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Phytochemical study and evaluation antibacterial activity of flavonoid excerpts: Male spathe of date palm

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ABSTRACT

Date palm (Phoenix dactylifera L.) contains a wide array of flavonoids, but little is known about their antimicrobial effects. In this paper a phytochemical and biological study of flavonoids extracted from the male spathe grown in Touggourt, Algeria at flowering Stage is conducted. Analysis by TLC chromatography of the three flavonoids extracts(diethyl ether, Ethyl-Acetate and n-Butanol) indicate that the n-Butanol phase is rich in flavonoids compounds compared to other fractions. The antimicrobial activity was determined on the strains(Streptococcus sp, Staphylococcus sp, Escherichia coli, Pseudomonas species, Condida) using the disk diffusion method and all samples showed an inhibitory effect on the microorganisms

Key words: *Phoenix dactylifera* L, flavonoids, antimicrobial activity.

INTRODUCTION

In recent years, research efforts are under-way on the possibilities of utilization of natural source of bioactive compounds for the dietary management of certain chronic diseases such as diabetes, obesity, cardiovascular diseases, and cancer[1]. There is an increasing body of evidence that many of the today's diseases are caused by the oxidative stress, which is the result of imbalance between formation and neutralization of reactive free radicals. These free radicals are continuously produced and neutralized in our body so as to maintain the constant internal environment i.e. redox state. These reactive free radicals are generated due to endogenous source for example by-products of normal metabolic processes for ATP production [2]

Date fruits are rich in phenolic compounds possessing antioxidant activity. The pollen grains of date palm have been used in Egyptian local practices to improve fertility in women, and in some locations in Arabia, date pits are roasted and used in lieu of coffee as a hot beverage.

Relatively few pharmacological studies have been conducted on dates. For example, it has been shown that, depending on the type of extract used, date fruit and pit extracts significantly increase or decrease gastrointestinal transit (GIT) in mice [3]

Date fruit is considered as a folk remedy for the treatment of atherosclerosis, hypertension, diabetes and cancer. The fruit pulp is rich in phytochemicals like phenolics, sterols, carotenoids, anthocyanins, procyanidins, and flavonoids [4]

Some flavonoids are more selective towards cancer cells and may have the potential for reducing side-effects compared with other anticancer drugs[5]. The fruit pulp is rich in phytochemicals like phenolics, sterols, carotenoids, anthocyanins, procyanidins, and Flavonoids. Some studies have shown that flavonoids, polyphenols and tannins are able to altering proliferation of cancer cell [5]

The inflorescence of date palm tree, in its early stages of growth is enclosed in a hard covering/envelope known as spathe which splits open as the flowers reach maturation. The spathes are removed during pollination and insemination of date. Spathe has a specific fragrance particularly when it is fresh and is utilized in large scale in production of Tarooneh hydrodistilled water. This water contains volatile components and is widely consumed as a beverage to improve heart functioning in local and traditional health practice. It also possesses analgesic and anti inflammatory effects [4]

MATERIALS AND METHODS

2-1-Plant Material

The spathes of *Phoenix dactylifera* L used for this study were collected in Touggourt south-east of Algeria in Avril 2012 at flowering stage. They were free of physical damage and injury from insects and fungal infection. The dried material was milled and stored in air tight container and kept at 4˚C until further analysis[6]

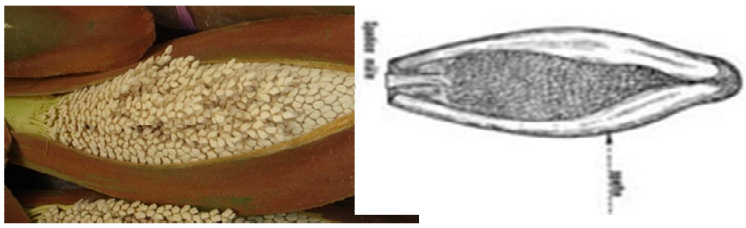

Figure 1: spathes of Phoenix dactylifera L

2-2-Phytochemical Screening

The dry extracts were subjected to various chemical tests in order to detect the presence of different phyto-constituents.

- **flavonoids**:, the addition of KOH (1%) to alcoholic extract led to the formation of yellow color indicating the presence of flavonoids.
- **Phenolic compounds**:5 mL of aqueous filtrate of each plant extract was added to2 drops of 1% of ferric chloride. A blue-green color indicated the presence of phenolic compounds.

2-3-Extraction

Liquid-liquid and solid-liquid extraction are the most commonly used procedures prior to analysis of polyphenolics and simple phenolics in natural plants. They are still the most widely used techniques, mainly because of their ease of use, efficiency, and wide-ranging applicability. Commonly used extraction solvents are alcohols (methanol, ethanol), acetone, diethyl ether, and ethyl acetate[7]

Soxhlet extraction is frequently used to isolate flavonoids from solid samples. In most cases, aqueous methanol or acetonitrile is used as solvent. In the literature, reported extraction times vary up to 12 h using this extraction mode.
Polarity is an important consideration in extraction. Less polar flavonoids (e.g., isoflavones, flavanones, methylated flavones, and flavonols) are extracted with chloroform, dichloromethane, diethyl ether, or ethyl acetate, while flavonoid glycosides and more polar aglycones are extracted with alcohols or alcohol–water mixtures. Glycosides have increased water solubility and aqueous alcoholic solutions are suitable. The bulk of extractions of flavonoid-containing material are still performed by simple direct solvent extraction[8].

Fifteen grams (15 g) of dried male spathe were placed in a soxhlet apparatus with methanol. Extraction was performed with 200mL of an appropriate solvent for 4 h. After extraction, a rotary vacuum evaporator at 40 ˚C was

used in order to remove solvent. In this experiment six solvents of different polarity were used: water, methanol, n-butanol, diethyl Acetate, diethyl ether and petroleum ether[9,10] .

After evaporation the solvent the residue was dissolved in boiling water to obtain an aqueous extract. Petroleum ether fraction was discarded due to its high content in fatty substances.

2-4-Determination of total flavonoids

Flavonoid content in the methanolic extract was determined by aluminum chloride colorimetric method [11] . Briefly, 0.50 mL of methanolic extract was diluted with 1.5 mL of distilled water and 0.50 mL of 10% (w/v). Aluminum Chloride was added along with 0.10 mL of 1 M potassium acetate and2.80 mL of distilled water. This mixture was incubated at room temperature for 30 min. The absorbance of resulting reaction mixture was measured at 430nm UV spectrophotometer (Hitachi U-2001). Quantification of flavonoids was done on the basis of standard curve of quercetin prepared in 80% methanol and results were expressed in milligram quercetin equivalent (QE) per 100 g of spathe. [3,6]

2-5-Antibacterial activity:

2-5-1-Preparation of extract solution:

Test solutions were prepared by dissolving the extracts in DMSO

2-5-2-Antibacterial activity:

- **Bacterial Cultures**:

The bacterial strains used in this study were *Staphylococcus sp and Escherichia coli* obtained in Microbiology laboratory, Touggourt Hospital, Ouargla-Algeria. The bacterial strains were grown and maintained on nutrient agar slants.

- **Screening for antibacterial activity**

The disc diffusion method was used to evaluate the antibacterial activity. Mueller Hinton (Pasteur Institute, Algiers, Algeria) agar was prepared in the plates as the media for the test microorganisms. Sterile filter paper discs (Whatman No. 1) left to dry under the laminar flow cabinet overnight. The bacterial inoculum was spread evenly onto the surface of the Mueller Hinton agar plates using a sterile glass L-form rod before the extract discs were positioned on the inoculated agar surface. Each extract was assayed in triplicate. Sterile distilled water served as negative control. All the plates were incubated for 48 h at 37° C. The antibacterial activity was interpreted from the size of the diameter of zone inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the discs [12].

2-6-TLC analysis

Aliquots of standards and crude extracts were spotted on TLC plate (silica gel). Thereafter; they were kept at 100°C for 30 minutes for activation and were then cooled at room temperature prior to loading of sample and developed in different mobile phases below. Components were visualized under ultraviolet light (254 and 365 nm)and detected by spraying the TLC plates with reagent. Flavonoids were verified in extracts after concomitant running with standards and they were visible as yellow and orange fluorescent spots [13].

RESULTS AND DISCUSSION

3-1-Extraction yield

The extraction yield calculated by the formula

The Percentage extractive value (yield %) = $\left(\frac{Weight\ of\ dry\ extract}{Weight\ taken\ for\ extraction}\right)$ *x 100*

Table 1.Yield and Color of Extracts

Extract	Yields (%)	Color
Overallflavonoid fraction	19.4	brown /redbrick
Diethyl ether fraction	1.74	red glass
Ethyl acetate fraction	4.30	Glass/ brown
Butanolic fraction	9.42	Dark redbrick
Lastwatery fraction	3.91	Light redbrick

3-2-Antimicrobial Activity

The results obtained from antimicrobial assay are presented in Tables 2,3,4,5 According to Table 5. the extracts of spathes showed variable ranges of antimicrobial activities against 5 microbes.

Table 2. Inhibition zone diameter (mm) of Diethyl ether extract(DE=5mg/mL)

Microorganisms	inhibitory concentration				
	DE	DE/2	DE/4	DE/8	DE/16
Escherichia coli	9	7	0	0	0
Staphylococcus aureus	18	16	15	7	6
Pseudomonas aeruginosa	9	8	7	7	0
Streptococcus sp	12	9	9	9	6
Condida	14	10	9	8	0

Table 3. Inhibition zone diameter (mm) of Ethyl acetate extract (EA=5mg/mL)

Microorganisms	inhibitory concentration				
	EA	EA /2	EA /4	EA /8	EA /16
Escherichia coli	12	11	9	8	0
Staphylococcus aureus	20	13	9	9	0
Pseudomonas aeruginosa	12	12	10	6	0
Streptococcus sp	12	10	8	7	6
Condida	10	9	9	8	8

Table 4. Inhibition zone diameter (mm) of n-butanolextract(nB=5mg/mL)

Microorganisms	inhibitory concentration				
	nB	nB/2	nB/4	nB8	nB/16
Escherichia coli	13	12	10	10	9
Staphylococcus aureus	11	11	10	0	0
Pseudomonas aeruginosa	16	11	8	8	0
Streptococcus sp	14	12	11	11	9
Condida	11	10	9	8	8

Table 5 : Minimum inhibition concentration (MIC) of different extracts

Microorganisms	MBC (mg/mL)	Extracts		
		Diethyl ether	Ethyl acetate	n-butanol
Escherichia coli	2	0.1	0.05	0.05
Staphylococcus aureus	2	0.1	0.05	0.05
Pseudomonas aeruginosa	2	-	-	0.5
Streptococcus sp	2	0.1	0.05	0.05
Condida	2	0.06	0.06	0.06

Flavonoids present in plants possess diverse health benefits, which includes antioxidant and radical scavenging activities, reduction of certain chronic diseases, prevention of some cardiovascular disorders and certain kinds of cancerous processes assessed the flavonoid content in the male spathe during the flowering stage [14].

To obtain the active component related to the antibacterial activity, date was extracted with MeOH and fractionated successively with n- butanol, EtOAc and Diethyl ether. Antibacterial activities of these fractions against Microorganisms were tested. The n- butanol fraction showed significant MIC values in comparison with the other fractions.

The extraction yields were 1.74%, 4.30% and 9.42% for Diethyl ether, Ethyl acetate and n-butanol respectively.

3-3-Analysis of Flavonoids TLC Methods

TLC was used for the analysis of the flavonoid exudates. was used for the development of the exudates on silica gel plates[12]. the dry TLC plates were observed under UV light at 365 nm and characterize groups of compounds

Table 6 : Showing the results of the TLC for Diethyl ether extract

Solvent system	Reagents	Number of spots	Rf value	probable compounds [10]
diethyl acetate / formic acid /acetic acid / water (100/26/26/11) v/v	UV254 nm	3	Three graySpots 0.96 ; 0.88 ;0.83	
	UV365 nm	5	0.96 Brown / yellow ; 0.91 Blue 0.88Mauve; 0.85Green 0.83Violet	0.96 Brown / yellow ; quercetin0.91Blue; coumaric acid 0.91Blue; Flavonoïds

Table 7 : Showing the results of the TLC for ethyl acetate extract

Solvent system	Reagents	Number of spots	Rf value	probable compounds [10]
diethyl acetate / formic acid /acetic acid / water (100/26/26/11) v/v	UV254 nm	5	FivegraySpots 0.94; 0.90; 0.80; 0.83; 0.76	
	UV365 nm	8	0.94red glass ; 0.91 Green;0.90 Blue;0.80 gray; 0.83 Violet; 0.81Black ; 0.77Violet; 0.76 Blue	0.90 Blue;coumaric acid 0.75 Violet; flavonoid-glycoside

Table 8 : Showing the results of the TLC for n-butanol extract

Solvent system	Reagents	Number of spots	Rf value	probable compounds [10]
diethyl acetate / formic acid /acetic acid / water (100/26/26/11) v/v	UV254 nm	3	FivegraySpots 0.82; 0.77; 0.46	-
	UV365 nm	6	0.83 Blue ; 0.82 green;0.77 Orange ;0.66 Blue; 0.50 yellow ; 0.46 Orange	0.83 Blue:caffeic acid /chlorogenic acid 0.46 Orange /0.50 yellow;flavonoid-glycoside

Results obtained show that in comparison with Number of spots and Rf value in similar conditions, more than three spots in the chromatograms, These results indicated that the extracts rich by compounds.

CONCLUSION

The flavonoids of spathe allowed the inhibition of some microorganisms that may be causal agents of human urinary, intestinal and respiratory tract infections; indicating that they could be used to cure these diseases, the flavonoid extracts showed the best antibacterial activity and acute toxicity effect which encouraged its use.

Comparatively minor attention has been given to date palm products other than utilization of all parts of the date palm may offer socio-economic benefits to date farmers and other sectors. The results of the present study may highlight the potential importance of spathe of date palm as a product rich in essential flavonoids. Future researches are needed to explore other characteristics of spathe of date palm.

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