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Effectiveness of Antimicrobials Extracted from Date Kernel Powders on Molds, Yeast and Bacterial Growth

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ABSTRACT

Background: Antimicrobials are agents that kill or prevent the growth of microorganisms like fungi, bacteria, viruses, or parasites. These agents (bioactive compounds) are obtained from plants. Incorporated into foods to extend their shelf life by inhibiting the effectiveness corruption microbe.

Methods: Antimicrobials were extracted from date kernel powder by soaking them in cold water, hot water and ethanol alcohol for 24 and 48 h. The effectiveness of antimicrobials against the growth of a number of microbes was evaluated by measuring the inhibition zone diameter (IZD) and minimum inhibitory concentration of antimicrobials (MIC) by using Well diffusion method.

Result: Antimicrobial extracts by ethanol showed high inhibition with an average inhibition zone diameter of 12.35 mm, which is higher than the inhibition zone diameter resulting from antimicrobials extracted by hot water (6.03 mm) and cold (2.74 mm). The minimum inhibitory concentration (MIC) of the produced antimicrobials that was able to inhibit the growth of *Klebsiella pneumoniae*, *Staphylococcus aureus and Fusarium oxysporum* was 10 mg/mL and the corresponding inhibition zone diameters were 10.46, 10.03 and 11.00 mm, respectively. The growth of *Candida albicans* and *Aspergillus niger* was inhibited at concentrations of 2.5 and 5 mg/mL respectively, with inhibition zones ranging from 6.92 to 11.00 mm.

Key words: Date kernel powder, Inhibition zone diameter, Minimum inhibitory concentration.

INTRODUCTION

Microbial food spoilage occurs as a result of bacteria, yeast and mold growth in foods. It is a source of concern for public health and safety officials (Aar-Abbas and Halkman, 2004), which is one of the greatest challenges in food preservation because of health risks and economic losses (Nasar-Abbas and Halkman, 2004). To overcome concerns about the harmful effects of these microbes on health. Chemical agents were used as industrial preservatives and it resulted in the development of resistant microbial strains, accumulation of toxic chemical residues in food and adverse or allergic reactions in consumers Ashraf *et al*. (2018). So many studies have focused extracting antimicrobials from safe, healthy, biodegradable,and effective natural sources that extend food shelf life (Manso *et al*., 2022). including alkaloids, flavonoids, phenols, tannins, terpenes, saponins and polyamines, displaying strong antifungal and antibacterial activities (Veg *et al*., 2009). Alsoufi and Aziz (2026) as studied by (Djahida and Houcin, 2021) antimicrobial properties extracted from *Thapsia garganica*.

Antimicrobial refers to any naturally occurring or synthetic chemical substance or agent produced or derived from plants or microorganisms that can kill bacteria or inhibit their growth and reproduction. Bialonska *et al*. (2010) by preventing bacteria from building their cell walls or other cell components Nasar-Abbas and Halkman (2004) also stop microbial reproduction by inhibiting the production of essential protein for the replication process Studies have also shown that kernels contain a variety of chemical compounds believed to possess antimicrobial, antioxidant, antifungal and antibacterial properties (Saleem *et al*., 2009).

This study adopted the use of date pit seeds as a primary source for extracting antimicrobials, which are a major source of environmental pollution when burned.

Therefore, the study aimed to achieve the following:

- Evaluating the effectiveness of plant extracts as a natural means of combating microbial growth.
- Studying the effect of the extraction solvent type on the effectiveness of the antimicrobial extracts.
- Evaluating the effect of soaking time and solution type on the quantity and effectiveness of microbial extracts against microbial growth.

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MATERIALS AND METHODS

Experimental design

The experiment was designed to determine the effect of the type of extraction solvent and the soaking time of date kernel powder on inhibiting the growth of different strains of microorganisms. 48 samples were collected to measure the minimum concentration of antimicrobials that inhibits microbial growth and to measure the diameter of the inhibition zone using a special ruler for these measurementmicrobes were provided by the General Company for Pharmaceutical Industries and laboratory tests were performed on Klebsiella pneumonia (10031 ATCC) American Culture Collection). *Staphylococcus aureus* (29737 ATCC), Candida albicans (1023 ATCC) and Saccharomyces cerevisiae (9763 ATCC*), Aspergillus niger*. (1015 ATCC) and Fusarium spp (76721 ATCC). ATCC: American Type Culture Collection, the world's largest biobank stores and distributes microbial reference strains to ensure consistency of results across laboratories. It is a reference strain, specifically requested by name in international protocols (Cooper and Denny, 1997). (United States Department of Health and Human Services) Control neomycin sulfate (10 mg /ml) for Bacteria Nystatin (100 IU) for Fungi using Dimethyl Sulfoxide (DMSO).

Preparation of date kernel powder

The date kernel are removed from the fruit and then cleaned. Of dirt and dust by water then drying by oven for 3 date 37c then ground into a fine powder, The resulting powder is sealed in containers, often under refrigeration. According (Venkatesan *et al*., 2021; Imran *et al*., 2021).

Antimicrobial extraction

Fifty grams (50 g) of ground date kernel powder were mixed with 100 mL of cold water (5-7°C), hot water (80°C), ethanol (95%) as extraction solvents. The mixtures were placed on a mechanical shaker (Griffin, England) for 24 h and 48 h to assess extraction efficiency.

After shaking, the mixtures were filtered using a vacuum filtration unit. The solvent was evaporated under reduced pressure using a rotary evaporator at 40°C. The resulting crude extracts were weighed calculate extraction efficiency (% yield) using the formula (Venkatesan *et al*., 2021):

$$\text{Extraction efficiency} = \frac{\text{Weight of dried extract}}{\text{Weight of dry kernel powder}} \times 100$$

The dried extracts were stored in airtight containers at 4°C until analysis.

Preparation of crude antimicrobials several concentrations of crude antimicrobials were prepared to determine the lowest concentration that inhibits microbial growth as follows: (1.25, 2.5, 5, 10, 12.5 and 15 mg/mL) of each solvent extract were prepared using DMSO as a diluent the following concentrations were prepared (Gajic *et al*., 2022).

Antimicrobial activity assay

The effectiveness of extracted antimicrobials is affected by the type of extraction solvent, the concentration of the antimicrobials and the type of plant- (AL-Saadi, 2016). Effectiveness was assessed by determining the minimum inhibitory concentration (MIC) and the average diameter of the inhibition zone. using the well diffusion method, Mueller-Hinton agar (for bacteria) or Sabouraud Dextrose agar (for fungi) were used to inoculate sterile Petri dishes with a 1 mL aliquot of saline suspension containing roughly 10 CFU/mL of each microorganism (Coorevits *et al*., 2015). Following even spreading, 100 µL of each extract concentration was added to six wells (6 mm in diameter) that were cut into the agar using a sterile cork borer. DMSO and reference antibiotics (neomycin or nystatin) were present in the control wells. For bacteria, plates were incubated at 37°C for 24 hours and for fungi, at 28°C for 48 hours. A digital caliper was used to measure the inhibition zone diameters (IZD) in) (Ghuman *et al*., 2016; Mulla *et al*., 2016; Wikler *et al*., 2006).

Statistical analysis

Every experiment was carried out in triplicate and the results were presented as mean ± standard deviation). One-way Analysis of Variance (ANOVA) was used in the statistical analysis to identify significant differences between treatments.

RESULTS AND DISCUSSION

The effectiveness of extracted antimicrobials influenced by the type of microbe, the type of plant, the type of organic solvent used in extraction and the duration for soaking the ground raw material in organic solvents (Kothari *et al*., 2019).

It expresses the effectiveness of extracted antimicrobials ted from plants by minimal inhibitory concentration (MIC) and the diameter of zone of inhibition. In general, MIC is considered the most basic laboratory measurement for determining antimicrobial activity against microorganisms.

Effectiveness of extracted antimicrobials against molds growth

The effectiveness of antimicrobial of date kernel extracts against mold growth was evaluated by determining the minimum concentrations of date kernel powder extracts and estimating the average diameter exacted of inhibition formed by the molds. The lowest concentration of date kernel powder extracts that inhibits *Aspergillus* activity after 24 hours of soaking in cold and hot water was 15 mg/ml, with inhibition diameters of 11.8±0.00 mm and 13.00±0.00 mm (Table 1). The results also showed that the minimum concentration of antimicrobial extract of ate kernel powder extracts that inhibits *Aspergillus* activity is 2.5 mg/ml, with an inhibition diameter of 10.0 mm. As for the minimum concentration of alcohol-soaked date kernel

powder extracts that inhibits *Fusarium*, it is 10 mg/ml. With an inhibitory diameter regarding the increased soaking time of 48 hours (Table 2) we observed that the lowest concentration of date kernel powder extracts in cold and hot water and alcohol after 48 hours of soaking showed inhibitory indicators at a concentration of 2.5 mg/ml. inhibitory diameter of 0.92 mm was observed. The concentration increased to 15 mg/ml and the inhibitory diameter to 12.46 mm for cold water and 12 mm for the extract. The ethanol extracts were more effective in inhibiting *Aspergillus* rot. At a concentration of 15 mg/ml, the inhibitory diameter was 21.0 mm. As for inhibiting *Fusarium* rot, the lowest concentration was 10 mg/ml.

Effectiveness of extracted antimicrobials against bacteria

Antimicrobial extracts were also shown to inhibit bacterial growth, among which were *Staphylococcus aureus* and *Klebsiella pneumonia*e bacteria (Table 3 and 4). On *K. pneumoniae*, ethanol extract had a maximum inhibition zone diameter (IZD) of 14.0±0 mm at 15 mg/mL with a 24-hour soaking duration and with a MIC value set at 10 mg/mL, IZD measured 11.46±0.057 mm. Likewise, ethanol extracts on *S. aureus* had an IZD value of 14.13±0.057 mm at 15 mg/mL. However, at 10 mg/mL MIC value, IZD measured 10.3±0.0 mm. Hot-water extracts were less efficient, with MICs of 12.5 mg/mL *(K*. pneumoniae: IZD 9.5±0 mm), suggesting that ethanol is more efficient as a solvent for extracting antibacterial agents. As evidenced previously, that aqueous extracts remain active at biologically active temperatures as high as 100°C for 30 minutes, it can be suggested that high temperatures do not reduce these active components. The antibacterial property of ethanol extracts remained consistent and had equal efficacy or greater efficacy compared with antibiotic gentamycin sulfate. Even extending the soaking duration for 48 hours did not significantly differ IZD values (Table 4), suggesting that it has more importance to focus on solvent and amount effects compared with duration. These findings support previous research suggesting that ethanol-extracted phytochemicals have more efficacy and at times surpass gentamycin sulfate.

Effectiveness of extracted antimicrobials against yeasts

C. albicans and *S. cerevisiae* are inhibited by date kernel powder ethanol extracts (Table 5 and 6). IZDs rose with extract concentration after 24 hours of soaking. For instance, ethanol extracts at 15 mg/ml generated an IZD of 14.7±0 mm against S. cerevisiae and 14.53±0.0 mm against *C. albicans*. Alcohol is a better solvent for extracting

Table 1: Evaluation of the effectiveness of antimicrobials extracted of soaked ground date kernels for 24 hour on mold growth.

Type of microbe	Concentration (mg/ml)	Mean inhibition zone diameter (mm)+S D\solvent			P value	F value	Control
		Ethanol	Hot water	Cold water			
Aspergillus	2.5	10.0±0.0	0.0±0.0	0.0±0.0		0.018	12
	5	12.0±0.0	0.0±0.0	0.0±0.		0.018	
	10	14.0±0.0	0.0±0.0	0.0±0.0			
	12.5	17.0±0.0	0.0±0.0	0.0±0.0		0.018	
	15	20.0±0.0	13.0±0.0	11.8±0.0			
Fusarium mold	10	9.0±0.0	0.0±0.0	0.0±0.0			
	12.5	11.0±0.0	9.73±0.057	0.0±0.0		0.02	
	15	13.0±0.0	12.0±0.0	0.0±0.0			14

The numbers represent the mean of three readings bacterial growth.

Table 2: Evaluation of the effectiveness of antimicrobials extracted from ground kernels soaked in hot/cold water for 48 hr on inhibition of mold growth.

Types of microbe	Concentration	Mean inhibition diameter (mm)+S D/solvent			C	P value	F value
		Ethanol	Hot water	Cold water			
Asperglius	2.15	6.92±8.0	0. 0±0.0	0,0±0.0	12	0.05	0.018
	5	9.3±0.8	0.0±00	0.00±0.0			0.020
	10	16.0±0.0	0.0±0.0	0.0±0.0			
	12.5	19.0±0.0	0.0.0±0.0	0.0±0.0			
	15	21.0±0.00	12.0±0.0	12.46±0.057			0.020
Fusarium mold	10	10.0±0.80	0.0±0.0	0.0±0,0	14	0.05	
	12.5	13.0±0.7	11.0±0.00	0.0±0.0			
	15	15.0±0.6	14.0±0.00	13.46±0.06			

The numbers represent the mean of three readings bacterial growth.

bioactive substances like phenolics and flavonoids, as evidenced by the lower effectiveness of water extracts (Table 5). The inhibitory effects against *C. albicans* gradually increased at 48 hours (Table 6) and at the highest concentration of 15 mg/ml ethanol extract, the zone of inhibition reached 0.0±16.7 mm. The inhibition zones for *S. cerevisia*e grew, reaching 0.057±15.86 mm at 15 mg/ml ethanol. Ethanol extracts consistently showed the highest inhibition, followed by hot water, while cold water extracts were least effective. Extended soaking enhanced antimicrobial activity due to increased extraction of bioactive compounds.

The effectiveness of antimicrobials extracted from plants, such as ground dates, is affected by the type of microbe, extraction solvent and temperature of the extraction solvent (Kothari *et al*., 2019).

Also published that the extracts' antimicrobial activity with ethanol is more successful at stopping the microorganisms' growth (Hussain, 2019). This may be attributed to the fact that the alcoholic extracts contain some

Table 3: Evaluation of the effectiveness of extracted antimicrobials from ground date kernels after 24 and 48 hours of soaking.

Type of microbe	Concentration	Mean inhibition zone diameter (mm)			Control	P value	F value
		Ethanol	Hot water	Cold water			
Klebsiella pneumoniae	10	11.46±0.057	0.0±0.0	0.0±0.0			0.02
	12.5	13.13±0.057	9.5±0.0	0.0±0.0			0.02
	15	14.0±0.0	11.23±0.057	0.0±0.0			0.018
Staphylococcus aureus	10	10.3±0.0	0.00±0.00	0.0±0.0	13		0.01
	12.5	11.8±0.0	9.33±0.057	0.0±0.0			0.02
	15	14.13±0.057	11.0±0.0	0.00±0.0			0.02

values are means±SD of triplicate readings.

Table 4: Evaluation of the effectiveness of extracted antimicrobials from ground date kernels after 48 hours of soaked.

Type of microbe	Concentration	Mean inhibition zone diameter (mm)+S D\solvent			Control	P value	falue
		Ethanol	Hot water	Cold water			
Klebsiella pneumoniae	10	10.0±0.0	0.00±0.00	0.00±0.00	14		0.02
	12.5	12.10±0.0	0.00±0.00	0.00±0.00			0.02
	15	13.70±0.0	0.00±0.00	0.00±0.00			0.018
		14.63±0.057	9.16±0.057	0.00±0.00			
		16.10±0.0	11.46±0.057	0.00±0.00			
15		10.10±0.0	0.00±0.00	0.00±0.00			
Staphylococcus aureus	10	12.90±0.0	0.00±0.00	0.00±0.00	13		0.01
	12.5	13.90±0.0	10.0±0.0	0.00±0.00			0.02
	15	16.56±0.0	12.73±0.057	0.00±0.00			0.02

The numbers represent the mean of three readings bacterial growth.

Table 5: Evaluation of the effectiveness of antimicrobials extracts of ground kernels soaked in hot or cold water after 24 hr on yeast growth.

Microbes	Mean inhibition zone diameter (mm±SD)			Cncentration (mg/ml)	P value	F vaule
Candida albicans	11.0±0.00	0.00±0.00	0.00±0.00	5	0.018	
	12.03±0.057	0.00±0.00	0.00±0.00	10	0.021	
	12.9±0.00	9.6±0.00	0.00±0.00	12.5	0.018	
	14.53±0.057	11.9±0.00	0.00±0.00	15	0.02	
Saccharomyces cerevisiae	9.56±0.057	0.00±0.00	0.00±0.00	2.5	0.021	0.005
	10.9±0.057	0.00±0.00	0.00±0.00	5	0.018	
	12.43±0.057	0.00±0.00	0.00±0.00	10	0.021	
	13.53±0.057	9.5±0.00	9.63±0.057	12.5	0.023	
	14.7±0.0	11.8±0.00	12.03±0.00	15	0.02	

The numbers represent the mean of three readings inhibition zone diameter and the standard deviation.

Table 6: Evaluation of the effectiveness antimicrobials extracted of soaked gound kernels after of 48 h of soaking on yeast growth.

p		Contra mean inhibation zon diameter			Con\mg\ml	Microbe
		Ethanol	Hot water	Cold water		
0.018		0.00±9.50	0.±0.0	0.0±0.0	5	Candida albican
0.021		0.11±11.23	0 00±	0.0±0.0	10	
0.023	12	0.057±14.16	0.057±10.03	0.0±9.80	12.5	
0.02		0.00±16.70	0.11±11.76	0.0±10.90	15	
0.0102		5.25±6.06	0.00±0.00	0.0±0.00	2.5	Saccharom yces
2	14	6.06±7.0	0.00±0.00	0.00±0.0	5	cerevisiae
0.430		0.00±12.40	0.00±0.00	0.00±0.00	10	
0.046		0.00±13.90	0.057±9.63	0.057±9.63	12.5	
0.023		0.057±15.86	0.057±11.46	0.00±10.0	15	

The numbers represent the mean of three readings bacteria growth.

chemicals that have biological effects and properties to inhibit microbial cell growth. Also due to the high solubility of phenolic and flavonoid compounds, ethanol extracts demonstrated superior inhibition across molds, bacteria, and yeasts in line with previous reports (Kothari *et al*., 2019).

In line with earlier research on aqueous plant extracts, the finding that hot water extracts were more successful than cold water extracts implies that thermal energy improves the release of active compounds.

This confirms that high temperatures do not destroy the raw compounds extracted from date pits and is consistent with what was published by some studies that the active components of aqueous extracts were not damaged by high temperatures, even when heated to 100°C for 30 minutes. It was published by (Atikya *et al*., 2014). The diameter of the inhibition zone or bacterial sensitivity of the raw extracts of guava leaves in boiling water was 22 mm. It was also noted that the diameter of the inhibition zone was (17 mm) (Nasar-Abbas and Halkman, 2004).

Date kernel powder's potential as a natural antimicrobial agent was confirmed by the MIC values for ethanol extracts, which were either better or equal to those of gentamycin sulfate. *Aspergillus* and *Candida albicans* had the highest IZDs, while molds and yeasts were generally more sensitive than bacteria. Consequently, the findings encourage the use of date kernel extracts in food and pharmaceutical applications as a sustainable and environmentally beneficial substitute for artificial properties.

CONCLUSION

The type of microorganism, the extraction solvent and the soaking time all had a significant impact on the antimicrobial activity of date kernel extracts. *Fusarium oxysporum, Staphylococcus aureus* and *Klebsiella pneumoniae* showed more resistance to the extracts. Ethanol and hot-water extracts demonstrated more potent antimicrobial effects than cold-water extracts. The efficacy of the extracts produced after soaking the date kernels for 24 and 48 hours did not differ significantly. The ethanol extracts were more effective in inhibiting *Aspergillus* rot. At a concentration of 15 mg/ml, the inhibitory diameter was 21.0 mm. As for inhibiting Fusarium rot, the lowest concentration was 10 mg/ml.

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Conflict of interest

They declare no conflicts of interest regarding the publication of this work. No sponsorship or funding had an impact on the study's design, data collection, analysis, publication decision, or manuscript preparation.

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