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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF DATE PALM FRUIT EXTRACTS (PHOENIX DACTYLIFERA L)

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ABSTRACT

Phoenix dactylifera L., also known as date or date palm, a plant flower in the palm family, Arecaceae, cultivated for its edible pleasant fruit. The proximate outcome showed that the date palm fruit has high carbohydrate content (84.6%), followed by crude fibre (8.66%), while the fat content was the lowest (0.21%). The date palm fruit contains high β -Carotene (87.91µg) and lower lycopene (0.006µg). The antimicrobial susceptibility of the test organisms, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus cereus* and *Aspergillus niger* to extracts of date palm fruit revealed that all the microorganisms were susceptible to the extracts.

Keywords: *Phoenix Dactylifera L.* (Date Palm), Antioxidant, Antimicrobial Susceptibility, *Staphylococcus Aureus*, *Escherichia Coli*, *Salmonella Typhi*, *Bacillus Cereus* and *Aspergillus Niger*

1. INTRODUCTION

Phoenix dactylifera L., also known as date or date palm, is a plant flower in the palm family, Arecaceae, cultivated for its edible sweet fruit. *P. dactylifera* is the type species of genus *Phoenix*, which contains 12–19 species of wild date palms, and is the major source of commercial production Rahimi (2015). Date is such a staple food from the Middle East and the Indus Valley, being existing for thousands of years. Archaeological evidence of date cultivation in Arabia from the 6th millennium BC was examined by availability. Patel et al. (2012).

Anmnual global production of dates amounts to a total of 8.5 million metric tons, nations from the Middle East and North Africa seem the largest producers of it. The plant species of *dactylifera* "date-bearing" emanate from the Greek ter *daktylos*, which connotes "date" (also "finger") and *fero*, meaning "I bear". The fruit is known as a date. The fruit's English name (through Old French), as well as the Latin all came from the Greek word for "finger", *dáktulos*, due to elongated shape of the fruit. Fossil revealed how the date palm has been in existence for at least many years Upadhyay et al. (2014). Dates have always included in staple food of the Middle East and the Indus valley for thousands of years. The ancient Hebrew turn the fruit into wine, vinegar, bread, and cakes, fruit stones to is also used to fatten livestock and the wood for making utensils. They are very high in some essential nutrients and have a variety of advantages and usage. Dates have an excellent nutrition profile since they are dried, their calorie content is higher than most fresh fruit Grover & Patni (2013). Dates usually yields various antioxidants that have a number of health benefits to offer, including a reduced risk of several diseases. Antioxidants protect body cells from free radicals, which are not stable molecules that may result in harmful reactions in the body, thereby causing disease. According to Shori (2015), and Savoia (2012), there are numerous varieties of date palm fruit. The botanical name of the date palm, *Phoenix dactylifera L.*, is presumably derived from a Phoenician name "phoenix", which means date palm, and "dactylifera" derived from a Greek word "daktulos" meaning a finger Abreu et al. (2012). The "dactylifera" originates from the Hebrew word "dachel" which describes the fruit's shape. Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. Diseases can spread, directly or indirectly, from one person to another. Infectious diseases is next to leading cause of death world Cassir et al. (2014).

Various plants usually serve as herbal medicine for the treatment of infectious diseases. Plants vary with the respect to their potency for healing diseases and their specificity as antimicrobial agents can be ascertained Odeyemi et al. (2017). Human populations are affected by bacterial and fungal infections due to uncontrolled growth and improper food habits and also there is increase in immune compromised Agyare et al. (2013). Antibiotics are medicines used to prevent or cure microbial infections. Antibiotics have proven to be powerful drugs for the control of infectious diseases and remain one of the most discoveries in modern medicine Hussah (2019). Their extensive and indiscriminate use has, however, imposed a selective pressure upon bacteria, leading to the emergence of antimicrobial resistance.

2. MATERIALS AND METHODS

2.1. STUDY AREA

The fruit of *Phoenix dactylifera* was obtained from Lafenwa market in Abeokuta, Ogun State. Lafenwa is located in Abeokuta, which is the capital city of Ogun State, Nigeria; an approximately 61km / 38m away from other regions.

Figure 1

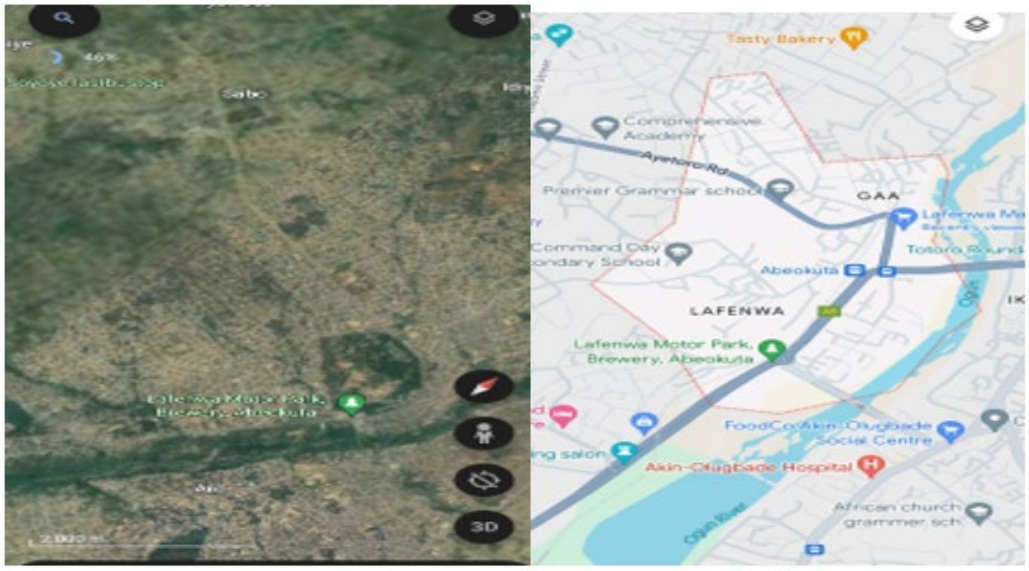

Figure 1 Study Area and Location Map

2.2. SAMPLE PREPARATION AND STORAGE

The fruits of *Phoenix dactylifera* was air-dried for two weeks and pulverised into fine powder in a Marlex electroline 750 watts milling machine. The powder of each were kept in air tight container to retain its potency and avoid loss of odour.

2.3. TEST ISOLATES

The test organisms used for this study were the clinical isolates collected from the Department of Medical Microbiology and Parasitology, Sacred Heart Hospital, Lantoro, Abeokuta. The isolates are *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Salmonella typhi* and *Aspergillus niger*. The collected organisms on sterile agar slant and incubated at 37^{o}C for twenty hours, which were even kept as stock culture on slant in the refrigerator set at 4^{o}C.

2.4. PREPARATION OF FRUIT EXTRACTS

The fruit was rinsed, air dried and grinded into fine powder with an electric blender. Extraction of *Phoenix dactylifera* fruit extraction was done by soxhlet method. Ten gram (10g) each of dry powder of date fruit was added to the sample chamber of the soxhlet apparatus containing 100 mL of water, ethanol, and methanol. The extraction was done for 48 hours till the green colour of the plant materials disappeared after which the extract was collected and stored in airtight bottles and tested for antimicrobial activity.

2.5. METABOLITE CONSTITUENTS OF DATE PALM FRUITS

Proximate analyses were done on date palm fruit pulverised into powder to determine the biochemical properties of the fruit and the effects on some microorganism and ailment.

2.5.1. ESTIMATION OF CRUDE FIBRE

Five grams (5g) of sample was weighed on an analytical balance and transferred to the volumentary flask. One hundred milliliter (100mL) of 1.25% Sulphuric acid was measured and poured into the volumentary flask. The acid with the sample was boiled under reflux for 45minutes. A sieve was used to trap the residue of the boiled sample. The trapped residue was washed in quantifiable proportion of hot water but allowed to drain. The residue above was transferred to the volumentary flask and boiled again with 100millitre of 1.25% sodium hydroxide solution (NaOH) for another 45 minutes. A sieve was again used to trap the residue of the boiled sample. The trapped residue was also washed in several portion of hot water and allowed to drain. The residue was transferred into a weighed crucible where it was transferred into an oven to obtain a constant weight at 105^{o}C for 3 hours. The sample in the crucible was taken into the muffle furnace where it was burnt. The ash left was weighed and the crude fibre was determined, thus;

$$= \frac{W2-W3 \times 100}{W1} \quad (1)$$

W1=Weight of the sample used

W2= Weight of the Crucible + sample after boiling, washing and drying

W3 = Weight of crucible + ash

2.5.2. DETERMINATION OF FAT CONTENT

An empty beaker was weighed on analytical balance and noted. One (1) gram of the sample was weighed into a separating funnel. Twenty (20) milliliter of 96 % ethanol was added into the funnel and shake gently. Allow to cool. Ten milliliter of Concentrated Sulphuric acid (H2SO4) was added. Twenty (20) milliliter of petroleum ether was added to extract and shake well. For emulsion to separate well,20 milliliter of ethanol and 20 milliliter of petroleum ether was added for better extraction as many times as possible. The separated fat extract was decanted. All the extracts were combined and evaporate to dryness. The fat extract was weighed and calculated.

$$= \frac{W2-W1 \times 100}{W3} \quad (2)$$

W= Weight of the sample used

W1= Weight of empty beaker

W2= Weight of empty beaker + fat after evaporation (ADA, 2005)

2.5.3. DETERMINATION PROTEIN CONTENT

One (1) gram of the sample was weighed into a Kjeldahl digestion flask. Fifteen gram (15g) of potassium sulphate and 0.5g of copper II sulphate penthydrate were

added. Thirty milliliter (30mL) of Concentrated Sulphuric acid (H2SO4) was added. The sample was heated in the fume cupboard to digest at 50°C until floating ceased. Then boiled at 80°C until it is cleared. Two hundred (200) milliliter of distilled water and 25mL of Sodium thiosulphate were added and mix. Anti-bumps were added and 50% of 110mL of NaOH was carefully added. The flask was connected to the distillation apparatus and boiled at 80°C. One hundred and fifty (150) milliliter of the distillate was collected. Five (5) drops of methyl red indicator was added to the distillate and titrated with 0.1M of HCl.

$$\%\text{Protein} = \frac{\text{Titer value} \times 0.0014 \times 6.25(\text{Jones conversion Factor}) \times 100}{\text{Weight of sample used (g)}}$$

(ADA, 2005) (3)

2.5.4. DETERMINATION OF MOISTURE CONTENT

Two clean crucibles were dried in an oven and cooled in a dessicator. The two cooled crucibles were weighed and recorded. One gram (1g) of the sample was weighed in duplicate. The crucibles with its contents were transferred into a hot air oven set at 105°C to dry for 3hours. Using a pair of tongs, the crucibles were transferred into a dessicator, allowed to cool, weighed and recorded.

$$\text{Moisture} = \frac{\text{Loss of weight on drying x100}}{\text{Weight of sample used}}$$

(ADA, 2005) (4)

2.5.5. DETERMINATION OF ASH CONTENT

Two clean crucibles were dried in an oven and cooled in a dessicator. One gram (1g) of the sample was weighed in duplicate into the crucibles and recorded. The crucibles with its contents were transferred into a muffle furnace set at 550°C until fully ashed for 5 hours. Using a pair of tongs, the crucibles were transferred into a dessicator, allowed to cool, weighed and recorded.

$$\%\ \text{Ash} = \frac{\text{Loss of weight on ashing x100}}{\text{Weight of sample used}} = \frac{(W2-W3)\ X100}{(W2-W1)} \quad (5)$$

Weight of the empty crucible W1

Weight of crucible + sample before drying W2

Weight of crucible + sample after drying W3

Weight of sample taken W2-W1

Loss of weight on ashing W2-W3 ADA (2005)

2.5.6. DETERMINATION OF CARBOHYDRATE CONTENT

The carbonhydrate content of the sample was determined using this formula:

Total Carbonhydrate= 100-(Weight of Crude fiber +Weight of protein+ Weight of ash+ Weight of moisture+ moisture content Weight of total fat). (6)

Where 100= Total weight of the sample. ADA (2005)

2.6. DETERMINATION OF ANTIMICROBIAL ACTIVITY DATE PALM FRUIT EXTRACT

Phoenix dactylifera (date palm fruit) was examined through agar well diffusion method. The adjustment of microbial cultures was made at 0.5 McFarland turbidity standards and innoculated on Mueller Hinton agar (MHA, oxoid) plate (diameter 9cm) in bacteria and Potato Dextrose agar (PDA) for fungi. The plate was flooded with 1ml of each of the standardized test organism, swirled and excess inoculum was carefully decanted. A sterile cork borer was used to make wells (6mm in diameter) on the agar plates. The extract was reconstituted in 50% dimethyl sulfoxide to give a concentration of 200 mg/ml. The culture plate was inoculated with the test microorganism. Each well was labeled appropriately. Control experiments were also carried out where the holes were filled with 200 mg of Ciprofloxacin (bacteria) and Fluconazole (Fungi) as positive controls. However, each extract was tested in triplicates. These were later left on the bench for one hour to give room to diffusion of the extracts. Thereafter, the plates were incubated at 37 ^{0}C and 28^{0}C for 48 hours for bacteria and fungi respectively. Measurement of the zone of inhibition around each of the wells determined antimicrobial activity for the extract.

2.7. STATISTICAL ANALYSIS

The experiments with samples were performed in three segments and, where applicable; statistical analysis of the data obtained was done using one-way analysis of variance (ANOVA), and the difference between samples were determined by Duncan's multiple range test. The data were expressed through mean which is the product of standard deviation and values, being significantly considered at $P < 0.01$.

3. RESULTS AND DISCUSSION

Table 1 shows the proximate analysis of the date palm fruit that include the moisture content, fat content, ash content, crude fibre, crude protein, carbohydrate. Date palm fruit *Phoenix dactylifera is* high in carbohydrate content with 84.6%, which is source of energy followed by crude fibre that has 8.66%. It has 2.48% crude fibre, 2.27% ash content, 1.78% moisture content, 0.21% fat content.

Table 1

Table 1 Proximate Analysis (%) of Dates Palm Fruit

Parameters	Proximate (%)
Moisture	1.78
Fat	0.21
Ash	2.27

Crude Fibre	8.66
Crude Protein	2.48
Carbohydrate	84.6

Antimicrobial Activities of Date Palm Fruit Extract by Agar Well Diffusion Test.

Table 2 shows the antimicrobial susceptibility *of* the test organisms, *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus cereus* and *Aspergillus niger* to the aqueous, ethanoic extract and control. All the above-mentioned microorganisms were susceptible to the above-mentioned extracts. The individual reactions of the test organisms against each solvent were discussed with their respective mean zone diameter. Table 2, also reveals the inhibition mean zone of the growth of the microorganism to the extracts. Ethanoic in date palm fruit have the highest antibacterial activity against *Staphylococcus aureus* having the mean zone diameter of 28mm followed by Aqueous 21mm and compared with the control of 29mm. Ethanoic in date palm fruit have the highest antibacterial activity against *Escherichia coli* having the mean zone diameter of 29mm followed by the Aqueous of 19mm compared with the control of 28mm. Ethanoic in date palm fruit have the highest antibacterial activity against *Salmonella typhi* having the mean zone diameter of 22mm followed by the Aqueous which is 16mm compared with control of 29mm. Ethanoic in date palm fruit have the highest antibacterial activity against *Bacillus cereus* having the mean zone diameter of 22mm followed by the Aqueous which is 17mm compared with control of 27mm. Ethanoic in date palm fruit have the highest antibacterial activity against *Aspergillus niger* having the mean zone diameter of 29mm followed by the Aqueous which is 22mm compared with the control of 28mm.

Table 2

Table 2 Antibacterial Activity of Date Palm Fruit Extract (Phoenix dactylifera) against some pathogen (mm)

Antimicrobial activity of the extract (mm)			Control
Organisms	Aqueous	Ethanolic	Antibiotics
1. *S. aureus*	21.00	28.00	29.00
2. *E. coli*	19.00	29.00	28.00
3. *S. typhi*	16.00	22.00	29.00
4. *B. cereus*	17.00	22.00	27.00
5. *A. niger*	22.00	29.00	27.00

Control (Antibiotics for Bacteria-Ciprofloxacin, Fungi- Fluconazole)

Minimum Inhibitory Concentration of the Date Palm Fruit (*Phoenix dactylifera)* Extract

Table 3 below shows the results of the Minimum inhibitory concentration of the date palm fruit extract against the test organism and antimicrobial susceptibility of the test organisms, *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus cereus and Aspergillus niger with* aqueous, ethanoic solvent. Result of this study showed that all the microorganisms were susceptible to the above mention extract. Aqueous of date palm fruit extract showed the highest MIC value for *Staphylococcus aureus* having the value of 13mg/mL followed by ethanoic 5mg/mL compared with 5mg/mL obtained from the control at the same concentration. Aqueous of date palm fruit extract showed the highest MIC value for *Escherichia coli* having the value of 19mg/mL followed by ethanoic 3mg/mL compared with 3mg/mL obtained from the

control at the same concentration. Aqueous extract of date palm fruit extract showed the highest MIC value for *Salmonella typhi* having the value of 25mg/mL followed by ethanoic 19mg/mL compared with 2mg/mL obtained from the control at the same concentration. Aqueous of date palm fruit extract showed the highest MIC value for *Bacillus cereus* having the value of 25mg/mL followed by ethanoic of the point 9mg/mL compared with 5mg/mL obtained from the Control at the same concentration. Aqueous *of date palm fruit* extract showed the MIC value for *Aspergillus niger* the value of 6mg/mL followed by ethanoic 3mg/mL compared with 3mg/mL obtained from the control at the same concentration. Minimum Inhibitory Concentration **(**MIC) refers to the minimum concentration for antimicrobial drug that impedes visible growth of microorganism after overnight incubation with media. The small MIC value indicates that less fruit extract (antimicrobial drug) is necessary for inhibiting growth of the organism, therefore, the fruit extract (antimicrobial drug) with lower MIC value are more effective.

Table 3

Table 3 Minimum Inhibitory Concentration Activity of Extracts of Date Palm Fruit (mg/mL)

Extracts		Control	
Organisms	**Aqueous**	**Ethanolic**	**Antibiotics**
S. aureus	13.00	5.00	5.00
E. coli	19.00	3.00	3.00
S. typhi	25.00	19.00	2.00
B. cereus	25.00	9.00	5.00
A. niger	6.00	3.00	3.00

Control (Antibiotics for Bacteria-Ciprofloxacin, Fungi- Fluconazole).

Minimum Bactericidal/Fungicidal Concentration Activity of Extracts of Date Palm Fruit

Table 4 below shows result of the minimum bactericidal/fungicidal concentration (MBC/MFC) of the test organism. *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus cereus* and *Aspergillus niger* with aqueous, ethanoic solvent. Result of this study showed that all the microorganisms were susceptible to the above mention extract. Aqueous of date palm fruit *e*xtract showed the highest MBC value for the *Staphylococcus aureus* with value of 19mg/mL followed by ethanoic 13mg/mL compared with 6mg/ml obtained from the control at the same concentration. Aqueous of date palm fruit extract showed the highest MBC value for the *Escherichia coli* with the value of 13mg/mL followed by ethanoic 5mg/mL compared with 3mg/ml obtained from the Control at the same concentration. Aqueous of date palm fruit extract showed the highest MBC value for the *Salmonella typhi* with the value of 25mg/mL followed by ethanoic 19mg/mL compared with 2mg/mL obtained from the control at the same concentration. Aqueous of date palm fruit extract showed the highest MBC value for *Bacillus cereus* with the value of 25mg/mL followed by the ethanoic 9mg/mL compared with 6mm/mg obtained from the control at the same concentration. Aqueous of date palm fruit extract showed the highest MBC value for the *Aspergillus niger* with the value of 13mg/mL followed by the ethanoic with 9mg/mL compared with 5mg/mL obtained from the control at the same concentration.

Table 4

Table 4 Minimum Bactericidal Concentration of the Extracts of Date Palm Fruit (mg/mL) Against Some Test Microorganism.

Extracts	Control		
Test Organisms	**Aqueous**	**Ethanolic**	**Antibiotics**
S. aureus	19.00	13.00	6.00
E. coli	13.00	5.00	3.00
S. typhi	25.00	19.00	2.00
B. cereus	25.00	9.00	6.00
A. niger	13.00	9.00	5.00

Control (Antibiotics for Bacteria-Ciprofloxacin, Fungi- Fluconazole).

4. CONCLUSION

In this comprehensive study of the antimicrobial activities of date palm extracts, we have uncovered compelling evidence of their potent inhibitory effects against a range of test organisms, including *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus cereus,* and *Aspergillus niger*. Both aqueous and ethanoic extracts of date palm fruit exhibited significant antimicrobial susceptibility, demonstrating their potential as natural agents for combating microbial infections. Ethanoic extracts, in particular, stood out with their remarkable antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, showcasing mean zone diameters of 28mm and 29mm, respectively. This performance surpassed that of the control, underlining the efficacy of date palm fruit extracts in inhibiting bacterial growth. Moreover, the Minimum Inhibitory Concentration (MIC) values further emphasized the superiority of ethanoic extracts, as they exhibited notably lower values compared to the control. These lower MIC values imply that ethanoic extracts require less fruit extract as an antimicrobial agent to hinder microbial growth, suggesting their potential as potent and cost-effective natural alternatives for combating bacterial and fungal infections. The findings provide valuable insights into the antimicrobial potential of date palm extracts, paving the way for further research and potential applications in the development of novel antimicrobial agents and therapeutics.

CONFLICT OF INTERESTS

None.

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