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Bioactive ingredients of different extracts of *Vitex agnus-castus L*. Fruits from Morocco and their antioxidant potential

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Abstract

The current study aimed to examine the effect of different solvents on phenols and flavonoid contents and evaluate the antioxidant activities of different extracts. At first, the Soxhlet extracts were performed with four solvents (ethanol, methanol, ethyl acetate, and water) and were examined for their polyphenolic content, flavonoid content, and antioxidant potentials using three assays (DPPH, FRAP, TAC). The dosage of phytochemical compounds (polyphenolic and flavonoid contents) revealed that the highest values were established in ethanol extract ($p<0.05$). Additionally, the strongest antioxidant activity measured by TAC and DPPH assays was established in ethanol extract with 400±00 mg AAE/g DW and 0.36 ± 0.07mg/mL, respectively, while the methanol extract showed the best antioxidant activity as measured by FRAP with an IC_{50} value of 2.98 ± 0.2 mg/mL; the lowest value was observed in ethyl acetate extract. The Vitex fruits possess remarkable antioxidant potential, which may enhance their protective effects.

Keywords: *Vitex agnus- castus,* Antioxidant activity, Flavonoids, Polyphenols.

1. Introduction

The failure of antioxidant defense systems is associated with oxidative stress, which induce the overproduction of reactive oxygen species (ROS) (McCord, 2000). ROS reacts with different molecules such as membrane lipids, proteins, and DNA leading to the pathogenesis and progression of various diseases including cancer, cardiovascular diseases, diabetes, tumors, rheumatoid arthritis, and epilepsy (Halliwell and Guterridge, 2007; Ho et al., 1992). Thus, there is a growing interest in finding natural substances with antioxidant properties. Medicinal herbs are considered as a good source of bioactive compounds. They contain a large variety of functional substances such as, polyphenols, flavonoids, vitamins, which provide protective effects (Hamli et al., 2017; Rodríguez et al., 2013).

Vitex agnus-castus L. (VAC) is a medicinal plant that grows in the Mediterranean region, Europe, and Asia; (Ahangarpour et al., 2016). It belongs to the Verbenaceae family, commonly known as the chaste tree (Yushchyshena and Tsurkan, 2014). The uses of this medicinal herb are shown to have many biological activities. The fruits of this plant have been used to treat various female conditions such as uterine cramps, menstrual disorders, lactation, and acne (Chhabra and Kulkarni, 2011). By-products of Vitex (essential oils and extracts) possess several biological activities including antimicrobial and antifungal activities (Ahmad et al., 2016; Arokiyaraj et al., 2009; Asdadi et al., 2015), while the aromatic leaves of this plant are used as a spice.

V. agnus-castus L contain different bioactive ingredients such as Iridoid glycosides, Flavonoids, Diterpenes and Essential fatty acids (Dugoua et al., 2008). *V. agnus-castus* is locally named "Angarf-lkrwaa" in the Imouzzer Ida Outanane region; the plant used as a sedative, antispasmodic, and an aphrodisiac (Abdelhai Sijelmassi, 1991).

The chemical contents and antioxidant capacities of different extracts of *Vitex agnus-castus* were determined and compared.

2. Materials and Methods

2.1. Plant material

Fruits of VAC were collected from Khenifra area (Latitude: 32°56′05″ Nord; Longitude: 5°39′42″ Ouest; altitude: 827 m), (Middle Atlas, Morocco) in June-October 2015 (flowering period). Professor A. Bari as a botanist identified our plant material (Department of Biology, FSDM, USMBA, Fez (Morocco).

2.2. Extract preparation

Before extraction, the Vitex fruits were washed and then dried. Next, the dried fruits were powdered. The extraction was performed with four solvents (ethanol, methanol, ethyl acetate, and water), and the solid to liquid ratio was 1/20 using Soxhlet extractor for 8 h. The rotary evaporator was used to concentrate all extracts then stored in a refrigerator at 4°C.

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2.3. Dosage of total polyphenolic content (TPC)

The determination of TPC of extracts was assessed by the method described by Jadouali et al., (2018) and detailed by Hamli et al., (2017) using Folin-Ciocalteu. Results of TPC were calculated as mg GAE/g DW.

2.4. Determination of Total flavonoid content (TFC)

To quantify TFC, we have chosen a modified Zhishen et al., (1999) method as detailed by Hamli et al., (2017). Results were expressed as mg RE/g DW.

2.5. Total antioxidant capacity test (TAC)

The test chosen to determine the TAC of extracts is based on the method proposed by Prieto et al., (1999). Briefly, each extract (25 µL) was appended to 1 mL of phosphomolybdate solution. The color intensity was read at wavelength 695 nm after incubating the mixture reaction at 95 °C for 90 min used ascorbic acid as the standard calibration. Results were expressed as mg AAE/g DW.

2.6. DPPH assay

To examine the capacity to quench free radicals, we have chosen DPPH assay as described by Wu et al., (2003). 0.1 mL of the extract/standard and 1.5 mL of DPPH solution (0.1 mmol) were mixed, then the mixture was intubated 30 min in the darkness. Decline in the intensity of coloration produced was read at 517 nm. The DPPH scavenging activity was estimated by the following equation:

$$\%Inhibition = [(A_C - A_S)/A_C] \times 100$$

Where Ac is the absorbance of the control, and As is the absorbance of the sample. BHT served as a positive control.

2.7. FRAP assay

The FRAP of extracts was examined using the technique of Oyaizu (OYAIZU and M., 1986). Briefly, 200 µL of the sample, 500 µL of phosphate buffer (0.2M, pH 6.6), and 500 µL of potassium ferricyanide [K_3Fe (CN) 6]1% were mixed. Then, 500 µL of Trichloroacetic (TCA) 10% was added and mixture was incubated during 20 min at 50°C. The supernatant (2, 5 mL) after centrifugation, 500 µL of deionized water, and 100 µL of FeCl3 (10%) were mixed. 700 nm was the wavelength used to read the absorbance. The outcomes were deducted as the dose of extract inhibiting 50% of FRAP.

2.8. Statistical analysis

Statistical analysis was carried out by ANOVA one-way followed by Tuckey-test, using the Graph Pad Prism 5 (Microsoft Software). Differences at $P < 0.05$ were considered significant. The outcome was also subjected to multivariate analysis (principal component analysis).

3. Results

3.1. Effects of solvent on extraction yield, polyphenol, and flavonoid contents

Table 1 resumes the results of yields. The extraction yield of water (24%) was significantly the highest followed by ethanol (7.2%), methanol (6.3%), and ethyl acetate (2.04%).

Table 1: Yield, total phenolic content and total flavonoids of different extracts

Extract	Yield (%)	Phenols **(mg GAE/g DW)**	Flavonoids **(mg RE/g DW)**
Ethanol	7.2	62.66 ± 2.5[a]	58.16 ± 1.3 [a]
Methanol	6.3	46.66 ± 2.6[b]	31.7 ± 0.7[b]
Ethyl acetate	2.04	21.50 ± 1.8[d]	12.08 ± 1.1[c]
Water	24	50.46 ± 1.2[cb]	16.07 ± 0.81[d]

a: comparison between the ethanol extract and all extracts, b: comparison between the methanol extract and all extracts, c: comparison between the ethyl acetate extract and all extracts, d: comparison between the water extract and all extracts.

Extraction process of active ingredients was assessed by different solvents. As shown in (Table 1), the quantity of phenols of various extracts, measured by Folin–Ciocalteu method, varied significantly from 21.50 ± 1.8 to 62.66 ± 2.5 mg GAE/g DW.

Results of flavonoids ranged from 12.08 ± 1.1 to 58.16 ± 1.3 mg RE/g DW. It is clear that the ethanol extract significantly contained the highest value of flavonoids (58.16 ± 1.3 mg RE/g DW), while ethyl acetate extract (12.08 ± 1.1) established the lowest value of TFC.

3.2. Antioxidant activities

3.2.1. Total antioxidant capacity (TAC)

Results are documented in Figure 2; they revealed that the best value of TAC was established in ethanol extract with a value equal to 408.33 ± 4.33(mg Eq AAE/g DW), while the ethyl acetate extract have the lowest antioxidant capacity (147.4 ± 2.04 mg Eq AAE/g DW).

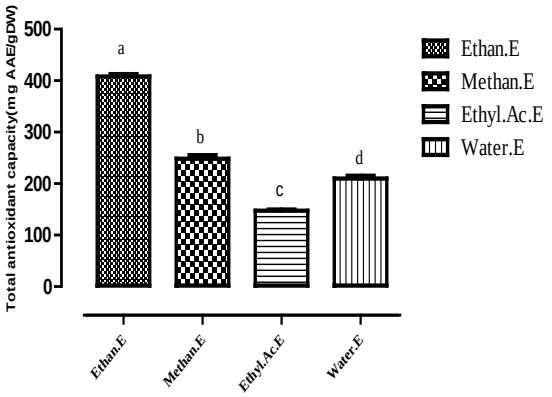

Figure 1. TAC of different extracts of V. fruits.

3.2.2. Antioxidant ability by DPPH

Results of DPPH assay are presented in Table 2. The IC_{50} values of all tested samples through the DPPH scavenging ability test ranged from 0.36 ± 0.07 mg/mL to 2.04 ± 0.21 mg/mL. All extracts inhibited the DPPH radical as follows: ethanol > water > methanol > ethyl acetate. The highest activity was obtained from ethanol extract (IC_{50} = 0.36 ± 0.07 mg/mL), while the lowest ability was registered from ethyl acetate extract (IC_{50} = 2.04 ± 0.21 mg/mL). However, the BHT exhibited the best antioxidant ability compared to all samples studied (0.10 ± 0.03 mg/mL).

3.2.3. Ferric reducing power assay

The highest and lowest reducing power values were obtained by methanol and aqueous extracts (2.98±0.2 - 3.54±0.22 mg/mL), respectively. Also, the best ability tested by FRAP assay was established by BHT with value of 0.12 ± 0.01 mg/mL.

Table 1: Antioxidant activities of different extracts

Extract	DPPH	FRAP
Ethanol	0.36 ± 0.07^{d}	3.29 ± 0.41^{c}
Methanol	0.71 ± 0.33^{bd}	2.98 ± 0.2^{dc}
Ethyl Acetate	2.04 ± 0.21^{a}	3.14 ± 0.21^{bcd}
Water	0.52 ± 0.02^{cd}	3.54 ± 0.22^{abc}
BHT	0.10 ± 0.03^{ed}	0.12 ± 0.01^{e}

a: comparison between the ethanol extract and all extracts, b: comparison between the methanol extract and all extracts, c: comparison between the ethyl acetate extract and all extracts, d: comparison between the water extract and all extracts, e: comparison between the BHT and all extracts.

3.3. Correlation of Antioxidant Activities with Flavonoids and Phenols Content

To find the influence bioactive compounds (phenols and flavonoids) on the antioxidant potential of *Vitex agnus castus* extracts, we studied the relation between the antioxidant tests and the content of bioactive ingredients (polyphenols and flavonoids) using correlation test. Based on the data presented in Table 3, positive correlation was recorded between phytochemicals and their ability to scavenge free radicals ($P < 0.01$). The IC_{50} DPPH negatively correlated with TFC ($r^2 = -0.87$) and phenols ($r^2 = -0.96$).

Table 3: Pearson correlation coefficients between compounds and antioxidant activities.

Antioxidant activities	Flavonoids (TFC)	Phenols (TPC)
CAT	0.89 **	0.95 **
DPPH	-0.87 **	-0.96 **
FRAP	-0.44	-0.34

* Correlation is significant at the P<0.05 level.

** Correlation is significant at the P<0.01 level.

4. Discussion

The present work was designed to evaluate, for the first time, the TPC, TFC, and antioxidant capacities of *Vitex agnus castus* fruits extracts, which grow in wild habitats in Khenifra area, Morocco. Therefore, from outcomes obtained from different extracts, water gives the highest yield in comparison with other solvents. Our findings are similar to those obtained by Sağlam et al., (2007). In the literature, the nature of solvent affects the yield of extraction (Do et al., 2014). Furthermore, several factors affect the extraction process such as the extraction method, sample particle size, nature of phytochemicals (Stalikas, 2007).

Regarding phenolic quantity, the most proper solvent to extract bioactive compounds was ethanol as previously documented by Latoui et al. (2012). Furthermore, the amount of active ingredients in plants is highly related to numerous biological parameters such as genotype, organ, ontogeny, and environmental conditions. Besides, the solvent used, polymerization, and interaction of active substances govern the solubility of phenolic compounds (Ksouri et al., 2008).

In the same context, the ethanol was the highly extractible solvent for flavonoids, while the lowest value was established in the ethyl acetate extract. The minimum yield of flavonoids extracted (7.12 ± 0.08 µg QE / mg of extract) was also documented in the ethyl acetate extract of *Vitex agnus castus* fruits from Manisa, Turkey (Sarikurkcu et al., 2009). Thus, the yield of flavonoids from fruits is controlled by the nature of the solvent (Gao and Liu, 2005). Hirobe et al., (1997) reported that the bark root of plant (Vitex) also contained flavonoids.

In summary, according to the results obtained from all extracts, ethanol was the most proper organic solvent to extract molecules with antioxidant potential (Table 1).

An extract is qualified to have a strong antioxidant effect if the $IC_{50} < 5$ mg/mL (Abdillah et al., 2015). The methanolic extract of VAC fruits collected from Antalya possesses strong antioxidant activity (Gökbulut et al., 2010), which is in accordance of our data. The existence of some anthraquinones could be responsible for this high reducing power (Yen and Chuang, 2000). Therefore, the methanolic extract could be rich in this class of secondary metabolites.

The TPC and TFC correlate positively with antioxidant ability evaluated by TAC and negatively with DPPH, FRAP. Therefore, the activity of eliminating free radicals from extracts could be attributed to the content of phenols and flavonoids. Phenol compounds can contribute to antioxidant activity, and they have been considered as anti-inflammatory, anticancer, anticholinergic enzymes , antiviral, and antibacterial agents (Atki et al., 2019; Banji et al., n.d.; Jaiswal and Thakur, 2017; Ojo et al., 2017; Rakass, 2018; Sagbo et al., 2017; Shawarb et al., 2017).

The correlation linked the FRAP and the bioactive substances was not significant, which indicates that the FRAP assay in these extracts measures the activity of certain phytochemical classes other than phenols and flavonoids (Yen and Chuang, 2000).

5. Conclusion

Bioactive ingredients and the assessment of antioxidant quality of *Vitex agnus castus* collected from Khenifra were conducted in the present work. The outcome shows that chemical composition was affected by nature of solvent, and ethanol is evaluated as a proper solvent for extraction of molecules, which exhibits the best antioxidant ability. The outcomes revealed the possible application of evaluated *Vitex agnus castus* fruits extracts as the best source of bioactive molecules with health benefits.

References

Sijelmassi A. 1991. Plantes médicinales du Maroc - broché - Abdelhaï Sijelmassi - Achat Livre - Achat & prix | Soldes fnac.

Abdillah S, Tambunan RM, Farida Y, Sandhiutami NMD and Dewi RM. 2015. Phytochemical screening and antimalarial activity of some plants traditionally used in Indonesia. *Asian Pac. J. Trop. Dis.* **5**: 454–457.

Ahangarpour A, Najimi SA and Farbood Y. 2016. Effects of Vitex agnus-castus fruit on sex hormones and antioxidant indices in a d-galactose-induced aging female mouse model. *J. Chin. Med. Assoc*. **79**: 589–596.

Ahmad B, Hafeez N, Ara G, Azam S, Bashir S and Khan I. 2016. Antibacterial activity of crude methanolic extract and various fractions of Vitex agnus castus and Myrsine africana against clinical isolates of Methicillin Resistant Staphylococcus aureus. *Pak. J. Pharm. Sci.* **29**: 1977–1983.

Arokiyaraj S, Perinbam K, Agastian P and Kumar RM. 2009. Phytochemical analysis and antibacterial activity of Vitex agnus-castus. *Int. J. Green Pharm.* **3**.

Asdadi A, Hamdouch A, Oukacha A, Moutaj R, Gharby S, Harhar H, El Hadek M, Chebli B and Idrissi Hassani LM. 2015. Study on chemical analysis, antioxidant and in vitro antifungal activities of essential oil from wild Vitex agnus-castus L. seeds growing in area of Argan Tree of Morocco against clinical strains of Candida responsible for nosocomial infections. *J. Mycol. Medicale.* **25**: e118-27.

Atki YE, Aouam I, Kamari FE, Taroq A, Lyoussi B and Abdellaoui A. 2019. Antioxidant activity of two wild teucrium species from Morocco. *IJPSR*. **10**(6): 2723-2729.

Banji A, Goodluck B, Ouluchi O and Stephen F. 2018. Antimicrobial and antioxidant activities of crude methanol extract and fractions of Andrographis paniculata leaf (Family: Acanthaceae) (Burm. f.) wall. Ex Nees. *Jordan J. Biol. Sci.* **11**(1): 23-30.

Chhabra GS and Kulkarni KS. 2011. Vitex agnus castus - an overview. *J. Nat. Remedies.* **11**

Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S and Ju Y-H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *J. Food Drug Anal*. **22**: 296–302.

Dugoua J-J, Seely D, Perri D, Koren G and Mills E. 2008. Safety and efficacy of chastetree (Vitex agnus-castus) during pregnancy and lactation. *Can. Pharmacol. Clin.* **15**: e74-9.

Gao M and Liu C-Z. 2005. Comparison of techniques for the extraction of flavonoids from cultured cells of Saussurea medusa Maxim. *World J. Microbiol. Biotechno.* **21**: 1461–1463.

Gökbulut A, Özhan O, Karacaoğlu M and Şarer E. 2010. Radical scavenging activity and vitexin content of Vitex agnus-castus leaves and fruits. *FABAD J Pharm Sci.* **35**: 85–91.

Halliwell B and Guterridge JMC. 2007. **Free Radicals in biology and medicine.**

Hamli S, Kadi K, Addad D and Bouzerzour H. 2017. Phytochemical screening and radical scavenging activity of whole seed of durum wheat (Triticum durum Desf.) and barley (Hordeum vulgare L.) varieties. *Jordan J. Biol. Sci.* **10**.

Hirobe C, Qiao Z-S, Takeya K and Itokawa H. 1997. Cytotoxic flavonoids from Vitex agnus-castus. *Phytochemistry*. **46**: 521–524.

Ho C-T, Chen Q, Shi H, Zhang K-Q and Rosen RT: 1992. Antioxidative effect of polyphenol extract prepared from various Chinese teas. *Prev. Med.* **21**: 520–525.

Jadouali SM, Atifi H, Bouzoubaa Z, Majourhat K, Gharby S, Achemchem F, Elmoslih A, Laknifli A and Mamouni R. 2018. Chemical characterization, antioxidant and antibacterial activity of Moroccan Crocus sativus L petals and leaves. *J Mater Env. Sci.* **9**: 113–118.

Jaiswal P and Thakur MK. 2017. The Investigation on Total Phenolic Content and in Vitro Antioxidant Potential of Different Plant Parts of Nyctanthes arbortristis (Night Jasmine). *Int. J. Pharm. Sci. Res*. **8**: 3547–51.

Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M, Smaoui A and Abdelly C. 2008. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. *C. R. Bio.* **331**: 865–873.

Latoui M, Aliakbarian B, Casazza AA, Seffen M, Converti A and Perego P. 2012. Extraction of phenolic compounds from Vitex agnus-castus L. *Food Bioprod. Process.* **90**: 748–754.

McCord JM. 2000. The evolution of free radicals and oxidative stress. *Am. J. Med*, **108**: 652–659.

Ojo OA, Ajiboye BO, Ojo AB, Olayide II, Akinyemi AJ, Fadaka AO, Adedeji EA, Boligon AA and de Campos MMA. 2017. HPLC-DAD Fingerprinting Analysis, Antioxidant Activity of Phenolic Extracts from Blighia sapida Bark and Its Inhibition of Cholinergic Enzymes Linked to Alzheimer's Disease. *Jordan J. Biol. Sci.* **10.**

Oyaizu M. 1986. Studies on products of browning reactions : antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr*. **44**: 307–315.

Prieto P, Pineda M and Aguilar M. 1999. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.* **269**: 337–341.

Rakass S. 2018. Antioxidant activity of Butternut squash Skin: Effect of different extracting solvents. Moroc. *J. Chem.* **6(3)**: 548-556.

Rodríguez ML, Estrela JM and Ortega Á. 2013. Natural polyphenols and apoptosis induction in cancer therapy. *J Carcinog Mutag S.* **6**.

Sagbo IJ, Afolayan AJ and Bradley G. 2017. Antioxidant, antibacterial and phytochemical properties of two medicinal plants against the wound infecting bacteria. *Asian Pac. J. Trop. Biomed.* **7**: 817–825.

Sağlam H, Pabuçcuoğlu A and Kıvçak B. 2007. Antioxidant activity of Vitex agnus-castus L. extracts. *Phytother. Res. Int. J. Devoted Pharmacol. Toxicol. Eval. Nat. Prod. Deriv.* **21**: 1059–1060.

Sarikurkcu C, Arisoy K, Tepe B, Cakir A, Abali G and Mete E. 2009. Studies on the antioxidant activity of essential oil and different solvent extracts of Vitex agnus castus L. fruits from Turkey. *Food Chem. Toxicol*. **47**: 2479–2483.

Shawarb N, Jaradat N, Abu-Qauod H, Alkowni R and Hussein F. 2017. Investigation of antibacterial and antioxidant activity for methanolic extract from different edible plant species in Palestine. *Moroc. J. Chem*. **5**: 573–579.

Stalikas CD. 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci*, **30**: 3268–3295.

Wu H-C, Chen H-M and Shiau C-Y. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomber austriasicus). *Food Res. Int.* **36**: 949–957.

Yen G-C and Chuang D-Y. 2000. Antioxidant properties of water extracts from Cassia tora L. in relation to the degree of roasting. *J. Agric. Food Chem*. **48:** 2760–2765.

Yushchyshena O and Tsurkan O. 2014. Phenolic compounds content in Vitex agnus-castus L. and V. cannabifolia Sieb. growing in Ukraine. *J Med Plants Stud*. **2:** 36–40.

Zhishen J, Mengcheng T and Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*, **64**: 555–559.