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Evaluation of Some Active Components of Palm Leaves (*Phoenix dactylifera*) Ethanol and Aqueous Extracts and Study of Their Antibacterial Effects

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A B S T R A C T

Introduction: Date palm (Phoenix dactylifera L) leaves are a widely available natural resource in Libya. Previous studies have demonstrated that various parts of the date palm (Phoenix dactylifera L) contain bioactive secondary metabolites with potential therapeutic applications, including antibacterial, antioxidant, and anti-inflammatory effects.

Aims: This study investigated the bioactive secondary metabolites present in three commonly cultivated date palm (Phoenix dactylifera L) leaf varieties in Libya: Tabuni, Hamuri, and Ammi. The aim was to conduct a phytochemical screening and evaluate the antibacterial activity of selected Libyan date palm leaf extracts.

Methods: Phytochemical screening was performed to identify the active constituents in the palm leaves. This was followed by quantitative assessment of specific compounds (moisture and Ash content) and evaluation of their biological efficacy against bacterial isolates including Staphylococcus aureus and Escherichia coli using the zone of inhibition method.

Results: Qualitative phytochemical analysis of ethanol and aqueous extracts showed that the leaves are rich in active compounds such as carbohydrates, proteins, phenols, flavonoids, tannins, steroids, terpenes, and terpenoids, while resins and alkaloids were not found in any of the dissolving extracts. The study also found that the leaves contained a moderate percentage of moisture and ash. The highest moisture percentage was recorded in Ammi leaves (9.96%), which is very close to Hamuri leaves (9.03%). On the other hand, the lowest percentage was recorded in Tabuni leaves at 6.45%. The highest ash content was found in Ammi leaves at 5.9% and the lowest in Tabuni leaves at 3.825%. Hamuri leaves had an ash content of 4.7%. The alcoholic extract of palm leaves showed good antibacterial activity against Staphylococcus aureus at all concentrations, with the largest growth inhibitory zones observed at a concentration of 100 mg/ml for all samples. The only exception was related to the Tabuni extract at the concentration of 12.5 mg/ml. In contrast, the results showed insensitivity of Escherichia coli to the extracts of the study.

Conclusion: Palm leaf extracts show antibacterial activity; more studies are needed to quantify this activity. The Hamuri and Ammi extracts demonstrated superior antibacterial activity compared to Tabuni, especially against Staphylococcus aureus, with phytochemical richness varying among varieties. The lack of activity against Escherichia coli is consistent with the greater resistance of Gram-negative bacteria due to their outer membrane structure.

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Keywords: *Date palm leaves, Moisture content, Phytochemicals and Antibacterial Activity*

1. Introduction

The date palm (Phoenix dactylifera L), one of the oldest fruit trees in the Arab world, is widely grown for its edible fruit [1]. In Libya, date palms are grown in the gardens of the southern and northern region for shading fruit plants. Many pharmaceutical companies are interested in developing plant-based treatments, mainly due to the growing belief that Green Medicine is more reliable and safer than expensive synthetic drugs that can have adverse side effects [2]. According to a World Health Organization (WHO) report, eighty percent of the world's population currently receives primary health care through herbal medicine [3]. Of the 25 most popular drugs sold in the world, approximately 42% are natural or derived from herbal products [4]. From ancient times to the present, most peoples and civilizations have relied entirely or largely on herbal therapy. Medicinal plants represent a source of enormous economic value throughout the world. Our country possesses a rich botanical diversity, with a wide variety of plant species growing in different regions. Plant phenolics present in fruits and vegetables have received considerable attention due to their potential antioxidant activity [5]. Crude extracts of palm leaves have been chemically analyzed for the presence of phytochemicals that may be responsible for their medicinal use in traditional medicine to control diabetes, hyperlipidemia and treat bronchopneumonia [6,7]. There have been many studies on palm leaves, especially in Arab countries such as Iraq, Algeria and Saudi Arabia [8-11]. However, this study focuses on three types (Tabuni, Hamuri and Ammi) which are widely used in Libya and have not been previously studied. Specifically, this study aims to evaluate the antibacterial activity of Libyan palm against specific bacterial strains, as well as to determine its antioxidant potential using standardized in vitro assays. By combining phytochemical profiling with biological evaluation, this work seeks to provide scientific evidence supporting the medicinal use of Libyan palm leaves in treating microbial infections.

2. Materials and Methods

2.1. Plant samples collection:

Plant leaf samples (Tabuni, Hamuri, and Ammi) were collected from city of Misurata in northern Libya during the period from November to May (2022-2023). The samples were preserved by [air-drying in the shade / refrigeration at 4°C / immediate processing] until analysis. The plant samples were identified in the Department of Botany, while the bacterial strains were identified in the Department of Microbiology, Faculty of Science, Misurata University.

2.2 Basic preparations:

Leaves were used for each type of palm used in the study. They were cleaned of dirt and insects, then washed well with water and dried in air away from moisture and sunlight at room temperature for 15 days. The plant material was then finely ground into powder using an electric mill (Silver Crest brand, Germany) and stored in dark, tightly sealed bottles away from light, moisture and heat until used. In this study, the soaking (solid-liquid) extraction method was used. Ten grams of dry material was soaked in 100 ml of solvent (water or ethanol) for 72 hours at laboratory temperature with continuous stirring with an electric stirrer. The extracts were then filtered using filter paper. The extracts were collected and the solvents were evaporated on a rotary evaporator at 40°C. The final product obtained was a crude extract, which was stored at a temperature gradient of 7°C until use [12].

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2.3 Phytochemical detection of active components of palm leaves:

Chemical testing of the palm leaf extract was carried out using standard procedures to identify the components as follows:

2.3.1 Tannin Detection:

The extract (0.5 g) was dissolved in 10 ml of distilled water, then the mixture was filtered. Four drops (0.3 ml) of a 1% ferric chloride solution were added to 2 ml of the filtrate. The appearance of a blue-black, green, or blue-green precipitate was considered evidence of the presence of tannins [13].

2.3.2 Steroid Detection:

Acetic acid (2 ml) was added to 0.2 g of the extract and cooled with ice, then concentrated H_2SO_4 was carefully added. A color change from purple to blue or bluish-green indicated the presence of an asteroidal ring [14].

2.3.3 Detection of terpenoids:

A quantity of 100 mg of the extract was dissolved in ethanol, after which 1 ml of acetic anhydride was added, followed by the careful addition of concentrated sulfuric acid. Color change from pink to purple was considered indicative of the presence of terpenoids [14].

2.3.4 Detection of saponins:

One gram of extract was boiled with 5 ml of distilled water and then filtered. Distilled water (3 ml) was added to the filtrate, and the mixture was shaken vigorously for approximately 5 minutes. Subsequently, 5 ml of silver nitrate solution was added to 5 ml of the extract in a test tube, and the tube was placed in a boiling water bath for 5 minutes. The appearance of a silver mirror on the inner walls of the test tube indicated the presence of saponins. In a separate test, 1-3 ml of mercuric chloride solution was added to 5 ml of the extract; the formation of a white precipitate served as a good indicator of the presence of saponins [15].

2.3.5 Detection of flavonoids:

Add 3 ml of extract and a few drops of NaOH solution to the test tube. The formation of an intense yellow color, which became colorless with the addition of a few drops of dilute HCl, indicated the presence of flavonoids [16].

2.3.6 Detection of alkaloids:

Ten grs of the extract were boiled in 50 ml of water acidified with 4% HCl, filtered and 0.5 ml of the supernatant was mixed with Mayer's reagent in a watch glass. A white precipitate indicates the presence of alkaloids [17].

2.3.7 Phenol detection:

To 2 ml of the test solution, 0.5 ml of FeCl3 solution (w/v) was added, the formation of an intense color indicated the presence of phenols [17].

2.3.8 Resin detection

Five mgs of raw extract were dissolved in ml of acetone, treated with 5 ml of 4% hydrochloric acid. The presence of resins is indicated by the appearance of transparent turbidity in the solution [14].

2.3.9 Carbohydrates Molisch Test: One ml of extract, 3 drops of Molisch reagent and 2 ml of H2SO4 concentrate were added to the test

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tube, carefully keeping the test tube slightly bent. The formation of a purple ring at the junction indicated the presence of glycosides [16].

2.3.10 Ninhydrin test:

To 5 ml of extract, 2 drops of freshly prepared 0.2% Ninhydrin reagent were added and heated. The appearance of a blue colour indicates the presence of amino acids [16].

2.3.11 Detection of glycosides

Kilani-Keller test:

A mixture of glacial acetic acid (2 ml) with two drops of a 2% FeCl3 solution is added to 5-2 mg of crude plant extract, then treated with 1 ml of concentrated sulfuric acid H2SO4. A brown ring forms between the layers with the appearance of purple and green solution in the layers, indicating the presence of cardiac glycosides [16].

2.4. Quantitative assessment:

2.4.1 Moisture

Two g were dried at 105°C for 3 hours in an oven until constant weight. After this, it was cooled in a desiccator, cooled in a desiccator-formator for 15 minutes. The percentage of moisture content was calculated using the following equation:

% Moisture = Weight 1-Weight 2/ Weight 1 X 100

Where; Wt1: weight (g) of the plant sample before drying and wt2: weight (g) of the plant sample after drying [18].

2.4.2 Ash content

Exactly 2g of air-dried leaves were placed in a quartz crucible and heated at 550°C for 3 hours in a muffle furnace to constant weight, the percentage of ash content was calculated using the equation:

% Ash = Mass of ash (g) / Mass of dry sample (g) X 100 [18].

2.5 Estimation of biological effectiveness:

2.5.1 Antimicrobial efficacy study:

The effectiveness of plant extracts against some types of pathogenic bacteria (*Staphylococcus aureus, Escherichia coli)* was evaluated to find out their ability to inhibit and stop the growth of these bacteria, all raw alcoholic extracts (ethanol) were prepared by dissolving them in DMSO solution (it does not affect the extracts nor the microbial growth) to obtain the desired concentration in our study (100- 75- 50 – 25- 12.5%). The bacteria suspensions were adjusted compared to a 0.5 MacFarland stander solution and using cotton swabs to spread on Miller Hinton Agar plates. By using sterile flamed cork borer, wells with diameter of 6mm were made in inoculation agar plates. 50µl of the different concentration were transfer into the wells, and the plates were then incubated at 37^0C for 24 hours. The effective of the extract was determined by measuring the diameter of the inhibition zone around the wells compared to DMSO used as negative control [19].

3. Results

As shown in Table 1, ethanol and aqueous extracts of the studied leaves contained flavonoids, phenols, carbohydrates, saponins, proteins, terpenoids, tannins, and glycosides, while resins and alkaloids were absent in all extracts. Crude ethanol and aqueous extracts were

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generally rich in terpenes, tannins, flavonoids, carbohydrates, glycosides, and phenolic compounds. However, the Hamuri ethanol extract showed relatively low phenol content compared to Tabuni and Ammi, which were rich in phenols. Saponins were more abundant in Ammi extracts than in Hamuri. These phytochemical findings confirm the presence of therapeutically important secondary metabolites (flavonoids, phenols, tannins, terpenoids) in the studied leaf extracts.

Table (1): Chemical Compounds in Extracts of Three Plant Species Using Different Solvents.

Functional group	Detector	Hamuri Solvent		Tabuni solvent		Ammi solvent	
		Ethanol	Water	Ethanol	Water	Ethanol	Water
Carbohydrates	Molish	++	+++	+	+++	+++	+++
Squirrels	Ninhydrin	-	+++	-	+++	-	+++
Phenols	Folin-Ciocalteu	+	+++	+++	+++	+++	+++
Tannins	FeCl3	++	+++	+	+++	++	+++
Flavonoids	Alkaline	++	+++	++	+++	+	+++
Alkaloids	Mayer	-	-	-	-	-	-
Saponins	foaming	+	++	++	+++	+++	++
Glycosides	Keller-Killani	+++	+++	+++	+++	++	++
Resins	/	-	-	-	-	-	-
Steroids	Liebermann-Burkhard	+++	+++	+++	+++	+++	+++
Terpenoids	Salkoski	+++	+++	+++	+++	+++	+++

The concentration was indicated as (+++) obvious change, (++) relative change, (+) very slight change, and (-) no change

Moisture content ranged from 6.45% (Tabuni) to 9.96% (Ammi), while ash content ranged from 3.82% (Tabuni) to 5.90% (Ammi). Tabuni consistently demonstrated the lowest values for both parameters, suggesting comparatively lower inorganic residue and better dry stability, whereas Ammi exhibited the highest levels of both moisture and Ash content as shows in Figure 1 and Figure 2.

Figure 1. Percentage (%) of moisture in different palm leaves **Figure 2: Percentage (%) of Ash in different palm leaves**

The results obtained and shown in the table (Table.3) and the following figures (3-7) showed that the extract has an inhibitory effect on bacterial growth. S-aureus and did not show an inhibitory effect on E-coli growth, as the area of inhibition was measured for each type of bacteria.

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Table 3: Inhibitory area against Staphylococcus aureus

The focus(mm) / Type of leaves	Concentration of Staphylococcus aureus				
	100	50	25	12.5	6.25
Hamouri	16	13.5	12.5	10.5	9
Common	15.5	14	12.5	10.5	9.5
Tabuni	12.5	10.5	9.5	-	-

where (-) indicates no inhibition

In Table3 the three extracts showed concentration-dependent antibacterial activity against Staphylococcus aureus, with inhibition zones decreasing as concentrations decreased. Hamuri extract recorded the highest inhibition at 100 concentration (mean 16 mm) and was the only extract that maintained clear inhibition even at the lowest concentration of 6.25 (mean 9 mm), making it the most effective and broadest in range. Common extract showed very similar results to Hamuri at concentrations 50, 25, and 12.5 (13.5, 12.5, and 10.5 mm respectively). At concentration 6.25, it was slightly better than Hamuri (9.5 mm vs. 9 mm). Tabuni extract was clearly the weakest, with the highest inhibition at concentration 100 not exceeding 12.5 mm, and its effect completely disappeared at concentrations of 12.5 and below (no inhibition). Figures 3 and 4 illustrate the concentration-dependent antibacterial activity of Tabuni extract and Common extract, respectively, against *Staphylococcus aureus*.

Figure 4 Zones of inhibition resulting from a range concentrations of common extract showing its antibacterial effect on *staphylococci*

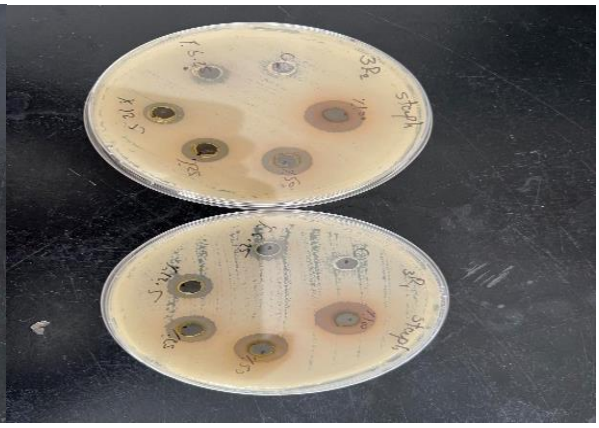

Figure 3 Zones of inhibition resulting from a range of of concentrations of Tabuni extract showing its antibacterial effect on *staphylococci*

Figures 5 and 6 demonstrate the antibacterial activity of Hamuri extract against two different bacterial species: the Gram-positive Staphylococcus aureus and the Gram-negative *Escherichia coli*.

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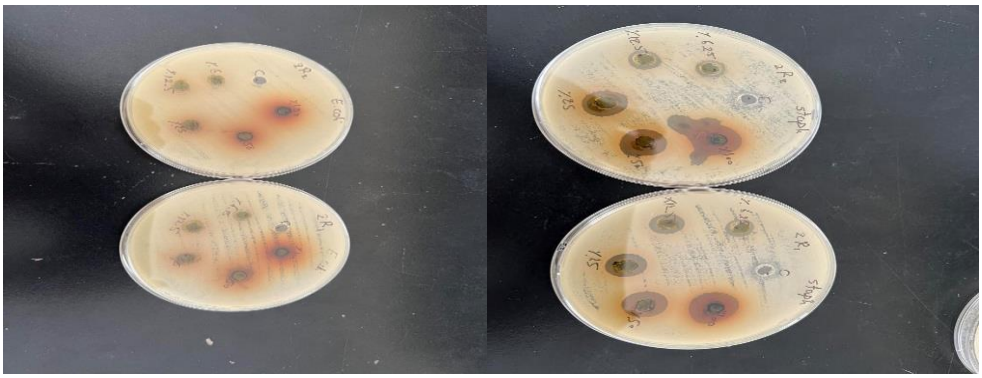

Figure 6: Zones of inhibition resulting from a range of concentrations of Hamuri extract showing antibacterial effect on *Escherichia coli*

Figure 5: Zones of inhibition resulting from a range of concentrations of Hamuri extract showing its its antibacterial effect on *staphylococci*.

Figure 7 illustrates the concentration-dependent antibacterial activity of Common extract and Tabuni extract against the Gram-negative bacterium *Escherichia coli*.

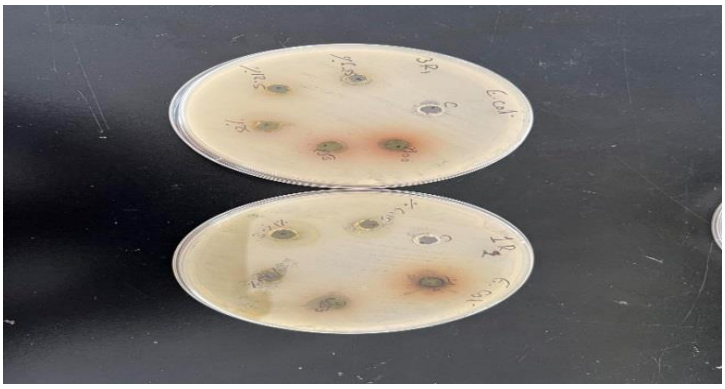

Figure 7: Zones of inhibition resulting from a range of concentrations of common extract ((R3) and Tabuni extract (R1) show antibacterial effect on *E. coli*.

4. Discussion

This study investigates the phytochemical composition and antibacterial activity of different date palm leaf extracts. Understanding these properties is important because date palm leaves are a widely available natural resource that may contain bioactive compounds with potential medical applications, including antioxidant, analgesic, antispasmodic, and bactericidal effects. Identifying which types of leaves possess the most effective antibacterial activity against pathogens such as Staphylococcus aureus can support the development of natural, plant-based therapeutic agents. A group of previous studies have confirmed the presence of various phytochemical compounds in date palm leaves. One study [16] discovered that date palm leaves contain flavonoids, phenols, saponins, and steroids. Another study on the same

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plant [20] showed that three types of palm leaves contain carbohydrates, steroids, and tannins. The present study is consistent with another investigation [21] in confirming that date palms contain flavonoids, phenols, steroids, saponins, and tannins, but disagrees with the presence of alkaloids. An additional study [22] found in palm leaf extracts the presence of various active substances, including tannins, flavonoids, saponins, phenols, carbohydrates, and terpenoids, which clarified the absence of steroids and the presence of alkaloids, differing from the results of this study. Our research has shown that date palm leaves do not contain alkaloids or resins, a difference that may be attributed to the influence of environmental conditions. Tannins, which are a group of phenolic compounds, are known to form irreversible complexes with proline-rich protein [23,24], resulting in inhibition of cellular protein synthesis [25]. Tannins react with proteins to produce a typical tanning effect, which is important for the treatment of inflamed or ulcerated tissues. Herbs containing tannins have an astringent effect and are used to treat intestinal disorders such as diarrhea and dysentery [26] when administered to animals, hence their widespread use in medicine for drug development [26]. They also have analgesic, antispasmodic, and bactericidal effects [27]. In addition, this study showed the presence of flavonoids in the form of phenolic compounds. The main group of compounds that act as primary antioxidants or free radical scavengers used for medical purposes, such as catechol, hydroquinine, and resorcinol, are phenolic salicylates, used as analgesics and antipyretics. Table (2) shows that the highest moisture percentage was recorded at the Aami plant (9.96%), which is very close to the humidity percentage at the Hamuri plant (9.03%). These results are consistent with the moisture percentage obtained in a study [28] which estimated humidity at 9%. They also correspond to the average moisture content reported in another study on areca palm leaves [29] of 9.35%. On the other hand, the lowest percentage was recorded at the Tabuni plant (6.45%), which is similar to the calculated moisture percentage in a study [30] where moisture was recorded at 7%. The highest ash content was found at the Aami plant (5.9%), and the lowest at the Tabuni plant (3.825%). The Hamuri plant had an ash content of 4.7%, which is very close to the value of 4.26% estimated in a study [31]. Another study [32] found lower values, with an ash content of about 2.9%. This difference can be explained by the different environments in which the plants grow.

The effect of the ethanol extract on some types of pathogenic bacteria was studied. The effect of the ethanol extract on some types of pathogenic bacteria was studied. The results obtained and shown in the table (Table.3) and the following figures showed that the extract has an inhibitory effect on bacterial growth. *S. aureus* and did not show an inhibitory effect on *E. coli* growth, as the area of inhibition was measured for each type of bacteria. From the results obtained in the table (Table 3), we note the presence of inhibitory activity of the three extracts against bacteria. *S. aureus* and the lack of inhibition against *E. coli* bacteria, as it was observed after 24 hours of incubation at a temperature of 37 degrees, a variation in the effect of the extracts at different concentrations, and this effect is evident in the appearance of a transparent area surrounded by the pits containing the extracts, which is called the inhibition zone, and this is confirmed by measurements of the diameters of the inhibition zones. The largest inhibition diameter was found in the case of bacteria. *S. aureus* was found in the Hamuri plant (16 mm), followed by the common plant (15.5 mm), then the Tabuni plant (12.5 mm) with the least effect, and did not have any effect on *E. coli* bacteria in any of the three extracts. The results of the current study are also consistent with a study by [22]. In another study conducted by [30]. For the same type of plant, Hamuri showed similar results, and the result was (14.5 mm) for *S. aureus* bacteria and did not show results against *E. coli* bacteria, which is not consistent with this study, which showed inhibition of the same bacteria. The current study is also consistent with the study [33]. In the absence of inhibition of the leaf extract against *E. coli* and also did not agree on the presence of inhibition of *S. aureus* bacteria. The study [34] showed, results are close to the results of the study, as there is an inhibitory effect on bacteria. *S. aureus* leaf extract and its ineffectiveness against *E. coli* of the same plant.

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5. Conclusion

Phytochemical screening of palm leaf extract revealed the presence of alkaloids, flavonoids, phenols, sterols, tannins, terpenoids, as well as carbohydrates and proteins. Moisture and ash were also assessed. The phytochemicals contained in palm leaf extract may be used for pharmacological purposes. Palm leaf extract shows effecacy against Staphylococcus aureus in a dose-dependent manner but ineffective against Escherichia coli, Staphylococcus aureus indicating a selective antibacterial action.

ETHICAL APPROVAL

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

Author A designed the study and performed the phytochemical screening. Author B wrote the manuscript and revised it critically. Author C conducted the antibacterial activity tests and analyzed the data. All authors reviewed and approved the final version.

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