WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 09, 1623-1634. Research Article ISSN 2277–7105

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF LEAF, FLOWER AND SEED EXTRACTS OFERYTHRINA VARIEGATA L.

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Article Received on
21 March 2018,
Revised on 10 April 2018,
Accepted on 01 May 2018
DOI: 10.20959/wjpr20189-12178

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ABSTRACT

The aim of study was to examine the phytochemical screening, total phenols, total flavonoids and antioxidant activity of leaf, flower and seed extracts of *Erythrina variegata* collected from Thiruvallur district, Tamil Nadu. The phytochemical analysis of leaf, flower and seed extract of *Erythrina variegata* revealed the presence of significant secondary metabolites such as steroids, quinones, cardiac glycosides, saponins, tannins, phenols, flavonoids, terpenoids and alkaloids. The aqueous leaf extract of *Erythrina variegata* was found maximum in total phenol and flavonoid contents were 19.2 mg GAE /g and 11.9 mg QE /g followed by seed and flower extract. The leaf, flower and seed extracts of *Erythrina variegata* were evaluated for antioxidant activities by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) radical scavenging assay. Among five different solvent extract used, maximum antioxidant activity was found in the aqueous leaf extract of *Erythrina variegata* (91.3 %) followed by seed and flower extract. The powerful antioxidant activity is attributed to the greater amount of total phenols and flavonoids compound in the aqueous leaf extracts of *Erythrina variegata*.

KEYWORDS: *Erythrina variegata*, phytochemical screening, Phenol, flavonoid, antioxidant activity.

INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Joshi et al., 2011). Most of the people in rural and urban areas of the world are dependent on the medicinal plants for the treatment of infectious diseases. Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Dietary phytochemicals are considered as an effective tool to cure body disorder. They play important roles as therapeutic agents in prevention of many diseases (Kareem et al., 2010).

A growing body of evidence indicates that secondary plant metabolites play important roles in human health and may be nutritionally important (Jeeva et al., 2012). Phytochemical screening of various plants has been reported by many workers (Mojab et al., 2003; Parekh and Chanda, 2008). These studies have revealed the presence of numerous chemicals including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Hagerman et al., 2008).

Reactive oxygen or nitrogen species (ROS, RNS) are important free radicals which complicates the human functions. ROS such as the superoxide anion radical (O2 -) and hydroxyl radicals (OH) are physiological metabolites. They are produced as an outcome of the respiration in the aerobic organisms but their excessive levels have been linked to the onset of diseases such as cancer, stroke and diabetes (Anthony et al., 2013). Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders (Rice-Evans et al., 1996). Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. The crude extracts of medicinal plants, spices and other plant materials, rich in phenolics and flavonoids are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Chu et al., 2000). In addition, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. (Miller, 1996).

The genus *Erythrina* comprises of about 110 species of trees and shrubs. It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m (800ft) in elevation. The most attractive type, var. *variegata*, is grown for its variegated leaves, as well as its seasonal showy red flowers (Muthukrishnan et al., 2014). *Erythrina variegata* is belongs to the family Fabaceae or Indian coral tree, is in an average size and grows rapidly in the deciduous forests all over India. The studies on phytochemical of Erythrina variegata have demonstrated alkaloids and flavonoids as major constituents. *E. variegata* parts (leaves, flowers, barks and roots) have been used in the natural medicines as nervine sedative, febrifuge, anti-asthmatic and antiepileptic. Traditionally, it has potential effects to heal some of the diseases like convulsion, fever, alzheimer, inflammation, bacterial infection, cough, ulcer, cuts and wounds (Cui et al., 2009).

MATERIALS AND METHODS

Collection of material: The healthy leaves, flowers and seeds of *Erythrina variegata* (Fig 1) were collected during the middle of February 2016 from Thiruvallur district, Tamil Nadu, India. The collected parts were brought to the laboratory and maintained at PG & Research Department of Botany, Presidency College, Chennai and heathy leaves, flowers and seeds used for further experimental studies.

Preparation of the plant extract: Preparation of the extracts was done according to the methods prescribed by Ramachandra Kumar et al., 2017. The dried leaf, flower and seed powder of *Erythrina variegata* plant materials were extracted with acetone, ethanol (75%), chloroform, petroleum ether and aqueous extract for 1 minute using an ultra turax mixer (13,000 rpm) and soaked overnight at room temperature. The extracts were then filtered through what man No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in an airtight container in the refrigerator below 10°C.

Phytochemical screening of *Erythrina variegata*: The phytochemical screening of leaf, flower and seed extracts of *Erythrina variegata* were assessed by standard methods (Savithramma et al., 2011). Phytochemical screening was carried out on the leaf, flower and seed extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides,

coumarins and steroids. General reactions in these analyses revealed the presence or absence of these compounds in the leaf extracts tested.

Estimation of total phenol content of *Erythrina variegata*: Total phenolic content in the leaf, flower and seed extracts was determined by the Folin– Ciocalteau colorimetric method (Slinkard, K and Singleton, 1977). For the analysis, 0.5 ml of aliquot of sample was added to 0.5 ml of Folin–Ciocalteau reagent (0.5 N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (2%) was added, and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolics contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of total flavonoid content in *Erythrina variegata*: Total flavonoids content in the ethanolic leaf, flower and seed extracts was determined by the aluminium chloride colorimetric method (Mervat et al., 2009). 0.5 ml of leaf, flower and seed extracts of *Erythrina variegata* at a concentration of 1mg/ ml were taken and the volume was made up to 3ml with methanol. Then 0.1ml AlCl3 (10%), 0.1ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

Qualitative analysis of antioxidant activity of *Erythrina variegate* : The antioxidant activity of leaf, flower and seed extracts of *Erythrina variegata* was determined by following the method as described by George et al., (1996). 50µL of leaf, flower and seed extracts of *Erythrina variegata* were taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of Free radical scavenging activity of *Erythrina variegate:* The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Leaf, flower and seed extract of *Erythrina variegata* 100µl were mixed with 2.7ml of methanol and

then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee et al., 2005). Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula: % DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100.

RESULTS AND DISSCUSION

In the present study, the phytochemical analysis of five different solvent extracts such as ethanol, chloroform, petroleum ether, acetone and aqueous studied, showed that the aqueous leaf extract of *Erythrina variegata* were rich in secondary metabolites such as phenol, tannins, saponins, terpenoids, steroid, flavonoids, cardiac glycosides, coumarins and alkaloids followed by seed and flower extracts (Table 1,2,3). The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, steroids, etc.,[Britto, J.D. and Sebastian, 2011] Thus, the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.[Doss et al., 2009].

Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992). In our study, total phenol content (TPC) of *Erythrina variegata* leaf, flower and seed extract was estimated by using FolinCiocalteau colorimetric method and represented in terms of gallic acid equivalent (GAE). The result of the present study showed that the phenol contents of the aqueous leaf extracts was found to be maximum (19.2 mg mg GAE/g) followed by seed extract (14.6 mg GAE /g) flower extract (13.8 mg GAE/g) Table.4. Phenolic compounds are important plant antioxidants which exhibited considerable scavenging activity against radicals. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds (Huang et al., 2009). Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The values of flavonoid content varied from plants. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003). The

result of the present study showed that the flavonoid contents of *Erythrina variegata* leaf, flower and seed extract was estimated by using aluminium chloride colorimetric method and represented in terms of quercetin equivalent (QE). The maximum amount of flavonoid content was found in *Erythrina variegata* aqueous leaf extract (11.9 mg QE /g) followed by seed extract (12.7 mg QE /g) and flower extract (10.1 mg QE /g) Table.4.

Erythrina variegata leaf, flower and seed extracts were further analysed for the presence of antioxidants. Table 6 shows the qualitative antioxidant analysis in the leaf, flower and seed extracts of *Erythrina variegata* collected from Thiruvallur district, Tamil Nadu. The results revealed that the strong positive response was obtained in the aqueous leaf extract followed by seed and flower extracts (Table.5). Scavenging activity for free radicals of DPPH has been widely used to evaluate the antioxidant activity of natural products from plant and natural sources. Among five different solvent extracts leaf, flower and seed extract of *Erythrina variegata*, the aqueous leaf extract of *Erythrina variegata* recorded the most effective DPPH radical scavenging activity (91.3 %) followed by seed extract (79.5%) and flower (77.9%). (Figure 2,3,4). *Erythrina variegata* value being very close to synthetic antioxidant (BHT) as positive control (98.4 %). The leaf, flower and seed samples of *Erythrina variegata*, aqueous leaf, seed and flower extracts recorded higher percentage of free radical scavenging activity followed by ethanolic, acetone, chloroform and petroleum ether.

In conclusion, phytochemical composition, total phenol, flavonoid contents and antioxidant activity of medicinal plants are very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. The present communication attempts to assess the status of phytochemicals, total phenol, flavonoid content and antioxidant activity. Thus from our findings, it is concluded that the aqueous leaf extracts from dry powdered of *Erythrina variegata* has a superior level of antioxidant activity. The powerful antioxidant effect is attributed to the greater amount of phenol and flavonoid compound in the leaf extracts of *Erythrina variegata*.

Table 1: Phytochemical

Phytochemicals Tested	Leaf Extracts of *Erythrina variegata*				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	++	+	-	-	+
Saponins	++	-	+	-	+
Quinones	++	++	+	+	++
Terpenoids	++	+	+	+	+
Steroids	++	+	+	+	++
Flavonoids	++	+	-	-	+
Phenol	++	++	+	+	+
Alkaloids	+	+	-	-	-
Glycosides	+	-	-	-	-
Cardiac glycosides	+	+	-	-	+
Coumarins	++	+	+	+	+
Antho cyanin	-	-	-	-	-
Beta cyanin	+	+	+	+	+

screening from leaf extracts of *Erythrina variegata.*

Key : + = positive, ++ = strong positive, - = negative

Table 2: Phytochemical screening from flower extracts of *Erythrina variegate.*

Phytochemicals Tested	Flower Extracts of *Erythrina variegata*				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	++	-	+	-	+
Saponins	++	++	+	+	+
Quinones	++	+	+	+	+
Terpenoids	+	+	+	+	++
Steroids	+	+	-	-	+
Flavonoids	++	++	+	+	+
Phenol	+	+	-	-	-
Alkaloids	+	-	-	-	-
Glycosides	+	+	-	-	+
Cardiac glycosides	+	+	-	+	-
Coumarins	+	+	-	-	+
Antho cyanin	+	+	-	-	-
Beta cyanin	++	+	-	-	+

Key : + = positive, ++ = strong positive, - = negative

Table 3: Phytochemical screening from seed extracts of *Erythrina variegate.*

Phytochemicals Tested	Seed Extracts of *Erythrina variegata*				
	Aqueous	Ethanol	chloroform	Petroleum ether	Acetone
Tannins	+	+	+	+	+
Saponins	+	+	-	+	-
Quinones	++	+	-	-	+
Terpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Flavonoids	++	+	+	+	+
Phenol	++	+	+	+	+
Alkaloids	-	+	-	-	+
Glycosides	-	-	-	-	-
Cardiac glycosides	++	+	+	+	+
Coumarins	+	++	-	+	+
Antho cyanin	-	-	-	-	-
Beta cyanin	+	+	-	-	-

Key : + = positive, ++ = strong positive, - = negative

Table 4: Quantification of Phytochemicals from leaf, flower and seed extract of *Erythrina variegate.*

Erythrina variegata	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE /g)
Leaf	19.2	11.9
Flower	13.8	10.1
seed	14.6	12.7

Table 5: Qualitative antioxidant activity of leaf, seed and flower extracts of *Erythrina variegate.*

S.No	Extractions	*Erythrina variegata*		
		Leaf	Flower	Seed
	BHT (standard)	++	++	++
S1	Aqueous	++	+	+
S2	Acetone	+	+	+
S3	Ethanol	++	+	+
S4	Chloroform	+	Semi positive	Semi positive
S5	Petroleum ether	+	Semi positive	Semi positive

a. Leaf b. Seed c. Flower

Figure 1: Mother plant of *Erythrina variegate.*

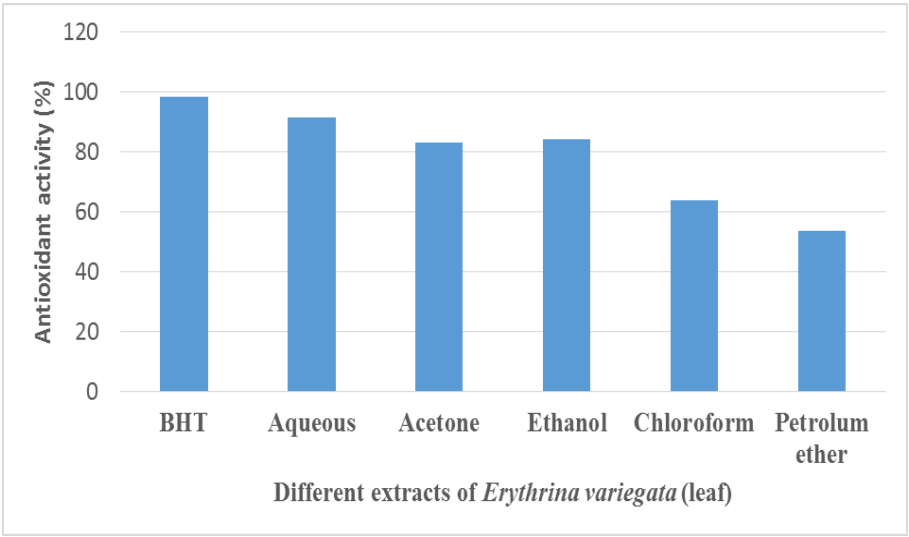

Figure 2: Quantitative antioxidant activity of leaf extracts of *Erythrina variegate.*

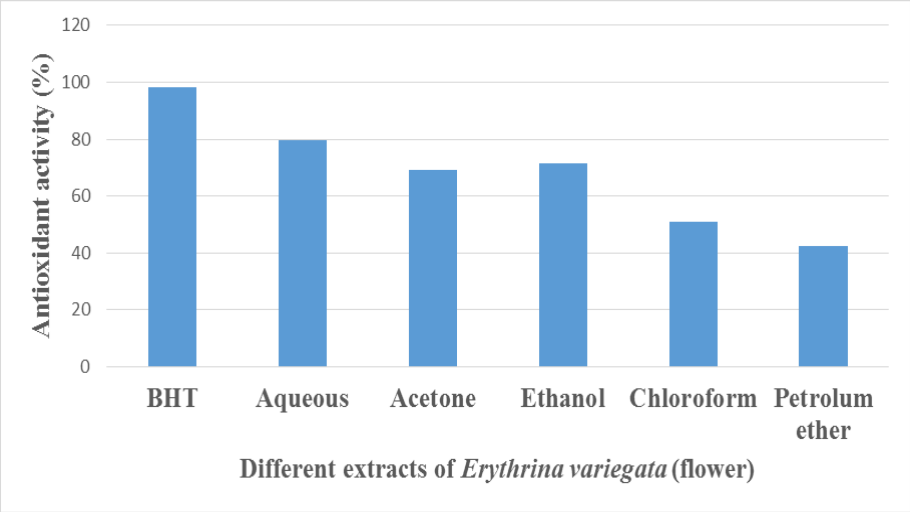

Figure 3: Quantitative antioxidant activity of flower extracts of *Erythrina variegate.*

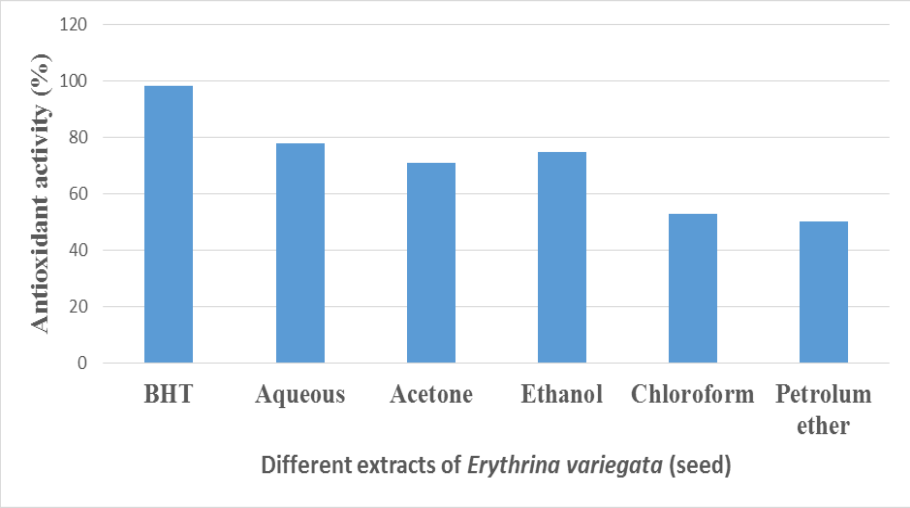

Figure 4: Quantitative antioxidant activity of flower extracts of *Erythrina variegate.*

REFERENCE

1. Joshi, B., Sah, GP., Basnet, BB., Bhatt, MR., Sharma, D., Subedi, K., Pandey, J and Malla, R. Phytochemical extraction and antimicrobial properties of different medicinal plants: Ocimum sanctum (Tulsi), Eugenia caryophyllata (Clove), Achyranthes bidentata (Datiwan) and Azadirachta indica (Neem). Journal of Microbiology and Antimicrobials, 2011; 3(1): 1-7.
2. Kareem, KT., Kareem, SO., Adeyemo, OJ and Egberongbe, RK. In vitro antimicrobial properties of Brideliaferruginea on some clinical isolates. Agric Bio J North Am, 2010; 1(3): 416-420.

3. Jeeva, S., Marimuthu, J., Domettila, C and Anantham, B., Mahesh, M. Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar, India. Asian Pacific Journal of Tropical Biomedicine, 2012; 2(1): S30-S33.
4. Mojab, F., Kamalinejad, M., Ghaderi N and Vahidipour H.R. Phytochemical screening of some species of Iranian plants. Iran. J. Pharm. Res, 2003; 2: 77-82
5. Hagerman, AN., Rield, KM., Jones, GA., Sovik, KN., Ritchard, NT., Hartzfeld, PW and Riechel, T.L. High molecular weight plat polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem, 1998; 46: 1887-1892.
6. Parekh, J and Chanda, S. Phytochemical screening of some plants from Western region of India. Plant Arch, 2008; 8: 657-662.
7. Anthony, AJ., Sharaibi, Olubunmi, S and Kazeem, M. Phytochemical Analysis and in vitro Antioxidant Activity of Nymphaea lotus L.. International Journal of Pharmacology, 2013; 9: 297-304.
8. Rice-Evans, C.A., Miller, N.J and Paganga, G. Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med, 1996; 20: 933–956.
9. Chu, Y.H., Chang, CL and Hsu, HF. Flavonoid content of several vegetables and their antioxidant activity. Journal of the Science of Food and Agriculture, 2000; 80:561–566.
10. Miller, A.L Antioxidant flavonoids: structure, function and clinical usage. Altern. Med. Rev, 1996; 1: 103-111.
11. Muthukrishnan, S, Palanisamy, S, Santhanam, I and Kaveriyappan, G.Phytochemical screening and antimicrobial activity of *Erythrina variegata*, World Journal of Pharmacy and Pharmaceutical Sciences, 2014; 3(6): 680-690.
12. Cui, L., Thuong, PT., Fomum, ZT and Oh, WK. A new erythrinan alkaloid from the seed of Erythrina addisoniae. Arch. Pharmacal. Res, 2009; 32: 325-328.
13. Ramachandra Kumar, MR., Ravikumar, S., Janarthanam, B. Phytochemical screening and antioxidant activities of seed and callus extracts of *Mucuna pruriens*. International Journal of Current Research, 2017; 9(12): 63047-63052.
14. Savithramma N, Linga Rao, M and Ankanna S. Screening of traditional medicinal plants for secondary metabolites. Int J Res Pharm Sci, 2011; 2(4): 643-647.
15. Slinkard, K and Singleton, V.L. Total phenol analysis: Automation and comparison with manual Methods. Am J Enol Vitic, 1977; 28(1): 49-55.
16. Mervat, MM., Far, El., Hanan, A and Taie, A. Antioxidant Activities, Total Anthocyanins, Phenolics and Flavonoids Contents of Some Sweetpotato Genotypes under

Stress of Different Concentrations of Sucrose and Sorbitol. Aust. J. Basic & Appl. Sci, 2009; 3: 3609-3616.

17. George H., Teng CM., Wu CL and Ko FN. Marchantin H as a natural antioxidant and free radical scavenger. Arch. of Biochem. and Biophys, 1996; 334: 18- 26.
18. Lee, SE., Hwang, HJ and Ha, JS. Screening of medicinal plant extracts for Antioxidant activity. Life Sci, 2003; 73: 167179.
19. Britto, JD and Sebastian, SR. Biosynthesis of silver nano particles and its antibacterial activity against human pathogens. Int J Pharm Pharm Sci, 2011; 5: 257-259.
20. Doss, A., Mubarak, HM. and Dhanabalan, R. Antibacterial activity of tannins from the leaves of Solanum trilobatum Linn. Indian J Sci Technol, 2009; 2(2): 41-3.
21. Shahidi, F. Wanasundara PK. Phenolic antioxidants.Crit Rev Food Sci Nutr, 1992; 32: 67-103.
22. Huang, Z., Wang, B., Eaves, D.H., Shikany, J.M. and Pace, R.D. 2009. Total phenolics and antioxidant capacity of indigenous vegetables in the Southeast Unites States: Alabama Colloboration for cardiovascular equity project. Int. J. Food Sci. Nutr, 2009; 60: 100-108.
23. Kessler, M., Ubeaud, G. and Jung, L. Anti- and prooxidant activity of rutin and quercetin derivatives. J. Pharm. Pharmacol, 2003; 55: 131- 142.