Perspective

Vitex agnus-castus L.: Main Features and Nutraceutical Perspectives

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Abstract: Medicinal plants are used worldwide due to their lower risk of side effects and eco-friendly, cost-effective production when compared to chemical drugs, encouraging researchers to further exploit the therapeutic potential of the former. One of the most popular medicinal plants is Vitex agnus-castus L., grown in tropical and sub-tropical regions, to which different health benefits have already been attributed. In this perspective article, the in vitro and in vivo therapeutic properties of V. agnus-castus L. have been analyzed and reviewed with a special focus on its health-promoting effects and potential nutraceutical applications.

Keywords: chaste tree; Vitex agnus-castus L.; bioactive compounds; in vitro studies; in vivo studies; nutraceuticals; health-promoting properties.

1. Introduction

Medicinal wild plants and herbs have been considered worldwide for centuries as valuable tools in the management of different diseases, due to their ease of use and improved cost-effectiveness when compared to chemical remedies obtained from synthesis [1]. Plants have recently been exploited...
for nutraceutical purposes, as they play a key role in the development of food and plant-derived phytochemicals with medicinal properties, to be used in health conditions as preventive or curative tools [2–23]. A popular medicinal plant with recognized beneficial effects on human health is *Vitex agnus-castus* L., belonging to the Lamiaceae family (formerly included in the Verbenaceae family), native to the Mediterranean area and diffused in Europe, Asia, and North Africa [24,25]. It has been used by people in Italy, Iran, Greece, and Egypt for over 2500 years, mainly to treat gynecologic disorders [26]. It is a globally famous plant known by different names, e.g. Fruit de gattilier (French), Sauzgatillo (Spanish), Mönchspfefferfrüchte (German), Panj-angosht (Persian), Frutto di Agnecasto (Italian), and Chaste tree (English) [27].

The term *agnus-castus* combines the Greek (αγνοςκατος, meaning pure, chaste) and the Latin (*castus* from “castitas” meaning chastity), repeating the term chaste for “pure”, which refers to the anaphrodisiac properties of this plant and its use by monks to maintain celibacy (thus the “monk’s pepper” synonym). The word “castus” has been used for centuries to remark further the meaning or purity associated with this plant. Homer, the semi-legendary author of the Iliad and the Odyssey epic poems, defines *agnus-castus* as a “tendril for braiding”, explaining the origin of the genus “Vitex”. Dioscorides, the Greek physician, used to suggest *V. agnus-castus* to decrease libido. Pliny the Elder, the famous Roman author, naturalist, and natural philosopher, commented that this herb was scattered on the beds of Athenian women when husbands went to war to ensure their loyalty. Pietro Andrea Mattioli, an Italian physician and botanist of the XVI century, commented in his text “Compendium de Plantis Omnibus una cum Earum Iconibus” (1571) with reference to the properties of *V. agnus-castus* L. that: “… it forces the impulses of Venus when eaten either fried or raw … . . . . . . . . . it is believed that not only eating or drinking it will make chaste men but even lying on it . . . . . . . . . V. agnus-castus* is also known as “monk’s pepper” since the fruits of this plant have a bitter taste, and the plant used to be cultivated by monks in their gardens as an anaphrodisiac, according to a legend, to help them not betray their vow of chastity.

*Vitex agnus-castus* L. fruits have been traditionally consumed as food to enhance milk volume and to treat flatulence and diarrhea as well as cyclic breast pain, menopause, acne, infertility, premenstrual dysphoric disorder and other menstrual disorders (amenorrhea, dysmenorrhea) [28].

The European Medicines Agency and the German Health Commission have reported many health benefits of this medicinal plant, including regulation of the menstrual cycle and treatment of premenstrual syndrome, and mastalgia [29]. This paper is focused on a perspective analysis of the health-promoting effects of *V. agnus-castus* L. and its nutraceutical potential. There are many reported data on the different beneficial health-promoting potentials of this plant, including antioxidant, immunomodulatory, cytotoxic, antimitogenic, antimicrobial, antifungal, antinociceptive, opioidergic, antiepileptic, and anti-inflammatory properties, as well as benefits for osteopenic syndromes, as shown in Figure 1 [30–34].
Figure 1. Scheme of the beneficial properties of *Vitex agnus-castus* L.

2. Main Substances of Nutraceutical Interest in *Vitex agnus-castus* L.

The chemical composition of *Vitex agnus-castus* L. includes many different chemical compounds, among which are: vitexilactone, rolundifuran, ketosteroids, diterpenoids (vitexlactam, vitexilactone, viteagnusin I, and rotundifuran), flavonoids (orientin, kaempferol, penduletin, luteolin, artemetin, vitexin, and casticin), and iridoids (agnuside, agnusoside, agnucastosid A/B, and aucubin) [35–37]. The identification and quantification of agnuside (Figure 2), together with *p*-hydroxy benzoic acid, can be achieved by high-performance liquid chromatography HPLC) [38]. This method has been validated for extracts of the species *Vitex negundo* L. and *Vitex trifolia* L., with limits of quantification and detection of 25 µg/mL and 10 µg/mL, respectively [38].
According to the findings from RP-HPLC-DAD analysis, potential related, with vitetrifolin D and vitexlactam C being the most promising [45]. In another study, the method of supercritical carbon dioxide was used to detect the compounds dihydroselarene, α-caryophyllene, sabinene, and 1,8-cineole [46]. Ono et al. applied HPLC coupled with NMR analysis to determine the chemical constituents of *V. agnus-castus* L. fruits, reporting that viteagnuside was the main compound present [47].

**3. An Updated Snapshot of In Vitro and In Vivo Studies on *Vitex agnus-castus***

In-depth knowledge of the phytochemical composition of any potential medicinal plant is the first step for the determination of its beneficial health properties. In the following, the main findings...
regarding beneficial health effects from in vitro and in vivo studies are reported. This information gives an updated picture of the many activities of the compounds contained in this plant, outlining its possible prospective application in the prevention and even in the treatment of pathological conditions.

3.1. Health-Promoting Activities of Vitex agnus-castus L. In Vitro

The in vitro health-promoting potential of *V. agnus-castus* L. has been reported in the past [48]. Table 1 summarizes the main results of in vitro studies. *V. agnus-castus* fruit extract showed in vitro anticancer activity against HL-60 cells by arresting the cell cycle at the G2/M phase and by inducing apoptosis [49]. Abdel-Lateef et al. [50] analyzed the chemical composition of *V. agnus-castus* L. leaf extract and found it to be composed of phenolic acids, flavonoids, and iridoids, which could significantly prevent HepG2 cell proliferation by inducing apoptosis through caspase-3 activation, with the butanolic fraction being the most active (IC$_{50}$ = 13.42 ± 0.17 µg/mL). The cytotoxic effect of agnuside was assayed in a colon cancer cell line (COLO 320 DM), showing an IC$_{50}$ value of 15.99 µg/mL, and 76.1% cytotoxic activity at 200 µg/mL [51]. Cytotoxic activity of *V. agnus-castus* L. fruit extract against another human colon carcinoma cell line (COLO 201) was shown, by inducing activation of caspase 3/9 leading to apoptosis [52]. Ribat et al. [53] described antiproliferative and anticancer properties of *V. agnus-castus* L. fruit methanol extract, showing that normal rat embryonic fibroblast cells were much less sensitive than AMN3 cells (mouse mammary gland adenocarcinoma cell line), with IC$_{50}$ values of 1324 µg/mL and 129 µg/mL, respectively. A synergistic effect was observed with co-administration of 5-fluorouracil and 10 µg/mL vitex (ethanolic extract of *V. agnus-castus* L. fruits), using colon cancer cell lines [54]. Casticin extracted from *V. agnus castus* L. showed cytotoxic and immunomodulatory properties by inhibiting phytohemagglutinin (PHA) induced T-cell proliferation, phagocytosis, and chemotaxis [55]. Sarac et al. reported 56.18% and 72.25% antimutagenic activities for the ethanolic extracts of *V. agnus castus* L. seed and leaf at the doses of 2.5 mg/plate and 0.125 mg/plate, respectively [56].

### Table 1. In vitro reported activities for *V. agnus-castus* L.

| Condition          | Plant Part                  | Extract                  | Activity             | Effect                                                                 | Reference |
|--------------------|-----------------------------|--------------------------|----------------------|------------------------------------------------------------------------|-----------|
| In vitro           | Fruits                      | Ethyl acetate            | Antioxidant activity | Lipid peroxidation was inhibited by casticin with an IC$_{50}$ value of 0.049 mM. | [57]      |
| In vitro           | Leaves                      | Supercritical CO$_2$     | Antifungal activity  | The antifungal potential of essential oil with an MIC value of 0.64 µL/mL. | [58]      |
| In vitro           | Leaves                      | Hydrodistillation        | Antifungal activity  | The antimutagenic activity of *V. agnus-castus* leaf extract against *Salmonella typhimurium*. | [56]      |
| In vitro           | Aerial parts                | Methanolic extract (Cr. MeOH Ext.) | Antimicrobial activity | The *V. agnus-castus* leaf essential oil showed an antibacterial effect against *Staphylococcus aureus* with an MIC value of 0.31% w/w. | [59]      |
| In vitro           | Seed                        | n-hexane                 | Antifungal activity  | Essential oil was effective against *Candida* species with an MIC50 value of 1.75 mg/mL. | [60]      |
| In vitro           | Ripened fruits and fruitless aerial parts | Ethanol | Anticancer activity | Cytotoxic effect of *V. agnus-castus* fruit extract against MCF-7 cancer cells with IC$_{50}$ = 88 µg/mL. | [61]      |
| In vitro           | Fruits                      | Chloroform–methanol (250:1) | Anticancer activity | The cytotoxicity was due to differentiation of the hematopoietic cell line. | [62]      |
| In vitro           | Fruits                      | Ethanol                  | Anticancer activity  | Enhanced apoptosis and decreased intracellular ROS levels. | [49]      |
| In vitro           | Leaves                      | Methanol                 | Antioxidant effect   | The free radical scavenging effect of *V. agnus-castus* methanol extract with an IC$_{50}$ value of 126.79 mg/mL. | [63]      |
The health of humans, animals, and the food chain is directly and indirectly influenced by various pathogens. Thus, extensive studies have been conducted to control such microorganisms using different approaches, among which the use of cost-effective and more effective natural plant extracts or essential oil has attracted further attention; for example, *V. agnus-castus* L. has been studied against several pathogenic species such as *Staphylococcus aureus*, *Escheria coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. Habbab et al. [64] evaluated the antifungal activity of *V. agnus-castus* flower and leaf essential oils against *Aspergillus flavus* and *Penicillium expansum*, as well as the antibacterial activity of *V. agnus-castus* L. seed and leaf essential oils against *P. aeruginosa*, *E. coli*, and *Klebsiella pneumonia*. *Vitex agnus-castus* L. essential oil was shown to exhibit antibacterial activity against *Staphylococcus aureus* [65]. Afarin et al. [66] observed in vitro antimicrobial activity for *V. agnus-castus* L. essential oil at doses of 112.5 and 56.25 µg/mL against *Candida albicans* and *S. aureus*, respectively. Katiaree et al. [67] found radical scavenging (IC₅₀ = 27.16 µg/mL) and antifungal properties for *V. agnus-castus* L. essential oil. Others reported that *V. agnus-castus* L. essential oil showed antifungal potential against *Sclerotinia sclerotiorum* and *Verticillium dahlia* with LC₅₀ values of 3.322 µg/mL and 1.063 µg/mL, and 9.729 µg/mL and 7.313 µg/mL, respectively [68]. Stojković et al. [69] reported antimicrobial potential for *V. agnus-castus* L. fruit and leaf essential oils attributed to the presence of α-pinene and 1,8-cineole. The administration of active ethyl acetate extract of *V. agnus-castus* L. leaf exhibited antibacterial potential against methicillin-resistant *S. aureus* (MIC = 0.312 mg/mL) owing to steroids, terpenoids, and flavonoids [70]. *Vitex agnus-castus* L. leaf essential oil eliminated the cariogenic bacteria *Streptococcus mutans* (MIC = 15.6 µg/mL) [71]. In another study, alcoholic and aqueous extracts had an antifungal effect on *C. albicans* isolated from clinical vaginal infections [72]. *Vitex agnus-castus* L. seed essential oil showed antifungal activity against *Candida* species (IC₅₀ = 1.072 mg/mL) and also antioxidant potential [73].

The anticancer activity of *Vitex agnus-castus* L. seed extracts against MCF-7 cells showed DNA-damaging, cytotoxic, and apoptotic effects, and this extract also showed antioxidant properties [74]. Rashed et al. [75] stated that the antioxidant effect of ethyl acetate extract of *V. agnus-castus* could be attributed to flavonoids and tannins, as the main compounds. The antioxidant effect of *V. agnus-castus* L. fruit and leaf extract was confirmed by the decolorization of the radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay [76]. Matlaq et al. described a H₂O₂ scavenging effect for *V. agnus-castus* L. extract with antioxidant activity of 93.5 ± 0.8% [77]. In another study, using Ethanolic extract of *V. agnus-castus* L. leaf, containing mainly flavonoids and phenols as active ingredients, it was revealed that there was a direct correlation between these compounds and antioxidant properties [78]. Ahmad et al. [79] reported that vitexcarpan, isolated from the aerial parts of *V. agnus-castus* L. by extraction using ethyl acetate, showed anti-inflammatory activity in activated human neutrophils. The presence of casticin in *V. agnus-castus* L. extract exhibited anti-inflammatory potential with an efficacy of 69.51% and an IC₅₀ value of 302.1 µg/mL [80].

*Vitex agnus-castus* L. fruit extract showed antiangiogenic activity due to the presence of antiangiogenic compounds, confirmed by phytochemical analysis [81].

### 3.2. Health-Promoting Activities of *Vitex agnus-castus* L. in Animals

There are numerous therapeutic effects of *V. agnus-castus* L. described in animal models. Table 2 reports the results of the main animal model studies. Oral administration of *V. agnus-castus* L. extract (200 mg/kg) in Sprague–Dawley rats with a mammary tumor for 15 days improved the oxidative status of the mammary tumor tissue and treated tumor regression properties [82].
### Table 2. Animal model reported activities for *V. agnus-castus* L.

| Condition | Plant Part | Extract | Activity | Effect | Reference |
|-----------|------------|---------|----------|--------|-----------|
| Animal model | Leaves | Hydrodistillation | Antinociceptive activity and analgesic effect | The analgesic activity was due to the activation of muscarinic receptors of the cholinergic system and endogenous opioidergic system. | [83] |
| Animal model | Stems and leaves | Ethanol | Treatment of polycystic ovary syndrome | *V. agnus-castus* exhibited hypoglycemic, antioxidant, and antihyperlipidemic activities in rats. | [84] |
| Animal model | Fruits | Chloroform, methanol, and water | Antihyperlipidemic activity | The *V. agnus-castus* extract (500 mg/kg for 28 days) decreased the levels of VLDL, LDL, TG, and TC. | [85] |
| Animal model | Fruits | Hexane, ethyl ether, and n-butanol | Protected against nonalcoholic fatty liver disease | Prevented oxidative stress and treated nonalcoholic fatty liver disease. | [86] |
| Animal model | Berries | Ethanol | Anti-inflammatory activity | Inhibited the production of reactive oxygen species, the release of cytokines, and the formation of leukotriene. | [87] |
| Animal model | Leaves | Chloroform | Antiangiogenic activity | Prevented growth of psoriasis, cataract, and tumor. | [88] |
| Animal model | Fruits | Ethanol | Antiaging effects | Improved α-galactose-induced aging symptoms, including enhanced serum LH and FSH levels, follicle degeneration, and endometrial atrophy. | [89] |
| Animal model | Fruits | Methanol | Antiepileptic activity | Reduced stage 5 duration and after-discharge duration. | [90] |
| Animal model | Fruits | Ethanol | Osteoprotective effects | Enhanced biomechanical stability of bone via connectivity density in the orchidectomized rats and improved the trabecular microarchitecture. | [91] |
| Animal model | Leaves | Methanol | Anti-inflammatory effect | The *V. agnus-castus* methanol extract (400 mg/kg) reduced IL-6 and TNF-α levels. | [92] |
| Animal model | - | Methanol, n-hexane, and ethyl acetate | Antioxidant and antiapoptotic effects | Vitexilactone extracted from *V. agnus-castus* reduced caspase-3 and apoptosis marker expression in Sprague–Dawley rats. | [93] |

The administration of casticin extracted from *V. agnus-castus* L. fructus (1, 2, and 10 mg/Kg per day) for two weeks protected inflammatory lung diseases in a mouse model due to an anti-inflammatory effect by decreasing epithelium thickness, perivascular inflammatory cells, and peribronchial infiltration, as well as reduced the numbers of total cells, lymphocytes, macrophages, and neutrophils [94]. The administration of *V. agnus-castus* L. fruit extract (165 mg/Kg/day) in rats controlled prostate cancer by inducing apoptosis and showed an anti-inflammatory effect by inhibiting cyclooxygenase-2 activity [95]. Webster et al. reported that *V. agnus-castus* attenuates pre-menstrual syndrome [96].
Vitex agnus-castus administered for five days protected mice against lipopolysaccharide (LPS)-induced acute lung damage, due to an antioxidant effect [97]. The methanol extract of V. agnus-castus leaf showed an antiangiogenic effect in an ex vivo rat aorta, and also antioxidant activity with an IC50 value of 126.79 µg/mL [88].

Moreover, administration of V. agnus castus Ethanolic extract (8 and 80 mg/Kg/day) for three months in ovariectomized rats improved memory and learning via decreasing uterine weight and increasing the estrogen receptor ERα gene expression, respectively, suggesting a solution for memory loss in postmenopausal women [98]. The hydroalcoholic extract of V. agnus-castus fruits (600 mg/Kg twice a day) reduced age-related changes in a female mice model after 7 days [99].

V. agnus-castus extract was administered to a mouse model of permanent middle cerebral artery occlusion for 30 days, and it was observed that the anti-inflammatory and estrogenic activities reduced stroke injuries. Moreover, this study also reported neuroprotective activity by reducing matrix metalloproteinase-9 (MMP-9), increasing interleukin 10, and improving adhesive removal and wire hanging test performance [100].

3.3. Health-Promoting Activities of Vitex agnus-castus L. in Humans, with Particular Regard to Clinical Trials

Many clinical trials confirm the health-promoting effects of V. agnus-castus L. [101–108]. Table 3 reports the results of the main clinical trial studies. Naseri et al. [109] reported a reduction in menopausal symptoms after taking V. agnus-castus L. extracts. The authors allocated the participants into two placebo groups and a Vitex-treated group, and then assessed menopausal symptoms before and after an 8-week intervention using the Greene Questionnaire [110]. After the intervention, the Vitex group showed a reduction in vasomotor dysfunction, anxiety, and total menopausal disorder. Yavarikia et al. [111] administered V. agnus-castus L. capsules to female participants three times a day for four months, and obtained data with the Higham five-stage chart (for 1 month before the treatment and 4 months during the treatment) [112] and a demographic questionnaire. They found a 47.6% decrease in bleeding in the V. agnus-castus L. group. Oral administration of ethanolic extract of V. agnus-castus L. (4.0 mg), dried as film-coated tablets, in Chinese women with premenstrual syndrome (PMS) reduced the PMS score of the third cycle from 27.10 to 14.59 in the case group [113]. Daily administration of V. agnus-castus L. extract-containing tablets in Chinese women with premenstrual syndrome (PMS) decreased the PMS scores of symptoms such as pain, food cravings, and water retention [114]. A self-assessment questionnaire was completed for the symptoms of PMS in women after taking 40 drops of V. agnus-castus L. extract for 6 days, the results of which showed mild to moderate relief in PMS symptoms [115]. In a recent clinical trial, it was reported that the administration of V. agnus-castus L. in women with polycystic ovary syndrome reduced the level of dehydroepiandrosterone sulfate (DHEA-S) and normalized the menstrual cycle [116]. Healthy menopausal women co-administrated V. agnus-castus L. and Nigella sativa with citalopram once a day for 8 weeks showed superior scores in three of the Menopause-specific Quality of Life Questionnaire (MENQOL) domains: psychosocial (p = 0.001), physical (p = 0.036), and vasomotor (p < 0.001) [117].
Vitex agnus-castus L. extracts have reportedly exhibited many health-promoting effects, but some limitations have been shown for human health such as agitation, headache, nausea, tachycardia, fatigue, dry mouth, urticaria, and gastrointestinal problems [78]. In a study by Owolabi et al. [127], it was also reported that increasing the dose and long-term administration of the Vitex genus induced toxicity in rats.

| Condition | Activity | Administration | Effect | Reference |
|-----------|----------|----------------|--------|-----------|
| Clinical trial | Treatment of vasomotor symptoms. | The administration of *V. agnus-castus* (40 mg) once a day for a month in women with postmenopausal symptoms. | Improvement of sleep satisfaction. | [118] |
| Clinical trial | Treatment of mastalgia. | The administration of *V. agnus-castus* in patients with mastalgia. | Reduction in prolactin level after three months. | [119] |
| Clinical trial | Treatment of premenstrual syndrome. | Administration of *V. agnus-castus* extract (20 mg) once a day for three menstrual cycles in Japanese women. | The symptoms of premenstrual syndrome were improved. | [120] |
| Clinical trial | Treatment of premenstrual syndrome. | The administration of *V. agnus-castus* (40 mg) once a day for three months in migrainous women with premenstrual syndrome. | The symptoms of premenstrual syndrome were reduced in 66 women. | [121] |
| Clinical trial | Treatment of premenstrual syndrome. | The administration of *V. agnus-castus* extract Ze 440 (20 mg) once a day. | The symptoms of premenstrual syndrome were relieved in women. | [122] |
| Clinical trial | Treatment of menopausal syndrome. | The administration of *V. agnus-castus* extract (40 drops) once a day for 8 weeks in women. | Hot flashes were positively influenced by Vitex in women. | [123] |
| Clinical trial | Treatment of premenstrual syndrome. | The co-administration of *V. agnus-castus* with *Hypericum perforatum* twice a day for 16 weeks in women with premenstrual syndrome. | Symptoms such as hydration clusters and anxiety were alleviated. | [124] |
| Clinical trial | Treatment of premenstrual syndrome. | The administration of *V. agnus-castus* extract (40 drops) for 4 months. | The pregnancy rate, endometrial thickness, ovulation, and fertility were increased in women. | [125] |
| Clinical trial | Prolactin-inhibiting activity. | The daily administration of *Agnus-castus* extract (BP1095E1) at a concentration of 480 mg for two weeks in healthy male subjects. | Decreased prolactin profile levels. | [126] |
4. Conclusions

Considering the naturally occurring bioactive compounds with therapeutic potential that it contains, *V. agnus-castus* L. is one of the best-selling and most widely used medicinal herbs worldwide. Nevertheless, there is a need for further in vivo and in vitro research and more clinical trials to assess the beneficial health compounds content of this plant and the related mechanisms of action in the treatment of various diseases, as it is so far been largely limited to animal model trials and in vitro studies. The clinical trial studies in humans focus on a limited number of health conditions, suggesting the need to exploit what is observed in animal trials and possible in prospective assess also possible applications in humans. The beneficial properties of *V. agnus-castus* L. trigger interest in the possibility of developing novel nutraceutical formulations, which can help to support health conditions before the need of a pharmacological therapy, in particular for individuals who do not qualify for a conventional drug-based treatment. It should be noted also that there is a need for more comprehensive clinical trials over a long timeframe in order to draw definitive conclusions about the findings related to the various effects and consequences of a long-term consumption of *V. agnus-castus* L.

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