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Estimation of the radical scavenging activity 2,2-diphenyl-1-picrylhydrazyl, total phenols and total flavonoids of *Vitex agnus-castus L.* methanolic leaf extract in Baghdad.

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تقدير فعالية الكسح الجذري بالمركب ٢،٢- ثنائي فينيل-١ بيكريل هيدرازيل والفينولات الكلية والفلافونويدات الكلية لمستخلص أوراق الميثانول لنبات كف مريم في بغداد

احمد محمد محمود قسم الصيدلة، كلية الفارابي الجامعة، بغداد، العراق

Abstract

Chaste tree, is a plant distributed in few regions like the Middle East and Southern Europe. Conventionally used for the treatment of several health issues and symptoms, such as hormonal changes premenstrual ones and spasmodic dysmenorrhea, certain menopausal conditions, poor lactation, and pimples (acne). Several reports have told that Vitex agnus-castus contained phytochemical that really interesting to be studied like phenols and flavonoids, and others. The work aims to estimate the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay activity of methanol extracts from leaves of Vitex agnus-castus in Iraq, and see if it contains appreciate level of bioactive ingredient of phenols and flavonoids. Dried samples were submitted to extraction with methanol using soxhlet and total flavonoids, and phenols were tested in the sample. DPPH free radical levels were significantly lowered in a concentration-dependent mode, there was no statistically significant difference ($p > 0.05$) between our extract and ascorbic acid in percent of inhibition of free radical. The IC50 found to be 725.4 µg/ ml as compared with the standard ascorbic acid (246.53 µg/ml), and the phenols level was 43.43 ± 1.12 (mg GAE/g extract) mg of Gallic Acid Equivalents (GAE) per g of extract, while the flavonoid was 14.7 ± 0.25 (mg QE/g) mg quercetin equivalence per gram dried extract. Therefore, Iraqi Vitex agnus-castus L. can be considered as encouraging candidates for natural herb sources of antioxidants with high value.

Keywords: Vitex agnus castus, Antioxidant, DPPH, Total phenols, Total Flavonoids

الخلاصة

كف مريم، المعروف باسم شجرة العفة، هو نبات منتشر في مناطق معينة مثل الشرق الأوسط وجنوب أوروبا. يُستخدم تقليديًا لعلاج العديد من المشكلات والأعراض الصحية، مثل التغيرات الهرمونية، تلك التي تحدث قبل الدورة الشهرية وعسر الطمث التشنجي، وبعض حالات انقطاع الطمث، وضعف الرضاعة، والبثور (حب الشباب). اثبتت العديد من التقارير أن كف مريم يحتوي على مادة كيميائية نباتية مثيرة للاهتمام حقًا لدراستها مثل الفينولات والفلافونويدات وغيرها. عملنا يهدف إلى تقدير نشاط فحص الكسح الجذري لمستخلصات الميثانول من أوراق نبات كف مريم في العراق، ومعرفة ما إذا كان يحتوي على مستوى جيد من العناصر الفعالة من الفينولات والفلافونويدات. تم تقديم العينات المجففة للاستخلاص بواسطة الميثانول باستخدام جهاز الاستخلاص السوكسليت، وتم اختبار الفينولات والفلافونويدات الكلية في العينة المستخلصة. حيث تم تخفيض مستويات الجذور الحرة بشكل ملحوظ في الوضع المعتمد على التركيز ($P < 0.05$) , ولايوجد فرق ملحوظ احصائيا بين مستوى فعالية المستخلص مقارنة مع حامض الأسكوربيك في النسبة المئوية لخفض مستوى الجذور الحرة. ووجد أن IC50 يبلغ ٧٢٥.٤ ميكروغرام / مل مقارنة

بحمض الأسكوربيك القياسي (٢٤٦.٥٣ ميكروغرام / مل)، وكان مستوى الفينولات ٤٣.٤٣ ± ١.١٢) ملغم / GAE جم مستخلص (ملغم من مكافئات حمض الغال (GAE) لكل جرام من المستخلص، بينما كان الفلافونويد (١٤.٧ ± ٠.٢٥) ملجم / QE جم (ملجم مكافئ كيرسيتين لكل جرام من المستخلص المجفف) ولذلك يمكن اعتبار نبات كف مريم العراقي مرشحاً مشجعاً لمصادر الأعشاب الطبيعية لمضادات الأكسدة ذات القيمة العالية.**الكلمات المفتاحية:** عشبة العفة، عشبة كف مريم، مضادات الاكسدة، الفينولات الكلية، الفلافونويدات الكلية.

1. Introduction Body organs should be protected from harmful effect done by free radical through antioxidant supplements. (Lobo et al, 2010). Nowadays, many patients and consumers claims from few side effects as a result of artificial antioxidants, so it is important to shift towards plants phytochemicals in our daily intake. (Halliwell B. 1996). At the present time, herbal product jump to spearhead, while some chemical products like penicillin and old molecules loses the effectiveness gradually according to many reasons, because plants support us with unlimited active natural ingredients with different mechanism of action, so it is expected to gain broad efficacy from plant extract. (Enneb H, etal. 2015). Many previous studies state the safety and efficacy of herbal extract to be used as prophylactic or treatment for variety of diseases like liver toxicity. (Ahmed. M Al-Yassen et al, 2022). Vitex agnus-castus L., chasteberry or chaste tree, a shrub that native with Asia and Mediterranean region. Family: LAMIACEAE, Genus: Vitex, Species: Vitex agnus-castus L. (Souto, E.B.; 2020). Vitex agnus castus L. has been used for decades for different purposes, in the section of medicine and treatment, Vitex agnus castus L. with its different plants parts (Leaf, fruit and seeds) used to control pain, regulate lactation, reduce inflammation, urine flow, mastalgia (van Die MD et al, 2013), and many females' conditions like acne v. uterine irregularities, lactation and menstrual events, (Chhabra GS and Kulkarni KS. 2011).

Fig. 1- Vitex agnus castus shrub. (Deborah Richardson, 2023).

At commercial health care, Vitex had been practice in the management of vision problems, pimples, scorpion and snakes attack, many pain distress, stomach pain, RA, inflammatory conditions, and to stimulate delayed menses (Lataoui M. et al, 2012; Niroumand M. et al,2018). Vitex agnus pose a many bioactive ingredients as in fruit many phytochemicals recognized as iridoids flavonoids, terpenoids, while polyphenols may be notice in leaves. (Li S.-H. et al, 2002), lately, biotic extracts and essential oils from Vitex agnus castus L. present hopefully activities such as antibiotics, free radical scavenging and antitumor. (Ricarte L. P. et al, 2020) This work, aim to study the radical scavenging activity of Vitex agnus-castus L. herb extract and the level of some bioactive constituents.

2. Experimental Work

2.1. Herb preparation and extractionThe plant leaves harvested from orchard in Baghdad in June and authenticated by botanical expert, the date of collection were labeled. The herb part (leaf) washed then oven dried, after that powdered. 33 gm of herb powder mixed with one solvent methanol and the ratio about 1to 20 with soxhlet apparatus and last about 8-9 hr., rotary evaporator was necessary to gain consistent concentrated extract. The final result kept at 4°C refrigerator prior to work.

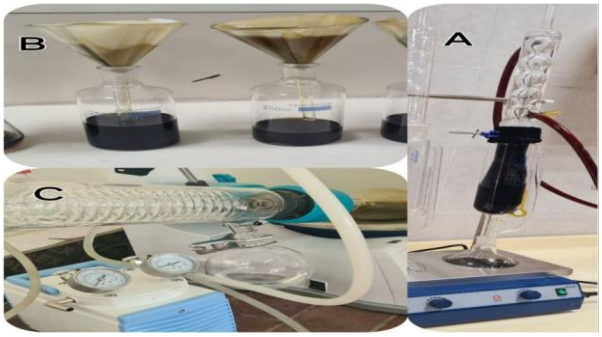

Fig. 2 – A – Soxhlet, B – Extract Filtration, C – Rotary Evaporator

2.2.DPPH assay

The procedure of DPPH assay run as explained by Wu et al, (2003) to test the ability to diminish free radicals. 1 ml of 0.1Mm of 1, 1-diphenyl -2-picrylhydrazyl solution mixed with standard/extract, then prepare for intubation for half hr. in dark room, the result of free radical control evaluated by the equation (Wu H-C. et al, 2003):Q= 100 × (A0-AC) / A0 Where A0= Absorbance of control AC=Absorbance of the two samples after 30min incubation.

2.3. Determination of Total flavonoid content (TFC)

Afify et al. (2012) method was chosen to detect and evaluate the TFC. Standard calibration done with quercetin and TFC (mg). (1mg/mL) 0.5ml of sample with 10% of 1milliliter aluminium chloride and 1Ml of potassium acetate (1ml) and distilled water about 2.5ml. UV-spectrophotometer on 415nm used to read the absorbance of mixture

2.4. Total polyphenolic content (TPC).

By use Folin-Ciocalteu (FC), compound reagent that elaborated by Jadouali et al, TPC has been estimated and represented as Gallic Acid Equivalent GAE per gram of our sample. (Jadouali SM, et al, 2018)

2.5 Experimental design

The results were reported as means ± standard deviation (SDV) and t test to compare between means. The statistical analysis was carried out by using SSPS 16.0, the level of significance was set at $P<0.05$

3. Result

3.1.DPPH assay

As known Antioxidants are molecules with the capacity to either imped or postpone the oxidation. (Pisoschi, AM et al, 2011). Reactive oxygen species (ROS) or ambient oxygen are present when this reaction occurs. An antioxidant protects the body from the damaging effects of free radicals.DPPH compound is rational and non-dimerizing free radical because the delocalization of own spare electron at molecule. The violet color with absorption at 520 nm may clearly indicate delocalization of the DPPH molecule. (Pisoschi, AM et al, 2009).

Below Figure (1) demonstrate a comparison of antioxidant activity between *Vitex agnus castus L.* extract and ascorbic acid.The methanol extract of Vitex agnus castus L. showed a significant dose-dependent inhibition of DPPH activity, by 50% inhibition (IC50) at dose concentration of 725.4 µg/ ml as compared with the standard ascorbic acid (246.53 µg/ml).There was no statistically significant difference ($p >0.05$) between our extract and ascorbic acid in percent of inhibition of free radical.

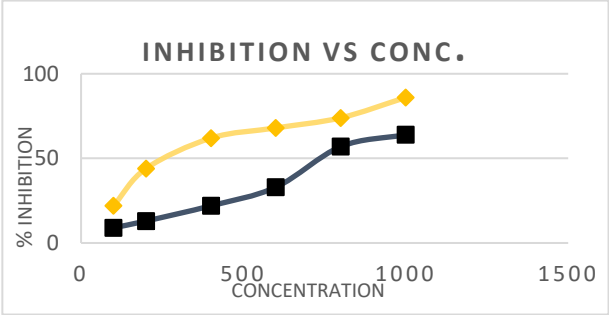

Fig 3. Show curve of concentration with percent of inhibition and ic50 of Vitex agnus castus L. leaf methanolic extract. (Yellow line show Ascorbic acid, while Blue line present the extract).

3.2. Total polyphenolic content (TPC).

The TPC test, commonly known as the Folin Ciocalteu (FC) technique, the procedure well established and includes the FC reagent to oxidize phenolic compounds. The reaction results in a blue-colored reduced FC reagent, which could be detected at 760 nm. (Singleton, V.L et al, 1999). 43.43 ± 1.12 (mg GAE/g extract) mg of Gallic Acid Equivalents (GAE) per g of extract.

3.3. Total Flavonoid Content (TFC).

The total flavonoid content was measured using the aluminum chloride colorimetric technique in 2002 that shown by Chang et al. (Chang et al, 2002). TFC content in this study was 14.7±0.25 (mg QE/g) mg quercetin equivalence per gram dried extract.

4.Discussion

Extreme free radicals that created by oxidative stress and radiation can really damage biomolecules and body systems. (Jorgensen LV et al, 1999; Halliwell B et al, 1993).Many previous works define antioxidant activity as the proportion (percent) of scavenging or inhibiting the free radical DPPH. Because there is no absolute definitive assessment of a compound's antioxidant capacity, findings are reported relative to ascorbic acid, a

reference antioxidant, to get good view to extract activity. (Aini F. et al, 2019)Six serial concentrations were used to accomplish the free radical inhibition activity of methanol extract. DPPH free radical levels were significantly lowered in a concentration-dependent mode. The statistical analysis revealed no significant variance between ascorbic acid and methanol extract scavenging results. Also this is agreeing with many previous studies that use leaf and fruit extract from vitex agnus castus. (Hayder B Sahib et al, 2014; Hajdu Z. et al, 2007)Also many preceding studies upon the examination of phytochemical of Vitex agnus-castus revealed the presence of different active molecules like flavonoids, glycosides, diterpenoids, essential oils and steroids. (Borges A. R. et al, 2012) The results of total phenols and flavonoids revealed interesting level of these biomolecules that may explain and clarify the antioxidant activity behind this herb generally and the extract specifically. (Rice-Evans C.A. et al, 1996). Many articles try to explain the suggested mechanism od antioxidant of Polyphenols, it tells it may deactivate the active species and precursors of free radicals, lowering the degree and rate of oxidation. Typically, they function as direct radical scavengers of lipid peroxidation chain processes (chain breakers). Chain-breakers provide an electron to the free radical, neutralizing it and transforming it into a stable (less reactive) radical, thereby stopping the chain reaction. (Pietta P.G. 2000; Guo J.-J. et al, 2009). A polyphenol skeleton assembles a class of low molecular weight molecules known as flavonoids. So it is predicting to be found in lower quantity than phenols upon extraction in general. (Kumar S and Pandey AK. (2013)While flavonoids have a broad range of actions, including 1- chelating metal 2- suppress ROS 3- activation of the body's own antioxidant enzymes and 4- pospone the enzymes that involved in the production of free radicals. (Procházková D. et al, 2011). Many types of bioactive flavonoid exist in vitex agnus castus and the major one is casticin. This substance is well known for its antioxidant and anti-inflammatory effects, which are mostly used in conventional medicine. And hence the antioxidant activity of this herb may related to such secondary active metabolite. (Carbone K. et al, 2023).

5.Conclusion

Chasteberry tree is well known and studies plant that also promoted for variety of traditional uses like premenstrual symptoms, anti-inflammatory and pain. And many studies work on different parts of Vitex agnus castus top flower, fruit and leaf.Agents of antioxidants properties offer promising activities in decrease oxidative stress, aid in tumor suppression and brain health.Vitex agnus castus cultured in Iraq exhibit significant antioxidant effect and worth next level studies for angiogenesis and tumor suppression since it contains appreciate levels of bioactive phytochemicals like phenols and flavonoids.

Conflicts of Interest

The authors report no financial or any other conflicts of interest in this work.

Ethical Clearance

An in-vitro study

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