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Original Research Article

Identification, antioxidant and cytotoxic potentials of casticin in *Vitex agnus-castus* fruit from different geographical regions of Turkey

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Abstract

Purpose: *To evaluate the antioxidant and cytotoxic effects, and casticin contents of Vitex agnus-castus (VAC) fruit extract from five geographical regions of Turkey.*
Methods: *Casticin determination in VAC fruit extracts was performed using HPLC-DAD method. The method was validated for linearity, sensitivity, selectivity, accuracy and precision. The methanol extracts of the VAC fruits were analyzed for antioxidant effects using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical scavenging capacity, metal-chelation capacity assay, radical cation scavenging capacity (ABTS•+), and total phenolic content. The cytotoxic potential of the extracts against NRK-52E and HeLa cells were determined using MTT and LDH assays.*
Results: *The casticin content of the extracts ranged from 0.048 and 0.152 %. Total phenol content was in the range of 36.67 ± 0.7 to 74.20 ± 1.02 mg gallic acid equivalent (GAE)/100 mg of extracts. High-to-moderate antioxidant properties were observed in the extracts. Cytotoxicity data demonstrated that all extracts showed high cytotoxic effects on HeLa cell line when compared to the NRK-52E cell line ($p < 0.05$).*
Conclusion: *These results suggest that VAC fruits (Monk's pepper fruits) from Aegean sea coasts have a potential for use in the preparation of phytopharmaceutical products.*

Keywords: *Vitex agnus-castus, Casticin, Antioxidant, Cytotoxic activity, Anticancer*

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INTRODUCTION

Monk's pepper (*V. agnus-castus* L.) is a plant that grows in the Mediterranean region. The fruits have long been used in traditional medicine for the treatment of gynecological problems all around the world [1]. Currently, the fruit extract of VAC is an approved medication for the treatment of menstrual disorders, premenstrual syndrome (PMS), mastalgia and mastodynie. The beneficial effects of VAC in gynecological treatments have gained the attention of the pharmaceutical

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industry [2,3]. This well-known aromatic shrub grows extensively in coastal areas of Turkey and its fruits are used in Anatolian folk medicine as diuretic, digestive, antifungal, and anxiety medication, and as remedy for stomach ache [4]. Several studies on biological activities of VAC have reported its antioxidant, antimicrobial, antifungal, cytotoxic, and anti-inflammatory properties [5-7].

Previous studies demonstrated that VAC contains flavonoids (casticin), iridoids, diterpenes and essential oil [5,8,9]. Casticin is a chemotaxonomic index for the genus *Vitex,* and also a major phytochemical in VAC fruits. It is a lipophilic flavonoid which has a strong chromophore that makes it easily detectable with UV. Therefore, casticin has been chosen for the standardization of VAC extracts [10]. According to the European Pharmacopoeia of 2007, the dried drug should contain a minimum of 0.08 % casticin [11]. Whether as a food supplement or as medicine, VAC extracts are commercially available and quite popular for the treatment of premenstrual syndrome (PMS), with most of them standardized according to casticin amount [12].

Most of diseases are associated with free radical damage. These include cardiovascular diseases, cancer, autoimmune disorders, and neurodegenerative disease. Medicinal plants have a great potential in the prevention and effective suppression of many diseases. These beneficial effects attract the attention of researchers to study the pharmacological potential of plants. Numerous investigations have been reported on antioxidant and cytotoxic activities of *Vitex* species [5,13].

In this study, quantitative analyses of casticin contents of methanol extracts of five samples of VAC from different coastal areas of Turkey were performed using HPLC-DAD. In addition, the extracts were subjected to *in vitro* antioxidant and cytotoxic assays by determination of their capacities to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS•+) radical, and metal-chelation capacity. The total phenolic contents of the extracts were also identified using spectrophotometric techniques.

EXPERIMENTAL

Chemical agents

Casticin was purchased from Sigma-Aldrich, Germany. All solutions were of HPLC grade and the compounds used for biological activity were of analytical grade.

Plant material

The fruits of Monk’s pepper were obtained from 5 different coastal areas, namely Manavgat-Western Mediterranean coast (GILAM no: 680), Bodrum-South Aegean Sea (GILAM no: 681), Altınoluk-Mid Aegean Sea (GILAM no: 682), Zonguldak-Western Black Sea coast (GILAM no: 683), Enez-North Aegean Sea (GILAM no: 684) (voucher specimen nos. in brackets). The VAC fruits of were authenticated by Professor Gunay Sariyar and were dried at room temperature (RT) in the dark.

Preparation of extracts

Five grams of dried and powdered fruit samples were sonicated with 50 mL of methanol in an ultrasonic bath for 20 min at RT and filtered. This procedure was repeated twice [11]. The filtrates were pooled and subjected to rotary evaporation at 30 °C, resulting in a residue which was kept at 4°C prior to use.

Quantification of casticin in *V. agnus-castus* samples using HPLC-DAD

HPLC-DAD conditions

HPLC analysis was performed on a Shimadzu 10A instrument (SAMI, Japan). The analytical HPLC column (Teknokroma Nucleosil 100 C18 column, 3 µm, 125mmx0.3mm (TR-N1318C12CL)) was used with gradient mobile phase system. The binary solvent mixture from Solvent A [phosphoric acid: water, 4:996 (v/v)] and solvent B [acetonitril: methanol, 30:70 (v/v)] was used for HPLC analysis.

The mobile phase was used in gradient elution: 0–13 min, 70 → 35 % A; 13 – 16 min, 35 → 0 % A; and 16 – 20 min, 0 → 70 % A. at mobile phase speed of 1.5 mL/min, injection volume of 10 µL, and column temperature set at 40 °C. Casticin was detected at 348 nm. The procedure was subjected to validation with respect to sensitivity, linearity, selectivity, accuracy and precision according to ICH provisions [14]. The data analysis procedure was performed with Shimadzu LC Solutions software.

A casticin standard calibration curve was prepared with casticin levels 0.1 – 0.0625 mg/mL. The standard casticin and extracts were disolved in methanol. All samples and solvents were subjected to filtration through 0.45 µm

pores prior to HPLC analyses. All analyses were done in triplicate.

Estimation of phenolics

Total phenolics were estimated using the procedure described earlier [15]. The total phenolics of each extract was expressed in milligrams of gallic acid equivalent per gram extract (mg GAE/g extract) using a calibration curve with gallic acid (standard solution). The total phenolic content was calculated as shown in Eq 1:

Absorbance = 2.395x – 0.027 .... (1) ($R^2 = 0.9995$)

The absorbance was measured at 760 nm, and the data are presented as mean of triplicate analyses.

DPPH$^•$ scavenging effect

The DPPH• radical scavenging effect of each sample was measured using a method described by Fu et al. [16]. Serial dilutions were prepared with the stock solution (5 mg/mL) of each extract. Moreover, 0.1 mM DPPH• solution in methanol was prepared and 3.9 mL solution of DPPH• and 0.1 mL of different concentrations of each extract were added. After 30 min. in the dark, the absorbance value was measured spectrophotometrically at 517 nm.

ABTS$^{•+}$ radical cation scavenging activity

The ABTS$^{•+}$ radical scavenging activity was assayed according to the method developed by Re *et al* [17]. This assay is based on the formation of free radical cation ABTS$^{•+}$ in a reaction between ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM), at RT, in the dark, for 12 – 16 h. In the assay, 3.96 mL of ABTS$^{•+}$ solution was made up to 4.00 mL with extract. After 6 min., optical density of the mixture was measured at 734 nm.

Assay of capacity of extracts to chelate metals

The metal chelating capacity towards ferrous ions (Fe2+) was measured using the procedure of Dinis *et al* [18]. Samples and EDTA (200 µL) were mixed with 2mM $FeCl_2$ (50 µL), followed by addition of 200 µL of ferrozine (5 mM) and incubation at RT. Then, the total volume was adjusted to 4 mL with methanol. The solution remained at RT for an additional period of 10 min. Finally, the absorbance of the solution was recorded at 562 nm. These assays were done in triplicate for each sample.

Evaluation of cytotoxic activity

Cell culture and sample treatment

Rat kidney proximal tubular epithelial cell line (NRK-52E) (CRL-1571^{TM}), and Human cervix adenocarcinoma cell line (Hela) (CCL-2™, both from ATCC, USA) were used. Rat kidney proximal tubular epithelial cells were maintained on DMEM: nutrient mixture F12 (DMEM/F12), while Human cervix adenocarcinoma cells were maintained on DMEM. The culture media contained 10 % FBS and streptomycin/penicillin mixture (each at 100 U/mL). The cells were seeded in 96-well plates at a density of 10^4 cells per well, and incubated at 37 °C in a 5 % CO_2 and 95 % O_2 humidified cell incubator for 24 h. The studied concentrations of samples were 0.0125 – 0.2 mg/mL. After 24 h of incubation, the toxic effects of the samples assessed with lactate dehydrogenase and MTT assays.

Determination of cytotoxicity with MTT assay

The methanol extracts of each sample was incubated with cells for 24 h. Then, 30 µL of the MTT solution (5 mg/mL in PBS) was applied to each well. The cells with MTT solution were kept in the incubator at 37 °C for 60 min. Following incubation, the supernatant was removed, and the residue formed was dissolved in DMSO, with shaking for 5 min at 150 rpm [19]. Thereafter, absorbance of the sample (Abs) and the solvent control (Abs_0) were measured at 590 nm against 670 nm, in a microplate reader. The cytotoxicity index (CI) values were calculated using the formula in Eq 2:

$$CI (\%) = 100 - \{(Abs \times 100/(Abs_0)\} \quad ....(2)$$

Determination of cytotoxicity using LDH assay

In present work, Roche Cytotoxicity Detection Kit (Mannheim, Germany) was used to determine cytotoxicity of extracts. After incubating cells with the extracts for 24 h, the culture media were collected separately from cultures and centrifuged to remove cell debris. The assay was conducted immediately by mixing the media with the assay reagent prepared by mixing two separate solutions (diaphorase/NAD+ mixture and iodotetrazolium chloride/sodium lactate mixture) [20]. This was incubated for 0.5 h in the dark, and optical density was read at 490 nm. Triton-X100 (10%) was used as positive control for maximum activity. The results were compared with positive control, and CI was computed as shown in Eq 3.

CI (%) = 100 -{(Abs x 100/(Abs_0)} ……... (3)

The IC_{50} value expresses the concentration of samples that produces a 50 % inhibition of LDH. Negative (untreated, culture medium) and solvent (1 % DMSO) controls were used in all assays.

RESULTS

Extract yields

The extract yield of VM, VB, VA, VZ and VE samples were 55.3, 88.9, 54.4, 66.8 and 60.4 mg/g of dry weight, respectively. Among all the extracts obtained from VAC fruits collected from 5 different locations, the methanol extract of VB had the highest percentage extract yield. The percentage yields of the extracts from VAC are presented in Table 1.

Levels of casticin in *V. agnus-castus*

The HPLC method was used for quantification of casticin from fruits of VAC. The identification of the casticin peak was done by comparison of UV spectra of extracts with the UV spectrum of standard casticin. Absorption maxima of casticin standard in methanol was seen at the wavelength of 348 nm. The casticin concentration of extracts was obtained with the regression equation: y= 1E + 07x + 8432.7, which was linear (r^2 = 0.9991).

This method was developed to obtain the best resolution. The PDA chromatogram and UV spectra of casticin are given in Figure 1. The association between casticin peak area and concentrations was linear. The levels of casticin in the methanol extracts of samples are presented in Table 1, while LC chromatograms are shown in Figure 2.

Validation

The HPLC-DAD method was subjected to validation to see if its efficacy was consistent with the appropriate standard for the analysis of casticin from VAC. The HPLC-DAD validation followed the ICH guidelines "Validation of Analytical Procedures: Text and Methodology Q2(R1)". The results of validation procedure are given in Table 2.

Total phenolic contents

Table 1 shows that the total phenolic contents of samples varied amongst the extracts. The methanol extract of VM had the largest levels of phenolics (74.20 ± 1.02 mg GAE/g extract). The order of total phenolic content of the other extracts was VB extract (60.00 ± 0.01 mg GAE/g extract) > VA extract (40.67 ± 0.9 mg GAE/g extract) > VZ extract (40.00 ± 0.06 mg GAE/g extract) >VE extract (36.67 ± 0.7 mg GAE/g extract).

Antioxidant capacity of extracts

The results of the antioxidant assays of samples are given in Table 3. The samples showed varying degrees of antioxidant capacity. The IC_{50} value of positive controls was measured as vitamin C (IC_{50}:0.09 ± 0.006 mg/mL) and BHT (IC_{50}:0.32 ± 0.03 mg/mL). The VAC extracts exhibited varying DPPH radical scavenging capacities with VM showing the highest DPPH neutralizing capacity (IC_{50}: 0.84 ± 0.02 mg/mL). Compared with other samples, methanol extracts of VB (IC_{50}: 1.25 ± 0.23 mg/mL) and VZ (IC_{50}: 1.74 ± 0.04 mg/mL) showed moderate DPPH free radical scavenging potential while VA and VE produced poor effects with IC_{50} values of 2.26 ± 0.02 mg/mL and 2.70 ± 0.18 mg/mL, respectively.

The ABTS radical cation scavenging activity of 10 μg/mL methanol extracts are presented in Table 3. The VM sample exhibited the strongest ABTS radical neutralizing capacity (14.95 ± 0.0 %), while the weakest activity was seen in VE sample (6.33 ± 0.9 %). The 10 μg/mL extracts of VB, VZ and VA produced 12.87 ± 0.4 %, 9.37 ± 0.7 %, 6.48 ± 0.6 % inhibition, respectively. None of the extracts was found to be as active as the positive control.

Table 1: Extract yield and total phenolic contents of *V. agnus-castus* fruits

Sample name	Location	Extract yields (%)	Value of casticin (%)	Total phenolics (mgGAE/g extract)
VM	Western Mediterranean coast	55.3	0.048 ± 0.004	74.20±1.02
VB	South Aegean Sea coast	88.9	0.152 ± 0.018	60.00±0.01
VE	North Aegean Sea coast	60.4	0.088 ± 0.009	36.67±0.7
VA	Mid Aegean Sea coast	54.4	0.110 ± 0.003	40.67±0.9
VZ	Western Black sea coast	66.8	0.064 ± 0.003	40.00±0.06

Data are mean of triple measurements (n = 3) ± standard deviation; GAE–Gallic acid equivalents

Table 2: Validated parameters for the developed HPLC-DAD method

Compound	Linear range (mg/ml)	Intercept ± SE [a]	Slope ± SE	r^{2} [b]	Sensitivity		Precision [e] (RSD%)		Recovery [f] (R%) (n=6)
					LOD [c] (mg/ml)	LOQ [d] (mg/ml)	Intraday (n=6)	Interday (n=9)	
Casticin	0.00625 – 0.1	4.61E+03 ± 1.35E+04	1.27E+07 ± 5.37E+06	0.9992	0.0083 ± 0.01	0.0252 ± 0.03	2.6	8.9	96.0 – 112.0

a: Standard error, b: Coefficients of correlation, c: Limit of detection, the lowest analyte concentration that produces a response detectable above the noise level of the system, d: Limit of quantification, the lowest level of analyte that can be accurately and precisely measured, e: Relative standard deviation, expressed as %, f: Average of recoveries at two spike levels (0.1-0.05 mg/ml)

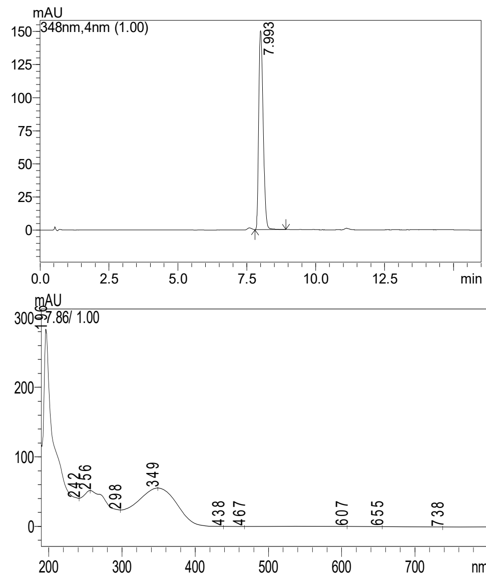

Figure 1: PDA chromatogram (a) and UV spectrum (b) of casticin standard

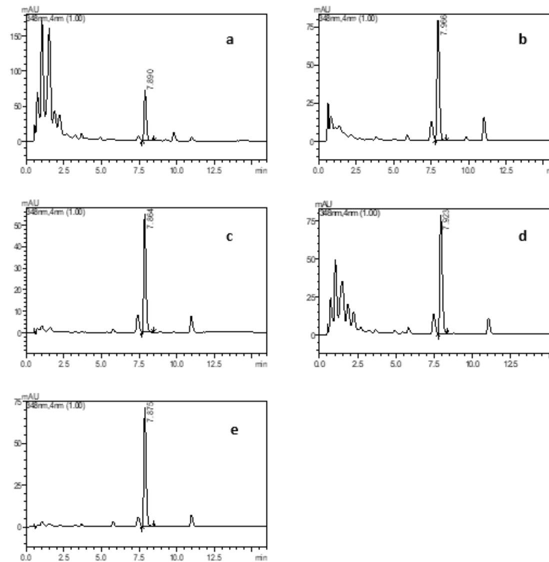

Figure 2: LC chromatogram of the samples (A: Manavgat, B: Bodrum, C: Altınoluk, D: Zonguldak, E: Enez)

Table 3: Antioxidant capacities of *V.agnus-castus* fruits, which were collected from five different regions

Extracts/standard	DPPH (IC_{50}: mg/mL)	ABTS (%) (10 μg/mL)	Metal chelating activity (%) (50 μg/mL)
VM	0.84± 0.02^{a}	14.95±0.03^{a}	17.50±0.8^{a}
VB	1.25± 0.23^{b}	12.87±0.4^{b}	8.53±0.6^{b}
VE	2.70± 0.18^{c}	6.33±0.9^{c}	5.91±0,03^{c}
VA	2.26± 0.02^{d,c}	6.48±0.6^{d,c}	14.35±0.6^{d}
VZ	1.74± 0.04^{e}	9.37±0.7^{e}	13.87±0.5^{e,d}
Ascorbic acid	0.09± 0.006^{f}	-	-
BHT	0.32± 0.03^{g}	54.39±0.1^{f}	-
EDTA	-	-	96.51±0.2^{f}

Values are mean of triplicate determination (n = 3) ± standard deviation. $P < 0.05$ according to Tukey's Multiple Comparison test.

Table 4: IC_{50} values of the extracts of the *V. agnus-castus* samples and casticin in HeLa and NRK-52E cell line

	LDH (mg/ml)		MTT (mg/ml)	
Sample	HeLa	NRK-52E	HeLa	NRK-52E
VM	0,057	0,044	0,096	0,038
VB	0,004	0,083	0,036	0,137
VE	0,033	0,027	0,035	0,023
VA	0,022	0,018	0,030	0,029
VZ	0,029	0,059	0,063	0,060
Casticin	0 ,0062 ug/ml	0,0297 ug/mL	0,01 ug/mL	0,034 ug/mL

Table 3 show results of Fe^{2+} chelating ability of the samples and EDTA. None of the extracts exhibited strong ferrous ion chelating activity. Among the studied extracts, VM samples exhibited the highest metal chelating activity which was lower than the metal chelating activity of the positive control. The hierarchy of metal chelating activity of the methanol extracts was VM (17.50 ± 0.8%) > VA (14.35 ± 0.6 %) > VZ (13.87 ± 0.5 %) > VB (8.53 ± 0.6 %) > VE (5.91 ± 0.03 %).

Cytotoxic activity of extracts

The results of cytotoxic potential of VAC extracts against the two cell lines, as determined with MTT and LDH assays are summarized in Table 4.

The IC_{50} value of the casticin against HeLa cell line was 0.01 ug/mL in MTT assay, while its IC_{50} value determined as 0.006 μg/mL with LDH assay.

DISCUSSION

In the present study, the concentration of casticin in the methanol extracts were determined by HPLC-DAD. During the HPLC-DAD analysis, one unknown compound detected in a small amount interfered with the casticin peak. Based on its retention time and UV spectra, this compound was identified as penduletin, which is another lipophilic flavonoid found in small quantities in the fruit extract. The developed HPLC-DAD method provides high resolution between casticin and penduletin. Casticin is employed for standardisation of VAC preparations [10]. Due to its significance in pharmaceutical industries, quantitative analysis of casticin was determined in the methanol extracts of VAC fruits from five different parts of Turkey. These regions are different coastal areas of Turkey with variabilities in climate and geographic conditions. It is well known that chemical composition and quantities of some compounds of plants can be affected by their growth conditions.

The amounts of casticin in the methanol extracts from VAC samples (VM, VB, VB, VZ and VE) were 0.048, 0.152, 0.110, 0.064 and 0.088 %, respectively. When the samples were compared with each other, VB was found to have the highest percentage of casticin. The quantity of casticin in the sample of VA was very similar to that of sample from VB, and both samples were taken from an area near the Aegean Sea. Previous studies on the fruits of VAC showed casticin levels ranging from 0.025 to 0.212% [21]. The results from the present study showed that samples from VB, VA and VE contained more than 0.08% of casticin, while samples from VM and VZ contained low amounts of casticin. From the validation results, the developed HPLC method achieved acceptable linearity, sensitivity, selectivity, accuracy as well as precision during the simultaneous analysis of casticin in the methanol extracts.

In recent years, investigation on natural antioxidants has increased considerably. There are several reports on antioxidant properties of VAC. According to previous studies, VAC extracts showed remarkable antioxidant activity [13,22]. In the present study, the methanol extract of VM which had the highest amount of phenolics, manifested the best radical scavenging and metal-chelating capacities. As is well known from the literature, phenolic contents contribute to strong antioxidant capacity. These results indicate a high correlation between total phenolic contents and antioxidant activity (metal chelating, DPPH and ABTS assays) of the methanol extracts VM and VE. However, total phenolic level was not correlated with and antioxidant potential (metal chelating, DPPH and ABTS assays) in the methanol extracts VB, VZ and VA. These results show that VM samples exhibited a significant antioxidant activity. Further studies are needed to investigate fruits from Western Mediterranean coast of Turkey. Many studies have been done on cytotoxic activity of VAC extracts and their major compounds [7,23-25]. Several studies have demonstrated that the extracts of VAC showed remarkable cytotoxic activity against various human cancer cell lines [23-25]. In this study, besides the extracts, the effect of casticin was also investigated on NRK and HeLa cells. Casticin, as a major component of VAC, has been researched on for anticancer activity against different types of cell lines [23,24]. Furthermore, significant cytotoxic effect was observed for all extracts and casticin against HeLa cell line, but the effect against NRK cell line was weak in all tests. From the MTT and LDH results, the extract of VB exhibited the highest cytotoxic activity against HeLa cells but this extract had no cytotoxic effect against NRK-52E cells at the concentration of, or below 0.083 mg/mL. Consequently, the high level of casticin may be responsible for cytotoxic effect of VB extract. On the other hand, in the other extracts, there was no correlation between cytotototoxic activity and percentage of casticin. As a result, all methanol extracts exhibited considerable cytotoxic activity against HeLa cell lines.

CONCLUSION

This is the first report on casticin contents of Turkish samples of VAC fruits and their cytotoxic and antioxidant potential, and is a significant contribution to the body of existing knowledge, especially that VA and VB extracts contain high concentrations of casticin compatible that meets European Pharmacopoeia standards. Thus, these fruits can be used in the pharmaceutical industry for the preparation of VAC drugs. However, the cytotoxicity observed in the VB extract requires further studies.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The study was designed by Gizem Gülsoy Toplan and Gunay Sariyar. Gizem Gülsoy Toplan, Esra Eroglu Ozkan, Turgut Taskın and Mahmoud Abudayyak collected and analysed the data. The manuscript was written by Gizem Gülsoy Toplan and Afife Mat. All authors have read and approved the manuscript for publication.

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REFERENCES

1. *Padmalatha K, Jayaram K, Raju NL, Prasad MNV, Arora R. Ethnopharmacological and biotechnological significance of Vitex. Biorem Biodiv Bioavail 2009; 3:6–14.*
2. *Schellenberg R, Zimmermann C, Drewe J, Hoexter G, Zahner C. Dose-dependent efficacy of the Vitex agnus castus extract Ze 440 in patients suffering from premenstrual syndrome. Phytomedicine 2012; 19:1325–1331.*
3. *Meena AK, Singh U, Yadav AK, Singh B, Rao MM. Pharmacological and Phytochemical Evidences for the Extracts from Plants of the Genus Vitex – A Review. Int J Pharm Clin Res 2010; 2:1–9.*
4. *Baytop T. Türkiye de Bitkilerle Tedavi. Istanbul: Nobel Tıp Kitabevleri; 1999. 480 p.*
5. *Barnes J, Anderson LA, Phillipson DJ. Herbal medicines: a guide for healthcare professionals. 3th ed. Pharmaceutical Press; 2003. 721p.*

6. Saglam H, Pabuçcuo A, Kıvçak B. Antioxidant Activity of Vitex agnus-castus L. Extracts. Phytother Res 2007; 2:1059–1060.
7. Duymuş HG, Çiftçi GA, Yıldırım ŞU, Demirci B, Kırımer N. The cytotoxic activity of Vitex agnus castus L. essential oils and their biochemical mechanisms. Ind Crop Prod 2014; 55:33–42.
8. Kırmızıbekmez H, Demir D. Iridoid Glycosides and Phenolic Compounds from the Flowers of Vitex agnus-castus. Helv Chim Acta 2016; 1212:518–522.
9. Kuruüzüm-Uz A, Ströch K, Demirezer LÖ, Zeeck A. Glucosides from Vitex agnus-castus. Phytochemistry 2003;63(Pt 8):959–964.
10. Hoberg E, Meier B, Sticher O. Quantitative high-performance liquid chromatographic analysis of casticin in the fruits of Vitex agnus-castus. Pharm Biol 2001; 39:57–61.
11. European Pharmacopoeia. Herbal Monographs, Agnus Castus Fruit (Agni casti fructus). 6th ed. 2007. p. 1116 – 1117.
12. Mari A, Montoro P, Pizza C, Piacente S. Liquid chromatography tandem mass spectrometry determination of chemical markers and principal component analysis of Vitex agnus-castus L. fruits (Verbenaceae) and derived food supplements. J Pharm Biomed Anal 2012; 70:224–230.
13. Gökbulut A, Özhan O, Karacaoğlu M, Şarer E. Radical Scavenging Activity and Vitexin Content of Vitex agnus-castus Leaves and Fruits. FABAD 2010; 35:85–91.
14. Branch SK. Guidelines from the international conference on harmonisation (ICH). J Pharm Biomed Anal 2005; 38:798–805.
15. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. Am J Enol Vitic 1977; 28:49–55.
16. Fu W, Chen J, Cai Y, Lei Y, Chen L, Pei L, Zhou D, Liang X, Ruan J. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch. et Sav.) Ching. J Ethnopharmacol 2010; 3:521–528.
17. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999; 26:1231–1237.
18. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994; 315:161–169.
19. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 1988; 48:589–601.
20. Legrand C, Bour JM, Jacob C, Capiaumont J, Martial A, Marc A, Wudtke M, Kretzmer G, Demangel C, Duval D. Lactate dehydrogenase (LDH) activity of the number of dead cells in the medium of cultured eukaryotic cells as marker. J Biotechnol 1992; 25:231–243.
21. Hoberg E, Meier B, Sticher O. Quantitative High-Performance Liquid Chromatographic Analysis of Casticin in the Fruits of Vitex agnus-castus Quantitative High-Performance Liquid Chromatographic Analysis of Casticin in the Fruits of Vitex agnus-castus. Phytochem Analysis 2000; 11:327-329.
22. Sarikurkcu C, Arisoy K, Tepe B, Cakir A, Abali G, Mete E. Studies on the antioxidant activity of essential oil and different solvent extracts of Vitex agnus castus L. fruits from Turkey. Food Chem. Toxicol 2009; 47:2479–2483.
23. Chen D, Cao J, Tian L, Liu F, Sheng X. Induction of apoptosis by casticin in cervical cancer cells through reactive oxygen species-mediated mitochondrial signaling pathways. Oncol. Rep 2011; 26:1287.
24. Tang SY, Zhong MZ, Yuan GY, Hou SP, Yin LL, Jiang H, Yu ZY. Casticin, a flavonoid, potentiates TRAIL-induced apoptosis through modulation of anti-apoptotic proteins and death receptor 5 in colon cancer cells. Oncol Rep 2013; 29:474–480.
25. Yang F, He K, Huang L, Zhang L, Liu A, Zhang J. Casticin inhibits the activity of transcription factor Sp1 and the methylation of RECK in MGC803 gastric cancer cells. Exp Ther Med 2017; 13:745-750.