*ASN 53rd Annual Conference Proceedings (Sub-Theme: Agricultural Engineering, Processing & Value Addition)*

# Quantitative and Correlation of the Bioactive Phytochemicals in Fruits of Date Palm (*Phoenix dactylifera* L) Accessions in Nigeria

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**Abstract**

Fruits are known to have different levels of antioxidant properties due to the presence of multiple types of chemical compounds in varying amounts. The phytochemical properties of twenty-one date fruits accessions from the experimental field of Nigeian Institute for Oilpalm Research (NIFOR) Date palm research substation, dutse, jigawa state, were employed and also quantified in order to substantiate the antioxidant and medicinal claims. The phytochemical analysis of saponins, alkaloids, tannins, flavonoids, and phenols was carried out using Atomic Absorption Spectrophotometer (AAS).the result revealed the total phenolic and tannin content to be highest in accession R5P8 (73.92mg/100g) and R7P1 (5.540mg/100g) there were no significant differences between accession R1P10 and R4P29 with the same value (12.10mg/100g). The lowest saponin content was recorded in accession R1P18 (203mg/100g) and the lowest alkaloid content was recorded in accession R5P20 (0.234mg/100g).Among the phytochemicalsin all the accessions, a very strong correlation was observed between tannin and saponin content while a very weak correlation was observed between flavonoids and Alkaloids.There is an overview of pharmacological properties of date palm. Date fruit has potent constituents that have therapeutic implication in prevention of diseases through anti-oxidant, anti-inflammatory,anti-tumor and ant diabetic effect. The accessions studied with considerable amount of phytochemicals could serve as a tool for future breeding programmes

**Keywords: Accession, Datepalm, Breeding, Phytochemicals, Correlation**

## Introduction

The medicinal values of some plants lie in some chemical substances that produce definite physiological actions in human body. The most important of these bioactive constituents are alkaloids, tannis, flavonoids and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants (Okwu, 1999 & 2001). An Ethno botanical and ubiquitous plant serves as rich resources of natural drugs for research and development (Kong *et al,* 2008). However, Human body is characterized by continuous production of free radicals and other reactive oxygen species due to aerobic metabolism. At the same time antioxidants and antioxidant enzymes exert synergistic action in removing the free radicals (Uttara *et al*., 2009). According to the polyphenol content, different fruits show different antioxidant capacities (Saura-calixto and Goni, 2006) and recent research has confirmed that vegetables and fruits play an important role in the prevention and treatment of different diseases caused by oxidative damage ( Hung*et al*., 2015). Flavonoids are the largest group of phenols found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine and play a role against oxidative stress, inflammation, allergy, viral infection, and cancer (Middleton, 1998).

**Materials and Methods**

**Plant materials:** Fresh fruit sample without any physical and microbial damage were collected among the genepools in the experimental field of Nigerian Institute for Oilpalm Research (NIFOR) date palm research substation, dutse,Jigawa state. The samples were packed and sealed in thick polythene bag. Each of the samples was given an entry number, information regarding the gene pool name and palm number until use.

**Sample Preparation:** The fruits were washed properly first with tap water and then with distilled water to remove dirts, they were then depulped (removal of seeds from fruits). the fruits were then shade dried at room temperature for two weeks and they were den grinded into powder. The powder was then passed through a 0.5mm metallic mesh. The resultant fine crude powder were then used for phytochemical investigation.

**Test for Saponin Contents**: Total saponins were determined according to the method described by Makkar *et al*., (2007). Vanillin reagent (0.25 ml; 8%) were added to each of the solution followed by sulphuric acid (2.5 ml; 72% v/v). The reaction mixtures were mixed well and incubated at $60^0$C in a water bath for 10 minutes. After incubation, the reaction mixtures were cooled on ice and absorbance at 544 nm (UV visible spectrophotometer) were read against a blank that does not contain the extract. The standard calibration curve was obtained from suitable aliquots of saponin (0.5 mg/ml in 50% aqueous methanol).

**Test for Alkaloids:** The total alkaloids concentration was quantified based on the reaction between alkaloid and bromocresol green (BCG) spectrophotometric method. The pH of the phosphate buffer solution were adjusted to neutral with 0.1 N NaOH and 1 ml of the solutions were transferred to a separating funnel, and then 5 ml of BCG solution along with 5 ml of phosphate buffer was added. The mixtures were shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm (Shams *et al*., 2008; Sharif *et al*., 2014).

**Test for Tannins:** The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the solution was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteuphenol reagent, 1 ml of 35 % $Na_2CO_3$ solution and diluted to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of Gallic acid (20, 40, 60, 80 and 100 µg/ml) was prepared in the same manner described earlier. Absorbance for test and standard solutions was measured against the blank at 725 nm with UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract (Marinova *et al*., 2005)

**Test for Flavonoids:** The total flavonoid content was determined by aluminium chloride colorimetric assay as described by Zhinshen *et al*., (1999). The aliquots of the samples 0.5 ml and standard solutions (0.01-1.0 mg/ml) of Quercetin was added with 2 ml of distilled water and subsequently with 0.15 ml of sodium nitrite (5 % $NaNO_2$ w/v) solution was added. After 6 minutes, 0.15 ml of (10 % $AlCl_3$ w/v) solution was added. The solutions were allowed to stand at 6 minutes and after that 2 ml of sodium hydroxide (4 % NaOH w/v) solution was added to the mixtures. The final volume was adjusted to 5 ml with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 minutes. The absorbance of each mixture was determined at 510 nm against the same mixture. The total flavonoid content was determined as mg quercetin equivalent per gram of sample with the assistance of calibration curve of quercetin.

**Test for Phenols:** The total phenol content was estimated and measured spectrophotometrically by Folin-Ciocalteu colorimetric method, using Gallic acid as the standard and expressing results as Gallic acid equivalent (GAE) per gram of sample. Different concentions (0.01-0.1 mg/ml) of Gallic acid was prepared in methanol. Aliquots of 0.5 ml of the test sample and each sample of the standard solution was taken, mixed with 2 ml Folin-Ciocalteu reagent (1:10 in deionized water) and 4 ml of saturated solution of sodium carbonate (7.5 % w/v). The tubes were covered with silver foils and incubated at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using methanol as blank. The total phenol was determined with the assistance of standard curve prepared from Gallic acid (Ainsworth and Gillespie, 2007).

**Results and Discussion**

The results obtained for the phytochemical screenings are shown (), from the result, accession R5P8 had the highest phenolic content of 73.92mg/g. this value was significantly the same with R1P10 with value of 72.220mg/g but significantly different from all other accessions. The lowest was obtained in accession R13P1

with the value of 15.10mg/g. this value was significantly different from all other accessions. The saponin contents was highest in accession R16P31with the value of 888mg/g, this value was significantly different from all other accessions .the lowest saponin content was observed from accession R1P18 with the value 203.0mg/g, this value was significantly different from all other accessions. The accession R13P5 had the highest alkaloid content with the value of 10.684mg/g, this value was significantly different from all other accessions. The lowest alkaloid content was obtained from accession R16P13 with value of 0.23mg/g, this value was significantly the same with accession R5P20 and R1P18 with the values 023mg/g but significantly different from all other accessions. The results of the tannin contents showed that accessions R5P8 had the highest tannin content with a value of 5.54mg/g, this value is significantly different from all other accessions. The least was observed in the accessions ZARIA with the value 0.0005mg/g though there were no significant differences in accessions R16P13, R13P9, R6P20 with the value 2.140mg/g but they are significantly different from the values among all other accessions. The flavonoid content was highest in accession R6P20 with the value of 12.18mg/g, this value was significantly the same with R1P10 and R4P29 but significantly different from all other accessions. The lowest was observed in R13P5 with the value of 2.77mg/g, this value is significantly the same with accessions R2P4 and R3P22 with the values 2.88mg/g and 2.85mg/g respectively. The phenol content was positively correlated with Tanin, flavonoid and saponin with their values (0.325335, 0.300383, 0.054061) while it was negatively correlated with Alkaloid having the value (-0.01975). however, a very strong correlation was observed between tannin and saponin (0.420871). in addition, flavonoid and saponin had a very weak correlation, though Alkaloid was also observed to have a weak correlation with all the phytochemicals studied (Table 1). Date fruits are considered as staple fruits and they are widely cultivated in semi-arid regions The phytochemical analysis of the studied Date fruit showed the presence of Alkaloids, Tannins, flavonoids, Phenols and saponins maybe due to significant contributions of the secondary metabolites.

However, the phenol contents of the date fruits recorded in the study was between 15.0mg/g and 73.92mg/g. This is in accordance with the reports of mohammed *et al* (2014) on Sudanese date fruits (35.82-199.34 mg/g) though lower than the findings reported by Al-turki *et al* (2010) on the Tunisian date fruits, and krishmony *et al* (2018) on Palmyra palm, The differentiations to colour, sensory and antioxidant properties among the studied accessions might be due to the presence of phenols, this is in conformity with the report of Robinson J.C (1996) on Banana and Plantain, Eleazu *et al* (2011) on Plantain. Phenols are very important plant constituents because of their free radicals scavenging ability which in turn due to the presence of hydroxyl groups in them. Dietery Phenols From date consumption may supply substantial antioxidants which, in turn, may provide health promoting and disease preventing effects (Tohidi, Rahimmalek & Arzani, 2017). This implies that the fruits of datepalm are rich source of anti oxidants because studies have shown that anti oxidants capacity of plants are tightly correlated with phenol compounds. The tannin content was between 0.005mg/g-5.540mg/g which might be due to the genetic variations in astringency contents among the studied accessions, the presence of tannin content also shows that date fruits could quicken the healing of wounds and inflamed mucus membrane, This is in line with the findings of Ogbonna *et al*., (2016) on banana, Ojobor *et al.* (2018) on coconut, sadiq *etal*., (2013) on Datepalm, Saha *et al*., (2017) on date palm. Tannins play an important role in the prevention of cancer and also used for the treatment of inflamed and ulcerated tissue (Mota *et al*., 1985; Aiyegoro and Okoh, 2010). So date palm fruits could be used as medicine in the treatment of many health challenges.The flavonoid contents obtained in this study was between 2.77mg/g – 12.18mg/g which is a considerable amount present in fruit crops. This is in conformity with the findings of saha *et al.,* (2017) on date palm, Tapas *et al.*, 2008) on *Phoenix sylvestris,* Ojobor *et al.,* (2018) on Coconut. However, it is an evident that date fruits can act as potent antioxidants and metal chelators. The presence of flavonoids in fruits have long been recognized to possess anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities, with this date palm is an excellent crop to possess medicinal values. The pronounced variations observed in saponin contents of the studied date palm fruits is in agreement with the report of Saha *et al* 2017, they observed significant differences in the saponin contents among all the fruits of the studied date cultivars. Similar results have been reported by Hasan *et al* (2012) on some Medicinal plants in Nigeria, Tiwari *et al.,* 2014) on *Gymnema sylvestre*. However, from the results of saponin content in the fruits recorded in this study (217mg/g-888mg/g), shows that date palm fruit could be used as a traditional medicine for many health beneficial effects, Studies have shown that Saponins are also used as a major constituent of traditional Chinese medicine (Liu and Henkel, 2002). The alkaloid content was between 0.23mg/g-10.684mg/g. A high amount of alkaloids recorded in this study indicate that date fruit

could play a wide range of physiological actions on human health care such as antibiotics, anticancer and different degenerative diseases. This is in agreement with the findings of Ogbonna *et al* (2016) on banana, Kasolo *et al* (2010) on Moringa. Alkaloids are in great demand for pharmaceutical formulations especially for the lethal diseases such as cancer and inflammatory disorders. Alkaloids are heterogeneous group of naturally occurring compounds found in the leaves, bark, roots or seeds of plants. They are the most effective plant substance used therapeutically as analgesic, antimicrobial and antibacterial agents. The positive correlation observed between phenol and flavonoid and Phenol and saponin are in agreement with the findings of Chaudhuri *et al.,* (2013) where they also observed a linear positive correlation between phenol and flavonoid (0.967) and phenol and saponin (0.960), the positive correlation between tannin and saponin (0.985) and tannin and flavonoid (0.989) are also in agreement with the findings of chaudhuri *et al*., 2013 but the values are higher when compared with the ones obtaind in this study (Table 1), the negative correlation observed between flavonoid and saponin (-0.09262) in this study disagrees with the findings of chaudhuri *et al.,* 2013, where a complete correlation (1.00) was obtained between flavonoid and saponin.

**Conclusion**

It can be concluded that Nigerian date fruits may be considered as a potential source of antioxidants, since they contain important phytochemicals that posses bioactive properties and maybe used as external therapeutic supplements.

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**Figure 1: phytochemicals of some of the studied parameters**

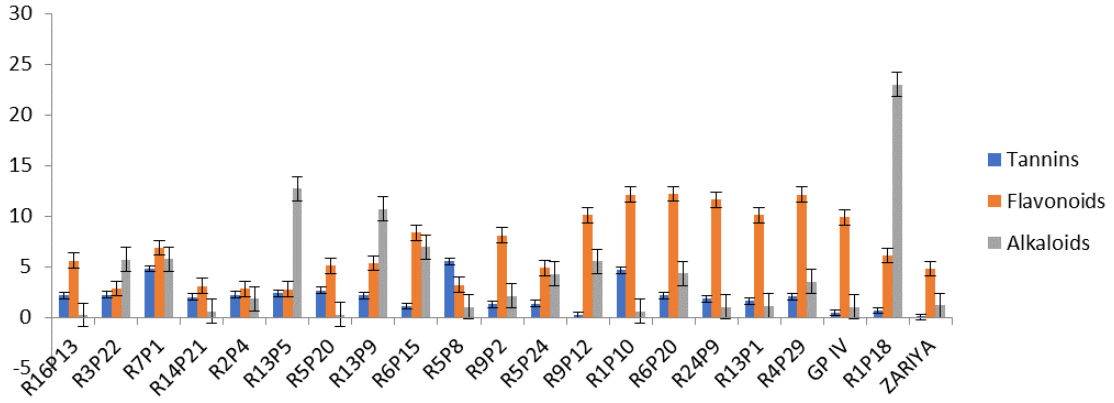


**Figure 2: phytochemicals of some of the studied parameters**

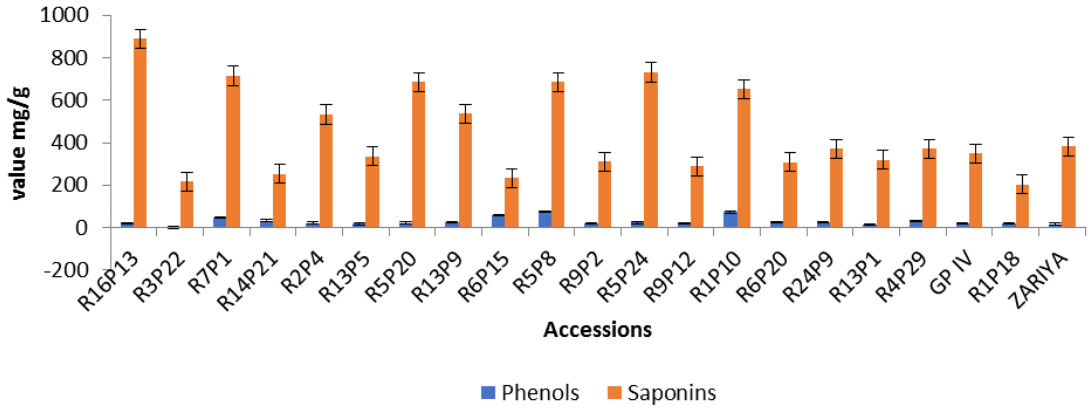


**Table 1: Correlation Coefficient of the Accessions based on Phytochemicals**

| | *Phenols* | *Tannins* | *Flavonoids* | *Saponins* | *Alkaloids* |
|---|---|---|---|---|---|
| Phenols | 1 | | | | |
| Tannins | 0.325335 | 1 | | | |
| Flavonoids | 0.300383 | 0.07277 | 1 | | |
| Saponins | 0.054061 | 0.420871 | -0.09262 | 1 | |
| Alkaloids | -0.01975 | -0.12149 | -0.24924 | -0.26323 | 1 |