



Antioxidant activity of Vitex Agnus Castus subcritical CO₂ extracts from two different regions of Albania

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Keywords

Medical plant
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GC-FID

Abstract

Medicinal plants include various types of botanicals used in herbalism, known and employed for their variety of medical usefulness. Recently, there has been a great interest in obtaining natural antioxidants from plant materials, as studies show that most of them possess significant antioxidant activity, and some of their phytotherapeutic potential properties include antimicrobial activity, antineoplastic, antidiabetic, anti-atherosclerotic, immunomodulatory, and in some cases, renoprotective or hepatoprotective effects are observed. The aim of this study is to differentiate the biological activity of the same plant (*Vitex agnus castus*) as a result of geographical variety. The plants used for this study are harvested in two separate Albanian regions, (Milot and Qeparo) extracted with subcritical CO₂ extraction method. The extracts were analyzed for antioxidant activities using the DDPH assays and the composition of the botanical extract was analyzed with 436-GC FID detector (Scion Instrument). The major compound detected in black and green fruits of *Vitex agnus castus*, collected in Milot, was (E)-trans-β- respectively 22.01% and 20.47% and in CO₂ extracts of Qeparo fruits (E) - Farnesene 20.1%. The plants collected in northern Albania resulted with a higher antioxidant activity compared to the plants collected in southern Albania. A significant difference was also noticed between the two maturation stages of the *Vitex agnus castus* fruit, the black fruit resulted to be more active than the green.

Introduction

For years, medicinal plants have been a valuable source of therapeutic agents, and still many of today's drugs are plant-derived natural products or their derivatives [1]. Natural products, especially those of vegetable origin, have always been an important source of therapeutic agents. About 25%–30% of drugs available for the treatment of diseases are derived from them [2]. Medicinal plants are the richest natural resource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [3]. *Vitex agnus castus* is one of 250 species of *Vitex* genus native to the Mediterranean region and Asia. *Vitex agnus castus* L. was formerly classified within the family of Verbenaceae, but now the phylogenetic classification located it in Lamiaceae. It is cultivated in warm temperate regions of the world, and obtained primarily from Mediterranean countries, especially Albania and Morocco [4].

The fruits have been applied for more than 2500 years in ancient Egypt, Greece, Iran, and Rome for a variety of gynecologic problems. It has been used for its claimed activity for reduction of libido [5]. The fruit of *Vitex agnus castus* has been recommended as carminative, energizer, sedative, antiepileptic [6], anaphrodisiac, emmenagogue agent and antispasmodic [7]. Fruits of *Vitex agnus castus* have been also used in the treatment of many female conditions, including menstrual disorders (amenorrhea, dysmenorrhea), premenstrual dysphoric disorder (PMDD), corpus luteum insufficiency, hyperprolactinemia, infertility, acne, menopause, disrupted lactation, cyclic breast pain, cyclical mastalgia and inflammatory conditions, diarrhea and flatulence. Other effects of this plant include tumoricidal, antioxidant, immunomodulatory, antimicrobial, antifungal, fracture healing, digestive complaints, infertility, acne and also for lactation support [8,9].

Material and Method

The plants used for this study are harvested in two separate Albanian regions, (Milot and Qeparo) extracted with subcritical CO₂ extraction method. In Milot, there were collected two types of plant fruit in two different maturation stages, the black and the green fruit. These samples were then placed in a shady room to be naturally dried. After they were completely dried, the fruits were separated from the flowers. The fully dried samples were cut into small pieces before extraction procedure began.

Antioxidant activity assay.

The DPPH radical scavenging antioxidant assay was employed to estimate antioxidant potential of *Vitex agnus castus* fruit extracts, obtained by subcritical extraction method. The interaction between the latter and the DPPH in an ethanol solution is monitored through the measuring of absorbance at 517 nm using spectroscopy.

Chromatographic analysis

The botanical extract was obtained by subcritical CO₂ extraction method and further analyzed for volatile compounds by chromatograph model 436-GC, flame ionization detector (FID) by Scion Instrument, using nonpolar-low polar column (60m x 0.32 mm, 0.1um df) with following parameters: injection temperature 180°C, oven temperature 70°C, detection temperature 280°C, carrier gas flow 1ml/min, volume of injected extract 2 ul [10].

Results

Table 1. IC₅₀ and R_{sq} valeus of each extraction

Sample	IC ₅₀	Rsq
CO ₂ extract of black fruit collected in Milot	0.01104	0.9904
CO ₂ extract of green fruit collected in Milot	0.01969	0.9714
CO ₂ extract of fruit collected in Qeparo	0.04758	0.9852

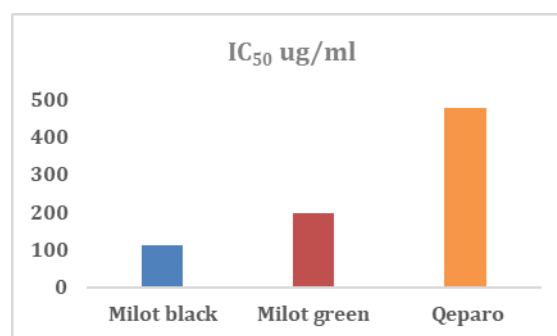


Figure 1. Comparison of the IC₅₀ of different regions plant harvesting. Analyzed with [Inhibitor] vs. Response (three parameters). Valeus represent means±SD.*p<0.033; **<0.002; ***<0.001

Table 2. GC-FID results of subcritical CO₂ extracts of black and green fruit of *Vitex agnus castus* collected in Milot.

No.	Black Milot		Compound	Green Milot		Compound	Black Milot		Green Milot		Compound
	No.	[%]		No.	[%]		No.	[%]	No.	[%]	
1	5.91	4.87	(E)-Caryophyllene	11	4.53	4.52	α-Terpinyl acetate	21	1.43	1.3	Globulol
2	22.1	20.47	(E)-trans - β- Farnesene	12	0.87	0.99	α -trans-Bergamotene	22	1.57	1.51	Ledol
3	1.1	1.63	(Z)-Farnesene	13	0.61	0.63	β -Acorenol	23	0.31	0.61	Limonene
4	8.79	6.41	1,8-Cineole	14	7.55	13.53	Bicyclogermacrene	24	3.5	2	Manool
5	2.05	2.73	7- α -Hydroxy-manool	15	0.79	0.87	Bicyclo-vetivenol	25	2.33	1.44	Myrcene
6	1.12	1.02	α- Terpinene	16	3.69	3.58	β-Phellandrene	26	0.81	0.94	Pimaradiene
7	2.82	2.72	α-Gurjunene	17	0.63	1.62	β -Pinene	27	2.06	2.17	Sabinene
8	1.4	1.39	α-Phellandrene	18	1.47	1.18	cis-Sabinene hidrate	28	3.11	2.07	Spathulenol
9	3.96	2.84	α-Pinene	19	1.02	1.04	Citronellyl-acetate	29	0.31	0.69	Terpin-4-ol
10	10.3	11.28	α-Terpineol	20	0.29	0.75	epi- α -Cadinol7	30	0.98	0.75	Trans-γ-Cadinene
								31	2.59	2.45	Viridiflorol

Table 3. GC-FID results of subcritical CO₂ extracts of fruit of *Vitex agnus castus* collected in Qeparo

No.	%	Compound	No.	%	Compound	No.	%	Compound
1	1.04	cis-Sabinene hidrate	11	12.37	α -Terpineol	21	0.94	Citronellyl-acetate
2	0.44	Limonene	12	1.11	Ledol	22	3.65	β -Phellandrene
3	2.33	7- α -Hydroxy-manool	13	1.10	α -Phellandrene	23	1.00	Globulol
4	4.28	Sabinene	14	1.07	(Z)-Farnesene	24	2.75	epi- α -Cadinol7
5	10.30	Bicyclogermacrene	15	1.76	Viridiflorol	25	4.71	α -Pinene
6	0.59	Trans- γ -Cadinene	16	0.54	β -Acorenol	26	1.25	β -Pinene
7	3.00	Spathulenol	17	7.22	1,8-Cineole	27	0.64	Pimaradiene
8	0.65	α -trans-Bergamotene	18	5.37	Manool	28	1.21	Myrcene
9	0.48	α -Terpinol	19	5.37	α -Terpinyl acetate	29	1.32	α -Gurjunene
10	0.58	Terpin-4-ol	20	2.32	(E)-Caryophyllene	30	0.60	Bicyclo-vetivenol
						31	20.01	(E)-Farnesene

Discussion

The GC-FID analysis was carried out using standards of the main compounds. The results were interpreted based on the studies done on *Vitex agnus castus* in Albania and nearby regions.

The major compounds detected in CO₂ extracts of black and green fruits of *Vitex agnus castus* collected in Milot, was (E)-trans- β -Farnesene respectively 22.01% and 20.47% a sesquiterpene, a group typically extracted with CO₂; α -Terpineol 10.3-11.28%; Bicyclogermacrene 7.55-13.53%. Regarding Qeparo fruits, extract major compounds found were (E)-Farnesene 20.01%; α -Terpineol 12.37%; Bicyclogermacrene 10.3%. There are no significant differences between black fruit and green fruit content of compounds. However, the green fruit extracts had a slightly higher percentage of compounds. CO₂ extraction has an advantage compared to other organic solvent extraction methods when it comes to the isolation of compounds with high volatility. The antioxidant activities were measured using the DPPH assays. In general, all the extract samples showed a high antioxidant activity. In the DPPH assay, the elevated antioxidant responses are probably due to the phenolic components of the studied extracts. The extract of the black fruit of *Vitex agnus castus* collected in Milot showed higher DPPH radical scavenging activity than the green one and Qeparo fruits.

Conclusion

Our survey data obtained shows that there is indeed a correlation between the region where the plant was harvested and the maturation of the plant, with the extract biological activity. The extract of the black fruit of *Vitex agnus castus* collected in Milot showed higher DPPH radical scavenging activity compared to the green one and fruits collected in Qeparo. No significant differences were found on the concentration of major component regarding volatile compounds identified by GC-FID.

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