Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2015; 3(9):790-795 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI :** 10.36347/sajb.2015.v03i09.010

Research Article

Extraction yield and antiooxidant activity of biomolecule and bioactive fractions from seed and peel parts of *Pithecellobium jiringa*

Yanti*, F. Irnawati, M. Vivian, Y.R.E. Wulandari
Faculty of Biotechnology, Atma Jaya Catholic University, Jakarta 12930, Indonesia

***Corresponding author**
Yanti
Email: yanti@atmajaya.ac.id

Abstract: *Pithecellobium jiringa*, known as jengkol in Indonesia, is a tropical plant of the *Leguminosae* family in Southeast Asia regions. *P. jiringa* seed has been used in Indonesian typical culinary due to its high nutrition contents, meanwhile its peel is generally applied for natural food dye. In this study, we investigated antioxidant potentials of biomolecule and bioactive fractions from seed and peel parts of *P. jiringa* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Biomolecule fractions (protein and polysaccharide) were extracted using precipitation techniques, while bioactive fractions, i.e. polyphenol and essential oil, were obtained from solvent extraction. All fractions were tested for their antioxidant potentials at various concentrations (10-250 µg mL^{-1}). Ascorbic acid and Trolox were used as standard drugs. The optimal extraction yield was reached by polyphenol fractions from both *P. jiringa* seed and peel with values of 22.68% and 16.80%, respectively. Among all tested fractions, polyphenol fraction from *P. jiringa* peel at lowest concentration (10 µg mL^{-1}) exerted the highest antioxidant activity (>80%) with the dose-independent profile. Its efficacy was slightly higher than Trolox. Meanwhile, protein and polysaccharide fractions from both seed and peel parts also had ~50% dose-independent antioxidant activity, similar to ascorbic acid. Thus, *P. jiringa* fractions from peel and seed parts may be potentially used for alternative natural antioxidant candidates with lowest dosage and high efficacy *in vitro*.
Keywords: antioxidant activity, *Pithecellobium jiringa*, jengkol, polyphenol fraction, DPPH assay.

INTRODUCTION

Oxidative stress is an imbalance condition between the production, removal of reactive oxygen species (ROS), and the repair of damaged molecules [1]. Oxidative stress is generally caused by free radicals from daily food consumption and the environment. Several degenerative diseases induced by oxidative stress, including diabetes mellitus, hypertension, cancer, Alzheimer's, atherosclerosis, and heart attack, have been increasing and become the focus of investigation.

The presence of antioxidants can keep the body in ward free radicals and capture the excess reactive material inside the cell [2]. Antioxidants act in several ways, such as inhibiting ROS formation, either preventing or inhibiting activation of phagocytes, binding of transition metal ions, preventing the formation of OH, and decomposing lipid hydroperoxide by repairing the damage [3]. Antioxidants are substances that exist in low concentrations and significantly reduce or prevent the oxidation of cells, such as proteins, fats, carbohydrates, and DNA. Natural antioxidants can be used at high concentration because there are no side effects to the body [4].

Pithecellobium jiringa, popularly known as jengkol in Indonesia, belongs to the *Leguminosae* family and a tropical plant in Southeast Asia regions. It has been traditionally used for culinary and folk medicine for treatment of skin ailment, chest pain, gum pains, toothache, hypertension, and diabetes [5]. Nutritional composition of a 100 g of edible *P. jiringa* seed consists of 6.2 g protein, 16.9 g carbohydrate, 0.2 g fat, and 1.3 g fiber [6]. *P. jiringa* seed also has vitamins, minerals, essential oil, alkaloids, steroids, glycosides, tannin, and saponin. Stem and fruit parts of *P. jiringa* are rich in polyphenol contents, including gallic acid with high antioxidant effect. Meanwhile, *P. jiringa* peel has flavonoid contents used for natural food coloring [7]. This study was aimed to explore the bioefficacy of polyphenol, protein, biopolysaccharide, and essential oil fractions from seed and peel parts of *P. jiringa* as alternative natural antioxidant candidates.

MATERIALS AND METHODS

Collection and preparation of samples

P. jiringa samples (code: DJ01 for peel and DJ03 for seed) were collected from traditional market at Balige, North Sumatera (Indonesia). It was dried under the sun and separated into sees and peel. Each dried part

of *P. jiringa* seed and peel were crushed separately until it became powder.

Extraction of biomolecule fractions - protein and polysaccharide

Two biomolecule fractions include protein and polysaccharide fractions from seed and peel parts of *P. jiringa* were extracted with precipitation methods. For protein fraction, extraction is based on the isoelectric precipitation [8]. Each part was weighed 100 g, crushed, and mixed with 25% (w/v) distilled water. A 1 M NaOH was added until pH of solution reached 8.6, and stirred for 30 minutes at temperature of 50^{o}C. The solution was centrifuged at 3000×g for 30 minutes and the supernatant was taken. The supernatant was added with 2 M HCl until pH of 4.5 for protein precipitation, followed by centrifugation at 1500×g for 20 minutes. The pellet was then dried in incubator at 37°C overnight.

For polysaccharide fraction, extraction of *P. jiringa* seed and peel was done according to the modified method of Oliviera *et al.* [9]. A 100 g of each crushed sample was mixed with 10% w/v of water and incubated in waterbath shaker at a temperature of 90^{o}C for 3 hours. After 3 hours, the liquid phase was taken and centrifuged at 4307×g and 4°C for 30 minutes. The supernatant was dried using rotary evaporator at 56-60°C and 72 atm pressure to evaporate the solvent water. The solution was then adjusted to pH 3 by the addition of 100% trichloroacetic acid (TCA) and incubated at 4°C for 30 minutes. The solution was centrifuged at 7656×g, 4°C for 30 minutes for separation of polysaccharide fraction. The pellet was taken and dried in incubator at 60°C overnight.

Extraction of bioactive fractions - polyphenol and essential oil

There are two bioactive fractions, i.e. polyphenol and essential oil fractions, from *P. jiringa* seed and peel that were extracted by using the modified methods of Liaw *et al.* [10] and Yanti *et al.* [11]. Each of *P. jiringa* seed and peel powder (100 g) was extracted with 70% ethanol (for polyphenol fraction) and hexane (for essential oil fraction) with 20% (w/v) sample:solvent ratio, then stirred for overnight. Ethanol and hexane residues were evaporated to separate the polyphenol and essential oil fractions using a Rotary Evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland), followed by freeze-dried using freeze-drying apparatus for polyphenol fraction production.

Sample preparation

Protein and polysaccharide fractions from *P. jiringa* seed and peel at stock concentration (10^3-10^5 µg mL^{-1}) and working concentration (10-250 µg mL^{-1}) were diluted in 50 mM phosphate buffer pH 7.6. Meanwhile, polyphenol and essential oil fractions from *P. jiringa* seed and peel at similar concentrations with biomolecule fractions were dissolved in dimethyl sulfoxide (DMSO). Standard drugs of ascorbic acid and Trolox at concentration of 10-100 µg mL^{-1} were dissolved in distilled water and DMSO.

DPPH radical scavenging activity

Free radical scavenging activity of all *P. jiringa* fractions was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) by the method of Gaikwad *et al.* [12]. A 125 µL of sample was added to 96 well-microplate and mixed with 50 µL of DPPH reagent. The mixture was incubated for 30 minutes at room temperature prior to absorbance measurement with ELISA reader at 517 nm. Control was 125 µL of DMSO 25% and 50 µL of DPPH reagent, meanwhile blank was 175 µL of DMSO 25%. Positive control was ascorbic acid and Trolox. Antioxidant activity was calculated using the following formula:

$$\textit{Radical scavenging activity}\ (\%) = \frac{\text{Control+Blank}-\text{Sample}}{\text{Control}} \text{x}100\%$$

Statistical analysis

All data were done triplicate from samples and standards. The data were presented as average ± deviation standard. Statistical analysis of untreatment and *P. jiringa* fraction treatment was performed by analysis of variance (SPSS 11.0 for Windows).

RESULTS

Extraction yields of fractions from *P. jiringa* seed and peel

Table 1 showed that among all fractions from seed and peel parts of *P. jiringa*, polyphenol fractions from both parts resulted in the highest extraction yields with 22.68% (for seed) and 16.80% (for peel). In addition, polysaccharide fractions from *P. jiringa* seed had approximately 7.64% of extraction yield.

Antioxidant activity of fractions from *P. jiringa* seed and peel

All biomolecule and bioactive fractions from seed and peel parts of *P. jiringa* were also evaluated for their ability as antioxidant agents using DPPH assay (Table 2). All fractions demonstrated the tendency to suppress the free radicals in the dose-independent manner as well as reference drug of ascorbic acid. Among all fractions from both *P. jiringa* seed and peel, polyphenol fraction from *P. jiringa* peel at lowest concentration (10) possessed the highest antioxidant effect (>80%). Its effect was similar to reference drug of Trolox. Interestingly, both protein and polysaccharide fractions from *P. jiringa* seed and peel at lowest concentration also exerted antioxidant activity ~50%, respectively. These fractions were dose-independent pattern and in linear with the antioxidant potency of ascorbic acid.

Table-1: Extraction yields of fractions from *P. jiringa* seed and peel

Fraction	Initial weight (g)	Final weight (g)	Yield (% w/v)
P. jiringa seed			
Protein	100	0.320	0.320
Biopolysaccharide	100	7.640	7.640
Polyphenol	100	22.680	22.680
Essential oil	100	1.672	1.672
P. jiringa peel			
Protein	100	0.320	0.320
Biopolysaccharide	100	0.720	0.720
Polyphenol	100	16.800	16.800
Essential oil	100	1.840	1.840

Table-2: Antioxidant activity of biomolecule and bioactive fractions from *P. jiringa* seed and peel

Sample	Antioxidant Activity (%)							
	Concentration (µg mL^{-1})							
	10	25	50	75	100	150	200	250
***P. jiringa* seed**								
Protein	52.77 ± 1.42	48.82 ± 3.45	48.88 ± 2.22	45.69 ± 6.57	40.73 ± 6.88	41.37 ± 5.22	41.26 ± 8.85	41.49 ± 1.10
Polysaccharide	50.93 ± 6.07	46.11 ± 8.85	39.21 ± 6.14	38.24 ± 4.38	38.00 ± 2.37	35.13 ± 3.56	33.92 ± 4.03	31.98 ± 4.08
Polyphenol	38.85 ± 2.85	43.32 ± 7.10	49.35 ± 2.01	39.97 ± 5.32	41.84 ± 8.45	43.69 ± 5.58	38.45 ± 3.21	50.39 ± 4.76
Essential oil	36.08 ± 5.18	41.65 ± 1.81	26.51 ± 1.75	26.88 ± 1.91	29.10 ± 1.01	29.93 ± 0.11	31.21 ± 1.50	30.52 ± 2.82
***P. jiringa* peel**								
Protein	50.32 ± 4.46	46.26 ± 1.52	51.10 ± 1.52	44.70 ± 6.38	44.60 ± 4.72	46.76 ± 2.37	50.67 ± 5.32	46.67 ± 5.75
Polysaccharide	57.27 ± 3.95	51.09 ± 1.80	48.69 ± 1.56	47.84 ± 0.81	51.34 ± 0.50	53.49 ± 0.31	54.35 ± 0.25	55.61 ± 1.22
Polyphenol	84.14 ± 2.12	88.03 ± 0.48	85.82 ± 0.61	89.23 ± 0.78	92.43 ± 0.45	89.32 ± 2.20	91.21 ± 3.58	91.44 ± 2.66
Essential oil	30.55 ± 0.19	26.51 ± 1.71	22.10 ± 3.81	22.62 ± 1.73	27.69 ± 2.52	21.15 ± 2.85	22.30 ± 2.84	20.33 ± 1.06
Reference drugs*								
Ascorbic acid	73.81 ±2.90	69.52 ±1.38	65.29 ±0.27	66.54 ± 1.89	64.36 ±1.00			
Trolox	59.87 ± 3.81	89.25 ± 0.80	91.69 ± 0.50	93.42 ± 2.31	93.51 ± 1.14			

DISCUSSION AND CONCLUSION

Free radical reactions occur both in the human body and food systems. In humans, over production of ROS may caused oxidative stress and several degenerative diseases [2]. The presence of antioxidants plays an important role to prevent cell damages caused by free radicals. However, long term effect of the consumption of synthetic antioxidants in food and pharmaceutical products, such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), may cause carcinogenicity. Therefore, the use of natural antioxidants offer a promising alternative natural sources with high effectiveness and less harmful effect for humans [13, 14].

This study was focused on screening of biomolecule fractions (protein and polysaccharide) and bioactive fractions (polyphenol and essential oil) from seed and peel part of *P. jiringa* by employing various precipitation methods. Our results demonstrated that among all 4 fractions, only polyphenol fraction from seed and peel of *P. jiringa* had the highest extraction yields (Table 1). Several reports showed that bioefficacy of plant extracts is mostly linked to their phenolic compounds [15, 16]. In linier with our study, extraction of phenolic contents from plant extracts with ethanol solvent may result in the optimal yield of extraction due to the solvent capability on enhancing the yield production of phenolic and flavonoid contents from extracts [17].

In contrast, protein fractions extracted from both parts of *P. jiringa* using isoprecipitation method showed the lowest extraction yields of all fractions. It is thought that extraction yield of protein is also related with low protein contents in *P. jiringa* seed and peel parts. Moreover, protein extraction from plants was known to be challenging due to the large quantities of secondary compounds and proteolytic enzymes [18]. The action of proteolytic degradation by enzymatic proteins also caused the loss of high molecular weight proteins [19]. Meanwhile, according to Subhadrabandhu [6], *P. jiringa* seed has higher soluble and insoluble polysaccharides in comparison to other components.

In this study, antioxidant activity of all *P. jiringa* fractions was tested by DPPH method using the principle of electron transfer from DPPH, a stable free radical reagent with violet colour [20]. The DPPH color will be reduced if antioxidant molecules are present. Table 2 showed that polyphenol fractions from *P. jiringa* peel exerted the highest antioxidant activity (>80%) starting from the lowest concentration (10 µg mL^{-1}) and its efficacy was higher than those of reference drugs (ascorbic acid and Trolox), indicating its potential candidate for an alternative natural antioxidant. However, this polyphenol fraction produced a dose-independent antioxidant effect *in vitro*. *P. jiringa* peel is known to contain flavonoid compounds [7]. In comparison with our data, ethanolic

extract from edible shoots of *Archidendron jiringa* had IC_{50} of 33.52 µg mL^{-1} [21]. It is noted that *A. jiringa* shoots contained bioactive compounds, such as phenolics, flavonoids, terpenoids, and alkaloids, that may act as antioxidants.

Several antioxidant mechanisms by phenolic antioxidants have been reported. The configuration, total number of hydroxyl groups, structural molecules, and amount of phenolic compounds influence the roles of polyphenolic antioxidants [22, 23]. In Japan, apple polyphenol extract with high antioxidant effect has been widely applied for food additive and nutritional supplements [24]. In terms of antioxidant efficacy, phenolic compounds derived from plants exhibit their antioxidant roles in several ways, including by scavenging free radicals, chelating metals, reducing action, and inhibiting lipid peroxidation [25, 26].

DPPH results also demonstrated that biomolecules including protein dan polysaccharide fractions from *P. jiringa* seed and peel had similar IC_{50} values at 10 µg mL^{-1}, suggesting their potentials as free radical scavengers (Table 2). Antioxidant activities of both fractions were dose-independent. The IC_{50} value is the concentration of antioxidant which reduces free radical DPPH about 50% [27]. As comparison, protein crude of *Lucas linifolia* had IC_{50} at 175 10 µg mL^{-1} with dose-dependent effect of antioxidant [28]. The unstability of protein may affect the antioxidant activity. Disruption of tertiary protein structure can increase solvent accessibility of amino acid residues to scavenge more free radicals (Elias *et al.* 2008). Proteins are included in enzymatic antioxidants that can protect cell damages from free radicals by changing free radicals to other molecules [29].

Polysaccharides are common structural and storage polymers with approximately of 75% dry mass in plants [30]. The edible *P. jiringa* seed also contains major carbohydrate compared to other nutrition [6]. Several antioxidants derived from plant polysaccharide have been reported. Kong *et al.* [31] showed that polysaccharide fraction from pulp tissue of *Litchi chinensis* Sonn. at 1 mg mL^{-1} exerted 25% of antioxidant activity. *Astragalus membranaceus* polysaccharide at 0.5 mg mL^{-1} showed ~80% of antioxidant effect [32]. Compared to our data, polysaccharide fractions from *P. jiringa* seed and peel are strongly effective as free radical scavengers at low dose.

Essential oil had the lowest dose-independent antioxidant activity (30-35%) compared to those of other fractions from *P. jiringa* seed and peel (Table 2). The low bioefficacy is linked to the number of total phenolics, total polar lipids, composition of polar lipids, and structural diversity of phenolics in oil [24]. At concentration of 200 µg mL^{-1}, essential oil of *Ocimum basilicum* leaves showed ~35% of dose-dependent antioxidant activity, respectively [33]. The highest antioxidant activity (<50%) of essential oil was reached at concentration of 25 µg mL^{-1}. Other study showed that essential oil from *Zataria multifloral* at concentration of less than 25 µg mL^{-1} exhibited >50% antioxidant activity [34]. Antioxidant activity of essential oil is strongly affected by its chemical structure. For example, trans-anethole can easily neutralize free radicals and form a stable molecule, while estragole can neutralize free radicals, but it makes itself a radical [35].

Antioxidants are grouped into non-enzymatic antioxidants, such as ascorbic acid and phenolics, and enzymatic antioxidants, such as superoxide dismutase and catalase. Reference drugs used in this study include ascorbic acid and Trolox. Our results showed that both standards had quite similar antioxidant efficacy, except for the dose pattern (Table 2). Ascorbic acid, known as a water soluble antioxidant, at 6 had 71 % antioxidant activity [36]. This result is slightly effective than our present data. It seems that the purity of ascorbic acid may contribute to the dose-independent profile. In contrast, Trolox, a water soluble analogue of vitamin E [37], showed dose-dependent antioxidant profile at range concentrations of 10-100 µg mL^{-1} (Table 2).

In conclusion, polyphenol fractions from both seed and peel parts of *P. jiringa* produced the highest extraction yields, and polyphenol fraction from *P. jiringa* peel exerted the highest antioxidant activity with dose-independent profile. For further research, isolation and identification of nutraceutical compounds in polyphenol fraction from *P. jiringa* peel that responsible for antioxidant effect are needed.

Acknowledgement

This research was funded by 2014 Atma Jaya Competitive Grant from Institute for Research and Community Service, Atma Jaya Catholic University, Jakarta, Indonesia.

REFERENCES

1. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB; Oxidative stress, inflammation, and cancer: how are they linked? Free Radical Biology & Medicine, 2010; 49(11): 1603-1616.
2. Wu DF, Cederbaum AI; Alcohol, oxidative stress, and free radical damage. Alcohol Research & Health, 2003; 27(4): 277-284.
3. Gupta VK, Sharma SK; Plants as natural antioxidants. Natural Product Radiance, 2006; 5(4): 326-334.
4. Mohamed TK, Issoufou A, Zhou H; Antioxidant activity of fractionated foxtail millet protein hydrolysate. International Food Research Journal, 2012; 19(1): 207-213.

5. Muslim NS, Nassar ZD, Aisha AF, Shafei A, Idris N, Majid A, et al.; Antiangiogenesis and antioxidant activity of ethanol extracts of Pithecellobium jiringa. BMC Complementary and Alternative Medicine, 2012; 12: 210-220.
6. Subhadrabandhu S; Under-utilized Tropical Fruits of Thailand. Edn 1, FAO Rap Publication, Bangkok, 2001; 70.
7. Lim TK; Edible Medicinal and Non-Medicinal Plants: Fruits, Vol 2, Springer, New York, 2012: 544-548.
8. Bollag DM, Edelstein SJ; Protein Methods. Edn 1, Wiley-Liss, New York, 1991; 33.
9. Oliviera R, Marques F, Azeredo J; Purification of polysaccharides from a biofilm matrix by selective preciputation of proteins. Biotechnology Techniques, 1999; 13: 391-393.
10. Liaw MY, Natan FA, Widiyanti P, Ikasari D, Indraswati N, Soetaredjo FE; Extraction of neem oil (Azadirachta indica A. Juss) using n-hexane and ethanol: studies of oil quality, kinetic and thermodynamic. Journal of Engineering and Applied Sciences, 2008; 3(3): 49-54.
11. Yanti, Pramudito TE, Nuriasari N, Juliana K; Lemon pepper fruit extract (Zanthoxylum acanthopodium DC.) supresses the expression of inflammatory mediators in lipopolysaccharide-induced macrophages in vitro. American Journal of Biochemistry and Biotechnology, 2011; 7(4): 190-195.
12. Gaikwad P, Barik A, Priyadarsini KI, Rao BSM; Antioxidant activities of phenols in different solvents using DPPH assay. Research on Chemical Intermediates, 2010; 36 (9): 1065-1072.
13. UmemuraT, Kodama Y, Nishikawa A, Hioki K, Nomura T, Kanki K, et al.; Nine-week detection of six genotoxic lung carcinogens using the rasH2/BHT mouse model. Cancer Letters, 2006; 231(2): 314-318.
14. Lobo V, Patil A, Phatak A, Chandra N; Free radicals, antioxidants and functional foods: impact on human health. Pharmacognosy Reviews, 2010; 4(8): 118-126.
15. G, Rocha JBT; Antioxidant in foods: a new challenge for food processors. In: Leading Edge Antioxidants Research, Edn 1, Nova Science Publishers, New York, 2007; 35-64.
16. Salas PG, Soto AM, Carretero AS, Gutiérrez AF; Phenolic compound - extraction systems for fruit and vegetable samples. Molecules, 2010; 15(12): 8813-8826.
17. Molehin OR, Adefegha SA; Antioxidant and inhibitory effects of aqueous and ethanolic extract of Tapinanthus bangwensis leaves on Fe^{2+}-induced lipid peroxidation in pancreas (in vitro). International Food Research Journal, 2015; 22(1): 269-274.
18. Plomion C, Bousquet J, Kole C; Genetics, Genomics, and Breeding of Conifers. Edn 2, CRC Press, New York, 2011; 323-357.
19. Valcu CM, Schlink K; Reduction of proteins during sample preparation and two-dimensional gel electrophoresis of woody plant samples. Proteomics, 2006; 6 (5): 1599-1605.
20. Garcia EJ, Oldoni TLC, Alencar SM, Reis A, Loguercio AD, Grande RHM; Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Brazilian Dental Journal, 2012; 23(1): 22-27.
21. Bunawan H, Dusik L, Bunawan SN, Amin NM; Botany, traditional uses, phytochemistry amd pharmacology of Archidendron jiringa: a review. Global Journal of Pharmacology, 2013; 7 (4): 474-478.
22. Heim KE, Tagliaferro AR, Bobilva DJ; Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. The Journal of Nutritional Biochemistry, 2002; 13(10): 572-584.
23. Ishtiaque S, Siddiqui MA, Siddiqi R, Naz S; Antioxidant potential of the extracts, fractions and oils derived from oilseeds. Antioxidants, 2013; 2(4): 246-256.
24. Shoji T, Akazome Y, Kanda T, Ikeda M; The toxicology and safety of apple polyphenol extract. Food and Chemical Toxicology, 2004; 42(6): 959-967.
25. Alia M, Horcajo C, Bravo L, Goya L; Effect of grape antioxidant dietary fiber on the total antioxidant capacityand the activity of liver antioxidant enzymes in rats. Nutritional Research, 2003; 23(9): 1251-1267.
26. Adefegha SA, Oboh G; Enhancement of total phenolics and antioxidant properties of some tropical green leafy vegetables by steam cooking. Journal of Food Processing and Preservation, 2011; 35(5): 615-622.
27. de Oliveira AMF, Pinheiro LS, Pereira CKS, Matias WN, Gomes RA, Chaves OS, et al.; Total phenolic content and antioxidant activity of some Malvaceae family species, Antioxidants, 2012; 1(1): 33-43.
28. Ramakrishna H, Murthy SS, Mama DR, Tharani DR, Murthy GP; Hydroxy radical and DPPH scavenging activity of crude protein extract of Leucas linifolia: A folk medicinal plant. Asian Journal of Plant Science and Research, 2012; 2(1): 30-35.
29. Boonmee A, Srisomsap C, Karnchanatat A, Sangvanich P; An antioxidant protein in Curcuma comosa Roxb. rhizomes. Food Chemistry, 2011; 124 (2): 476-480.
30. Zhang Z, Jin J, Shi L; Antioxidant activity of the derivatives of polysaccharide extracted from a Chinese medical herb (Ramulus mori). Food Science and Technology Research, 2008; 14(2): 160-168.
31. Kong F, Zhang M, Liao S, Yu S, Chi J, Wei Z; Antioxidant activity of polysaccharide-enriched fractions extracted from pulp tissue of Litchi

chinensis Sonn. Molecules, 2010; 15(4): 2152-2165.
32. Hao S, Zaobao W; Effects on exercise endurance capacity and antioxidant properties of astragalus membranaceus polysaccharides (APS). Journal of Medicinal Plants Research, 2010; 4(10): 982-986.
33. Khelifa LH, Brada M, Brahmi F, Achour D, Fauconnier ML, Lognay G; Chemical composition and antioxidant activity of essential oil of Ocimum basilicum leaves from the northern region of Algeria. Topclass Journal of Herbal Medicine, 2012; 1(2): 25-30.
34. Khalighi-Sigaroodi F, Ahvazi M, Hadjiakhoondi A, Taghizadeh M, Yazdani D, Khalighi-Sigaroodi S, et al.; Cytotoxicity and antioxidant activity of 23 plant species of leguminosae family. Iran Journal of Pharmaceutical Research, 2012; 11(1): 295-302.
35. Shahat AA, Ibrahim AY, Hendawy SF, Omer EA, Hammouda FM, Abdel-Rahman FW, et al.; Chemical composition, antimicrobial and antioxidant activities of essential oil from organically cultivated fennel cultivars. Molecules, 2011; 16 (2): 366-1377.
36. Cholisoh Z, Utami W; Antiradical activity of ethanolic (70%) stinky bean (Archidendron jiringa) extract. Pharmacon, 2008; 9(1): 33-40.
37. Sagach VF, Scrosati M, Fielding J, Rossoni G, Galli C, Visioli F; The water-soluble vitamin E analogue Trolox protect against ischaemia/ reperfusion damage in vitro and ex vivo. Pharmacological Research, 2002; 45(6): 435-439.