ABSTRACT

The phytochemical and anti-nutrient compositions of Phoenix dactylifera fruits commonly sold in Abakaliki, Ebonyi State, Nigeria were carried out using spectrophotometric method. The result of phytochemical analysis revealed the amount of phenols, tannins, flavonoids, terpenoids, alkaloids, steroid and saponins as 4.73 ± 24 mg/100g, 9.42 ± 72 mg/100g, 3.45 ± 39 mg/100g, 8.67 ± 59 mg/100g, 4.88 ± 59 mg/100g, 5.4 ± 43 mg/g and 4.3 ± 43 mg/g respectively in the fruit sample. The amount of oxalate, phytate, haemaglutinin and trypsin inhibitor detected in the fruit sample are 1.32 ± 0.04 mg/100g, 1.32 ± 0.0 mg/100g, 0.85 ± 0.09 Hu/mg, 0.60 ± 0.3 IUI/mg respectively. This shows that the fruit is rich in phytochemicals and this explains the use of this fruit in ethno-medicine for the management of ailment and the analyzed anti-nutrient compositions are below the toxic level which indicates that the fruit does not interfere with the absorption of essential nutrients.

Keywords: Phytochemical, Antinutrient, Phoenix dactylifera and Spectrophotometric method.

INTRODUCTION

Phoenix dactylifera (Date fruit) is one of the oldest cultivated plants and has been used as food for about 6000 years [1]. It is an important food crop in Middle East and is considered to be one of the most important fruit tree particularly in North African, Middle Eastern and Asian countries. The fruit contributes to the economy and social life within these regions [2] and it is considered as a vital component of their diet [3]. Date fruits are well known as a staple nutritious food and source of wealth for many years [4]. Because of its high nutritional value, great yields and its long life, the date palm has been mentioned as the “tree of life” [5]. The fruits of the date palms are
consumed throughout the world. Dates are being consumed in modern cultures for their pleasant flavour, odour and their biting texture in addition to their use for flavouring foods, beverages and medication [6]. Date fruits are considered as major source of carbohydrate which include simple sugars like glucose and fructose and sucrose [7]. They are good sources of dietary fibre and some important minerals which include iron, potassium, selenium, calcium and vitamins and it also contains vitamin C, B1, B2, A, riboflavin and niacin but it is low in fat and protein contents [8].

The protective effects of fruits against chronic diseases are attributed to bioactive non-nutrients called phytochemicals. Phytochemicals have gained increased interest among researchers and clinicians due to their antioxidant activity, cholesterol-lowering properties, and other potential health benefits such as chemoprevention of cancer, prevention of diabetes, and cardiovascular diseases. Anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients [9]. Nutrition studies focuses in food sources and beverages and other varieties of foods, especially grains, beans, legumes and other foods. Fruits and vegetables are consumed because of their high nutrients and antioxidants potential. However, *Phoenix dactylifera* fruit is one of the common fruits consume by Hausas more especially among the Muslim and non-Muslim communities in Abakaliki, Ebonyi State, Nigeria. Therefore, this study was carried out to evaluate phytochemical and anti-nutrient compositions of *Phoenix dactylifera* fruit which is one of the major fruits sold in Hausa quarter in Abakaliki, Ebonyi State, Nigeria.

**MATERIALS AND METHODS**

**Materials**

**Collection and Preparation of *Phoenix dactylifera* Fruits**

The dried fruits of *Phoenix dactylifera* were purchased from Hausa Quarter Abakaliki, Ebonyi State and were identified by a taxonomist in the Department of
Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. A part of the plant was also kept in the herbarium for reference purposes. The fruit of *Phoenix dactylifera* were dried at room temperature and ground to powdered form using electrical grinding machine. The paste sample was then stored in an airtight container and kept in the refrigerator until required.

Figure 1: *Phoenix dactylifera* Fruits

**Chemical and Reagents:** The chemicals and reagents used for the analyses were of analytical grades.

**Methods:** Quantitative Phytochemicals were determined by the following methods:

**Determination of flavonoids:** This was determined by the method of Harborne (1973) [10].

**Principle:** Flavonoids reacts with dilute ammonia (NH3) to produce a coloured complex which can be measured spectrophotometrically at 470nm.

**Procedure:** Exactly 1g of the sample was weighed and macerated in 20mls of ethylacetate. It was filtered using Whatman filter pepper. Five mills of the filtrate was pipetted and added with 5mls of dilute ammonia. After that the upper layer was collected and absorbance was read at 490nm.
Determination of Tannins: This was determined by the method of Harborne (1973) [10].

Tannins reduce phosphotungstomolybdic acid in alkaline solution to produce highly coloured blue solution, the intensity of which is proportional to amount of tannins. The intensity is measured in spectrophotometer at 720nm.

**Procedure:** Exactly 1g of the sample was weighed and macerated in 20mls of distilled water and filtered. Exactly 5mls of the filtrate was pipetted and 0.3ml of 0.1n ferric chloride in 0.1NHCl was added. 0.3ml of 0.0008m potassium ferricyanide was equally added and absorbance at 720nm was measured against the blank.

**Determination of Glycoside**

This was determined by the method of Trease and Evans (1989) [11].

**Procedure:** Exactly 1g of the sample was weighed and macerated in 20mls of distilled water. After macerating, 2.5ml of 15% of lead acetate was added and filtered, then 2.5 mills of chloroform was added and stirred vigorously. The layer was collected and evaporated to dryness. The residue was dissolved with 3mls of glacial acetic acid. 0.1ml of 5% ferric chloride and 0.25mls of concentrated H₂SO₄ was then kept in the dark for 24hours. Absorbance was read at 530nm against the blank.

**Determination of phenol:** This was determined by the method of Harborne (1973) [10]

**Principle:** Phenols react with phosphomolybdic acid in folin-ciocalteau reagent in alkaline medium to produce a blue coloured complex (Molybdenum blue) which can be estimated spectrophotometrically at 650nm.
Procedure: Exactly 1g weighing was of the sample and macerated in 20mls of 80% ethanol and filtered with Whatman filter paper. 5mls of the filterate was pipetted and 0.5mls of folin ciocalteus reagent was added. It was allowed to stand for 2 minutes and 2mls of 20% sodium carbonate was added. Then absorbance was read at 650nm.

Determination of Steroids: This was determined by the method of Harborne (1973) [10]

Procedure: Exactly 1g of the sample was weighed and macerated in 20mls of ethanol and filtered. Then 2mls of the filtrate was pipetted and 2mls of colour reagent was added and allowed to stand for 30mins. Then absorbance was taken at 550nm.

Determination of Terpenoids: This was determined by the method of Harborne (1973) [10]

Procedure: A known quantity of Phoenix dactylifera was weighed and macerated in 20mls of ethanol and was filtered properly with a whatman filter paper. 1ml of the filtrate was pipetted and 1ml of 5% phosphomomolybdic acid was added. Gradually, 1ml of sulphuric acid was equally added and allowed to stand for 30mins. Finally 2mls of ethanol was added and absorbance was read at 550nm against the blank.

Determination of Alkaloids: This was determined by the method of Harborne (1973) [10]

Principle: H$_2$SO$_4$ reacts with alkaloids in the presence of formaldehyde to form a coloured complex which is read spectrophotometrically at 565nm.

Procedure: Exactly 1g of the sample was weighed and macerated in 20mls of ethanol in 20% sulphuric acid (1:1) and then filtered. 1ml of the filtrate was pipette and 5mls of 60% H$_2$SO$_4$ was added. After 5mins, 5ml of 0.5% formylladehyde in 60% H$_2$SO$_4$ was also added, mixed and allowed to stand for 3hours. Absorbance was read at 565nm.
**Determination of Saponin:** This was determined by the method of Harborne (1973) [10].

**Principle:** Saponin reacts with anisaldehyde and ethylacetate to give a coloured complex which is read spectrophotometrically at 430nm.

**Procedure:** Exactly 1g of the sample was weighed and macerated in 20mls of petroleum ether and decanted into a beaker. It was washed again with 10ml of petroleum ether. The filtrate was combined and was allowed to evaporate to dryness. The residue was dissolved in 6mls of ethanol and 2mls of it was taken into a test tube. 2mls of chromogen solution was added and allowed to stand for 30mins. Absorbance was read at 550nm.

**Determination of Cynogenic Glycosides:** This was determined by the method of Trease and Evans (1989) [11].

**Principle:** Cyanogenic glycosides react to alkaline picrate under boiling temperature to produce a colour that is read spectrophotometerically at 490nm.

**Procedure:** Exactly 1g of the sample was weighed and macerated in 50mls of distilled water. It was filtered properly with whatman filter paper. 1ml of the filtrate was pipette and 4mls of alkaline picrate solution was then added. It was then boiled for 5mins and allowed to cool. The absorbance was then read at 490nm.

**Quantitative Analysis of Anti-nutrients:** Anti-nutrient analysis of date fruits were carried out by the method of Association of Official Analytical Chemist (AOAC, 1997) [12] as follows:

**Determination of Haemagglutinin**

**Procedure:** A known weight of the sample was taken and dispersed in a 10mls normal saline solution and buffered at pH 6.4 with a 0.01m phosphate buffer solution and was allowed to stand at room temperature for 30mins and then centrifuge to obtain the extract. Exactly 0.1ml of the
extract was diluted in a test tube and 1ml of tryspinezed rabbit blood was added. Then control was mounted on the test tube containing the blood cells and the both test tubes was allowed to stand for 4 hours at room temperature, 1ml of normal saline was added to all the test tubes and allowed to stand for 10mins, after which the absorbance was read at 620nm specifically against the blank.

**Determination of Trypsin Inhibitor**

**Procedure:** Exactly1.0g of the sample was weighed and dispersed in 50mls of 0.5ml NaCl solution. The mixture was stirred for 30mins at room temperature and centrifuged. The supernatant was filtered with the Whatman filter paper and the filtrate is taken. 10mls of the filtrate was pipetted into a test tube and 20mls of the standard trypsin solution was added. A blank solution of 10mls of the same substrate was prepared in a test tube. The content of the test tubes was allowed to stand for at least 5mins and then measured spectrophotometrically at 410nm.

**Determination of Oxalate**

**Procedure:** Exactly 2.0g of the sample was weighed into a conical flask. 20mls of 30% HCl was added and was allowed to stand for 5mins. 4.0g of ammonium sulphate was equally added. It was stirred gently to dissolve and was allowed to settle. Supernatant was decanted into a 25mls volumetric flask and the volume was made up with 30% HCl. It was transferred into 50mls volumetric flask equal volume of diethyl ether. The pH was adjusted to 7.0 with either NH₄OH or CH₂COOH and was centrifuged at 3000rpm. It was equally decanted into a 250mls conical flask and titrated with 0.1MKMnO₄. The absorbance was read at 490nm.

**Determination of Phytate**
Procedure: Exactly 0.5g of the sample was weighed into a 500 ml round bottom flask, and extracted with 100mls of 2.4% HCl for 1hr at room temperature and filtered. 5mls of the filtrate was pipetted and diluted to 25mls with water. From the diluted sample, 10mls was taken into a test tube through amber let resin grade 200-400 mesh to elude inorganic phosphates. 15mls of 0.7 M sodium chloride was added and the absorbance was read at 520nm.

RESULTS

The result of the phytochemical analysis revealed that a Phoenix dactylifera fruit has high level of phenol, tannin, flavonoid, alkaloids, glycoside, oxalate, phytate, hemaglutinin and trypsin inhibitor as shown in Figures 2 and 3. The result of anti-nutrient composition also revealed the presence of phytate, haemaglutinin, oxalate and trypsin inhibitor as shown in Table 1.

![Figure 2: Phytochemical Compositions of Phoenix dactylifera Fruits in mg/100g.](image-url)
Figure 3: Phytochemical Compositions of *Phoenix dactylifera* Fruits in mg/g

Table 1: Anti-nutrient Composition of *Phoenix dactylifera* Fruits

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Phytate</th>
<th>Oxalate</th>
<th>Haemaglutinin</th>
<th>Trypsin Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values</td>
<td>1.32±0.04 mg/100g</td>
<td>1.32±0.51 %</td>
<td>0.85±0.09 HU/mg</td>
<td>0.60±0.03 IUI/mg</td>
</tr>
</tbody>
</table>

Discussion

The result revealed that *Phoenix dactylifera* fruit is a good source of phenols and other phytochemicals as shown in Figure 2 and 3. Plants have the ability to synthesis wide range of chemical compound that are used to performed important biological functions and to defend against attack from predators such as insects, fungi and herbivorous animals [13]. These phytochemicals, often secondary plants metabolites have biological properties such as anti-oxidant activity, anti-microbial effect, modulation of detoxification enzymes, and stimulation of the immune system [14]. These
metabolites are said to be useful to the plants, but some are toxic to plants including man [14].

The high level of phenol (4.73±24mg/100g) in Phoenix dactylifera fruits cannot be neglected as phenols exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protective agent against free radical mediated diseases processes [15]. This result supported the reports of Aja et al. (2015) [16], which reported high levels of phenols and other phytochemicals in Dissotis rotundifolia and Cajanus cajan leaves and seeds. Aja et al. [2011] [17], had revealed that Moringa oleifera leaf is a good source of phytochemicals which is in line with the present result on date fruits. This result also correlated with the report of Nwali et al. (2012) [18] which showed that Bryophylum pinnatum leaf contained low levels of phytochemicals.

The results of this study revealed that Phoenix dactylifera contains tannins (9.42±72mg/100g) as shown in Figure 2. This is below the toxic level. It is believed that tannic acid has anti-bacterial and astringent properties which have action upon mucous tissue such as tongue and inside the mouth; the indigestion of tannic acid causes constipation and can be used in the treatment of diarrhea. Tannins are polyphenols that are obtained from various parts of different plant belonging to multiple species. Tannins can also be effective in curbing hemorrhages and as well restrict bare swellings [19]. This result supported the result of Aja et al. (2010) [20] which almost the same value of tannins and other phytochemical in dry and samples of Talinum triangulare leaves.
Flavonoids concentration found in fruit of Phoenix dactylifera is 3.45±39 mg/100g. Flavonoids has been reported to exert multiple biological properties which including antimicrobial, cytotoxicity, anti-inflammatory as well as anti-tumor activities but the best describe property of flavonoids is the capacity to act as powerful antioxidant which can protect the human body from free radicals and reactive oxygen species [21]. This result supported the reports of Aja et al. (2015) [22] which reported high levels of phenols and other phytochemicals in Dissotis rotundifolia and Cajanus cajan leaves and seeds. Aja et al. (2011) [17] had revealed that Moringa oleifera leaf is a good source of phytochemicals which is in line with the present result on date fruits. This result also correlated with the report of Nwali et al. (2012) [18] which showed that Bryophyllum pinnatum leaf contained low levels of phytochemicals.

Terpenoids are commercially important because of their use as flavours and frangrances in food fruits and cosmetics industries [23]. Alkaloids level found in Phoenix dactiphyfera is not that significant. Alkaloids have many pharmacological activities including anti-hypertensive and anti-arrhythmic effects, anti-malarial activity (Quinine), and anticancer action [24]. Steroid have many biological importance in the human cell, some steroids are signaling molecules which tell one part of the body to do something at a particular time, such as the female sex hormone, steroid that control menstruation and female fertility. The immune system also use steroids, which in this case are called, adrenocorticosteroids are regulator of metabolism, and move glucose and other nutrient around the body. Testosterone, a male steroids hormone promotes the developments of male genitalia and body hair.

Saponins are found to be active in plants cells. It functions as being anti microbial, to inhibit moulds and to protect plants from insect attack. Saponins may be considering as a part of plant defense system, and as such
have been included in a large group of protective molecules known as phytoprotectants [25]. Glycoside plays numerous roles in living organisms. Many plants store chemicals in the form of inactive glycosides. They can be activated by enzymes hydrolysis which causes the sugar part to be broken off, making the chemical available for use [26]. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body [27]. The cardiac glycoside mainly used therapeutically in the treatment of cardiac failure due to their anti-arrhythmic effect; these effects are caused by the ability to increase cardiac output. The level of glycoside found in Phoenix dactylifera fruits is negligible.

The results obtained from Table 1 revealed low levels of anti-nutrients in Phoenix dactylifera fruits. The anti-nutrients such as tannin, phytate, oxalate, trypsin inhibitor, haemagglutinin and cyanogenic glycosides were observed to be very low in concentration in Phoenix dactylifera fruit even below permissible toxicity levels [15]. This indicates probable lack of interference with the availability of mineral elements. This result supported the report of Aja et al. (2015) [21] which reported low levels of these anti-nutrients in Parkia biglobosa fruits from Abakaliki, Ebonyi State, Nigeria. The anti-nutrients are natural or synthetic compounds that interfere with the absorption of nutrients. Nutrition studies focus on those anti-nutrients commonly found in food sources and beverages. The level of Oxalate obtained was relatively low compared to 4.00% reported for Parkia biglobosa fruits by Aja et al., (2015) [21]. The result obtained by Oke, (1969) [26] was in correlation to the result obtained in this study. The phytate composition in Phoenix dactylifera fruits observed was in correlation with the result obtained from the fruit of Parkia biglobosa (0.05 %) by Aja et al.(2015) [21]. Phytate chelate metal ions such as calcium, magnesium, zinc, copper, iron and molbdenium to form insoluble complexes that are not readily absorbed from gastrointestinal. Phytic acid also inhibits the action
of gastrointestinal tyrosinase, trypsin, pepsin, lipase and amy lase [21]. Trypsin inhibitors composition of Phoenix dactylifera fruits was observed to be low (0.60±0.03IU/mg). The observed low level of trypsin inhibitor is correlation to the work done by Aja et al. (2015) [21]. Trypsin inhibitors have been implicated in reducing protein digestibility and in pancreatic hypertrophy action. Haemagglutinin level found in Phoenix dactylifera fruits was beyond the toxic level as shown in Table 1. Haemagglutinin is a glycoprotein found on the surface of influenza viruses. It is responsible for binding the virus to cells with sialic acid on the membrane, such as cells in the upper respiratory tract or erythrocytes (Russel et al., 2008). It is responsible for the fusion of the viral envelope with the endosome membrane.

**Conclusion:** The phytochemical and anti-nutrient screening of Phoenix dactylifera fruit revealed that the fruits are good source of phenol, flavonoids, steroids, terpenoids and alkaloids. The results obtained from the study that the anti-nutrients in Phoenix dactylifera fruits are below the permissible toxic level.

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