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Isolation and identification of campesterol, stigmasterol and beta-sitosterol in Iraqi date palm pollen

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Abstract :

date palm pollen-DPP (Phoenix dactyliferaL.,Palmae) one of the significant herbal medicine In ancient Egypt, the suspension of DPP was reported as a traditional remedy to cure men infertility in folk medicine and the male flowers of palms can also be eaten directly by women as a fresh vegetable to promote fertility. DPP grains contain a logical amounts of secondary metaboiltes such as sterol, Flavonoids, triterpenoid, tannins...etc. Multiple types of sterols got identified in DPP so the current study include the isolation of sterol from Iraqi date palm pollen-DPP Phoenix dactylifera L. and then identified the present of the important famous sterol campesterol, stigmasterol and beta-sitosterol by HPLC on it.

The HPLC analysis for sterols Iraqi DPP revealed that it contain 650.272 µg/g of campesterol , 241.626 µg/g of stigmasterol and 178.899 µg/g for β-sitosterol with Unknown one peak .

Key word: date palm pollen , sterol , campesterol, stigmasterol, beta-sitosterol.

الخلاصة :

يعتبر طلع النخيل (Phoenix dactyliferaL.,Palmae) احد الادوية العشبية المهمة في مصر القديمة واعتبر المحلول العالق لطلع النخيل احد العلاجات التقليدية لمعالجة العقم عند الرجال في الطب الشعبي و يمكن ايضا تناول أزهار النخيل الذكرية مباشرة من قبل النساء كخضروات طازجة لتعزيز الخصوبة تحتوي حبيبات طلع النخيل على كميات منطقية من المستقلبات الثانوية مثل الستيرول ، الفلافونويد ، تيربينويد ثلاثي ، التانينات ... إلخ.

تم التعرف على أنواع متعددة من الستيرولات في طلع النخيل لذا فإن الدراسة الحالية تشمل عزل الستيرول من حبوب لقاح النخيل العراقية ، ومن ثم التعرف على وجود ستيرول كامبستيرول الشهير وستيغماستيرول وبيتا سيتوستيرول بواسطة HPLC عليه.

أظهر تحليل HPLC لستيرول طلع النخيل العراقي أنه يحتوي على 650.272 ميكروغرام / غرام من كامبيسترول، 241.626 ميكروغرام / غرام من ستيغماستيرول و 178.899 ميكروغرام / غرام لـ β-sitosterol مع قمة واحدة غير معروفة.

Introduction :

Datepalm(PhoenixdactyliferaL.,Palmae) one of the oldest trees cultivated in earth, also it has been used as food source around 6000 years ago(1) . Date palms can be found in Middle eastern countries like Saudi Arabia and Egypt(2) . Date palm is dioeciously having parts of the both genders originating from a unique seed but half part is female only i.e. fruit bearing(3) . In Egypt people believed that pollens can consolidate fertility and it is an aphrodisiac. Many components got isolated and identified from the pollen including phenolic acids, procyanidins, saponins, lipids, flavonoids, triterpenes, sterols and carbohydrates(4) . Its contain many phytochemical compounds like sterols, carotenes ,flavonoids, triterpenoid, tannins...etc (5) . This compounds were reported from many researcher to have great medicinal use as antioxidant (6), anti-inflammatory, antitumor (7), antibacterial (8), antifungal, neuroprotective (9) and hepatoprotective agents (4).

Phytosterols is another main type of lipid soluble phytochemicals exist in DPP (Fig. 1). The grains of the pollen are the main cistern of phytosterols (Duke 2001; Duke and Beckstrom Sternberg, Accessed 2007) (10) and it has been used for a long time to cure many hormone related health cases. Kikuchi and Miki (1978) isolated the mixture of crystalline plant sterol from the eatable flesh of dates first and then they classified them as β-sitosterol, stigmasterol, campesterol, and isofucosterol (11) (Fig. 2). Despite that, the dissimilarity in phytosterol composition between the steroles cultivars of the date and fruit ripening's stages remains hazy and it is a significant avenue for future research. Not only sterols included, but also date pits contain estrogen, ergasterol, esterone , and brassicasterol, in a newly

published study, multiple phytosterols came to be identified in further species of date palm (P. theophrasti) in the eatable fragment of the fruit (10). They carry Campesterol, , γ -sitosterol , 4-methylcholest-4-en-3-one , spinasterone ,

(E)- 24-propylidenecholesterol, stigmastan-3, 5-diene, stigmasta-5,22-dien-3β-ol, β-sitosterol, lupenone, lupeol, 24-methylenecy-cloartanol, cholest-4-en3-one , stigmast-4- en-3-one, and cholesta-3,5-diene [12].

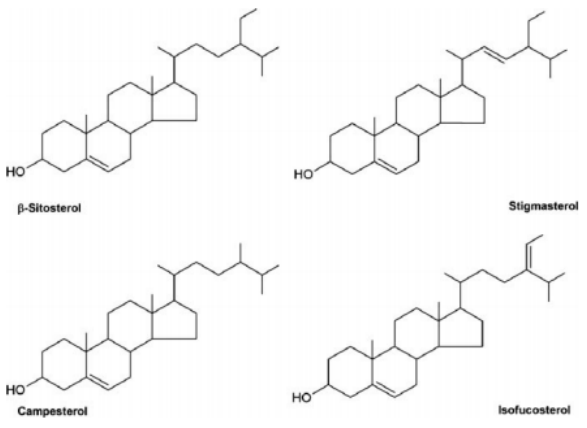

Fig. 1 : Phytosterols identified in DPP

Material and methods:

Date pollen palm (Phoenix dactylifera L.) Multiple of El-Ghannmi Ahmar has been collected from the city of Samarra in the governorate of Salah Al-Din in,Iraq separated by using a fine gauze sieve from the kernels e and kept in an incubator at 35°C for a period of three hours.

Saponification :

1 - Deliver 5 ml sample into dry test tube .
2 - Add 25.0mL of 5 % potassium hydroxide Ethanol
3 - Gently heat the test tube occasionally shaking in a hot water bath for 30 min.
4 - After heated, immediately cool it , take the precipitate soap for analysis .

Preparation of extracts:

10 mg of the extract were dissolved 1 ml HPLC grade methanol by vortexing ,a 2.5 um disposable filter has been used to pass the mixture through it, and then the mixture has been stored at 4o C for more analysis , after than 20 ul of the sample injected into HPLC system depending on the above , ideal separation conditions .

Methods

Analysis of steroid in the sample :

According to the optimum condition the standard phytosterols got separated on FLC (fast liquid chromatographic) column.

The standard phytosterols were separated on FLC (fast liquid chromatographic) column under the optimum condition

- Column : Nuclear C-18 , 3 µm the size of the particle is (50x4.6 mm I.D) column .
- Mobile phase : 0.01 M phosphate buffer : acetonitrile , (20:80 v/v) .
- Detection : UV at 210 nm .
- Temperature : ambient
- Flow rate : 1.2 ml / min.

The Volume of the Injection: 20 µl

The identification of the concentrations of sterols happened by depending on the following equation :

Concentration of sterols(µg/ml) =

Area of sample X C X D

Area of stand.

Result and discussion :

HPLC method was used for qualitative and quantitative content of phytosteroids in the Iraqi DPP. Three standard sterols (compesterol , stigmasterol , β - sitosterol) were used as standard phytosteroids, and the chromatograms for the 3 standards were shown in Fig. 2 . The retention time-Rt of the 3 standard steroids were (3.95, 4.86, and 6.45) min respectively and the area under curve were 124461, 147834, and 200745 respectively tab. 1

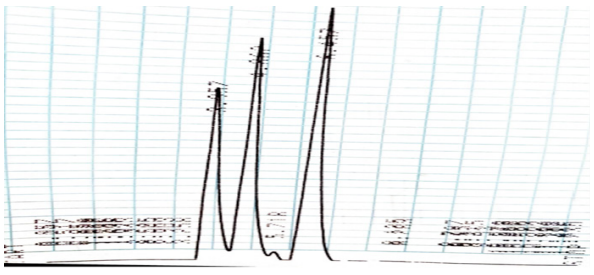

Fig 2 : HPLC Analysis of Three Standard Phytosterols (compesterol, Stigmasterol and β-Sitosterols)

Tab. 1 : Rt and the area below the curves for standard sterol

Sterols	Rt	Area under curve
Compesterol	3.95	124461
Stigmasterol	4.86	147834
B- sterol	6.45	200745

The chromatograms of DPP showed 4 peaks Fig.(3) with different Rt (1.95, 3.987, 4.853, and 6.433) min. also each peak's area were 64839, 161876, 71441 and 71826 which summarized in tab. 2

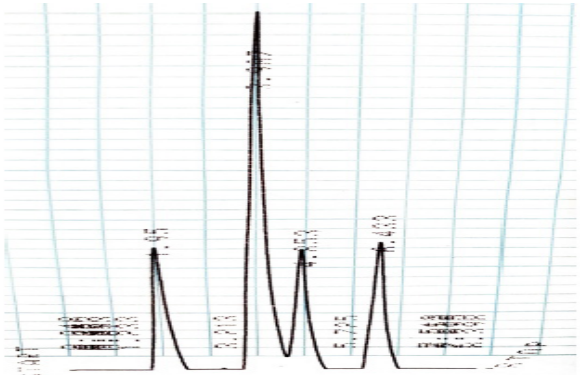

Fig. 2 : HPLC Analysis of Sterol in Iraqi DPP

Tab. 2 : Rt , area under curves and conc. of identified sterol in DPP

Identified sterol	Rt.(min.)	Area under curve	Conc. µg /g
Unknown	1.95	64839	-
Campesterol	3.987	161867	650.272
Stigmasterol	4.853	71441	241.626
β - sitosterol	6.433	71826	178.899

By comparing between retention times for sample and standard we found that 3 peaks can characterized , DPP contain 650.272 µg /g campesterol , 241.626 µg /g stigmasterol and 178.899 µg /g β - sitosterol , the 4th peak cannot characterize it because there is no standard for it .

The results that has been obtained in the current study are consistent with the results of Abbas and Ateya(13) which indicated the existence of Estrone ،Estradiol، Estriol in DPP in Egyptian Phoenix dactylif-

eraL., Palmae , also Al-Samarrai in her study noticed existence of Estriol،Estrone، Estradiol and β-sitosterols in Alghanimy ahmar Phoenix dactylifera L., Palmae(14) . The ß-sitosterol was identified in the pollen by HPLC in this study but Mohamed et al(15) identified cholesterol, ß-sitosterol and stigmasterol in Sewi pollen by TLC .

Conclusions :

The phytochemical screening showed that Iraqi DPP Phoenix dactyliferaL., contain high concentration of sterols (campesterol, stigmasterol and beta-sitosterol) can isolated it and characterized by HPLC technique.

Recommendations :

According to the result of this study above , we may recommend the following :

1- Study the phytochemical structure of other Iraqi DPP cultivars (such as Smasmii, Wardii , Ghanmi Akhdar, Khukri, Dekel G and Dekel S).

2 - Study the other sterols in Phoenix dactyliferaL., Palmae and their effects at health .

References :

1 - Krueger, Robert R. "Date Palm Genetic Resource Conservation, Breeding, Genetics, And Genomics In California" (PDF). The Conference Exchange. Retrieved 2018-03-26 .

2 - Tengberg, M. (November 2012). "Beginnings and early history of date palm garden cultivation in the Middle East". Journal of Arid Environments. 86: 139-147. Bibcode:2012JArEn..86..139T. doi:10.1016/j.jaridenv.2011.11.022 .

3 - El Bassam, Nasir. Handbook of bioenergy crops: a complete reference to species, development and applications. Routledge, 2010.

4 - Singab AN, El-Taher EMM, Elgindi MR, Kassem MES. Phoenix roebelenii O›Brien DNA profiling, bioactive constituents, antioxidant and hepatoprotective activities. Asian Pacific Journal of Tropical Disease, 2015; 5, 552-558.

5 - Saxena, Mamta, et al. «Phytochemistry of medicinal plants.» Journal of pharmacognosy and phytochemistry 1.6 (2013).

6 - Kchaou, W., Abbès, F., Blecker, C., Attia, H.,Besbes, S. Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (Phoenix dactylifera L.). Industrial crops and products, 2013; 45: 262-269.

7 - Rahmani AH, Aly SM, Ali H, Babiker AY, Srikar S. Therapeutic effects of date fruits (Phoenix dactylifera) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour

activity. International Journal of Clinical and Experimental Medicine, 2014; 7.

8 - Al-zoreky, Najeeb S., and Abdulla Y. Al-Taher. «Antibacterial activity of spathe from Phoenix dactylifera L. against some food-borne pathogens.» Industrial crops and products 65 (2015): 241-246.

9 - Pujari RR, Vyawahare NS,Thakurdesai PA. Neuroprotective and antioxidant role of Phoenix dactylifera in permanent bilateral common carotid occlusion in rats. Journal of Acute Disease, 2014; 3, 104-114.

10 - Vayalil, Praveen K. «Date fruits (Phoenix dactylifera Linn): an emerging medicinal food.» Critical reviews in food science and nutrition 52.3 (2012): 249-271.

11 - Kikuchi, Nobu, and Taihei Miki. «The separation of date (Phoenix dactylifera) sterols by liquid chromatography.» Microchimica Acta 69.1-2 (1978): 89-96.

12 - Liolios, C. C., G. T. Sotiroudis, and I. Chinou. «Fatty acids, sterols, phenols and antioxidant activity of Phoenix theophrasti fruits growing in Crete, Greece.» Plant foods for human nutrition 64.1 (2009): 52-61.

13 - Abbas, Fawkeya A., and Abdel-Monem Ateya. «Estradiol, esteriol, estrone and novel flavonoids from date palm pollen.» Aust J Basic Appl Sci 5.8 (2011): 606-14.

14 - Al-Samarrai ,R.R.››Study of Date Palm Pollen on the Level of Sex Hormones and Antioxidant Parameters in Blood of Adult Male Rabbits›› theses of philosophe in biochemistry. Tikrit University , collage of education .2013 .

15 - Mohamed, S. M.; H. A. Bosila; S. F. E. Sharabasy I. A. Ibrahim and K. A. Refay. "Phytochemical screening of some in vivo and in vitro date palm tissue". Al-Ain, United Arab Emirates, 2001;35:25-27.