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Research Article

ESTIMATION OF PRIMARY METABOLITES AND ANTIOXIDANT POTENTIAL OF *EUPHORBIA HIRTA* AND *LUCAS ASPERA*

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ABSTRACT

In the present investigation, medicinal plants viz *Euphorbia hirta* and *Lucas aspera* were selected 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 proteins (280mg/gdw) was observed in stem and the minimum content was total soluble sugars (2.2mg/gdw) in root of *L.aspera* while in *E. hirta* maximum quantity of lipids (333.3mg/gdw) was observed in stem which was at par with roots and the minimum amount of phenol (0.75mg/gdw) was observed in roots.. Leaves of *L. aspera* was found to be better antioxidant (150 µmol Fe (II)/g) and leaves in *E. hirta* (160 µmol Fe (II)/g) when observed by FRAP assay while roots of *L. aspera* (20.77 mg/g fresh weight) and roots again in *E. hirta* (16.27 mg/g fresh weight) was more potent when analyzed by LPO. In peroxidase roots of *L.aspera* (0.56 mg/g fresh weight) and stem in *E. hirta* (0.176 mg/g fresh weight) were found to be potent.

INTRODUCTION

Most of ayuervedic medicine obtained from plants, so they are unique source of chemical compounds. Herbal drugs are of great importance to human population [1]. Plants are worldwide used for the management of various diseases.

Euphorbia hirta, known in the Philippines as "Tawa-tawa" or "gatas-gatas," is a hairy herb grown in open grasslands, roadsides and pathways. The plant belongs to Euphorbiaceae. This family occurs mainly in the tropics, with the majority of the species in the Indo-Malayan region and tropical America a good second. There is a large variety in tropical Africa, but it is not as abundant or varied as in these two other tropical regions. However, *Euphorbia* also has many species in non-tropical areas such as the Mediterranean Basin, the Middle East, South Africa, and southern USA.

It was reported that [2] ethanol, methanol, chloroform and Aqueous (water) extracts of leaf, stem, root and whole plant of *Euphorbia hirta L. (Euphorbiaceae*) were used to evaluate antibacterial activity. These plant metabolites according to their composition are grouped as alkaloids, glycosides, corticosteroids, essential oils etc. *Euphorbia hirta, (family Euphorbiaceae)* is an herb found in many parts of the world. In Sanskrit it means "Dugadhika" According to the Doctrine of Signatures, the plant has a reputation for increasing milk flow in women, because of its milky latex, and is used for other female complaints as well as diseases of the respiratory tract. The plant has been reported as increase in urine output, antidiarrheal, antispasmodic, anti-inflammatory.

Leucas aspera is a species within the *Leucas* genus and the Lamiaceae family. Although the species has many different common names depending on the region in which it is located, it is most commonly known as Thumbai.

Leucas cephalotes is an annual herb growing widely in India. It is used as anti-filarial, anti-inflammatory, antioxidant, hepato-protective, antimicrobial & anti-diabetic.

World is endowed with a rich heritage of medicinal plants [3]. The use of medicinal agents presumably predates the earliest recorded history. The medicinal plants are widely used by the traditional practitioners for various ailments. *Leucas lavandulaefolia (Labiatae)* commonly known as 'Gumo' is a well-known plant used in the Indian system of medicine. Various parts of this plant have been used in traditional medicine. The plant include phytochemicals are acacetin, chrysoeriol, linifoliside, linifoliol, chrysoeriol-6'' (OAc)-4'-β-glucoside, lupeol and taraxerone.

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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) [4]. When oxidants increase in human body, 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 body [5].

MATERIALS AND METHODS

Carbohydrates

Total Soluble Sugars

Extraction

The dried experimental plant material (50 mg each) was homogenized in pestle and mortar with 20 mL of 80% ethanol separately and left overnight. Each sample was centrifuged at 1200 rpm for 15 minutes; the supernatants were collected separately and concentrated on a water bath using the established method [6]. 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 mL of 52% perchloric acid [7]. Later, 6.5 mL of water was added to each sample and the mixture was shaken vigorously for 5 minutes.

Quantitative Estimation

1mL of aliquot of each sample was used for the estimation of carbohydrates using the phenol sulphuric acid as per prescribed protocol [8] A standard regression curve of standard sugar (glucose) was prepared. A stock solution of glucose (100µg mL^{-1}) was prepared in distill 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 conc. sulphuric added 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 water bath at 26-30^{0}C for 20 minutes. The characteristics yellow orange colour was developed. The optical density was measured at 490 nm using spectrophotometer (Carl Zeiss, 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 Lambert Beer's Law. All samples were analyzed in the same way as described above and contents of total soluble sugars and starch were calculated by computing optical density of each o the samples with standard curve.

Proteins

Extraction

The test sample (50mg each) were separately homogenized in 10 mL of cold 10% trichloroacetic acid (TCA) for 30 min and kept at 4^{0}C for 24 hours. 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 80^0 on a water bath 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 overnight at room temperature [9].

Quantitative Estimation

Each of the above samples (1 mL) was taken and the total protein content was estimated using the spectrophotometer through optimized method [10]. A regression curve was prepared. A stock solution of BSA (Sigma Chem. Co., St. Louis, USA) was prepared in 1N NaOH (1mgL^{-1}). Eight concentrations (ranging from 0.1 to 0.8 mgL^{-1}) were separately measured in test tube and volume of each sample was made to1 mL by adding distilled water. To each, 5 mL 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 % $CuSO_4$ .5H_2O in 1% Sodium potassium tartarate) was added and kept at room temperature for 10 minutes. In each sample 0.5mL 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 minutes at 750 nm using spectrophotometer against blank. Five replicates of each concentration were taken and average value was plotted against their respective concentrations to compute 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 replicates of each concentration were taken and their mean value was calculated.

Lipids

Extraction and Quantification

The test sample were dried, powdered and 100mg was macerated with 10 mL distilled water, transferred to a conical flask containing 30 mL of chloroform and methanol [11]. 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 were separated, the lower layer of chloroform, which contained all the lipids, was carefully collected in the preweighed glass vials and the colored aqueous layer of methanol which contained all the water soluble substances and thick interface layer were discarded in each test sample. The chloroform layers dried *in vacuo* and weighed. Each treatment was repeated thrice and their mean values were calculated.

Phenols

Extraction

The deproteinized test materials (200mg each) were macerated with 10 mL of 80% ethanol for 2 hours, 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 spectrophotometer method [12]. It includes 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 the test tube and volume was raised to 1mL by addition of 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 2 mL of 20% sodium carbonate solution was added and then 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 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. The concentrations in the test samples were calculated by referring the respective optical density of test sample against standard curve of tannic acid.

Antioxidant activity

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 method was performed by the established protocol [13].

Reagents

- Acetate buffer, 300mM/L pH 3.6 (3.1 g sodium acetate x H_2O and 16 mL conc. Acetic acid per 1 mL of buffer solution).
- 10mM 2, 4, 6-tripyridyl –s- triazine (TPTZ) in 40 mM 1 HCl.
- 20mM $FeCl_3$ x 6 H_2O in distilled water

FRAP working solution: 25 mL acetate buffer (1), 2.5mL TPTZ solution and 2.5mL $FeCl_3$ x 6 H_2O solutions. The working solution must be always freshly prepared. Aqueous solution of known Fe (II) concentration was used for calibration (in a range of 100-1000µ mol/L).

Assay; - Blank: FRAP reagent
Sample: FRAP reagent -1.5mL, plant extract- 50mL

Procedure

Plant sample (1g) were cut into small pieces and mashed with a cool mortar and pestle using quartz sand and 9 mL cool 0.1M phosphate buffer was added. (pH 7.6, containing 0.1mM EDTA). This mixture was filtered through a filter paper and centrifuged at 15,000 rpm for 10 min. The supernatant was used for the measurements. The volume was make up to 5mL and O.D. was taken at 593nm.

Calculation: The relative activities of samples were assessed by comparing their activities standard curve of ferrous sulphate.

Lipid Peroxidation (LPO) Assay
Reagents and Test sample solutions

- Ethanol
- Trichloroacetic acid (20% w/v in water)
- Butylated hidroxytoluene (0.01% w/v in ethanol)
- Thiobarbituric acid (0.5% w/v in 20% TCA)

Procedure

The level of lipid peroxidation was measured in terms of malondialdehyde content of a product of lipid peroxidation as per described [14]. 1g fresh plant sample was homogenized with 25 mL of ethanol in pre chilled mortar and pestle and refrigerated centrifuged at 10000rpm for 20 minutes at 4ºC. The clear supernatant was taken as the enzyme extract. In one test tube 1mL of enzyme extract was added in 0.8 mL trichloroacetic acid (TCA) and 0.2 mL of butylated hidroxytoluene. In second test tube 1mL of enzyme extract was added in 0.8 mL thiobarbituric acid (TBA) and 0.2 mL of butylated hidroxytoluene. The mixtures were incubated at 95ºC for 25 min. The reaction was stopped by cooling in ice bath for 15 min. Reaction tubes were centrifuged at 10,000g for 10 min and supernatants were used to determine the absorbance at 532 nm and 400nm. The value for non-specific absorption at 600 nm was subtracted

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.

Pyrogallol+ H_2O_2 Peroxidase ⟶ Purpurogallin

Reagents and Test sample solutions

- Phosphate buffer (0.1M, pH- 6.8)
- Phosphate buffer (125µmol, pH- 6.8)
- Pyrogallol (50µmol)
- H_2O_2 (30%)

Procedure

Plant sample (200mg) was homogenized 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 prescribed method [15] with the following modifications. 2.4mL of phosphate buffer, 0.3mL of pyrogallol and 0.2mL of H_2O_2 was added. The amount of purpurogallin formed was determined by taking the absorbency at 420nm immediately after adding 0.1mL enzyme extract.

RESULTS AND DISCUSSION

Quantification of Primary metabolites

In the present investigation it was observed that *L. aspera and E.hirta* contain many primary metabolites like carbohydrates, proteins, phenols and lipids. In the present investigation, various plant parts (leaves, stem and root of *L. aspera*) and(leaves, stem and roots of *E. hirta)* were estimated for their primary metabolites viz, total soluble sugar, starch, lipid, protein and phenol (Table 1and 2).

Table 1 Primary metabolites from *L. aspera*

Phytochemical Name		*L. Aspera*		
		Leaf (mg/gdw)	Stem (mg/gdw)	Root(mg/gdw)
Proteins		256	280	180
Lipids		146.6	23.3	83.3
Phenols		3.50	3.95	3.50
	TSS	2.36	2.36	2.2
Carbohydrates	Starch	2.39	2.36	3.98

Table 2 Primary metabolites from *E.hirta*

Phytochemical Name		*E.Hirta*		
		Leaf (mg/gdw)	Stem (mg/gdw)	Root(mg/gdw)
Proteins		196.0	145.5	137.23
Lipids		223.3	333.33	333.33
Phenols		4.23	1.5	0.75
	TSS	3.6	4.5	1.62
Carbohydrates	Starch	2.81	1.62	2.4

Proteins*:* The maximum amount of protein was observed in stem of *L.aspera* (280mg/gdw) while minimum was in roots of *E. hirta* (137.23mg/gdw) (Table1 and 2 respectively).High level of protein content indicates their nutritive value.
Lipid: The observed quantity for lipid was higher in stem of *E. hirta* (333.33mg/gdw) which was at par with roots while minimum in stem of *L.aspera* (23.3mg/gdw) (Table1 and 2).
Phenol: The amount of phenol was highest in leaves and minimum in roots of *E. hirta* (4.23mg/gdw and 0.75 mg/gdw respectively) (Table1 and 2).

Carbohydrate

Total soluble sugar: In the present study, among all the samples, stem of *E. hirta* exhibited highest content (4.5mg/gdw) while minimum was in roots of same plant *(*1.62mg/gdw) **(Table1 and 2).**

Starch: the maximum content of starch was observed in roots of *L.aspera* (3.98mg/gdw) and minimum in stem of *E. hirta* (1.62mg/gdw (Table1 and 2).

Determination of antioxidant activity

The antioxidant potential of different plant parts of *L. aspera* and *E. hirta* was done using FRAP, LPO and Peroxidase assay. Leaves of *E.hirta* was found to be better antioxidant potential when sample analyzed by FRAP .Peroxidase and LPO method. When samples of *L. aspera* were analyzed by FRAP, LPO, it was observed that leaves had higher antioxidant activity and sample analysed by Peroxidase roots found to be better antioxidant potential (Table3 and 4).

Table 3 Total antioxidant (FRAP) activity of *Lucas aspera & Euphorbia hirta*

S.No	Extracts	FRAP (µmol Fe (II)/g)
	Lucas Aspera	**150**
1	Leaves	58
2	Stem	108
3	Root	
	Euphorbia Hirta	
1	Leaves	**160**
2	Stem	
3.	Root	130
		170

Abbreviation: FRAP: Ferric reduction activity potential; *Expressed in units of µmol Fe (II)/g.*

Table 4 Levels of Lipid Peroxidation and Peroxidase in *L.Aspera* mg/g fresh wt.)

S. No	Samples	LPO units mg/g fresh wt Mean ± SD	Peroxidase units mg/g fresh wt mg Mean ± SD
1.	Leaves	46.48	0.389
2.	Stem	28.65	0.260
3.	Roots	20.77	0.56

The present result showed potential of these plants as herbal drug for treating human from metabolism in which free radicals are involved, like cancer, cardiovascular effects, and aging.

Table 5 Levels of Lipid Peroxidation and Peroxidase in *E.Hirta*

S. No	Samples	LPO units mg/g fresh wt Mean ± SD	Peroxidase units mg/g fresh wt mg Mean ± SD
1.	Leaves	33.32	1.42
2.	Stem	31.15	0.176
3.	Roots	16.27	0.334

CONCLUSION

In the present study, it was found that *L. aspera and E.hirta* are rich source of Protein, lipid and carbohydrate which are the major contributors of balanced diet. When children not intake sufficient amount of protein by their food, they suffer from protein-energy under nourishment which may cause severe mal-function.

Both plants can serve as a boon for curing malnutrition in a developing country where many people suffer from malnutrition because *L. aspera and E.hirta* 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 and source of novel drugs.

References

1. Franstisek S (1998). The natural guild to medical herbs and plants. Tiger Bks. *Int*. 6 - 15. Jendanssik I, Gof P (1997).Bilirubin, colorimetric method. *Biochem. Z* 1938: 297: 81.
2. Kumar O.A.; Naidu M.; and Raja Rao K.G.; (2012). Antibacterial evaluation of snake weeds (euphorbia hirta l.) *J. Phytol.* 2(3): 08-12
3. Makhija I.K.; Chandrashekar K.S.; Richard L.; and Jaykumar B.; (2011). Phytochemical and Pharmacological Profile of Leucas lavandulaefolia: A review. *Res. J. Med. Plant,* 5: 500-507.
4. Ebrahimzadeh M, Nabavi SM, Nabavi SF, Bahramian F and Bekhradnia AR (2010). Antioxidant and free radical scavenging activity of *H. officinalis* L. var. *angustifolius, V. odorata, B.hyrcanca* and *C. speciosum. Pak. J. Pharm. Sci.*, 23(1): 29-34.
5. Finkel T and Holbrook NJ (2000). Oxidants, oxidative stress and the biology of aging. *Nature,* 408: 239-247.
6. Loomis WE and Shull CA. 1973. Methods in Plant Physiology. McGraw Hill Book Co., New York, USA

7. McCready RM, Guggoiz J, Silviera V, and Owens HS. 1950. Determination of starch and amylase in vegetables. *Anal. Chem*. 22:1156-1158.
8. Dubois M, Gills KA, Hamilton JK, Rebers PA and Smith F. 1951. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28: 350-356
9. Osborne DJ. 1962. Effect of kinetin on protein and nucleic acid metabolism in *Xanthium* leaves during senescence. *Plant .Physiol*. 37: 595-602.
10. Lowry OH, Rose HN, Broug J, Farr AL and Randall RJ. 1951. Protein measurement with the Folin-phenol reagent. *J. Biol. Chem*. 193:265-275.
11. Jayaraman J. 1981. Laboratory Manual in Biochemistry. Wiley Eastern Limited, New Delhi. pp 96-97
12. Bray HG and Thorpe WV. 1954. Analysis of phenolic compounds of interest in metabolism. *Meth. Biochem. Anal.* 1:27-52.
13. Varga I. S. Z., Matkovics B., Sasvari M. and Salgo L. (1998). Comparative study of plasma antioxidant status in normal and pathological cases. *Curr. Topics. Biophys*. 22: 219-224.
14. Hodges DM, DeLong JM, Forney CF and Prange RK. 1999. Improving the thiobarbituric acid reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta. Med*. 207: 604 - 611.
15. Chance B. and Maehly A.C. (1955). Assay of catalase and peroxidases. Methods. *Enzymol*. 2: 764 -775.

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