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Antibacterial activities and essential oil composition of chaste tree (*Vitex agnus cactus L.*)

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Essential oil composition and antibacterial activity of chaste tree (*Vitex agnus-castus* L.) have investigated. Twenty seven components were identified in the essential oil that represented 91.8 % of the oil. The major components of the essential oil were alpha-pinene (16.22%), Eucalyptaol (1,8 cineole) (20.12 %), α terpenylacetate (6.12 %), Sabinene (8.33%), Limonene (5.85 %), β-farnesene (5.48%) and 4-terpineol (6.17%). After determining the phenolic contents of the essential oils in the seeds and leaves of our plant, by disc diffusion method, Gr (-), The antimicrobial effects of *Escherichia coli* ATCC 25922, Gr (-) *Pseudomonas aeruginosa* ATCC 27853, Gr (+) *Staphylococcus aureus* ATCC 25923 and Standard Gr (+) *Micrococcus lute67* NCIMB 132 were investigated. It has been proven that this active ingredient have a positive effect against bacteria and fungi and 1.8 cineole compound has been found to be effective against the *Aspergillus niger* pathogen. However, it was seen that the inhibition zone formed by the seed oil on the *Pseudomonas aeruginosa* bacterium is higher than on the *Escherichia coli* bacterium.

Keywords: GC-MS; Essential oils composition; Chaste tree (*Vitex agnus castus)* L, ; Antimicrobial activity.

INTRODUCTION

Chaste tree (Chaste berry) has its original latin name as *Vitex agnus-castus* Linn. (VAC) plant has been used in medicine for more than 2000 years. (Meier et al. 1994, Asdadi et al. 2015). It is seen that it was first mentioned in the inscriptions of Hippocrates in the 4th century BC (Odenthal 1998). It is known that its fruits are used as an infusion (2-5%) as a diuretic, gas remover and sedative (Fakir et al. 2014). In addition, moderate cytotoxic and pro-apoptotic effects of VAC extracts in human cancer cells have been demonstrated (Sezik et al. 2013). It has been suggested that VAC can be used as a valuable tool in the treatment of osteoporosis, benign enlargement of the prostate and prostate cancer in men (Ingjatovic, 2012). In vivo studies of VAC extracts have been shown to decrease prolactin levels, increase healing in bone fractures, and have osteopenia preventive effects (Sezik et al. 2013). Essential oils, also called essential oils, are complex mixtures obtained from plants or parts of these plants by distillation or pressing (Evren and Tekgüler 2011). Since essential oils obtained from plants can easily pass through the cell membrane, they can be easily absorbed from organs such as the skin and lungs. In addition, the pharmaceutical value of essential oils taken into the body as medicine or food additives is quite high and their biological activation is still a matter of curiosity (Erdogan, 2012). Essential oils are used in different fields such as cosmetics, perfumery, medicine and food industry. Their use and acquisition have reached the present day starting from the Roman period (Kılıç, 2008). In addition, the effects of spices and essential oils, which are used in many fields such as food, pharmaceutics, perfumes and cosmetics, have been tested in many studies in terms of their antimicrobial effects, starting from the 1980s. The effects of spices and their derivatives (extracts, essential oils and components) are generally used as antibacterial and antifungal effects in vitro (Arslan and Karabulut 2005). The reasons such as the places where the plants from which essential oil is obtained are grown, climatic conditions and stress factors affect the amount of decrease or increase in the ratio of active substances they contain. (Summer and Reproduction 2015). In the literature studies we have done, many studies (antifungal, antibacterial, antitumor, hormonal, prostate cancer, etc.) related to the *Vitex agnus-castus* L. species have been found. In addition, the secondary component richness in its content has been proven in most studies and the areas of use are specified. In addition to all these, antibacterial studies were carried out with the disk diffusion method in order to show the diversity of essential oils in its content by total phenolic and GC/MS analysis and to see the antibacterial effects of these components.

The aim of this research is to obtain essential oils from the aerial parts of *Vitex agnus- castus,* to determine their content and to determine their antibacterial activity.

MATERIALS AND METHODS

Plant Material

Plant samples of chaste tree (leaves and seeds) were collected from the natural environment of the province of Denizli (Karahayıt, 253 m) in Turkey in April 2017.

Preparation of extracts

Plants brought to the laboratory environment were dried without losing their morphological characteristics (Baydar et al. 2008; Yaşar 2005). The dried plants were cut into small pieces with the help of a blender (Waring Commecial Blender, USA) and made ready for the extraction process. 100 ml solvent was used for each 10 g sample (Faresin et al. 2000). Ethanol (Merck, Germany) was used as the solvent (Dülger et al. 2002; Ono et al. 2011). Plants cut into small pieces were extracted with ethanol in a Nucleon Water Bath for 6 hours at 55 ºC. This process was repeated at least 2 times. The resulting mixture was filtered through Whatman No 1 filter paper. Then, the solvent in the solution was evaporated in a Rotary Evaporator (IKA RV10, Germany) at 50 °C. The remaining water in the extract was extracted by freezing in a lyophilizer (Freeze Dryer) machine (Labconco Freezone 6) (Faresin et al. 2000). The obtained extracts were stored at -20 ºC until the time of use in the experiment (Liu and Yang 2012). The total amount of phenolic compounds was determined (gallic acid equivalent) (Singleton and Rossi 1965).

Essential oil extraction method

The volatile oil leaf and seed parts of *V.agnus cactus* were obtained by Clevenger apparatus (3h.) with the hydrodistillation methods, *V. Agnus castus* aerial parts produced (v/W) essential oil yields. The obtained oils were taken into dark colored bottles and stored at +4 °C until the time of use (Umay 2007, Batish et al. 2012).

Gas chromatography/Mass spectrometry analysis

The instrument used for GC/MS analysis is Agilent Brand gas chromatography/mass spectroscopy (AGILENT 5975 C AGILENT 7890A GC). Program: MSDCHEM. Column: CPWAX 52CB (50 m*0.32 mm*0.20) (Ulusoy et al. 2009).

Antimicrobial assay

Antimicrobial activities of extracts obtained from seeds and leaves were determined by standard disc diffusion method (Collins et al., 1995; Murray et al. 1995). Densities of microorganisms were prepared using 0.5 ml Mc Farland standard (108 CFU/ml). 250 µl of liquid culture of bacteria was taken and inoculated on Müeller Hinton Agar (Difco). Standard sterile discs of 6 mm diameter (Schleicher & Schell) impregnated with 10 µl of extract were placed on the medium. Then, sterile Ampicillin (AM, 10 mcg, Bioanalyze) and Penicillin (P, 10 U, Bioanalyze) antibiotics were placed on the media for comparison. The media were incubated at 37±0.1°C for 24 hours. At the end of the incubation, the diameters of the inhibition zones formed around the discs were measured in mm. All the media were cooked at 121°C for 15 minutes. sterilized. Nutrient Broth medium was used to activate the bacteria, and Mueller Hinton Agar medium was used to determine the antimicrobial activity.

RESULTS AND DISCUSSION

The total phenolic content of the plant extracts is 781,22 µg gallic acid equivalents /g Fresh weight. *Vitex agnus castus* extract absorbance was measured at 765 nm. The antioxidant activity efficiency were also calculated. The highest antioxidant activity efficiency in leaf extract (1.013 µg/g) and least efficiency in seed extracts were 0.124 µg/g).

GC/MS study

The GC/MS study of Chaste tree leaves has shown many phytochemicals which contributes to the medicinal activity of the plant. The major components which present in the leaves of the plant *V.agnus-castus* were α-pinene (RT: 4.2, 16.22%), Eucalyptaol (1,8 cineole) (RT:6.9, 20.12 %), α-terpenylacetate (RT: 11, 6.12 %), Sabinene (RT:5.7, 8.33%), Limonene (RT:6.7, 5.85 %), β-farnesene (RT:10.7, 5.48%) and 4-terpineol (RT: 10.3, 6.17%).

Antimicrobial effects

The antimicrobial activities of different extracts tested by agar dilution method were shown in Table 1.

Table 1: Antimicrobial effects of chasteberry (VAC) leaf and seed extracts

Microorganism	VAC Leaves (mm)	VAC Seed (mm)	A 10	P 10
E. Coli	4	3	3	nt
	2	1	1,5	1
	2,5	2	1,5	nt
S.aureus	1,1	1	3,3	1,1
	5	1,2	3	nt
	3	0,8	2	0,6
P. aeureginosa Gr (-)	nt	nt	1	1
	0,8	0,6	2,1	1,5
	nt	nt	nt	nt
M.lutesus Gr (+)	nt	nt	nt	nt
	nt	nt	nt	nt
	nt	nt	nt	nt
	nt	nt	nt	nt

VAC: *Vitex agnus cactus;* nt: No tested; Referans Antibiyotikler: A: Ampiclin(10mg), P: Penicillin (10U)

The antimicrobial effect of essential oils obtained from the leaves and seed of Chaste tree was investigated and its chemical content was examined. Inhibition zones of

extracts measured between 3 and 4 mm. These results show that; Leaf and seed essential oils of chasteberry have an antimicrobial effect. In the chemical content of the oil obtained from the unripe fruits of the plant, the most sabinene and 1,8 cineol compounds were found, respectively. In the oil obtained from the leaves, the main compound was found to be Eucalyptaol (1.8 cineole) (RT: 6.9). It has been shown that 1,8 cineole compound is effective against the *Aspergillus niger* pathogen, which has been proven to have a positive effect against bacteria and fungi and causes problems during the storage of apples (Stojkovic et al. 2011). In a study by Arora et al. 2013, the antimicrobial activities of 2 seed samples (S1 and S2) of *G.max* and methanol and hydroalcoholic extracts were investigated. While the extracts did not show any effect on Gr(-) *P.fluorescens*, *P. alcaligenes* has been reported to show antimicrobial activity (Arora et al. 2013). In a study by Vaizogullar et al. 2016; It was determined that *H.annuus*-Tanay extracts showed antimicrobial activity only against *S.aureus* ATCC 25923 bacteria.

Antimicrobial activity of sunflower oil and black cumin oil on *Listeria monocytogenes* bacteria was determined by disk diffusion method. It was stated that the highest antimicrobial activity was observed in black cumin oil (31.5 mm), while an inhibitory effect was not observed in sunflower oil (Nair et al. 2013). The antibacterial studies of Senatore et al. (1996). also supported our study and similar results were observed. However, it is seen that the inhibition zones (cm) on Gr(-) bacteria are more. It has been observed that the antibacterial effect of the leaf oil is higher than the seed oil. However, it is seen that the inhibition zone formed by the seed oil on the *Pseudomonas aeruginosa* bacterium is higher than on the *Escherichia coli* bacterium. Looking at this result, we can say that the antibacterial effect of chaste seed is more on Gr (-) bacteria.

In our study, *Vitex agnus castus* seed and leaf essential oils were used on Gr (-) *Escherichia coli* ATCC 25922, Gr (-) *Pseudomonas aeruginosa* ATCC 27853, Gr (+) *Staphylococcus aureus* ATCC 25923 and Gr (+) MicroCIMB luteus 132. When we look at Gr (-) bacteria from the essential oils applied to *Escherichia coli* bacteria, it is seen that the leaf oil forms more inhibition zones than the seed. It was observed that seed oil formed more inhibition zones in *Pseudomonas aeruginosa* bacteria. It is seen that the inhibition zone formed by the oil of the oil is more on *Staphylococcus aureus* bacteria, which is Gr (+). Thanks to the rich components of the chasteberry plant, the antibacterial work we have done shows that the leaf and seed oils of Chaste tree (*Vitex agnus-castus)* L. are effective against Gr (+) and Gr (-) bacteria.

CONCLUSION

In this study, the antimicrobial activity and essential oil composition effects of chaste tree (*Vitex agnus-cactus*) VAC seed extract was investigated. *V. agnus castus* extracts containing the most phenolic component amount is the leaf extract containing the lowest phenolic component amount is the seed extract. It has been observed that the antibacterial study of Chaste tree plant, leaf and seed oils are effective against Gr (+) and Gr (-) bacteria.

CONFLICT OF INTEREST

Conflict of interest We declare that there is no conflict of interest. There are neither ethical nor financial conflicts of interest involved.

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AUTHOR CONTRIBUTIONS

Yeşim Kara: Conceived the idea, planned for the study and writing the article.

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