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Phytochemical, Proximate and Antifungal Studies on *Phoenix dactylifera* L.

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Abstract : *Date fruits are one of the very common fruits known to man having a lot of vitamins and minerals necessary for the wellbeing of an individual. In this study, the aqueous, ethanolic, n-hexane and diethylether extracts of date fruits (Phoenix dactylifera L.) obtained from a local market were analyzed for their phytochemical and proximate composition as well as antifungal properties on two different fungi species (Loramyces macrosporus and Aphanomyces laevis). Phytochemical screening showed that there was the presence of alkaloids, flavonoids, tannin, saponin, teterpenoid, steroids and phenols while the proximate analysis showed the abundance of carbohydrate as well as low concentrations of protein, crude fat and moisture. The aqueous extract of the fruit had a higher phytochemical profile than the other extracts hence was used in the antimicrobial studies. Antifungal studies using varying concentrations (200 mg/ml, 400 mg/ml, 1000 mg/ml) of the aqueous extract of the fruit showed that increase in the concentration of the extract, was directly proportional to the increase in zones of inhibition observed in the fungi tested. The extracts of the date fruits showed a correlation between their phytochemical composition and the fungal resistivity. These findings can be seen as additional knowledge on the importance of having dates in our diet and also as a natural antifungal agent for daily use.*

Keywords: *Date fruit, Fungistatic, Medicinal plant, Phytochemistry*

I. Introduction

A wide array of natural foods has been known to exhibit many antimicrobial properties and have been screened and discovered to have many antimicrobial compounds. As industrialisation and technology continues to grow, there is a need to pay a close attention to human health and human how nature's gifts can be used to combat microbes that cause increasing health issues. For centuries, *Phoenix dactylifera* L., commonly known as the date palm, has been cultivated for its fruit by the Arabs although the exact origin has not been verified (Manickavasagan *et al*, 2012). In Nigeria, it is commonly found in the Northern region and sparsely found in the Southern and Western states. Date palm leaves are often used to make mats, baskets, crates and fans for religious purposes. The petiole of the plant is used as a source of cellulose pulp. Mature leaves are used as insulating boards, saddles, ropes, coarse clothes and hats due to their richness in fibre. They also represent a source of fuel and raw material for making fishing implements and objects such as walking sticks and brooms. The fruits are berries with varying shape, size, colour and chemical composition (Zaid and de Wet 2002; Al-Yahyai and Kharusi 2011). They are very sweet, rich in carbohydrates, dietary fibres and certain essential amino acids, vitamins and minerals. Previous studies have shown that the date fruit contains anthocyanins, phenolics, sterols, carotenoids, procyanidins and flavonoids and it is known to show free radical scavenging, antioxidant, antimutagenic, antimicrobial, antidiarrheal, anti-inflammatory, gastroprotective, hepatoprotective, nephroprotective, anticancer, laxative, and immunostimulant activities (Block *et al*, 1992; Tapiero *et al*, 2002; Al-Qarawi et al., 2003; El Hadrami *et al*, 2005; Foroogh *et al*, 2008; Manjeshwar *et al*, 2011). The date fruit has found a niche in science and its experimentation; it is one of the most researched fruits as it has so many properties that make it suitable as not only a delicacy but as a medicine too. In this study, we report the phytochemical and nutritional composition as well as antifungal activity of extracts of date fruit as part of our exploration for new bioactive compounds.

II. Materials And Methods

2.1 Collection of Samples

The Date fruits were bought from one of the local markets in Lagos popularly known as Idi-Araba Market (Latitude 6°31.152'N and Longitude 3°20.932'E). Qualitative characters of the fruit (type, shape and colour) were assessed and described following Radford *et al* (1974).

2.2 Preparation of plant extracts with Distilled Water

Fruits of *Phoenix dactylifera* L. were shade dried for 14 dys and then oven dried at 60° C to a constant weight. The dried samples were ground to powder using, weighed and divided into four parts of 150 g each. Each part

was soaked in water, ethanol, n-hexane and diethylether for a period of 72 h respectively. The extracts were then filtered with What-man's filter paper and the filtrates concentrated at 40° C using a rotary evaporator.

2.3 Phytochemical Analysis of the Plant Extract

The quantitative and qualitative phytochemical analysis of the plant sample was carried out for the presence of anthraquinones, tannins, saponnins, steroids, cardiac glycosides, flavonoids, terpenoids, phenols and alkaloids following Sofowora (1993).

2.3.1 Qualitative Analysis

Test for Tannins: 1 ml of extract added to 1 ml of 3% $FeCl_3$. Greenish-black coloration shows presence of Tannin.

Test for Saponins: 2 g of powdered sample boiled with 20 ml of water for 5 mins at 100° C, filtered. 10ml of the filtrate was added to 5 ml of water and shaken to observe for stable froth, add 3 drops of olive oil and shake vigorously for formation of emulsion.

Test for Terpenoids: 1 ml of extract added to 1 ml of chloroform and few drops of concentrated hydrochloric acid underplayed to form a layer and observed. Brownish coloration indicated presence of Terpenoid.

Test for Steroids: 1 ml of extract added to 1 ml acetic anhydride was added to few drops of sodium hydroxide. Colour change from violet to blue green indicates presence of steroid.

Test for Flavonoids: 1 ml of extract was added to 1 ml of dilute NaOH. Presence of cloudy precipitate shows the presence of flavonoid.

Test for Alkaloids: The powdered sample of the date fruit, 0.2 g was boiled with 2 ml of 2% hydrochloric acid, filter and add 2 drops of Dragendorff. Orange-red precipitate showed the presence of Alkaloid.

Test for Anthraquinones: One ml of extract was added to few drops of 1 M sodium hydroxide, observed. Red coloration shows presence of combined anthraquinone.

Test for Cardiac glycosides: Extract measured at 0.1 ml was added to 1ml of glacial acetic acid and 1 drop of 3% $FeCl_3$ underlayed with 1 ml of concentrated hydrochloric acid was observed. Brown ring at the interphase showed presence of cardiac glycoside.

Test for Phenols: 2 ml of extracts was added to 5 ml of water and warmed between 45-50° C, 2 ml of 3% $FeCl_3$ was added to it and observed. Formation of green or blue colour shows presence of phenol.

2.3.2 Quantitative Analysis

Flavonoids: To 1 ml of the extract, 4 ml of water was added and incubated for 5 mins. 0.3 ml of 5% sodium nitrate and 0.3 ml of 10% aluminum chloride was added to the solution and incubated again for 6 mins. 2 ml of 1 M sodium hydroxide and 2.4 ml of dH_2O was added to the solution and absorbance at 510 against blank Quercetin as standard.

Alkaloids: 20 ml of 10% acetic acid in ethanol was added to 0.5 g of powdered sample and incubated for 4 h. The solution was filtered and filtrate concentrated to reduce to ¼ in a water bath. Concentrated ammonium hydroxide was added, filtered with an already weighed filter paper and rinsed with 1% ammonium hydroxide and dried in an oven. The weight of the filter paper is retaken and recorded.

Tannin: 1 ml of extract, 5 ml of water and 4 drops of tannin reagent were mixed in a cuvette and the absorbance was read at 720 nm.

Saponin: To 1 g of sample, 25 ml of isobutyl alcohol was added, shaken and filtered with filter paper. After 5 mins 10 ml of 40% magnesium carbonate was filtered against 1 ml of the filtrate and 2 ml of 5% $FeCl_3$. The volume was made up to 50 ml with distilled water and incubated for 30 mins. Absorbance was read at 380 nm.

Terpenoids: 20 ml of chloroform methanol was added to 0.5 g of powdered sample, shaken, incubated for15 mins and centrifuged for 15 mins. The supernatant was decanted and rewashed with 20 ml of chloroform methanol and re-centrifuged. The precipitate was dissolved in 40 ml of 10% sodiumdodecyl sulphate solution and 1 ml of 0.01 M iron iii chloride, incubated for 30 mins and absorbance at 510 nm was taken.

Steroids: 3 ml of 0.1 M sodium hydroxide, 2 ml of chloroform, 3 ml of ice cold acetic anhydride and 2 drops of concentrated sulphuric acid was added to 2 ml of extract. Absorbance at 420 nm was taken.

Combined Anthraquinone: 5 ml of aqueous methanol was added to 1 g of sample, incubated for 10 mins and filtered. 1 ml of the extract, 1 ml of 2% 3-5 DNS (Dinitrosalicyclic acid) and 1 ml of 5% sodium hydroxide as boiled for 2 mins at 95° C until brick red precipitate observed (N.B: a change in color from yellow to brick red shows positive presence of combined anthraquinone)

A filter paper was weighed and used to filter the boiled sample then oven dried at 50° C and reweighed.

$$\% \text{ combined anthraquinone} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Weight of sample}} \times 100$$

Cardiac Glycosides: 10 ml of 70% alcohol was added to 1 g of sample, left to sit for 2 h and filtered. 4 ml of 12.5% lead acetate and 8 ml of 4.77% disodium hydrogen phosphate was added to the filtrate and made up to 32 ml with distilled water. 5 ml of Buljets reagent was then added and the absorbance at 495 nm read.
Phenol: One ml of extract had 0.4 ml proline added and left to sit for 5 mins. 4 ml of 75% Na2Co3, 4.6 ml of dH_20 was added and incubated for 1 h 30 mins and the absorbance of 565 nm was taken against the standard prepared at ranges of 0, 50, 100, 150, 200, 250 mg/L.

2.4. Determination of Antimicrobial Activity

2.4.1. Organisms

The microorganisms used in this study consist of two test fungi *Loramyces macrospores* and *Achyla racemosa.*

2.4.2. Media

Sabouraud dextrose agar (SDA) was used in the culturing and antifungal screening of the date fruit extracts. This was prepared according to the manufacturer's instructions and Ampicillin was used as a standard antibacterial agent to avoid the growth of bacteria in the medium.

2.4.3 Antimicrobial Agents

The chemotherapeutic agent used in the test as positive control was Fulcin 10µg/ml.

2.4.4 Antimicrobial Screening of Plant Extracts

This was carried out using the agar diffusion well antifungal assay method. The pure fungi inoculum was uniformly cut out and dissolved in 5 ml of saline solution to release the spores from the fungi culture into the solution. Sterile Petridishes each 90 mm containing SDA were used. The SDA plates had 0.5 ml of the saline solution containing the fungal spores placed in them and spread with a glass spreader for even distribution around the medium. The medium was then bored with a cork borer to make holes of 6 mm in diameter. The experiment had aqueous date fruit extracts of varying concentrations (200 mg/ml, 400 mg/ml and 1000 mg/ml) as the test experiment, the control was divided into Positive control, using 500 g of a known antifungal drug, Fulcin with trade name REFUCIL and negative control using distilled water. The different concentration of extracts, 200 mg/ml, 400 mg/ml and 1000 mg/ml, were taken in 0.5 ml by a plastic dropper and placed in the 6 mm diameter holes. The petridishes were then labeled, sealed and incubated for 72 h at 28°C under aerobic conditions (All manipulations were done in sterile conditions). After 72 h of incubation, inhibition of the fungi zones was obtained by a meter rule as inhibition zone diameter (mm).

2.5. Proximate Analysis

2.5.1. Ash (Blight and Dye Method): 1 g of sample was weighed into a clean oven dried crucible and placed in a blast furnace of 400-600° C for 4 h until whitish grey ash was obtained from the crucible. The crucible was then reweighed.

$$\% \text{ ash} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Weight of sample}} \times 100$$

2.5.2. Moisture (Blight and Dye Method): 1 g of sample was placed in a clean oven dried petridish and kept in an oven for 65° C for 6 h to dry and kept in a desiccator afterwards. The sample was weighed after drying to get the final weight.

$$\%\text{moisture} = 100 - \frac{(\text{Final weight} - \text{Initial weight})}{\text{Weight of sample}} \times 100$$

2.5.3. Carbohydrate Determination: 1 g of sample homogenized in a mortar with 20 ml of 2.5% of sulphuric acid was poured in a boiling tube and boiled for 10 mins. The solution was filtered and filtrate was made up to 250 ml with distilled water. I ml was taken out of the 250 ml of filtrate and added to 9 ml of water to make 10 ml of solution. 1 ml was taken to a clean boiling tube and added 4 ml of 0.1% anthrone reagent and boiled for 10 mins. It was allowed to cool and the absorbance was read at 620 nm.

$$\text{i.e.} \quad \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.5.4. Lipid Determination (Blight and Dye Method)

1 g of sample was weighed and homogenized with chloroform methanol then centrifuged for 10 mins. The beaker was dried in an oven and weighed. The filtrate was decanted and collected and poured into a separating funnel and 10ml of normal saline was added to it. The lower layer of the solution was collected in a beaker and dried in an oven before being weighed.

$$\%\text{lipid} = \frac{\text{Final weight} - \text{weight of beaker}}{\text{Weight of beaker}} \times 100$$

2.5.5. Protein Determination

Digestion: 1 g of sample was added to 1 g of sodium sulphate, 0.1 g of copper sulphate as well as 20 ml of concentrated sulphuric acid and digested in a fume cupboard, slowly heating until digestion becomes clear pale green. This was allowed to cool and the volume of digested solution was adjusted to 250 ml with distilled water. The solution was poured in a bottle and covered.

Distillation: The distillation apparatus was steamed and 10 ml of the digest was added into the apparatus. It was allowed to boil and sodium hydrochloride added so that the ammonia is not lost. 50 ml of 20% Boric acid was added to the distillate containing mixed indicator (methyl red and blue) changes to green.
Titration: The alkaline ammonium was titrated directly with 0.1 M hydrochloric acid. The value, which is the volume of acid used, was recorded.

$$\text{Protein content} = \frac{(t5 - t6)}{1000} \times 0.1 \times 14 \times \frac{200}{30} \times \frac{100 \times 6.25}{\text{weight of sample}}$$

Where:
t5 = titre of sample
t6 = titre of blank

III. Results And Discussion

3.1. Morphology

Interactions with the sellers of the date fruit showed that the fruits were bought from Saudi Arabia due to the fact that the date palm plantations in the north are not enough to cater for the needs of the northerners who relish this fruit talk more of transporting it to other parts of the country. The date fruits bought from the Idi-araba local market is a single, oblong, one seeded berry with a terminal stigma and a fleshy pericarp. The dark brown fruit has an average weight and length of 3.46 g and 2.3 inches respectively. The seed obtained from splitting the fruits into two is oblong, ventrally grooved with a hard endosperm with an average weight of 1.06 g.

3.2. Phytochemistry

Both qualitative and quantitative phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, phenols and combined anthraquinone while cardiac glycosides were absent (Table 1). These phytochemical constituents are also in varying quantities (Fig. 1).

Table 1: Qualitative phytochemical characteristics of *Phoenix dactylifera*

Extracts	Alkaloid	Flavonoid	Tannin	Saponin	Terpernoid	Steroids	Combined Anthraquinone	Phenol	Cardiac Glycoside
AQUEOUS	+	+	+	+	+	+	+	+	-
	+	+	+	+	+	+	+	+	-
ETHANOL	+	+	+	+	+	-	+	+	-
	+	+	+	+	+	-	+	+	-
DIETHYL ETHER	+	+	+	+	+	+	-	+	-
	+	+	+	+	+	+	-	+	-
N-HEXANE	+	+	+	+	+	+	-	-	-
	+	+	+	+	+	+	-	-	-
KEY: + = PRESENT ; - = ABSENT									

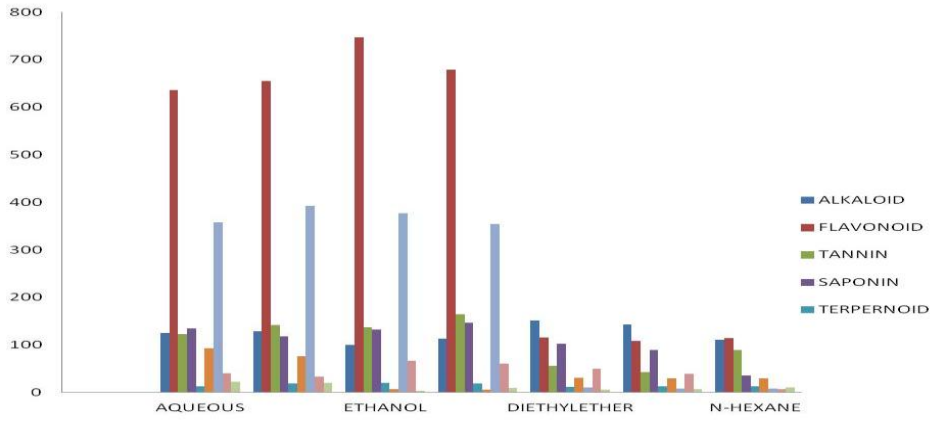

Figure 1: Chart showing the quantitative phytochemical analysis (in grams) of date fruit

3.3. Proximate Analysis

The proximate analysis of the date palm shows us that it is very high in carbon, hydrogen and oxygen (C, H, O) which are the starting block of carbohydrates (71.5%) and contains low amount of proteins and lipids , 4.85% and 2.75% respectively. Moisture on the other hand is at 14.4% which is quite low, this is due to the process of sun drying before transportation of these fruits to different parts of the world to prevent spoilage (Fig 2).

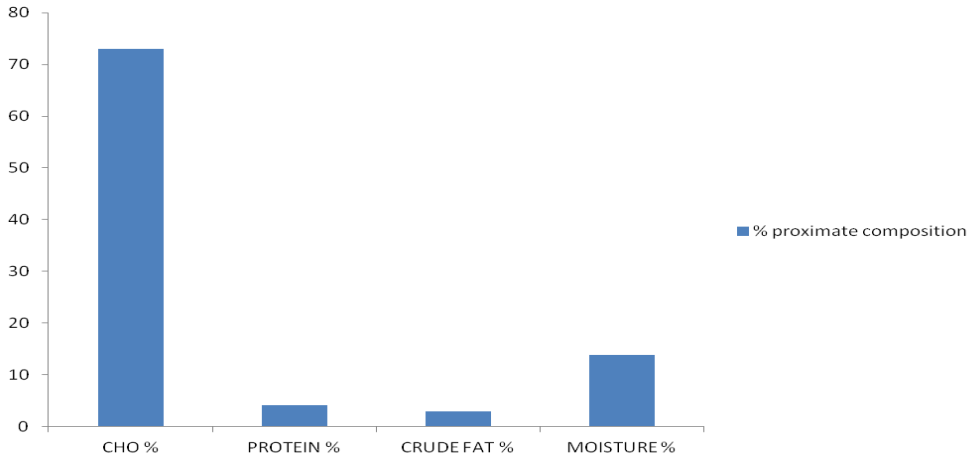

Figure 2: Chart showing the proximate composition (in percentage) of date fruit

3.4 Antifungal Screening

The screening for the antifungal activities of different concentrations, 200 mg/ml, 400 mg/ml and 1000 mg/ml of the aqueous extract of date fruit compared with a standard fungicide, Fulcin showed these results: 11.0 mm ±0.5 (A101) and 10.5 mm ±0.5 (B101) in the 200 mg/ml date aqueous extract concentration, 12.0 mm ±0.5 (A101) and 13.0 mm ±0.5 (B101) in the 400 mg/ml date aqueous extract concentration, 14.0 mm ±0.5 (A101) and 15.0 mm ±0.5 (B101) in the 1000 mg/ml date aqueous extract concentration, no inhibition zones for water which was used as the negative control, inhibition zones of 14.5 mm ±0.5 in (A101) and 15.0 ±0.5 in (B101) for Fulcin which is the positive control (Fig 3).

Figure 3: Antifungal activities of different concentration of aqueous extract of the date fruit in mm.

The phytochemical analysis of this fruit has shown us that it is rich in different bioactive constituents, most especially in a group of plant chemicals known as phenols. These phenols have been found to be toxic and thus inhibit the growth of microorganisms in this case, fungi. The reason why some of these phytochemicals

inhibit fungi growth is not fully known. It is possible that they act as inhibitors for enzyme systems as a result of their structural formulae but other reactions may be involved (Christie, 1965). The antifungal analysis of the date palm showed that increase in the concentration of the extract, was directly proportional to the increase in zones of inhibition of the fungi tested. Phytochemicals exert antimicrobial activity through different mechanisms; tannins for example, act by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes (Scalbert, 1991) in microbial cells. Li *et al.* (2003) reported that tannins have remarkable activity in cancer prevention and anticancer, this suggests that date fruit could be a possible source of important bioactive molecules for the treatment and prevention of cancer. Alkaloids known for their toxicity against cells of foreign organisms was also observed in the date fruit (Ganguly and Sainis, 2001). Saponins, a well reported antimicrobial compound was also recorded.

IV. Conclusion

From the result of this study, it can be concluded that the date fruits are a good source of minerals, and medicinally active constituents and they have good antifungal properties. The date fruit can be used as a natural antifungal agent for various food, fruits and their products. It is hereby recommended as a valuable and healthy food with a lot of health benefits and a viable raw material for the fungicide production industry.

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