

ANTIOXIDANT ACTIVITY ASSESSMENT OF TAMUS COMMUNIS L. ROOTS

ZERARGUI FATIMA¹, BAGHIANI ABDERRAHMANE¹, KHENNOUF SEDDIK², ARRAR LEKHMICI¹

¹Laboratory of Applied Biochemistry, Faculty of Nature and Life Sciences, University Setif, Setif 19000, Algeria, ²Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature and Life Sciences, Univ. Setif, Setif 19000, Algeria

Email: Fatima.zerargui@yahoo.fr

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ABSTRACT

Objective: The aim of this study was to evaluate the antioxidant activities of *Tamus communis* L. (*T. communis*) roots. This plant is used in folk medicine in the treatment of rheumatism, arthritis and lumbago.

Methods: The extraction of polyphenols has been followed by the determination of total polyphenols and flavonoids. The methanol extract (MeOHE) was chromatographed on a silica gel column. The column was eluted with chloroform and then with chloroform/methanol mixtures of increasing polarity, 6 main fractions (F1--FVI) were collected and grouped according to their TLC (Thin layer chromatography). Several methods were used to evaluate the *in vitro* antioxidant activity such as DPPH (2, 2'-diphenyl-1-picrylhydrazyl), bleaching of β -carotene for 24 h, lipid peroxidation and anti-hemolytic activity. An *in vivo* approach was carried out on mice treated with CE (crud extract) at a dose of 100 mg/Kg/day for 21 consecutive days, and one group was treated with vitamin C (Vit C 50 mg/kg) as a standard drug. To determine the improvement of antioxidant potential, basic biochemical parameters were used in tissue (liver), plasma and whole blood.

Results: The results showed that the highest level of polyphenols was recorded in FII (73.143 \pm 0.009 mg GA-Eq/g extract (Gallic Acid Equivalent)/g of extract) followed by MeOHE (69.786 \pm 0.10 mg GA-Eq/g extract). Total flavonoid contents of extracts/fractions showed that MeOHE contains the highest level. Inhibition of DPPH radical showed that MeOHE (0.1187 \pm 0.025 mg/ml) and CE (0.2363 \pm 0.019 mg/ml) have the most efficient antioxidant activity. All the extracts were capable of inhibiting the bleaching of β -carotene by scavenging linoleate derived free radicals, and all the studied extracts have a very important antihemolytic activity ($p < 0.001$) and revealed an extension of hemolysis half time from 70.34 \pm 2.15 min of CTL (AAPH (2,2-azo-bis (2-amidinopropane) dihydrochloride)) and 207 \pm 5.65 min for F1. The *in vivo* assay was exhibited a significant decrease (54.09%) of the content of malondialdehyde (MDA) in the liver and increased glutathione (GSH) and catalase (47.30% and 46.87% respectively).

Conclusion: These findings suggest that the extract obtained from *T. communis* have active substances contributing to the increase in natural antioxidant potential.

Keywords: *Tamus communis* L., Polyphenols, Flavonoids, DPPH, Antihemolytic activity, Catalase, GSH.

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INTRODUCTION

Free radicals are one of the causes of chronic and degenerative diseases. To scavenge reactive oxygen species (ROS), the human body has a complex system of natural enzymatic and non-enzymatic antioxidants including superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT), however, excessive generation of ROS enhances lipid peroxides (LPO) and could deplete these antioxidant enzymes [1]. Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery. According to Sofowora [2], people used plants for healing, and this is certainly due to poverty but also to the richness of our flora in medicinal plants. Phenolic substances and flavonoids are increasingly recognized as the major bioactive components contributing to the antioxidant potency of many herbs [3, 4] and there are evidence that the consumption of polyphenolic compounds from natural sources may lower the risk of serious oxidative injuries such as atherosclerosis, inflammatory processes, cancer and cardiovascular diseases as a result of their antioxidant activity [5-7]. The recent trend of the science is to explore the antioxidant potential of natural compounds.

Plants contain many constituents with local physical impact on body tissues, and the topical use of herbal remedies is among the most noticeable in the simplest traditions of healthcare [8]. One of the plants commonly used in the region is *T. communis* L. (*Dioscoreaceae*) commonly known as black bryony. Both the

rhizomes and the berries of the plant have been traditionally used as an effective rubefacient and for the treatment of rheumatism, artrosis, lumbago and dermatosis [9]. The fleshy aerial parts of the plant are also consumed as food [10].

Studies on this plant revealed a higher concentration of phenolic compounds and flavonoids [11-13]. The objective of this work was to investigate *in vitro* and *in vivo* antioxidant activity of extracts/fractions of plant roots in order to clear its ethnomedicinal importance.

Accordingly, in the present work we employed *in vitro* and *in vivo* assays to evaluate the antioxidant activity effect of *Tamus communis* extract which to the best of our knowledge has not been explored.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical reagent grade and obtained from Sigma-Aldrich and Prolabo. The assay kits (Spireact, Spain). Among the equipment used: a rotary evaporator (BÜCHI), centrifuge 3-K30 (Sigma), Shimadzu 1601 spectrophotometer and the UV-Vis spectrophotometer double beam (TECHCOMP). A microplate reader (BioTek).

Animals

Swiss albino's mice weighing 20–25 g were purchased from Pasteur Institute of Algiers, Algeria. They were housed in the animal care center of the Faculty of biology, Setif University, under controlled environmental conditions of 24 °C and 12 h light-dark cycle. The experimental protocol was approved by the Ethics Committee of the

university of Sétif 1. All procedures were performed in compliance with laws and institutional guidelines.

Plant material

T. communis was harvested from natural resources from Setif, a province in the northeast of Algeria, during the spring (May–June) mainly at flowering stage. The authentication was confirmed by Prof. Oudjhih University of Batna, Algeria. A specimen was deposited at the Laboratory of Botany, Faculty of natural and life sciences, University Ferhat Abbas Setif 1, Algeria.

Extract preparation

Roots were cut into pieces, air-dried and powdered. The ground material (2 Kg) was extracted with 85 % methanol (10 litres) incubated for 5 d at room temperature. The extract was filtered through Whatman No. 4.1 filter paper to obtain particle free extract. The combined methanolic extract was pooled and concentrated and dried under vacuum. Finally, extracts were stored at 4 °C and used to explore their antioxidant activity [14].

Fractionation by column chromatography

The residual solid material (169g) was extracted thoroughly with methanol. The methanol soluble fraction (138g) was chromatographed on a silica gel column. The column was eluted with chloroform and then with chloroform/methanol mixtures of increasing polarity. A total of 52 fractions (400 ml each) were collected and grouped according to their TLC behavior into 6 main fractions (I–VI).

Determination of total phenolics

Total phenolic content was determined with the Folin-Ciocalteu's reagent (FCR) according to the published method [15]. Each sample (0.5 ml) was mixed with 2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na₂CO₃ (7.5%, v/v) solution. The absorbance was then measured at 765 nm after incubation at 30 °C for 90 min. Results were expressed as mg gallic acid equivalent (mg GA-Eq/g dried extract).

Determination of flavonoid

Flavonoids were quantified using aluminum chloride reagent AlCl₃ [16]. Flavonoids were expressed as quercetin equivalent (Q-Eq). 1 ml of *T. communis* samples were dissolved in methanol, then 1 ml of AlCl₃ (2 % in MeOH) was added, after incubation for 10 min, the absorbance was measured at 430 nm.

In vitro methods

Determination of free radical scavenging activities

The hydrogen atom or electron donation abilities of *T. communis* extracts were measured by the bleaching of the purple-colored methanol solution of 2,2'-diphenyl picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent [17]. An aliquot of 50 µl of various concentrations of the two compounds was added to 5 ml of a 0.004% solution of DPPH in methanol. After an incubation period of 30 min at room temperature, the absorbance was read against a blank at 517 nm with the aid of a Techom UV/VIS-8500 spectrophotometer. The inhibition of DPPH free radical in percent (I %) was calculated from the equation.

$$I\% = [(A \text{ blank} - A \text{ sample}) \times 100] / A \text{ blank}$$

Where A blank is an absorbance of the control reaction (containing all reagents except the tested compound) and A sample is the absorbance of the tested compound. The IC₅₀ values, the sample concentration providing 50% inhibition, were calculated from a plot of I% versus extract concentration. Assays were carried out in triplicate.

β-carotene bleaching assay

In this assay, the antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation according to the method of Kartal *et al.* [18]. A stock solution of β-carotene/linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform, 25 µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely

evaporated using a vacuum evaporator. Then, 100 ml distilled water, saturated with oxygen (for 30 min at 100 ml/min), were added with vigorous shaking; 2.5 ml of this reaction mixture were dispersed into test tubes and 350 µl portions of the extracts prepared at 2 mg/ml concentrations, were added. Then emulsion was incubated for 24 h at room temperature and the absorbance (490 nm) was recorded at different time intervals. The same procedure was repeated with the synthetic antioxidant, BHT and a blank (containing only methanol). The relative antioxidant capacity (RAC) was calculated as follow.

$$RAC = 100 (A_{t=24h \text{ sample}} / A_{t=24h \text{ BHT}})$$

Where A_{t=24h sample} is the absorbance of the test compounds after 24h and A_{t=24h}

BHT is the absorbance of BHT after 24 h.

Ferrous ion chelating activity

Ferrous ion chelating activity was measured by inhibition of the formation of iron (II)-ferrozine complex after treatment of test material with Fe²⁺ following the method of Le *et al.* [19]. The reaction mixture contained 500 µl of extracts or the standard chelator EDTA, 100 µl of FeCl₂ (mM in water) and 900 µl of methanol. The control contained all the reaction reagents except the extracts or EDTA. The mixture was shaken well and allowed to react at room temperature for 5 min. 100 µl of ferrozine (5 mmol in methanol) was then added, the mixture was shaken again, followed by further reaction at room temperature for 10 min to complex the residual Fe²⁺ ion. The absorbance of the Fe²⁺-ferrozine complex was measured at 562 nm against a methanol blank. The chelating effect was calculated as a percentage, using the equation below.

$$\% \text{ chelating activity} = 100(A \text{ control} - A \text{ sample}) / A \text{ control}$$

Where control is the absorbance of the control reaction mixture without the test compounds, and A sample is the absorbance of the test compounds.

Reducing power determination

The determination of the reducing power was conducted according to the method developed by Oyaizu [20]. The solution of plant extracts (1 ml, 0-10 mg/ml) was spiked with 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of potassium ferricyanide (1%). The mixture was then placed in a 50 °C water bath for 20 min. After cooling rapidly, 1 ml of 10 percent trichloroacetic acid (TCA 10%) was added and centrifuged at 3000 rpm (revolutions per minute) for 10 min. The supernatant (1 ml) was then mixed with 2 ml of distilled water and 0.1 ml of ferric chloride (0.1%). The absorbance at 700 nm was recorded for the reaction for 10 min. Increasing optical density (OD) of the reaction mixture indicated an increase of the reducing power.

Blood total antioxidant capacity

The global potential of antioxidantizing defense was measured by KRL test (kit of dosage of the Free Radicals) according to the protocol described by Girard *et al.* [21]. The principle of the test is to submit whole blood to a thermo-controlled free radical Aggression. All families of antioxidant present in the blood are mobilized to fight off the oxidant attack and to protect the integrity of erythrocytes resulting in the delay of hemolysis.

Briefly, Aliquots of 80 µl of diluted blood was deposited in a 96-well microplate with 136 µl of AAPH (300 mmol in solution), 2 µl of samples (0.1 mg/ml) and then incubated at 37 °C. The kinetic of hemolysis was followed using a 96-well microplate reader by measuring optical density decrease at 630 nm. The blood resistance to free radical attack is expressed by the time needed to hemolysis 50% of the red blood cells (half-Hemolysis Time, HT₅₀ in mn). The hemolysis of red blood cells without samples was used as a control and was presumed to be 100%.

In vivo models

Animal's treatment

After an adaptation period of 6 d, the mice were randomly divided into three groups of 6-8 animals. The first group was given 100

mg/kg of an extract of *T. communis* (CE) by intraperitoneal route; the second group was given 50 mg/ml of Vit. C as a reference antioxidant for comparison, the control group CTL received the same volume of normal saline solution (NaCl 0.9%). After twenty-one days all animals were sacrificed.

Blood collection

Blood (1 ml) was collected by cardiac puncture after diethyl ether anesthesia. A subsample of whole blood 100 μ l was immediately diluted in 2.4 ml of PBS (NaCl 125 mmol, sodium phosphate 10 mmol, pH 7.4). The remaining quantity of the blood was centrifuged (1500g/5mn/4C °) to separate serum. The serum was kept at -20 °C for subsequent determination of antioxidant status.

Dissection and homogenization

The liver was dissected out, washed in ice-cold saline, Portions of the tissue from liver were blotted, weighed and homogenized with 0.15M KCl and centrifuged at 800 g for 10 mn at 4 °C. The lipid extract obtained using the method of Folch *et al.* [22]. It was used for the estimation of Thiobarbituric acid reactive substances (TBARS).

Plasma antioxidant capacity

DPPH radical scavenging activity

The capacity of the plasma to trap, the DPPH radical was estimated according to the method of Hasani *et al.* [23] with some modifications. Briefly, 50 μ L of plasma were added to 950 μ l of the DPPH methanolic solution (4 mg/100 ml of methanol). After 30 min of incubation in the darkness and at room temperature followed by centrifugation, the absorbance of the supernatant is measured at 517 nm. The plasma antioxidant power was then calculated (see the part *in vitro*). DPPH solution without serum is used as the control value.

Reducing power assay

The total antioxidant capacity of serum was determined by measuring its ability to reduce Fe³⁺ to Fe²⁺ by the same method described *in vitro* and prescribed by Oyaizu [20].

Determination of antioxidant status in the liver

Lipid peroxidation

Thiobarbituric Acid Reactive Substances (TBARS) assay is the method of choice for screening and monitoring lipid peroxidation, a major indicator of oxidative stress. 0.25 ml of TCA 20% (m/V) was added into 0.25 ml of the homogenate. Then 0.25 ml of TBA (0.67%) was added to the sediment, shaken and incubated for 15 min in a boiling water bath at 100 °C. Then, 2 ml of *n*-butanol was added, and the solution which was then centrifuged at 3000 rpm for 15 mn, cooled and the supernatant absorption was recorded at 530 nm using a UV-Visible spectrophotometer (Shimadzu, Japan). The calibration curve was obtained using different concentrations of 1,1,3,3-tetramethoxypropane as standard to determine the concentration of TBA-MDA adducts in samples [24].

Reduced glutathione (GSH) estimation

The method illustrated by Ellman [25] can be used for determination of antioxidant activity. It is a method based on an assessment of the reduction of DTNB Ellman's reagent (5,5'-dithiobis (2-nitrobenzoic acid)) per group (HS) to form the 2-nitro-5-mercaptobenzoic, the

latter is characterized by an intense yellow color, which allows the spectrophotometric quantification at 412 nm. The tissue homogenate (in 0.1 M phosphate buffer pH 7.4) is taken and added with an equal volume of 20% trichloroacetic acid (TCA) containing 1 mmol EDTA to precipitate the tissue proteins. The mixture is allowed to stand for 5 mn prior to centrifugation for 10 mn at 2000 rpm. The supernatant (200 μ l) is then transferred to a new set of test tubes and added with 1.8 ml of the Ellman's reagent (0.1 mmol) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution. Then all the test tubes are made up to the volume of 2 ml. After completion of the total reaction, solutions are measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from known GSH.

Catalase (CAT) activity estimation

The enzymatic activity of catalase was determined in the liver tissue by the method of Claiborne [24], whose principle is based on the diminution of the absorbance at 240 nm which is due to the decomposition of hydrogen superoxide (H₂O₂) in the presence of catalase. In a quartz cuvette, 50 μ l of the homogenate was mixed with 2.95 ml of a solution of H₂O₂ in 19 mmol/ml prepared in potassium phosphate buffer pH 7.4 (0.1M). The change in absorbance is monitored for two mn. The enzymatic activity of catalase is expressed as rate constant for the reaction order of 0 per gram of tissue. The enzymatic activity of catalase is calculated using the following formula:

$$K = 2.303 \times \log (A1/A2)/T$$

K: rate constant of a first order reaction
1, T: Interval time in a minute, A1: Absorbance at t=0 and A2: Absorbance at t1. The enzymatic activity of catalase = K/N.

N represents the amount in grams of tissue in the volume used for the test sample.

Blood total antioxidant capacity

The same protocol used *in vitro* with some modifications by replacing the radical AAPH [2,2'-azo-bisazo-bis (2-amidinopropane) HCl] by the tert-butyl hydroperoxide (t-BHP: 166 μ M) [25].

Statistical analysis

Data obtained *in vitro* and *in vivo* are expressed as mean \pm SD and mean \pm SEM respectively. The sigmoid hemolysis curves were fitted by computer analysis Software (Graph Pad. Prism. V5.00). Differences between the control and the treatments in these experiments were tested for significance using analysis of variance followed by Dunnet's/Tukey's test. A probability P value less than 0.05 were considered to be statistically significant. *P<0.05, **<0.01 and ***<0.001, when compared with control.

RESULTS AND DISCUSSION

Determination of total polyphenol and flavonoids contents

In this study, an attempt was made to quantify and identify polyphenols in *T. communis*. The obtained results (table 1) show that there was a wide range of polyphenols concentration in different extracts. The highest level of polyphenols was recorded in FII (73.143 \pm 0.009 mg GA-Eq/g extract) followed by MeOHE (69.786 \pm 0.10 mg GA-Eq/g extract). Also, total flavonoid contents of extracts/fractions showed that MeOHE contains the highest level (19.387 \pm 0.11 mg R-Eq/g extract and 8.080 \pm 0.07 mg Q-Eq/g extract).

Table 1: Total phenolic and flavonoid content in extracts/fractions of *T. communis*

Extracts	Total phenolic content (mg GA-Eq/g extract)	Total flavonoid content	
		mg Q-Eq/g extract	mg R-Eq/g extract
CE	29.571 \pm 0.11 ^a	2.091 \pm 0.05 ^a	3.404 \pm 0.030 ^{a*}
MeOHE	69.786 \pm 0.10 ^b	8.080 \pm 0.07 ^c	19.387 \pm 0.11 ^{b*}
FI	34.500 \pm 0.03 ^a	0.321 \pm 0.07 ^b	0.962 \pm 0.02 ^{a*}
FII	73.143 \pm 0.09 ^b	0.755 \pm 0.04 ^b	2.092 \pm 0.007 ^{a*}
FIII	37.500 \pm 0.06 ^a	0.621 \pm 0.04 ^b	1.479 \pm 0.03 ^{a*}
FIV	30.5 \pm 0.06 ^a	0.141 \pm 0.09 ^b	0.487 \pm 0.07 ^c
FV	32.071 \pm 0.03 ^a	0.394 \pm 0.07 ^b	1.070 \pm 0.26 ^{a*}
FVI	29.214 \pm 0.03 ^a	0.359 \pm 0.02 ^b	0.954 \pm 0.06 ^{a*}

Each value represents the mean \pm SD (n = 3). Total phenolic content was expressed as mg gallic acid equivalent/g dried extract. Total flavonoid content was expressed as mg quercetin and rutin equivalent/g dried extract. Lines with different letters indicate activities significantly different (p \leq 0.05)

According to other studies, our results suggest that our samples are rich in phenolic compounds and flavonoids [28]. Therefore, our results suggest that there is not a direct relationship between extraction yield and phenolic content in this kind of samples, probably due to the extraction of other methanol soluble components, such as sugars.

In vitro methods

DPPH assay

The DPPH radical scavenging assay is an easy rapid and sensitive method for the antioxidant screening of plant extracts. A number of methods are available for the determination of free radical scavenging activity, but the assay employing the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received the maximum attention owing to its ease of use and its convenience [29]. The results of radical scavenging showed that, MeOHE (0.1187±0.025 mg/ml) and CE (0.2363±0.019 mg/ml) possessed strong radical scavenging effect compared with the fractions which the most active fraction is FII (1.040±0.070 mg/l) followed by FVI (1.14±0.12 mg/ml), FV (1.210±0.087 mg/ml) and FIII (1.23±0.09 mg/ml) (fig. 1). It was observed a significant difference ($P < 0.001$) among the IC_{50} of all fractions and CE and MeOHE, which means that the radical scavenging activities of these fractions were less than standards.

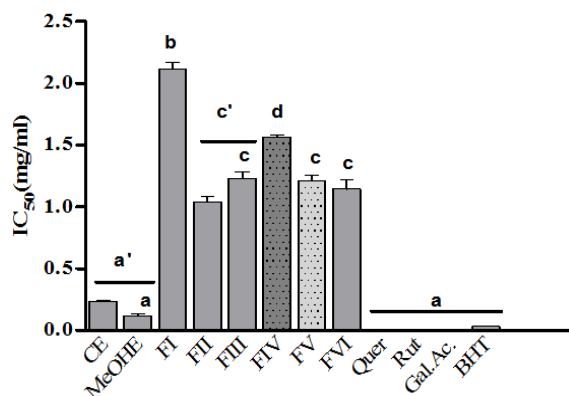


Fig. 1: DPPH radical scavenging activity of *T. communis* extracts/fractions and standards. Data are presented as IC_{50} values. Each value represents the mean±SD (n = 3). Bars with different letters indicate activities significantly different ($p \leq 0.05$)

These results prove that the activity is significant in comparison with the work on the same plant [30].

β -carotene bleaching assay

In this assay, oxidation of linoleic acid produces hydro peroxide-derived free radicals which attack the chromophore of β -carotene, resulting in a bleaching of the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of β -carotene may be described as a free radical scavenger and primary antioxidant [31]. As can be seen in fig. 2, all the extracts were capable of inhibiting the bleaching of β -carotene by scavenging linoleate derived free radicals. The inhibition extent of lipid oxidation by *T. communis* extracts/fractions, when compared to BHT, which had 95.53±1.57% at the same concentration (2 mg/ml), showed marked activity effects. The high inhibition ratios by the MeOHE were showed for 91.47±1.48 %, similar to that of BHT ($P > 0.05$), and CE (79.31±0.75%), followed by others fractions, the less active is the FIV (45.07±1.97 %). No significant differences exist between the activity effects of FI (52.16±2.75%), FIII (52.68±1.38) and FV (51.23±0.08) ($P > 0.05$).

The high activity of MeOHE is most likely attributed to their phenolic and flavonoid content [32], the low activity of the others fractions like fraction FIV is probably explained by its high polarity. Indeed, Frankel and Meyer [33] have suggested that the polarity of an extract is

important in water: oil emulsions, a polar extract exhibit most important antioxidant properties as they are concentrated within lipid-water interface, thus helping to prevent radical formation and lipid oxidation of β -carotene. While polar extract are diluted in the aqueous phase and are thus less effective in protecting lipids.

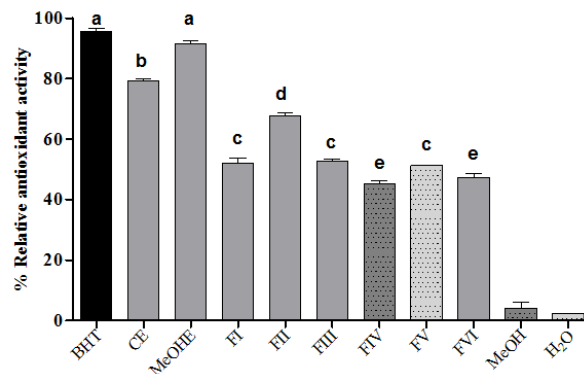


Fig. 2: Relative antioxidant activities of extracts/fractions of *T. communis* and BHT in β -carotene/linoleic acid system after 24h (values are the average of three measurements±SD), bars with different letters indicate activities significantly different ($p \leq 0.05$)

Reducing power assay

The reducing power of the extracts may be due to the biologically active compounds in the extract which possess potent donating abilities [34]. The reducing power is widely used to evaluate the antioxidant activity of plants extracts. Earlier authors have observed a direct correlation between antioxidant activities and reducing the power of certain plant extracts [35]. As shown in fig. 3, higher absorbance value indicates a stronger reducing power of the samples. In the same contest, the reducing capacity (RC) of the MeOHE was significantly at 20 mg/ml than that of BHT and Vit. C ($P < 0.001$), which exhibited the strongest reducing power. Significant correlations were observed between RC of CE, MeOHE and FII ($r^2 = 0.9202$). The rest of the fractions (FI, FIII, FIV, FV and FVI) are shown inactive.

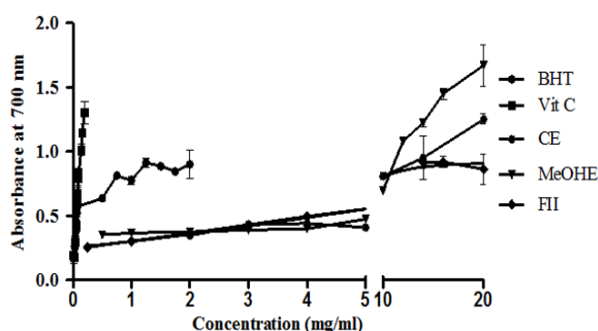


Fig. 3: Comparison of the RC of CE, MeOHE and FII with Vit. C and BHT. Data are the Mean values±SD (n=3)

Ferrous ion chelating activity

It has been well recognized that transition metal ions such as those of iron and copper are important catalysts for the generation of the first few free radicals to initiate the radical chain reaction or the radical mediated lipid peroxidation [36]. Chelating agents may inhibit radical generations by stabilizing transition metals, consequently reducing free radical damage. To better estimate the potential antioxidant activities of the *T. communis* extracts/fractions,

the chelating activity of each extract was evaluated against Fe^{2+} . The results are shown in fig. 4; the results obtained show that the extracts exert a chelating effect in a dose-dependent manner. However, the chelating effect of maximum 77.87% and 77.27% by the CE and MeOHE is achieved at high concentrations (5.625 mg/ml and 3.125 mg/ml respectively). EDTA exerts a chelating effect of 73.143% at a concentration of 14.687 $\mu\text{g}/\text{ml}$. The difference between the activity of these two extracts CE, MeOH E and EDTA used as a standard is highly statistically significant ($p < 0.001$). The other fractions showed no chelating activity of iron. Iron chelating ability of EDTA was higher than that of phenolic compounds [37].

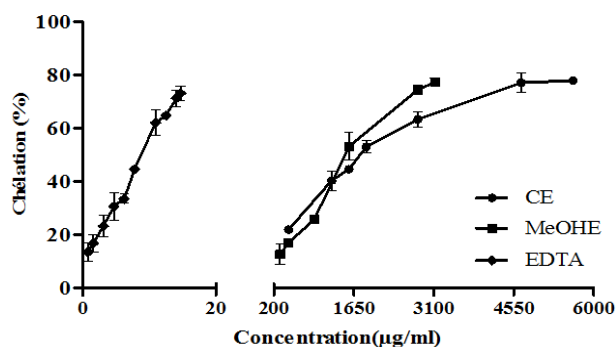


Fig. 4: Metal chelating activity of *T. communis* extracts and standard (EDTA) Data were presented as mean \pm SD (n= 3)

Antihemolytic activity

Hemolysis of RBCs is a very good model for studying free radical induced oxidative damage to membranes and to evaluate the antioxidant activity of compounds [38, 39]. Erythrocytes are known as main targets of oxidative stress due to the presenting of membrane polyunsaturated fatty acids the linolenic and arachidonic acids [40]. In this study, lipid oxidation of mice erythrocyte by AAPH (300 mmol) induced peroxy radicals leads to hemolysis.

The results of showed that all the extracts/fractions can effectively protect erythrocytes against hemolytic injury induced by AAPH. The results presented in fig. 5 show that all the studied extracts had a very important antihemolytic activity ($p < 0.001$) and revealed an extension of hemolysis half time from 70.34 \pm 2.15 mn of CTL (AAPH) to 207 \pm 5.65 min of FI but the % hemolysis of FII and FIII was 24.73% and 25.92% respectively at 208 min.

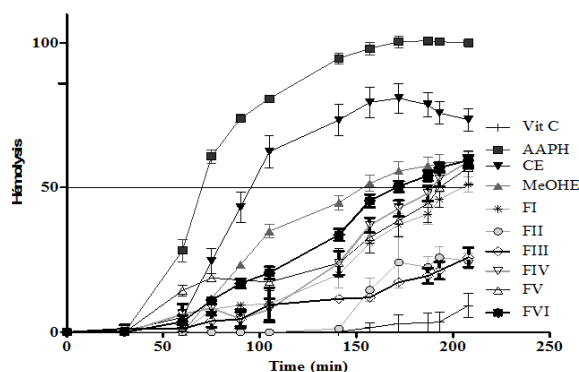


Fig. 5: Comparison of % Hemolysis of *T. communis* extracts/fractions and Vit C with CTL. Values were expressed as mean \pm SD (n= 3)

The antihemolytic action on erythrocytes is at least partly due to the antioxidative property of phenols [41]. The studies by Zhang and co-

workers [42], examining Jasmine green tea polyphenolics *in vitro* and *in vivo*, reported enhanced protection afforded RBC from hemolysis. Other studies showed that Xue-Sai-Tong injections, herbal medicine injection generally used as anti-coronary medicine, contain some unknown ingredient showed a positive correlation with hemolytic activity [43]. However, the injection S. sediforme extracts showed a strong and significant anti-hemolytic effect *in vitro* and *in vivo* assay, where the injection of the CrE (50 mg/Kg) could strongly inhibit hemolysis (%HI = 96.37 \pm 9.26 %) [44].

In vivo models

Analysis of the tissue parameters (liver)

The liver is a highly sensitive organ which plays a major role in maintenance and performance of the homeostasis in our body. It is the chief organ where important processes like metabolism and detoxification take place. Thus the liver is prone to injury due to the chronic exposure to drugs, environmental toxicants and other xenobiotic [45]. The liver disorders are one of the serious health problems, the reason for which it was chosen for this work.

Lipid peroxidation

One of the most often used biomarker to investigate the oxidative damage on lipid is TBARS a major lipid peroxidation product. It can react with the free amino group of proteins, phospholipids, and nucleic acids leading to structural modification [46]. According to the provided data in tableau 2, a significant decrease in TBARS level in liver was observed by CE (100 mg/ml) of *T. communis* (35.04 \pm 6.73 mole/g of tissue) and Vit C (50 mg/ml) (17.63 \pm 6.07 mole/g of tissue) compared with their respective control normal (NaCl) (64.78 \pm 7.14 mole/g of tissue). From the result of our investigation, *T. communis* has the potential to prevent this cell death due to lipid peroxidation by inhibiting the process [46]. This finding supported reports in the literature that vitamin C is a strong reducer of MDA concentration. Vit C can protect the cell membrane and cytosolic component of cells against the damage of oxidant [48].

Propagative lipid peroxidation is a degenerative process that affects cell membranes and other lipid-containing structures under conditions of oxidative stress [49]. As mentioned above, the observed results may be due to the high polyphenolic content of the plant extract. Accordingly, a lower level of oxidative stress and improvement in antioxidant status was found in many types of research after polyphenol-rich food administration mainly based on the reduction of thiobarbituric acid reactive substance levels (TBARS) [50,51]. According to Soma Gupta *et al.* [52] it was shown that a relationship existed between the administration of a supplement rich in polyphenols and decreased MDA levels.

GSH and catalase

The *in vivo* antioxidant assay showed that the extract increased the activity of GSH and catalase. GSH is an intra-cellular reductant and plays a major role in catalysis, metabolism, and transport. It protects cells against free radicals, peroxides, and other toxic compounds [53]. Catalase is a ubiquitous enzyme that catalyzes the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of both normal aerobic metabolism and pathogenic ROS production [54].

The results showed (table 2) that the GSH determined in three different groups of mice tested, showed that the content of GSH of the untreated mice equal to 3.34 \pm 1.12 $\mu\text{moles}/\text{g}$ tissues increased in the group of mice treated with Vit C (4.79 \pm 1.84 $\mu\text{moles}/\text{g}$ tissues) and significantly in those who were treated by the CE roots of *T. communis* (7.06 \pm 0.30 $\mu\text{moles}/\text{g}$ tissues).

Similar results are observed about the activity of catalase, where the fraction of catalase increased in the mice treated with the CE. It increased from 0.32 \pm 0.14 U/mg protein for the control group to 0.15 \pm 0.03 U/mg protein for the group treated with the CE ($p < 0.001$) and 0.18 \pm 0.01 U/mg protein for the group treated with the Vit C (table 2).

Table 2: The liver tissue antioxidant parameters, effects of CE, Vit C and CTL

	TBARS level (mole/g of tissue)	Activity of GSH (μ moles/g tissues)	Activity of catalase (U/mg protein)
CE	35.04 \pm 6.73	7.06 \pm 0.30	0.15 \pm 0.03
Vit. C	17.63 \pm 6.07	4.79 \pm 1.84	0.18 \pm 0.01
CTL	64.78 \pm 7.14	3.34 \pm 1.12	0.32 \pm 0.14

Data were presented as means \pm SEM (n = 6)

It appears that the increase of the antioxidant capacity of the tissue is probably attributed to elevated levels of exogenous antioxidants such as flavonoids and phenolic compounds, also or other constituents acquired following treatment with the CE of *T. communis*, whose antioxidant properties have already been demonstrated in the *in vitro* assay. This finding supported reports in the literature [55], that The flavonoids rich extract from *R. tomentosum* fruits significantly enhanced the activities of antioxidant enzymes (SOD, CAT and GSH) of mice after they were administered with the extract. The results suggested that the flavonoids rich extract possesses potent antioxidant properties.

Plasma antioxidant capacity

In fact, the plasma contains a network of endogenous antioxidants such as (albumin, bilirubin, reduced glutathione and uric acid) as well as exogenous antioxidants derived from food. These antioxidants may act in a complementary and synergistic manner to provide better protection against ROS. The increase in plasma antioxidant capacity is probably attributed to the elevated levels of exogenous antioxidants acquired following treatment with CE of *T. communis* this is evidenced by the results obtained.

The results of the total antioxidant power activity of the crude extract (CE) of *T. communis*, the Vit C and the normal control (CTL) showed that, the reducing capacity of the *T. communis* with OD value of 1.031 \pm 0.253, was significantly ($p < 0.05$) than that of CTL (0.655 \pm 0.134) but there was no significant difference in the CTL to the total antioxidant power activity of the Vit C. Our results showed that *T. communis* present a strong antioxidant capacity *in vivo* compared to that of *S. sediforme* [44] and of *Anchusa azulea* [56].

In the same contest, the DPPH test showed that the % I of CE (33.20 \pm 2.13 mg/ml) and Vit C (33.55 \pm 2.66 mg/ml) was not significantly different with CTL (32.11 \pm 1.80 mg/ml), CE at a dose of 100 mg/kg-1 per day did not significantly alter the serum DPPH.

Several studies have shown improved antioxidant capacity of plasma after dietary supplementation [57-60] and especially in this study [61], the addition of catechins or tea extract to human plasma and tea consumption by humans were also reported to increase the total antioxidant capacity of plasma. According to *Adefolaju et al.* it was shown that a diets supplemented with *T. triangularis* will improve hepatoprotection against oxidative liver damage.

Blood total antioxidant capacity

These antioxidants are mobilized during an ROS attack induced *ex vivo* by t-BHP, to protect erythrocytes integrity and then delay their hemolysis [44]. From the obtained sigmoid hemolysis curves, it appears that treatment with the CE of *T. communis* and the Vit C causes a delay of hemolysis which is evident in a shift of the curves to the right. The HT₅₀ values reveal an extension of half hemolysis time in treated groups compared with controls (CTL). Increasing the HT₅₀ (HT₅₀ = 75.66 \pm 4.45 mn and 75.33 \pm 6.52mn) of the two groups of mice treated with *T. communis* (100 mg/kg) and Vit C (50 mg/kg) respectively, is statistically not significant compared to the group control (HT₅₀ = 70.66 \pm 6.08mn). These results indicate that a dose of 100 mg/kg of CE of *T. communis* is statically no significant but extrapolation of results obtained *in vivo* is difficult to interpret in the light of the metabolism of antioxidants.

CONCLUSION

This article is focused on *in vitro* and *in vivo* methods of antioxidant evaluation. The *in vitro* methods report that nearly all the extracts/fractions and standard antioxidants used for comparison

studies have clearly showed differences from test to test due to different reaction principles. Nevertheless, the results of scavenging activity on DPPH radicals, reducing power, inhibition of carotene bleaching, and inhibition of lipid peroxidation of all the samples revealed antioxidant activity, and Our study provides the evidence that *T. communis* extracts have strong anti-hemolytic effect results reflecting membrane protection. The *in vivo* assay was performed to confirm this strong activity of the extract.

An *in vivo* approach was conducted in attempting to administer to male mice of CE (100 mg/kg). The analysis of parameters of the antioxidant potential found in hepatocyte a significant decrease in the content of a major product of lipid peroxidation (MDA) in the group of mice treated with the untreated group. The content of GSH and catalase increased much more in the group treated with CE reflecting a better improvement of antioxidant potential than the group treated with Vit C. These endogenous antioxidant enzyme systems play a vital role as enhancers of the immune system and the management of oxidative stress. Plasma antioxidant status and antihemolytic capacity improved slightly in both treatment groups by the CE and Vit C; this indicates that *T. communis* extract can improve the baseline of the defense mechanism against possible oxidative stress, thus decreasing susceptibility to diseases related to oxidative stress as cardiovascular and liver diseases. However, as far as we know this is the first study *in vivo* dealing with roots *T. communis*, and the interpretation of results is difficult because each method has its specificities and limits, and reference values for each parameter are often poorly defined. The broad range of antioxidant activity of extracts indicates the potential of the bark as a source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits. Though other antioxidants are probably present in these extracts/fractions, phenolic compounds might have made a significant contribution to their bioactivity.

In fact, it is very difficult to attribute the antioxidant effect of total extracts to one or a few active principles, because extracts always contains a mixture of different chemical compounds. Besides the major components, minor components may also make a significant contribution to the antioxidant activity of extracts. Further studies are required to gain more insight into the possible mechanisms of action of this plant extract. As a conclusion, this study reveals that *T. communis* is a good candidate for a rich source of natural antioxidant compounds and further studies based on the present results will help to develop the new drugs for antioxidant therapy.

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CONFLICTS OF INTERESTS

Declared none

REFERENCES

1. Cadirci E, Suleyman H, Aksoy H, Halici Z, Ozgen U, Koc A. Effects of *Onosma Armenia* cum root extract on ethanol-induced oxidative stress in stomach tissue of rats. *Chem Biol Interact* 2007;170:40-8.
2. Sofowora A. Research on medicinal plants and traditional medicine in Africa. *J Altern* 1996;2:365-72.
3. Fattahi M, Nazeri V, Torras-Claveria L, Sefidkon F, Cusido RM. Identification and quantification of leaf surface flavonoids in wild-growing populations of *Dracocephalum kotschyi* by LC-DAD-ESI-MS. *Food Chem* 2013;141:139-46.

4. Misbah H, Aziz AA, Aminudin N. Antidiabetic and antioxidant properties of *Ficus deltoidea* fruit extracts and fractions. *BMC Complementary Altern Med* 2013;13:118-29.
5. Roome T, Dar A, Ali S, Naqvi S, Choudhary MI. A study on antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of *Aegiceras corniculatum* (stem) extracts. *J Ethnopharmacol* 2008;118:514-21.
6. Bing Peng, Rui-Feng Bai, Ping Li, Xu-Yang Han, Hong Wang, Chong-Chong Zhu, *et al.* Two new glycosides from *Dryopteris fragrans* with anti-inflammatory activities. *J Asian Nat Prod Res* 2016;18:59-64.
7. Rajalakshmi K, Mohan VR. Antiinflammatory activity of stem and leaf of *Myxopyrum serratum* A. W. Hill (Oleaceae). *Int J Curr Pharm Res* 2016;7:190-2.
8. Murugesan S, Bhuvanewari S. Evaluation of antioxidant activity of methanol extracts of red algae *Chondrococcus hornemannii* and *Spyridia fusiformis*. *Int J Appl Pharm* 2016;5:8-9.
9. Leporatti ML, Ivancheva S. Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy. *J Ethnopharmacol* 2003;87:123-42.
10. Dogan Y. Traditionally used edible wild greens in the Aegean region of turkey. *Acta Soc Bot Pol* 2012;81:329-42.
11. Boumerfeg S, Baghiani A, Messaoudi D, Khennouf S, Arrar L. Antioxidant properties and xanthine oxidase inhibitory effects of *Tamus communis* L. root extracts. *Phytother Res* 2009;23:283-8.
12. Rafael M, Barros L, Carvalho AM, Ferreira ICFR. Topical anti-inflammatory plant species: bioactivity of *Bryonia dioica*, *Tamus communis* and *Lonicera periclymenum* fruits *Industrial Crops Prod* 2011;34:1447-54.
13. Barreira João CM, Pereira E, Dueñas M, Carvalho AM, Santos-Buelga C, Ferreira I CFR. *Bryonia dioica*, *Tamus communis* and *Lonicera periclymenum* fruits: characterization in phenolic compounds and incorporation of their extracts in hydrogel formulations for topical application. *Complete* 2013;49:169-76.
14. Arrar L, Benzidane N, Krache I, Charef N, Khennouf S, Baghiani A. Comparison between polyphenol contents and antioxidant activities of different parts of *Capparis spinosa* L. *Pharmacogn Commn* 2013;3:70-4.
15. Wong SP, Leong LP, William Koh JH. Antioxidant activities of aqueous extracts of selected plants. *Food Chem* 2006;99:775-83.
16. Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, *et al.* Oxygen species scavenging activity of phenolic extract from horwthorn fresh plant organs and pharmaceutical preparation. *Arzneimittel-Forschung/Drug Res* 1996;46:1086-9.
17. Cuendet M, Hostettmann K, Potterat O. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helv Chim Acta* 1997;80:1144-52.
18. Kartal N, Sokmen M, Tepe B, Daferera D, Polissiou M, Sokmen A. Investigation of the antioxidant properties of *Ferula orientalis* L using a suitable extraction procedure. *Food Chem* 2007;100:584-9.
19. Le K, Chiu F, Ng K. Identification and quantification of antioxidants in *Fructus lycii*. *Food Chem* 2007;105:353-63.
20. Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucosamine. *J Nutr* 1986;44:307-15.
21. Girard A, Madani S, Boukourt F, Cherkaoui-Malki M, Belleville J, Prost J. Fructose-aenriched diet modifies antioxidant status and lipid metabolism in spontaneously hypertensive rats. *Nutrition* 2006;22:758-66.
22. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497-509.
23. Hasani P, Yasa N, Vosough-Ghanbari S, Mohammadirad A, Dehghan G, Abdollahi M. *In vivo* antioxidant potential of *Teucrium polium*, as compared to α -tocopherol. *Acta Pharm* 2007;57:123-7.
24. Satho K. Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* 1978;90:37-43.
25. Ellman G. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70-7.
26. Claiborne A. Catalase activity. In: *Handbook of Methods for Oxygene Radical Research*. Greenwald RA. Ed. CRC Press: Boca Raton, FL; 1985. p. 283-4.
27. Manna C, D'angelo S, Migliardi V, Loffredi E, Mazzoni O, Morrira P, *et al.* Protective effect of the phenolic fraction from virgin olive oils against oxidative stress in human cells. *J Agric Food Chem* 2002;50:6521-6.
28. Baghiani A, Boussoulim N, Boumarfeg S, Trabsa H, Aouachria S, Arrar L. *In vivo* free radical scavenging, antihemolytic activity and antibacterial effects of *anchusa azurea* extracts. *Int J Med Res* 2013;46:1502-10.
29. Nur Alam Md, Jahan Bristi N, Rafiquzzaman Md. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharm J* 2013;21:143-52.
30. Küçükboyacı N, Nilüfer N, Dural T, Körorglu A, Ğçcan G, Aydin A. Evaluation of antioxidant and antimicrobial activities of *Tamus communis* L. ssp. *cretica* (L.) Kit tan and its mineral composition. *Turk J Pharm Sci* 2015;12:113-22.
31. Liyana-Pathirana CM, Shahidi F. Antioxydant propreties of commercial soft and hard winter wheats (*Triticum aestivum* L.) and their milling fractions. *J Sci Food Agric* 2006;86:477-85.
32. Sutar C, Kalaichelvan VK. Evaluation of antioxidant activity of leaf extracts of *Holoptelea integrifolia* (Roxb) planch ravindra. *Int J Appl Pharm* 2014;6:3-6.
33. Frankel EN, Meyer AS. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J Sci Food Agric* 2000;80:1925-40.
34. Arulpriya P, Lalitha P, Hemalatha S. *In vitro* antioxidant testing of the extracts of *samanea saman* (Jacq.). *Merr Der Chem Sin* 2010;1:73-9.
35. Tung YT, Wu JH, Huang CY. Antioxidant activities and phytochemical characteristics of extracts from *Acacia confusa* bark. *Bioresour Technol* 2009;100:509-14.
36. Zhao H, Fan W, Dong J, Lu J, Chen J, Shan L, *et al.* Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. *Food Chem* 2008;107:296-304.
37. Andjekovic M, Camp JV, Meulenaer BD, Depaemelaere G, Socaciu C, Verloo M. Iron-chelating properties of phenolic acids berry catechol and galloyl groups. *Food Chem* 2006;98:23-31.
38. Chwalek M, Lalun N, Bobichon H, Ple' K, Voutquenne-Nazabadioko L. Structure-activity relationships of some hederagenin diglycosides: haemolysis, cytotoxicity and apoptosis induction. *Biochim Biophys Acta* 2006;1760:1418-27.
39. Kunwar A, Mishra B, Barik A, Kumbhare LB, Pandey R, Jain VK, *et al.* 3,3_-Diselenodipropionic acid, an efficient peroxy radical scavenger and a GPx mimic, protects erythrocytes (RBCs) from AAPH-induced hemolysis. *Chem Res Toxicol* 2007;20:1482-7.
40. Nabavi SM, Nabavi SF, Eslami S, Moghaddam AH. *In vivo* protective effects of quercetin against sodium fl uoride-induced oxidative stress in the hepatic tissue. *Food Chem* 2012;132:931-5.
41. Sasikumar JM, Maheshu V, Smilin AG, Gincy MM, Joji C. Antioxidant and antihemolytic activities of common Nilgiri barberry (*Berberis tinctoria* Lesch.) from south India. *Food Res J* 2012;19:1601-7.
42. Zhang A, Zhu QY, Luk YS, Ho KY, Fung KP, Chen Z. Inhibitory effects of jasmine green tea epicatechin isomers on free radical-induced lysis of red blood cells. *Life Sci* 1997;61:383-94.
43. De-Qianga D, Zheng X, Guanga Y, Jian Feng Z, Ying-Kun Q, Jing-Xian Y, *et al.* Prediagnostic methods for the hemolysis of herbal medicine injection. *J Ethnopharmacol* 2011;138:445-50.
44. Trabsa H, Boumarfegue S, Baghiani A, Naoual Boussoulim, Krache I, Khennouf S, *et al.* Anti-haemolytic, antioxidant and xanthine oxidase inhibitory effect of sedum sediforme shoot extracts. *J Med Plants* 2014;47:1502-10.
45. Amacher DE. A toxicologist's guide to biomarkers of hepatic response. *Hum Exp Toxicol* 2002;21:253-62.
46. Pandey KB, Rizvi SI. Markers of oxidative stress in erythrocytes and plasma during aging in humans. *Oxid Med Cell Longevity* 2010;3:2-12.
47. Devaki T, Raghavendran HRB, Sathivel A. Hepatoprotective nature of alcoholic seaweed extract on acetaminophen-induced hepatic oxidative stress. *J Health Sci* 2004;50:42-6.
48. Sapakal VD, Shikalgar TS, Ghadge RV, Adnaik RS, Naikwade NS, Magdum CS. *In vivo* screening of antioxidant profile: a review. *J Herb Med Toxicol* 2008;2:1-8.
49. Najeeb Q, Bhaskar N, Masood I, Wadhwa S, Kaur H, Ishaq S. Malondialdehyde (MDA) superoxide dismutase (SOD) levels-

- distinguishing parameters between benign, malignant pleural effusions. *Free Radicals Antioxid* 2012;2:8–11.
50. Boaventura BCB, Di Pietro PF, Klein GA, Stefanuto A, de Morais EC, de Andrade F, *et al.* Antioxidant potential of mate tea (*Ilex paraguariensis*) in type 2 diabetic mellitus and prediabetic individuals. *J Funct Foods* 2013;5:1057–64.
 51. Athiroh N, Permatasari N, Sargowo D, Widodo MA. Antioxidative and blood pressure lowering effects of *Scurrula atropurpurea* on deoxycorticosterone acetate-salt hypertensive rats. *BGM* 2014;6:32-6.
 52. Gupta S, Reddy MV, Harinath BC. The role of oxidative stress and antioxidants in aetiopathogenesis and management of oral submucous fibrosis. *Indian J Clin Biochem* 2004;19:138-41.
 53. Aydemir T, Öztürk R, Bozkaya LA. Effects of antioxidant vitamins A, C, E and trace elements Cu, Se on CuZnSOD, GSHPx, CAT and LPO levels in chicken erythrocytes. *Cell Biochem Funct* 2000;18:109-15.
 54. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* 2002;30:620–50.
 55. Wu P, Ma G, Li N, Deng Q, Yin Y, Huang R. Investigation of *in vitro* and *in vivo* antioxidant activities of flavonoids rich extract from the berries of *Rhodomyrtus tomentosa* (Ait.) Hassk. *Food Chem* 2015;173:194-202.
 56. Baghiani A, Boussoualim N, Boumerfeg S, Trabsa H, Aouachria S, Arrar L. *In vivo* free radical scavenging, antihemolytic activity and antibacterial effects of *anchusa azurea* extracts. *Int J Med Med Sci* 2013;46:1113-8.
 57. Gladine C, Morand C, Rock E, Gruffat D, Bauchart D, Durand D. The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. *Anim Feed Sci Technol* 2007;139:257–72.
 58. Oliveira C, Amaro LF, Pinho O, Ferreira IM. Cooked blueberries: anthocyanin and anthocyanidin degradation and their radical-scavenging activity. *J Agric Food Chem* 2010;58:9006–12.
 59. Vicente SJ, Ishimoto EY, Torres EA. Coffee modulates the transcription factor Nrf2 and highly increases the activity of antioxidant enzymes in rats. *J Agric Food Chem* 2014;12:116–22.
 60. Avila-Nava A, Calderón-Oliver M, Medina-Campos ON, Zou T, Gu L, Torres N, *et al.* Extract of cactus (*Opuntia ficus indica*) cladodes scavenges reactive oxygen species *in vitro* and enhances plasma antioxidant capacity in humans. *J funct foods* 2014;10:13–24.
 61. Bors W, Michel C, Stettmaier K. Structure–activity relationships governing antioxidant capacities of plant polyphenols. *Methods Enzymol* 2001;335:166–80.
 62. Adefolaju G, Ajao M, Olatunji L, Enaibe B, Musa M. Hepatoprotective effect of aqueous extract of water leaf (*Talinum Triangulare*) on carbon tetrachloride (CCL4) induced liver damage in wistar rats. *Int J Pharm Appl* 2008;8:1-6.
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