nm and at 20°C.



Procedure

Test samples (200mg) was macerated with 10mL of phosphate buffer and refrigerated centrifuged at 10000 rpm for 20 minutes. The clear supernatant was taken as the enzyme extract.

The activity was assayed after the method¹⁸. with the slight modifications. 2.4mL of phosphate buffer, 0.3mL of pyrogallol and 0.2mL of H₂O₂ was added. The optical density was taken at 420nm immediately after adding 0.1mL enzyme extract.

Lipid Peroxidation (LPO) Assay

Procedure

The level of lipid peroxidation was measured in terms of malondialdehyde content of a product of lipid peroxidation using established protocol¹⁹.

RESULTS AND DISCUSSION

Quantification of Primary metabolites

The present investigations quantify that *L. pyrotechnica* and *C. olitorius* contain many primary metabolites like carbohydrates, proteins, phenols and lipids. In the present investigation, various plant parts (fruit and shoot of *C. olitorius*) and (root and shoot of *L. pyrotechnica*) were estimated for their

primary metabolites viz, total soluble sugar, starch, lipid, protein and phenol (Table 1 and 2).

Proteins

The maximum amount of protein was observed in shoot of *C. olitorius* (16.5±0.28mg/gdw) while minimum was in shoot of *L. pyrotechnica* (14±0.18mg/gdw) (table1 and 2 respectively). High level of protein indicate their food value.

Lipid

The observed quantity for lipid was higher in shoot of *L. pyrotechnica* (140±0.94mg/gdw) while minimum in root of *L. pyrotechnica* (20±0.33mg/gdw) (Table2). In case of *C. olitorius* fruits have more lipid content than shoot (Table1).

Phenol

The amount of phenol was highest in fruit of *C. olitorius* (4.45±0.24mg/gdw) (table1) and minimum was reported in root of *L. pyrotechnica* (0.22±0.003mg/gdw) (Table2).

Carbohydrate

Total soluble sugar: in the present study, among all the samples, shoot of *L. pyrotechnica* exhibited higher total soluble sugar level (5.5±0.22mg/gdw) (Table2) and minimum in shoot of *C. olitorius* (1.6±0.09mg/gdw) (Table1).

Starch

the maximum content of starch was observed in root of *L. pyrotechnica* (4±0.16mg/gdw) and minimum in shoot of *L. pyrotechnica* (1±0.05mg/gdw) (Table2).

Determination of antioxidant activity

The antioxidant action of shoot and fruit of *C. olitorius* L. and shoot and root of *L. pyrotechnica* find out by using FRAP, LPO and Peroxidase methods. The results of antioxidant activities of *C. olitorius* L. and *L. pyrotechnica* are present in Table 3 and Table4 fruit of *C. olitorius* L. was found to be better antioxidant potential when sample analyzed by FRAP while shoot was showing better potential in Peroxidase and LPO method. When samples of *Leptadenia pyrotechnica* were analyzed by FRAP, Peroxidase, LPO it was observed that shoot had higher antioxidant activity than root (Table3 and 4).

The present result showed potential of these plants as herbal drug for treating human from diseases like cancer, cardiovascular effects, and aging which were caused by free radicals.

CONCLUSION

In the present study, it was found that *L. pyrotechnica* and *C. olitorius* are rich source of Protein lipid and carbohydrate.. When children not intake sufficient amount of protein by their food, they suffer from protein-energy under nourishment. The both plants can serve as a boon for curing malnutrition in a developing country where many people suffer from malnutrition because *L. pyrotechnica* and

C. olitorius easily available in rural areas. Both studied plants possessed significant antioxidant activity. Therefore it can be concluded that both plants effective in treating various diseases that are caused by free radicals produced in body by extreme oxidative stress, thus further advance research provide an alternative source of antioxidant agents.

Table 1: Primary metabolites from Shoot and Fruit of *C. olitorius* L.(in mg/gdw)

Primary metabolites	Shoot	Fruit
Protein	16.5±0.28	15.5±0.27
Lipid	110±0.52	130±0.78
Phenol	3.350±0.13	4.450±0.24
TSS	1.6±0.09	2.9±0.11
Starch	1.5±0.08	2.5±0.10

Values are the mean ±SEM(n=3 variable in each group).
*P<0.05;**P<0.001 compared with the control;P<0.001

Table 2: Primary metabolites from Shoot and Root of *L. pyrotechnica*.(in mg/gdw)

Primary metabolites	Shoot	Root
Protein	14±0.18	15±0.22
Lipid	140±0.94	20±0.33
Phenol	0.26±0.004	0.22±0.003
TSS	5.5±0.22	3±0.12
Starch	1±0.05	4±0.16

Values are the mean ±SEM(n=3 variable in each group).
*P<0.05;**P<0.001 compared with the control;P<0.001

Table 3: Antioxidant activity of *C. olitorius* L

Antioxidant assay	Shoot	Fruit
FRAP (mM/g/fresh wt)	1.01±0.11	2.32±0.19
Peroxidase (mM/g/fresh wt)	0.63±0.08	1.33±0.16
LPO(mM/g/fresh wt)	33.79±1.23	11.47±0.87

Values are the mean ±SEM(n=3 variable in each group).
*P<0.05;**P<0.001 compared with the control;P<0.001

Table 4: Antioxidant activity of *L. pyrotechnica*

Antioxidant assay	Shoot	Root
FRAP (mM/g/fresh wt)	1.90±0.14	1.26±0.15
Peroxidase (mM/g/fresh wt)	0.036±0.002	0.072±0.006
LPO(mM/g/fresh wt)	81.84±2.59	61.53±1.58

Values are the mean ±SEM(n=3 variable in each group).
*P<0.05;**P<0.001 compared with the control;P<0.001

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