BJP

Bangladesh Journal of Pharmacology

Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2016; 11: 301-307
ISSN: 1991-0088

Phytochemically evaluation and net antioxidant activity of Tunisian *Melia azedarach* leaves extract from their ProAntidex parameter

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Article Info

Received: 16 December 2015
Accepted: 14 January 2016
Available Online: 15 March 2016

DOI: 10.3329/bjp.v11i2.25981

Cite this article:
Akacha M, Karima Lahbib, Boughanmi NG. Phytochemically evaluation and net anti-oxidant activity of Tunisian *Melia azedarach* leaves extract from their ProAntidex parameter. Bangladesh J Pharmacol. 2016; 11: 301-07.

Abstract

Phytotherapy is a discipline which is interested in the design, preparation and interpretation of Structure Activity Relationship of the natural bioactive molecules. In this context, ethanolic leaves extract of *Melia azedarach* was phytochemically analyzed on the bases of HPLC and GC–MS. Extract was tested for *in vitro* anti-oxidant activities by 1,1-diphenyl-2-picrylhydrazyl (DPPH), H_2O_2, hydroxyl radical scavenging activity, Ferric Reducing Power (FRP) and ferrous ion chelating abilities methods. The anti-oxidant activity of the extract was analyzed simultaneously with their pro-oxidant capacity. The ratio of pro-oxidant to the anti-oxidant activity (ProAntidex) represents a useful index of the net free radical scavenging potential of the synthesized compounds. Tested extract showed significant anti-oxidant activity with a moderate ProAntidex.

Introduction

Nowadays, an increasing demand to evaluate the antioxidant properties of plant extracts (McClements and Decker, 2000) have been observed for their safe use for scientific research as well as for industrial purposes excluding many synthetic antioxidants which have possible activity as promoters of carcinogenesis. Phenolic compounds have been found to be strong antioxidants able to scavenge or suppress ROS and RNS formation by inhibiting some enzymes or chelating metals involved in free radical production, and upregulate or protect antioxidant defense, thereby preventing carcinogenesis (Gouvêa, 2004; Halliwell, 2007; Huang et al., 2010). Among the numerous families with recognized botanical pharmacological activities, those belonging to *Meliaceae* family deserve mention. *Melia azedarach* L. an invasive deciduous tree is one of species belonging to *Meliaceae* family. Although native to India and China, it is found in Africa, Australia, America and Arab countries (Khan et al., 2011). This tree is widespread in Tunisia as an ornamental shade tree characterized by abundant foliage and it will be interesting if it will successfully managed.

Leaves are used for extraction since they are known to have many biological activities: antidiarrheal, de-obstruent and diuretic (Joy et al., 1998). They are also used to treat skin diseases like scabies and for brushing teeth, loosening or pain of tooth, rheumatic pain, and fever and as insecticidal (Rahmatullah et al., 2010). Furthermore, they are applied externally on burns, used as mouth wash for gingivitis; pyrexia and bloody piles hysteria (Hussain et al., 2008, Kaneria et al., 2009) snake bite, (Handa et al., 2006) diabetes, cure pimples and blood purifier (Sultana et al., 2011). Moreover, chemical composition and bioactivity differences between species naturalized in different regions have been observed (Gottlieb et al., 2001; Szewczuk et al., 2003, Orhan et al., 2012) so phytochemical investigation of ethanolic Tunisian *M. azedarach* leaves extract was conducted by the quantification of phenolics and fatty acid profiles achieved by respectively high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) as well as antioxidant activities.

Materials and Methods

Plant materials and extraction

M. azedarach mature leaves were collected in July and August from Bizerte, North of Tunisia Lat. (37.27 N, Long. 9.87 E), and shade dried. Then these were powdered in an electric grinder.

Powder was extracted with absolute ethanol (20 g/100 mL) using a soxhlet apparatus for 12 hours.

Collected solution was filtered through Whatman No. 1 filer paper. The alcoholic extract was evaporated to dryness under reduced pressure using rotary vacuum evaporator R-114 (Buchi, France). *M. azedarach* leaves extract was conserved in dark place and at -4°C until use. Dry fraction of alcoholic extract was weighed and its yield was 14% w/w with respect to dried powder.

Phytochemical study of M. azedarach leaves ethanolic extract

Estimation of total phenolic content

Total phenolic contents of the extract were determined using Folin-Ciocalteu reagent (Falleh et al., 2011). Gallic acid was used to calibrate the standard curve. The extract was analyzed in triplicate and the results were expressed in milligrams of gallic acid equivalents per gram of dry weight (mg GAE /g).

Estimation of total flavonoid content

Total flavonoids content were estimated as Falleh et al. (2011). Absorbance was determined at 510 nm and flavonoid concentration was calculated according to the equation obtained from (+) catechin graph and was expressed as mg catechin equivalents per gram dry weight (mg CE/g).

Determination of proanthocyanidines contents

The proanthocyanidines content was determined according to the method of Falleh et al. (2011). 50 µL AIE were mixed with 3 mL vanillin (4%; w/v). Then, 1.5 mL 12M hydrochloric acid was added to the mixture. After incubation for 15 min at 25°C, absorbance was measured at 500 nm. Results were expressed as mg Catechin/g of dry weight (mg C/g).

HPLC conditions for phenolic compounds analysis

The identification of phenolic compounds was done using HPLC system (consisting of a vacuum degasser, an auto sampler, and a binary pump with a maximum pressure of 600 bar; Agilent 1260, Agilent technologies, Germany) equipped with a reversed phase C18 analytical column of 4.6 x 100 mm and 3.5 µm particle size (Zorbax Eclipse XDB C18). The DAD detector was set to a scanning range of 200-400 nm. Column temperature was maintained at 25°C. The injected sample volume was 2 µL and the flow rate of mobile phase was 0.4 mL/min .Mobile phase B was milli-Q water consisted of 0.1% formic acid and mobile phase A was methanol.

GC–MS conditions for fatty acid analysis

GC analysis was performed on HP 5MS Agilent 6890 column (length 30 m, diameter 0.25 mm, film thickness 0.25 µm, 5 mol% phenylmethylpolysiloxane) Agilent part number 1991S-433 under the following conditions; a volume of 1 µL of each extract was injected, splitless into the column, in the constant flow mode with 70ev ionization energy. The carrier gas was helium. GC oven temperature started at 50°C to and was held for 2 min at 270°C. The injector and detector temperatures were set at 270 and 280°C, respectively. The mass range was scanned from 50 to 550 amu.

Identification of the components was assigned by comparison of their retention times and mass spectra by comparison of their mass spectra with Wiley and Nist libraries.

Evaluation of antioxidant activity of extracts

DPPH assay

The reduction capability of DPPH radical is determined by the decrease in absorbance at 517 nm induced by anti-oxidants as described by Braca et al. (2001). Ascorbic acid was the reagent used as standard. Experimentally, various dilutions of the ethanolic solution of extract or standard (0.01-1 mg/mL, in triplicate) were added to DPPH solution (0.035 mg/mL). The absorbance of the mixture was read at 517 nm with ethanol as blank. A control sample with no added test compounds was also analysed. Radical scavenging activity was expressed as a percentage and calculated using the formula: $\%Scavenging = [(A_{cont} - A_{test}) / A_{cont}] \times 100$

Where, A_{cont} is the absorbance of the control, and A_{test} is the absorbance of the sample in the presence of test-extract

The result was presented as IC_{50} (The concentration of test extract required for scavenging 50% of the DPPH radical).

H_2O_2 scavenging activity

The ability to scavenge hydrogen peroxide was determined according to the method of Yen and Chen. (1995). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4), then an ethanolic solution of the test-extract or standard at various concentrations (0.01–1 mg/mL) was added. Absorbance of hydrogen peroxide at 230 nm was determined 19 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging was calculated from the equation: $\%\ Scavenging = [(A_{cont} - A_{test}) / A_{cont}] \times 100$

Where, A_{cont} is the absorbance of the control, and A_{test} is the absorbance of the sample in the presence of test –extract or standard

The result was presented as IC_{50} (The concentration of

test extract required for scavenging 50% of the H_2O_2 stable ROS).

OH· scavenging activity

OH· scavenging activity was measured by the deoxyribose method (Halliwell et al., 1987) and compared with that of ascorbic acid. The OH^- scavenging activity of ethanolic extract was carried out by measuring the competition between deoxyribose and the compounds that generate hydroxyl radicals from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. Attack of the hydroxyl radicals on deoxyribose led to formation of thiobarbituric acid-reactive substances (TBARS) which were measured by the method of Ohkawa et al. (1979). The OH^- scavenging activity was calculated from the equation: OH^- scavenged (%) = ((Acont –Atest) / A cont)* 100

Where, A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample

The result was presented as IC_{50} (the concentration of test extract required for scavenging 50% of the OH^- radical).

Ferrous ion chelating ability (FIC) (**Video clip 1 2 3**)

The FIC ability of ethanolic extract was determined according to the method of Singh and Rajini. (2004) and compared with that of ascorbic acid. Extract at various concentrations (0.01-1 mg/mL) was added to $FeSO_4$ (0.1 mM) and ferrozine (0.25 mM). The tubes were shaken well and left to stand for 10 min. The absorbance was measured at 562 nm. The ability of each sample to chelate ferrous ions was calculated relative to the control consisting of only iron ferrozine, using the following formula:

% FIC = $[(A_{cont} - A_{test}) / A_{cont}] \times 100$

Where, A_{cont} is the absorbance of the control, and A_{test} is the absorbance of the sample in the presence of test compound

The result was presented as IC_{50} (The concentration of test extract required for chelate 50% of the iron ion).

Ferric reducing power (FRP)

The reducing power of the extract was determined according to the method of Oyaizu (1986). The solution of compound (1 mL) at various concentrations (0.01-1 mg/mL) was mixed with 2.5 mL of phosphate buffer (0.2 M) and 2.5 mL of 1% potassium ferricyanide and incubated at 50°C for 20 min. To this mixture, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3,000 rpm for 20 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride and the UV absorbance was measured at 700 nm using a spectrophotometer. Increase of absorbance of the reaction mixture indicates increase in reducing power. The percentage increase in reducing power was calculated relative to the control prepared without adding test compound, using the following formula:

Increase in reducing power (%) = $[(A_{test} - A_{cont}) / A_{cont}] \times 100$

Where, A_{test} is the absorbance of the sample and A_{cont} is the absorbance of the control. Index = IC_{50} (mg/mL) reducing power/IC_{50} (mg/mL) DPPH scavenging assay

Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Statistical differences were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's HSD test when significant differences were observed ($p<0.05$).

All analyses were performed using STATISTICA version 5.00 (Stat Soft- France, Tulsa, OK, USA) for Windows.

Results

Phytochemical analyze of ethanolic extract

Data demonstrate that *M. azedarach* leaves ethanolic extract contain 93.40 mg GA/ g DW of total phenols. It was found that extract was more rich in flavonoids (77.0 ± 5.118 mg C/g DW) than tannins (15.0 ± 0.6 mg C/g DW).

Identification of phenolic compounds by HPLC

The results obtained revealed eight phenolic compounds which were phenolics acids and flavonoids as shown in Figure 1 and Table I. The major compound was one of the main catechin metabolites dihydroxybenzoic acid commonly known as protocatechic acid (72.2%) then respectively the flavonoid isoquercitrin (23.5%), syringic acid (gallic acid derivate, 1.4%), chlorogenic (hydroxycinnamic acid; ester of caffeic and quinic acids, 1%) and sinapic acids (hydroxycinnamic

Table I

Identification and quantification of phenolic compounds

Retention time (min)	Identification	Quantification (µg/mL)
7.95	Gallic acid	2.6
14.93	Catechine hydrate	29.3
15.33	Chlorogenic acid	5.7
17.55	Syringic acid	3.2
19.96	Sinapic acid	6.0
21.79	Protocatechuic acid	120.9
21.96	Isoquercitrin	608.2
25.67	Isorhamnetin	0.3

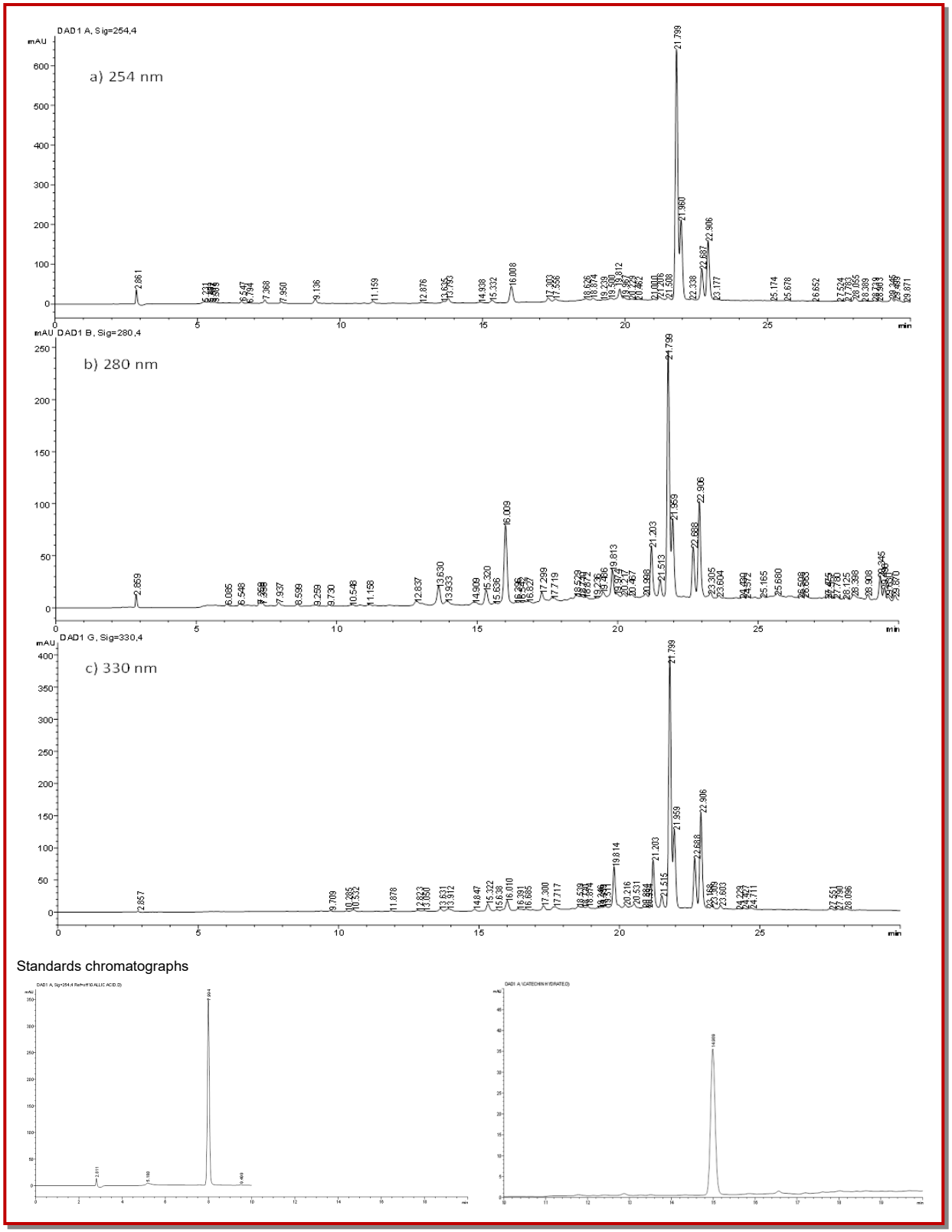

Figure 1: chromatograms of identified and quantified phenolic compounds

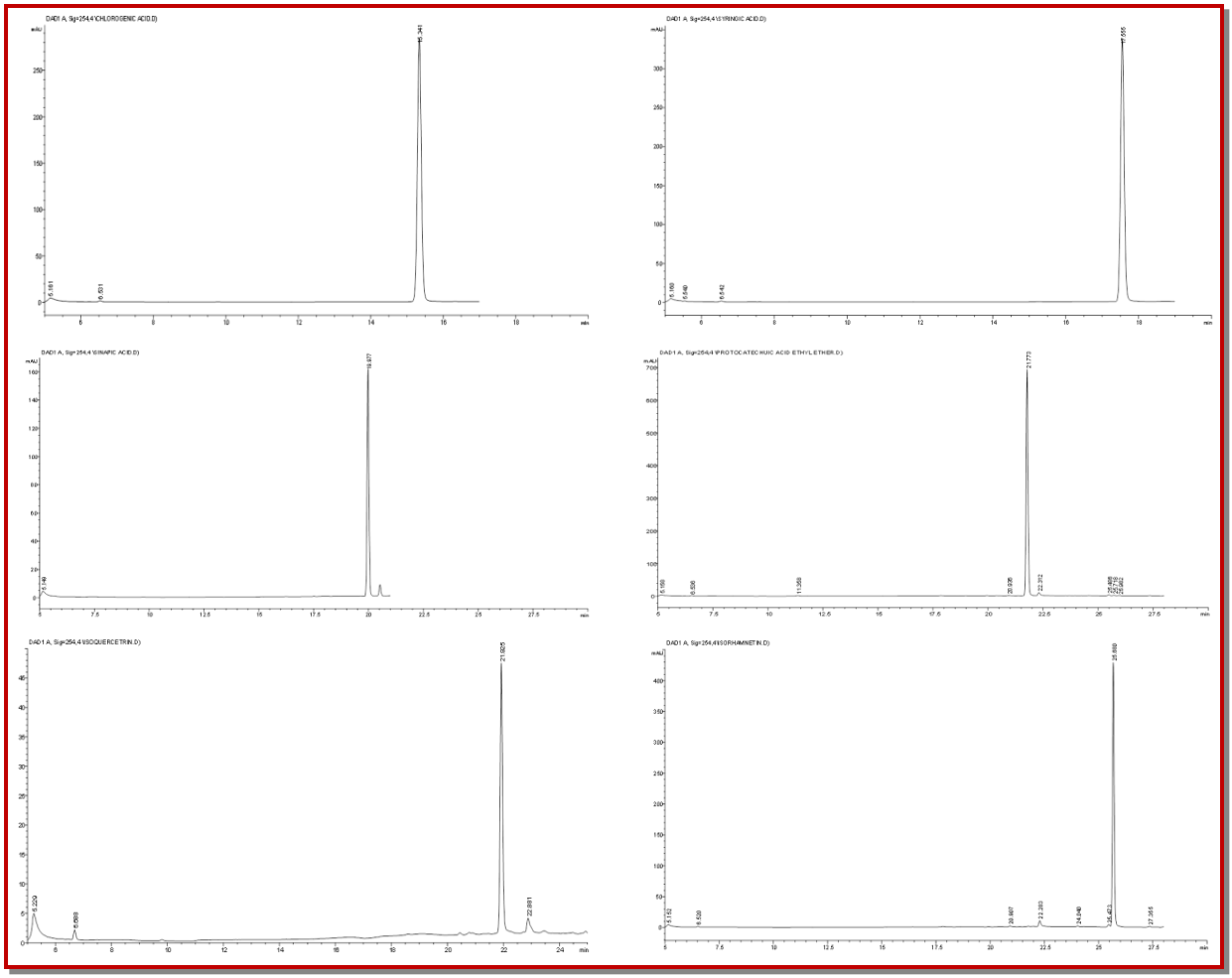

Figure 1: Chromatograms of identified and quantified phenolic compounds (Cont.)

Table II

Fatty acids composition of the extract

Ethanolic extract fatty acid	Retention time (min)	Area (%)
Stearic acid (C18:0)	35.12	32.6
Palmitoleic acid (C16:1)	34.65	30.6
Palmitic acid (C16:0)	31.48	25.5
Oleic acid (C18:1)	34.77	10.5
Linoleic acid (C18:2)	34.54	0.3
Myristic acid (C14:0)	27.53	0.3
Margaric acid (C17:0)	33.33	0.1
Nonadecanoic acid (C19:0)	38.45	0.1

acid, 0.7%), gallic acid (phenolic acid, 0.5%) and finally the flavonoid catechine hydrate (0.5%).

Identification of fatty acids by GC-MS

The analysis of ethanolic leaves extract of *M. azedarach* by gas chromatography revealed higher amount of fatty acids (Table II). Higher amounts of stearic acid (32.6%) followed by palmitic acid (25.5%) were found as saturated fatty acids. Among unsaturated fatty acids, palmitoleic, linolenic acid (30.6%), followed by oleic acid (10.5%) and linoleic acid (0.3%) were found.

Biological investigation

The ethanolic extract exhibited an appreciate anti-oxidant capacity (Table III). Results shown that the extract exhibited a DPPH scavenging activity with an IC_{50} at 1.0 ± 0.05 mg/mL. Similarly, the H_2O_2 scavenging activity was set at IC_{50}: 0.5 ± 0.006 mg/mL and the $OH^{\cdot}$ at 1.2 ± 0.2 mg/mL. The extract exhibited moderate reducing and chelating activities with IC_{50} at 1.2 ± 0.2 and 0.2 ± 0.02 respectively. All antioxidant activities were been lower than that of ascorbic acid.

Evaluation of prooxydant activity of extracts

In plant pro-oxidant and antioxidant effects are due to the balance of free radical scavenging activity and reducing power of iron ions which might drive the Fenton reaction. In this reaction, Fe^{2+} react with H_2O_2

Table III

ProAntidex was devised using the ratio of pro-oxidant to the antioxidant activities

	Extract (IC_{50}, mg/mL)	Vitamin C (IC_{50}, mg/mL)
	1.0 ± 0.1	0.04 ± 0.00
H_2O_2 scavenging activity	0.5 ± 0.0	0.06 ± 0.00
OH· scavenging activity	1.2 ± 0.2	0.01 ± 0.00
FIC ability	1.2 ± 0.2	0.01 ± 1.02
Reducing power activity	0.2 ± 0.0	0.00 ± 0.00
ProAntidex	0.1 ± 0.0	0.09 ± 0.01

resulting in production of OH· that is the most harmful ROS. The predominance of reducing power over the free radical scavenging activity result in the pro-oxidant effect (Karima et al., 2015). More the index was lower, more the extract was an antioxidant than pro-oxidant. The extract exhibited a moderate proantidex compared to ascorbic acid.

Discussion

In the present study, we focus on phytochemically, phenolics profile and fatty acids composition of ethanolic leaves *M. azedarach* extract naturalised in Tunisia and its probable correlations with their anti-oxidant activity. *M. azedarach* leaves ethanolic extract was screened for their antioxidant activity with 1,1-diphenyl-2-picrylhydrazyl (DPPH), H_2O_2 and OH•-scavenging activities, ferric reducing power (FRP) assay and ferrous ion chelating (FIC) methods. Based on profile of phenolics, we speculate that antioxidant activity of *M. azedarach* extract may be partly related to their phenolic acids content/composition such as chlorogenic, gallic protocatechuic and sinapic acids and flavonoids: Catechine hydrate, isoquercitrin and isorhamnetin.

In fact, Siger et al. (2013) and Gaspar et al. (2010) showed antioxidant activity of sinapic acid as one of the four most common hydroxycinnamic acids and its derivatives. Further, Mehra et al. (2013) found that (+)-catechin hydrate has antioxidant activity and is effective in reducing oxidative stress. The major phenolic component in *M. azedarach* leaves extract which is protocatechuic acid (PCA) has antioxidant action as proven by Li et al. (2011). The flavonoid isorhamnetin isolated from sea buckthorn marc possess antioxidant properties as shown by Pengfei et al. (2009) as well as the flavonoid isoquercitrin isolated from *Camellia sasanqua* (Sukito and Tachibana, 2014). Vellosa et al. (2011) investigated among others isoquercitrin anti-oxidant and cytotoxic activities. Besides, Oleic and linoleic acids as well as their derivatives were reported to exert potent antioxidant effect in different assays (El-Din et al., 2007; Hur et al., 2007; Orhan et al., 2011).

With regard to the mechanism of antioxidant activity, one can speculate that *M. azedarach* ethanolic leaves extract act as direct antioxidant agent by the *scavenging* activity of radicals which may be attributable to its hydrogen-donating ability (Chen and Ho, 1995; Gordon, 1990), Moreover, *M. azedarach* ethanolic leaves extract as iron chelator, exerts a secondary antioxidant effect by chelating the bivalent iron necessary for the formation of hydroxyl radicals in the Fenton reaction (Dombrecht et al., 2004).

These results suggested that *M. azedarach* leaves extract exhibited an antioxidant activity may be by both chelating metal transition ions as well as by scavenging free radicals. Observed biological activities could be attributed not solely to the studied phenolic contents and/or fatty acids profile, but might be attributable to other unidentified substances or to synergistic interactions between identified ones.

Conclusion

The current study made the link between phytochemically composition of plants extract and oxidative stress. The results emphasize the ability of *M. azedarach* ethanolic leaves extract, as scavenger and iron chelator, to exert direct and secondary antioxidant effect by preventing the Fenton reaction.

Financial Support

Self-funded

Conflict of Interest

Authors declare no conflict of interest

References

Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Anti-oxidant principles from *Bauhinia terapotensis*. J Nat Prod. 2001; 64: 892–95.

Chen CW, Ho CT. Anti-oxidant properties of polyphenols extracted from green and black tea. J Food Lipids. 1995; 2: 35–46.

Dombrecht EJ, Cos P, Vanden Berghe D, Van Offel JF, Schuerwegh AJ, Bridts CH, Stevens WJ, De Clerck LS. Selective *in vitro* anti-oxidant properties of bisphosphonates. Biochem Biophys Res Commun. 2004; 314: 675–80.

El-Din B, Nariman K, Stanley T. Concentration-dependent anti-oxidant activities of conjugated linoleic acid and α-tocopherol in corn oil. J Sci Food Agric. 2007; 87: 2715-20.

Falleh H, Ksouri R, Medini F, Guyot S, Abdelly C, Mange C.

Anti-oxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule* L. Ind. Crops Prod. 2011; 34: 1066-71.

Gaspar A, Martins M, Silva P. Dietary phenolic acids and derivatives: Evaluation of the anti-oxidant activity of sinapic acid and its alkyl esters. J Agric Food Chem. 2010; 58: 11273–80.

Gordon MH. The mechanism of the anti-oxidant action *in vitro*. In: Food anti-oxidants. Hudson BJF (eds.). 1990, pp 1-18. Elsevier, London.

Gouvêa CMCP. Oxidações biológicas e atividade anti-oxidante de compostos de origem vegetal. In: Carvalho JCT (Ed.), Fitoterápicos Anti-inflamatórios: Aspectos Químicos, Farmacológicos e Aplicações Terapêuticas. Tecmedd, Ribeirão Preto, 2004.

Halliwell B. Biochemistry of oxidative stress. Biochem Soc T. 2007; 35: 1147–50.

Handa SS, Rakesh DD, Vasisht K. Compendium of medicinal and aromatic plants Asia. 2006, pp 73-74.

Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. Nutr Cancer. 2010; 62: 1–20

Hur, SJ, Park, GB, Joo ST. Biological activities of conjugated linoleic acid (CLA) and effects of CLA on animal products. Livestock Sci. 2007; 110: 221-29.

Husain SZ, Malik RN, Javaid M, Bibi S. Ethonobotanical properties and uses of medicinal plants of Morgah biodiversity park, Rawalpindi. Pakistan J Bot. 2008; 40: 1897-911.

Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal plants. Kerala Agriculture University. Aromatic and medicinal plants research station. 1998, pp 193-94.

Kaneria M, Baravalia Y, Vaghasiya Y, Chanda S. Determination of antibacterial and anti-oxidant potential of some medicinal plants from Saurashtra region, India. Indian J Pharm Sci. 2009; 71: 406–12.

Karima Lahbib, Mohamed Tarhouni, Soufiane Touil. Evaluation of net anti-oxidant activity of mono- and bis-mannich base hydrochlorides and 3-keto-1,5-bisphosphonates from their ProAntidex parameter. J Mol Struct. 2015 ; 1091: 152–58.

Khan AV, Ahmed QU, Mir MR, Shukla I, Ali AK. Antibacterial efficacy of the seed extracts of *Melia azedarach* against some hospital isolated human pathogenic bacterial strains. Asian Pac J Trop Biomed. 2011; 1: 452–55.

Li X, Wang X, Chen D, Chen S. Anti-oxidant activity and mechanism of protocatechuic acid *in vitro*. Funct Food Health Dis. 2011; 1: 232–44.

Ling LT, Yap SA, Radhakrishnan AK, Subramaniam T, Cheng HM, Palanisamy UD. Standardized *Mangifera indica* extract is an ideal anti-oxidant. Food Chem. 2009; 113: 1154–59.

McClements DJ, Decker EA. Lipid oxidation in oil-in water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J Food Sci. 2000; 65: 1270–82.

Mehra P, Garg M, Koul A, Bansal DD. Effect of (+)-catechin-hydrate on oxidative stress induced by high sucrose and high fat diet in male Wistar rats. Indian J Exp Bot. 2013; 51: 823-27.

Orhan I, Ozcelik B, Şener B. Evaluation of antibacterial, antifungal, antiviral, and anti-oxidant potentials of some edible oils and their fatty acid profiles. Turk J Biol. 2011; 35: 251-58.

Orhan IE, Guner E, Ozturk N, Senol FS, Erdem SA, Kartal M, Sener B. Enzyme inhibitory and anti-oxidant activity of *Melia azedarach* L. naturalized in Anatolia and its phenolic acid and fatty acid composition. Indian Crops Prod. 2012; 37: 213-18.

Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr. 1986; 44: 307–15.

Pengfei L, Tiansheng D, Xianglin H, Jianguo W. Anti-oxidant properties of isolated isorhamnetin from the sea buckthorn marc. Plant Foods Hum Nutr. 2009; 64: 141–45.

Rahmatullah MM, Khatun A, Morshed N, Neogi PK. A randomized survey of medicinal plants used by folk medicinal healers of Sylhet division, Bangladesh. Adv Nat App Sci. 2010; 4: 52-62.

Siger A, Czubinski J, Dwiecki K, Kachlicki P, Nogala-Kalucka M. Identification and anti-oxidant activity of sinapic acid derivatives in *Brassica napus* L. seed meal extracts. Eur J Sci Lipid Tech. 2013; 115: 1130–38.

Singh N, Rajini PS, Free scavenging activity of an aqueous extract of potato peel. Food Chem. 2004; 85: 611-16.

Sukito A, Tachibana S. Isolation of hyperoside and isoquercitrin from *Camellia sasanqua* as anti-oxidant agents. Pakistan J Biol Sci. 2014; 17: 999-1006.

Sultana S, Khan MA, Ahmad M, Bano A, Zafar M, Shinwari ZK. Authentication of herbal medicine neem (*Azadirachta indica* A. Juss.) using taxonomic and pharmacognostic techniques. Pakistan J Bot. 2011; 43: 141-50.

Szewczuk VD, Mongelli ER, Pomilio AB. Antiparasitic activity of *Melia azedarach* growing in Argentina. Mol Med Chem. 2003; 1: 54-57.

Vellosa JCR, Regasini LO, Khalil NM, Da Silva Bolzani V, Khalil OAK, Manente FA, Netto HP, de Faria Oliveira OMM. Anti-oxidant and cytotoxic studies for kaempferol, quercetin and isoquercitrin Ecl. Quím., São Paulo. 2011, pp 7-20.

Yen GC, Chen HY. Anti-oxidant activity of various tea extracts in relation to their anti-mutagenicity. J Agric Food Chem. 1995; 43: 27-32.

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