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In vitro antimutagenic activity of *Vitex agnus-castus* L. essential oils and ethanolic extracts

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ABSTRACT

This study investigated the antimutagenic activity of the essential oil of the leaves and the ethanolic extract of the seeds of *Vitex agnus-castus*, a common plant with both medicinal and economic value. Ames *Salmonella*/microsome mutagenicity tests showed the essential oil of *V. agnus-castus* leaves at 0.125, 0.0125, and 0.00125 mg/plate concentrations and the ethanolic extract of *V. agnus-castus* seeds at 2.5, 0.25, and 0.025 mg/plate concentrations to have antimutagenic effects on *Salmonella typhimurium* TA98 and TA100. Moreover, neither the essential oils nor the ethanolic extracts exhibited any mutagenic activity themselves. To our knowledge, this is the first study to be conducted on the antimutagenic activities of *V. agnus-castus* essential oils and extracts and provides important data for the fields of medicine and pharmaceuticals.

1. Introduction

Research has shown that mutation rates have risen due to the effects of human civilization and global environmental pollution. Moreover, increasing evidence has shown that somatic cell mutation plays a role in carcinogenesis as well as in the development of genetic disorders such as atherosclerosis and heart disease (Stockwell, 1988). Any agent that increases DNA damage or cell proliferation can also increase the rate of mutation, which, in turn, can alter cell regulatory control, leading to uncontrolled cell division and cancer (Zaveri et al., 2011). Conversely, antimutagens, such as those present in plants as secondary metabolites, may help strengthen cell defenses against environmental mutagens and stress (Kaur et al., 2010). Thus, the interest in studying the effects of plant compounds on DNA has increased in recent years.

The species *V. agnus-castus* – once phylogenetically classified in the Verbenaceae family, but now situated within the Lamiaceae family (Chantaranothai, 2011) – is a small tree or shrub native to the Mediterranean region. Mostly found along dense coastal areas and rivers, *V. agnus-castus* is grown as an ornamental plant in tropical and subtropical regions around the world (Tutin et al., 1972), including in Western Anatolia, where it can be found in the parks and gardens of most cities (Orçun, 1975). The plant has a variety of economic uses: the prized "agnus castus honey" is obtained from this plant; its thin, flexible branches make it ideal for basket weaving; its leaves are used for dying fabrics and wools for rug and carpet weaving; and both the leaves and fruit are ground and sprinkled over woollen fabrics to protect them from moths (Baytop, 1984).

Commonly known as 'vitex' or 'chaste tree', *V. agnus-castus* has been used for medicinal purposes for at least two thousand years (Baytop, 1984). While Hippocrates recommended chaste tree for treating injuries and inflammation (Chantaranothai, 2011), Vitex fruit has primarily been used in folk medicine to treat obstetric and gynecological complaints (Kunio et al., 2003), for example, to relieve uterine cramps and regulate menstruation, as a lactogen (Saglam et al., 2007), and to prevent premature labor (Baytop, 1984; Chhabra and Kulkarni, 2011). In addition to obstetric and gynecological treatment, *V. agnus-castus* has also been used as a diuretic, an anorectic, a hypnotic, and in the treatment of dyspepsia (Blumenthal, 2000); as an antifungal and anti-anxiety medication (Baytop, 1984; Chhabra and Kulkarni, 2011); and for the treatment of acne (Pearlstein and Steiner, 2008; Chhabra and Kulkarni, 2011). Moreover, infusions of *V. agnus-castus* fruits and flowering tops are widely used as a sedative, antispasmodic and an aphrodisiac (Sijelmassi, 1991). Secondary effects are rare, and may include rashes, gastrointestinal disorders, headaches and increased menstrual flow (Saglam et al., 2007).

According to Brazier and Levine (2003), the success of *V. agnus-castus* in regulating amenorrhea, infertility and menopause may be

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attributed to phytoesterogenic compounds. Phytochemical analyses of *V. agnus-castus* have revealed the presence of phenolic acids and their derivatives, glycosides, flavonoids, annins, iridoids, diterpenoids, steroids and essential oils (Belie et al., 1961; Males et al., 1998; Hoberg et al., 1999; Hajdu et al., 2007; Saglam et al., 2007; Borges et al., 2012). GC/MS analysis of essential oil isolated from *V. agnus-castus* leaves using hydrodistillation identified 32 components representing 94.3% of the total oil content, with the major components as follows: α-terpineol (11.5%), trans-β-farnesene (7.7%), 2-amino-6,7-dimethylpteridin-4-ol (6.5%), terpinen-4-ol (6.3%), γ-cadinene (6.3%), and terpinolene (5.6%) (Omikorede et al., 2012).

Whereas previous studies have shown pharmaceutical and biological activity of *V. agnus-castus* to include antimicrobial (Maltaş et al., 2010; Azizuddin and Choudhary, 2011; Ghannadi et al., 2012) and antioxidant functions (Saglam et al., 2007; Sarikurkcu et al., 2009; Maltaş et al., 2010), this study is the first to evaluate essential oil of *V. agnus-castus* leaves and ethanolic extract of *V. agnus-castus* seeds for antimutagenic activity in order to facilitate their use in the fields of agriculture and phytomedicine.

2. Materials and methods

2.1. Plant material

Leaves and seeds of *V. agnus-castus* plants were collected from Muğla, Turkey, air-dried at room temperature for 7 days, and then stored for analysis at a later date.

2.2. Essential oil and ethanolic extract preparation

Essential oil of *V. agnus-castus* leaves was obtained by hydrodistillation using a clevenger apparatus. Ethanol extract was obtained from ground seeds using a Soxhlet apparatus. Following evaporation, the extract was diluted in ethanol/water (1:1, v/v), and the essential oil was diluted in DMSO/water (1:9, v/v). The essential oils and extracts were stored in sterile opaque bottles under refrigerated conditions until use.

2.3. Bacterial strains

Mutagenicity and antimutagenicity tests were performed using *Salmonella typhimurium* TA98 and TA100. Both strains were analyzed according to Mortelmans and Zeiger (2000) for histidine and biotin requirements alone and in combination, rfa mutation, excision repair capability, presence of plasmid pKM101 and spontaneous mutation rates. Bacterial stock cultures were inoculated in nutrient broth and incubated at 37 °C for 12–16 h with gentle agitation (Oh et al., 2008).

2.4. Mutagenic and antimutagenic activity

2.4.1. Viability assays and determination of test concentrations

Cytotoxic doses of the essential oils and extracts were determined according to Mortelmans and Zeiger (2000). The toxicity of the essential oils and extracts toward *S. typhimurium* TA98 and TA100 was determined as described in detail elsewhere (Santana-Rios et al., 2001; Yu et al., 2001).

2.4.2. Mutagenicity and antimutagenicity tests

Mutagenicity and antimutagenicity of *V. agnus-castus* were examined using the plate incorporation method (Maron and Ames, 1983) described in detail by Sarac and Sen (2014). Known mutagens 4-nitro-o-phenylenediamine (4-NPD) 3 μg/plate) and sodium azide (NaN_3) (8 μg/plate) were used as positive controls for *S. typhimurium* TA98 and *S. typhimurium* TA100, respectively. DMSO/water (1:9, v/v) and ethanol/water (1:1, v/v) were used as a negative control for essential oils and ethanolic extracts, respectively. Essential oil was prepared at concentrations of 0.125, 0.0125 and 0.00125 mg/plate, whereas ethanolic extract was prepared at concentrations of 2.5, 0.25 and 0.025 mg/plate. Mutagenicity inhibition (%) was calculated using the following equation:

$$\text{Inhibition} = [(M - S_1) - (M - S_0)] \times 100$$

where M = number of revertants/plate induced by mutagen alone; S_0 = number of spontaneous revertants; and S_1 = number of revertants/plate induced by the extract plus the mutagen.

Antimutagenicity was recorded as follows: strong: 40% or more inhibition; moderate: 25–40% inhibition; low/none: 25% or less inhibition (Negi et al., 2003; Evandri et al., 2005).

2.5. Statistical analysis

Experiments were performed in triplicate, and results were recorded as mean ± SD. Data was entered into a Microsoft Excel database and analyzed using SPSS.

3. Result and discussion

This study examined essential oil of *V. agnus-castus* leaves and ethanolic extract of *V. agnus-castus* seeds for their potential antimutagenicity toward *S. typhimurium* TA98 and TA100. The findings are summarized in Tables 1 and 2.

Considering that mutations are important early steps in carcinogenesis, short-term genetic tests such as the *Salmonella*/reversion assay and DNA strand scission assay have been used successfully to identify mutagens/carcinogens as well as antimutagens/anticarcinogens (Rausher et al., 1998; Horn and Vargas, 2003). This study used the Ames *Salmonella*/microsome mutagenicity test,

Table 1
Results of antimutagenicity assays of essential oils of *V. agnus-castus* leaves for *S. typhimurium* TA98 and TA100 bacterial strains.

Test items	Concentration (mg/plate)	Number of revertants			
		TA98		TA100	
		Mean ± S. error	Inhibition%	Mean ± S. error	Inhibition%
Negative control		9.33 ± 4.5[b]		9.33 ± 2.51	
4-NPD[a]	3	196 ± 14.84		–	
NaN_3[a]	8	–		356 ± 15.57	
Essential oil	0.125	58.2 ± 4.86	70.31	98.8 ± 5.49	72.25
	0.0125	66.2 ± 2.77	66.22	117.2 ± 3.19	67.08
	0.00125	72.2 ± 3.76	63.16	140.4 ± 5.68	60.56

[a] 4-NPD and NaN_3 were used as positive controls for *S. typhimurium* TA98 and TA100 strains, respectively.
[b] Values expressed are means ± SD of three replications. Regression analysis for mutagenicity inhibition (%) and plant extract concentrations (log values) (R^2: 0.99) was performed using Microsoft Excel.