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PHARMACOLOGICAL EVALUATION OF *PHOENIX DACTYLIFERA* L. SEED EXTRACTS REVEALED ANALGESIC, ANTI-INFLAMMATORY AND ANTISPASMODIC ACTIVITIES

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Abstract

This study aimed to explore the pharmacological potential of *Phoenix dactylifera* L. seeds. The acetic acid-induced writhing test was employed to assess analgesic activity, the carrageenin-induced edema test was used to evaluate anti-inflammatory potential, and the charcoal-induced spasm test was conducted to examine antispasmodic activity. The organic fractions of *P dactylifera* L. seeds exhibited significant dose-dependent suppression of pain in the acetic acid-induced writhing test at 150 and 300 mg/kg, surpassing standard medications. The n-hexane partition of the seeds demonstrated a 53.7% reduction in writhing response compared to the traditional medication's 66.67%. The seed's methanolic and chloroform extracts exhibited notable pain suppression (51.8% and 44.4%, respectively). Similarly, the n-hexane partition of commercial seeds displayed the highest percentage inhibition (62.9%), followed by chloroform (58.8%) and methanol (56.48%). In the carrageenin-induced paw edema test, the methanol extract of Ajwa original seeds showed the highest reduction (71%) compared to the standard drug (79.4%) after 3 hours at a dosage of 300 mg/kg. The chloroform and n-hexane extracts also demonstrated significant reductions (64.6% and 62.5%, respectively). The methanol partition of market seeds exhibited the highest reduction (71.4%), followed by n-hexane (59.2%) and chloroform (35.1%). Additionally, the methanol extract of Ajwa original seeds showed the highest inhibitory percentage (65.5%) in the charcoal-induced spasm test at 300 mg/kg, followed by n-hexane (61.8%) and chloroform (49%). In the market seeds, the methanol partition demonstrated the highest inhibition (67.7%), followed by n-hexane (62.5%) and chloroform (52.39%). Phytochemical analysis confirmed the presence of flavonoids, glycosides, sugars, and lipids. The methanol extract contained the highest amount of sugar, while flavonoids and lipids were predominant in the chloroform and n-hexane extracts, respectively. These findings highlight the nutritive and curative properties of Phoenix dactylifera L. seeds as a valuable source of natural products for treating diseases, further study is recommended to isolate potential compounds and check it on clinical trials.

Key words: Anti-inflammatory; Antimicrobial activities; Pharmacology; Phytochemicals; *Phoenix dactylifera*.

Introduction

Throughout human civilization, medicinal plants and their products have played a crucial role in the treatment of various diseases, both internal and external, with minimal or no side effects (Halberstein, 2005). The extensive range of pharmacological activities and structural diversity found in plants has led to their utilization in various medical industries for the isolation and characterization of different compounds (Khan *et al*., 2021; Zhang *et al*., 2023). Medicine, whether natural or synthetic, encompasses any internally administered substance that exerts biochemical or physiological effects on cells, tissues, organs, or the entire body (Raza *et al*., 2022). Pharmacology, as a scientific discipline, is dedicated to the study of the actions of medicines. Natural compounds exhibit both beneficial and adverse effects on living systems, as explored within the field of pharmacology (Zhu *et al*., 2022). As medications constitute a vital component of human disease treatment, pharmacology emerges as a fundamental branch of medicine (Khan *et al*., 2022).

Phoenix dactylifera L. fruit and seeds have been used by Muslims for centuries to heal a variety of diseases, both conventionally and religiously (Parvin *et al*., 2015). Later, as science and technology advanced, examinations on a variety of therapeutic plants gave proof for previously held theories (Marwat & Rehman, 2011). The *Phoenix dactylifera* L. (Ajwa dates) is ones of the Quran's most frequently referenced fruits (Alyahya *et al*., 2022). The date palm is an old plant, used in traditional medicine to cure a variety of illnesses and problems (El Hadrami & Al-Khayri, 2012). It is consumed as principal foods and ingredients that form the basis diet of people in Middle East and the world at large (Al-Juraisy & Al-Saigh, 2010; Friedman *et al*., 2010). It is high in important nutrients, like vitamins, and minerals that are required for development, growth, and overall wellbeing (Al-Farsi *et al*., 2007; Li *et al*., 2023). Date palm fruit is commonly available fruit which is cheap and affordable; being a natural remedy comes with little or no side effect (Rahmani *et al*., 2014).

Bendiab *et al*., (2021) reported date fruit is not only recognized for its nutritional benefits, but for its therapeutic benefits. Algerian dates' pulp, seed, and whole fruit were studied for phenolic components and antioxidant and anti-inflammatory activity. Eltahir *et al*., (2019) studied the preventive properties of the Ajwa dates extract against the diclofenac sodium (DFS)-induced nephrotoxicity. Uddin *et al*., (2020) studied the role of *Phoenix dactylifera* L. in CNS disorders. Maryam *et al*., (2015) reported that the aqueous extract of *P. Dactylifera* L. fruit has anti-inflammatory and analgesic potential. Conducted an activity on rats, concluded that feeding or oral administration of aqueous extract in high doses is more effective in the reduction of pain and inflammation of an acute injury in rats (Luo *et al*., 2022; Feng *et al*., 2022). Ali *et al*., (2016) evaluate the phytochemical and the medicinal properties of dates (*Phoenix dactylifera*). Date fruit's antioxidant, anti-tumor, anti-inflammatory, and anti-diabetic qualities offer therapeutic implications for disease prevention (Ahmad *et al*., 2016). The pharmacological effect of *P. dactylifera* support its traditional use in a variety of diseases, as well as its importance in Islamic teachings, according to the study. Dates offer health benefits, and our findings imply that, with the discovery and isolation of active components, dates might be a commercially viable therapy for a variety of ailments, including cardiac, gastrointestinal and neurological problems (Rahmani *et al*., 2014).

El-Mousalam *et al*., (2016) reported that in Type II diabetic rats, the aqueous type and the methanolic extract of the Palm date seeds and fruits protect against the diabetic nephropathy. Investigated the effects of the aqueous extracts of black pepper and the ajwa seed on hyperglycemic rats and found that they had both preventative and ameliorative effects (Alyahya *et al*., 2022). Sumaira *et al*., (2017) studied the pharmacological and nutritional properties of ajwa dates. Ajwa fruit pits are high in dietary fibers, lipids, proteins, and minerals, according to the study. Ajwa dates are eaten not just for their nutritional value, but also for their therapeutic properties against a variety of diseases. Ajwa flesh and its pits are also high in the phenolic and the flavonoids, which provide a varieties of health benefits due to its strong antioxidant characteristics, according to phytochemical study. The purpose of the present study was to investigate different phytochemical, pharmacological and biological bioassays from the *Phoenix dactylifera* L. seeds.

Material and Methods

Plant material: The plant sample Ajwa dates (*Phoenix dactylifera*) is an instinctive plant, cultivated in the western Saudi Arabian region of Al-Madinah. Ajwa dates seeds powder were obtained from commercially available Ajwa seeds powder and fresh Ajwa dates fruit (Fig. 1).

Experimental animals: The experiments were conducted using mixed-sex albino mice as the experimental organisms. The mice were procured from the Veterinary Research Institute in Peshawar, Khyber Pakhtunkhwa, Pakistan. In accordance with biosafety protocols and ethical considerations, prior approval was obtained from the Department's ethical committee before commencing the experimental work and utilizing mice as research subjects.

Preparation of extract: For the extraction process, 20g grinded powder of *Phoenix dactylifera* L. seeds was dissolved in 200 mL (solvent like methanol, n-hexane, and chloroform) and kept in shaker for 72 hours, then filtered through Whitman filter paper, the filtrates were kept in water bath for 4 days at 45ºC for the evaporation to obtain methanol, n-hexane, and chloroformed extracts. When we obtain these extracts then 0.24g extract was dissolved in 10 mL distal water to form 300mg/kg dose and 0.12g dissolved in 10 mL of distal water to form 150mg/kg dose. Methanolic, n-hexane and chloroform doses were prepared by those manners. Then these solvents doses were used for pharmacological activities (Dirar *et al*., 2019).

Fig. 1. The *Phoenix dactylifera* L. (Ajwa)'s dry seeds (A) and powder (B).

Pharmacological activities

Evaluation of analgesic activity: The acetic acid-induced writhing test was used to measure the analgesic activity. For low or high analgesic potential, the results of groups 3rd, 4th, 5th, 6th, and up to 14th were compared to group 2nd (standard drug). The percentage inhibition (%) is calculated using the formula below (Dirar *et al*., 2019).

$$\% \text{ Inhibition} = \frac{\text{Number of writhes (control)} - \text{Number of writing in test drug}}{\text{Number of writing in test drug}} \times 100$$

Anti-inflammatory activity: To evaluate the anti-inflammatory action by the fractions of *Phoenix dactylifera* L. (Ajwa dates) seeds. The anti-inflammation potential of standard drugs (Diclofenac sodium) was compared tofor reduction of paw edema in mice. The percent inhibition (%) is determined using the formula below (Dirar *et al*., 2019; Mansourabadi *et al*., 2016).

$$\% \text{ Reduction} = \frac{\text{Vt} - \text{Vn}}{\text{Vt} - \text{Vo}} \times 100$$

where,
VT (volume after inflammation induction);
Vn (volume after 1, 2 and 3 hours);
Vo (Normal paw volume).

Antispasmodic activity: Following the standard protocol of (Hussain *et al*., 2017) kept the mice in average environmental conditions then divided them into test, positive, and control groups to evaluate the anti-spasmodic efficacy of *Phoenix dactylifera* L. The plant extracts (methanol, n-hexane and chloroform extract) solutions of original and market available seeds were given to mice groups at 150mg/kg and 300mg/kg, respectively.

The mice were given a 10% deactivated charcoal solution orally and the drugs were immediately given orally. After 1 hour of dosing, all the animals in each group were dissected and the intestine was cut out, and the charcoal travelled from the stomach to the rectum was measured and compared to the standard, that's atropine for low or high antispasmodic potential.

$$\text{The intestinal transit (\%)} = D/L \times 100.$$

As D (the charcoal meal length) and L (total intestinal length) in cm.

Phytochemical analysis

Quantitative analysis

Determination of total flavonoids content: Total flavonoids in plant extracts were screened using a colorimetric approach based on aluminum chloride reagent (Daffodil *et al*., 2013).

Determination of total lipid content: Total lipid contents of plant extract were determined according to the procedure adopted by (Vogel & Bonner, 1956).

Determination of total sugar content: Methanol and n-hexane extracts were used for detection of total sugar (Wildman & Parkinson, 1979).

Antioxidant Activity (DDPH Assay): Antioxidant properties of plant extracts (methanolic, chloroform, and n-hexane) were investigated using the stable DPPH technique. Check the OD (spectrometry) at 517 nm, using the ethanol as a blank. The following formula was used to calculate the percent inhibitions of the test model's radical scavenging activity (Najafian & Moradi, 2017).

$$\% \text{ Inhibition of DPPH Assay calculated by} = A\text{-}A0/A \times 100$$

where the A stands for Absorbance of the blank sample and the 'A0' stands Absorbance of the tested sample.

Statistical analysis

In each test, three different replicates were used. The mean (±) and standard error of the mean (S.E.M., n = number of experiment) are used to express the data. The data was evaluated using a one-way ANOVA (mean ± SEM and mean ± SD) and formerly subjected to the (Duncan test), with $p<0.05$ value found statistically significant.

Results

Ajwa date proximate analysis: In Ajwa's Dates a proximate analysis there are some variation patterns between Ajwa original seeds powder (Ajwa-O is evident for fat, fibers, protein, ash, moisture and carbohydrates values (Table 1). Ajwa-O seeds have higher moisture, ash and fiber contents, than Ajwa-M seeds, however Ajwa-M seeds have higher fats, protein and carbohydrate contents. Similarly, compared to Ajwa-M, the fat content of 1.98 %, the Fat content of Ajwa-O is (1.72%). Ajwa-O seeds have a higher moisture content (9.02%) than Ajwa-M seeds (8.84%). Ajwa-O seeds had a greater Ash content (1.29%) than Ajwa-M seeds (1.09%). Ajwa-O seeds have a lower carbohydrate content (83.11%) than Ajwa-M seeds (88.13 %). In Ajwa-O has a fiber content of 48.02%, whereas Ajwa-M has a fiber content of 45.32%. Ajwa-M has a high protein content of 1.85% then Ajwa-O seeds (1.58%).

Table 1. Proximate composition in the two variety Ajwa.

Proximate analysis (%)	Ajwa-O	Ajwa-M
Fat	1.72 %	1.98 %
Ash	1.29 %	1.09 %
Moisture	9.02 %	8.84 %
Carbohydrates	83.11 %	88.13 %
Fiber	48.02 %	45.32 %
Protein	1.58 %	1.85 %

Elemental analysis of Ajwa seeds: In Ajwa seeds various elements contents were checked (Table 2). It was noted that iron (Fe), calcium (Ca), sodium (Na), magnesium (Mg), sodium (Na), phosphorus (P), calcium (Ca), potassium (K) and manganese (Mn) were found in varied amounts in the original seeds, compared to other elements, K (210.4 mg/50 g) had the highest concentration, followed by Mg (33.6 mg/50 g) and P (71.98 mg/50 g).

Just like the original seeds, the marketable Ajwa seed powder has the highest concentration of K, P and Mg with 501.97mg/50g, 79.05 mg/50g and 57.05mg/50g, respectively. Calcium concentrations in Ajwa original seeds (Ajwa-O) grinded with mechanical grinder were higher (102.27 mg/50g) compared to Ca concentration in commercial Ajwa seed powder. However, the concentrations of Na were also significantly higher (23.3 mg/50 g) compared to commercial seed powder (15.54 mg/50 g).

In commercially available powder, magnesium 57.05 mg/50 g, phosphorous 79.05 mg/50 g, potassium 501.97 mg/50 g and the manganese 0.69 mg/50 g were identified in higher concentration, but (Fe), (Ca) and (Na) were found in high quantity in Ajwa powder made by mechanical grinder.

Antibacterial activity of Ajwa seeds: Ajwa seeds extract showed significant antibacterial activity against gram positive and negative bacterial strains. Among all microorganisms examined, *S. Aureus* growth was inhibited significantly (19 mm), followed by *E. Faecalis* and *K. Pneumoniae* with 16 and 16 mm inihibtion, respectively at 80 mg/mL (Table 3).

Pharmacological study

Analgesic activity of Ajwa seeds 1: At a dose of 300 mg/kg, the number of writhing was significantly reduced in the methanol, n-hexane and chloroform fractions (13±0.7, 12.5±0.5 and 15±0.4). At a dosage of 150 mg/kg, the methanol and chloroform fractions both showed a considerable reduction in writhing (14.25±0.6, and 15±0.3 respectively).

The inhibition (%) of the n-hexane fraction at a dose of 300mg/kg was greater (53.7%) when it is compared with the other fraction (51.8%, 44.4%) methanol and chloroform (Fig. 2). These percentage inhibitions were compared to aspirin (standard drugs) with 66.67% inhibition which is not equivalent but comparable. The significant inhibition were found in all the dose as compared to standard drugs.

Analgesic activity of Ajwa seeds 2: An analgesic activity was assessed using the acetic acid induced writhing method. At a dose of 300 mg/kg, the number of writhing was significantly reduced in the n-hexane, methanol, and chloroform fractions (10±0.12, 11.75±2.2, and 11.1±0.8), respectively. At a dose of 150 mg/kg, the number of writhing was significantly reduced in the chloroform, n-hexane, and methanol fractions (12.3±0.9, 14±0.32and 12.25±1.4) respectively (Fig. 3). When compared to the other fractions (58.8 % and 56.48 %) chloroform and methanol, the inhibition percentage of the n-hexane fraction at a dose of 300mg/kg was greater (62.9 %). This inhibition was compared to the standard drug (aspirin) which inhibits at 66.67%, which is not equivalent but comparable. The significant inhibition was found in all dose as compared to standard drugs.

Table 2. Showing the analysis of elements present in Ajwa seeds powder.

Element	Ajwa (O) mg/50g	Ajwa (M) mg/50g
Mg	33.6	57.05
P	71.98	79.05
K	210.4	501.97
Mn	0.67	0.69
Ca	102.27	32.505
Na	23.3	15.54

Table 3. In seed extract presence, a zone of inhibition exists against bacterial strains.

Test organisms	Seed extract (The diameter of the Zone of Inhibition in mm)	
	For 40 mg/mL	For 80mg/mL
S. Aureus	13	19
E. Faecalis	11	16
K. Pneumoniae	12	16
E. coli	10	15
P. Aeruginosa	9	14

Anti-inflammatory activity of Ajwa seeds 1: After 1hr the sample administration the paw edema was observed (1.39±0.13, 1.29±0.05, and 1.35±0.8), after 2hrs of sample administration the paw edema was observed (1.35±0.11, 1.21±0.09 and 1.26±0.2), and after 3hrs the paw edema was noticed (1.29±0.07, 1.18±0.10and 1.23±0.1) at a dosage of 150mg/kg in methanol, n-hexane and chloroform fraction correspondingly (Table 4**)**. After 1hr of sample administration the significant inhibition of paw edema was noticed (1.29±0.10, 1.33±0.04, and 1.44±0.4), the paw edema noticed after 2 hours of sample administration. (1.25±0.11, 1.23±0.08, and 1.39±0.4), and after 3hrs of drug administration the paw edema was observed (1.24±0.03, 1.22±0.06and 1.21±0.2) at a dose 0f 300mg/kg in methanol, n-hexane and chloroform fraction respectively. After 3 hours at a dose of 300 mg/kg, the methanol fraction showed a highly significant percentage inhibition of 71 percent against carrageenan-induced edoema, followed by chloroform (64.6%) and n-hexane (62.5%). In the Diclofenac sodium-treated group, the percentage of inhibition was determined to be 79.4 percent. The proportion of inhibition of the standard medication is comparable to that of methanol, n-hexane, and chloroform (fractions).

Anti-inflammatory activity of Ajwa seeds 2: The anti-inflammatory efficacy of the different fractions of *Phoenix dactylifera* L. (Ajwa seeds) in mice treated with carrageenan-induced paw edema was highly evident. Paw edema was observed after 1 hour of treatment. (1.27±0.07, 1.24±0.13 and 1.27±0.9), after 2 hours of drug administration the paw edema was observed (1.18±0.06, 1.21±0.08 and 1.25±0.1), and the paw edema was noticed after 3 hours of medication delivery (1.16±0.08, 1.19±0.07 and 1.22±0.6) at a dose of 150mg/kg in methanol, n-hexane and chloroform fractions correspondingly. After 1 hour of delivery of drug, there was a considerable reduction in paw

edema (1.36±.07, 1.23±0.04, and 1.22±0.3), The paw edema was noticed after 2 hours of delivery of drug (1.23±0.08, 1.20±0.09 and 1.21±0.3), and after 3hrs of drug administration the paw edema was observed (1.16±0.05, 1.19 ±0.07 and 1.20±0.5) at a dose 0f 300mg/kg in methanol, n-hexane and chloroform fraction, respectively (Table 5). The methanol fraction showed a highly substantial percentage inhibition of 71 percent against the edoema generated by carrageenan after 3 hours at a dose of 300mg/kg, followed by n-hexane 59.2% and chloroform 35 percent. The proportion of inhibition in the Diclofenac sodium-treated group was determined to be 78.7%. The inhibition percentage of the standard drug is comparable to that of the (methanol, n-hexane, and chloroform) fraction.

Antispasmodic activity (Ajwa seeds 1): After 15 minutes of oral delivery of extract at dosage of 150 and 300 mg/kg, there was a significant increase in charcoal flow. All of the mice were sacrificed and the results were recorded. At a dose of 150 mg/kg, methanol, n-hexane and chloroform fractions significantly increased charcoal flow (27±1.9, 23±2.0 and 23±0.1), respectively. While at a dose of 300 mg/kg, the methanol, n-hexane and chloroform fractions showed a highly substantial increase in charcoal flow (33.25±2.0, 32±0.2, and 25±0.4), respectively (Fig. 4). At a dose of 300 mg/kg, the methanol fraction had the highest inhibition percentage (65.5%), followed by the n-hexane chloroform fraction (61.8%) and (49%). The percentage inhibition of all fractions was comparable to that of the standard the atropine sulphate, which had a percentage inhibition of 77.75%.

Antispasmodic activity (Ajwa seeds 2): At a dose of 150 mg/kg, the methanol, n-hexane, and chloroform fractions significantly increased charcoal flow (21±1, 24±0.3, and 23±0.2), respectively. Similarly, at a dose of 300 mg/kg, the methanol, n-hexane and chloroform partition showed significant increase in charcoal flow (34±0.3, 35±0.1, and 24.1±0.1), respectively. Among all, methanol partition had the highest inhibition percentage (67.7%) at 300mg/kg, followed by for the n-hexane chloroform partitions with 62.5% and 52.39%, inhibition (Fig. 5). The percentage inhibition of all fractions were comparable to that of the standard (atropine sulphate), which had a inhibition of 77.75 %.

Phytochemical investigation

Quantitative phytochemical analysis

Quantitative phytochemical analysis of total flavonoid contents: A spectrophotometric approach was used to evaluate the quantity of the flavonoids in various plant extracts of the species *Phoenix dactylifera* L. It was noted that chloroform had the highest concentration of flavonoids, followed by n-hexane, while methanol had the lowest concentration (Fig. 6).

Quantitative phytochemical analysis of total lipid contents: Using a spectrophotometric technique, the content of lipids in *Phoenix dactylifera* L. plant extracts was measured (Fig. 7). Maximum lipid concentration was found in chloroform, followed by n-hexane and methanol extract, with the lowest lipid concentration found in methanol extract.

Quantitative phytochemical analysis of total sugar contents: Using a spectrophotometric approach, the concentration of total sugar in several plant extracts of the species *Phoenix dactylifera* L. was measured. Among all, methanol extract had the highest sugar concentration, followed by n-hexane, while chloroform had the lowest concentration (Fig. 8).

Table 4. % Inhibition of anti-inflammatory activity of different fraction of *Phoenix dactylifera* L. (Ajwa seeds 1).

Treatments	Concentration	Reduction (%)		
		1hr	2hrs	3hrs
Control	10 mL	…	…	…
Standard/Diclofenac sodium	10mL	29.7%	49%	79.4%
Methanol	150 mg/kg	20%	31.4%	48.5%
Methanol	300 mg/kg	40%	66%	71%
n-hexane	150 mg/kg	34.6%	51%	57%
n-hexane	300 mg/kg	20.8%	58.3%	62.5%
Chloroform	150 mg/kg	11.6%	32.5%	39.5%
Chloroform	300 mg/kg	29.2%	36.9%	64.6%

Here, 1hr (= 1 hour), 2hrs (2 hours) and 3hrs (= 3 hours)

Table 5. Percent inhibition of Anti-inflammatory activity of different fraction of *Phoenix dactylifera* L. (Ajwa seeds 2).

Treatments	Concentration	%Reduction		
		1hr	2hrs	3hrs
Control	10 mL	…	…	…
Standard (Diclofenic sodium)	10 mL	29.7%	48.9%	78.7%
Methanol	150 mg/kg	24.3%	48.6%	54.05%
Methanol	300 mg/kg	23.8%	54.7%	71.4%
n-hexane	150 mg/kg	24.2%	33.3%	39.4%
n-hexane	300 mg/kg	44.4%	55.5%	59.2%
Chloroform	150 mg/kg	12.8%	18%	25.6%
Chloroform	300 mg/kg	29.7%	32.4%	35.1%

Here, 1hr (= 1 hour), 2hrs (2 hours) and 3hrs (= 3 hours)

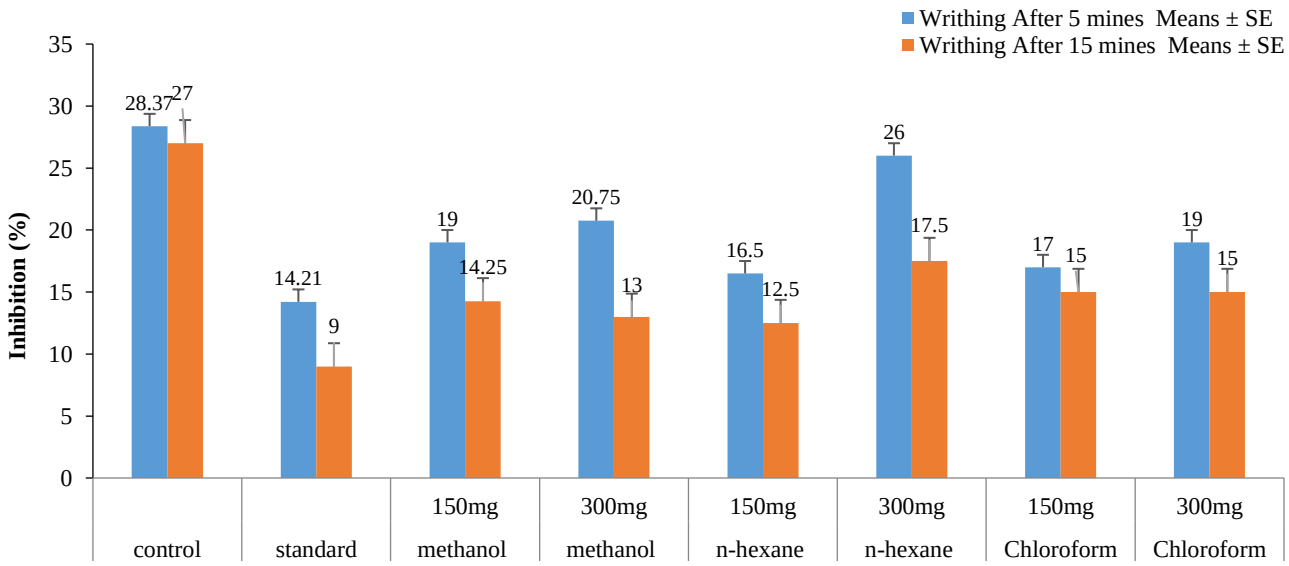

Fig. 2. Shows the decreasing of writhing graphically in different (fraction) of Ajwa seeds.

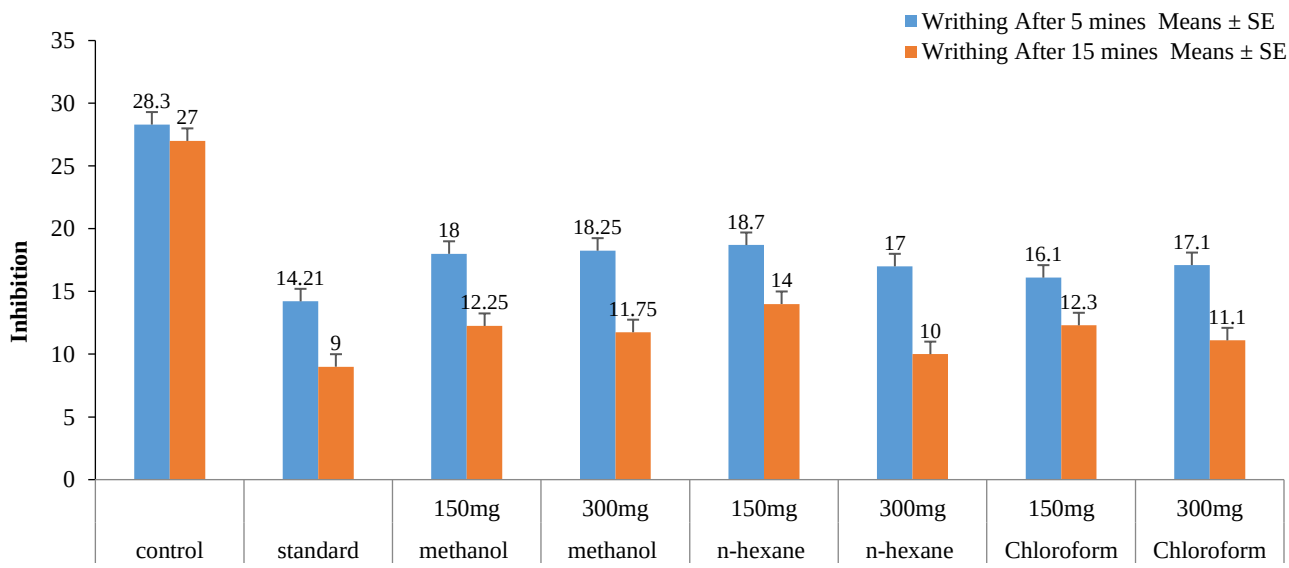

Fig. 3. Graph Showing the decreasing of writhing graphically in different fractions of *Phoenix dactylifera* L. (Ajwa seeds).

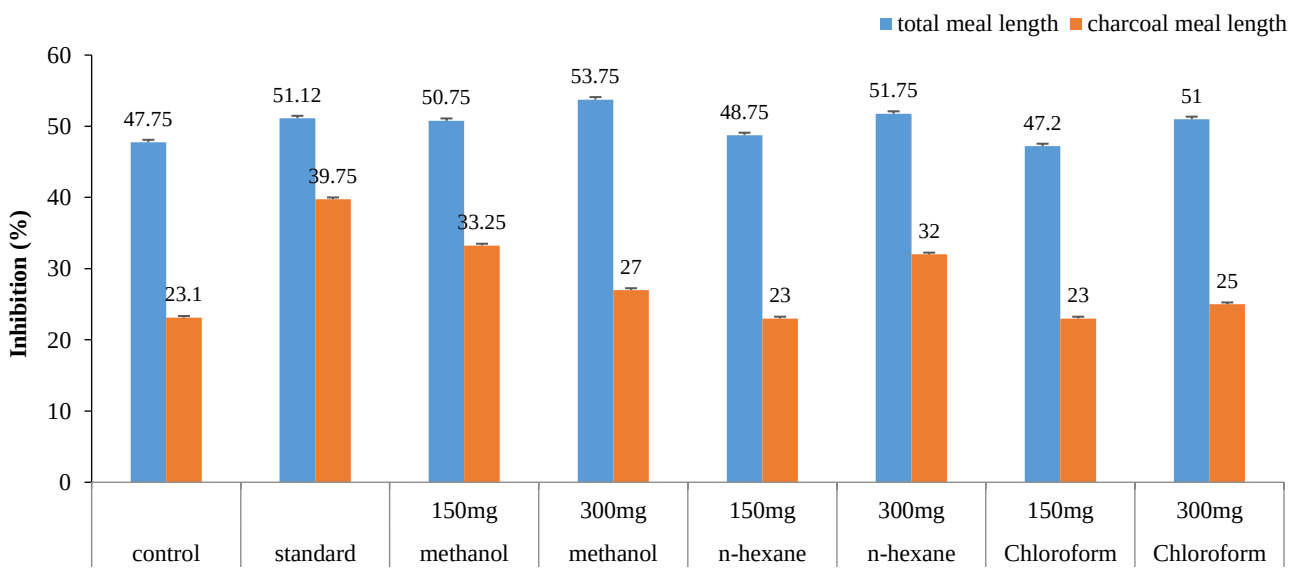

Fig. 4. Antispasmodic activity of different partition phases of Ajwa seeds 1.

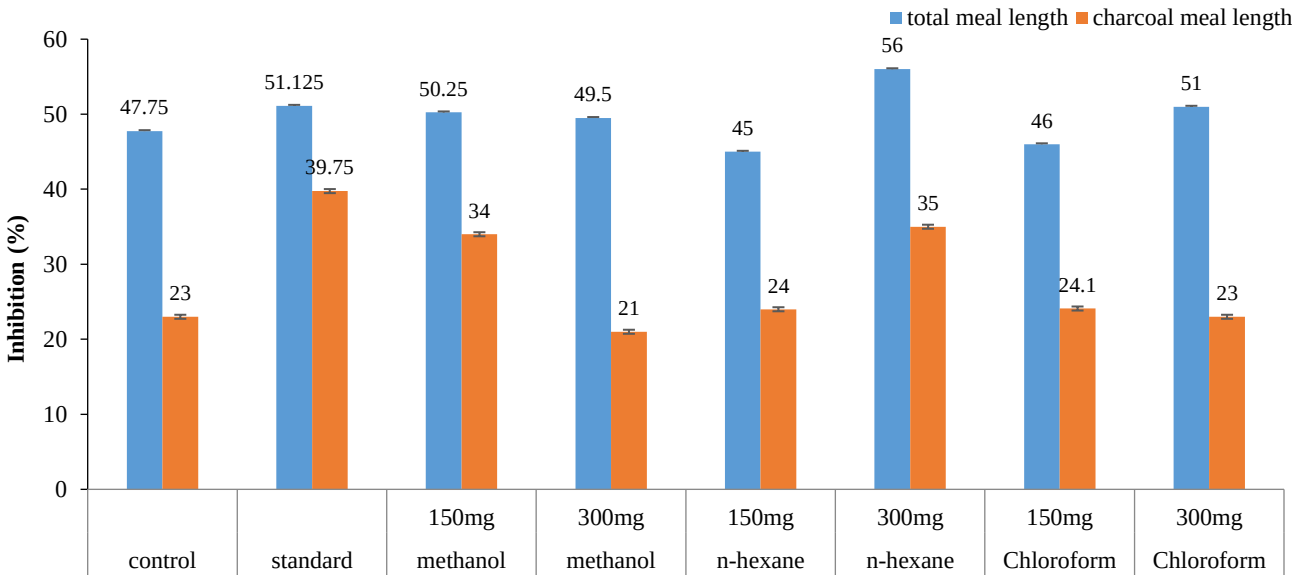

Fig. 5. Antispasmodic activity of different partition phases of Ajwa seeds.

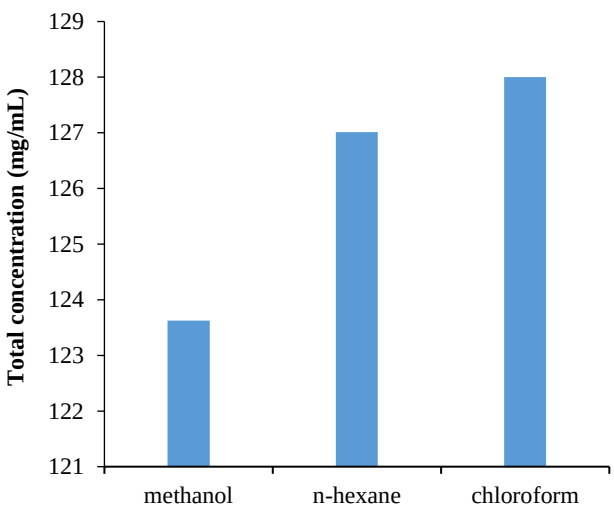

Fig. 6. Total flavonoid contents in the different partition phases of *Phoenix dactylifera* L.

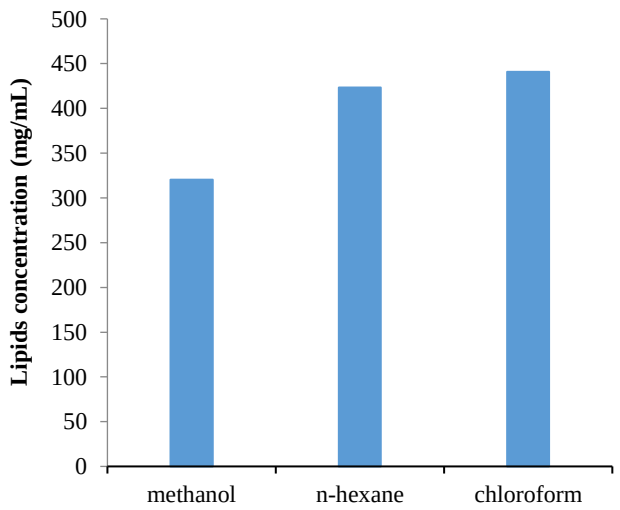

Fig. 7. The total lipid contents in different partition phases of *Phoenix dactylifera* L.

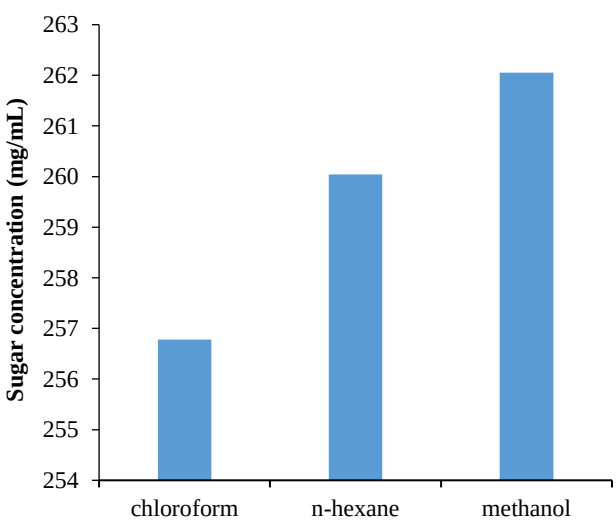

Fig. 8. The total sugar content in the different partition phases of *Phoenix dactylifera* L.

Discussion

The pharmacological activities like the analgesic, the anti-inflammatory, and the antispasmodic and the phytochemical analysis of methanol, n-hexane and chloroform fractions of *Phoenix dactylifera* L. (Ajwa) seeds were investigated in this study. Ajwa dates original seeds powder was obtained from fresh Ajwa dates fruit brought from Madina and commercially available Ajwa seeds powder were bought from local market. Potassium, magnesium, and calcium are all essential components of the human cardiovascular system. These might be a realistic reason for people in different regions of the world consuming Ajwa seed powder and using it to treat cardiovascular problems. Syed *et al.*, (2021) found that Ajwa dates can help lower blood pressure by regulating endothelial activities. As we learn more about the mechanisms of action of Ajwa dates seed powder, it becomes increasingly important to understand the components.

A sensitive procedure for evaluating peripherally acting analgesics is acetic acid produce abdominal constriction reaction (Ghebreselassie *et al*., 2011; Qiu *et al*., 2018). At an amount of 300 mg/kg, the analgesic activity of writhing model induced by acetic acid, n-hexane, methanol, and chloroform extract of Ajwa (original seeds) exhibited considerably reduced writhing (12.5±0.5, 13±0.7, and 15±0.4) accordingly. When it is compared to the reference medicine (aspirin), which stopped 66.67 percent of writhing produced by acetic acid, n-hexane showed highest percentage inhibition of writhing, 53.7% at a dose of 300 mg/kg. Similarly at a dosage of 300 mg/kg, the n-hexane, methanol, and chloroform fractions of Ajwa (market seeds) showed considerably reduced writhing (10±0.12, 11.75±2.2, and 11.1±0.8) respectively. Then-hexane showed the highest percentage inhibition of writhing at 62.9% at a dosage of 300 mg/kg when related to the conventional painkiller aspirin, which stopped 66.67 percent of the writhing. The acetic acid-activate writhing response test is a commonly used to assess the peripheral analgesic activity of any part in an animal model, with acetic acid serving as the primary pain inducer (Klöck *et al*., 2009; Wang *et al*., 2023).

The higher dose test medication's total central analgesic efficacy is comparable to that of the conventional medicine aspirin. High concentrations of flavonoid compound present in greater doses prevented the release and synthesis of PGA (prostaglandins) mediated effect (Wang *et al*., 2010; Wang *et al*., 2017; Wu *et al*., 2023). When compared to standard (66.67%) the organic fraction of *Phoenix dactylifera* L. demonstrated considerable dose-dependent inhibition. As a result, it's probable that the fractions of *Phoenix dactylifera* L. have an analgesic effect through these mechanisms, though the actual mechanism of action has yet to be discovered.

When tissue is exposed to trauma or injury, inflammation is a common phenomenon (Uhegbu *et al*., 2011). Paw edema caused by carrageenan was employed as an anti-inflammatory model to investigate the drug's anti-inflammatory effects (El-Shenawy *et al*., 2002; Liu *et al*., 2022). In carrageenan induce paw edema different fractions of Ajwa (original seeds) methanol, n-hexane, and chloroform at a dose of 300 mg/kg showed maximum inhibition of (71%), (62.5%), and (64.6%) with the value of (1.24±0.03, 1.22±0.06 and 1.21±0.2 respectively), when it is compared to the commercial drug (diclofenac sodium) which showed (79.4%) inhibition. The Carrageenan is a substance which causes the production of serotonin, histamine, and kinins, as well as bradykinins, which cause inflammation. Flavonoids, which have an anti-inflammatory action, are responsible for the inhibition of these substances (Mahajan *et al*., 2009). While the different fraction of Ajwa (market seeds powder) methanol, n-hexane, and chloroform at a dose of 300 mg/kg showed maximum inhibition of (71.4%), (59.2%), and (35.1%) with the value of (1.16±0.05, 1.19 ±0.07 and 1.20±0.5) correspondingly, when comparing to the conventional medicine (diclofenac sodium), which exhibited inhibition of (79.4%).

A charcoal meal test in mice was used to assess the antispasmodic effect. Different partitions of the *Phoenix dactylifera* L. seeds were more efficient in controlling the intestinal motility. However, fifteen minutes of oral administration of the medication at a dose of 300 mg/kg, a significant increase in charcoal flow was noticed. Similarly, at a dose of 300 mg/kg, the n-hexane, methanol and chloroform fractions all showed a significant increase in charcoal flow (32±0.2, 33.25±2.0 and 25±0.4, respectively). Interestingly, at300 mg/kg, the methanol fraction showed the highest inhibition (65.5%) compared to n-hexane (61.8%) and chloroform fraction (49%) correspondingly.

The inhibition (%) of all partitions were equivalent, but not similar, to that of the standard medication atropine sulphate, which inhibited 77.75 percent. Charcoal decreases K^+ channel function while raising intracellular Ca^{2+} contents, which are important for contractile responses. Smooth muscle contraction is caused by increased K^+ efflux (Ghayur *et al*., 2005; Zheng *et al*., 2023). Contractile responses are caused by smooth muscle contraction induced by a rise in intracellular Ca^{2+} concentrations, and *S. Paniculata'*s antispasmodic impact promoted calcium channel blockage or K^+ channel opening (Qadir, 2015). Increasing Ca^{2+} concentrations and lowering the K^+ channel induced increased gastrointestinal motility in mice. The current study shows that using a dose following approach, *Phoenix dactylifera* L. (Ajwa) seeds, both original (brought from Madina) and market seeds (commercially available), can increase K^+ concentrations while reducing the Ca^{2+} channel. All of these studies show that *Phoenix dactylifera* L. (Ajwa) seeds are a rich source of novel spasmolytic medicines that can also be employed for antispasmodic action.

Many plants' the biological activity is influenced by secondary metabolites such as alkaloids, saponin, glycosides, terpenoids, and tannins (Zhang *et al*., 2021; Ortega-Lozano *et al*., 2023). Phenols and flavonoids are medicinally active antioxidant chemicals. Insecticidal, antifungal, antibacterial, and anti-constipative actions are among the most well-known therapeutic properties of phytochemicals (Shah & Yadav, 2015). The medicinal properties of plants were revealed as a result of the existence of specific phytochemical ingredients. As a result of the phytochemicals found in the test plant, it can be concluded that these plants are good source of phytoconstituents, can be used for the creation of new drugs.

Phosphorus' presence highlighted its relevance in daily living, particularly for the heart. While potassium is usually believed to be a significant element due to antihypertensive effect (Yamada & Inaba, 2021). Calcium is an essential mineral for life and an integral part of a balanced diet. Magnesium also helps to regulate the blood pressure, relax the blood vessels, and produce energy (Chen *et al*., 2020). According to the findings, (Ajwa) seed powder, either the original seeds taken from the ajwa fruit or commercially available seeds powder, all contain the essential elements responsible for treating conditions such as congestive heart failure, hypertension, and cardiac arrhythmias (Chen *et al*., 2020).

Furthermore, date seed extract has been shown to protect against reactive oxygen species damage. Antioxidants found in extract may be responsible for the inhibitory activity against several reactive oxygen species (Anwar *et al*., 2021; Lin *et al*., 2023). The Ajwa seed

extract has a strong antibacterial action against gram-negative bacteria and gram-positive, according to our findings. Antioxidant enzymes are a key defense mechanism against the pathology of a variety of diseases. Glycation, on the other hand, may inhibit these enzymes (Anwar *et al*., 2014).

Date seed is thought to have a wide range of nutrients. As only the flesh of the date fruit is edible, just the flesh is eaten, and the seed half is thrown. However, date seed is claimed to be even healthier, with high levels of minerals, healthy the fatty acids, and a high energy value (Idowu *et al*., 2020).

Conclusion

This study proved the analgesic, the anti-inflammatory, and the antispasmodic potential of *Phoenix dactylifera* L. (Ajwa) seeds, both Ajwa original (seeds derived from fresh Ajwa fruit brought from Madina) and Ajwa Market (bought from local market). Because of the differing grinding techniques, they have different textures. But the results of the original and commercially available seeds powder (chloroform, n-hexane, and methanolic fractions) are chemically comparable, there is little difference between them. It reduces the pain in acetic acid-induce writhing, carrageenan-induced edema, while the charcoal-induced spasms. At doses of 150 mg/kg and 300 mg/kg, the organic fraction of *Phoenix dactylifera* L. were found to be more effective in the management of pain, edema, and antispasmodic agents. As a result, the current study scientifically validated and supported its usage in ethnomedicine. Furthermore, the presence of phytochemicals in medicinal plants is influenced by biological processes. As different secondary metabolites were found in distinct organic fractions of *Phoenix dactylifera* L. seeds, it is recommended that the phytoconstituent be analyzed using HPLC and LCMS.

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