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The impact of extraction methods on isolation of pharmacologically active compounds from *Vitex agnus-castus* - a review

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ABSTRACT

Importance of pharmacologically active natural compounds from plant sources has been re-evaluated in recent years and it has become one of the most active research fields. Despite great advances in medicine, plants still play important roles in healthcare due to their useful natural components such as antioxidants. The main purpose of this review was to present updated information about chemical composition of Vitex agnus-castus (VAC) and its role in healthcare. According to several studies, chemical composition of VAC is characterized by presence of different secondary metabolites, among which iridoids, flavonoids, diterpenoids, essential oils (EO) and ketosteroids are dominant. Differences in chemical composition are observed as a result of different geographic origin and applied extraction method. In this review, the chemical composition of EO and VAC extracts obtained by different extraction methods, including conventional and non-conventional methods, are given and discussed.

Keywords: *Vitex agnus-castus, extraction, SC-CO_2, chemical composition*

INTRODUCTION

Chaste tree or *Vitex agnus-castus* (VAC) is an aromatic, deciduous and very decorative shrub, which was formerly classified in the family of *Verbenaceae*, but now the phylogenetic classification assorts it in the *Lamiaceae* [1,2]. There are more than 250 species of *Vitex* genus in the world. This genus is an important natural source of food and medicine products around the world [2]. Its genuine habitat are arid and semi arid area of Mediterranean, Western Asia, Southern Europe, West Asia, Crimea, Caucasia, Iran, North Africa, Central Asia and India [3]. VAC is growing in areas where the Mediterranean climate is dominant, rather rocky places, wetlands such as stream banks and valleys. VAC can also be found on limestone slopes, in sunny and warm areas [4,5]. It can be grown as shrub with small and narrow leaves with purple-black berries on the top [5, 6]. VAC is a plant that blooms in the period from July to September. Leaves are characterized by aromatic flavor, which repels mosquitoes, while spikes are generally close to or at the ends of branches and they are weakly aromatic, varying in color from purple to light purple. Branches have followed by fleshy fruit containing four seeds that are similar to black pepper [4]. Seed is oval to almost globular, with a diameter of up to 5 mm. Hydro-distillation of VAC fruits yielded a mobile, fragrant pale-yellow to yellow EO with agreeable, interesting, somewhat peculiar top-note, reminding one of cannabis and eucalyptus. After a few minutes, it revealed an aromatic floral, warm but fresh, somewhat peppery, sweet, spicy fragrance with lemon-like, woody undertones [7].

Well-known medicinal plant

VAC is a well-known medicinal plant and it is probably one of the most important herbs used by medical herbalist for centuries. Hippocrates use it in the treatments of injuries and inflammation and for treatment of several female hormonal disorders [3,8]. The specific epithet of this plant comes from the Latin words "*castitas*" (chastity) and "*agnus*" (lamb). The plant is also known under the common name "*chaste tree*" because of its ability to decrease sexual desire and promote chastity in women and celibacy in monks. The monks use it as a spice in cooking and the plant is therefore known as "monk's pepper" [6,9].

Synonyms for VAC are: Baccae agni casti, Fructus agni casti, Semen agni casti, Monk’s pepper, chaste berry, poire sauvage.

Common names on other languages are as follows: *English*- Chaste tree, *French*- Fruit de gattilier, *Italian*- Frutto di Agnocasto, *Spanish*- fruto de agnocasto, *German*- Mönchspfefferfrüchte [6,10].

Extraction methods of pharmacologically active compounds from plant material

Selection of appropriate method of extraction leads to a better qualitative and quantitative isolation of bioactive compounds from plant material. Extraction of plant materials can be done by various extraction procedures, which include conventional and non-conventional extraction methods.

There are several conventional methods of extraction, such as infusion, decoction, digestion, maceration, percolation, Soxhlet extraction and hydro-distillation [11,12]. Hydro-distillation is the oldest and the most common method of extracting EO since it is economically viable and safe. Although distilling equipment has gradually improved through the years, the method for extracting EO from the plant has changed very little. The process of production of EO has several stages that begin with transportation of raw materials from the field to the warehouse, followed by grinding and distillation. Consumption of water vapor, pressure and duration of distillation are specific for each plant species [12,13]. Conventional extraction methods, such as Soxhlet, are still considered as reference methods for comparison with newly developed methodologies [11].

Non-conventional methods have been developed during the last 50 years and they are more environmental friendly due to decreased use of the synthetic and organic chemicals, reduced operational time and better yield and quality of extract. They include: ultrasound, pulsed electric field, enzyme digestion, microwave heating, ohmic heating, accelerated solvents and supercritical fluid extraction (SFE). Followed by an appropriate extraction process it is possible to conduct further separation, identification and characterization of bioactive compounds [11]. Product quality requirements in food, cosmetic and pharmaceutical industries have made the SFE of EO an attractive technology compared to conventional processes, such as organic solvent extraction and hydro-distillation [14], and because of these facts the further discussion will be related to this technology. Advantage of SFE implementation, such as the absence of toxic residues in the final product, is especially useful for extraction of valuable bioactive compounds like flavors, colorants and removing undesirable compounds, organic pollutants, toxins and pesticides, namely. SFE is process that can increase the productivity and the selectivity in extraction of desired compounds. That is novel technique, in which the characteristics of the final product can be easily altered by changing the process parameters such as temperature, pressure and addition of co-solvent. Influencing the operating conditions it is possible to increase the salvation power, so this technology is a good option for the recovery of several types of substances [15]. This method is highly suitable for extraction of natural agents and valuable components from plant materials, enabling preservation of heat-sensitive compounds without degradation [4]. These components are often present in plants in low concentrations and are chemically sensitive. Several solvents can be used in SFE, investigated as the supercritical solvents, such as supercritical CO_2 (SC-CO_2), propane, ethane, hexane, pentane and butane [15]. SC-CO_2 as a solvent is nontoxic, readily available, cheap, nonflammable and has low critical points of temperature and pressure [16]. Compressed SC-CO_2 is the most commonly used solvent because it enables better separation of thermally labile compounds under the lower temperature of the extraction. The technical and environmental advantages of SFE technology include the fact that SC-CO_2 is generally recognized as safe (GRAS) solvent [17]. Based on the successful and widely-known applications, SFE is being used to extract EO and nutraceuticals from botanical substrates. It points to possible expansion to more and more industrial applications [18]. After separation of fruit and leaves from the main plant, content of plant natural compounds is changed, so it is necessary choose the right extraction method, optimize extraction time and choose a proper solvent in order to obtain a certain chemical composition of the extract [19]. To illustrate the wide applicability of SFE few diverse examples are presented below.

Chemical composition of VAC extracts obtained by conventional extraction methods

Fruits, flowers and leaves of VAC are reported to contain different bioactive compounds, like iridoids and iridoid glycosides (i.e. aucubin and agnuside), flavonoids (i.e. casticin, penduletin and kaempferol), terpenoids (i.e.

vitexlactam and rotundifurane) and diterpenoids, EO (i.e. limonene, α- and β-pinene), ketosteroids [4,20,21,22,23], rolundifuran, vitexilactone [5] and all of these compounds can contribute to therapeutic and medicinal properties [24]. Due to both intrinsic (sexual, seasonal, ontogenetic and genetic variations) and extrinsic (ecological and environmental aspects) factors of *Lamiaceae* essential oil-bearing plants, the yield of EO and its chemical composition vary considerably [25].

The VAC EO obtained with hydro-distillation from raw material from **Budapest, Hungary** was investigated by Cossuta et al. [4]. The identified the main volatile compounds of obtained EO as α-pinene, limonene, 1,8-cineol, β-caryophyllene, *trans*-β-farnesene, α-humulene and spathulenol. Compared to samples provided by different extraction processes the ratio of the identified components was nearly the same. They conducted study where they were using ethanol and n-hexane as solvents. Results showed that the yields of the ethanolic extracts were 2.8 times higher than those provided by *n*-hexane. Hexane extracts were characterized with a strong smell, brownish-yellow color and easy spread consistency, while ethanol (96%, v/v) extracts were dark-green-black colored and highly viscous. Concentration of other compounds such as rotundifurane, β-sitosterol, β-amyrin and casticin was higher in n-hexane extracts [4].

The studies conducted by Novak et al. [26] have shown results from the **Vinagrella, Mallorca**, **Spain** of the hydro-distilled VAC leaves, immature and mature fruits. The main compounds of the EO were 1,8-cineole, sabinene, α-pinene, β-phellandrene and α-terpinyl acetate, *trans*-β-farnesene and bicyclogermacrene. The same compounds were present in all plant organs, but leaves showed difference from the fruits in the concentrations of all compounds except α-terpinyl acetate and <epi-13>manoyl-oxide. The compositions of immature and mature fruits were equal.

According to the investigation conducted by Sorensen et al. [7] the major components in the studied EO of VAC seeds from **Cretan, Greece** were 1,8-cineol, sabinen, α-pinene, β-farnesene, β-caryophyllene oxide and β-caryophyllene. Other components were present at lower levels, as β-sitosterol whose presence can be due to essentially geographical conditions and climate. In some reports, major compounds have been determined as 1,8-cineole, β-farnesene, and/or sabinene.

In comparison to **Egyptian** EO the most interesting difference is presence of α-cadinene and β-cadinene. Also, components which were not detected in Cretan studies, were found in Egyptian EO, α-terpineol, as the major component of Egyptian EO, being one of them. When comparing EO from **Russian** and **Cretan origin**, they differentiated in the presence of α- and β-pinene and limonene content which were very high in Russian EO. The quantitative composition of the Creatan EO was found to be similar to the EO from Yugoslavia [7].

Phytochemical analysis of the of VAC EO from an **Amazon origin** identified several components, mainly 1,8-cineole, (E)-β-farnesene, sabinene, α-pinene, α-terpinyl acetate, β-caryophyllene and bicyclogermacrene [27].

According to the research conducted by Stojkovic et al. [28] EO of leaves and immature and mature fruits collected in **Montenegro,** were rich sources of α-pinen. The main components in EO of leaves were 1,8-cineole, *trans*-β-farnesene, α-pinene, *trans*-β-caryophyllene and terpine-4-ol among other 46 compounds which were present. The analysis of the EO of VAC mature fruit detected 51 components, the main components of the EO were 1,8-cineole, sabinene, α-pinene and *trans*-β-famesene. In the EO of the immature fruits 50 compounds were identified, among which sabinene and 1,8-cineole were the main ones [28].

The leaves EO of a **North-Central Nigerian**-grown VAC had 34 compounds present in the EO and the oil yield was 0.8% v/w. The main components in EO were β-pinene, α-pinene, cis-ocimene, 1,8-cineole, terpinen-4-ol, β-phellandrene and α-terpineol. This study indicated that the most abundant components of VAC seeds EO were caryophyllene oxide, n-hexadecane and α-terpinyl acetate. Studies in Iran indicated the same, where the chemical composition of EO VAC fresh leaves from **Maraghe, East Azerbaijan** province (Iran) shown that the major component was α-pinene. The combination of cyclohexenes had the highest percentage amongst the other constituents. This combination included, 1-methyl-4- (1-methylethenyl), caryophyllene, sabinene and β-sesquiphellandrene. A previous studies in Iran indicated that the most abundant components of VAC seeds EO were caryophyllene oxide, n-hexadecane and α-terpinyl acetate [29].

The most abundant constituents in EO of VAC growing in **north Brazil** were 1,8-cineole, *trans*-β-farnesene, sabinene, α-pinene, α-terpenyl acetate, β-caryophyllene and bicyclogermacrene. The major compounds in VAC leaves were 1,8-cineole and sabinene [28].

The analysis of the EO of VAC from **Manisa, Turkey** resulted in detection of 27 components. Major components of the EO were 1,8-cineole, sabinene, α-pinene, α-terpinyl acetate and (Z)-β-farnesen [30]. From the fruits of VAC collected from **Romanshorn, Switzerland**, one new diterpene was isolated, 6β,7β-diacetoxy-13-hydroxy-labda-8,14-diene, as well as two known diterpenes, rotundifuran and vitexilactone [31].

The fruits of VAC collected in **Israel** were powdered and extracted with methanol. During study of the cytotoxic principles from this plant, four new flavonoids, luteolin 6-*C*-(4''-methyl-6''-*O*-*trans*-caffeoylglucoside), luteolin 6-*C*-(6''-*O*-*trans*-caffeoylglucoside), luteolin 6-C-(2''-*O*-*trans*-caffeoylglucoside), and luteolin 7-*O*-(6''-*p*-benzoylglucoside) together with four known ones, 4',5- dihydroxy-3,3',6,7-tetramethoxyflavone, luteolin, artemetin and isorhamnetin were isolated and applied to cytotoxic bioassay [32]. Phytochemical investigation was performed on defatted methanol extract of VAC fruits in order to further detect and isolate additional chemical constituents in VAC for different bioassays. 24 compounds were isolated and identified using spectroscopic methods which included a new labdane-type diterpene named viteagnusin I together with 23 known compounds, including 9 isolates, which were identified for the first time from the genus *Vitex*, i.e. 3-*epi*-maslinic acid, 3-*epi*-corosolic acid, *p*-hydroxyphenylethanol-*p*-coumarate, 5,7,3′,5′-tetrahydroxyflavanon, 5,3′,5′ trihydroxymethoxylflavanone, ilelatifol D, 3,7-dimethylquercetin, ficusal and balanophonin. Isolates 3-*O*-methylkaempferol, 3-methylquercetin and vladirol are reported for the first time from the species VAC. Other compounds reported from VAC include casticin, penduletin, 8-epi-manoyl oxide, ferulic acid, apigenin, aromadendrane-4α, 10α-diol, 1- glyceryl linoleate, luteolin and kaempferol [33].

Chemical composition of VAC using non-conventional extraction method - SFE

Research conducted by the Cossuta et al. [4] determined the optimal conditions for SC-CO_2 extraction that achieve maximum yield and recovery of the diterpene rotundifuran and flavonoid casticin, among other minor components from the fruit of VAC from **Budapest, Hungary**. Results show the effects of SFE conditions on the extraction yield and recoveries of certain compounds. Different minor components were rotundifurane, β-sitosterol, β-amyrin and casticin. Comparing the extraction process SC-CO_2 and extraction with ethanol it can be concluded that 3-4 times higher concentration of the minor components could be obtained by using SC-CO_2. High-value biologically active extracts obtained with SC-CO_2, contain valuable components like EO (β-caryophyllene, 1,8-cineol, etc.), diterpenes (rotundifurane), triterpenes (β-sitosterol, β-amyrin) and flavonoids (casticin) [4].

Research of Mele et al. [17] investigated the use of near critical liquid CO_2 for the extraction of VAC mature fruits from **Albania**. Two procedures utilizing near critical liquid CO_2 were tested: the extraction of plant material via continuous solvent recycling and the extraction by a Soxhlet-type process via periodic solvent recycling. The results were compared with data obtained from the traditional Soxhlet extraction process using three different solvents, namely *n*-hexane, dichloromethane and methanol. They observed that, for obtaining the same extraction yield from 1 g of plant material, a lower amount of solvent is needed when continuously recycling liquid CO_2 is applied, than Soxhlet extraction mode. Recycling the liquid CO_2 continuously is the faster mode of extraction thanks to the permanent contact of the solvent and plant material during the continuous displacement of the liquid CO_2. That it is not possible during the periodic Soxhlet extraction. The particle size has also a big influence on extraction process. Using larger particle sizes of the VAC fruits 3 times longer extraction period was needed to achieve yields comparable to those where the fruits are grinded in the powdered form. Extracting the VAC fruits with liquid CO_2, *n*-hexane, dichloromethane and methanol, casticin and rotundifuran were obtained. It shows that flavonoid casticin is selectively extracted with *n*-hexane using traditional Soxhlet process, and diterpene rotundifuran is selectively extracted by near critical liquid CO_2 in continuous recycling mode [17].

Antioxidant activity

VAC containes high levels of phenolic components so can serve as an excellent natural source of antioxidans. It can be assumed that these components are involved in antioxidant activity of EO [29]. Antioxidant activity is related to the presence of some compounds in the EO and the main role of such compounds is reducing free radicals [34]. α-pinene and sabinene possess a significant antioxidant activity. Furthermore, β-sitosterol is of a great interest due to its antioxidant activity and impact on health. Consequently, EO of VAC seeds have demonstrated an antioxidant activity similar to BHT (Butylated hydroxytoluene). It can be explained by the presence of β-sitosterol which is well known for its antioxidant activity [25].

Antifungal and antimicrobial activity

Growing interest of EO from plants due to their antibacterial and antifungal properties is by virtue of for their useful role as natural additives in foods [35]. Generally recognized as safe, EO can be used in any foods and, as long as their maximum effects is attained with the minimum change in the organoleptic properties of the food. *In vitro* studies report a high efficacy of EO and extracts against food-borne pathogens and spoilage bacteria due to the presence of bioactive substances such as flavonoids, terpenes, coumarines and carotenes [28,36,37]. The results of general screening antifungal activity of VAC EO showed highest antifungal activity against *Aspergillus niger*, followed by *Candida albicans*, *C. tropicalis*, *C. parapsilosis* and *C. dubliniensis*. These four fungi were followed by *Alternaria* and the lowest antifungal activity was shown against *C. krusei*, *A. flavus* and *Penicillium* species. Antimicrobial activities of VAC have been investigated previously and results showed that fungi were more sensitive than the bacteria. There are many reports suggesting that antimicrobial activity depends on the contents of phenolic compounds. EO from all parts of VAC exhibited fungistatic and fungicidal effect [2,29,36,38].

Clinical Indications

Phytochemical studies of VAC [2,5] revealed that extracts from this plant regulate the function of the reproductive organs in women. It is also thought to exhibit a normalizing or balancing effect on hormone production, and to increase luteinizing hormone (LH) levels without affecting follicle stimulating hormone (FSH) in women. Some data suggest pharmacological properties such as antibacterial, antihistaminic, anti-inflammatory and antioxidant activities. It can be concluded that VAC extract can have a significant adverse effect on blood parameters and can lower total serum protein and cholesterol while the exact mechanism is not yet established [5]. Studies conducted on patients suffering from human prostate cancer and benign prostatic hyperplasia showed that VAC flowers contain components that inhibit proliferation and induce apoptosis in human prostate epithelial cell lines and that they also can be useful for its prevention [39]. Studies show long-term supplementation is required for therapeutic effect [6]. Several sources [40,41,42] report response to treatment ranges from 4-6 months depending on the condition and duration since diagnosis. Some recent clinical trials show therapeutic effects after three months of treatment and a gradual return of symptoms after treatment cessation.

Pharmaceutical forms for well-established use

European Medicines Agency assessment report on VAC in November, 2010. The assessment report at hand refers to the use of the mellowed and dried fruits of VAC in phytomedicine and gives a review of scientific data.

The assessment report gives the following chemical composition of fruit:

- Iridoidglycosids (about 1%) including agnuside and aucubin, agnucastosides A-C
- Flavonoids such as casticin (lipophilic) with a content of 0.02-2.0%, small amounts of penduletin, chrysoplenole D, vitexin and eupatorin; hydrophilic flavonoids of O- or C-glycosidic types as orientin, luteolin-7-glycoside and isovitexin
- EO with main components (15-25%) such as 1,8-cineole, limonene, α- and β-pinene; in smaller amounts (2-5%) bornyl acetate, campher, p-cymene and sabinene
- Triglycerides with α-linolenic, palmitic, oleic, stearic and linolenic acid
- Diterpenes such as rotundifuran (0.04-0.3%), vitexilactone (0.02-0.17%), vitetrifolines B [10].

Replacement of synthetic additives with natural ones is recent trend which is based on use of plant extracts. Nowadays, a large number of plant-based pharmaceutical excipients are available and many researchers have explored the usefulness of plant-based materials for such purposes [43]. Different medicinal products have been presented on market of European union (EU) under well-established use and under traditional use. According to the market overview for EU members, herbal preparations based on VAC have been present on the market for more than 30 years. Each EU member state has given detailed information about products based on VAC according to the form in which they are used and their daily dosage. The data shows that the form of oral drops (solution) was available on the market from 1968 until the late '90s, when other forms of products based on VAC like capsules, film tablets, film-coated tablets and dry extracts started to appear. Recommended daily dose of VAC is 30-40 mg of herb as such or as an extract in tablets or capsules, with possibility of rash appearance which can be associated with intake of VAC. Pharmaceutical forms for well-established use are herbal preparations in solid dosage form for oral use and for traditional use solid or liquid dosage forms, also for oral use [10]. The development of new technologies has led to new solutions in the formulation of the products and increased the production of new types of products [44]. New types of products called nutraceuticals have been recently developed. Their development is based on use of natural compounds in different formulations which are available in form of pills, capsules, tablets, powder and vials [44,45].

Warnings/Contraindications

The use of VAC is contraindicated in pregnancy and lactation due to unknown effects in early pregnancy and possible hormonal effects through breast milk. According to current evidence-based clinical trials there is no human studies that have been conducted to determine the safety of VAC during pregnancy and lactation [46].

CONCLUSION

The growing demands in extraction of plant bioactive compounds encourages continuous search for convenient extraction methods. Referring to this, the increasing economic significance of bioactive products and commodities rich in these bioactive compounds may lead to discovery of more sophisticated extraction methods in future. Up to know, SFE is associated with high investments and this is often mistakenly associated with technology that cannot compete with traditional extraction techniques. Increasing performance and consumers demands and regulatory constraints being placed on many products are fulfilled with SFE based processes. This review gave an insight on different chemical composition of VAC EO produced by different extraction methods with emphasis on extracts obtained by SFE. These compounds can be extracted in higher amounts with SC-CO_2 compared to traditional organic solvents; in addition the SC-CO_2 extracts do not contain any dangerous residual solvent after the extraction. Results of the studies show that quantity and quality of compounds depends on geographical origin of the plants. VAC isolates contain flavonoids, EO, diterpenes, and glycosides, thus representing an inexpensive source of phenolic compounds. Considering the EO obtained from different parts of VAC plants, it seems that α-pinen and 1,8-cineole were highly present in all parts of plant. VAC EO showed activity against all studied fungi species so it can serve as an excellent natural source for use in traditional medicine. Due to its significant antimicrobial activity, VAC EO could find its application in food and pharmaceutical industries. The components of EO sabinene, β-sitosterol and α-pinene have antifungal, antimicrobial and insect-repellent qualities. Based on these results, the VAC

EO has provided several successful clinical trials in the last decade, thus supporting its use for treatment of premenstrual syndrome. Since EO VAC also showed an antioxidant activity and remarkable phenol content, it can serve as an excellent natural source of antioxidant agents. Healthy and beneficial effects of EO VAC should be further analyzed because a list of the uses in food and pharmaceutical industry is long.

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