IN VITRO EVALUATION OF THE ANTIOXIDANT PROPERTY AND DPPH RADICAL SCAVENGING KINETIC BEHAVIOR OF ALGERIAN *QUERCUS ROBUR* L. LEAVES' SELECTIVE EXTRACTS

Elhassan Benyagoub[a,b]*, Nouria Nabbou[b,c], Miaad K. Alkhudhairy[d] and Amal Dine[c]

(Received 11 October 2021) (Accepted 01 August 2022)

ABSTRACT

Today, there is a growing demand for natural antioxidants. The unbalanced production and consumption of reactive oxygen species cause many disorders such as cancer, arteriosclerosis, Alzheimer's disease and aging. For this purpose, the present study was a part of the *in vitro* evaluation of the antioxidant activity of *Quercus robur* L. leaves' selective extracts, namely tannins and saponins groups. The antioxidant activity was evaluated by using two techniques: the DPPH radical scavenging activity method and the ferric reducing antioxidant power method. In addition, a kinetic behavior study of the antiradical activity was established. The obtained results show that tannins and saponins extracts have a significant free radical scavenging activity with IC50 values of 0.128 and 0.145 mg mL^{-1}, respectively. Moreover, the kinetic behavior of the scavenging ability of the studied extracts makes it possible to determine the antiradical efficiency, the antiradical power, the percentage of the remaining DPPH free radical, the T_{IC50} parameter, the half-reaction time and the equilibrium antiradical reaction time. These results showed that the tested extracts provided a significant antioxidant activity. This plant can keep an important value in pharmacy and herbal medicine, and act as natural agents in food applications.

Keywords: *Quercus robur* L. leaves, Antioxidant property, Selective extracts, Free radical

Abbreviations

Abs: Absorbance, ARE: Antiradical efficiency, ARP: Antiradical power, DPPH: 1, 1-Diphenyl-2-picrylhydrazyl, E: DPPH free radical scavenging effect, FRAP: Ferric ion reducing antioxidant power, IC50: Half-maximal inhibitory concentration, OD: Optical density, ORAC: Oxygen radical absorbance capacity, ROS: Reactive oxygen species, SD: Standard deviation, $t_{1/2}$ (half-reaction time): The time (in min) required by each antioxidant to decrease the concentration of DPPH to half of its initial concentration, T_{IC50} or Teq: Time reaches equilibrium at a half-maximal inhibitory concentration IC50, TPTZ: Tripyridyltriazine, TRAP: Total radical antioxidant parameter.

INTRODUCTION

Antioxidants can be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or spread of oxidative chain reactions[1]. Antioxidants can also protect the human body against free radicals and the effects of reactive oxygen species (ROS). They delay the lipid peroxidation and the progression of many chronic diseases and ailments at the origin of oxidative stress phenomena[2,3].

Today, natural antioxidants are being widely studied to explore compounds used for protection against some diseases related to oxidative stress and damage induced by free radicals such as cancer, aging, degeneration and neurological disorders, arthritis and cataracts[4]. Therefore, the plant kingdom (*Plantae*) offers a wide range of compounds exhibiting antioxidant activities. Polyphenols such as tannins, flavonoids and phenolic acids have been considered excellent natural antioxidants, and are one of the most diverse phytochemicals distributed in fruits, vegetables and herbs[5]. They are widespread and can be considered as the most abundant plant secondary metabolites, components of medicinal and other plants with diverse structures and magical properties[6,7].

[a] Department of Biology, Mohammed Tahri University of Bechar, Bechar - 08000, Algeria

[b] Architecture and Environmental Heritage Laboratory (Archipel), Department of Architecture, Mohammed Tahri University of Bechar, Bechar - 08000, Algeria

[c] Department of Process Engineering, Mohammed Tahri University of Bechar, Bechar - 08000, Algeria

[d] Community Health Department, College of Health and Medical Techniques, Al-Furat Al-Awsat Technical University, Kufa, Iraq

*For Correspondence: E-mail: benyagoubelhassan@gmail.com

https://doi.org/10.53879/id.59.11.13218

The *Quercus* genus (family of *Fagaceae*) includes over 600 species of evergreen or deciduous trees and shrubs distributed especially in the northern temperate regions and in the tropics' areas where they are generally confined into the high altitudes[8]. Several studies have shown that oak (*Quercus spp*) contains high polyphenols content[9-11]. Structurally, phenolic compounds comprise an aromatic ring, carrying one or more hydroxyl substituents, and range from simple molecules to highly complex polymerized compounds[12]. Tannins are polyphenolic antioxidant compounds that act against allergies, ulcers, tumors, platelet aggregation, and cardiovascular diseases and may reduce the risk of cancer[10]. The plant tannins' bioactivity is generally recognized as being largely dependent on their structure and in particular on their degree of polymerization[12]. Otherwise, saponins are increasingly used in medicine as anti-inflammatory, molluscicides, antimicrobials, antispasmodics, antidiabetics, antitumors, antioxidants agents, as well as adjuvants[13-15], but also in the food and the cosmetic industry as emulsifiers or sweeteners[16]. The vegetal extracts from *Quercus sp* species were found to possess interesting biological activities. On this basis, the current study aims to evaluate the antioxidant activity of the tannins and saponins extract of *Q. robur* L. leaves collected from the Algerian highlands.

MATERIALS AND METHODS

All chemicals used for analytical procedures were of analytical grade or of the highest available purity.

Plant material

The green leaves of all sizes and ages were randomly collected from the *Q. robur* L. in March (2021) from the Mezi mountain located in the Djeneine Bourezg of the Naâma province, Algeria. They were washed and dried at room temperature for one month in a dry and ventilated place. The dried leaves were powdered and stored in a dark and dry place until further use.

Selective extraction processes

Defatting process of plant material

This process was carried out by refluxing in hexane in the Soxhlet apparatus to degrease the plant material according to the method described by El-Hela et al.[17], and Benyagoub et al[18].

Extraction of selective extracts

The extraction of selective extracts of *Q. robur* leaves, namely 'tannins and saponins', was carried out by the methods described by Benyagoub et al[18]; Okwu et al.[19] and Lin et al[20].

Evaluation of antioxidant activity

DPPH assay

This assay was based on the principle of reduction of DPPH free radical by accepting a hydrogen atom from the scavenger compound; hence, the color changed from violet to yellow[21]. This method was carried out according to the technique described by Potbhare and Khobragade[22], and Singh et al[23]. Likewise, the antioxidant activity was measured at 517nm for the positive control (ascorbic acid) at the same concentrations as the selective extracts (0.5; 0.25; 0.125; 0.0625; 0.0312; 0.0156 and 0.0078 mg mL^{-1}).

The DPPH free radical scavenging effect (E) or scavenging ability, expressed in (%), was calculated using the following equation[23-25] :

$$E\ (DPPH\ \%) = \frac{\text{Abs(Control)} - \text{Abs(Sample)}}{\text{Abs(Control)}} \times 100$$

where,

Abs (Control): the absorbance value of the control reaction

Abs (Sample): the absorbance value in the presence of the tested Q. robur L. extract

The results can also be expressed as follow[24] :

$$ARP = \frac{1}{\text{IC50}}$$

where,

ARP: Antiradical power

IC50: Half-maximal inhibitory concentration

FRAP assay

This assay is often used to measure the antioxidant capacity of foods, beverages, and nutritional supplements containing polyphenols[23]. Ferric reducing antioxidant power assay (FRAP) is based on the reduction of a colorless (Fe^{3+} -TPTZ) complex into intense blue (Fe^{2+} -TPTZ), once it interacts with a potential antioxidant[26,27]. The antioxidant activity was measured at 700nm for the following selective extract concentrations: 1; 2; 3; 4; and 5 mg mL^{-1}. This method was carried out according to the technique described by Gulçin et al[28], Jadhav and Saudagar[29].

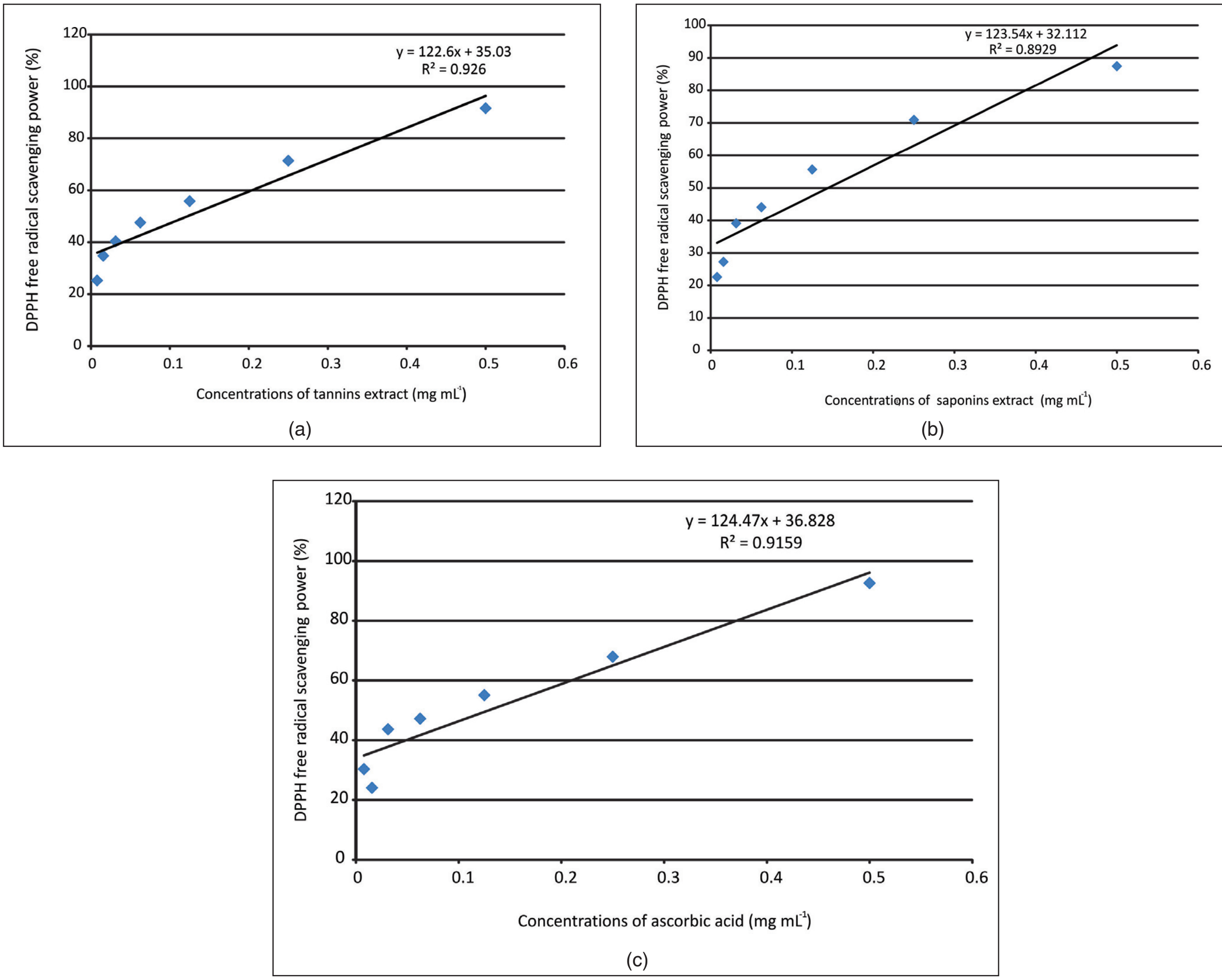


Fig. 1: DPPH free radical scavenging activity of *Q. robur* L. selective extracts (a, b) and ascorbic acid as a positive control (c)

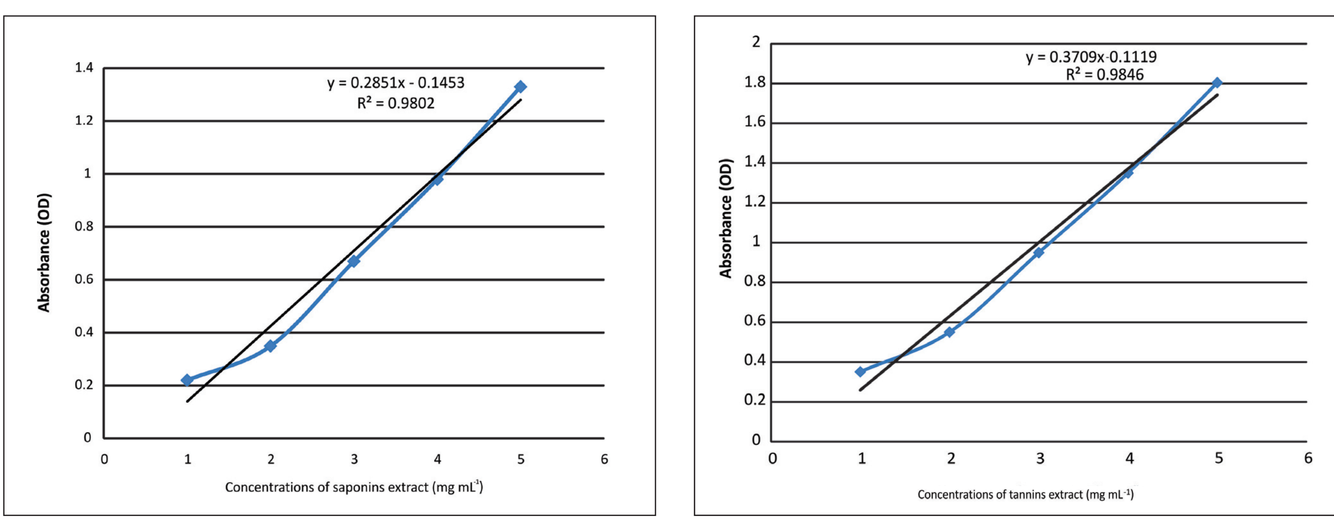
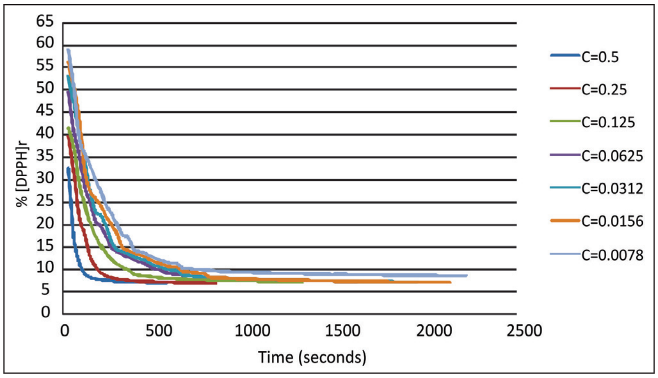
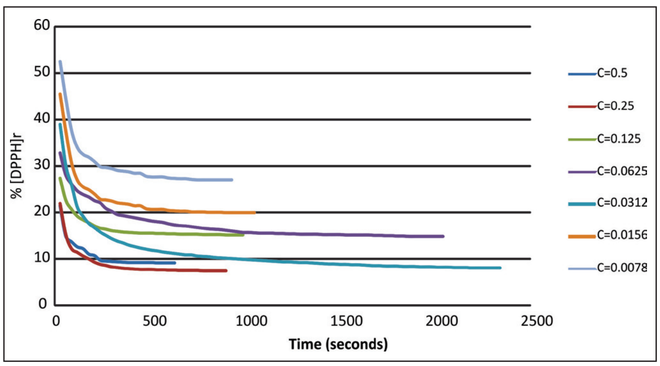


Fig. 2: Ferric-reducing power (FRAP) of *Q. robur* L. leaves' selective extracts



(a)



(b)

Fig. 3: Graphical illustration of DPPH radical scavenging kinetic behavior of *Q. robur* L. leaves' selective extracts as a function of time

(a): Antiradical power of tannins extract; (b): Antiradical power of saponins extract; C: Concentrations of selective extracts in (mg mL^{-1})

Table I: Extraction yield and the half-maximal inhibitory concentration values (IC50)

Selective extracts	IC50 (Mean value ± SD) (mg mL^{-1})	Extraction yield (Mean value % ± SD)[18]
Tannins	0.128±0.00282	7.93±0.35
Saponins	0.145±0.00424	16.94±0.76
Ascorbic acid (*)	0.104±0.00565	/

(*): Positive control; SD: Standard deviation

DPPH radical scavenging kinetic behavior of *Q. robur* L. leaves' extracts

From a stock solution, different concentrations of vegetal extracts were prepared and used in the kinetic study. Monitoring the kinetic of DPPH free radical scavenging activity was conducted by measuring the absorbance every 30 seconds until the absorbance became constant at the equilibrium time (Teq). Teq parameter makes it possible to classify the free radical scavenging reaction[30].

The T_{IC50} time was determined graphically, and both parameters (IC50 and T_{IC50}) were used to assess and classify the antiradical efficiency[31,32]

$$ARE = \frac{1}{\text{IC50} \times \text{TIC50}}$$

The DPPH reduction kinetics at different antioxidant concentrations was monitored over time by measuring the absorbance (optical density OD) every 30 seconds until a plateau was reached at the final time. The residual DPPH free radical ([DPPH]r) expressed in (%) was calculated by dividing DPPH's concentration at the equilibrium time (t=Teq) on its initial concentration at (t=0) ([DPPH]r ÷ [DPPH] ratio)[33]:

$$\%(\text{DPPH})\text{r} = \frac{[\text{DPPH}](\text{t} = \text{Teq})}{[\text{DPPH}]\ (\text{t} = 0)} \times 100$$

All the other parameters relating to the kinetic behavior of the scavenging ability of the studied extracts were determined graphically.

Statistical analysis

The experimental results, as well as the plotted graphs, were obtained by using Excel software. IC50 values were also calculated by linear regression analysis. The experimental results were analyzed by the Pearson correlation coefficient (R^2).

RESULTS

Selective extraction yield

The yield of selective extraction showed a value of 16.7 and 7.9 % for saponins and tannins extract, respectively, with a rate of fatty residues removal equal to 0.61 %[18] (Table I).

DPPH assay

The results of the DPPH free radical scavenging activity are given in Fig. 1 and Table I.

The graph analysis in the current study showed a proportional increase between the DPPH free radical scavenging and the concentrations of the tested extracts, where a straight line of type [y=ax+b] was obtained with a linear correlation coefficient R^2 equal to 0.8929; 0.9262 and 0.9159 for saponins, tannins as selective extracts, and the positive control (ascorbic acid), respectively.

Table II: DPPH free radical scavenging kinetic behavior of *Q. robur* L. leaves' selective extracts

Selective extract of *Q. robur* L. leaves	Concentration (mg mL^{-1})	DPPH in AO g^{-1}	% (DPPH) t=Teq	% $(DPPH)_r$ t=Teq	Teq (minutes)	$t_{1/2}$ (minutes)
Tannins extract	0.5	6250	93.00	07	08	0.763
	0.25	3125	92.91	7.09	12	1.243
	0.125	1562.50	92.83	7.17	20	1.676
	0.0625	781.25	92.76	7.24	25	1.693
	0.0312	390	92.68	7.32	30.5	1.772
	0.0156	195	92.60	7.40	32.5	1.801
	0.0078	97.50	91.35	8.65	35	2.129
Saponins extract	0.5	6250	92.60	7.40	09	1.124
	0.25	3125	91.97	8.03	13	01
	0.125	1562.50	90.88	9.12	20	1.20
	0.0625	781.25	85.19	14.81	36.5	1.324
	0.0312	390	84.88	15.12	32	1.816
	0.0156	195	80.05	19.95	12,5	1.617
	0.0078	97.50	72.95	27.05	15	1.601

Table III: Characteristic parameters of the DPPH free radical scavenging kinetics

Selective extracts	IC50 μg AO mL^{-1}	T_{IC50} (minutes)	ARP	ARE (×10^{-3})	ARE's classification
Tannins	128	2	0.0078	3.90	Medium
Saponins	145	4.25	0.0069	1.62	Medium

FRAP assay

The reducing potential of tannins and saponins selective extracts determined by the ferric ion reducing antioxidant power assay (FRAP) is illustrated in Fig. 2.

DPPH radical scavenging kinetic behavior of *Q. robur* L. leaves' extracts

The obtained results of the kinetic behavior of DPPH free radical scavenging are illustrated in Table II and Fig. 3.

Table III shows the DPPH free radical scavenging kinetics behavior results of the tested selective extracts where the tannins extract of *Quercus robur* L. was the most active than the saponins one.

DISCUSSION

Plants are an inexhaustible source of natural bioactive compounds. Secondary metabolites shall remain the subject of much research *in vivo* and *in vitro*, including the search for new natural constituents such as phenolic compounds for their possible uses as alternatives, especially for the protection against oxidative stress and the treatment of several diseases[34]. For this, the choice fell on tannins and saponins groups as selective extracts due to their pharmacological properties exerted, and the broad-spectrum use of the studied plant in traditional medicine.

The literature has revealed that most research on phenolic compounds of the European and the Asian oak species shows that the following parts: leaves, twigs, galls, and bark have high total phenols content, including tannins, proanthocyanidins, and flavonoids having strong antiradical properties[35-40]. Regarding the obtained extraction yield of selective extracts, the extraction yield depends on many factors, including the emulsion challenge during the liquid-liquid extraction steps and the total aqueous phase exhaustion[24].

An earlier study was done on the Algerian *Q. robur* L. species, where the qualitative phytochemical screening showed that the leaves part was rich in bioactive phytoconstituents such as alkaloids, anthraquinones, saponins, tannins and other components[41]. These results corroborate the study carried out by Uddin and Rauf[37] in Pakistan on the *Q. robur* L. leaves, which shows the presence of bioactive secondary metabolites such as steroids, terpenoids, saponins, tannins, and reducing compounds. According to Raja et al.[5] and Madhu et al.[42], this rich amount of phytochemicals can act as a free radical hunter and avoid free radical-mediated oxidation of biological molecules. The best-known antioxidants are β-carotene (provitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E) as well as phenolic compounds. Indeed, most synthetic or natural antioxidants contain hydroxyphenolic groups in their structures, and the antioxidant properties of polyphenols are ascribed in part to the ability of these natural compounds to scavenge the free radicals such as hydroxyl (OH) and superoxides (O_2) radicals[43-47].

For the DPPH free radical scavenging assay results, the leaves' selective extracts of the studied plant showed good antiradical activity, in particular, the tannins extract, which seems to be the most active with an IC50 value equal to 0.128 mg mL^{-1} compared to the saponins extract (IC50=0.145 mg mL^{-1}). The study conducted by Dróżdż and Pyrzynska[11] on the antioxidant activity of hydro-alcoholic extract of *Q. robur* bark collected in Poland, evaluated by the DPPH assay, reported a good antioxidant activity, while the study conducted by Uddin and Rauf, 2012[37] noted a moderate antioxidant power of the hexane, chloroform, ethyl acetate, and methanolic extracts of the oak leaves of Pakistani origin.

It is important to emphasize that many works described in the literature confirms that tannins and saponins extracts have good antioxidant power[14,48-53]. Galiňanes et al.[54] noted a good antioxidant activity (DPPH) of the 2 % Na_2SO_3 extract and the aqueous extract of the *Q. robur* bark collected in Spain, with an IC50 value equal to 0.063 mg mL^{-1} and 0.074 mg mL^{-1}, respectively.

Another study was done in Turkey on the *Q. coccifera* bark, conducted by Genç et al.[55], where a high DPPH free radical scavenging power was reported, with IC50 values of 0.04325 mg mL^{-1} and 0.06784 mg mL^{-1} for the methanolic and the aqueous extracts, respectively.

A study carried out by Rivas-Arreola et al. 2010[56] on the antioxidant screening of several extracts of *Quercus* species (*Q. resinosa, Q. eduardii* and *Q. sideroxyla*) collected in Mexico. The obtained results showed that the infusion extracts of *Q. resinosa* exhibit good antioxidant activity with an IC50 value of 0.220 mg mL^{-1}.

According to He et al.[36], the flavonoids extract of *Q. macrocarpa* leaves collected in China exhibited a stronger DPPH free radical scavenging activity than that of ascorbic acid as a positive control with IC50 varying between 0.0092 mg mL^{-1} and 0.0132 mg mL^{-1} versus 0.0426 mg mL^{-1}, respectively.

However, Valencia-Avilés et al.[57] studied the antioxidant activity of the *Quercus laurina*, *Q. crassifolia*, and *Q. scytophylla* barks part collected in Mexico. The results of the free radical scavenging method showed a very high antioxidant activity for methanolic extracts than for the aqueous ones of these species. Also, according to Rivas-Arreola et al.[56], the FRAP assay results showed that the infusion extract of *Q. resinosa* leaves exhibits a higher iron-reducing power than that of the *Q. eduardii* and *Q. sideroxyla* species, and from these properties, Azmaz et al.[40] suggest that the gall and the leaf extracts of *Q. infectoria* could be used as natural inhibitors in the food industry. Through processing these bibliographic data, it can be noted that the barks and leaves parts of the *Quercus* species have good antiradical activity.

The kinetic reaction of the antioxidant compound is really important as the free radical has a short half-life and there is structural heterogeneity within phenolic compounds, which results in different properties[21]. According to some studies, the tannins isolated from *Q. robur* consisted mainly of various gallic and ellagic acid esters of glucose. The structures that were partially determined included: grandinine, roburin E, castalagine, and vescalagin as major compounds as well as gallic acid, valoneic acid dilactone, monogalloyl glucose, digaloyl glucose, trigalloyl glucose, ellagic acid rhamnosides, quercitrin, and ellagic acid[58,59]. A lot of studies indicate that certain plant saponins have strong antioxidant activities. They could, therefore, be potential new antioxidant candidates, which could rely on their free radical scavenging abilities[52,53].

The phytochemical studies describing the isolation and the characterization of saponins extracted from the *Quercus robur* species have made it possible to isolate the following compounds: 2,3,19-trihydroxyolean-12-ene-24,28-dioic acid; 2,3,19-23-tetrahydroxyolean-12-ene-24,28-dioic acid; 28-D-glucopyranosyl-2,3,19-trihydroxyolean-12-ene-24,28-dioic acid; and 28-D-glucopyranosyl-2,3,19,23-tetrahydroxyolean-12-ene-24,28-dioic acid[60,61]. These compounds identified from tannins and saponins extracts were known to have broad antioxidant power[62,59].

Thus, we can notice that the tannins extract has higher reducing power than that of the saponins one. Tannins exhibited stronger iron-reducing activity than saponins extract (OD 1.8 vs. 1.33) at a concentration of 5 mg mL^{-1}, with a linear correlation coefficient (R^2=0.98). This difference could be attributed to their ability to release hydrogen atoms, change the free radical to a more stable form, or reduce the rate of auto-oxidation by scavenging initiating radicals as antioxidant mechanisms[63,64].

CONCLUSION

In vitro, DPPH, and FRAP methods showed high efficacy in evaluating the antioxidant activity of both tannins and saponins that were extracted from leaves of *Q. robur* L. The study's results show that *Q. robur* tannins extract exhibits strong antioxidant activity compared to saponins extract. According to the Teq and ARE's classification results, the kinetic behavior of the DPPH free radical scavenging activity of the selective extracts showed that the tested extracts have a medium kinetic action. These results prove the role of the tested extracts as scavengers of the free radicals and can be used as a source of new antioxidants at a low cost.

REFERENCES

1. Velioglu Y. S., Mazza G., Gao L. and Oomah B. D.: Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. **J. Agric. Food Chem.,** 1998, 46(10), 4113–4117. DOI: 10.1021/jf9801973
2. Pryor W. A.: The antioxidant nutrient and disease prevention –what do we know and what do we need to find out? **Am. J. Clin. Nutr.,** 1991, 53(1s), 391–393. DOI: 10.1093/ajcn/53.1.391S
3. Gardès-Albert M., Bonnefont-Rousselot D., Abedinzadeh Z. and Jore D.: Espèce réactives de l'oxygène: Comment l'oxygène peut-il devenir toxique? **Actual. Chim.,** 2003, 270, 91–96.
4. Cook N. C. and Samman S.: Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. **J. Nutr. Biochem.,** 1996, 7(2), 66–76. DOI: 10.1016/S0955-2863(95)00168-9
5. Raja W., Dubey A. and Verma P.: Evaluation of phytochemicals screening and antioxidant activity of *Vitis vinifera* (Grapes) fruit extract using Fenton reaction. **Eur. J. Biomed. Pharm. Sci.,** 2020, 7(7), 582–587.
6. Van Ruth S. M., Shaker E. S. and Morrissey P. A.: Influence of methanolic extracts of soybean seeds and soybean oil, on lipid oxidation in linseed oil. **Food Chem.,** 2001, 75(2), 177–184. DOI: 10.1016/S0308-8146(01)00195-9
7. Kurhekar J. V.: Tannins-antimicrobial chemical components. **Int. J. Technol. Sci.,** 2016, 9(3), 5–9.
8. Castro-Vázquez L., Alañón M. E., Ricardo-da-Silva J. M., Pérez-Coello M. S. and Laureano O.: Evaluation of Portuguese and Spanish *Quercus pyrenaica* and *Castanea sativa* species used in cooperage as natural source of phenolic compounds. **Eur. Food Res. Technol.,** 2013, 237(3), 367–375. DOI: 10.1007/s00217-013-1999-5
9. Sroka Z. and Franiczek R.: Antiradical and antimicrobial activity of extracts obtained from plant raw materials. **Adv. Clin. Exp. Med.,** 2008, 17(3), 275–283.
10. Ruiz-Aquino F., González-Peña M. M., Valdez-Hernández J. I., Revilla U. S. and Romero-Manzanares A.: Chemical Characterization and Fuel Properties of Wood and Bark of Two Oaks from Oaxaca, Mexico. **Ind. Crops Prod.,** 2015, 65, 90–95. DOI: 10.1016/j.indcrop.2014.11.024
11. Dróżdż P. and Pyrzynska K.: Assessment of polyphenol content and antioxidant activity of oak bark extracts. **Eur. J. Wood Wood Prod.,** 2018, 76(2), 793–795. DOI: 10.1007/s00107-017-1280-x
12. Stevanovic T., Diouf N. and Garcia-Perez M. E.: Bioactive polyphenols from healthy diets and forest biomass. **Curr. Nutr. Food Sci.,** 2009, 5(4), 264–295. DOI: 10.2174/157340109790218067
13. Sun H. X., Xie Y. and Ye Y. P.: Advances in saponin-based adjuvants. Vaccine. 2009, 27(12), 1787–1796. DOI: 10.1016/j.vaccine.2009.01.091
14. Hassan S. B., Gullbo J., Hu K., Berenjian S., Morein B. and Nygren P.: The nanoparticulate quilaja saponin BBE is selectively active towards renal cell carcinoma. **Anticancer Res.,** 2013, 33(1), 143–151.
15. Zhang W., Wang X., Tang H., Wang M., Ji L., Wen A. and Wang J.: Triterpenoid saponins from *Clematis tangutica* and their cardioprotective activities. **Fitoterapia,** 2013, 84, 326–331. DOI: 10.1016/j.fitote.2012.12.011
16. Güçlüc-Ustündağ O. and Mazza G.: Saponins: properties, applications and processing. **Crit. Rev. Food Sci. Nutr.,** 2007, 47(3), 231–258. DOI: 10.1080/10408390600698197
17. El-Hela A. A., Abdelhady N. M., El-Hefnawy H. M., Ibrahim T. A. and Abdallah G. M.: Anti-inflammatory effect, antioxidant potentials and phytochemical investigation of *Crataegus sinaica* boiss. roots growing in Egypt. **Eur. J. Pharm. Med. Res.,** 2016, 3(11), 128–137.
18. Benyagoub E., Nabbou N. and Dine A.: Antimicrobial effect of *Quercus robur* L leaves' selective extracts from Mezi mountain of Djeniene Bourezg (West of Algeria). **Curr. Bioact. Compd.,** 2020, 16(8), 1181–1190. DOI: 10.2174/1573407216666191226141609.
19. Okwu D. E.: Phytochemicals, vitamins and mineral contents of Two Nigerian medicinal plants. **Int. J. Mol. Med. Adv. Sci.,** 2005, 1(4), 375–381.
20. Lin Y. M., Liu J. W., Xiang P., Lin P., Ye G. F. and Da Sternberg L. S. L.: Tannin dynamics of propagules and leaves of *Kandelia candel and Bruguiera gymnorrhiza* in the Jiulong River Estuary, Fujian-China. **Biogeochem.,** 2006, 78(3), 343–359. DOI: 10.1007/s10533-005-4427-5.
21. Ahmad N. A., Jubri K., Ramli A., Abd Ghani N., Ahmad H. and Lim J. W.: A kinetic approach of DPPH free radical assay of ferulate-based protic ionic liquids (PILs). **Molecules,** 2018, 23, 3201. DOI: 10.3390/molecules23123201.

22. Potbhare M. and Khobragade D.: *In vitro* evaluation of antioxidant potential of Ayurvedic preparations Lauha bhasma and Mandura Bhasma. **Asian J. Pharm. Res.,** 2017, 7(2), 63-66. DOI: 10.5958/2231-5691.2017.00011.9.
23. Singh M., Patra S. and Singh R. K.: Chapter 15 - Common techniques and methods for screening of natural products for developing of anticancer drugs. In: Srivastava A. K, Singh R. K, Kannaujiya V. K, Singh D (eds.), Evolutionary diversity as a source for anticancer molecules. Academic Press, India 2021, pp. 323–353. DOI: 10.1016/B978-0-12-821710-8.00015-1.
24. Benyagoub E., Nabbou N. and Moghtet S.: Propriétés anti radicalaires d'une plante saharienne *Anastatica hierochuntica* L. issue d'une région de l'extrême Sud-Ouest algérien. **Rev. Bioresour.,** 2015, 5(2), 54–66. DOI: 10.12816/0045855.
25. Desnilasari D., Agustina W., Putri D. P., Iwansyah A. C., Setiaboma W., Sholichah E. *et al.*: The characteristics of probiotic drink based on Moringa leaves juice. **J. Food Technol. Ind. (Jurnal Teknologi dan Industri Pangan),** 2021, 32(1), 9–15. DOI: 10.6066/jtip.2021.32.1.9.
26. Spiegel M., Kapusta K., Kolodziejczyk W., Saloni J., Zbikowska B., Hill G. A. and Sroka Z.: Antioxidant activity of selected phenolic acids-ferric reducing antioxidant power assay and QSAR analysis of the structural features. **Molecules,** 2020, 25, 3088. DOI: 10.3390/molecules25133088.
27. Njoya E. M.: Chapter 31- Medicinal plants, antioxidant potential, and cancer. In: Preedy V.R, Patel V.B. (Eds.), Cancer: Oxidative stress and dietary antioxidants. 2nd (Ed.), Academic Press, London-UK 2021, pp. 349–357. DOI: 10.1016/B978-0-12-819547-5.00031-6.
28. Gulçin I., Huyut Z., Elmastas M. and Aboul-Enein H. Y.: Radical scavenging and antioxidant activity of tannic acid. **Arab. J. Chem.,** 2010, 3(1), 43–53. DOI: 10.1016/j.arabjc.2009.12.008.
29. Jadhav G. B. and Saudagar R. B.: Free radical scavenging and antioxidant activity of *Punica granatum* Linn. **Asian J. Res. Pharm. Sci.,** 2014, 4(2), 51–54.
30. Sánchez Moreno C., Larrauri J. A. and Saura Calixto F.: A procedure to measure the antioradical efficiency of polyphenols. **J. Sci. Food Agric.,** 1998, 76(2), 270–276. DOI:10.1002/(SICI)1097-0010(199802)76:2%3C270::AID-JSFA945%3E3.3.CO; 2-0.
31. Brand-Williams W., Cuvelier M. E. and Berset C.: Use of a free radical method to evaluate antioxidant activity. **Lebensm.-wiss. u.-Technol.,** 1995, 28(1), 25–30. DOI: 10.1016/S0023-6438(95)80008-5.
32. Scherer R. and Godoy H. T.: Antioxidant activity index (AAI) by the 2,2 diphenyl-1-picrylhydrazyl method. **Food Chem.,** 2009, 112(3), 654–658. DOI: 10.1016/j.foodchem.2008.06.026.
33. Hatano T., Edamatsu R., Hiramatsu M., Mori A., Fujita Y., Yasuhara T., Yoshida T. and Okuda T.: Effects of the interaction of tannins with co-existing substances. VI.: Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. **Chem. Pharm. Bull.,** 1989, 37(8), 2016–2021. DOI: 10.1248/cpb.37.2016.
34. Bazílio Omena C. M., Valentim I. B., Guedes G. S., Rabelo L. A., Mano C. M, Henriques Bechara E. J., Sawaya A. C. H. F., Salles Trevisan M. T., Costa J. G., Silva Ferreira R. C., Goulart Sant'Ana A. E. and Fonseca Goulart M. O.: Antioxidant, anti-acetylcholinesterase and cytotoxic activities of ethanol extracts of peel, pulp and seeds of exotic Brazilian fruits: Antioxidant, anti-acetylcholinesterase and cytotoxic activities in fruits. **Food Res. Int.,** 2012, 49(1), 334–344. DOI: 10.1016/j.foodres.2012.07.010.
35. Paaver U., Matto V. and Raal A.: Total tannin content in distinct *Quercus robur* L. galls. **J. Med. Plants Res.,** 2010, 4(8), 702–705. DOI: 10.5897/JMPR10.091.
36. He Y. Q., Ma Z. Y., Zhang J., Du B. Z. and Yao B. H.: Antioxidant activity of the chemical constituents from the leaves of *Quercus macrocarpa*. **Chem. Nat. Comp.,** 2011, 47(3), 472–473. DOI: 10.1007/s10600-011-9969-2.
37. Uddin G. and Rauf A.: Phytochemical screening, antimicrobial and antioxidant activities of aerial parts of *Quercus robur* L. **Middle-East J. Med. Plants Res.,** 2012, 1(1), 1–4. DOI: 10.5829/idosi.mejmpr.2011.1.1.1101.
38. Söhretoglu D., Sabuncuoglu S. and Harput U. S.: Evaluation of antioxidative, protective effect against H_2O_2 induced cytotoxicity, and cytotoxic activities of three different *Quercus* species. **Food Chem. Toxicol.,** 2012, 50(2), 141–146. DOI: 10.1016/j.fct.2011.10.061.
39. Popovic B. M., Štajner D., Zdero R., Orlovi S. and Galic Z.: Antioxidant characterization of Oak extracts combining spectrophotometric assays and chemometrics. **Sci. World J.,** 2013, 134656. DOI: 10.1155/2013/134656.
40. Azmaz M., Kilinçarslan Aksoy Ö., Katilmiş Y. and Mammadov R.: Investigation of the antioxidant activity and phenolic compounds of *Andricus quercustozae* gall and host plant (*Quercus infectoria*). **Int. J. Second. Metab.,** 2020, 7(2), 77–87. DOI: 10.21448/ijsm.674930
41. Benyagoub E., Nabbou N., Boukhalkhel S. and Dehini I.: The *In vitro* evaluation of the antimicrobial activity of *Quercus robur* L methanolic and aqueous leaves' extracts, from the Algerian high plateaus against some uro-pathogenic microbial strains. **Phytother.,** 2020, 18(5), 262–274. DOI: 10.3166/phyto-2019-0133.
42. Madhu C. H., Swapna J., Neelima K. and Shah M. V.: A comparative evaluation of the antioxidant activity of some medicinal plants popularly used in India. **Asian J. Res. Pharm. Sci.,** 2012, 2(3), 98–100.
43. Rice-Evans C. A., Miller N. J., Bolwell P. G., Bramley P. M. and Pridham J. B.: The relative antioxidant activities of plant-derived polyphenolic flavonoids. **Free Radic. Res.,** 1995, 22(4), 375–383.
44. Burda S. and Oleszek W.: Antioxidant and antiradical activities of flavonoids. **J. Agric. Food Chem.,** 2001, 49(6), 2774–2779. DOI: 10.1021/jf001413m
45. Antolovich M., Prenzler P. D., Patsalides E., McDonald S. and Robards K.: Methods for testing antioxidant activity. **Analyst J.,** 2002, 127, 183–198. DOI: 10.1039/B009171P.

46. Bartosz G.: Generation of reactive oxygen species in biological systems. **J. Toxicol. Env. Heal. B.,** 2003, 9(1), 5–21. DOI: 10.1080/08865140302420.
47. Khomdram S. D. and Singh P. K.: Polyphenolic compounds and free radical scavenging activity in eight *Lamiaceae* herbs of Manipur. **Not. Sci. Biol.,** 2011, 3(2), 108–113. DOI: 10.15835/nsb325638.
48. Muir A. D.: Antioxidative activity of condensed tannins. In: Shahidi, E. (Ed.), Natural Antioxidants Chemistry Health Effects and Application. AOCS Press, Champaign, Illinois, 1996, pp. 64–73.
49. Ryszard A. and Agnieszka T.: Antioxidant activity of extract of pea and its fractions of low molecular phenolics and tannins. **Polish J. Food Nutr. Sci.,** 2003, 53(1s), 10–15.
50. Sanches A. C. C., Lopes G. C., Nakamura C. V., Dias Filho B. P. and Mello J. C. P.: Antioxidant and antifungal activities of extracts and condensed tannins from Stryphnodendron obovatum Benth. **Rev. Bras. Cienc. Farm.,** 2005, 41(1), 100–107. DOI: 10.1590/S1516-93322005000100012.
51. Bang S. C., Lee J. H., Song G. Y., Kim D. H., Yoon M. Y. and Ahn B. Z.: Antitumor activity of *Pulsatilla koreana* saponins and their structure-activity relationship. **Chem. Pharm. Bull. (Tokyo),** 2005, 53(11), 1451–1454. DOI: 10.1248/cpb.53.1451.
52. Tapondjou L. A., Nyaa L. B., Tane P., Ricciutelli M., Quassinti L., Bramucci M., Lupidi G., Ponou B. K. and Barboni L.: Cytotoxic and antioxidant triterpene saponins from *Butyrospermum parkii* (*Sapotaceae*). **Carbohydr. Res.,** 2011, 346(17), 2699–2704. DOI: 10.1016/j.carres.2011.09.014.
53. Bi L., Tian X., Dou F., Hong L., Tang H. and Wang S.: New antioxidant and antiglycation active triterpenoid saponins from the root bark of *Aralia taibaiensis*. **Fitoterapia.,** 2012, 83(1), 234–240. DOI: 10.1016/j.fitote.2011.11.002.
54. Galiňanes C., Freire M. S. and González-Ǎlvarez J.: Antioxidant activity of phenolic extracts from chestnut fruit and forest industries residues. **Eur. J. Wood Prod.,** 2015, 73(5), 651–659. DOI: 10.1007/s00107-015-0936-7.
55. Genç Y., Yüzbaşioğlu M., Harput Ü. S. and Kuruüzüm-uz A.: Antioxidant activity and total phenolic content of *Quercus coccifera* L. **FABAD J. Pharm. Sci.,** 2012, 37, 17–22.
56. Rivas-Arreola M. J., Rocha-Gusman N. E., Gallegos-Infante J. A., Gonzalez-Laredo R. F., Rosales-Castro M., Bacon J. R., Cao R. T, Proulx A. and Intiago-Ortega P.: Antioxidant activity of Oak (*Quercus*) leaves infusion against free radicals and their cardioprotective potential. **Pak. J. Biol. Sci.,** 2010, 13(11), 537–545. DOI: 10.3923/pjbs.2010.537.545.
57. Valencia-Avilés E., García-Pérez M. E., Garnica-Romo M. G., Dios Figueroa-Cárdenas J. D., Meléndez-Herrera E., Salgado-Garciglia R. and Martínez-Flores H. E.: Antioxidant properties of polyphenolic extracts from *Quercus Laurina*, *Quercus Crassifolia*, and *Quercus Scytophylla* Bark. **Antioxidants,** 2018, 7(7), 81. DOI: 10.3390/antiox7070081.
58. Chatonnet P., Dubourdieu D. and Boidron J. N.: Comparative study of the characteristics of American white oak (*Quercus alba*) and European oak (*Quercus petraea* and *Q. robur*) for production of barrels used in barrel aging of wines. **Am. J. Enol. Vitic.,** 1998, 49(1), 79–85.
59. Buche G., Colas C., Fougère L. and Destandau E.: Oak Species *Quercus robur* L. and *Quercus petraea* Liebl. Identification based on UHPLC-HRMS/MS molecular networks. **Metabolites,** 2021, 11(10), 684. DOI: 10.3390/metabo11100684.
60. Arramon G., Saucier C., Colombani D. and Glories Y.: Identification of triterpene saponins in *Quercus Robur* L. *Q. Petraea* Liebl. Heartwood by LC-ESI/MS and NMR. **Phytochem. Anal.,** 2002, 13(6), 305–310. DOI: 10.1002/pca.658.
61. Marchal A., Waffo-Téguo P., Gammacurta M., Prida A. and Dubourdieu D.: Origins of the sweetness derived from the aging of dry wines: the role of oak triterpenoids. **IVES Tech. Rev. (Vine & Wine),** 2020. DOI: 10.20870/IVES-TR.2020.3286.
62. Burlacu E., Nisca A. and Tanase C.: A comprehensive review of phytochemistry and biological activities of *Quercus* Species. **Forests,** 2020, 11(9), 904. DOI: 10.3390/f11090904.
63. De Pooter H. L. and Schamp N. M.: Comparison of the volatile composition of Some Calamintha/Satureja Species. Progress in Essential Oils Research: Proceedings of the International Symposium on Essential Oils, Holzminden/Neuhaus, Federal Republic of Germany, Sept 18-21, 1986, 139–150. DOI: 10.1515/9783110855449-015.
64. Selvakumar K., Madhan R., Srinivasan G. and Baskar V.: Antioxidant assays in pharmacological research. **Asian J. Pharm. Technol.,** 2011, 1(4), 99–103.