Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

TOTAL POLYPHENOLIC CONTENT AND FREE RADICAL QUENCHING POTENTIAL OF *DIOSCOREA ALATA* L. TUBERS

AARTI NARKHEDE, JASKARAN GILL[1], KAJAL THAKUR[1], DIVYA SINGH[1], ELANGBAM SINGH[1], OMKAR KULKARNI, ABHAY HARSULKAR, SURESH JAGTAP*

Interactive Research School for Health Affairs (IRSHA), [1]Rajiv Gandhi Institute of Biotechnolgy, Bharati Vidyapeeth Deemed University, Pune Satara Road, Pune 411 043 India. Email: chiritatml@rediffmail.com

Received: 09 May 2013, Revised and Accepted: 16 Jun 2013

ABSTRACT

Objective: In the context of today's scenario of disease progression due to oxidative stress, there is a need to find out natural antioxidants of plant origin. Several plants have been used as food as well as medicine. *Dioscorea alata* L. (DA) is one of the plants routinely used as food and medicine and can be effectively utilized as source of natural antioxidants. DA is an important staple food as well as medicinal plant but its scientific authentication and mechanism of action has not been investigated.

Methods: We investigated ethanolic and water extracts of DA for its polyphenolic content and free radical quenching potential using *in vitro* system.

Results: Both the extracts showed high reductive potential and total antioxidant capacity. Similarly, it has effective free radical scavenging potential against 2, 2-diphenyl-1-picrylhydrazyl, nitric oxide and lipid peroxidation. Comparatively, potency of ethanol extract resembles with standard ascorbic acid, which may be due to presence of different classes of phenolic compounds. Water extract also showed presence of polyphenols except flavonols. There was positive correlation between total phenol, total flavonoids, total flavonol, total proanthocyanidins content and free radical scavenging activity of plant.

Conclusion: The data clearly demonstrated that DA tubers are potential source of natural antioxidants and may be a good candidate for pharmaceutical plant based products. Studies on its mechanism of action may yield a potential compound against oxidative stress.

Keywords: *Dioscorea alata*, Tubers, Polyphenol contents, Radical scavenging activity, Potential source.

INTRODUCTION

In recent years, there is an upsurge in the areas related to new developments in prevention of disease, particularly in the role of free radicals and antioxidants. The relation between free radicals and disease can be explained by the concept of 'oxidative stress'. In a normal healthy human body, the generation of pro-oxidants in the form of Reactive Oxygen Species (ROS) and Reactive Nitrogenous Species (RNS) are effectively kept in check by various antioxidants. Free radicals inherently react with certain chemicals in body and thereby interfere with the cell's ability to function normally. It is increasingly being realized that many of the modern human diseases are due to oxidative stress initiated by overproduction of ROS. This can result in tissue injury, disease progression and oxidative damage of proteins and nucleic acids. Lack of antioxidants to quench the excess reactive free radicals leads to different diseases like cancer, neurodegenerative and inflammatory disorders etc [1]. Therefore, recent studies are focussed on plants having high free radical quenching properties as a substitute to dietary intake of synthetic antioxidants which could cause genotoxicity and carcinogenicity at high concentrations [1].

Natural antioxidants from plant origin are more beneficial in reducing ROS levels, due to synergistic actions of wide range of biomolecules such as phenols, flavonoids, vitamin C, vitamin E and phytomicronutrients [2]. Among the entire tuber crops, *Dioscorea alata* L. (DA) is one of the medicinally potent plants. This plant is mostly studied for its starch granules and structure [3]. *D. alata* is known for its high carbohydrate content where starch is major carbohydrate reserve accounting for 85% of dry matter and is being studied as an alternative source of starch [4]. Five estrogenic compounds were isolated and identified from tubers of *D. alata* by Cheng et al [5]. The tubers are reported to have antihypertensive [6], bone protective [7], immunostimulatory [8] and anticlastogenic effects [9]. Dioscorin is found in *D. alata* which is a storage protein and has antihypertensive, enzymatic, immunomodulatory, lectin activities [10]. Since free radicals are major cause of most of the diseases and *D. alata* has a good medicinal value, study of polyphenolic content and free radical quenching properties of DA would yield important insights into the physiological role of tubers of *Dioscorea alata* L.

MATERIAL AND METHODS

Collection of plant material

Plant material was collected from cultivated pots from Maval (Pune) and authenticated. Their voucher specimens were deposited in herbaria of Medicinal Plants Conservation Centre (MPCC). *Dioscorea alata* L. (MPCC 0578).

Sample Preparation and Extraction

Fresh tubers were cut in small pieces and shade dried in the laboratory. The dried material was then pulverized using an electronic blender and stored in an air tight polythene bags for further use. Ethanol and water extracts were prepared using hot and cold extraction method respectively [11]. The ethanol extract (DAE) was concentrated to dryness under vacuum using rotary evaporator. Water extracts (DAW) were filtered through filter paper. The yield of dried ethanol extract as a percentage weight of dried plant powder was 11.79 % and 2.72 % for water extract of DA.

Chemicals

Quercetin, Aluminium chloride ($AlCl_3$), Catechin, 2, 4, 6-tripyridyl-s-triazine (TPTZ), 2, 2- Diphenyl-1-picrylhydrazyl (DPPH), Sodium nitroprusside (SNP) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Folin- ciocalteu reagent, Thiobarbaturic acid (TBA), N-1-Napthyl ethylene diamine dihydrochloride (NED) was purchased from Loba chemie, Potassium ferricyanide, Trichloroacetic acid, Butylated hydroxyl toluene (BHT), Potassium chloride were purchased from Merck, Mumbai. Gallic acid, Vanillin, Sulphanilamide was purchased from Sisco Research Laboratory. All reagents were of analytical grade and distilled water was used throughout.

Polyphenolic content

Determination of Total Phenolic Content

The total phenolic content of extracts was determined as described earlier [12]. Samples were analyzed at final concentration of 0.1 mg/ml. The standard curve was prepared using gallic acid and values were expressed as gallic acid equivalents.

Determination of Total Flavonoid and Flavonol content

The total flavonoids were determined using the method of Khan et al [13]. The plant extracts were evaluated at a final concentration of 1 mg/ml. Total flavonoid and flavonol content was calculated as quercetin equivalent (mg/g) using equation obtained from the regression curve.

Determination of Total Proanthocyanidin Content

The total proanthocyanidins were determined as previously described [14], where absorbance of red color was measured spectrophotometrically at 500nm. The total proanthocyanidin contents were expressed as catechin equivalent (mg/g).

Reducing ability Assays

Ferric Reducing Antioxidant Power assay (FRAP)

The ferric reducing power of the extracts was assayed by the method as described previously [15]. Absorbance of the sample extracts and standard was read at 593nm against reagent blank. The results expressed as Fe (II) equivalent (µ moles/ml).

Reducing activity or Reducing Power assay

The reducing power of extracts was assayed as described previously [12]. The absorbance was measured spectrophotometrically at 700 nm. The higher absorbance of the reaction mixture indicates strong reducing power of the plant extracts and compared with standard ascorbic acid.

Free radical scavenging assays

Total antioxidant capacity assay by phosphomolybdenum method

Total antioxidant capacity of extracts was assayed [12] and results were expressed as gallic acid equivalents.

2, 2- Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

In this method absorbance of the mixture was measured at 517 nm using spectrophotometer and ascorbic acid was used as standard [12]. The scavenging ability of the plant extract was calculated using following equation:

Scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$

Where, $Abs_{control}$ is the absorbance of DPPH in methanol; Abs_{sample} is the absorbance of sample or standard.

Anti-lipid peroxidation assay

Anti-lipid peroxidation assay was performed [15] in which the complex of TBA-MDA which is formed in this assay is selectively detected at 532nm against solution without $FeCl_3$ (normal) and without drug (induced).

Scavenging activity (%) = [(O.D. induced – O.D. sample)/ (O.D. induced – O.D. Normal)] x 100

Nitric oxide (NO) scavenging assay

To determine the nitric oxide radical scavenging activity the reaction mixture contained SNP (10mM) in phosphate buffered saline (pH 7.4) and different concentration of extract and it was incubated for 150 min at 25⁰C. Then 125µl from above reaction mixture was reacted with griess reagent, in which firstly with 1% sulphanilamide (0.1ml), and after 5 min incubation with 0.1% NED (0.1ml) in 5% phosphoric acid. After 5 min incubation, pink chromogen, indicating nitrite formation, was quantified by the Griess-Illosvoy reaction at 540nm [17]. The nitric oxide radicals scavenging activity was calculated according to the equation

Scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$

Statistical analysis

The observations were replicated thrice for each parameter, mean values were pooled and standard deviation (S.D.) was calculated.

RESULTS AND DISCUSSION

Polyphenolic content

Determination of total phenolic content

Maximum total phenolic content was recorded in DAE (2.69 ± 0.02 mg/g of GAE) while the low concentration was present in DAW which is 0.89 ± 0.03 mg equivalent GAE/g dry mass per 100 µg plant extract. Phenol antioxidant index is a combined measure of the quality and quantity of antioxidants and is responsible for effective free radical scavenging and antioxidative action. The antioxidant capacity of phenolic compounds has long been accepted for their ability to scavenge radicals and strong chain-breaking actions, thereby protecting cells against the detrimental effects of reactive oxygen species [16].

Determination of total flavonoids, flavonols and proanthocyanidins content

Flavonoids are phenolic substances which have important structure-activity relationships of the antioxidant activity. The concentrations of flavonoids, flavonols and proanthocynidins content were high in ethanolic extract and least in water extract (Table 1). Flavonoids are common as oligomers and polymers (condensed tannin or proanthocyanidins) and are divided into several classes like flavones, flavonols, isoflavones, anthocyanidins, flavonols and flavanones [2]. Their mechanism of action is through scavenging or chelation [12].

Table 1: Total flavonoid, flavonol and proanthocyanidin content per 1 mg/ml of extract

Drug	Total flavonoid content (mg QE/g)[b]	Total flavonol content (mg QE/g)[b]	Total proanthocyanidin content (mg CE/g)[c]
DAE[a,d]	0.75 ± 0.02	3.6 ± 0.03	5.93 ± 0.09
DAW[a,e]	0.14 ± 0.02	-	1.73 ± 0.06

[a]All experiments were done in triplicates and data were expressed as mean and standard deviation (SD).
[b]Data expressed in mg quercetin equivalent/g dry weight (mg QE/g)
[c]Data expressed in mg catechin equivalent/g dry weight (mg CE/g)
[d]DAE, *Dioscorea alata* ethanol extract
[e]DAW, *Dioscorea alata* water extract

Reducing ability Assays

Ferric reducing or antioxidant power assay (FRAP)

The trends for ferric ions reducing activities of *D. alata* extracts at same concentrations were observed (Table No.2). DAE showed relatively strong ferric ion reducing activity (12 ± 0.04) and low (4.6 ± 0.06) activity in DAW equivalent to Fe (II)/g dry mass. The ferric reducing power was higher in DAE indicating the hydrogen donating ability of the extract. In FRAP, intense blue color was monitored which is formed in non specific reaction where ferric tripyridyltriazine (Fe III TPTZ) complex is converted to ferrous form. This assay is implied for determining hydrophilic antioxidants [18].

Reducing activity or Reducing power assay

The dose-response increase in the reducing powers of tested samples were observed and correlated well with the increasing concentration (Table No. 2.). Results of antioxidative activity determined by Fe^{+3}/Fe^{+2} reducing activity showed that both extracts of *D. alata* possessed the iron (III) to (II) reducing activity and it was higher in DAE than DAW.

Table 2: Reducing capacity expressed with standard equivalents

Sample	Conc. (µg/ml)	FRAP (µM Fe(II)/gm)	Reducing Power assay (mg Asc AE/g)
DAE[a,d]	25	3.77 ± 0.06	0.26 ± 0.03
	50	6.03 ± 0.32	0.55 ± 0.01
	100	12.0 ± 0.04	0.87 ± 0.01
DAW[a,e]	25	2.13 ± 0.06	0.04 ± 0.03
	50	2.87 ± 0.06	0.15 ± 0.03
	100	4.60 ± 0.06	0.31 ± 0.03

[a]All experiments were done in triplicates and data were expressed as mean and standard deviation (SD).
[b]Data expressed in µM Fe (II) equivalent/g dry weight (µM Fe (II)/gm)
[c]Data expressed in mg Ascorbic acid equivalent/g dry weight (mg Asc AE/g)
[d]DAE, *Dioscorea alata* ethanol extract
[e]DAW, *Dioscorea alata* water extract

Free radical scavenging assays

Total antioxidant capacity assay by phosphomolybdenum method

The activities of both extracts tremendously increased with the concentration of the sample (Figure 1). Ethanolic extract exhibited comparatively high activity than water extract. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of gallic acid. This assay represents activity of all the compounds including polyphenols. The total antioxidant activity of the extract was calculated from the formation of green colored complex at acidic pH, which was measured spectrophotometrically at 695nm [12]. Interaction with the extract or standard increases the formation of green colored complex and ultimately increases the absorbance.

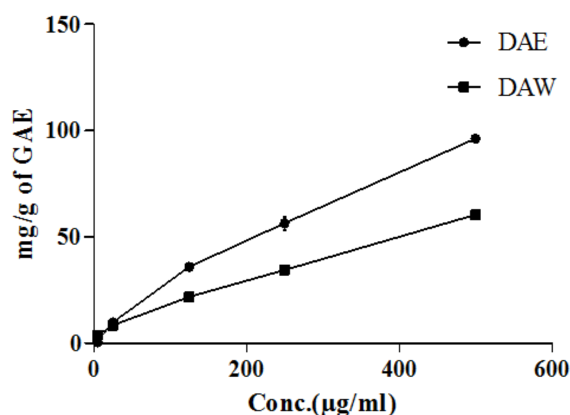

Fig. 1: Total antioxidant capacity

2, 2- Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

Being a stable free radical, DPPH is often used to determine radical scavenging activity of natural compounds. The reduction capability of DPPH was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants activity [14]. The ethanol extract of *D. alata* revealed inhibition of DPPH radical with a 50% inhibition (IC_{50}) at a concentration of 12.9 ± 0.32µg/ml, while water extract was shown to occur least activity (IC_{50} 70.6 ± 0.72µg/ml). The concentration dependent curve of DPPH radical scavenging activity of both extracts compared well with standard ascorbic acid (IC_{50} 20.1 ± 3.19µg/ml) (Fig. 2) and its polyphenolic content, which suggests that ethanol extract possesses high scavenging activity than standard and it has ability to scavenge DPPH radical and relatively its strong proton donating ability [14].

Anti-lipid peroxidation assay

The effects of both extracts of *D. alata* and standard, on non enzymatic peroxidation are shown in Fig. 3. The percentage inhibition of peroxide formation increased in a dose dependant manner. The highest activity was remarked for DAE (IC_{50} 33.7 ± 4.23µg/ml) and is more potent in inhibition of lipid peroxidation in goat liver homogenate than DAW (IC_{50} 161.7 ± 1.78µg/ml). Standard used was ascorbic acid (IC_{50} 5.5 ± 0.13µg/ml). Lipid peroxidation is probably the most extensively investigated free radical induced process. It induces lipid peroxidation in poly unsaturated lipid rich areas like brain and liver [19].

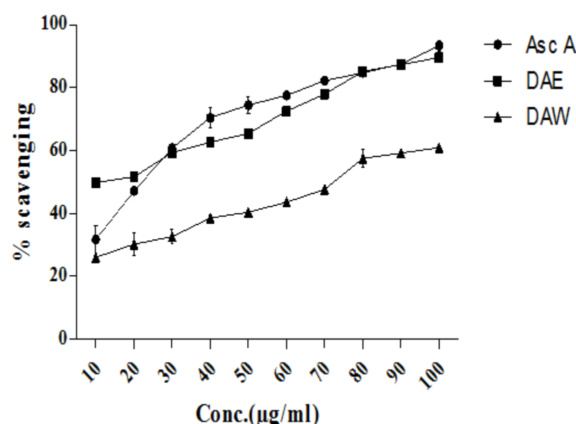

Fig. 2: Comparative DPPH scavenging activity of DA extracts

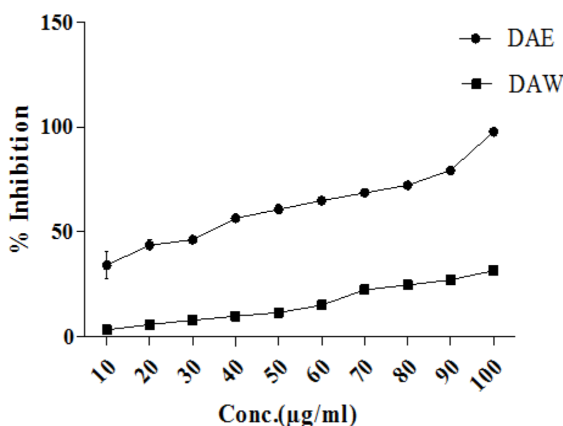

Fig. 3: Anti - lipid peroxidation assay

Nitric oxide scavenging assay

A trend in nitric oxide scavenging activities of *D. alata* extracts at different concentrations as compared with standard ascorbic acid is presented in Fig. 4. Suppression of released NO may be partially attributed to direct NO scavenging [20], as the extracts of *D. alata* decreases the amount of nitrite generated from the decomposition of SNP *in vitro*. The scavenging of NO by extracts increased in dose dependent manner. DAE showed maximum activity of 79.32% and DAW showed 60.33% at 1 mg/ml, whereas ascorbic acid at the same concentration exhibited 82.64% inhibition. Results revealed that

DAE has property to counteract with the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation *in vivo*.

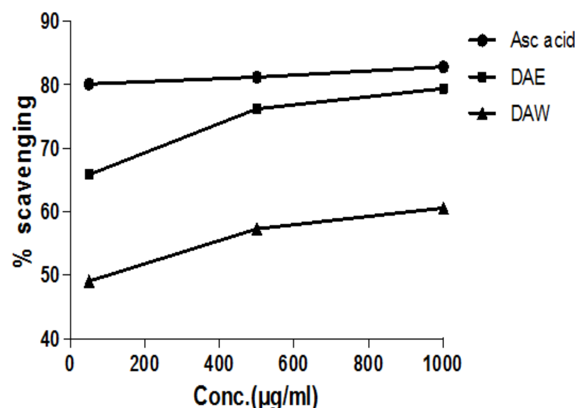

Fig. 4: Nitric oxide (NO) radical scavenging assay

On the basis of the results obtained in the present study, it is observed that ethanolic extract of *Dioscorea alata* L. tubers, which contains large amounts of flavonoids, flavonols, proanthocynidins and phenolic compounds, exhibits reducing power and free radical quenching properties. It is the extent of these phytochemicals present in these extracts being responsible for its marked antioxidant activity as assayed through various *in vitro* methods. These results are supported by some earlier work published viz. heat treatment could reduce the phenolic content and DPPH radical scavenging activity of *D. alata* tubers [21]. Additionally, hydroalchoholic extract of tubers of *D. alata* showed high phenolic content, reducing power and it gives protection against hypochlorous acid damage at low doses [22]. Thus, present investigation confirms its activity as natural antioxidant.

CONCLUSION

This study affirms the *in vitro* free radical quenching potential of crude extracts of the tubers of *D. alata*, with results comparable to those of the standard compounds such as gallic acid, ascorbic acid, quercetin and catechin. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further research is necessary to isolate the active components of these extracts including *in vivo* antioxidant activity. This needs to be assessed prior to commercial use of this plant.

REFERENCES

1. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn 2010; 4: 118–126.
2. Ndhlala A, Moyo M, Staden J. Natural antioxidants: fascinating or mythical biomolecules? Molecules 2010; 15: 6905-6930.
3. Tetchi FA, Dje KM, Dabonne S, Kouame LP. Morphological Characteristics of Starch Granules from Yam Tuber Parts of "Florido" and "Bètè-Bètè" Cultivars (*Dioscorea alata*) During the Post-harvest Storage. Pakistan Journal of Nutrition 2012; 11: 66-77.
4. Ahmed, F, Urooj A. *In vitro* starch digestibility characteristics of *Dioscorea alata* tuber. World J. Dairy & Food Sci. 2008; 3: 29-33.
5. Cheng W, Kuo Y, Huand C. Isolation and identification of novel estrogenic compounds in Yam tuber (*Dioscorea alata* Cv. Tainung No. 2). J Agric Food chem. 2007; 55:7350-7358.
6. Liu Y, Lin Y, Liu D, Han C, Chen C, Fan M et al, Effects of different types of yam (*Dioscorea alata*) products on blood pressure of spontaneously hypertensive rats. Biosci. Biotechnol. Biochem. 2009; 73: 1371-1376.
7. Chen H, Hong L, Lee J, Huang C. The bone-protective effect of a Taiwanese yam (*Dioscorea alata* L. cv. Tainung No. 2) in ovariectomised female BALB/C mice. J Sci Food Agric 2008; 89: 517-522.
8. Shang HF, Cheng HC, Liang HJ, Liu HY, Liu SY, Hou WC. Immuostimulatory activities of yam tuber mucilages. Botanical studies 2007; 48: 63-70.
9. Wang TS, Lii CK, Huang YC, Chang JY, Yang FY. Anticlastogenic effect of aqueous extract from water yam (*Dioscorea alata* L.). Joual of medicinal plant research 2011; 5: 6192-6202.
10. Lu Y, Chia C, Liu Y, Hou W. Biological activites and applications of Dioscorins, the major tuber storage proteins of yam. Journal of traditional and complementary medicine 2011; 2: 41-46.
11. Sudha P, Zinjarde S, Bhargava S, Kumar AR. Potent a-amylase inhibitory activity of Indian *ayurvedic* medicinal plants. BMC Complementary and Alternative Medicine 2011; 11: 1-10.
12. Narkhede AN, Nirmal PS, Tupe R, Kulkarni OP, Harsulkar AM, Jagtap SD. *In vitro* antioxidant, antiglycation and alpha amylase inhibitory potential of *Eulophia ochreata* L. Journal of pharmacy research 2012; 5: 2532-2537.
13. Khan RA, Khan MR, Sahreen S, Ahmed M. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (l.) Hill. Chemistry Central Journal 2012; 6: 1-7.
14. Igbinosa O, Igbinosa I, Chigor V, Uzunuigbe O, Oyedemi S, Odjadjare et al, E. Polyphenolic content and antioxidant potential of stem bark extracts from *Jatropha curcas* (Linn). Int. J. Mol. Sci. 2011; 12: 2958-2971.
15. Giramkar S, Ligade G, Narkhede A, Muharjee S, Takle S, Patil S, et al, Effect of pre- sterilization on physicochemical parameters and *in vitro* free radical scavenging potential of *Saraswatarishta*. Journal of Pharmacy research 2012; 5: 2657-2663.
16. Routray R, Kar M, Sahu R. Evaluation of antioxidant potential in selected leafy vegetables of Odisha, India. Int J Pharm Pharm Sci 2013; 5: 232-235.
17. Awah FM, Verla AW. Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *oscimum gratissimum* leaf extract. Journal of Medicinal Plants Research 2010; 4: 2479-2487.
18. Mukharjee S, Pawar N, Kulkarni O, Nagarkar B, Thopate S, Bhujbal A, et al, Evaluation of free radical quenching properties of standard *ayurvedic* formulation *vayasthapana rasayana*. BMC complementary and alternative medicine 2010; 11: 38-43.
19. Sakat S, Juvekar A, Gambhire M *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci 2010; 2: 146-155.
20. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complementary and Alternative Medicine 2010; 10: 1-8.
21. Chen JC, Yeh JY, Chen PC, Hsu CK. Phenolic Content and DPPH Radical Scavenging Activity of Yam-containing Surimi Gels Influenced by Salt and Heating. Asian Journal of Health and Information Sciences 2007; 2: 1-11.
22. Das A, Mandal DN, Chatterjee A. Study of antioxidant and reactive oxygen species scavenging activity of the edible tuber of “greater yam” (*Dioscorea alata* L.) From north-east India. Asian J Pharm Clin Res 2012; 5: 74-84.