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COMPARATIVE PHYTOCHEMICAL ANALYSIS OF *ALPINIA OFFICINARUM* RHIZOME AND *TERMINALIA CHEBULA* FRUIT

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ABSTRACT

Rhizomes and fruits form a drug and medicinal plants are the future as they lack side effects, each parts of plants require proper research to know its importance. Hence, the present study was undertaken to analyse. Alpinia officinarum rhizome and Terminalia chebula fruit both qualitatively and quantitatively. Qualitative phytochemical analysis showed positive result for most of the tests assessed. And FTIR showed positive result for flavonoid, saponin, tannin. Similarly, HPLC was tested for flavonoid and shows positive peaks for flavonoid. Both the samples tested showed good antioxidant scavenging activity, phenolics in large amount, while lesser nutrient content. The results obtained shows, that it has to be researched further for the purposive uses like skin disorders etc.

KEY WORDS

Antioxidants, Fruit, FTIR, HPLC, Rhizome

INTRODUCTION

Natural products are good resources for medicinal compounds. Plants were in use by 1.5 million practitioners for remedial measures. Around 2000 tonnes of herbs was consumed by 7800 drug synthesis unit, the top users were China and India.[1-4] For its anti-inflammatory, antibacterial, antifungal, antiviral, diuretic, and anticancer properties.[5-6] *Alpinia officinarum* rhizome and *Terminalia chebula* fruit contain flavonoids. Flavonoids like quercetin, kaempferol, galangin, protect against oxidative stress through antioxidative protein expression. Flavonoid alters the frequency of free radicals generated by radical scavenging, metal chelating activity, xanthine oxidase inhibition, increased endogenous antioxidants.[7-9] Presence of several phytochemicals allows its use in ayurveda, unani, homeopathy too. [10-12] **20**-50% tannins in *Terminalia chebula*, impart pharmacological activity.[13-15] Since, *Alpinia officinarum* rhizome and *Terminalia hebula* fruit possess pleiotropic properties, it was decided to study the phytochemicals both qualitatively and quantitatively. Hence, the present study was focused much on analytical (FTIR, HPLC), biochemical analysis (Phytonutrients, Secondary metabolites, Antioxidant activity) in *Alpinia officinarum* rhizome and *Terminalia hebula* fruit.

METHODS

Sample collection

Samples were purchased from local herbal shop at Salem, Tamil Nadu, India.

Extract preparation

Aqueous extract was prepared by dissolving 15g of powdered *Alpinia officinarum* rhizome and *Terminalia hebula* fruit powder in 200ml water or laboratory use and heated on a hot plate (constant stirring) at 30-40°C for 20minutes, cooled and filtered through filter paper and used for qualitative,[16-18] antioxidant, phytonutrient analysis by adopting standard procedures.

QUALITATIVE ANALYSIS

Test for Glycosides:

To the extract added aqueous NaOH. Formation of yellow color indicates glycosides.

Test for flavonoids:

In a test tube containing 0.5 ml of extract, 5-10 drops of dilute HCl and ZnCl or magnesium were added the

solution was boiled for a few minutes. Presence of reddish pink or dirty brown color confirms flavonoid.

Test for Saponins:

In a test tube containing 0.5ml of aqueous extract, a drop of sodium bicarbonate was added, shaken vigorously. Appearance of froth confirms saponins.

Test for Steroids:

To 2ml of chloroform extract, 1ml of concentrated H_2SO_4 was added carefully along the sides of the test tube. Appearance of red color in the chloroform layer confirms steroids.

Test for Carbohydrate

Molisch test:

To the extract added few drops of alcoholic alpha naphthol solution. Few drops of concentrated sulphuric acid was added along the sides of test tube to get a violet colored ring at the junction.

Fehling's test:

To the extract added equal amount of Fehling's A and B solution and then the tubes were kept in a boiling water bath. Brick red precipitation of cuprous oxide formation confirms reducing sugar.

Test for Tannin and Phenolic compound:

Ferric chloride test:

To the extract added ferric chloride. Greenish black color confirms a positive result.

Potassium dichromate test:

To the extract added potassium dichromate solution. Positive result was confirmed by brown precipitate formation.

Oil test:

Blue color denoted positive result when the extract was mixed 1ml of 1% copper sulphate and 10% sodium hydroxide.

Fourier transform infrared analysis

Sample preparation

For FT-IR analysis, the samples were ground in a mortar and pestle to reduce the average particle size to 1 or 2 microns. The Fourier transform infrared spectrum was recorded using FT-IR - AGILENT - Model Number: CAP-15T at Tamil Nadu Test House PVT Limited, Chennai.

HPLC

High performance Liquid Chromatography was performed at Tamil Nadu Test House PVT Limited, Chennai using AGILENT- Model Number :1100 With DAD.

SECONDARY METABOLITES

The phenol and flavonoid content of aqueous leaf extract was analysed.

Determination of Total phenol content

Total phenolic content was determined by Folil-ciocalteau method Nabavi et.al (2008).[19] To 0.1ml of extract add folin ciocalteau reagent (5 ml, 1:10 diluted with distilled water) stand for 5 min and aqueous $NaCo_3$ (4ml, 1M) was added, incubated for 15min.The phenol liberated was determined by colorimetric method at 765 nm. The standard curve was prepared, expressed in terms of Gallic acid equivalent (mg/g of dry mass).

Estimation of flavonoids

Aluminium chloride method Mervat et.al (2009)[20] was used for the determination of the total flavonoid content. To 0.1ml of extract added 0.1ml of $AlCl_3$ (10%). The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30min of incubation. The concentration of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

ANTIOXIDANT ASSAYS

Nitric oxide scavenging assay, reducing power, Total antioxidant assay was performed.

Nitric oxide scavenging activity

Nitric oxide scavenging activitywas estimated.[21] Sodium nitroprusside (10mM) in phosphate buffered saline was added to 0.1ml of extract and kept at RT for 150min, followed by addition of 0.5ml of Griess reagent. The absorbance was read at 546nm. Quercetin was used as a reference compound.

Reducing power assay

Reducing power assay was performed.[22]To 0.1ml of extract, phosphate buffer (2.5ml, 0.2M, P^H 6.6) and potassium ferric cyanide (2.5ml 1%), incubated for 20min at 50^0C, 1.0 ml of trichloro acetic acid (10%) was added to stop the reaction, centrifuged at 3000rpm for 10min. To 1.5ml of upper layer solution add 1.5ml distilled water and $FeCl_3$ (0.1ml, 0.1%), contents were mixed and incubated for 10 min and the absorbance was measured at 700nm. Vitamin C was used as a positive control.

Total antioxidant capacity

Total antioxidant capacity assay[23] is based on the reduction of Mo (V1) to Mo (V) by the analyte allowing green phosphate/Mo (V) complex at acidic pH. The total antioxidant activity is expressed as equivalents of ascorbic acid.

Analysis of phytonutrients

Total carbohydrates, proteins, amino acids were performed according to the standard prescribed methods.

Estimation of carbohydrate

The total carbohydrate was estimated.[24] To 0.1 ml of extract, add 4ml of anthrone reagent, heat for 8-10minutes in a boiling water,cooled and read at 630nm using spectrophotometer Schimadzu Model - UV 1800. Glucose forms a standard.

Estimation of protein

The total protein was estimated.[25] To 0.1ml of extract, add 2ml of alkaline copper reagent, mixed well and kept for 10minutes, followed by 0.2ml of Folin ciocalteau reagent (diluted in the ratio of 1: 2), incubated for 30minutes, read at 660nm using spectrophotometer Schimadzu - Model UV 1800. BSA was used as a standard. With the standard graph, unknown concentration was studied.

Estimation of aminoacids

The amino acid was estimated [26] To 0.1 ml of extract, add 1 ml of ninhydrin solution dissolved in Butanol: Acetone. Close the tubes to avoid evaporation. With mild stirring, heated at 80-100ºC,4-7 minutes. Cooled and read at 570nm. Tyrosine standard was used.

STASTICAL TOOL

The Mean and Standard deviation (S) was calculated by using the following formula:

Mean = Sum of x values / n (Number of values)

$$s = \frac{\sqrt{\sum(X-M)^2}}{n-1}$$

Antibacterial assay

Preparation of Inoculum:

The *Escherichia coli,* Bacillus *punilus, Salmonella typhimurium,* Staphylococcus *aureus* were pre-cultured in nutrient broth overnight in a rotary shaker at 37°c, centrifuged at10,000rpm for 6min, pelletwas suspended in double distilled water and the cell density was standardized spectrometrically (A nm).

Preparation of test sample:

For the anti-microbial tests, extracts were diluted in dimethyl sulfoxide (DMSO): Methanol (1/1: v/v) solvent to a concentration of 20mg/ml. Anti-bacterial activity was measured using agar dilution technique. Briefly the extracts were dissolved in dimethyl sulfoxide (DMSO, Merck) and serially diluted in molten Muller Hinton Agar (MHA, Sigma) in petridishes (100mm×15mm). The solvent did not exceed 1% concentration and did not affect the growth of the organisms. For bio-assays, suspension of approximately 1.5×10^8 bacterial cell /ml in sterile normal saline were prepared and about1.5ml of was uniformly seeded on Muller-Hinton-Agar medium with 3-4mm thickness in 12cm × 1.2cm glass petri dishes, left aside for 15min.and excess of suspension was then drained and discarded properly. Wells of 60mm in diameter and about 2cm apart were punctured in the culture media using sterile cork bores. Well were filled with 0.1ml of each 20µg/ml concentration of each sample (2µg/well) and incubated at 37 °c for 48hrs. Bio-activity was determined by measuring diameter of inhibition zones(DIZ) in mm.

RESULTS

The results obtained from the Qualitative and Quantitative analysis was shown below.

Qualitative analysis

Table 1 shows the result of phytochemicals present in *Alpinia officinarom* Rhizome and *Terminalia chebula* Fruit. Except saponin and phenol, oil all the other phytochemicals were present sufficiently in *Alpinia officinarom* rhizome. Similarly, in *Terminalia chebula* fruit except oil content remaining phytochemicals were sufficiently present.

Table 1. Qualitative analysis of Phytochemicals in *Alpinia officinarum* Rhizome and *Terminalia chebula* fruit

Phytochemicals Tested	*Alpinia officinarom* rhizome	*Terminalia chebula* fruit
Glycosides	++	+++
Flavonoids	+++	++
Saponins	+	+++
Steroids	++	+++
Phenols	+	+++
Alkaloids	++	+++
Molish's test	+++	++
Fehling's test	+++	+++
Oil test	+	+
Ferric chloride test	++	+++
Potassium dichromate test	+++	+++

+++ Stronger, ++ Moderate , + Mild reaction

Quantitative analysis using FTIR

Table 2 shows the results of *Alpinia officinarom* rhizome and *Terminalia chebula* Fruit. From the result, we could observe that, *Alpinia officinarom* rhizome contains higher amount of phenol and tannin. While in *Terminalia chebula* fruit phenol, flavonoid, tannin, saponin were observed in higher quantity with lesser amount of alkaloid.

Table 2. FTIR spectrum results of *Alpinia officinarum* Rhizome and *Terminalia chebula* fruit

Phytochemicals	Spectrum Results of *Alpinia officinarum* rhizome	Spectrum Results of *Terminalia chebula* fruit
Total phenol as gallic acid equivalent	3.6	85.3
Total tannin as tannic acid equivalent	1.2	60.3
Total flavonoids as quercetin equivalent	BQL (LOQ: 0.1)	43.2
Saponins	BQL (LOQ: 0.1)	15.12
Alkaloids	BQL (LOQ: 0.1)	2.2

BQL - Below Quantification Limit, LOQ – Limit of Quantification

Quantitative analysis using HPLC

The results of flavonoids analysed in *Alpinia officinarom* rhizome through HPLC was shown in Fig.1 and Table.3. Similarly, the HPLC results of *Terminalia chebula* fruit was depicted in Fig.2 and Table.4.

Quantitative analysis

The results of secondary metabolites, phytonutrients, antioxidant activity of ***Alpinia officinarum* rhizome and *Terminalia chebula* fruit** was shown in Table.5.

Table. 5 shows the results of biochemical parameters analysed in *Alpinia officinarum* rhizome and *Terminalia chebula* fruit. Both *Alpinia officinarum* rhizome and *Terminalia chebula* fruit contain higher antioxidant activities such as nitric oxide scavenging (34.50& 31.0mg/g, reducing power (39.75 and 52.50mg/g, total antioxidant activity 18.00 and 26.00mg/g). Among the secondary metabolites studied, phenolics was found to be higher in concentration in both the samples, while, the flavonoid content was low in both the samples. The nutrients like protein, carbohydrate and aminoacids were found to below in the experimented samples.

Antibacterial activity

Table.6 shows the results of antibacterial activity of *Alpinia officinarum* rhizome and *Terminalia chebula* fruit. The result shows that the antibacterial activity was found to be higher with *Alpinia officinarum* rhizome compared to *Terminalia chebula* fruit. *Alpinia officinarum* rhizome showed significant antibacterial activity in all the four-test organism. Whereas, in *Terminalia chebula* fruit, the antibacterial activity was low with *Bacillus punilus* and the remaining organisms show significant antioxidant activity. *Alpinia officinarum* rhizome and *Terminalia chebula* fruit showed strong inhibitory

activity on *S.aureus* causing bacteremia, sepsis and *Salmonella typhi* an intestinal bacteria.

Fig.1 HPLC chromatogram of *Alpinia officinarom* rhizome -Flavonoids

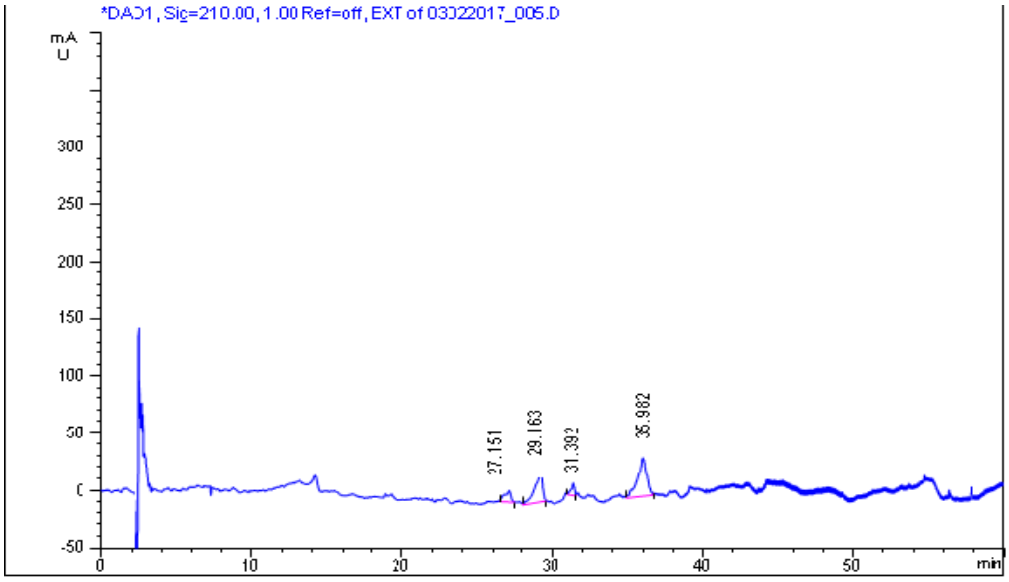

Table.3. Details of area percent / peak for *Alpinia officinarom* rhizome

Peak	RT (min)	Height	Area	Area%
1	27.151	9.844	294.636	10.816
2	29.163	27.167	903.698	33.175
3	31.392	10.474	165.125	6.062
4	35.982	33.205	1360.601	49.948

Fig.2. HPLC chromatogram of *Terminalia chebula* fruit – Flavonoids

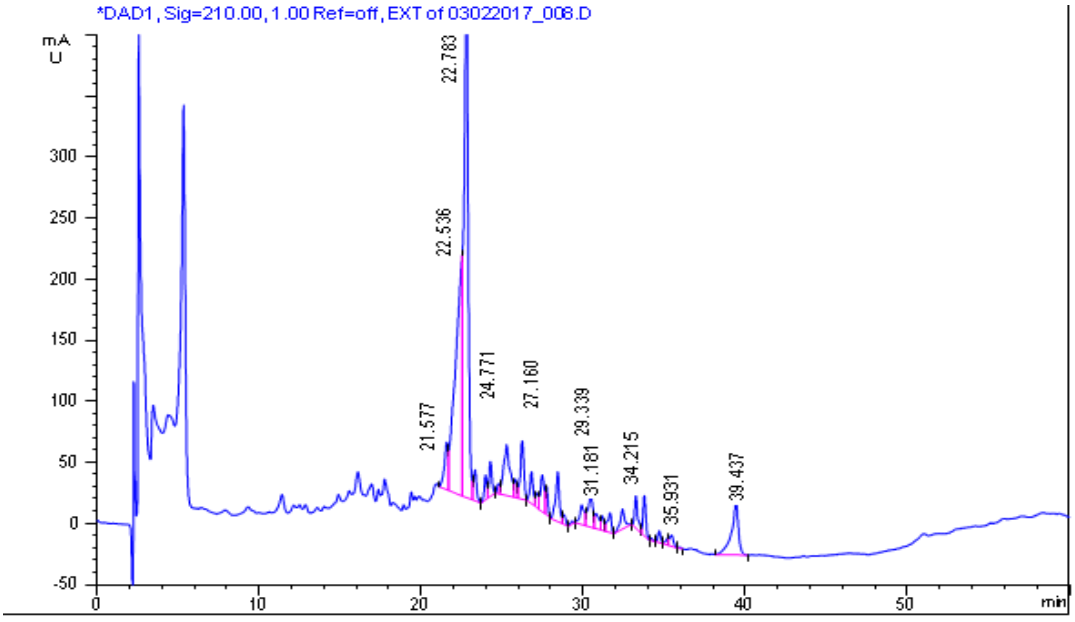

Table.4. Details of area percent / peak for *Terminalia chebula* fruit

Peak	RT (min)	Height	Area	Area%
1	21.577	39.134	0649.992	2.789
2	22.536	196.611	5524.666	23.703
3	22.783	426.811	7798.949	33.461
4	23.322	25.470	0337.523	1.448
5	23.974	20.326	0225.906	0.969
6	24.272	28.706	0365.563	1.568
7	24.771	08.317	0090.588	0.838
8	25.278	41.631	1126.203	4.832
9	25.820	15.300	0195.264	0.838
10	26.234	47.506	0716.616	3.075
11	26.813	25.897	0351.139	1.507
12	27.160	11.898	0158.656	0.681
13	27.476	30.156	0469.914	2.016
14	27.734	23.387	0254.322	1.091
15	28.435	40.571	0671.259	2.880
16	28.808	07.741	0101.080	0.434
17	29.339	02.326	0027.157	0.117
18	29.909	16.233	00298.795	1.282
19	30.476	27.970	0576.042	2.471
20	30.794	12.539	0230.170	0.988
21	31.181	12.098	0157.470	0.676

Table 5. Analysis of Secondary metabolites, Phytonutrients, Antioxidant activity in *Alpinia officinarum* rhizome and *Terminalia chebula* fruit

Compounds / Activity studied	*Alpinia officinarum* rhizome (mg/g)	*Terminalia chebula* fruit (mg/g)
Phytonutrients		
Protein	2.2 ± 0.70	2.4 ±0.00
Amino acid	1.3 ± 0.56	0.3 ±0.00
Carbohydrate	1.2 ± 0.00	4.5± 0.848
Secondary metabolites		
Total phenolics	33.0 ± 0.00	33.0 ±0.00
Flavonoids	2.00 ±0.00	01.65 ±0.00
Antioxidant activity		
Nitric oxide	34.50 ±0.00	31.0 ± 0.00
Reducing power	39.75± 0.00	52.5 ±1.22
Total antioxidant	18.00 ±2.12	26 .0 ± 1.41

Values are Mean ± SD for three experiments

6. Antibacterial activity of *Alpinia officinarum* rhizome and *Teminalia cebula fruit*

Test organism	NCIM	*Alpinia officinarum* rhizome zone diameter (mm)	*Terminalia chebula* fruit zone diameter (mm)
Escherichia coli	2065	1.7	1.6
Bacillus pumilus	2327	1.9	0.8
Salmonella typhi	2501	1.8	1.2
S taphylococcus aureus	5345	1.9	1.7

CONCLUSION

Alpinia officinarum rhizome and *Terminalia chebula* fruit possess antimicrobial and antioxidant activity which might be due to the presence of secondary metabolites in it. Analytical assessment also showed good amount of flavonoid content attributing for its pharmaceutical properties like application in the treatment of several infectious disorders.

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REFERENCES

1. 1.Ramakrishnappa K. Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity:Case studies from India. In: Biodiversity and the Ecosystem Approach in Agriculture. Forestry and Fisheries [online], FAO, 2002.
2. 2.Masood E. Medicinal plants threatened by over-use. Nature. 385(6617); 1997:570.
3. 3.Rawat RBS. Medicinal Plants Sector in India with reference to Traditional Knowledge and IPR issues [online]. Paper presented at International Seminar for the Protection of Traditional Knowledge, New Delhi,April 2002.
4. 4.Wang Zhen-Gang, Ren J. Current status and future direction of Chinese herbal medicine. Trends in Pharmacological Sciences. 2002; 23(8): 347-348.
5. 5.Lee JS, Kim KA, Jeong SH, Lee SG, Park HJ "Anti-inflammatory, anti-nociceptive, and anti-psychiatric effects by the rhizomes of *Alpinia officinarum* on complete Freund's adjuvant-induced arthritis in rats." J Ethnopharmacology. 2009; 126: 58-64.
6. 6.Konno K, Sawamura R, Sun Y, Yasukawa K, Shimizu T, Watanabe W, Kato M, Yamamoto RKM."Antiviral activities of diarylheptanoids isolated from *Alpinia officinarum* against respiratory syncytial virus, poliovirus, measles virus, and herpes simplex virus type 1 *in vitro*"Nat. Prod. Commun. 2011; 6(12): 1881-1884.
7. 7.Hanneken A, Lin FF, Johnson J, Maher P. Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death. Invest. Ophthalmol. Vis. Sci.47;2006: 3164–3177.
8. 8.Nijveldt RJ, Nood E van, Hoorn DE van, Boelens PG, Klaske van Norren A and Leeuwen PA van. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 2001; 74(4):418-25.
9. 9.Knishinsky R. Prickly Pear Cactus Medicine, Treatments for Diabetes, Cholesterol and the Immune System. Rochester: Healing Arts Press; 2004.
10. 10.Ratha KK, Joshi GC. Haritaki (Chebulic myrobalan) and its varieties. Ayu 2013; 34: 331-4.
11. Baliga MS, Meera S, Mathai B, Rai MP, Pawar V and Palatty PL. Scientific validation of the ethnomedicinal properties of the ayurvedic drug triphala: areview. Chin. J Integr. Med.2012; 18:946-54.
12. Baliga MS Triphala. Ayurvedic formulation for treating and preventing cancer: a review. J. Altern. Complement. Med. 2010; 16: 1301-8.
13. 13.CSIR, The Wealth of India, A dictionary of Indian raw material & industrial products, Vol. X, New Delhi: Publication and Information Directorate 2002;522-24.
14. 14.Varier. A dictionary of Indian raw materials & Industrial products, New Delhi: Publication and Information Directorate Council of scientific & Industrial research 2002; 387.
15. 15.Khare CP. Indian medicinal plants: An illustrated dictionary, Berlin, Springer-verlag: 2007; 652-3.
16. 16.Harborne JB. Phytochemical methods, 2nd edition, Chapman and Hall, New York. 1984
17. 17.Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 3rd edition, Nirali Prakashan, Pune. 1995
18. 18.Kokoshi CL, Kokoshi RJ, Sharma FJ. Fluoresence of powdered vegetable drugs under UV Radiation. J Am Pharm Assoc. 1958; 47: 715-717.

19. 19.Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A and Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of Parrotia persica Mey. Pharmacologyonline. 2008; 2: 560-567.
20. Mervat MM, Far EI, Hanan A and Taie A. Antioxidant activities, total Anthocyanins, phenolics and flavonoids contents of some Sweet potato genotypes under stress of different concentrations of sucrose and sorbitol. Aust. J. Basic Appl. Sci., 2009; 3: 3609-16.
21. 21.Ebrahimzadeh MA, Nabavi SF and Nabavi SM. Antioxidant activities of methanol extract of *Sambucus ebulus L*.flower. Pak. J. Biol. Sci., 2009d; 12(5): 447-450.
22. 22.Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agri. Food Chem., 1995; 43(1): 27-32
23. 23.Prieto P, Pineda M, and Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. Analytical Biochemistry. 1999; 269: 337-341.
24. 24.Hedge JE, Hofrelter BT. In: Carbohydrate chemistry, 17 Eds. Whistler RL, Be Miller JN, Academic press, New York. 1962
25. 25.Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with Folin phenol Reagent, J Biol Chem., 1951; 193: 265-275.
26. 26.Yemm EW, Cocking EC and Ricketts RE. The determination of amino acids with ninhydrin. Analyst, 1955; 80: 209-214.

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