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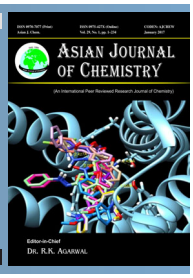

Phytochemical Study, Antioxidant activity and Kinetic Behaviour of Flavonoids Fractions Isolated from *Prunus persica* L. Leaves

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The present study was conducted to evaluate the antioxidant activity of flavonoids extract from the leaves of *Prunus persica* L. The selective extraction of flavonoids gave yields of 0.71, 1.5 and 4.8 % for the fractions ethyl ether, ethyl acetate and *n*-butanol, respectively. The antioxidant activity was investigated with three different methods: Bioautographic HPTLC test, 2,20-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and ferric reducing ability of plasma (FRAP) assay. It has been found that flavonoids revealed the positive reaction (yellow spots) on the TLC plates sprayed with DPPH methanol solution. Besides, the DPPH method showed that the fractions of flavonoids (bunanol, ethyl acetate and diethyl ether) exhibited a potent scavenging activity with IC_{50} = 0.22, 0.27 and 0.76 mg/mL, respectively. Furthermore, our findings revealed that the extracts under study provided higher reducing power.

Keywords: *Prunus persica*, Phytochemical study, Flavonoids, Antioxidant activity, Free radical.

INTRODUCTION

Natural antioxidant compounds have pharmacological potent with low or no side effects for use in preventive medicine and the food industry. The inclusion of antioxidants in the diet has beneficial effects on human health because they protect the biologically important cellular components, such as DNA, proteins and membrane lipids, from reactive oxygen species (ROS) attacks [1]. Today, natural antioxidants have been studied extensively for decades in order to find compounds protecting against a number of diseases related to oxidative stress and free radical-induced damage such as cancer, ageing, neurological degeneration, arthritis and cataracts [2-7].

Therefore, the plant kingdom offers a wide range of compounds exhibiting antioxidant activities. Essential oils and polyphenols such as tannins, flavonoids, phenolic acids have been considered as excellent natural antioxidants. They are widely distributed and can be considered as the most abundant plant secondary metabolites with highly diversified structures [8-10] *Prunus persica* (L.) Batch belongs to the family *Rosaceae* is a deciduous tree with a height of 5 to 10 m and is commonly cultivated in West Asia, Europe, India and north Africa [11]. There are about 100 genera and 3,000 species in Rosaceae family [12]. *Prunus* has nearly 200 species cultivated for their edible fruits and seeds [13]. The leaves are astringent, anthelmintic, insecticidal, vermicidal, demulcent, diuretic, expectorant, febrifuge, laxative, parasiticide, mildly sedative and are used in leucoderma and in piles. They are used internally in the treatment of gastritis, whooping cough, coughs and bronchitis [14]. Leaf paste is used to kill worms in wounds and fungal infections [15]. Its seeds are used in traditional medicine to treat amenorrhea and rheumatoid arthritis [16]. Furthermore, pharmacological studies on the seeds of *P. persica* have shown they have multiple activities, which include anticancer, antioxidant, hepatoprotective, antinociceptive and anticoagulant and inhibitory effects on platelet aggregation [17-19]. In South Algeria, the majority of population uses this plant as antitumor and anticancer. Phytochemical studies on this plant have reported various cyanogenic glycosides, glycerides, sterols and fatty acids [17,20,21].

The aim of the present was to screen the chemical composition of the leaves of *Prunus persica* L. as well as to evaluate *in vitro* the antioxidant power of the flavonoids extracts from the leaves of *Prunus persica* L. and to investigate their kinetic behaviour of DPPH radical scavenging activity.

EXPERIMENTAL

Prunus persica L. leaves were collected in October 2013 from Bechar Department (southern Algeria). A voucher specimen of the plant was identified and authenticated at the laboratory of botany at the biology Institute. The leaves were ground by an electrical mill mesh and powdered part was stored in a nylon bag in a deep freeze until the time of use.

Phytochemical screening: Chemical tests were carried out respectively on the diethyl ether, methanol and water extracts for the qualitative determination of phytochemical constituents as described in literature [22-24].

Extraction of flavonoids: A total of 100 g of defatted leaves powder were contacted with 400 mL of water/ethanol (50:50, v/v) in 500 mL capped flask with timely shaking and stirring for 24 h at room temperature (maceration). The obtained extract was filtered by using Whatmann filter paper and evaporated to dryness by using a rotary evaporator. The residue was dissolved then in boiled water and extracted respectively with diethyl ether (3 × 50 mL); 4 × 50 mL with ethyl acetate and 4 × 50 mL with *n*-buthanol. The organic layers (ethyl acetate) were dried on Na_2SO_4, filtered and concentrated to dryness to give crude extract of flavonoids [25].

Antioxidant activity

Thin layer chromatography-antioxidant assay: The flavonoids fractions of *Prunus persica* L. were subjected to TLC-antioxidant assay on a silica gel plate (20 × 20 cm, silica gel F254, Merck). The solvent system optimized for crude extracts of *Prunus persica* L. was methanol and chloroform (10: 90 v/v). The flavonoids fractions were loaded on a TLC silica gel plate and the plate was developed in a sandwich TLC chamber to a distance of 70 mm. After 15 min air-drying, the plate was sprayed with 0.004 % (w/v) DPPH reagent prepared in methanol.

The spots on the plates were observed after the plate had been heated at 60 °C for 30 min exactly after spraying [26].

DPPH radical scavenging activity: The antioxidant potential of the flavonoids fractions and methanolic crude extracts were determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Briefly, 100 µL of various concentrations of the each extract in methanol was added to 1.9 mL of a methanol solution of DPPH (0.004 %). The mixture was vigorously shaken and then allowed to stand at room temperature for 30 min in the dark. The absorbance of the mixture was measured at 517 nm by using (UV-VIS 1700 pharma spec SHIMADZU) spectrophotometer. A mixture of 100 µL of methanol and 1.9 mL of DPPH solution was used as the control. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of the control reaction (containing all reagents except the test compound) and A_s is the absorbance of the test compound. The commercial known antioxidant, ascorbic acid was used for comparison or as a positive control.

The tests were done in triplicate. The extract concentration providing 50 % inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration (0.5; 0.25; 0.125; 0.0625; 0.0312; 0.0156; 0.0078 mg/mL) [27,28].

The results may also be expressed as antiradical power (ARP) [29].

$$ARP = 1/IC_{50}$$

Reducing power determination (FRAP assay): The reducing power of tannins extract and ascorbic acid was evaluated according to the method of Oyaizu [30]. Different concentrations of tannins crude extract and ascorbic acid (1, 2, 3, 4, 5 mg/mL) in 1mL of distilled water were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1 % potassium ferricyanide separately and undergoes vortex. The mixture was made homogeneous and incubated at 50 °C for 20 min; aliquots of trichloroacetic acid (2.5 mL, 10 %) were added to the mixture, which was then centrifuged at 1500 rpm for 10 min (2.5 mL) and finally freshly prepared $FeCl_3$ solution 3 (0.5 mL, 1 %) was added to this and mixed uniformly. The absorbance of supernatant was measured at 700 nm.

The mean of absorbance values were plotted against concentration and a linear regression analysis was carried out. Increased absorbance of the reaction mixture indicated increased reducing power [31]. The ascorbic acid was used as positive control.

Kinetic analysis: About 5 mg of each extract was dissolved in 4 mL methanol [mother solution (2) 1.25 mg/mL]. From this solution, different concentrations were prepared (0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, 0.00780 mg/mL) and used for kinetic behaviour.

To follow the kinetic behaviour of DPPH radical scavenging activity of extracts under study, 1.5 mL of solution (2) was mixed with 1.5 mL of solution (1), the absorbance was measured after each 30 s until it becomes constant.

Some kinetic parameters were calculated such as the time at the steady state. The kinetic classification, according to this time has been reported as rapid < 5 min, intermediate 5-30 min and slow > 30 min [27]. Two factors, IC_{50} and T_{EC50}, are combined in order to obtain the antiradical effectiveness (AE) parameter [32]. This is calculated as:

$$AE = 1/IC_{50} \cdot T_{EC50}$$

The parameter T_{EC50} is defined as the time needed to attain a balance with an antioxidant concentration equal to IC_{50}. This time is calculated graphically [32].

RESULTS AND DISCUSSION

Phytochemical screening: Table-1 shows the phytochemicals detected in *Prunus persica* L. leaf extract. Tests for alkaloid salts, steroids, terpenoids, tannins, flavonoids, coumarins, anthracenosides, anthocyanosides and fatty acids were positive in water, diethyl ether and methanolic extracts. Whereas, saponosids, free quinones, anthraquinones, anthracenosides, starch and reducing sugars were not detected.

Extraction of the flavonoids crude extracts: In this study and using the protocol of Bruneton [25], the fractionation of the aqueous extract with diethyl ether, ethyl acetate and *n*-butanol lead to 4.80; 1.5 and 0.71 % yields, respectively. These contents of flavonoids were found to be lowest than those

TABLE-1
PHYTOCHEMICALS DETECTED IN *Prunus persica* L. LEAVES

Extracts	Phytochemicals	Result
Aqueous	Tannins	Present
	Saponins	Absent
	Alkaloids salts	Present
	Anthraquinones	Absent
	Reducing sugars	Absent
	Starch	Absent
Methanol	Flavonoids	Present
	Anthocyanosides	Present
	Anthracenosides	Absent
	Tannins	Present
	Alkaloids salts	Present
Diethyl ether	Free alkaloids	Absent
	Coumarins	Present
	Steroids	Present
	Terpenoids	Absent
	Free quinones	Absent
	Fatty acids	Present

reported by Dhingra *et al.* [33] who mentioned that the same extraction of flavonoids gave 25.14, 10.2 7.8 and 4.8 %, respectively, for the aqueous, butanol, ethyl acetate and hexane fractions. The variation in yield may be due to the polarity of the solvents used in the extraction process. Another study carried out on *Prunus persica* L. showed that methanol extract yielded 16.3 % of flavonoids [34].

Antioxidant activity of the flavonoids crude extracts: Antioxidants are known to eliminate oxidative stress by scavenging free radicals and protect biological macromolecules from their toxic effect. Therefore, in recent years evaluating plant antioxidant activity and their free radical quenching ability is considered to be an important task in pharmacological studies. However, between all the phytochemicals distributed plant secondary metabolites, flavonoids have attracted considerable interest because of their potentially beneficial effects in humans; they have been reported to have antiviral, antiallergic, antiplatelet, antiinflammatory, antitumor and antioxidant activities [35,36]. Many investigations have focused on these health-promoting effects and antioxidant activities of flavonoids, particularly their role in the chemoprevention of cancer [37,38]. Furthermore, Shimoi *et al*. [39] concluded that plant flavonoids, which show antioxidant activity *in vitro* also function as antioxidants *in vivo*. A strong relationship between these phytochemicals content and antioxidant activity has also been reported [40].

In the present investigation, the data obtained from the TLC-antioxidant assay revealed that the flavonoids extracts as for ascorbic acid exhibited an antioxidant effect. Yellow spots were observed after spraying the TLC plates with DPPH solution. Our results are in accordance with those reported by Molyneux [41].

The results of the DPPH radical scavenging activity of the flavonoids fractions and ascorbic acid are as shown in the graphical presentation in Fig. 1.

The results indicate that the radical-scavenging activity of the fractions under study increased in a dose-dependent manner. It seems that a good correlation exists between the percentage inhibition (% scavenging effect) assayed by DPPH and flavonoids fractions ($0.9755 \leq R^2$ (0.9934). The IC_{50} values of the butanol, ethyl acetate and diethyl ether fractions were calculated to be 0.22, 0.277 and 0.76 mg/mL, respectively, while that of the positive control, ascorbic acid, was 0.04 mg/mL).

The results revealed that the butanolic fraction was found to be more potent followed by ethyl acetate and diethyl ether fractions. Our results are in harmony with those reported by several workers. Dhingra *et al.* [33] and Christabel *et al.* [42] reported that the ethyl acetate fraction from fruits of *P. persica*

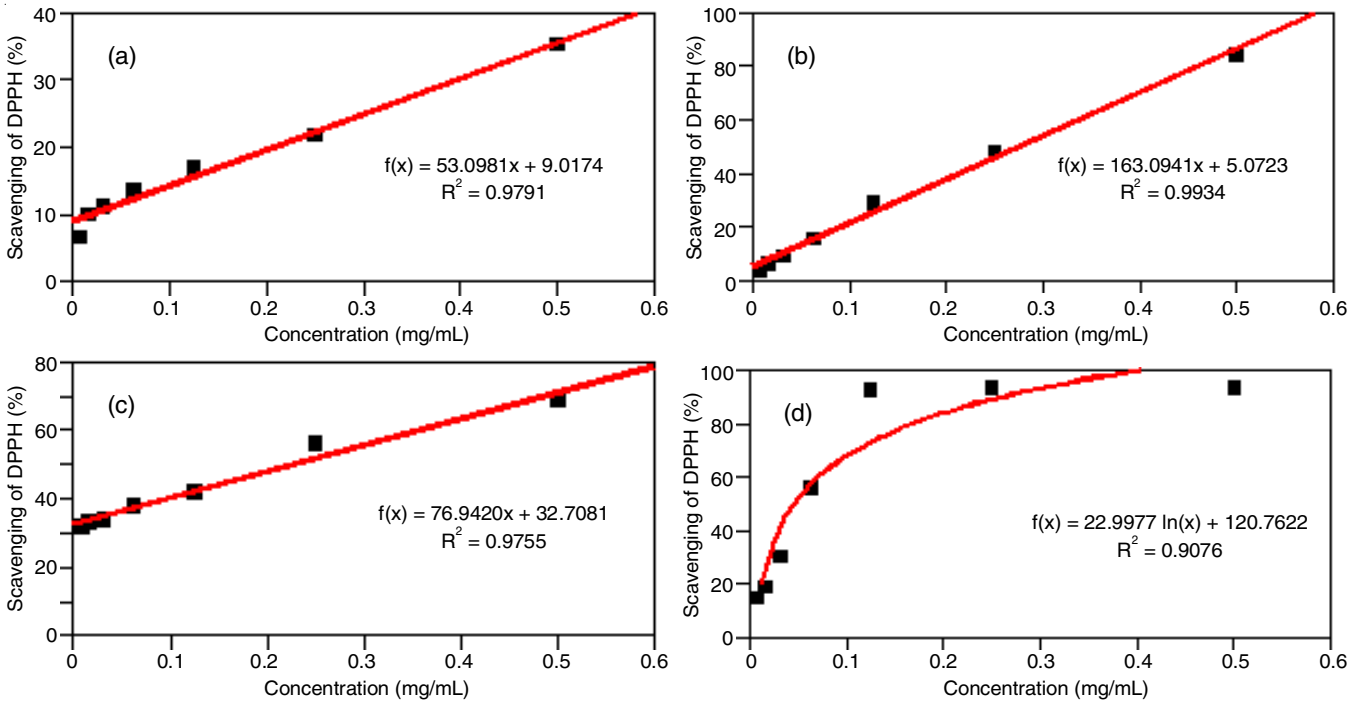

Fig. 1. DPPH free radical scavenging activity of the flavonoids fractions and ascorbic acid: (a) diethyl ether fraction; (b) ethyl acetate fraction; (c) butanolic fraction; (d) ascorbic acid fraction

L. exhibited an excellent antioxidant power with IC_{50} values of 0.184 and 0.29 mg/mL, respectively. Besides, Raturi *et al.* [43] showed that the inhibition percentages of the methanolic extract of pulp at concentrations of 50 and 100 µg/mL were found to be 57 and 93 %. Furthermore, Deb *et al.* [44] indicates that the aqueous extract of the *P. persica* presented at concentration of 100 g an antioxidant activity with IC_{50} = 72.79 and inhibition percentage of 58.42 %.

The potent antioxidant effect of our extracts under study may be due to the following data as described above.

It has been reported that the antioxidant activity of different flavonoids depends on the number and location of hydroxyl groups of the flavonoid ring system [45].

The structural requirement considered to be essential for effective radical scavenging by flavonoids is the presence of a 3',4'-dihydroxy, *i.e.*, *o*-dihydroxy group (catechol structure) in the B ring, possessing electron donating properties and being a radical target [31,46]. Also, 3-OH and 5-OH moieties and the 2,3-double bond in conjugation with 4-oxo function in the C ring, are also beneficial for the antioxidant activity of flavonoids [47]. In the absence of *o*-dihydroxy structure in B ring, hydroxyl substituents in a catechol structure on A-ring were able to compensate and become a larger determinant of flavonoid antiradical activity [48]. Fig. 2 summarizes the structural criteria that modulate the free radical scavenging activity of flavonoids.

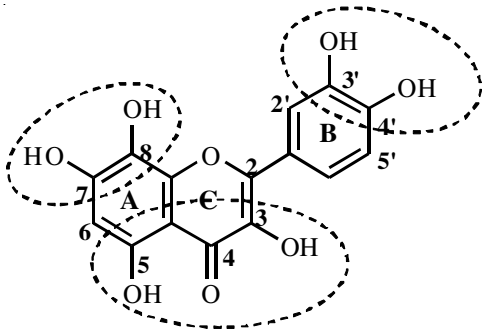

Fig. 2. Structural features of flavonoids with a high radical scavenging activity

The reducing potential of the flavonoids fractions was determined by the ferric reducing ability of plasma (FRAP) method and the results are depicted in Fig. 3.

In the present study, the reducing power assay is used to test the reducing capability of the flavonoids fractions isolated from the leaves of *P. persica* to convert the potassium ferricyanide (Fe^{3+}) complex to form potassium ferrocyanide (Fe^{2+}). The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm [30].

Fig. 3 shows the reducing power or absorbance of the flavonoids fractions and ascorbic acid as a function of their

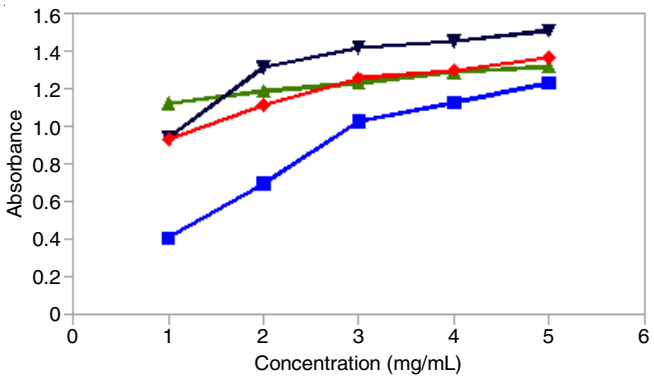

Fig. 3. FRAP assay of flavonoïds fractions and ascorbic acid: ● *n*-butanol; ● ascorbic acid; ● ethyl acetate; ● diethyl ether

concentration. The reducing power of the samples increased with concentration. According to the results, the most active sample was butanol fraction with an absorbance value of 1.51 at 5 mg/mL concentration. At this concentration value, this activity was followed by ethyl acetate fraction (1.36) and diethyl ether fraction (1.23). While the reducing power of ascorbic acid at 1 mg/mL was 1,157. Our findings are in analogy with those of Dhingra *et al.* [33] who confirm that the ethyl acetate and butanol fractions from fruits of *Prunus persica* L. have a pronounced ferric reducing power. Besides, Manzoor *et al.* [34] indicate that the pericarp of P.*persica* L. possessed an excellent reducing capacity in comparison with the pulp extract.

The results of antioxidant activity based on measurments of IC_{50} together with those based on kinetic data provide comprehensive information on the total antioxidant property of sample [49]. In this work, kinetic parameters were evaluated to clarify the antioxidant activity of the flavonoids fractions.

Fig. 4 represents the kinetic behaviour of DPPH free radical scavenging activities of flavonoids fractions as compared to that of ascorbic acid as standard antioxidant.

As can be observed in Fig. 4, at the concentration of 0.5 mg/mL, the time reaction at the steady state is 4.5 min for the butanolic fraction, while it takes the values of 6 and 11.5 min for the ethyl acetate and diethyl ether fractions, respectively. This finding reveals well that the rate reaction in case of butanolic extract is superior in comparison to that of the other ones. In the other hand, the reading of the Fig. 4 showed that the time reaction at the steady state and half time reaction ($t_{1/2}$) increase when the concentration of the fractions decrease. We observed also that for all the flavonoids extracts, the remaining DPPH percent decrease as function of time and when the concentration increase. It has been found that the ethyl acetate and butanolic extracts at concentrations of 0.0625; 0.125; 0.25 and 0.5 mg/mL were most efficient DPPH radical scavenger after 5 min where the remaining DPPH percents ranged between 6 and 13 %.

TABLE-2
REDUCTION KINETIC PARAMETERS OF DPPH•

Fractions	IC_{50} (mg/mL)	$T_{EC_{50}}$ (min)	Antiradical effectiveness	Antiradical power	Classification
Diethyl ether	0.76 ± 0.115	14	0.094	1.31	Intermediate
Ethyl acetate	0.27 ± 0.050	10	0.370	3.70	Intermediate
Butanol	0.22 ± 0.045	8	0.568	4.54	Intermediate
Ascorbic acid	0.04 ± 0.015	3	8.33	25	Rapid

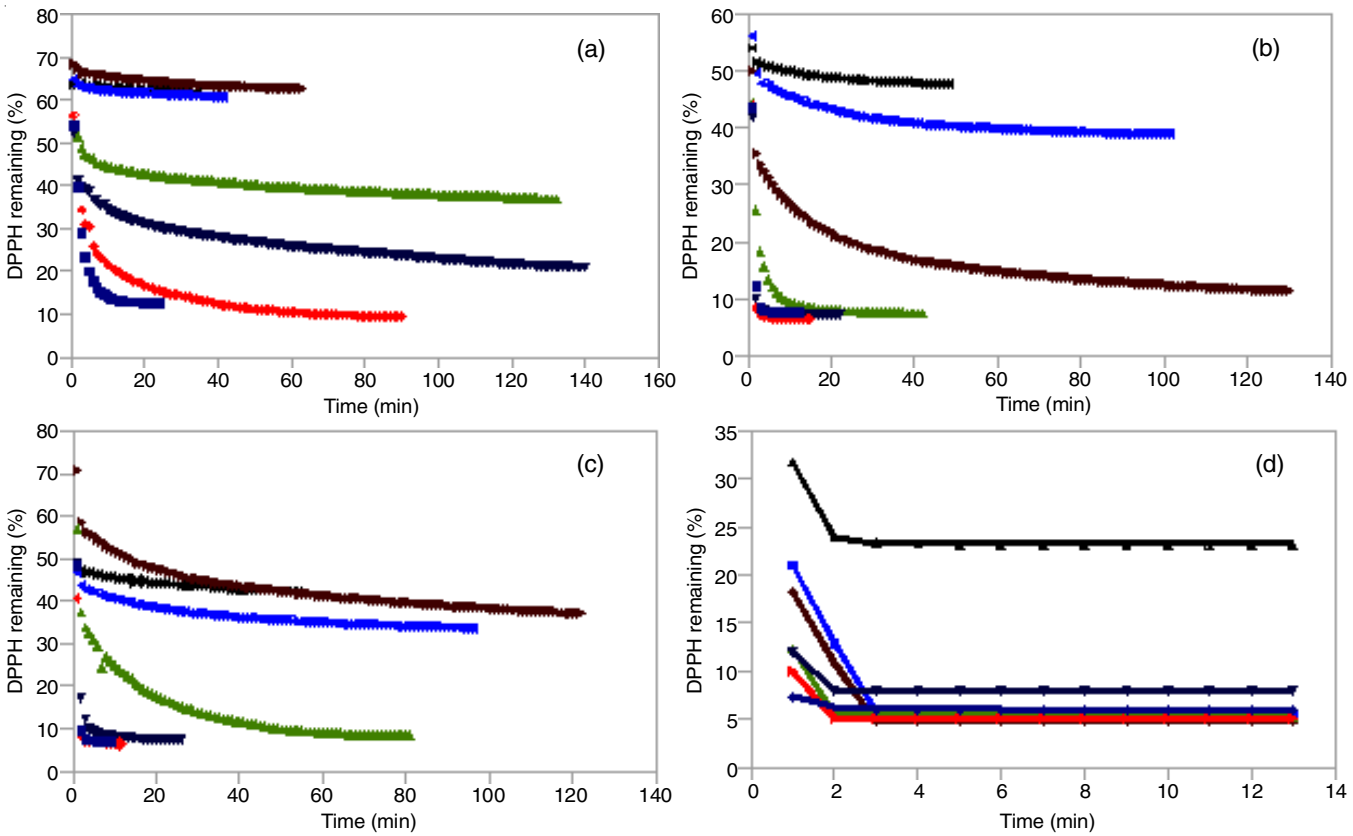

Fig. 4. Kinetic behaviour of flavonoids fractions: (a) diethyl ether fraction, (b) ethyl acetate fraction, (c) butanolic fraction, (d) ascorbic acid. Concentrations of flavonoids fractions in the medium are: ● 0.00780; ● 0.0156; ● 0.0312; ● 0.0625; ● 0.125; ● 0.25; ● 0.5 mg/mL

Therefore, the kinetic classification, according to the time at the steady state, has been reported as rapid < 5 min, intermediate 5-30 min and slow > 30 min [27]. Based on these data, the kinetic is rapid in case of butanolic fraction and intermediate for ethyl acetate and diethyl ether extracts (Table-2).

Ascorbic acid, on the other hand exhibits a very rapid initial step (Fig. 4) and the disappearance of the purple colour of DPPH$^{\bullet}$ occurs almost immediately upon contact between flavonoids. These observations on the scavenging rate of ascorbic acid are consistent with the observations reported by Sanchez-Moreno *et al.,* who classified this compound as displaying rapid kinetic behaviour [27].

REFERENCES

1. L. Su, J.-J. Yin, D. Charles, K. Zhou, J. Moore and L.L. Yu, *Food Chem.*, **100**, 990 (2007).
2. C. Sanchez-Moreno, *Food Sci. Technol. Int.*, **8**, 121 (2002).
3. F. Marc, A. Davin, L. Deglene-Benbrahim, C. Ferrand, M. Baccaunaud and P. Fritsch, *Med. Sci. (Paris)*, **20**, 458 (2004).
4. D. Huang, B. Ou and R.L. Prior, *J. Agric. Food Chem.*, **53**, 1841 (2005).
5. M. Suhaj, *J. Food Compos. Anal.*, **19**, 531 (2006).
6. M.B. Tadhani, V.H. Patel and R. Subhash, *J. Food Compos. Anal.*, **20**, 323 (2007).
7. D.I. Varban, M. Duda, R. Varban and S. Muntean, *Bull. UASVM Agric.*, **66**, 225 (2009).
8. D.M. Pereira, P. Valentão, J.A. Pereira and P.B. Andrade, *Molecules*, **14**, 2202 (2009).
9. J. Dai and R.J. Mumper, *Molecules*, **15**, 7313 (2010).
10. C. Koechlin-Ramonatxo, *Nutr. Clin. Metab.*, **20**, 165 (2006).
11. R.D. Gaur, Flora of the District Garhwal North West Himalayas, Transmedia, Srinagar, Garhwal, India, pp.227 (1999).
12. W.S. Judd, S. Christopher, A.C. Elizabeth, F. Kellogg, P. Stevens and J.M. Donoghue, Plant Systematics: A Phylogenetic Approach, Publishers Sunderland, Massachusetts, USA, edn 2, pp. 365 (1999).
13. A. Rheder, Manual of Cultivated Trees and Shrubs Hardy in North America, Macmillan Company, New York, pp. 425-481 (1940).
14. H.C. Yeung, Handbook of Chinese Herbs and Formulas, Institute of Chinese Medicine, Los Angeles (1985).
15. K.R. Kritikar and B.D. Basu, Indian Medicinal Plants, Bishen Singh Mahendra Pal Singh, Dehradun, vol. 1, p. 954 (1984).
16. F.S. Santamour, *Phytochemistry*, **47**, 1537 (1998).
17. H. Wu, J. Shi, S. Xue, Y. Kakuda, D. Wang, Y. Jiang, X. Ye, Y. Li and J. Subramanian, *Lebensm. Wiss. Technol.*, **44**, 2032 (2011).
18. H.J. Hwang, P. Kim, C.J. Kim, H.J. Lee, I. Shim, C.S. Yin, Y. Yang and D.H. Hahm, *Biol. Pharm. Bull.*, **31**, 1559 (2008).
19. H.S. Yun-Choi, S.O. Kim, J.H. Kim, J.R. Lee and H.I. Cho, *J. Nat. Prod.*, **48**, 363 (1985).
20. T. Kosuge, H. Ishida and M. Ishii, *Chem. Pharm. Bull. (Tokyo)*, **33**, 1496 (1985).
21. T. Fukuda, H. Ito, T. Mukainaka, H. Tokuda, H. Nishino and T. Yoshida, *Biol. Pharm. Bull.*, **26**, 271 (2003).
22. A. Sofowara, Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd, Ibadan, Nigeria (1993).
23. J.B. Harborne, Phytochemical Methods, Chapman & Hall, London, edn 3 (1978).
24. G.E. Trease and W.C. Evans, Trease and Evans' Pharmacognosy: A Physician's Guide to Herbal Medicine, Bailliere Tindall, London, edn 13 (1989).
25. J. Bruneton, Pharmacognosie, Phytochimie et Plantes Médicinales, La Voisier TEC et DOC, Paris, edn 2, pp. 268-277 (1993).
26. L.J. Subramanion, Z. Zakaria and S. Sreenivasan, *J. Med. Plants Res.*, **5**, 1941 (2011).
27. C. Sanchez-Moreno, J.A. Larrauri and F. Saura-calixto, *Int. J. Sci. Tech.*, **8**, 121 (1998).
28. R.M. Samarth, M. Panwar, M. Kumar, A. Soni, M. Kumar and A. Kumar, *Food Chem.*, **106**, 868 (2008).
29. W. Brand-Williams, M.E. Cuvelier and C. Berset, *LWT-Food Sci. Technol.*, **28**, 25 (1995).
30. M. Oyaizu, *Japanese J. Nutr.*, **44**, 307 (1986).
31. P. Cos, L. Ying, M. Calomme, J.P. Hu, K. Cimanga, B. van Poel, L. Pieters, A.J. Vlietinck and D.V. Berghe, *J. Nat. Prod.*, **61**, 71 (1998).

32. F. Sharififar, M.H. Moshafi, S.H. Mansouri, M. Khodashenas and M. Khoshnoodi, *Food Control*, **18**, 800 (2007).
33. N. Dhingra, R. Sharma and A. Kar, *Spectrochim. Acta A: Mol. Biomol. Spectrosc*., **132**, 582 (2014).
34. M. Manzoor, F. Anwar, Z. Mahmood, U. Rashid and M. Ashraf, *Molecules*, **17**, 6491 (2012).
35. V. Izzi, L. Masuelli, I. Tresoldi, P. Sacchetti, A. Modesti and F. Galvano, *Front. Biosci.*, **17**, 2396 (2012).
36. C.D. Kay, L. Hooper, P.A. Kroon, E.B. Rimm and A. Cassidy, *Mol. Nutr. Res.*, **56**, 1605 (2012).
37. A.M. Gonzalez-Paramas, C. Santos-Buelga, M. Duenas and S. Gonzalez-Manzano, *Mini Rev. Med. Chem.*, **11**, 1239 (2011).
38. M. Galleano, V. Calabro, P.D. Prince, M.C. Litterio, B. Piotrkowski, M.A. Vazquez-Prieto, R.M. Miatello, P.I. Oteiza and C.G. Fraga, *Ann. N. Y. Acad. Sci.*, **1259**, 87 (2012).
39. K. Shimoi, S. Masuda, B. Shen, B. Furugori and N. Kinae, *Mutat. Res.*, **350**, 153 (1996).
40. H.J.D. Dorman, M. Kosar, K. Kahlos, Y. Holm and R. Hiltunen, *J. Agric. Food Chem.*, **51**, 4563 (2003).
41. P. Molyneux, *Songklanakarin J. Sci. Technol.*, **26**, 211 (2004).
42. P.H. Christabel, S. Nishaa, M. Vishnupriya, J. Sasikumar and G. Gopalakrishnan, *World J. Pharm. Res.*, **5**, 1371 (2012).
43. R. Raturi, H. Singh, P. Bahuguna, S.C. Sati and P. Badoni, *J. App. Nat. Sci.*, **3**, 312 (2011).
44. L. Deb, R. Gupta, A. Dutta, A. Yadav, D. Bhowmik and S.K.P. Kumar, *Der Pharm. Sinica*, **1**, 157 (2010).
45. E.J. Lien, S. Ren, H.H. Bui and R. Wang, *Free Radic. Biol. Med.*, **26**, 285 (1999).
46. S.A.B.E. van Acker, M.J. de Groot, D.-J. van den Berg, M.N.J.L. Tromp, G. Donné-Op den Kelder, W.J.F. van der Vijgh and A. Bast, *Chem. Res. Toxicol.*, **9**, 1305 (1996).
47. C.G.M. Heijnen, G.R.M.M. Haenen, F.A.A. Van Acker, W.J.F. Van der Vijgh and A. Bast, *Toxicol. in Vitro*, **15**, 3 (2001).
48. A. Arora, T.M. Byrem, M.G. Nair and G.M. Strasburg, *Arch. Biochem. Biophys.*, **373**, 102 (2000).
49. P. Terpinc, M. Bezjak and H. Abramovic, *Food Chem.*, **115**, 740 (2009).