Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 1 pp. 23–29, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

LIPID PEROXIDATION INHIBITION AND ANTIRADICAL ACTIVITIES OF SOME LEAF FRACTIONS OF *MANGIFERA INDICA*

JELILI A. BADMUS[1]*, TEMITOPE O. ADEDOSU[1], JOHN O. FATOKI[1], VICTOR A. ADEGBITE[1], OLUWATOSIN A. ADARAMOYE[2] AND OYERONKE A. ODUNOLA[2]

[1]Department of Biochemistry, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria
[2]Department of Biochemistry, University of Ibadan, Ibadan, Nigeria

Abstract: This study was undertaken to assess *in vitro* lipid peroxidation inhibitions and anti-radical activities of methanolic, chloroform, ethyl acetate and water fractions of *Mangifera indica* leaf. Inhibition of Fe^{2+}-induced lipid peroxidation (LPO) in egg, brain, and liver homogenates, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl (OH^-) radical scavenging activities were evaluated. Total phenol was assessed in all fractions, and the reducing power of methanolic fraction was compared to gallic acid and ascorbic acid. The results showed that Fe^{2+} induced significant lipid peroxidation (LPO) in all the homogenates. Ethyl acetate fraction showed the highest percentage inhibition of LPO in both egg yolk (68.3%) and brain (66.3%), while the aqueous fraction exerted the highest inhibition in liver homogenate (89.1%) at a concentration of 10 μg/mL. These observed inhibitions of LPO by these fractions were higher than that of ascorbic acid used as a standard. The DPPH radical scavenging ability exhibited by ethyl acetate fraction was found to be the highest with IC_{50} value of 1.5 μg/mL. The ethyl acetate and methanolic fractions had the highest OH^- radical scavenging ability with the same IC_{50} value of 5 μg/mL. The total phenol content of ethyl acetate fraction was the highest with 0.127 μg/mg gallic acid equivalent (GAE). The reductive potential of methanolic fraction showed a concentration-dependent increase. This study showed that inhibition of LPO and the DPPH and OH^- radicals scavenging abilities of *Mangifera indica* leaf could be related to the presence of phenolic compounds. Therefore, the ethyl acetate fraction of the leaf may be a good source of natural antioxidative agent.

Keywords: lipid peroxidation, antiradical, *Mangifera indica,* radical, antioxidant agent

Lipid peroxidation is an accumulated effect of reactive oxygen species (ROS), which leads to deterioration of biological systems. It may be initiated by reactive free radicals, which subtract an allylic hydrogen atom from a methylene group of polyunsaturated fatty acid side chains. This is accompanied by bond rearrangement that results in stabilization by diene conjugate formation. The lipid radical then takes up oxygen to form peroxy species. (1).

Oxygen radicals and other reactive species are generated in biological systems either as by-products of oxygen reduction or by xenobiotic catabolism (2). These ROS such as superoxide anion (O_2^-), hydroxyl radicals (OH·), nitric oxide (NO) and peroxy radical (ROO·) are unstable and can attack key biomolecules such as lipids, proteins and nucleic acids (3). The consequences of oxidation of these biomolecules have been linked to a variety of different human disorders, including atherosclerosis, cancer and disease of the nervous system (4). Cells have a comprehensive array of antioxidant defense mechanisms to reduce free radical formation or limit their damaging effects (5). These mechanisms are not sufficient when the balance shifts to the side of free radicals generation (6), thus body requires antioxidant supplements to reduce oxidative damage and retard lipid peroxidation. Nowadays, the use of synthetic antioxidants is limited because of inherent toxicity associated with them at optimum concentration. The use of natural antioxidants of plant origin is receiving great attention. Phytochemical constituents of plants have been reported as scavengers of free radicals and inhibitors of lipid peroxidation (7). The leaf of *Mangifera indica* is used by traditional medicinal practitioners to treat a wide range of diseases. Its antiviral and antitumor (8–10) spasmolytic (11) antidiabetic (12, 13) and immunostimulating properties (14) have been established.

* Corresponding author: e-mail: jabadmus@gmail.com

Therefore, this study was designed to evaluate lipid peroxidation inhibitory potentials and antiradical activities of different fractions from the leaves of *Mangifera indica*

MATERIALS AND METHODS

Plant material

Fresh leaves of *Mangifera indica* were collected around the College of Health Sciences, LAUTECH, Ogbomoso and were authenticated by Dr. A.J. Ogunkunle, of the Department of Pure and Applied Biology, LAUTECH, Ogbomoso, Oyo state, Nigeria.

Chemicals

Ferrous sulfate, acetic acid, thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid (TCA), ferrous chloride, sodium acetate, Folin–Ciocalteu reagent, sodium carbonate, gallic acid, ascorbic acid, butanol, methanol, chloroform, ethyl acetate, *n*-hexane were procured from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade and purest quality available.

Extraction procedure

The powdered leaves of *Mangifera indica* (1 kg) were exhaustively extracted with 3 liters of 70% methanol. The methanolic filtrate was concentrated at room temperature to a dark brown mass. The concentrate (50 g) was subjected to solvent fractionation by first adding distilled water to make the concentrate to form a gel. This was followed by subsequent washing with *n*-hexane, chloroform and ethyl acetate. The obtained fractions (chloroform, ethyl acetate and aqueous) were separately concentrated under room temperature.

Preparation of tissues and egg yolk homogenates

Wistar strain albino rats were collected from the animal house of Department of Physiology, LAUTECH, Ogbomoso. They were decapitated under mild chloroform anesthesia and the cerebral tissue (whole brain) and liver were rapidly dissected, washed in cold washing buffer (1.15% KCl) to remove blood stain and then homogenized using Teflon head homogenizer in 0.15 M Tris-KCl buffer. Eggs from Teaching and Research Farm, LAUTECH, Ogbomoso were used for this study.

Inhibition of lipid peroxidation

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed using brain, liver and egg yolk homogenates as lipid-rich media, as described by Ruberto et al., (15). Briefly, 0.5 mL of brain homogenate (10% v/v) was added to 0.1 mL of the extract (10 μg/mL). The volume was then made up to 1.0 mL with distilled water. Thereafter, 0.05 mL of $FeSO_4$ was added and the mixture was incubated at 37°C for 30 min. Then, 1.5 mL of acetic acid was added, followed by 1.5 mL of TBA in SDS. The resulting mixture was vortex mixed and heated at 95°C for 1 hour. After cooling, 5 mL of butanol was added and the mixture was centrifuged at 3000 rpm for 10 min. The same procedure was repeated for liver and egg yolk homogenates. The absorbance of the organic upper layer was measured at 532 nm and the percentage inhibition was calculated with the formula:

$$\%\ \text{inhibition of lipid peroxidation} = \left(\frac{100 - A_{\text{sanple}}}{A_{\text{control}}}\right) \times 100$$

Hydroxyl radical ($OH^{\bullet}$) scavenging activities

All solutions were freshly prepared before the assay. One mL of the reaction mixture contained 100 μL of 2.8 mM 2-deoxyribose (dissolved in phosphate buffer (10 mM), pH 7.4), 500 μL solution of various concentrations of the extract (500–1000 μg/mL), 200 μL of 200 μM $FeCl_3$ and 1.04 μM EDTA (1:1 v/v), 100 μL of H_2O_2 (1.0 mM) and 100 μL of ascorbic acid (1.0 mM). After incubation period of 1 hour at 37°C, the extent of deoxyribose degradation was measured by TBA reaction (16). The % inhibition of hydroxyl radical was calculated and calculation of results was descripted above.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

DPPH free radical scavenging activity of the test solutions was determined using DPPH photometric method of Mensor et al. (17). When DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The change in color from deep violet to golden/light yellow can be measured at 518 nm. Briefly, 1 mL of 0.3 mM of DPPH solution was added to 1 mL each of the test solutions, and was incubated in the dark at room temperature for 30 min. The absorbance values were read at 518 nm, and converted into percentage antioxidant activity, using the formula below:

$$\text{DPPH scavenging effect (\%)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Total phenol content

This was estimated as described by McDonald et al. (18). The assay is based on the reduction of Folin–Ciocalteu reagent by the phenolic compounds. The reduced Folin–Ciocalteu reagent is blue and the absorbance was measured at 500–750 nm. Briefly, 1 mL each of the test solution was added to 0.2 mL each of Folin-Ciocalteu reagent and 2 mL of distilled water. One mL of 15% Na_2CO_3 was mixed with the solution. The solutions were incubated at 40°C for 30 min and the absorbance was read at 760 nm. Total phenol content was expressed as µg/mg of gallic acid equivalent (GAE).

Reductive potential

The method of Oyiazu (19) was employed for determination of the reducing power of fractions. One hundred fifty µL of various concentrations of the extract in 1 mL of distilled water was mixed with 2.5 mL each of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Two and a half mL of 10% trichloroacetic acid (TCA) was added and the mixture was centrifuged at 1000 × g for 10 min. Thereafter, 2.5 mL of the upper layer of solution was mixed with 2.5 mL of distilled water and 0.5 mL of 1% $FeCl_3$. The absorbance was read at 700 nm.

Statistical analysis

All the experimental results were the mean (n = 6) ± standard deviation (SD) of three parallel measurements of two independent experiments conducted on separate days using freshly prepared reagents. The results were analyzed statistically using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. The regression curve analysis was used to evaluate IC_{50} values using Graphpad prism version 5.02 statistical software. The IC_{50} value is a concentration of fraction required to scavenge 50% free radicals and is inversely proportional to the activity of fraction.

RESULTS

Inhibition of lipid peroxidation

Brain: The percentage inhibition of lipid peroxidation by methanolic and ethyl acetate fractions were significantly higher ($p < 0.01$) when compared with ascorbic acid (standard) as shown in Table 1. Chloroform and aqueous fractions showed no significant difference when compared with ascorbic acid lipid peroxide inhibition. Ethyl acetate fraction significantly inhibited Fe^{2+}-induced lipid peroxidation in brain ($p < 0.01$) when compared with chloroform and aqueous fractions. This result showed that ethyl acetate fraction inhibited lipid peroxidation better than any of the fractions and standard used.

Liver: Using liver homogenate as a medium of peroxidation, percentage lipid peroxidation inhibition by ascorbic acid was observed to be significantly ($p < 0.01$) higher compared with methanolic and chloroform fractions, while there was no significant difference between ascorbic acid and ethyl acetate fractions. Aqueous fraction showed significant lipid peroxidation inhibition ($p < 0.01$) as compared with ascorbic acid. Ethyl acetate inhibition of lipid peroxidation was significantly higher as compared with methanolic ($p < 0.05$) and chloroform ($p < 0.01$) fractions, while significantly lower inhibition was observed when compared with aqueous ($p < 0.01$) fraction. Percentage inhibition of lipid peroxide in liver homogenate by the fractions followed this order; aqueous > ascorbic acid = ethyl acetate > methanol > chloroform as presented in Table 1.

Table 1. Percentage inhibition of lipid peroxidation of leaf extracts of *Mangifera indica,* at 10 µg/mL, using brain, liver and egg yolk homogenates as media.

Media	Fractions				
	Methanol (%)	Chloroform (%)	Ethyl acetate (%)	Aqueous (%)	Ascorbic acid (%)
Brain	61.63 ± 1.20**	46.51 ± 1.33##	66.28 ± 2.56**	39.54 ± 2.11##	48.84 ± 2.21
Liver	58.96 ± 2.20**##	48.47 ± 2.22**##	71.57 ± 2.21##	89.08 ± 3.14	72.49 ± 3.12
Egg yolk	56.87 ± 2.34##	58.64 ± 1.76#	68.27 ± 3.12	43.60 ± 2.34**##	63.34 ± 2.65

Values are expressed as the mean of triplicate measurements ± standard deviation (SD). Significant at *$p < 0.01$ compared to ascorbic acid. Significant at #$p < 0.05$ and ##$p < 0.01$ compared to ethyl acetate.

Table 2: Percentage hydroxyl radical scavenging activities of *Mangifera indica* leaf fractions.

Concentration (µg/mL)	Methanol (%)	Ethyl acetate (%)	Chloroform (%)	Aqueous (%)
50.0	57.02 ± 0.043	56.93 ± 0.024	39.92 ± 0.225	52.21 ± 0.020
100.0	67.78 ± 0.006	62.11 ± 0.046	64.36 ± 0.024	60.31 ± 0.011
150.0	77.50 ± 0.010	68.95 ± 0.020	73.72 ± 0.026	74.03 ± 0.045
200.0	85.10 ± 0.001	75.34 ± 0.038	78.13 ± 0.054	79.34 ± 0.042
250.0	93.47 ± 0.006	83.66 ± 0.018	90.55 ± 0.110	82.23 ± 0.006

Values are expressed as the mean of triplicate measurements ± standard deviation (SD).

Table 3a: Percentage DPPH scavenging activities of *Mangifera indica* leaf fractions.

Concentration (µg/mL)	Methanol (%)	Ethyl acetate (%)	Chloroform (%)	Aqueous (%)
1.00	10.52 ± 0.021	19.30 ± 0.006	3.50 ± 0.000	5.26 ± 0.142
2.50	24.56 ± 0.015	66.67 ± 0.032	15.79 ± 0.006	33.33 ± 0.025
5.00	54.38 ± 0.032	84.21 ± 0.205	19.30 ± 0.035	52.63 ± 0.023
10.00	78.94 ± 0.021	89.47 ± 0.000	28.07 ± 0.020	89.47 ± 0.006
15.00	91.23 ± 0.000	92.98 ± 0.000	33.33 ± 0.023	92.98 ± 0.000

Values are expressed as the mean of triplicate measurements ± standard deviation (SD)

Table 3b: Percentage (%) DPPH radical scavenging effect (standard)

Concentration (µg/mL)	Vitamin C (%)	Gallic acid (%)
2.00	47.10 ± 0.23	43.28 ± 1.20
3.00	56.22 ± 1.22	53.73 ± 1.22
4.00	71.64 ± 1.33	56.73 ± 2.23
5.00	80.68 ± 1.12	65.17 ± 1.66

Table 4. Total phenol content of *Mangifera indica* leaf fractions expressed as µg/mg gallic acid equivalent (GAE)

Fractions (extract)	Total phenol content (µg/mg GAE)
Methanolic	0.106
Aqueous	0.111
Ethyl acetate	0.127
Chloroform	0.089

Egg yolk: As presented in Table 1, ascorbic acid significantly showed higher lipid peroxidation inhibition compared with aqueous fraction only ($p < 0.01$) in egg yolk, while no significant difference was observed when compared with methanolic, chloroform and ethyl acetate fractions. Ethyl acetate fraction showed significant higher inhibition when compared with methanolic ($p < 0.01$), chloroform ($p < 0.05$) and aqueous ($p < 0.01$) fractions.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging ability calculated as IC_{50} from Table 2 reveals that methanolic, ethyl acetate, aqueous and chloroform fractions have IC_{50} values of 5 µg/mL, 5 µg/mL, 26 µg/mL and 66 µg/mL, respectively. These results implied that methanolic and ethyl acetate fractions have the highest $OH^{\bullet}$ radical scavenging abilities followed by aqueous and chloroform fractions.

DPPH scavenging activity

DPPH scavenging ability calculated as IC_{50} from Tables 3a and 3b shows that ethyl acetate has IC_{50} of 1.5 µg/mL followed by ascorbic acid (2.32 µg/mL), gallic acid (2.83 µg/mL), aqueous (6.0 µg/mL), methanolic (6.5 µg/mL) and chloroform (22.5 µg/mL) fractions. The result revealed that ethyl acetate fraction has the highest DPPH scavenging ability.

Total phenolic content

Table 4 shows that ethyl acetate fraction has the highest total phenolic content (0.127 µg/mg GAE) followed by aqueous, methanolic and chloro-

Table 5. Reducing power of methanolic fraction of *Mangifera indica* leaves compared to vitamin C and gallic acid.

Concentration	2.2 μg/mL	4 μg/mL	5.3 μg/mL	6.7 μg/mL
Methanolic extract	0.030 ± 0.001	0.049 ± 0.005	0.067 ± 0.008	0.095 ± 0.004
Vitamin C	0.965 ± 0.043	1.102 ± 0.002	1.119 ± 0.006	1.266 ± 0.013
Gallic acid	0.214 ± 0.004	0.345 ± 0.009	0.629 ± 0.029	0.867 ± 0.043

form fractions with 0.111, 0.106 and 0.089 μg/mg gallic acid equivalent (GAE), respectively.

Reducing power

Table 5 shows reductive power of methanolic fraction as compared with ascorbic acid and gallic acid. The reducing power of methanolic fraction of *Mangifera indica* leaf was found to increase with increasing concentration of methanolic fraction which is comparable with used standards (ascorbic acid and gallic acid).

DISCUSSION

Many reports support the use of antioxidant supplements in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases (20). The process of lipid peroxidation has been suggested to proceed *via* a free radical chain reaction (21), which has been associated with cell damage in biomembranes (22). The damage has been shown to precipitate different diseases like cancer, cardiovascular diseases and diabetes. Incubation of brain, liver and egg yolk homogenates in the presence of $FeSO_4$ causes a significant increase in lipid peroxidation. The abilities of the fractions of *Mangifera indica* to inhibit the process of lipid peroxidation were tested using the method of Ruberto et al. (15). The ethyl acetate fraction exhibited the highest inhibition of lipid peroxidation in brain homogenate with 66.3%, followed by methanol (61.6%), chloroform (46.5%) and aqueous (39.5%) fractions. High percentage inhibition of lipid peroxidation was observed in aqueous fraction with 89.1% in liver homogenate followed by ethyl acetate fraction (71.6%), methanol fraction (59.0%) and chloroform fraction (48.5%). The percentage inhibition of lipid peroxidation induced by ethyl acetate fraction was the highest in egg homogenate (68.3%), followed by chloroform (58.6%), methanol (56.9%) and aqueous fractions (43.6%). The activities of ethyl acetate (in brain and egg yolk homogenates) and aqueous (in liver homogenate) fractions in inhibiting lipid peroxidation were higher compared to ascorbic acid (48.8%, 63.3% and 72.5% in brain, egg yolk and liver homogenates, respectively). The relatively high inhibition of lipid peroxidation at 10 μg/mL observed in liver homogenates by all fractions compared to that of brain and egg yolk homogenates could be attributed to the presence of glutathione and other antioxidants in hepatic cells, while brain cells rely on surrounding astrocyte cells to provide usable glutathione precursors and also limited access to the bulk of antioxidants produced by the body (23). High lipid content and lower endogenous antioxidant components could also be ascribed to lower lipid peroxidation inhibition exhibited by all fractions in egg homogenate. Interestingly, aqueous fraction exhibited the lowest inhibitory activities in both brain and egg homogenates and the highest activity in the liver. This could probably be due to the fact that aqueous fraction performs better in the presence of some enzymic antioxidants in hepatic cells. This implies that aqueous fraction reinforced hepatic endogenous antioxidants better than other fractions. High lipid peroxidation inhibitions showed by ethyl acetate in all the media could be related to the presence of phenolic compound, which has been shown to be correlated to the antioxidant activity of natural plant product (6).

Hydroxyl radicals are considered to be one of the rapid initiators of lipid peroxidation process, abstracting hydrogen atoms from polyunsaturated fatty acid, which brings about peroxidic reactions of membrane lipids (24) and also, from each of the carbon atom of the sugar moiety of DNA causing oxidative damage to DNA. These effects have been implicated in mutagenesis, carcinogenesis and aging (3). Ferric-EDTA incubated with H_2O_2 and ascorbic acid at pH 7.4, produces hydroxyl radicals and was detected by their ability to degrade 2-deoxyribose into fragments, on heating with TBA at low pH forming a pink chromogen (16, 25). Addition of various fractions of *Mangifera indica* caused the removal of hydroxyl radicals and prevented the degradation of 2-deoxyribose. Fractions of *Mangifera indica* were found to exhibit a concentra-

tion-dependent hydroxyl radical scavenging activities indicated as percentage inhibition in Table 2. The observed IC_{50} values of methanol, ethyl acetate, chloroform and aqueous fractions were 5 μg/mL, 5 μg/mL, 66 μg/mL, and 26 μg/mL, respectively. The highest activity was observed in both methanol and ethyl acetate, followed by aqueous and chloroform fractions. The result showed that various fractions of *Mangifera indica* are powerful scavengers of OH^- radical and could therefore prevent $OH^{\bullet}$ radical related pathophysiological diseases.

DPPH is a free radical stable at room temperature, and produces a purple color solution in methanol. It is reduced in the presence of antioxidant molecule, giving rise to a yellowish methanol solution. One of the mechanisms involved in antioxidant activity assay is the ability of a molecule to donate a hydrogen atom to a radical, and the propensity of the hydrogen donation is the critical factor involved in free radical scavenging (26). The DPPH radical scavenging activities of fractions of *Mangifera indica* (Table 3a) showed that the ethyl acetate fraction had the highest activity (IC_{50} = 1.5 μg/mL), followed by aqueous (IC_{50} = 6.0 μg/mL), methanol (IC_{50} = 6.5 μg/mL), and chloroform (IC_{50} = 22.5 μg/mL) fractions. Ethyl acetate fraction had higher activity compared to ascorbic acid (IC_{50} = 2.32 μg/mL) and gallic acid (IC_{50} = 2.83 μg/mL).

Phenolic compounds may contribute directly to antioxidative action (6). They are regarded to be the most important antioxidative components of plants; hence correlations between the concentrations of plant phenolics and the total antioxidant capabilities have been reported (27). The results in Table 4 show the phenolic contents of *Mangifera indica* expressed as μg/mg gallic acid equivalent. The ethyl acetate fraction had the highest total phenolic contents (0.127 μg/mg GAE), followed by aqueous, methanolic and chloroform fractions (0.111, 0.106 and 0.089 μg/mg GAE, respectively). Phenolic content of ethyl acetate fraction corresponds to its lipid peroxidation inhibition, hydroxyl radical and DPPH radical scavenging activities. This supports earlier reports, correlating the presence of phenolic compounds to antioxidative actions (6, 27).

The reducing power of a compound is related to its electron transfer ability, and may therefore served as a significant indicator of its potential antioxidant activity (28). For the measurements of the reductive ability, the Fe^{3+} to Fe^{2+} transformation in the presence of methanol fraction of *Mangifera indica* leaves was investigated. The reductive capability of methanol fraction of *Mangifera indica* (Table 5) was compared to ascorbic acid and gallic acid (standards). The reducing power increased with increasing amount of extract but at a lower rate compared to the standards. This implies that H ion donating potential of the methanolic extract is lower compared to that of the standards.

CONCLUSION

Involvement of free radicals appears to be the feature of most human diseases (29). Therefore, the inhibition of lipid peroxidation and radical scavenging power of plants might be important in fighting diseases by conferring protection against free radical damage to cellular DNA, lipids, and proteins. The results from this work showed that *Mangifera indica* fractions have potentials as free radical scavengers. Further studies to isolate and characterize components responsible for the observed activities are necessary.

REFERENCES

1. Dzingiral B., Muchuweti M., Murenje T., Chidewe C., Benhura M.A.N., Chagonda L.S.: African J. Biochem. Res. 1, 137 (2007).
2. Chance B., Sies H., Boveris A.: Physiol. Rev. 59, 527 (1979)
3. Halliwell B., Gutteridge J.M.C.: Free radicals in biology and medicine, 3rd edn., Oxford University Press, Oxford 1999.
4. Cross C.E., Halliwell B., Borish E.T., Pryor W.A., Ames B.N., Harman D.: Ann. Int. Med. 107, 526 (1987)
5. Sato M., Ramarathnam N., Suzuki Y., Ohkubo T., Takeuchi M., Ochi H.: J. Agric. Food Chem. 44, 37 (1996).
6. Gülcin I., Buyukokuroglu M E., Oktay M., Küfrevioglu O I.: J. Pineal Res. 33, 167 (2002).
7. Beutner S., Bloedom B., Frixel S., Hernandez Blanco I., Hoffmann T., Martin H-D., Mayer B. et al.: J. Sci. Food Agric. 81, 559 (2001).
8. Guha S., Ghosal S., Chattopadhyay U.: Chemotherapy 42, 443 (1996)
9. Ngo T.: Sci. Technol Publ. (Vietnam) 563, (2001).
10. Zhu X., Song J., Huang Z., Wu Y., Yu M.: Zhongguo Yao Li Xue Bao (China) 14, 452 (1993).
11. Tona L., Kambu K., Ngimbi N., Mesia K., Penge O., Lusakibaza M., Cimanga K. et al.: Phytomedicine 7, 31 (2000).
12. Ichiki H., Miura T., Kubo K., Ishihara E., Komatsu Y., Tanigawa M., Okada M.: Biol. Pharm. Bull. 21, 1389 (1998).

13. Miura T., Ichiki H., Hashimoto I., Iwamoto N., Kato M., Kubo M., Ishihara E. et al.: Phytomedicine 8, 85 (2001).
14. Garcia D., Leiro J., Delgado R., Sanmartin M L., Ubeira F M.: Phytother. Res. 17, 1182 (2003).
15. Ruberto G., Baratta M T., Deans S G., Dorman H J.: Planta Med. 66, 687 (2000).
16. Halliwell B., Gutteridge J., Aruoma O.: Anal. Biochem. 165, 215 (1987).
17. Mensor L.L., Menezes F.S., Leitao G.G., Reis A.S., dos Santos T.C., Coube C.S., Leitao S.G.: Phytother. Res. 15, 127 (2001).
18. Mcdonald S., Prenzler P D., Autolovic M., Robards K.: Food Chem. 73, 73 (2001).
19. Oyaizu M.: Jpn. J. Nutr. 44, 307 (1986).
20. Rose. G., Hamilton P.J.S., Colwell L., Shipley M.J.: J Epidemiol. Commun. Health 36, 102 (1982).
21. Halliwell B.: Free Radic. Biol. Med. 7, 645 (1989).
22. Usuki R., Endo Y., Kaneda T.: J Jpn. Soc. Food Sci. Technol. 28, 583 (1981).
23. Perry S.W., Norman J.P., Litzburg A., Gelbard H.A.: J. Neurosci. Res. 78, 485 (2004).
24. Kitada M., Igarashi K., Hirose S., Kitagawa H.: Biochem. Biophys. Res. Commun. 87, 388 (1979).
25. Aruoma O., Laughton M., Halliwell B.: Biochem. J. 264, 863 (1989).
26. Miliauskas G., Venskutonis P.R., van Beek T.A.: Food Chem. 85, 231 (2004).
27. Moskovitz J., Yim K.A., Choke P.B.: Arch. Biochem. Biophys. 397, 354 (2002).
28. Sanchez-Moreno C.: Food Sci. Technol. Int. 8, 121 (2002).
29. Deighton N., Brennan R., Finn C., Davies H V.: J. Sci. Food Agric. 80, 1307 (2000).

Received: 12. 01. 2010