

Antioxidant Activity and HPTLC Analysis of *Pandanus odoratissimus* L. Root

J.M. Sasikumar, U. Jinu and R. Shamna

Department of Biotechnology, Karpagam University, Coimbatore - 641 021, India

Abstract: The methanolic and aqueous extracts of *Pandanus odoratissimus* L. root were examined for their contents of phenolics and flavonoids as well as for their *in vitro* antioxidant activities using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical quenching assay and reducing power. The methanolic root extract yielded 56.24 ± 0.02 mg/g GAE of phenolic content and 81.25 ± 0.046 mg/g CE of flavonoid content. Higher antioxidant potential was observed in both DPPH scavenging assay ($EC_{50} = 48.3 \pm 0.002 \mu\text{g mL}^{-1}$) and reducing capacity (OD at $1000 \mu\text{g mL}^{-1} = 0.787$) by the methanolic root extract than by the aqueous extract. A positive correlation was found between phenolics and flavonoid contents and antioxidant properties of the extracts. These findings indicate that polyphenolic components may have antioxidant potential, as reported in other medicinal plants.

Key words: *Pandanus odoratissimus* L. • Phenolics • Flavonoid • Antioxidant properties • HPTLC

INTRODUCTION

Antioxidants from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress. Several epidemiological, clinical and experimental data suggested that plant based antioxidants have beneficial effects on prevention on chronic diseases [1, 2]. As a result, there has been a keen interest in evaluating the role bioactive constituents from medicinal plants in reducing the risk of the aforesaid diseases. Recent studies have demonstrated the antioxidant activities and health benefits of the phenolic compounds occurring in plants [3, 4]. In the view of the aforesaid, in the present study, the contents of phenolics, flavonoids and antioxidant activities of methanol and aqueous extracts of root of *P. odoratissimus* were assessed using established assay procedures. We have used DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical quenching and reducing capacity assays to determine the antioxidant activities.

Pandanus odoratissimus L., a shrub, belongs to the family Pandanaceae. The root is used to treat skin diseases, ulcers, dyspepsia, diabetes, fever and leprosy [5]. Root juice is administered for 'Prameha', i.e. extreme heat constitution [6]. Four strong antioxidative (thiocyanate method) lignan type compounds and a new benzofuran derivative activities were isolated from the root parts of *P. odoratissimus* [7]. The methanolic leaf extract of *P. odoratissimus* were evaluated for its free

radical scavenging activities by DPPH, super oxide, nitric oxide and hydroxyl scavenging assays [8]. Our present work encompasses chemical profile and antioxidant activity to authenticate the medicinal properties of *P. odoratissimus* root.

MATERIALS AND METHODS

Preparation of Methanol Extract: Root parts of *P. odoratissimus* were collected from Kerala State, India. The taxonomic identification was done by Dr. R. Gopalan, Karpagam University. A voucher specimen has been deposited in Karpagam University, Coimbatore. The plant part collected were shade dried and then pulverized. The powder (100 g) was exhaustively extracted with methanol in the ratio of 1:5 (w/v) for 24 h using soxhlet apparatus. The extract was completely evaporated to dryness using rotary flash evaporator (Buchi type). Different concentrations of extracts were prepared from the resultant crude methanol and aqueous extracts to determine *in vitro* antioxidant activity.

Chemicals: All chemicals used including the solvents, were of analytical grade. 1,1-diphenyl-2-picryl hydrazyl (DPPH), butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) were purchased from Merck Co (Mumbai, India). Folin ciocalteu reagent, gallic acid, trichloroacetic acid (TCA), ferric chloride and ascorbic acid were purchased from SD fine Chemicals.

Estimation of Total Phenolics: Total phenolic content of root was measured based on Folin-Ciocalteu assay [9]. Briefly, 1.2ml of sodium carbonate (7.5% w/v) was added to the 5 gm of methanolic extract of root. After 30 min, absorbance was measured at 765nm with UV/Vis spectrophotometer (Elico, India). Total phenolic content was expressed as mg gallic acid equivalents (GAE)/g fresh weight.

Estimation of Total Flavonoids: The flavonoid content was examined by adopting the methodology of Chang *et al.* [10]. Briefly, 5 g of methanol extract of root were mixed with 3ml of methanol, 0.2ml of 10% AlCl₃, 0.2 ml of 1M potassium acetate and 5.6ml of distilled water. It was incubated at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415nm. Total flavonoid content was expressed as mg catechin equivalents (CE)/g fresh mass.

HPTLC Analysis for Polyphenols: A densitometric HPTLC analysis was performed for the development of characteristic finger printing profile. The *P. odoratissimus* methanolic extract of root was dissolved with HPLC grade methanol 100 mg/0.5ml. The solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis. Then, 2 µl of the samples were loaded as 7 mm band length in the 10 x 10 Silica gel 60F₂₅₄ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapor) with respective mobile phase (polyphenolic compound) and the plate was developed in the respective mobile phase (Toluene-Acetone-Formic acid 4.5:4.5:1) up to 90 mm. The developed plate was dried using hot air to evaporate solvents from the plate and sprayed with stannic chloride reagent. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at UV366 nm. Finally, the plate was fixed in scanner stage and scanned at 254nm. The Peak table, Peak display and Peak densitogram was identified [11].

Determination of Antioxidant Activity

DPPH Radical Quenching Activity: DPPH radical scavenging activity was determined by a standard method of Singh *et al.* [12]. Briefly, 5 ml of methanol solution of DPPH (0.1 mM) was added to 1 ml of the sample solutions at different concentrations (1000-25 µg mL⁻¹) and vortexed. The mixtures were allowed to stand at room temperature for 20 minutes. Changes in the absorbance of the samples were measured at 517 nm. The

scavenging activity of the extract was expressed as 50% effective concentration (EC₅₀). BHT and ascorbic acid were used as reference compounds. The inhibition percentage was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = \frac{1 - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100.$$

Butyl hydroxy anisole (BHA), butylated hydroxyl toluene (BHT) and ascorbic acid were taken as reference standards. A percentage inhibition versus concentration curve was plotted and the concentration of the extract required for 50% inhibition of radicals was expressed as EC₅₀ values (µg mL⁻¹).

Reducing Capacity: The reducing power of the methanol and aqueous extracts was assayed as described by Oyaizu [13]. Different volumes of the test samples were mixed with phosphate buffer (5ml, 0.2 M, pH = 6.6) and 5ml of 1% potassium ferric cyanide solution [K₃Fe (CN)₆]. The mixtures were incubated in a water bath at 50°C for 20 minutes. After the incubation period, 5 ml of 10% trichloro acetic acid was added to the mixture and the contents were centrifuged at 1000 rpm for 10 minutes. 5ml aliquots were mixed with 5ml of distilled water and 1ml ferric chloride (0.1%) and the absorbency of the reaction mixtures was measured spectrophotometrically at 700nm after reaction for 10 min. The higher the absorbency represents the stronger, the reducing power. Butyl Hydroxy Anisole (BHA) and Butyl Hydroxy Toluene (BHT) were taken as reference standards.

RESULTS AND DISCUSSION

The identification and investigation on antioxidants from medicinal plants is a fast expanding field of research and several antioxidants have been investigated such flavonoids and other phenolic compounds. In the view of the upsurging interest in the health benefits of the medicinal plants, we examined total phenolics and flavonoids and evaluated the antioxidant effects of crude methanolic and aqueous extracts of root of *P. odoratissimus*.

Estimation of Total Phenolics and Total Flavonoids: In the present study, we examined total phenolics (TF) using gallic acid as standard (R²=0.9968), flavonoids (TF) using catechin as standard (R²=0.9965) The content of total phenolics and flavonoids in methanol extract of *P. odoratissimus* root amounted to 56.24 ± 0.02 mg/g GAE and 81.25 ± 0.046 mg/g CE respectively.

Table 1: DPPH scavenging activity of methanol and aqueous extracts of *P. odoratissimus* root (EC₅₀ values)

Sample	EC ₅₀ (µg mL ⁻¹)*
Methanol extract	48.3 ± 0.002
Aqueous extract	> 1000
BHT	26 ± 0.005
BHA	53 ± 0.005
Ascorbic acid	11.24 ± 0.022

* Values represent mean ± S.D. of three replicates

Table 2: Peak table with Rf values, height and area of polyphenols and unknown compounds

Track	Peak	Rf	Height	Area	Assigned Substance
C	1	0.24	86.8	3242	unknown
C	2	0.3	91.9	3010.5	unknown
C	3	0.52	178.9	5266.6	Polyphenol
C	4	0.58	167.4	3998.1	Polyphenol
C	5	0.7	318.1	12169	Polyphenol

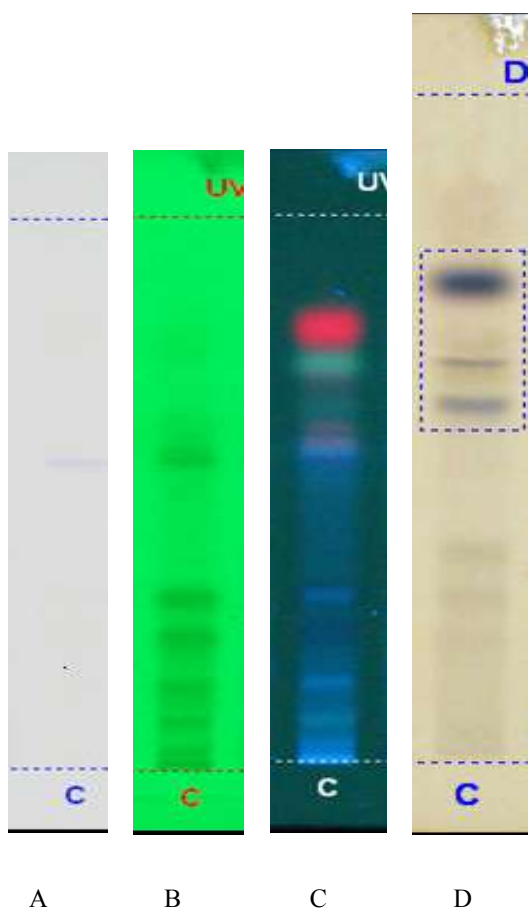


Fig. 1: Chromatograms of extract in HPTLC analysis (A) Before derivatization under day light, (B) Under UV 254 nm (C) Under 366 nm, (D) After derivatization under day light

HPTLC Fingerprinting Profile: HPTLC profile of methanol extract of *P. odoratissimus* root was recorded in Table 2. Blue, brown color zone was detected in UV after derivatization in the chromatogram. (Fig. 1,2.) confirms the presence of polyphenols. The extracts were run along with the standard polyphenols compounds. The root extract which shows the presence of polyphenols in the chromatogram as well as in UV after derivatization. The Rf value of the root extract was found to be 0.24, 0.3, 0.52, 0.58, 0.7 of peak 1, 2, 3, 4, 5 respectively. Among them peaks 3, 4, 5 were found as polyphenols. The peak height of the respective polyphenols was also given in the Table 2.

DPPH Radical Quenching Activity: The DPPH assay constitutes a quick and low cost method, which has frequently been used for the evaluation of the antioxidative potential of various natural products [14]. The methanol root extract exhibited a dose dependent higher radical quenching activity (Fig. 3.) with an EC₅₀ value of 48.3 ± 0.002 µg mL⁻¹. It is than the aqueous extract (>1000 µg mL⁻¹) and also higher than that of BHA (EC₅₀ = 53 ± 0.005 µg mL⁻¹). However, the activity was lesser than that of BHT (26 ± 0.005 µg mL⁻¹) and L-ascorbic acid (11.24 ± 0.022 µg mL⁻¹). The results of this study infer that the methanol extract reduces the radical to the corresponding hydrazine when it reacts with the hydrogen ions released from the samples which contain antioxidant principles (15).

Reducing Capacity: The reducing capacity of a compound may serve as a significant indicator of its

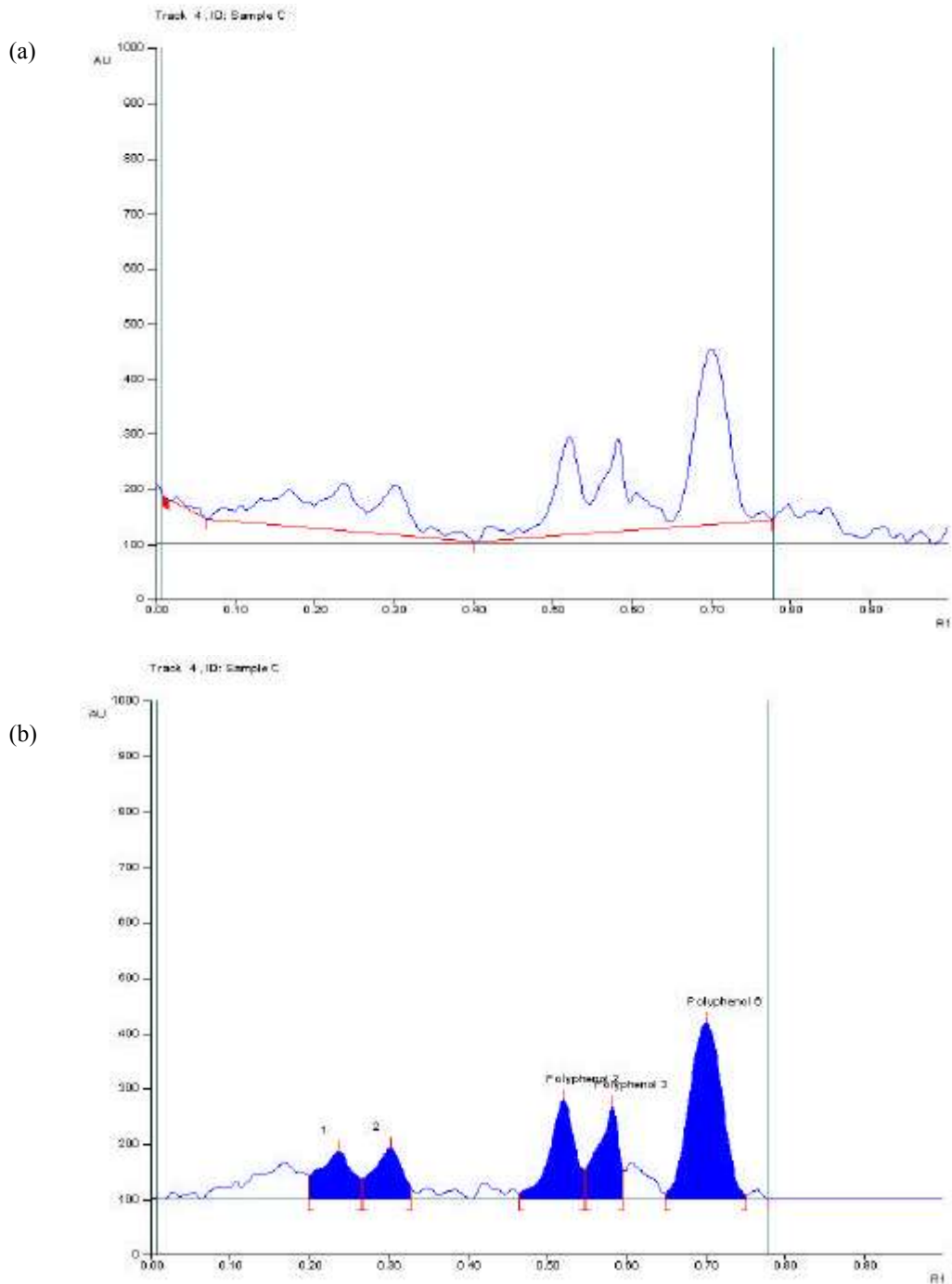


Fig. 2a,b: (a) Baseline display (Scanned at 500nm); (b) Peak densitogram display

potential antioxidant activity. The reduction of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) is measured by the intensity of the resultant blue-green solution which absorbs at 700 nm. As can be seen in Fig. 4 the extracts caused significant elevation of reducing power. The reducing power of methanol extract (0.787 at $1000 \mu\text{g mL}^{-1}$) was

relatively more pronounced than that of aqueous extract (0.324 at $1000 \mu\text{g mL}^{-1}$). The higher absorbance at high concentration indicates the strong reducing power potential. The figure also shows the reductive capabilities of the extracts compared with BHA (0.683 ± 0.008 at $1000 \mu\text{g mL}^{-1}$). The reducing power of the extract might

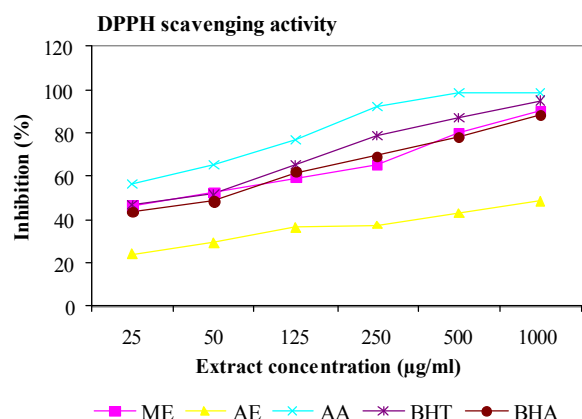


Fig. 3: DPPH radical scavenging activity of methanol and aqueous extracts of *P. odoratissimus* root; ME - methanol extract, AE - aqueous extract, AA - ascorbic acid, BHA - Butyl hydroxyl anisole, BHT - Butyl hydroxyl toluene

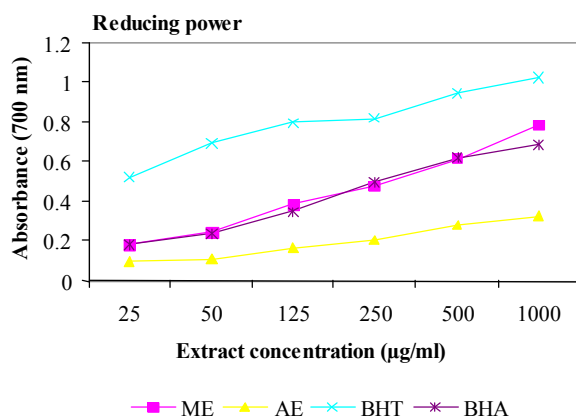


Fig. 4: Reducing capacity of methanol and aqueous extracts of *P. odoratissimus* root ME - methanol extract, AE - aqueous extract, BHA - Butyl hydroxyl anisole, BHT - Butyl hydroxyl toluene

be due to the di and mono hydroxyl substitutions in the aromatic ring which possess potent hydrogen donating abilities. The result revealed that the methanolic extract of *P. odoratissimus* possess a tendency to donate electrons and could react with free radicals to convert them into more stable products and to terminate radical chain reactions.

Correlations: All the findings from the experiments were used to find an association between TP, TF and antioxidant activities based on multiple regression analysis. The correlation matrix between DPPH radical scavenging activity ($R^2 = 0.897$) and TP was positive.

A linear correlation was also observed between DPPH activity ($R^2 = 0.988$) and TF. Significant correlations were observed between DPPH radical scavenging and reducing power, TP and TF. Many supportive reports emphasize the positive correlation between phenolic content and antioxidant efficacy [16, 17]. A positive correlation between antioxidant activity and polyphenol content was found, suggesting that the antioxidant capacity of the plant extracts is due to a great extent to their polyphenols [18, 19]. High positive correlations between antioxidant properties evaluated by all the assays and TP and TF indicate that the polyphenolic compounds are important contributors of antioxidant potential of *P. odoratissimus* root extract.

CONCLUSIONS

Recent years have seen an exponential increase in research antioxidant properties of medicinal plants. If it is accepted that higher intakes of natural antioxidants containing phenolics are associated with long term health benefits, then the results presented in this paper offer possible avenues toward health promotion by identifying those compounds. The health promoting properties of *P. odoratissimus* root may be due to its antioxidant properties and is also attributed to its multitherapeutic characteristics. Thus, *P. odoratissimus* might be useful in the development of raw materials of medicine. *In vivo* antioxidant study and toxicological tests on root of *P. odoratissimus* are warranted.

REFERENCES

- Halliwell, B., 1994. Free-radicals, antioxidant and human disease-curiosity, cause, or consequence. *Lancet*, 344: 721-724.
- Espin, J.C., C. Soler-Rivas and H.J. Wichers, 2000. *J. Agric. Food Chem.*, 48: 648-656.
- Prior, R.L., 2003. Fruits and vegetables in the prevention of cellular oxidative damage. *American J. Clinic. Nutrition*, 78: 570S-578S.
- Rice-Evans, C., N.J. Miller and G. Paganga, 1997. Antioxidant properties of phenolic compounds. *Trends in Plant Sciences*, 2: 152-159.
- Warrier, R.K., V.P.K. Nambiar and C. Ramankutty, 1995. *Indian Medicinal Plants: a compendium of 500 species: Orient Longman Ltd, Madras, India*
- Nandkarni, A.K., 1992. *Indian Materia Medica, Popular Prakashan, Bombay, India*, 1: 894-895.

7. Ting-Ting Jong and Shang-Whang Chau, 2000. Antioxidative activities of constituents Isolated from *Pandanus odoratissimus*. *Phytochemistry*, 40(7): 2145-2148.
8. Londonkar, R. and A. Kamble, 2009. Evaluation of free radical scavenging activity of *Pandanus odoratissimus*. *Intl. J. Pharmacol.*, (Citation not found).
9. Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen, 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.*, 47: 3954-3962.
10. Chang, C., M. Yang, H. Wen and J. Chern, 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food and Drug Analysis*, 10: 178-182.
11. Shah, C.R., B.N. Suhagia, N.J. Shah, D.R. Patel and N.M. Patel, 2008. Stability-indicating simultaneous HPTLC method for olanzapine and fluoxetine in combined tablet Dosage form. *Indian J. Pharmaceutical Sci.*, 70(2): 251-255.
12. Singh, R.P., K.N. Chidambara Murthy and G.K. Jayaprakash, 2002. Studies on antioxidant activity of pomegranate peel and seed extracts using *in vitro* models. *J. Agric. Food Chem.*, 50: 86-89.
13. Oyaizu, M., 1986. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. *Japan Journal of Nutrition*, 44: 307-315.
14. Molyneux, P., 2003. The use of the stable free radical diphenyl picryl hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol.*, 26(2): 219.
15. Sanchez-Mareno, C., 2002. Review: Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems. *Food Sci. Technol. Intl.*, 8: 121-137.
16. Kukic, J., S. Petrovic and M. Niketic, 2006. Antioxidant activity of four endemic *Stachys* taxa. *Biological and Pharmaceutical Bulletin*, 29(4): 725-729.
17. Canadanovic'-Brunet, J., G. Cetkovic', S. Djilas, V. Tumbas, G. Bogdanovic', A. Mandic', S. Markov, D. Cvetkovic', V. Canadanovic', 2008. Radical scavenging, antibacterial and antiproliferative activities of *Melissa officinalis* L. extracts. *Journal Medicinal Food*, 11(1): 133-143.
18. Ng, T.B., F. Liu and Z.T. Wang, 2000. Antioxidative activity of natural products from plants. *Life Sciences*, 66: 709-723.
19. Kiselova, Y., D. Ivanova, T. Chervenkov, D. Gerova, B. Galunska and T. Yankova, 2006. Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytotherapy Research*, 11: 961-965.