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Characterization of Antioxidant Activities of Wild Hawthorn Fruits Growing in Lebanon

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Abstract: Hawthorn fruits and flowers have been traditionally and clinically used in the treatment of cardiovascular irregularities with minimal side effects. Hawthorne extracts are thought to exert there beneficial effects largely due to their antioxidant capabilities. The pharmacological effects of *Crataegus* have mainly been attributed to their polyphenolic contents. To the best of our knowledge there has been no characterization of such capabilities in the hawthorn plants in the Middle East region. For this purpose, ripe fruits (red) from wild trees growing in a mountainous region of Lebanon were chosen for this study. The fruits were extracted with ethanol (80%) and following complete evaporation of alcohol, the extract was suspended in water and frozen until used. The extract (H-1) exhibited a total antioxidant capacity of EC_{50} = 0.16mg mL^{-1}. It also possessed free radical scavenging activities against the super-oxide anion and diphenylpicrlyhydrazyl (DPPH) generation of EC_{50} = 0.1 mg mL^{-1} and 1.0 mg mL^{-1}, respectively. H-1 was able to demonstrate effective protection against Fe^{+3}-induced lipid per-oxidation in human blood plasma mounting to 37.6±8.1 % in the concentration range of 0.14-1.0 mg mL^{-1}. Hence, the hawthorn source we used is generally similar in its qualities to others growing in other parts of the world.

Key words: hawthorn, middle east, fruits, antioxidants, characterization

INTRODUCTION

Hawthorn (*Crataegus oxycanatha*), is a member of the Rosaceae family[1]. It is a fruit-bearing plant that grows in Europe, Asis and the Eastern parts of North America. Its fruits are yellow, yellow-green, orange, or red berries, with the latter being the most common. Hawthorn has a long history of use in traditional medicine in China and Europe for the treatment of irregular heartbeat, high blood pressure, chest pain, hardening of arteries and congestive heart failure with minimal side effects[1]. Clinically, hawthorn extracts have been used to treat early stages of congestive heart failure[2-4], angina pectoris[5], hypertension and hypercholestemia[6]. The fruits reduce the formation of atheroma and thrombosis by significantly inhibiting thromboxane biosynthesis and platelet adhesion[7].

The main constituents of Hawthorn fruits are falvonoids (hyperoside, quercetin, quercitrin, hyperfine), flavon-C-glycosides (vitexin, isovetixin, orientin, isooerientin), catechins, amines, triterpene saponins and oligomeric procyanidins[8-11]. The pharmacological effects of Hawthorn is generally related to its flavonids and oligomeric proantho-cyanidin content, namely seven of which possess antioxidant activities. They are hyperoside, isoquercitin, epicatechin, chlorogenic acid, quercetin, rutin and protocatechuic acid[12,13]. Such antioxidant capabilities can reduce the production of free radicals, protecting against the subsequent damage to tissues and/or vascular systems[12].

Although wild Hawthorn plants grow relatively in abundance in the Middle East region, their fruits are not widely consumed. In addition and to the best of our knowledge, there are no published studies on the constituents of their parts (leaves, fruits, barks, or flowers). The present study, therefore, was carried out to characterize the antioxidant capabilities in fruits harvested from Hawthorn grown in the region and eventually to incite research in the area concerning this botanical plant.

MATERIALS AND METHODS

Preparation of extract (H-1): Ripe fresh (red) hawthorn fruits were picked from the mountain area around the city of Sh-heem, approximately 30 kilometers south of Beirut, Lebanon. Samples were immediately frozen at -20°C and shipped within one week to our laboratory at the Qassim University, Kingdom of Saudi Arabia where they were kept at -20°C. Three weeks later, plants were thawed and seeds were removed. The fruit flesh (95 g) was ground in coffee grinder, followed by extraction with 3 volumes of 80% ethanol for 2 h at room temperature while on a shaker. The residue was re-extracted twice; the first was the same as the aforementioned extraction and the

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second using 300 mL of 80% ethanol for 2 h. All extracts were pooled, filtered and then evaporated giving 20% yield. The extracted material was suspended in water followed by rotational shaking for 15 min to give a final stock concentration of 346.

Assays: Free radical scavenging activities of the H-1 extract against the super-oxide anion and its total antioxidant status were measured using commercial kits from Randox (Randox, Crumlin, Co. Antrium, UK). The latter is proportional to the concentration of antioxidants in the assayed extract[14]. The scavenging activity of the H-1 extract against the free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was performed using a 96-well microtitre procedure[15,16]. Each well contained 150 µL ethanol solution of DPPH (400 μM) and 50 of H-1 extract solution at the appropriate concentrations. Controls were prepared with 150 µL ethanol and water. The plates were incubated at 37°C for 30 min and the absorbance was measured at 515 nm using a microplate reader (Molecular Devices $Versa_{max}$, Sunnyvale, California, USA).

In order to measure the effects of H-1 extract on lipid peroxidation, blood was collected from apparently healthy young volunteers into EDTA-blood collection tubes, then centrifuges at 1,500 x g for 10 min and at 4°C. Ferric chloride at final concentration of 5.0 mM was used to induce lipid peroxidation in the freshly prepared plasma in the presence and absence of known concentrations of H-1 extract. The reaction time was 30 min at 37°C. Fe3+-induced lipid-peroxidation measurements were based upon quantifying the concentration of thiobarbituric-reactive species (TBARS) using Oxi-Tek assay kit (ZeptoMetrix, Buffalo, NY, USA). The flavonoid antioxidant, quercetin, was used as a positive control.

The % degree of protection, radical scavenging or suppression is calculated from the equation:

$$\% = [A_0 - (A_1/A_0)] \times 100$$

Where, A_0 is the respective measurement under control conditions (absence of H-1) and A_1 is the respective measurement in the presence of H-1.

Statistical Analysis: Data are expressed as means±S.E.M, unless otherwise indicated. Differences between control and measurements in the presence of H-1 extracts were compared by Student *t* test or one-way ANOVA as appropriate. A value of $p<0.05$ was considered to indicate significant difference between assayed groups.

RESULTS

Total antioxidant capacity of H-1 extract: The technique used to measure the total antioxidant capacity in the H-1 extract is based upon its ability to suppress the generation of the radical cation ABTS [2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]. Antioxidants in the extract cause suppression of this radical's generation to a degree which is proportional to their concentration. The obtained data (Fig. 1) for this suppression were used to determine the concentration of the extract required to suppress 50% of the ABTS cation (EC_{50}). The percent inhibition was plotted against the concentration of the H-1 extract and the EC_{50} was determined (0.16±0.007 mg mL^{-1}) using the Prism 3.0 curve fitting program.

Superoxide and DPPH scavenging activity of H-1 extract: The extracts ability to inhibit the generation of the superoxide radical ($O_2^{\cdot-}$) from xanthine by the xanthine oxidase was measured at different concentrations of H-1. The EC_{50} for this inhibition was calculated from the data in Fig. 2 and it gave a value of 0.10±0.006 mg mL^{-1}. On the other hand, the EC_{50} determined for the scavenging of the

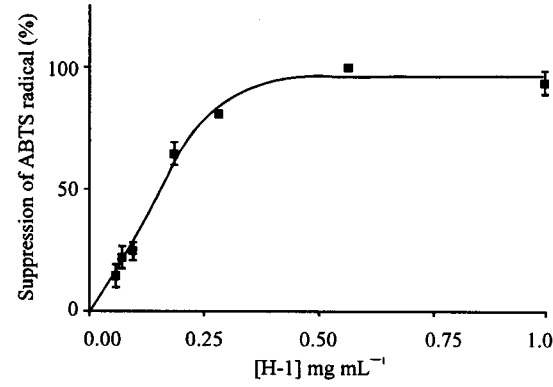

Fig. 1: Total antioxidant capacity of the H-1 extract expressed as % suppression of the ABTS cation radical. An EC_{50} of (0.16±0.007 mg mL^{-1}) was calculated from the curve fit

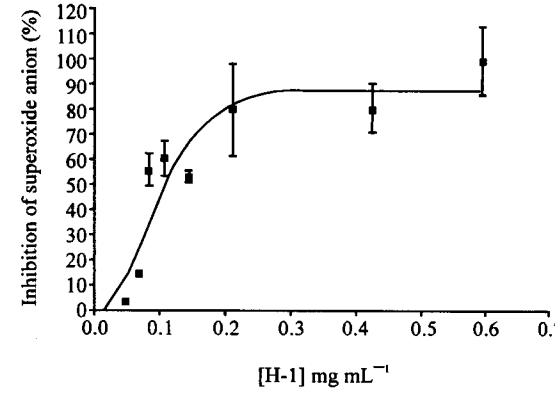

Fig. 2: H-1 scavenging of superoxide anion free radical. An EC_{50} = of 0.10±0.006 mg mL^{-1} was calculated from the curve fit

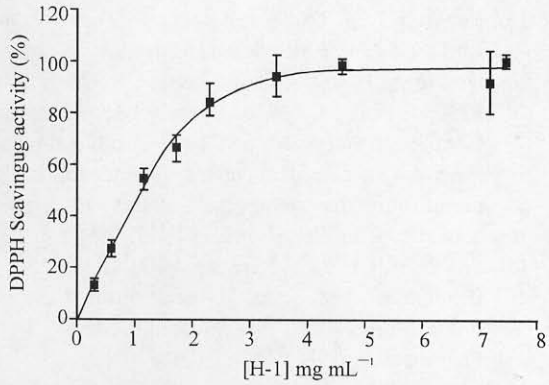

Fig. 3: The ability of H-1 extract to scavenge the free radical DPPH. An EC_{50} of 1.0±0.03 mg mL^{-1} was calculated from the best fit

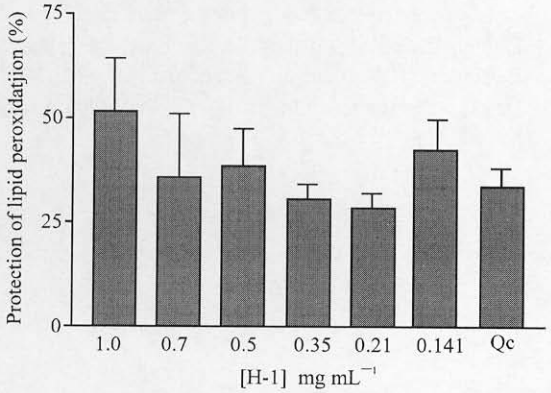

Fig. 4: H-1's capacity to protect against Fe^{+3}-induced lipid peroxidation in human plasma. A % inhibition of lipid peroxidation of 37.6±8.1 % in the concentration range of 0.14-1.0 mg mL^{-1} was determined without any significant change within this range ($P > 0.05$). The effect is comparable to that of quercetin (Qc: a positive control) at 0.5 mg mL^{-1}

DPPH activity by H-1 was 1.0±0.03 mg mL^{-1}, as calculated from data presented in Fig. 3.

Effects of H-1 on Fe^{3+}-induced lipid peroxidation in human plasma: The H-1 extract exhibited a significant protection against ferric chloride induced lipid peroxidation in human plasma mounting to 37.6±8.1 % in the concentration range of 0.14-1.0 mg mL^{-1} without any significant change within this range (Fig. 4), which is comparable to that of quercetin at 0.5 mg mL^{-1}.

DISCUSSION

Although hawthorn fruits, traditionally known in the greater Syria are as *za'roor*, is relatively abundant in the Middle East, it is consumed in a limited sense during its season of growth (September-November). The present study demonstrated clearly some beneficial characteristics of Hawthorn growing in parts of the Middles East i.e. in Lebanon. The plant possesses an antioxidant potential equivalent to an EC_{50} of 0.16±0.007 mg mL^{-1}, indicative of antioxidant capability that may reduce the initiation and propagation of free radicals and thus minimize free radical-induced damage to vascular systems. The general antioxidant capacity is asserted in the particular sense when the H-1's ability to scavenge specific free radicals was assessed. In this respect H-1 exhibited a superoxide anion-scavenging capacity of EC_{50} = 0.10±0.006 and a DPPH quenching capacity of EC_{50} = 1.0±0.03 mg.ml. These results are in line with those using other hawthorn extracts and in which the fruits and/or flowers' extracts were demonstrated to possess scavenging activities of the superoxide and the DPPH free radical species[13,16].

Recent studies have shown that hawthorn extracts and/or its phenolic compounds were capable of imparting significant inhibition of induced human low density lipoprotein (LDL) oxidation in vitro[12,16,17]. A hot-water extract of the dried hawthorn fruit inhibited lipid peroxidation in the concentration range of 0.5-1.0 mg mL^{-1}[16]. Our extract exhibited significant inhibition of 37.6±8.1 % in a relatively comparable concentration range 0.14 -1.0 mg mL^{-1}.

These results suggest an overall similarity in the antioxidant and in anti-lipid peroxidation qualities of our howthorn extract when compared to other sources of the plant grown in other parts of the world. Additional work is needed to further characterize other potential benefits of the hawthorn plant found in the Middle East.

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