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Antioxidant activity of different extracts of *Vitex agnus-castus* (L.) and phytochemical profile

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The main goal of this study was to evaluate antioxidant activity of *Vitex agnus-castus* aerial parts and also to investigate the main phytoconstituents in the plant extracts. N-hexane, dichloromethane, ethyl acetate and methanol 80% extract were tested for free radical scavenging activity on model reaction with stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). The results showed that ethyl acetate was the most active one as antioxidant agent and phytochemical analysis of the ethyl acetate extract revealed the presence of triterpenes, flavonoids, tannins, alkaloids and carbohydrates. The results suggest new chemical classes of natural antioxidant substances that could serve as selective agents for infectious diseases.

Key words*: Vitex agnus-castus,* aerial parts, Free radical scavenging, phytoconstituents.

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (El- Hela et al. 2010). There has been a growing interest in the discovery of antioxidant molecules for use in food and medicinal products, as substitutes for synthetic antioxidant substances. *Vitex agnus-castus* L. is a small tree from *Verbenaceae* family It is native in the whole Mediterranean regions but now grows as ornamental plant in tropical and subtropical regions of the world (Tutin et al., 1972). *Vitex agnus-castus* has been used in traditional medicine as a remedy for menopausal and stomach-ache, headache, influenza, diarrhea and syphilis, digestive aid, sedative and anti-infective (Neumann-Kuhnelt et al., 1993; Schellenberg 2001; Barbara 2001; Hamid et al., 2010). In addition, several biological activities including immunodulatory, antimicrobial and antioxidant have also been reported for the plant (Ekundayo et al., 1990, Mesaik et al., 2010; Sarikurkcu et al., 2009; Latoui et al., 2012). Previous studies on the phytochemical analysis of *V. agnus-castus* revealed the presence of glycosides, flavonoids, diterpenoids, steroids and essential oils (Hajdu et al., 2007; Belie et al., 1969; Males et al., 1998; Hoberg et al;., 1999; Borges et al., 2012). The present study was designed to search for new antioxidant drugs from natural source (*Vitex agnus-castus*) aerial parts and to identify the phytoconstituents in the active plant extract.

Materials and Methods

Experimental

Plant identification and collection

Fresh aerial parts of *Vitex agnus-castus* were collected from the Agricultural Research Centre, Giza, Egypt in May 2011 and the plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereeza Labib consultant of plant taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Agricultural Research Centre, Giza, Egypt.

Preparation of the extracts

Air dried powder of *Vitex agnus-castus* (400 g) were extracted with n-hexane, dichloromethane, ethyl acetate and methanol 80% solvents at room temperature by maceration method. Each extract was concentrated to dryness in vacuo to give 9.2 g, 7.8 g, 6.4 g and 24 g of n-hexane, dichloromethane, ethyl acetate and methanol 80% extracts, respectively.

DPPH assay

The scavenging reaction between (DPPH•) and an antioxidant (H-A) can be written as: DPPH • + H - A→ DPPH - H + A• (Anna et al., 2012). Antioxidants react with DPPH•, which is a stable free radical and is reduced to the DPPH-H and as consequence the absorbance decreased from the DPPH• radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant extract in terms of hydrogen donating ability. DPPH radical scavenging activity from the plant extract was measured by taking 100µg/ml of extract, 900µl of acetate buffer and 3 ml freshly prepared 100µM DPPH solution in methanol. Reagent blank was 1 ml buffer and 3 ml DPPH solution. The absorbance was measured after 90 min of incubation in dark at 517 nm. DPPH radical scavenging activity (%) was determined by following equation: DPPH radical scavenging: Activity (%) = A_b-A_s / A_b x 100. (A_s - absorbance of the test sample, A_b - absorbance control reaction).

Results and Discussion

The present study was focused on the evaluation of anti-oxidant activity of *V. agnus-castus* aerial parts extracts where ethyl acetate extract showed a significant anti-oxidant activity (Table 1). Also we investigated the presence of phytochemicals in the extracts of *V. agnus-castus* methanol extract and phytoconstituents are shown in table 2. The DPPH radical scavenging activity of *V. agnus-castus* aerial parts extracts were compared with that of known natural green tea (Table 1) where ethyl acetate extract showed a significant antioxidant potential (88.46%) and the other extracts were less active as antioxidant agents. As revealed by Ahmadi et al. 2007, DPPH method measures the ability of antioxidants present in scavenging the hydrophilic free radicals. In line to this theory, ethyl acetate extract has better ability in scavenge hydrophilic free radicals as compared to other *V. agnus-castus* extracts that might due to the presence of hydrophilic antioxidants. Furthermore, the

high antioxidant activity could be due to the increased in hydroxyl groups or antioxidant compounds found particularly in the *V. agnus-castus* ethyl acetate extract. Ethyl acetate extract is very rich with phenolic compounds (tannins and flavonoids). Flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003). The highest level of radical scavenging properties at low concentrations of flavonoids exhibits quercetin and in the following order luteolin, rhamnetin, isorhamnetin and apigenin (Kessler et al., 2003). Tannins are the most abundant antioxidants in the human diet and they exhibit many biologically important functions which include protection against oxidative stress and degenerative diseases, gallic acid showed strong antioxidant activity by preventing lipid per-oxidation **(**Shahrzad *et. al*., 2001).

Table 1: Antioxidant activity of *Vitex agnus-castus* extracts

Extracts	Concentration (%)	DPPH free radical scavenging effect (%)
Green tea extract	1%	96.41%
N-hexane extract	0.1%	39.92%
Dichloromethane extract	0.1%	59.45.3%
Ethyl acetate extract	0.1%	88.46%
Methanol extract	0.1%	61.39%

Table 2. Phytochemical analysis of different extracts of *Vitex agnus-castus* aerial parts.

Chemical Constituents	N-hexane	Dichloro methane	Ethyl acetate	Methanol
Carbohydrates and/or glycosides	-	-	+	+
Tannins				
a. Condensed tannins	-	-	+	+
b. Hydrolysable tannins	-	-	+	+
Alkaloids and/or nitrogenous bases	-	-	+	+
Flavonoids	-	+	+	+
Sterols and/or triterpenes	+	+	+	+
Saponins	-	-	-	-
Coumarins	-	-	-	-
(+) denotes the presence of the constituents, (-) denotes the absence of the constituents				

Conclusion

The presented results indicate that antioxidant potential of *V. agnus-castus* aerial parts ethyl acetate extract is due the presence of bio-active phytoconstituents as phenolic compounds (tannins and flavonoids) and these results also endorsed the ethnobotanical use of this plant from the collected territory due to presence of various chemicals.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

References

Anna G.G., Marlena D.M., Irena M. 2012. DPPH Radical Scavenging Activity And Phenolic Compound Content In Different Leaf Extracts From Selected Blackberry Species. Acta Biol. Cracov. Ser. Bot. 54 (2):32–38.

Ahmadi F., Kadivar M., Shahedi M. 2007. Antioxidant activity of Kelussia odor atissima Mozaff. in model and food systems," Food Chem. 105:57–64.

Barbara C.L. 2001. *Vitex agnus-castus* essential oil and menopausal balance: a research update. International Journal of Aromatherapy. 13:169-172.

Belie I., J. Bergant-Dolar, Morton R. A. 1961 Constituents of *Vitex agnus-castus* seeds. Part 1. Journal of Chemical. Society 2: 2523-2525.

Borges A. R., J. R. A. Aires, T. M. M. Higino, M. Graças, F. Medeiros, A. M. G. Citó, J. A. D Lopes, and R. C. B. Q. Figueiredo, Trypanocidal and cytotoxic activities of essential oils from medicinal plants of Northeast of Brazil. Experimental Parasitology, 132: 123-128, 2012.

El-Hela, A., Abdullah A. 2010. Chemical Composition and Biological Activities of Essential Oil of Salvia acetabulosa L. Grown in Egypt. Journal of Applied Sciences Research, 6(6): 690-695.

Ekundayo O., Laakso I., Holopainen M., R. Hiltunen, R. Oguntimein, and Kauppinen, V. 1990. The chemical composition and antimicrobial activity of the leaf oil of *Vitex agnus-castus*. Journal of Essential Oil Research 2: 115-119.

Hoberg E., J. Orjala, B. Meier, O. Sticher 1999. Diterpenoids from the fruits of *Vitex agnsu-castus*. Phytochemistry 52: 1555-1558.

Hajdu Z., J. Hohmann, P. Forgo, T. Martinek, T. Deruarics, M. Zupko, I., Falkay, G., Cossuta, D., Mathe I. 2007. Diterpenoids and flavonoids from the fruits of *Vitex agnus-castus* and antioxidant activity of the fruit extracts and their constituents. Phytotherapy Research 21: 391-394.

Hamid A.A, Usman L.A., Adebayo S.A., M. F. Zubair, Elaigwu S. E. 2010. Chemical constituents of leaf essential oil of North-central Nigerian grown *Vitex agnus-castus* L. Advances in Environmental Biology, 4:250-253.

Kessler M, Ubeaud G, Jung L 2003. Anti- and pro-oxidant activity of rutin and quercetin derivatives. J. Pharm. Pharmacol. 55: 131-142.

Latoui M., B. Aliakbarian, A. C. Casazza, M. Seffen, A. Converti, P. Perego 2012. Extraction of phenolic compounds from *Vitex agnus-castus* L. food and Bioproducts Processing 90:748-754.

Males Z., N. Blazevic, and A. Antolic 1998. The essential oil composition of *Vitex agnus-castus* F. rosea leaves and flowers. Planta Medica 64:286-287.

Mesaik M. A., S., Azizuddin, K. M. Murad, R.B. Khan, A. Tareen, A Atta-ur-Rahman, M. I. Choudhary 2009. Isolation and immunodolatory properties of a flavonoid and casticin from *Vitex agnus-castus.* Phytotherapy Research 23:1516-1520,

Neumann-Kuhnelt B., G. Stief, H. Schmiady, H. Kentenich 1993. Investigations on possible effects of the phototherapeutic agent Agnus-castus on the follicular and corpus luteum phases. Human Reproduction 8:110-116.

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

Schellenberg R. 2001. Treatment for the premenstrual syndrome with Agnus-castus fruit extract: a prospective, randomizedplacebo controlled study. British Medical Journal. 322:134-137,

Shahrzad S, Aoyagi K, Winter A, Koyama A, Bitsch I. 2001. Pharmacokinetics of gallic acid and its relative bioavility from tea in healthy humans. J. Nut. 22: 1207-1210.

Tutin T. G., Heywood V. H., N. A. Burges, D. H. Valentine, S. M. Walters, D.A. Webb. 1972 Flora Europaea, Vol. 3, Cambridge University Press, Cambridge, pp. 122-128.