

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The phytochemical screening of some polyphenolic compounds present in Phoenix dactylifera L. seeds cultivated in Iraq.

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ABSTRACT

Phoenix dactyliferaL. one of most important plant in world is represent source of food for human and animal since ages, the seeds of phoenix dactylifera represent source of food and have many pharmacological properties, this study concentrate on extraction of bioactive constituents in seeds of phoenixdactylifera which cultivated in Iraq phytochemical screening by chemical tests and qualitative and quantitative estimation of biological important polyphenolic compounds by HPLC and TLC. Extraction of bioactive constituents was carried out using 60%methanol, Phytochemical screening exposes the presence of sterols, tannin, phenolic compounds and flavonoids, alkaloids. Qualitative and quantitative estimation of polyphenolic compounds was done by high performance liquid chromatography (HPLC) and Qualitative estimation was done by thin layer chromatography (TLC).

Keywords: Phonixdactylifera, chemical test, HPLC, TLC

September-October

2017

RJPBCS

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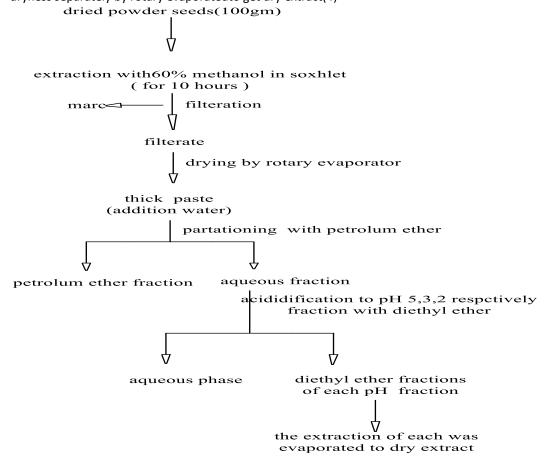
INTRODUCTION

Phoenix dactylifera L. whichknown as data palm, it is not very edible plant the native to dry regions of north Africa and southwest Asia. Wild grooves are repotedly still growing fresh or brackish in border area of Iraq and Iran and desert area of Jordan. its belong to monocotyledon plant from family Arecaceae which called also(Palmae) the fruit have five growth stage were name khababauk, kimri, khalal, rutaband tamer(10) it is use as source of food to human and animal the first domestication of data palm believed to have takenplaced at least 6000 years agoin Mesopotamia the land between the tigris and Euphrates rivers in Iraq(1)(2)phoenix dactylifera L. data palm seed used traditionally to wounds, lesions, inflammation demulcent laxative and relieve toothache and ague(9)also it have many medicine activity as Anticancer activity, Antidiarrheal Activity, Hepatoprotective activity, Antioxidant activity, Antiinflammatory activityantiviral activity, Antihyperlipidemic activity Nephroprotective activity(7). Data palm considered as a rich source of antioxidant compounds like phenolic acid and flavoniods. The main target of the present study is to investigate the phytochemical compounds dried powder seed extract in the phoenixdactylifera L.which Cultivated in Iraq by chemical tests and determine the presence of some polyphenolic compound by TLC and HPLC

MATERIAL AND METHODS

Plant material: plant collect from Agraden in Diyla during the month of (July – October) in year (2016) it was in khalal stage the variety of plant is barhee. the plant identified bydepartment of Pharmacognosy, college of pharmacy/Baghdad University.

Extraction: Amount of 100gm of The dried seeds powder of *Phoenix dactylifera L.* was extract with methanol 60% ($v\v$) insoxhlet apparatus for 10 hours . then the result was filtered and then concentrated by rotary evaporator to get dry extract , then was add 250 ml of water and partition with 250ml of petroleum ether to get rid of fat material (defatting step)the residual aqueous phase then acidified to pH 5 and extract with diethyl ether then gradually acidified to pH 3 and 2 each diethyl ether according to the pH was evaporated to dryness separately by rotary evaporated to get dry extract(4)





Phytochemical identification by chemical test: (5) (6)

Active constituent	Chemical Test	Result
phenolic compounds	ferric chloride 5%	Deep green
Tannin	%10lead acetate	White ppt
	potassiumdichromate 1%	Organ ppt
Flavonoids	1.aqueous sodium hydroxide	1.yellowish color
	then2. Add the 5%HCL	2. Disappear of color
sterols	1. Few acetic anhydride	1. pink color
	then2.added few drops sulfuric	2.green color
	acid	
Alkaloid	Dargendroff's reagent	Orange-brown ppt

HPLC analysis

Qualitative and quantitive estimation of expected (vanillic acid(VA), gallic acid (GA), cinammic acid(CA), catechin (cat), quercetin(Q), rutin (R), coumarin(Co), lutein (L) in their crude extracts obtained by the methanolic extraction at pH3 method the identification were made by comparison of retantion time at identical chromatography condition of analyzed sample and authentic standard.

HPLC conditions of exception poly phenol:

Mobile phase: isocratic: methanol- 0.05% phosphoric acid water (20:80)

Column : Shamadzu LC C18 (250mm x 4.6, 5mm partical size)

Column temperature: Ambient

Detector: UV- vis at 338 nm Injected volume: 20 *m*I

Injection concentraction: 1 ppm

Flow rate: 0.8ml/min

TLC Analysis:

Use of thin layer chromatography for identification of some polyphenolic compound (gallic acid (GA) , Vanillic acid (VA), catechin(cat), quercetin(Q)) present in the extract this method is qualitative estimation method and using t mobile phase

S1. THF:toluene :HCOOH :H2O 16:8: :2:1(8)

RESULT AND DISCUSSION

Phytochemical identification by chemical test: the results was given by table 1

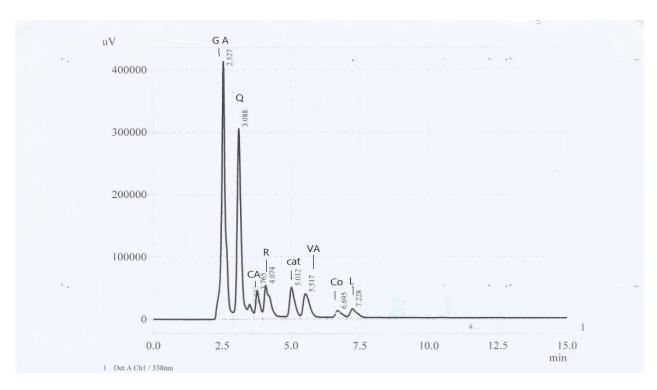
Table 1 result of chemical test

Active constituent	phenolic compounds	Tannin compound	Flavonoid compounds	Sterol compound	Alkaloid compound
result	+	+	+	+	+

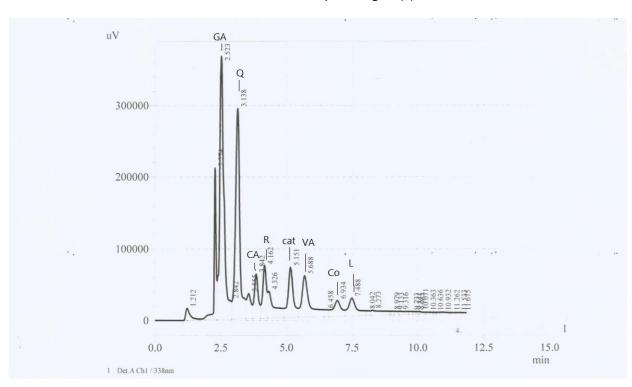
Analysis: Result of HPLC

The figure 1,2 represent the result of HPLC $\,$ and table 2 give the retention time of standards and compound the present in crude extract at PH $_3$





HPLC of standard compound Figure (1)



HPLC OF extract inpH3

Table 2: Comparation between retention

compound	Retention time of standard	Retention time in polyphenol extraction at pH3		
Gallic acid 2.527		2.523		
Quercetin	3.088	3.138		
Cinnamic acid	3.765	3.842		



Rutin	4.074	4.162
Catechin	5.012	5.151
Vanillic acid	5.517	5.688
Coumarin	6.695	6.458
lutein	7.228	7.488

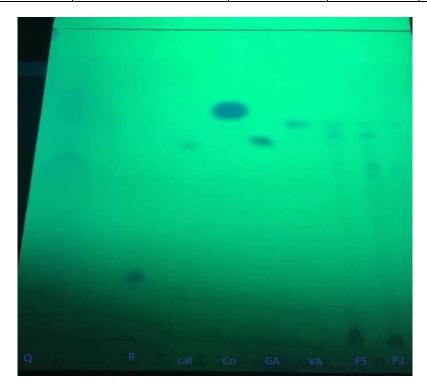
Quantitive concentraction ppm of poly phenolic compounds *in Phoenix dactylifera*L. dried seeds in extract(diethyl ether) at PH3:

Poly phenolic compound	Concentration (ppm)	Area uder the curve	
Gallic acid	1.046	4203643	
quercetin	1.0606	3420202	
Cinnamic acid	1.182	672818	
Rutin	0,8596	761674	
Catechin	1.459	1000158	
Vanillic acid	1.689	1216061	
coumarin	0,477	110319	
lutein	1,38	584802	

TLC Analysis: result

 R_f VALUE result from TLC Chromatography in S1 development system was show in table4 in three different PH at development solvent system S1.

bioactivecompound	reference standard	PH5	PH3	PH2
catechin	0.63	0.613	0.613	0.606
quercetin	0.57	0.563	0.56	0.56
Gallic acid	0.66	0.652	0.645	0.645
Vanillic acid	0.57	0.563	0.56	0.56



Development solvent systemTHF:toluene :HCOOH :H2O 16:8: :2:1



CONCLUSION

Phytochemical screening of cultivated Iraqi plant used widely in many countries as source of food and traditionalmedicine named data plam was done and the results show the presence of bioactive constituents (sterols, alkaloids, tannin, phenolic compounds and flavonoids) in methanolic extractat PH3of Iraqi: phoenix dactylifera L.dried seeds investigated by chemical tests and chromatographic HPLC analysis was carried out to qualify and quantify eight types of polyphenols (Quercetin, Gallic acid, vanillicacid, cinnamic acid, coumarin, lutein, catechin ,Rutin and) and the results reveal that vanillicacidl had the highest concentration followed by catechin while coumarin had the lowest concentaction

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