IN VITRO ANTIOXIDANT ACTIVITIES OF *Aloe vera* LEAF SKIN EXTRACTS

Sonia Miladi, Mohamed Damak *

Laboratoire de Chimie des Substances Naturelles, Faculté des Sciences de Sfax, BP 1171, 3000 Sfax, Tunisie

(Reçu le 12 Octobre 2007, accepté le 5 Juin 2008)

ABSTRACT: The ethanolic extract of *Aloe vera* leaf skin was fractionated by liquid-liquid partition using hexane, ethyl acetate, chloroform-ethanol and butanol. The total phenolic content of the four different fractions were determined by Folin-Ciocalteu method and their antioxidant activity was essayed through some in vitro models such as the antioxidant capacity by phosphomolybdenum method, β-carotene bleaching method, radical scavenging activity using 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay and reducing power assay. The chloroform-ethanol fraction showed the highest total phenolics (40.500 ± 0.041 µg gallic acid equivalents/g of extract), the highest scavenging activity and the greatest reducing power, followed by ethyl acetate, butanol and hexane extracts. However, the hexane fraction showed the highest antioxidant capacity (471.300 ± 0.013) and the highest antioxidant activity coefficient (AAC) by the β-carotene bleaching method.

Keywords: Antioxidant activity, *Aloe vera* leaf skin, total phenols, DPPH, Reducing power, β-carotene.

1. INTRODUCTION

Many diseases are caused by oxidative stress. Accelerated cell oxidation contributes to cardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease, and even a decline in energy and endurance [1-4]. Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effect by scavenging reactive oxygen species, activating a buttery of detoxifying proteins or preventing the generation of reactive oxygen species [5-7]. In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases [8, 9]. Natural antioxidants such as α-tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer adverse reactions but their antioxidant activities are lower than the synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) which have been restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens [10-12]. Therefore, there is a considerable interest in finding new and safe antioxidants from natural sources to replace these synthetic antioxidants [13, 14].

Recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids [15] which prevent free radical damage, reducing risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular disease [16]. The search for newer natural antioxidants, especially of plant origin has ever since increased.

* corresponding author, e-mail: mohamed.damakl@fss.rnu.tn , tél.: +216 74274600 , fax: +216 74274437

Aloe vera L. (syn.: *Aloe barbadensis* Miller) is a perennial succulent plant belonging to the Aloeaceae family (sub-family of the Asphodelaceae) [17]. From 400 *Aloe* species, *A. vera* is most widely accepted and used for various medical and cosmetic purposes [18-20]. The plant is made of turgid green leaves joined at the stem in a rosette pattern. Each leaf consists of two parts: an outer green rind (skin) and an inner clear pulp (gel). The plant contains a large amount of phenolic compounds [18, 21-26]. It also has a high content of 1,8-dihydroxyanthraquinone derivatives (aloe emodin) and their glycosides (aloins), which are used as cathartic [27-29]. Various studies have revealed that *Aloe vera* leaf skin *(AVLS*) possesses many pharmaceutical activities, including purgative [30], antibacterial [31, 32], anticancer [33-35], antifungal [36] and antioxidant [37-41].

The objective of our research work was to investigate the total phenolic content and the antioxidant properties of different fractions of the ethanol extract from *AVLS* by various methods including reducing power, total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and the β-carotene linoleate model system.

2. MATERIALS AND METHODS

2.1. Experimental materials

Mature fresh leaves of *Aloe vera* plant (Aloaceae) with an approximate length of 0.5-0.7 m were supplied in August 2006 by Mr. Abdallah Sakli Msakni (Chemist Biologist in Kairouan, Tunisia).

The compounds, 2,2-Diphenyl-1-picrylhydrasyl (DPPH), linoleic acid, polyoxyethylene sorbitan monopalmitate (tween 40), potassium phosphate, β-carotene, BHT (butylated hydroxytoluene), α-tocopherol and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Potassium ferricyanide, ferric chloride and Folin-Ciocalteu phenol reagent were purchased from Merck. Other reagents were of analytical grade. Visible spectra measurements were done using Anadéo visible spectrophotometer (Anadéo-Bibby).

2.2. Preparation of extracts

Fresh *Aloe vera* leaf skin (3 kg) was washed with distilled water and was extracted with ethanol by maceration at room temperature for 48 hours. After filtration, the ethanol was evaporated under reduced pressure to yield the ethanolic extract (79.80 g). This extract was dissolved in water, kept at 4°C for 12 hours and filtered again thus obtaining the aqueous extract (AE). This AE was then partitioned successively with hexane, ethyl acetate, chloroform-ethanol (2:1, v/v) and butanol, yielding respectively the hexane (5.11 %), ethyl acetate (11.21 %), chloroform-ethanol (6.01 %) and butanol (8.58 %) fractions.

2.3. Determination of the total phenolic content

The total phenolic content (TPC) of the *Aloe vera* leaf skin fractions were estimated by a colorimetric assay, according to the method described by Singleton and Rossi (1965) with some modifications [42]. Briefly, 1 ml of sample (1mg/ml) was mixed with 1 ml of Folin-Ciocalteu reagent. After 3 min, 1 ml of saturated Na_2CO_3 solution was added to the mixture folled by the addition of 7 ml of distilled water. The mixture was kept in the dark for 90 min, after which the absorbance read at 725 nm. The total phenol contents (TPC) was determined using a standard curve prepared with gallic acid. The estimation of the phenolic compounds was carried out in triplicate. The results were mean ± standard deviations and expressed as milligram of gallic acid equivalent/g of extract.

2.4. Determination of total antioxidant capacity

The total antioxidant capacity (TAOC) of hexane, ethyl acetate, chloroform-ethanol (2-1, v/v) and butanol fractions of *AVLS* was evaluated by the method of Prieto et al. [43]. An aliquot of 0.1 ml of sample solution (1 mg/ml) was combined with 1 ml of reagent solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions. The antioxidant capacity was expressed as the number of equivalents of α-tocopherol (µg/g of extract).

2.5. Reducing power assay

The reducing power was determined according to the method of Oyaizu [44]. Various concentrations of *AVLS* fractions (1 ml) were mixed with 1 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After 1 ml of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 2000 rpm for 10 min. The upper layer solution (2.5 ml) was mixed with 2.5 ml of deionised water and 0.5 ml of fresh ferric chloride (0.1%). The absorbance was measured at 700 nm: a higher absorbance indicates a higher reducing power.

2.6. DPPH radical scavenging assay

The antioxidant activity of the *AVLS* was measured in terms of hydrogen-donating or radical scavenging ability, using the DPPH method [45-47] with a minor modification. Briefly, 1.5 ml of DPPH solution (10^{-4} M, in 95% Ethanol) was incubated with 1.5 ml of *AVLS* fractions at various concentrations (0.01-1 mg). The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. The control was prepared as above without any extract. The absorbance of the solution was measured at 517 nm against a blank. The radical scavenging activity (RSA) was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left(1 - \frac{A_{\text{Sample(517nm)}}}{A_{\text{Control (517nm)}}}\right) \times 100$$

The assays were carried out in triplicate and the results were expressed as mean values ± standard deviation. The extract concentration providing 50% inhibition (EC_{50}%) was calculated from the graph of scavenging effect percentage against the extract concentration. α-tocopherol and BHT were used as standards.

2.7. Antioxidant assay using the β-carotene bleaching method

The oxidative losses of β-carotene/linoleic acid emulsion were used to assess the anti-oxidation ability of the *AVLS* fractions [48, 49]. One milligram of β-carotene was dissolved in 10 ml of chloroform and 1 ml β-carotene solution was mixed with 20 mg of linoleic acid and 200 mg of Tween 40 emulsifier in a round-bottom flask. The chloroform was removed by nitrogen gas and 50 ml of oxygenated distilled water was added slowly to the semi-solid residue with a vigorous shaking, to form an emulsion. An absorbance at 470 nm was immediately recorded after adding 2 ml of the sample to the emulsion, which was regarded as t = 0 min. The round-bottom flasks were capped and placed in an incubator at 50°C. The absorbance at 470 nm was determined every 15 min until 120 min. A second emulsion, consisting of 20 mg of linoleic acid and 200 mg of Tween 40

and 50 ml of oxygenated water, was also prepared and served as blank to zero the spectrophotometer.
All determinations were carried out in triplicate. The antioxidant activity coefficient (AAC) was calculated according to the following equation:

$$AAC = [(A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)})] \times 1000$$

Where $A_{A(120)}$ is the absorbance of the antioxidant at 120 min, $A_{C(120)}$ is the absorbance of the control at 120 min and $A_{C(0)}$ is the absorbance of the control at 0 min .
The assays were run in triplicate and the results expressed as mean values ± standard deviation. The AAC was calculated from the graph of absorbance against time. BHT and α-tocopherol were used as standards.

2.8. Statistical analysis

Experimental results were mean value ± SD of three parallel measurements. All statistical analysis was conducted using Microsoft Excel. Differences among treatments were determined using student's *t*-test. Differences at $P<0.05$ were considered to be significant.

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity and phenols content

The antioxidant activity of plant materials closely correlated with the content of their phenolic compounds [50]. Table I shows the phenolic concentration in the *AVLS* fractions, expressed as milligram of gallic acid equivalents (GAEs) per gram of extract.

Among the four fractions, the chloroform-ethanol fractions showed the highest amount of phenolic compounds (40.500 ± 0.041 mg/g) followed by the ethyl acetate extract (23.800 ± 0.058 mg/g), the butanol extract (16.900 ± 0.039 mg/g) and the hexane extract (9.600 ± 0.014 mg/g).

3.2. Total antioxidant capacity

The antioxidant capacity of the fractions was measured spectrophotometrically through phosphomolybdenum method, which was based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of fruit extracts of *AVLS* was found to decrease in this order: hexane extract > ethyl acetate extract > chloroform-ethanol extract > butanol extract (Table I).

Table I: Total phenolic content (TPC) and total antioxidant capacity (TAOC) in *AVLS* fractions

Samples	**TPC** (µg gallic acid equivalent /g of extract)	**TAOC** (µg d'α-tocopherol/g of extract)
Hexane fraction	9.600 ± 0.014	471.300 ± 0.013
Ethyl acetate fraction	23.800 ± 0.058	420.700 ± 0.010
Chloroform-ethanol fraction	40.500 ± 0.041	289.600 ± 0.014
Butanol fraction	16.900 ± 0.039	195.400 ± 0.005

The results are the average of three determination ± standard deviation.

3.3. Reducing power assay

Figure 1 shows the reducing power of the *AVLS* fractions and BHT, as a function of their concentrations. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, we can monitor the Fe^{2+} concentration. The reducing properties are generally associated with the presence of reductones [51], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [52].

The reducing power of *AVLS* fractions increased and correlated well with the increasing concentration. However, as anticipated, the reduction power of BHT was relatively more pronounced than that of *AVLS*. The reducing powers for the different fractions were in the following order: chloroform-ethanol > ethyl acetate > butanol > hexane fraction.

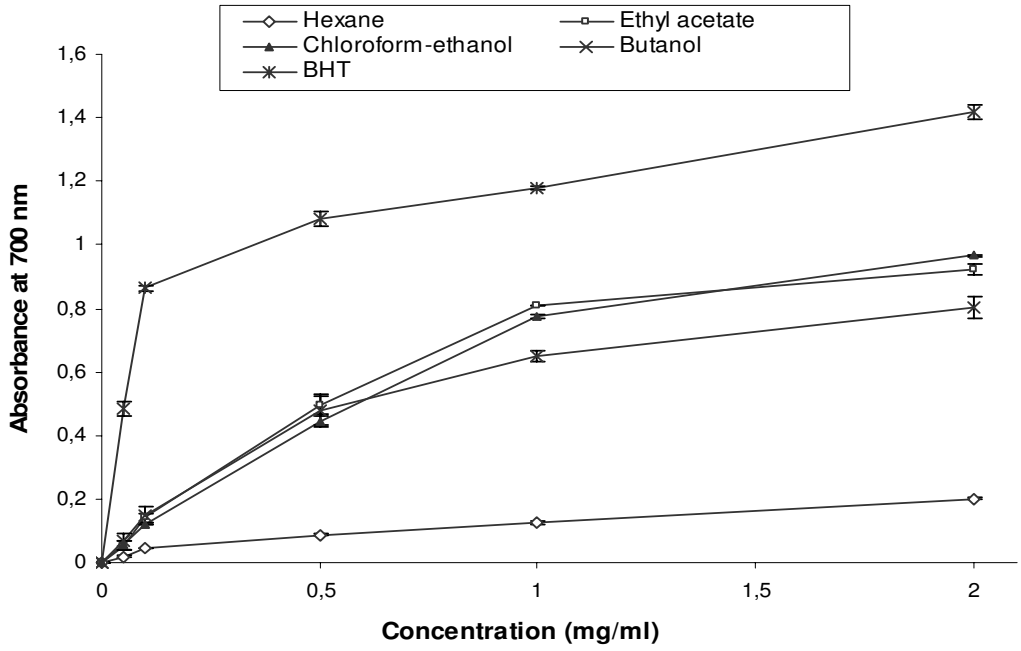

Figure 1: Reducing powers of *AVLS* fractions (higher absorbance indicates higher reducing power). Results are mean ± SD of three parallel measurements.

3.4. DPPH radical scavenging activity (RSA) assay

The RSA of the *AVLS* fractions was evaluated using an ethanolic solution of the stable free radical, DPPH. A freshly prepared DPPH solution exhibited a deep purple color with a maximum absorption at 517 nm. This purple color disappears when an antioxidant is present in the medium. Thus, antioxidants molecules can quench DPPH free radicals and convert them to a colorless product, resulting in a decrease in absorbance at 517 nm.

The RSA values of hexane, ethyl acetate, chloroform-ethanol and butanol fractions are presented in Figure 2; results are expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of an extract at 517 nm. RSA % was proportional to

the concentration of the extract. EC_{50} values (concentration of sample required to scavenge 50% of free radicals) of *AVLS* fractions, α-tocopherol and BHT are indicated in Table II.

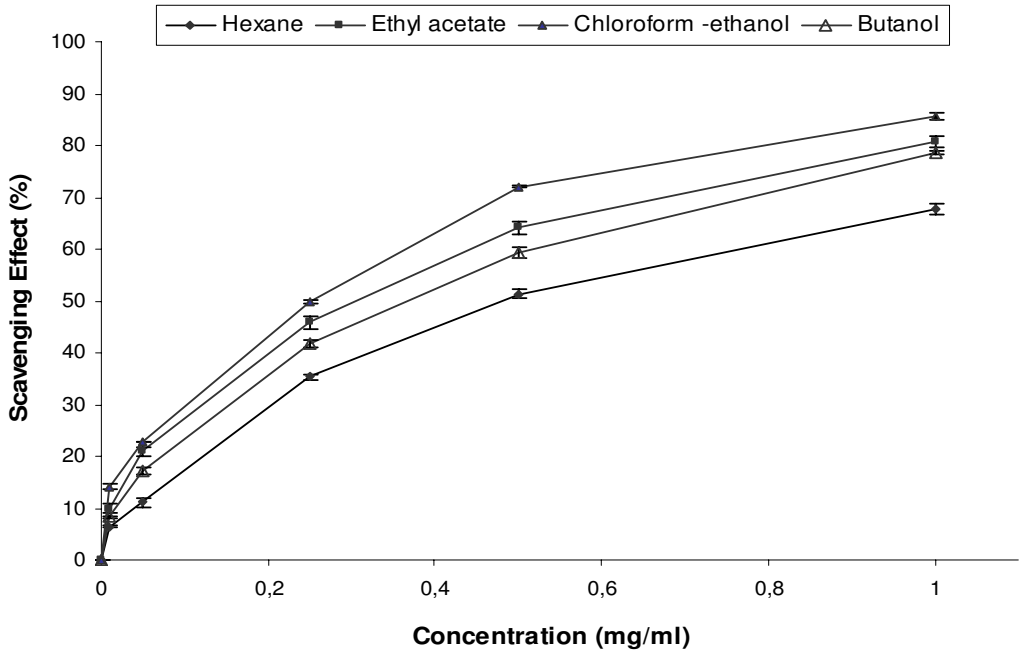

Figure 2: Scavenging activity (%) on DPPH radicals of *AVLS* fractions. Results are mean ± SD of three parallel measurements.

Among the four fractions the chloroform-ethanol fractions has the greater scavenging effect. From the analyses of Figure 2, we can conclude that the scavenging effects of leaf skin extract increase as the concentration increases. Chloroform-ethanol extract that contained the highest amount of total phenolics was found to be the most active radical scavenger followed by the ethyl acetate, butanol and hexane extracts. However the chloroform-ethanol extract was not as effective as the positive controls, BHT and α-tocopherol.

Table II: Comparison of antioxidant properties of *AVLS* fractions BHT and α-tocopherol

Sample	EC_{50} (µg/ml)	AAC
Hexane fraction	406.8	681
Ethyl acetate fraction	335.5	566
Chloroform-ethanol fraction	254.2	143
Butanol fraction	361.0	349
BHT	68.9	619
α-tocopherol	7.5	646

3.5. Antioxidant assay using the β-carotene bleaching method

The antioxidant activities of the *AVLS* fractions by the β-carotene bleaching method, in which the oxidation of linoleic acid takes place. Linoleic acid hydroperoxides attack the β-carotene

molecule and as a result, it undergoes a rapid decolorization. The corresponding decrease in absorbance can be monitored spectrophotometrically. The presence of antioxidant extracts can hinder the extent of β-carotene bleaching by acting on the linoleate-free radical and other free radicals formed in the system [54]. Accordingly, the absorbances decreased rapidly in samples without antioxidant whereas, in the presence of an antioxidant, they retained their color and thus absorbance, for a longer time. The absorbance of the emulsion decreased with time (Figure 3). *AVLS* fractions, BHT and α-tocopherol showed a variant anti-oxidation activity. The decreasing rate of absorbance for an emulsion sample with the added of hexane and ethyl acetate fractions was significantly lower than the samples with the addition of other fractions. The order of AAC at 2 mg/ml among the four fractions of *AVLS* was as follows: hexane > ethyl acetate > butanol > chloroform-ethanol. The AAC are summarized in table II.

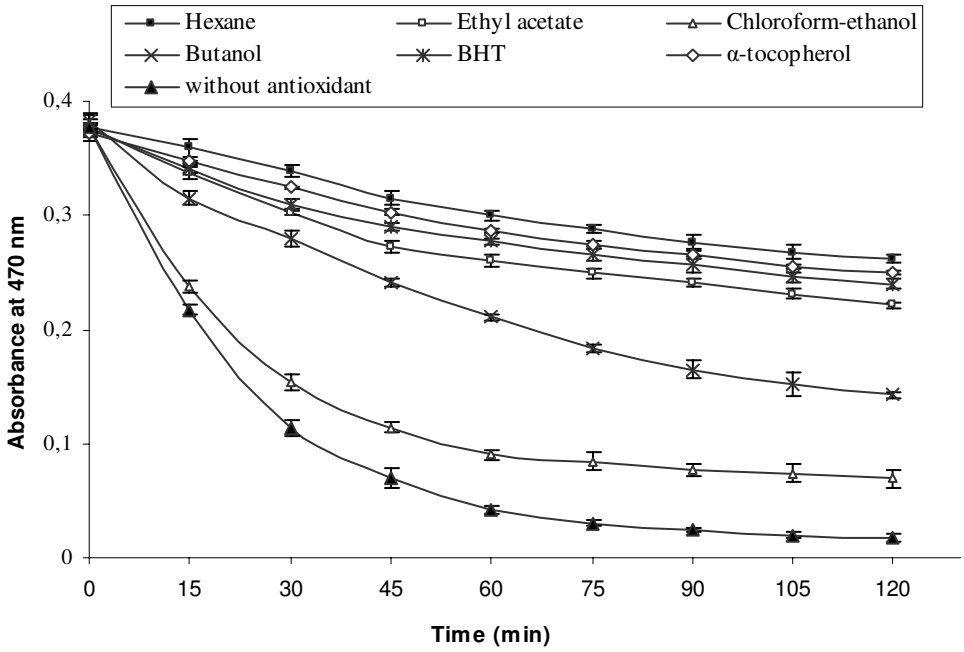

Figure 3: Change of absorbance at 470 nm with time for *AVLS* fractions (2mg/ml) in β-carotene / linoleic acid emulsion. Results are mean ± SD of three parallel measurements.

3.6. Conclusion

The chloroform-ethanol fraction of the ethanol crude extract of *AVLS* showed the highest antioxidant activity when evaluated by the DPPH and reducing power method compared with the other fractions. Whereas the hexane extract showed the highest antioxidant activity when determined by the phosphomolybdenum and the β-carotene-bleaching methods.
Research is in progressing to isolate and identify the antioxidant compoments in the chloroform-ethanol fraction.

Acknowledgement

The authors wish to thank Mr. Abdallah Sakli Msekni (Chemist Biologist in Kairouan-Tunisia) for supplying Aloe *vera* leaves.

References

[1] T. Finkel, N.J. Holbrook, *Nature* **2000**, *408*, 239.

[2] D. C. Radisky, D. D. Levy, L. E. Littlepage, E. L. Godden, D. G. Albertson, M. A. Nieto, Z. Werb, M. J. Bissel, *Nature*, **2005,** *436*, 123.

[3] N. Houstis, E. D. Rosen, and E. S. Lander, *Nature,* **2006**, *440*, 944.

[4] F. Fresquet, F. Pourageaud, V. Leblais, R. P. Brandes, J. P. Savineau, R. Marthan, B. Muller, *Brit. J. Pharmacol.*, **2006,** *148*, 714.

[5] B. Halliwell, J. M. C. Gutteridge, C. E. Cross, *J. Lab. Clin. Med.,* **1992,** 119, 598.

[6] B. Halliwell, *Annu. Rev. Nutr***., 1997**, *16*, 33.

[7] B. Halliwell, J. M. C. Gutteridge, Free radicals in biology and medicine. United Kingdom: Oxford University Press, **1999**.

[8] C. Kaur, H. C. Kapoor, *J. Food Sci. Tech.*, **2001**, *36*, 703.

[9] J. E. Kinsella, E. Frankel, B. German, J. Kanner, *Food Tech.*, **1993**, 4, 85.

[10] K. Imaida, S. Fukushima, T. Shirui, M. Ohtani, K. Nakanishi, N. Ito, *Carcinogenesis*, **1983**, *4*, 895.

[11] D. L. Madhavi, D. K. Salunkhe, In D. L. Madhavi, S. S. Deshpande, D. K. Salunkhe, Editors, *Food antioxidants*, Dekker, New York,**1996**, 239.

[12] M. Hirose, Y. Takesada, H. Tanaka, S. Tamano, T. Kato, T. Shirai, *Carcinogenesis*, **1998**, *19*, 207.

[13] G. Gazzani, A. Papetti, G. Masoolini, M. Daglia, *Food Chem.*, **1998**, *6*, 4118.

[14] M. Namiki, *Crit. Rev. Food Sci. and Nutr.*, **1990**, *20,* 273.

[15] A. T. Diplock, J. L. Charleux, G. Crozier-willi, F. J. Kok, C. Rice-Evan, M. Roberfroid, *Brit. J. Nutr.* **1998**, *80S*, S77.

[16] F. B. Hu, *Curr. Opin. Lipidol.*, **2000**, *13*, 3.

[17] U. Eggli, Ullustrated Handbook of Succulent plants: Monocotyledons. Springer, **2001**.

[18] T. Reynolds, *Bot. J. Linn. Soc.*, **1985**, *90*, 157.

[19] D. Grindly, T. Reynolds, *Journal of Ethnopharmacology*, **1986**, *16*, 117.

[20] Jr. J. S. Haller, *Bulletin of the New York Academy of Medicine*, **1990**, *66*, 647.

[21] N. Okamura, N. Hine, S. Harada, T. Fujioka, K. Mihashi. A. Yagi, *Phytochemistry*, **1996***, 43*, 495.

[22] N. Okamura, N. Hine, Y. Tateyama., M. Nakazawa., T. Fujioka, K. Mihashi. A. Yagi, *Phytochemistry*, **1997**, *45*, 1511.

[23] N. Okamura, N. Hine, Y. Tateyama., M. Nakazawa., T. Fujioka, K. Mihashi. And A. Yagi, *Phytochemistry*, **1998**, *49*, 219.

[24] E. Dagne, D. Bisrat, A. Viljoen B.E. Van Wyk, *Curr. Org. Chem.*, **2000***, 4*, 1055.

[25] C. Seong won, C.A. Myung-Hee, *Sem. Integ. Med.*, **2003**, *1,* 53.

[26] B. Sultana, F. Anwar, *Food Chem*., **2008**, *108*, 879.

[27] Y. Ishii, H. Tanizawa, Y. Takino, *Chem. Pharm. Bull.*, **1990**, *38***,** 197.

[28] Y. Ishii, H. Tanizawa, Y. Takino, *Biol. Pharm. Bull.*, **1994**, *17(4)*, 495.

[29] Y. Ishii, H. Tanizawa, Y. Takino, *Biol. Pharm. Bull.*, **1994**, *17(5)***,** 651.

[30] T. Akao, Q. M. Che, K. Kobashi, M. Hattori, T. Namba, *Biol. Pharm. Bull.*, **1996**, *19***,** 136.

[31] H. H. Wang, J. G. Chung, C. C. Ho, L. T. Wu, S. H. Chang, *Planta Med.*, **1998**, *64***,** 176.

[32] B. Tian, Y. J. Hua, X. Q. Ma, G. L. Wang, *Zhongguo Zhong Yao Za Zhi*, **2003**, *11***,** 1034.

[33] H. Z. Lee, S. L. Hsu, M. C. Liu, C. H. Wu, *Eur. J. Pharmacol.*, **2001**, *431*, 287.

[34] H. Z. Lee, *Brit. J. Pharmacol.*, **2001**, *134***,** 1093.

[35] T. Pecere, M. V. Gazzola, C. Mucignat, C. Parolin, F. D. Vecchia, A. Cavaggioni, G. Basso, A. Diaspro, B. Salvato, M. Carli, G. Palù, *Cancer Res.*, **2000**, *60*, 2800.

[36] D. Jasso de Rodríguez, D. Hernández-Castillo, R. Rodríguez-García, J. L. Angulo-Sánchez, *Ind Crop. Prod.*, **2005**, *21***,** 81.

[37] K.Y. Lee, S.T. Weintraub, B.P. Yu, *Free Rad. Biol.,* **2000**, *28*, 261.

[38] Y. Hu, J. Xu, Q. Hu, *J. Agr. Food Chem.,* **2003,** *51***,** 7788.

[39] Q. Hu, Y. Hu, J. Xu, *Food Chem.*, **2005**, *91***,** 85.

[40] J.H. Wu, C. Xu, C.Y. Shan, R.X. Tan, *Life Sci*., **2006**, *78*, 622.

[41] L. Chan-hui, W. chang-hai, X. Zhi-liang, W. Yi, *Process Biochem.,* **2007**, *42,* 961.

[42] V. L. Singleton, J. A. Jr. Rossi, *Am. J. Enol. Viticult.*, **1965**, *16*, 144.

[43] P. Prieto, M. Pineda, M. Aguilar, *Anal Biochem.*, **1999**, *269*, 337.

[44] M. Oyaizu, *Jpn. J. Nutr.,* **1986**, *44*, 307.

[45] W. Brand-Williams, M. E. Cuvelier, C. Berset, *Lebensm. Wiss. Technol.,* **1995**, *28*, 25.

[46] Y. Chen, M. F. Wang, R. T. Rosen, C. T. Ho, *J. Agr. Food Chem.*, **1999**, *47*, 2226.

[47] G. H. Naik, K. I. Priyadarsini, J. G. Satav, M. M. Banavalicar, P. P. Sohoni, M. K. Biyani, H. Mohan, *Phytochemistry*, **2003**, *63*, 97.

[48] S. Chevolleau, A. Debal, E. Ucciani, *Rev. Fr. Corps* Gras, **1992**, *39(1-2)*, 3.

[49] A. Moure, D. Franco, J. Sineiro, H. Dominguez, M. J. Nunez, J. M. Lema, *J. Agr. Food Chem.*, **2000**, *48*, 3890.

[50] M. Skerget, P. Kotnik, M. Hadolin, A. R. Hras, M. Simonic, Z. Knez, *Food Chem.*, **2005**, *89*, 191.

[51] S. Meir, J. Kanner, B. Akiri, S. P. Hadas, *J. Agr. Food Chem.***, 1995**, *43***,** 1813.

[52] V. L. Schimada, K. Fujikawa, K. Yahara, T. Nakamura, *J. Agr. Food Chem.*, **1992**, *50*, 4989.

[53] X. Pin-Der-Duh, *J. Am. Oil Chem. Soc.*, **1998**, *75*, 455.

[54] G. K. Jayaprakasha, R. P. Singh, K. K. Sakariah, *Food Chem.*, **2001**, *73*, 285.