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Hydrophilic and Lipophilic Antioxidant Capacity and Content of Phenolic Compounds in Fresh Khat Leaves (*Catha edulis* Forsk.)

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Abstract

Khat (*Catha edulis* Forsk.) is used in East Africa and Middle East as a mild stimulant and traditional remedy. At the same time, long-term use of khat may have adverse health effects. The stimulant action of the plant is related to the presence of amphetamine-like alkaloids, primarily cathinone. In addition, the khat leaves are rich in phytochemicals with health-promoting potential, such as polyphenols and ascorbic acid. In this work, the content of phenolic compounds and antioxidant activity of hydrophilic and lipophilic fractions were investigated in fresh leaves and stems from two distinct genetic forms of khat ("red" and "green"). The leaves were divided into three age groups in accordance with their proximity to the shoot tip. The leaves of khat showed very high hydrophilic antioxidant capacity, comparable to that of the tea leaves. No significant difference was found in this parameter between the leaves of red and green khat, as well as between the three leaf age groups studied. The samples also demonstrated considerable, but not outstanding lipophilic antioxidant capacity. It was suggested that returning to the old practice of consuming khat as infusion of dried leaves ('Abyssinian tea') would fully realize its health-promoting antioxidant potential and minimize the adverse effects.

Introduction

Khat (*Catha edulis* Forsk., Celastraceae) is an evergreen shrub or small tree native to East Africa and South Arabian peninsula (Yemen). Chewing young khat leaves causes a mild stimulant effect and is a popular social habit in the mentioned regions, as well as in the communities of East-African and Yemenite origin over the world. Khat consumption induces mild euphoria and is described to relief fatigue, increase alertness and reduce sleepiness. Its use for the treatment of depressive states has been practiced in Arabian peninsula since ancient times (Iwu, 1993). In addition, in Ethiopia processed leaves and roots of khat are traditionally used to treat various diseases, including influenza, cough, gonorrhea, asthma and other chest problems (Lemessa, 2001). Antimicrobial effect of khat extracts against oral pathogens was reported by Al-hebshi et al. (2006). At the same time, long-term use of khat may result in health problems affecting nervous, cardiovascular, gastrointestinal and reproductive systems (Dhaifalah and Santavy, 2004; Al-Habori, 2005).

The legal status of khat is controversial; it is considered a controlled drug in some countries

(e.g. USA and many European states) but is legal in others (such as UK, the Netherlands and Israel). The classification of drugs by their negative health and social effects (Nutt et al., 2007) positioned khat as the least harmful substance among the 20 drugs of potential misuse studied, far behind such legal habits as alcohol drinking (5-th place) and tobacco smoking (9-th place).

The stimulant effect of the plant is attributed to the presence of amphetamine-like phenylpropylamino alkaloids, cathinone and to a lesser extent cathine (Szendrei, 1980; Krizevki et al., 2007). Cathedulins are another group of khat alkaloids with poorly investigated biological effects (Al-hebshi and Skaug, 2005). In addition, khat leaves are rich in phenolic compounds including flavonoid glycosides and condensed tannins releasing flavonols kaempferol, quercetin and myricetin after acid hydrolysis (El Sissi and Abd Alla, 1966). High content of ascorbic acid (vitamin C) was reported in shoot tips and especially in leaves of khat (Mustard, 1952). The leaves also contain other vitamins (thiamin, niacin and riboflavin), carotenoids (ß-carotene), amino acids, minerals (calcium, iron) and fiber (Duke, 1992).

The information about prooxidant/antioxidant effects of khat is equivocal. Individuals regularly using khat, especially in combination with tobacco smoking, showed higher level of post-meal plasma lipid peroxidation as compared to control group not following these habits. At the same time, the lipid peroxidation level decreased during the khat chewing session following the meal consumption, suggesting certain antioxidant activity (Al-Zubairi et al., 2003). The authors associated these antioxidant properties with the presence of polyphenolic compounds in the khat leaves. Oral administration of total aqueous khat extract or of its alkaloid fraction exacerbated the oxidative stress in restrained rats due to the decreased activity of antioxidant enzymes, i.e. superoxide dismutase, catalase, glutathione-S-transferase (Al-Qirim et al., 2002). Similarly, khat induced an increase in reactive oxygen species and a depletion of intracellular glutathione in the cell cultures of human keratinocytes and fibroblasts, the reactions that could be opposed by addition of exogenous antioxidants (Lukandu et al., 2008). But on the other hand, the flavonoid fraction of the khat enhanced the activity of the antioxidant enzymes in rats and thus could provide a protection against the oxidative stress (Al-Qirim et al., 2002). In addition, this fraction demonstrated an anti-inflammatory activity (Al-Meshal et al., 1986). Methanolic extract of dried khat leaves showed *in vitro* high scavenging activity towards artificial free radical 1,1-diphenyl-2-picryldrazyl (DPPH) (Dudai et al., 2008). Thus, it seems that khat leaves simultaneously possess prooxidant (alkaloid) and antioxidant (phenolic) fractions.

In the present work, we have investigated the content of phenolic compounds and the activity of hydrophilic and lipophilic antioxidants extracted from fresh khat leaves and stems as affected by age and plant genotype.

Materials and Methods

Shoots of the two distinct genetic forms of *Catha edulis* known in Israel in accordance with their twig color as “red khat” and “green khat” were collected from the plants field-grown at the Neve Ya’ar Research Center in Northern Israel. The plant material was brought in a wet wrap to the laboratory at the Volcani Center (Central Israel) on the day of harvest and processed during the same day.

The following samples were prepared from the shoots of each genetic form as shown in Fig. 1: (1) upper leaves: one or two pairs of the youngest tender leaves near the shoot tip, normally taken for

chewing; (2) medium leaves: relatively young leaves located on the shoot below the previous group, approximately 2-4 cm from the shoot tip, occasionally used for chewing; (3) lower leaves: mature leaves located approximately 5-7 cm from the shoot tip, normally not used for chewing; (4) stems: upper relatively tender parts of the stem, approximately 3-4 cm in length, occasionally used for chewing. The samples were prepared in triplicate, each replication comprising material from 4-5 typical khat shoots.

Hydrophilic and lipophilic antioxidant fractions were isolated from the fresh samples using a stepwise extraction of the plant material with acetate buffer, acetone and hexane and repeated partition of water-soluble and water-insoluble portions as described previously (Vinokur and Rodov, 2006). The radical-scavenging activity of each fraction was measured by our modification of the TEAC (Trolox Equivalent Antioxidant Capacity) method based on decolorization of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{+}\cdot$) generated in acidified ethanol medium (Vinokur and Rodov, 2006). The method was further modified by using 0.06 mM potassium persulfate as a radical initiator instead of the 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). Total content of phenolic compounds was determined in the hydrophilic (acetone-water) fractions by the Folin-Ciocalteu method according to Singleton and Rossi (1965) and expressed in gallic acid equivalents (GAE). The analyses were performed in triplicate and the results statistically analyzed by calculation of confidence interval at the 95% confidence level.

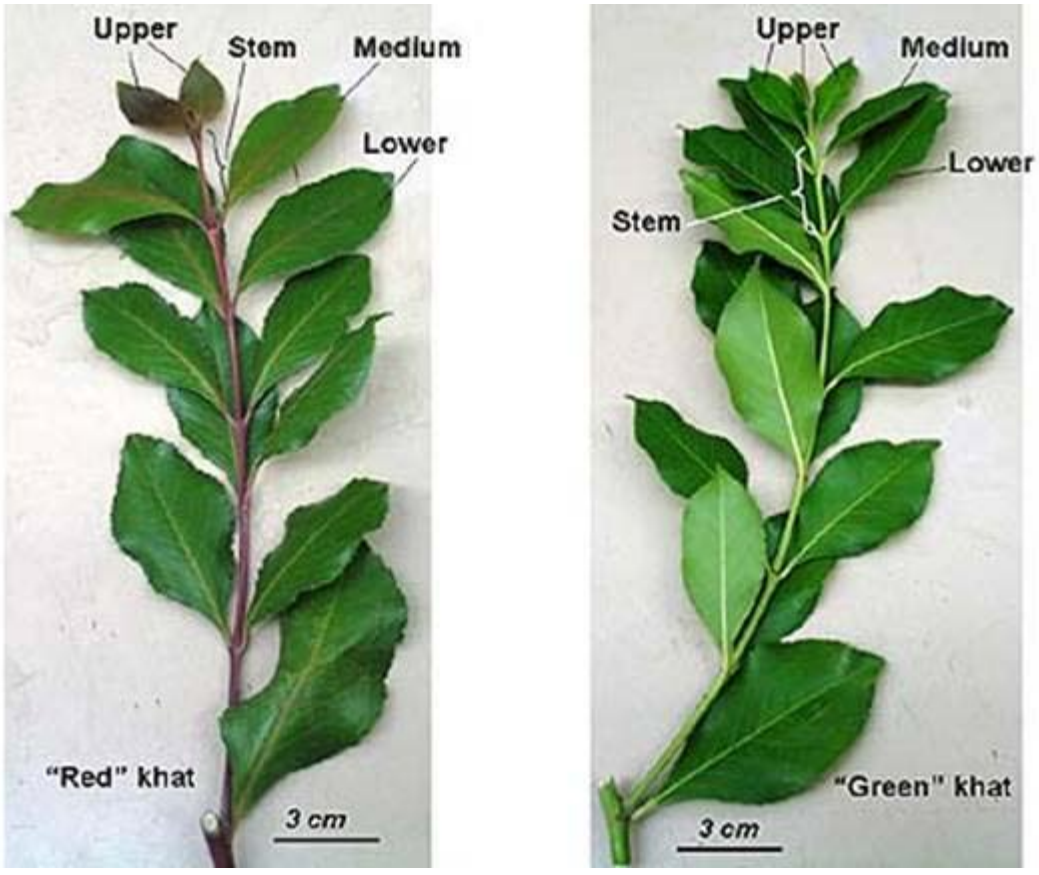

Figure 1. The appearance of "red" and "green" khat shoots and location of samples taken.

Results

The khat leaf extracts demonstrated very high hydrophilic antioxidant capacity, close to 300 µM Trolox equivalents per gram fresh weight. No significant difference in this parameter was found between the leaves of red and green khat forms, as well as between the leaves of different age (distance from the shoot tip). The hydrophilic activity in stems was significantly lower than that in leaves, although still rather high, close to 200 µM TE per gram fresh weight (Fig. 2).

The content of total phenolic compounds followed a pattern similar to that of the hydrophilic antioxidant capacity, especially in the "red khat" samples (Fig. 3). The content of phenolic compounds in the "green khat" leaves tended to be somewhat lower than in the red form. This difference was especially evident in the young (upper) leaves and tended to reduce in the leaves of older ages (medium and lower locations). On the contrary, the content of phenolic compounds in the stems of the "green khat" was slightly higher than that in the red form, both significantly than in the lower leaves.

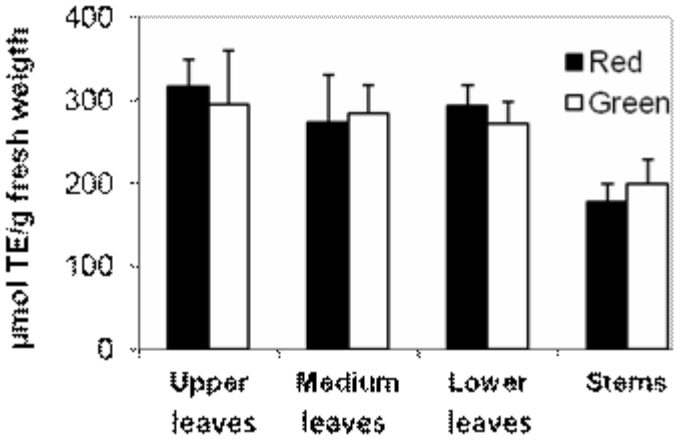

Figure 2. Activity of hydrophilic antioxidants in the leaves of 'red' and 'green' khat of the three age groups.

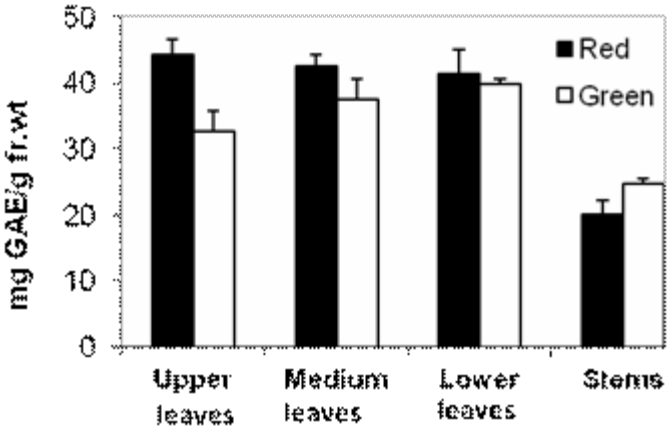

Figure 3. Total content of phenolic compounds in the leaves of 'red' and 'green' khat of the three age groups.

The values of lipophilic antioxidant capacity in the khat samples were much lower than those of

the hydrophilic one, in agreement with the typically observed situation (Wu et al., 2004). The youngest upper leaves tended to have less lipophilic antioxidants than the older ones. Even lower lipophilic activity was found in the stems, especially in the stems of the “red khat”. At the same time, the mature leaves of the “red khat” demonstrated the highest lipophilic antioxidant activity (Fig. 4).

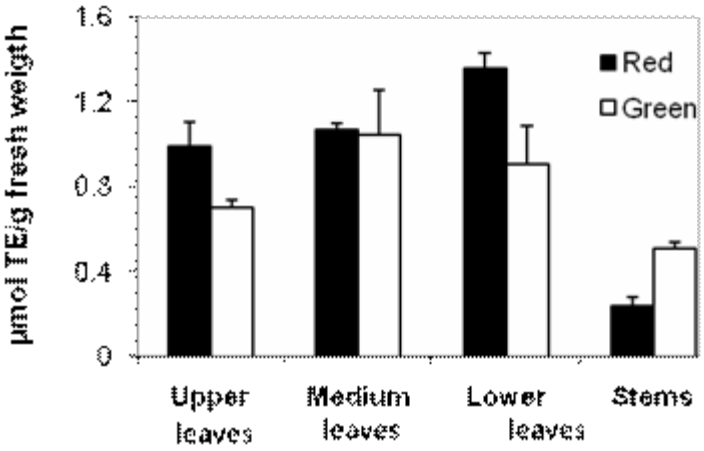

Figure 4. Activity of lipophilic antioxidants in the leaves of ‘red’ and ‘green’ khat of the three age groups.

Discussion

Our study has shown that fresh leaves of khat possess very high hydrophilic radical-scavenging capacity, comparable to that of the tea leaves (Chan et al., 2007) and correlating with the high content of phenolic compounds. This finding corroborates the recent report of Dudai et al. (2008) concerning the dried khat leaves. In addition, khat contains a noteworthy but not outstanding amount of lipophilic antioxidants, which at least partially can be attributed to the leaf carotenoids.

Similar amounts of phenolic antioxidants were found in the leaves of the three age groups tested. Lack of clear gradient of the antioxidant activity along the top 8 cm of the shoot is in a notable contrast to the distribution of the psychoactive alkaloid cathinone present almost exclusively in the youngest top leaves of the shoot (Krizevsky et al., 2007). It should be noted that the oldest leaves from the basal part of the shoot were not included in this trial since they are never chewed due to the rough texture. According to Dudai et al. (2008), these old leaves are characterized by reduced radical-scavenging capacity as compared to the younger ones.

The analysis of the information available indicates that most negative health consequences of the khat chewing (including the prooxidant and cytotoxic effects) are related to the alkaloids, primarily cathinon, while the phenolic compounds (e.g., flavonoids) are responsible for its positive antioxidant, anti-inflammatory and antimicrobial effects (Al-Meshal et al., 1986; Al-Qirim et al., 2002; Al-Zubairi et al., 2003; Al-hebshi et al., 2006). Indeed, alkaloids do not contribute to the radical-scavenging activity of the khat leaves (Dudai et al., 2008). Accordingly, the overall health effect of khat would depend on the balance between alkaloid and phenolic fractions.

Although nowadays chewing is the major way of consuming khat, its leaves were also used in the past for brewing a tea (Getahun and Krikorian, 1973; Al-hebshi and Skaug, 2005). Moreover, Paris and Moyse (1958) described the organoleptic quality of the ‘Abyssinian tea’ prepared from the dried

leaves of *Catha edulis* (5-15 g per liter) as “very agreeable”. At the beginning of the previous century the ‘Abyssinian tea’ gained popularity in Europe (Anonymous, 1911). Paris and Moyse (1958) demonstrated in animal trials that the preparation from the dried leaves had certain stimulant effect, but was less toxic than that from the fresh ones. It also had much lower toxicity than 'mate', the well-known and legal stimulant tea of South-American origin. The reduced toxicity of dried leaves as compared to the fresh ones might be due to the conversion of the cathinone into a less potent alkaloid cathin (Cox and Rampes, 2003).

We suppose that utilizing leaves from the upper part of the khat shoot (combination of the three types of leaves used in this study) for brewing a tea would result in a beverage with high antioxidant activity and reduced alkaloid content. Such tea would be mildly stimulant and fully realize the health-promoting antioxidant potential of the khat with minimized adverse effects. Both “red” and “green” forms of khat can be used for preparing such tea.

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