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Enzyme Inhibitory Activity of Certain Vegetables Indigenous in Iran as Potential Antiandrogens

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ABSTRACT: Androgenetic alopecia is the chief type of scalp hair loss regardless of gender, causing anxiety, depression, arterial stiffness, and cardiovascular disease. The areal parts of *Olea europaea* (OE) and *Trigonella foenum-graecum* (TF), as well as the bulbs of *Allium sativum* (AS) are vastly used in Persian folklore for a great range of culinary and medicinal purposes. This study was an attempt to evaluate 5α-reductase (5α-R) inhibitory activity of the ethanol-based extracts of three edible plant species traditionally used in Iran. In order to measure enzyme inhibitory potential, six samples were prepared: NADPH + enzyme + testosterone + extract (n = 3); enzyme blank (n = 1); positive and negative controls (n = 2). On the whole, AS and TF showed strong enzyme inhibitory activities. The findings indicated that the herbal extracts tested in the present study could be utilized to develop new remedies to manage testosterone-induced diseases such as androgenic alopecia.

Keywords: *Enzyme Activity, Ethanolic Extract, NADPH, Testosterone, Vegetable Plant*.

Introduction

Herbs are invaluable source with a vast range of features that unleash the potential of new safe treatments for various medical conditions. *Olea europaea* (OE) (synonyms: Zeitoon in Persian, Olive in English) is typically grown in the eastern Mediterranean basin, southeastern Europe, northern Iran, western Asia, as well as northern Africa (Ryan & Robards, 1998). Its fruit was a part of Iranian food and diet. Aerial parts of OE are utilized to treat hemorrhoids and rheumatism (Suntar *et al.*, 2010). Oleuropein along with other secoiridoids such as secologanoside, oleoside, 6′-E-p-coumaroyl-secologanoside (comselogoside), and 6′-O-((2E)-2,6-dimethyl-8-hydroxy-2-octenoyloxy) -secologanoside have been derived from the methanolic extract of boron deficient leaves (Karioti *et al.*, 2006). The most abundant substances in the ethanol based olive leaf extract was oleuropein, luteolin-4-O-glucoside, luteolin-7-O-glucoside, and apignin-7-O-glucoside (Luo, 2011). It has been observed that continuous application of olive oil hinders hair loss (Zargari, 1997).

Trigonella foenum-graecum L. (TF) (synonyms: Shanbalileh in Persian, Fenugreek in English), as an annual legume, is vastly cultivated across the world for medicinal, culinary and commercial purposes (Ahari *et al.*, 2009). It is valued for its seeds in Asia, Africa, and Mediterranean

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countries. It has been well documented that the biological and pharmacological effectiveness of fenugreek is related to its constituents, including steroids, N-compounds, polyphenolic substances, volatile constituents, amino acids, and so forth (Mehrafarin *et al.*, 2010). It is shown that the extract of fenugreek leaves is associated with hair growth properties and hair color preservation (Yadav & Kaushik, 2011).

Allium sativum L. (AS) (synonyms: Sir in Persian, Garlic in English) is originally from Asia, subsequently introduced to China, North Africa (Egypt), Europe, and Mexico. Allicin is the paramount alkaloid abundant in garlic, which causes vastly beneficial effects (McRae, 2006). A double-blind randomized controlled trial reported that the addition of garlic to topical betamethasone valerate significantly enhanced the therapeutic efficacy in case of alopecia areata (Hajheydari *et al.*, 2007). In another study on patients with single or multiple patches of alopecia areata, regrowth of terminal coarse hairs as well as negligible undesirable effects were observed following the topical use of garlic extract (Maluki *et al.*, 2009).

Steroid-5α-reductase (5α-R) is an androgen metabolizing enzyme that adjusts key processes related to the prostate, including the NADPH-dependent local conversion of testosterone to the more potent androgen dihydrotestosterone (DHT) (Krieg *et al.*, 1995; Levy *et al.*, 1995). As well-documented, high levels of DHT cause different diseases, such as androgenetic alopecia, hirsutism, , and male pattern of baldness (Urysiak-Czubatka *et al.*, 2014). Accordingly, 5α-R is a promising strategy for the treatment of such diseases through the inhibition of DHT synthesis (Brawley, 2003; Lieberman, 2003). Two certain isozymes—type I (5α-RI) and type II (5α-RII)—are recognized with different biochemical properties, tissue distribution behaviors, and reactions to pharmaceutical agents (Zhu & Sun, 2005). Since 5α-R type II is considered as the chief isoform in human prostate, selective anti-androgenic agents are more likely to result in desirable outcomes for the inhibition of DHT.

Finasteride is vastly applied as a main contributor to the 5α-R inhibition worldwide. It has been associated with several side effects though. Herbal agents with an inhibitory potential against 5α-R might be a key to overcome this issue. With focus on plant-derived 5α-R inhibitors, this *in vitro* study was intended to investigate three edible vegetables of Iran for their potential against 5α-RII.

Materials and Methods

In this study, three plant species with a related history of medicinal use in hair loss were selected to explore their comparative potential in preventing testosterone-induced diseases (Table 1).

- *Preparation of the extracts*

All the plant species were purchased from the local grocery stores in Tehran, Iran. Thereafter, they were identified through comparison with those raised in the Medical Plant Farm, Jahad Daneshgah, Islamic Republic of Iran. Moreover, their names have been checked with www.theplantlist.org. A voucher specimen for each plant has been deposited in the Herbarium of the Department of Pharmacology, Jahad Daneshgah, Tehran, Iran. The aerial parts of TF and OE, along with the cloves of garlic (AS) from a single bulb were utilized. Subsequently, the extraction was carried out using ethanol (100 mL; Merck, Germany, 96%) in triplicates. Briefly, the herbal parts were cleaned and crushed in ethanol for two days. Thereafter, the mixture underwent centrifugation at 200 ×g for 5 min. The supernatant was subsequently filtered and finalized using a rotary evaporator (LABOROTA 4000,

Heidolph, Germany). The resulting extracts were kept in sealed dark vials at 4 °C. The extracts were then analyzed for chemical compositions as described by Azimi et al. (2017) and the results are presented in Table 2.

Table 1. Summary of traditional use of the plants used in this study

Botanical Name	Traditional Uses
Olea europaea L.	alopecia, baldness (Acquaviva *et al.*, 2012; Ghanbari *et al.*, 2012; Hossain *et al.*, 2016)
Trigonella foenum-graecum L.	baldness, alopecia, hirsutism (Didarshetaban *et al.*, 2013; Gupta *et al.*, 2013; Marchese, 2012)
Allium sativum L.	alopecia, hirsutism (Ahmed, 2016; Goswami *et al.*, 2012; Noumi & Kolaipla, 2011)

Table 2. Chemical compositions of the extract (%)

Allium sativum[a]		*Olea europaea*[b]		*Trigonella foenum-graecum*[b]	
Methyl 2-propanol disulfide	2.86	Hydroxytyrosol	2.13	Aziridine, 1,2,3-trimethyl-, trans-	3.01
Dimethyl trisulfide	1.32	Tyrosol	0.76	2-Propen-1-amine, Nethyl-	3.23
Diallyl disulfide	7.00	Caffeic acid	0.21	1-Azabicyclo[2.2.2]octane, 4-methyl-	0.60
2-Ethylidene[1,3]dithiane	2.54	Ferulic acid	1.00	á-D-Glucopyranoside, methyl	72.13
Methyl 2-propanol trisulfide	10.11	Verbascoside	3.42	3-O-Methyl-d-glucose	15.98
3-Vinyl-1,2-ditiocyclohex-4-ene	8.67	Rutin	7.58	Dibutyl phthalate	0.35
3-Vinyl-1,2-ditiocyclohex-5-ene	6.43	Luteolin-7-O-glucoside	12.34	Heptanoic acid, 2-ethyl-	0.12
Di-2-propenyl trisulfide	23.03	Oleuropein	44.17	Hexane, 3-bromo-	0.10
Diallyl tetrasulfide	6.91	Luteolin-4-O-glucoside	11.05	1-Dodecyne	0.41
Benzeneacetaldehyde	5.62	Apigenin-7-O-glucoside	8.04	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-	0.18
--	--	Luteolin	1.46	Piperidine, 1,1'-methylenebis-	0.10
--	--	Apigenin	5.03	1-Octanol, 2-nitro-	0.12
--	--	sulfurous acid	0.61	Pentanal, 2-methyl-	0.22
--	--	Nitrous acid	1.03	Didodecyl phthalate	0.07
--	--	Methyl tartronic acid	0.32	1-Tridecyne	0.26
--	--	--	--	Squalene	0.77
--	--	--	--	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	0.71

[a] Total identified = 74.49%
[b] Total identified = 99.15%
[c] Total identified = 89.36 %

- *Enzyme Inhibition Activity*

Enzyme inhibition was evaluated via Sun *et al*. (1998) method. First, human prostate obtained from Imam Khomeini hospital in Tehran was cut into very small pieces. The prostate pieces were mixed with a 10 mL of medium which included sodium phosphate (20 mM; Sigma-Aldrich, USA), sucrose (0.32 M; Sigma-Aldrich, USA), and ethylenediamine tetraacetic acid (1 mM; EDTA; Sigma-Aldrich, USA) to achieve a homogenate. The mixture then underwent centrifugation in duplicate at 4000 rpm for 15 min. Bradford method was conducted using the ultimate supernatant to calculate the enzyme concentration (Bradford, 1976). The findings showed that the concentration of 5αRII was 372.38 µg/mL. Study samples (n=3) were prepared via mixing nicotinamide adenine dinucleotide phosphate (3 mL; 22 µM; NADPH; Sigma, USA), enzyme (1 mL), Tris–HCl buffer (4 mL; 0.5 M; Sigma, USA), testosterone (2 mL; 75 µM; Sigma, Germany), and ethanolic extracts (2 mL; 1 mg/mL). An enzyme blank, a negative control (2 mL of testosterone added to the blank), and a positive control (2 mL of 200 nM finasteride added to the negative control) were considered, as well (Nahata & Dixit, 2014). These samples were analyzed employing spectrophotometer at 340 nm over 30 min with a time interval of 10 min.

- *Statistical analysis*

The data were presented as mean±standard deviation (SD). Due to normal distribution, one-way ANOVA and Duncan's multiple range tests were utilized to determine significant differences at P-values lower than 0.05 (SPSS 19.0, IBM Inc., USA).

Results and Discussion

A number of three plants with vast applications in cuisine and traditional medicine by the Iranian were utilized to test their enzyme inhibitory activities. As shown in Table 3, the ethanol-based extraction procedure resulted in the extract yield ranging from 2.17% to 4.11%. The lowest and maximum yields were respectively related to OE and AS.

Table 3. Extraction yield of the ethanolic extracts

Samples	Extraction yield (%)
AS	4.11
OE	2.17
TF	3.35

The plant species were then examined for their inhibitory activities on 5αRII (Table 4). Generally, the inhibitory enzyme activity notably increased during 30 min ($p < 0.05$). In the case of the plant genera, an influential disparity was observed at each time point ($p < 0.05$). However, there was no significant difference between OE and AS at 10 min ($p > 0.05$). Additionally, TF showed the strongest potency against 5αRII ($p<0.05$). This test showed the superiority of AS at 30 min (36.15 ± 0.26 µg/mL). The decreasing order of the enzyme inhibitory activity was presented at each time point as followed:

10 min: TF> AS> OE
20 min: TF>AS>OE
30 min: AS> TF> OE

Table 4. Enzyme inhibitory activity of the ethanol extracts (µg/mL; mean±SD)

	Time (min)			
Samples	10	20	30	p-value
AS	8.23 ± 0.04^{b}	14.56 ± 0.08^{b}	36.15 ± 0.26^{a}	0.04
OE	6.84 ± 0.32^{b}	8.39 ± 0.28^{c}	9.20 ± 0.04^{c}	0.02
TF	20.16 ± 0.25^{a}	22.39 ± 0.13^{a}	25.57 ± 0.25^{b}	0.01

Different letters in each column indicated significant difference ($p<0.05$).

All of the plant extracts investigated here had a distinct ability to hinder 5α-R enzyme. Of note, this is the first study reporting the anti-androgenic effects of TF, AS, and OE. Also, the results of this investigation came up with another outcome; time had a statistically positive impact on the progress of enzyme inhibition, which was in broad agreement with Nahata and Dixit's (Nahata & Dixit, 2014) findings. This study indicated that AS and TF, as the first two plant species, possessed quite strong anti-androgenic potentials at each time point.

Conclusion

The findings indicated that the herbal extracts tested in the present study could not only be used to develop new remedies to manage testosterone-induced diseases such as androgenic alopecia, but also might be used in food formulation to improve their shelf life, particularly regarding the oxidation chain reaction due to the potential antioxidants present in the extracts, namely caffeic and ferulic acids.

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