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INTERNATIONAL JOURNAL OF PROGRESSIVE RESEARCH IN ENGINEERING MANAGEMENT AND SCIENCE (IJPREMS)
(Int Peer Reviewed Journal)
Vol. 04, Issue 12, Decembaer 2024, pp : 1756-1765

e-ISSN : 2583-1062
Impact Factor : 7.001

VITEX AGNUS-CASTUS: A HERB FOR THE AGES – EFFICACY, SAFETY, AND APPLICATION

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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 . Plants have recently been exploited for nutraceutical purposes, as they play a key role in the development of food and plant-derived phytocomplexes with medicinal properties, to be used in health conditions as preventive or curative tools . 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. It has been used by people in Italy, Iran, Greece, and Egypt for over 2500 years, mainly to treat gynecologic disorders . 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 Agnocasto (Italian), and Chaste tree (English) .

The term agnus-castus combines the Greek (ἀγνοῦς, 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) .

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 mastalgiaThis 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, antimutagenic, antimicrobial, antifungal, antinociceptive, opioidergic, antiepileptic, and anti-inflammatory properties, as well as benefits for osteopenic syndromes, as shown in Figure 1 .

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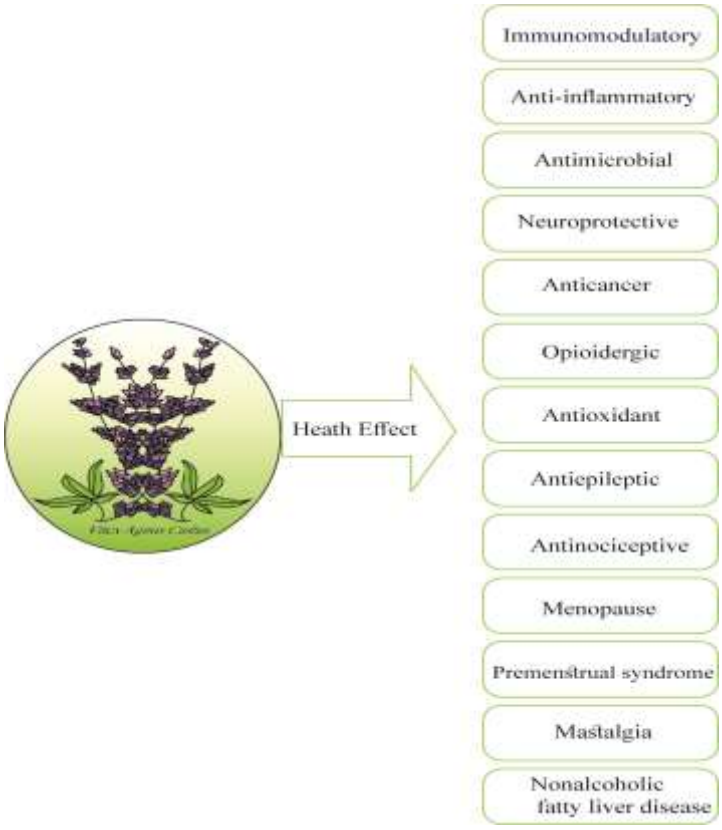
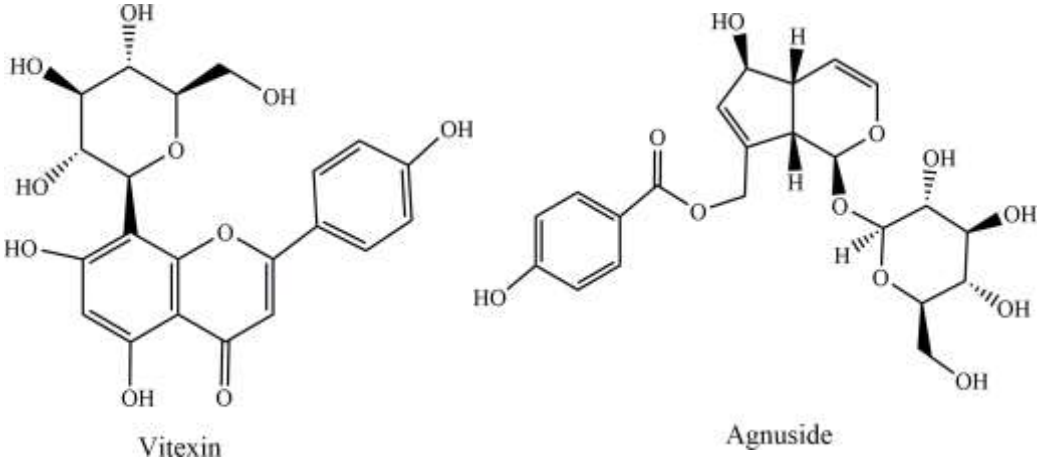


Figure 1. Scheme of the beneficial properties of Vitex agnus-castus L.

Main Substances of Nutraceutical Interest in Vitex agnus-castus

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) .The identification and quantification of agnuside (Figure 2), together with p-hydroxy benzoic acid, can be achieved by high-performance liquid chromatography HPLC) .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 .



(**a**) (**b**)

Figure 2. Chemical structure of vitexin (**a**) and of agnuside (**b**).

Gokbulut et al. measured the levels of vitexin (Figure 2), isolated from V. agnus-castus L. fruit and leaf extracts, using the RP-HPLC-DAD (diode array detector) technique, and the results showed that this flavonoid was present in considerable amounts (0.342 ± 0.0153% and 0.252 ± 0.0089%, respectively) .

Agnuside together with casticin (a tetramethoxyflavone) have been used as fingerprint markers to evaluate the quality of Japanese commercial products containing V. agnus-castus L. . Another marker validated for quality assessment was reported by Yahagi et al., using a liquid chromatography–mass spectrometry (LC-MS)-based metabolomic technique

and nuclear magnetic resonance (NMR) spectroscopy to detect 3-O-trans-feruloyl tormentic acid, which was isolated from the V. agnus-castus L. fruit extract . In another study, using a rapid ultra-high performance liquid chromatography diode array detector (UHPLC-DAD-QTOF-MS), seven markers of V. agnus-castus L. fruit extract were claimed to be used as reference compounds for quality validation of medicinal products containing this extract, in particular, vitetrifolin D (labdane diterpenoid), 5-hydroxykaempferol-3,6,7,4'-tetramethylether, casticin, isovitexin, and agnuside compounds . A study conducted to compare the chemical markers occurring in food supplements and in V. agnus-castus L. extracts, using the liquid chromatography electrospray triple quadrupole tandem mass spectrometry (LC/ESI/(QqQ)MSMS) method in multiple reaction monitoring (MRM) mode as a quantitative analysis, reported the presence of aucubin, orientin, luteolin-7-O-glucoside, agnuside, isovitexin, homoorientin, and casticin compounds . According to the findings from RP-HPLC-DAD analysis, V. agnus-castus L. leaf and fruit extracts contained chlorogenic and caffeic acid phenolic compounds with average concentrations of 0.27% and 0.32% (w/w), respectively .Li et al. isolated eighteen compounds from V. agnus-castus L. fruit extract, using 1D/2D NMR and mass spectrometry methods, and their chemopreventive potential was studied in Hepa 1c1c7 cells, which showed NADP(H): quinone oxidoreductase type 1 (QR1) induction potential related, with vitetrifolin D and vitexlactam C being the most promising . In another study, the method of supercritical carbon dioxide was used to detect the compounds dihydroselarene, α-terpinyl acetate, trans-caryophyllene, sabinene, and 1,8-cineole. 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 .

1. 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.

1.1. 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 . 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 .Abdel-Lateef 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 mg/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 . Cytotoxic activity of V. agnus-castus L. fruit extract against other another human colon carcinoma cell line (COLO 201) was shown, by inducing activation of caspase 3/9 leading to apoptosis . Ribat 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 . Casticin extracted from V. agnus castus L. showed cytotoxic and immunomodulatory properties by inhibiting phytohemagglutinin (PHA) induced T-cell proliferation, phagocytosis, and chemotaxis . 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 .

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. evaluated the antifungal activity of V. agnus-castus flower and leaf essential oils against Aspergillus flavus and Penicillium escpansum, 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 . Afarin 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. Katiraee found radical scavenging (IC_{50} = 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 LC_{50} values of 3.322 µg/mL and 1.063 µg/mL, and 9.729 µg/mL and 7.313 µg/mL, respectively .Stojkovic´ reported antimicrobial potential for V. agnus-castus L. fruit and leaf

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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. Vitex agnus-castus L. leaf essential oil eliminated the cariogenic bacteria Streptococcus mutans (MIC = 15.6 µg/mL). In another study, alcoholic and aqueous extracts had an antifungal effect on C. albicans isolated from clinical vaginal infections . Vitex agnus-castus L. seed essential oil showed antifungal activity against Candida species (IC_{50} = 1.072 mg/mL) and also antioxidant potential .

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. Rashed 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^{J}-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay Maltas¸ et al. described a H_2O_2 scavenging effect for V. agnus-castus L. extract with antioxidant activity of 93.5 ± 0.8% 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 Ahmad 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 IC_{50} value of 302.1 µg/mL.

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

1.2. 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 .

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. 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. Webster et al. Reported that v. Agnus-castus attenuates pre-menstrual syndrome.

Table 1. 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 opiodergic system.	[83]
Animal model	Steam and leaves	Ethanol	Treatement of polycystic ovary syndorme	v. agnus-castus exhibited hypoglycemic ,antioxidant activities in rats.	[84]
Animal model	Fruits	Choloroform , methanol and water	Antihyperlipidemic activity .	The v. agnus-castus Extract decrease the level VLDL, LDL, TG, TC	[85]
Animal model	Fruits	Hexane,ethyl ,ether,&n-butanol	Protected against non alcholic fat liver diseases	Prevented oxidative stress and treated alcoholic fat liver diseases	[86]

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Animal model	Berries	Ethanol	Anti-inflammetory Activity	Inhibited the production of reactive oxygen in species the release of cytokines .	[87]
Animal model	leaves	chloroform	Antiangiogenic activity	Prevent growth of psoriasis , cataract and tumor	[88]
Animal model	fruits	Ethanol	Antiaging effects	Improved, d-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 afterdischarge duration.	[90]
Animal model	leaves	Methanol	Anti-inflammatory activity	The V. agnus-castus methanol extract (400 mg/kg) reduced IL-6 and TNF-α levels.	[91]
Animal model	fruits	Methanol	Osteoprotective activity	Enhanced biomechanical stability of bone via connectivity density in the orchidectomized rats and improved the trabecular microarchitecture.	[92]

Vitex agnus-castus administered for five days protected mice against lipopolysaccharide (LPS)-induced acute lung damage, due to an antioxidant effects . The methanol extract of V. agnus-castus leaf showed an antiangiogenic effect in an ex vivo rat aorta, and also antioxidant activity with an IC_{50} value of 126.79 µg/mL . 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. 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 .

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.

1.3. 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. reports the results of the main clinical trial studies. Naseri 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 After the intervention, the Vitex group showed a reduction in vasomotor dysfunction, anxiety, and total menopausal disorder. Yavarikia 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) 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 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.

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 .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 dehy-droepiandrosterone sulfate (DHEA-S) and normalized the menstrual cycle. 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 ($p < 0.001$) .

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 . In a study by Owolabi it was also reported that increasing the dose and long-term administration of the Vitex genus induced toxicity in rats.

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Animal model	fruits	Methanol	Osteoprotective activity	Enhanced biomechanical stability of bone via connectivity density in the orchidectomized rats and improved the trabecular microarchitecture.	[92]

2. 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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