COMPARATIVE EVALUATION OF THE NUTRITIONAL, PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF THE DRIED POWDER AND ETHANOL EXTRACT OF *DENNETTIATRIPETALA* FRUIT

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Abstract
Fruits contain several chemical compounds essential for optimal metabolic functions in the biological system. There is an increasing consumption of pepper fruit (*Dennettiatripetala*) because of its health benefits. In this study, the nutritional, phytochemical and antioxidant properties of the ethanol extract and dried powder of *Dennettiatripetala* fruit was comparatively evaluated. The dried powder and ethanol extract samples were prepared from fresh ripe pepper fruit. The samples were subjected to nutritional (proximate), phytochemical and antioxidant analysis using standard methods. Results showed that the ethanol extract of *Dennettiatripetala* fruit contained significantly ($P<0.05$) higher amounts of moisture, ash, protein, crude fat, flavonoid, phenol, tannins, alkaloids and reducing power than the dried powder. However, the dried powder contained significantly ($P<0.05$) higher amounts of crude fibre, carbohydrate, and DPPH activity than the ethanol extract. Thus, comparatively, the ethanol extract was shown to be richer in nutrients, phytochemicals and highly valuable as a source of natural antioxidants which may help combat oxidative stress and inhibit degenerative disorders.

Key Words: Pepper fruit, dried powder, ethanol extract, proximate analysis, phytochemicals, antioxidants

Introduction

Plants remain an integral source of food and nutrients for humans and animals. They contain several chemical compounds that are essential for proper metabolic functions (Iseghohi et al, 2017;Omage et al, 2022). In Nigeria, a variety of fruits and vegetables are consumed daily as they form an integral part of our diet. However, the fleshy pulps of these fruits are often consumed while the seeds are thrown away. Fruits are known for high micronutrients concentrations including carotene or provitamin A, vitamin K, ascorbic acid, riboflavin, iron, iodine, and other mineral elements (Olusanya, 2008; Omage et al, 2019; Omage and Omage, 2020). Fruits and vegetables provide vitamins and minerals in quantities high enough to provide the body with its needs (Olusanya, 2008).There is an increasing consumption of pepper fruit in Nigeria because of its health benefits (Iseghohi et al, 2017; Omage et al, 2019; Omage and Omage, 2020; Omage et al, 2021; Omage et al, 2022). Studies have shown that pepper fruit contains important phytochemicals, which promote healthy growth and prevents cardiovascular diseases (Iseghohi, 2015; Iseghohi et al, 2017; Omage et al, 2019; Omage and Omage, 2020; Omage et al, 2021; Omage et al, 2022).

Pepper fruit (*Dennettiatripetala*) belongs to the Annonaceae family. It is a tropical plant that is mostly found in the West African region especially Nigeria, Ivory Coast and Cameroon. It is a pungent, peppery, spicy medicinal plant that is often greenish in colour when unripe and reddish or pinkish in colour when ripe. The edible mature fruit is mostly eaten raw but can also be used for preparing food and herbal medicines(Okwu 2005). The fruit, leaves, roots and barks of *Dennettiatripetala* plant are distinguished by their strong pungent spicy and peppery taste, fragrance and aroma (Achinewhu et al 1995) and are useful for medicinal purposes (Iwu,1989). The fruit is effective for ethno medical (traditional medical) purposes (Egharevba et. al., 2015).The presence of essential oils (oleoresins) in *Dennettiatripetala* is responsible for its taste aroma and pungency (Iseghohi, 2015). Pepper fruit is normally chewed as a fruity snack due to its peppery stimulating effects. In view of the myriad of benefits derived from the fruit, the form in which it is consumed may influence the availability of its nutrients. Thus, this study was undertaken to compare the nutritional, antioxidant and phytochemical constituents of the dried powder and ethanol extract of *DennettiaTripetala* fruit.

MATERIALS AND METHODS

Collection of Fruit and Sample Preparation

Fresh ripe samples of pepper fruit (*Dennettiatripetala*) were obtained from local farmers within Benin City, Edo state. The sample was chopped into bits, air-dried, and ground into fine powder. The powdered fruit was divided into two parts and weighed. One part was used as a sample (powdered/dried fruit) while 400g of the other part was soaked in 1600ml of absolute ethanol for 72hrs with occasional stirring (using a magnetic stirrer). The mixture was then filtered, and the filtrate (extract) evaporated to dryness (using a rotary evaporator) and weighed. This formed the second sample (ethanol extract). The percentage yield of the ethanol extract obtained was 8.17.

PROXIMATE ANALYSIS

Determination of moisture content

The moisture content of the samples was determined by the method of the Association of Official Analytical Chemist (A.O.A.C., 2003), where the moisture content was determined from the difference in weight after complete evaporation.

Determination of crude fiber content

The crude fiber content of the samples was determined using the method of (AOAC, 2003).

Ash Determination

The ash content of the samples was determined by Furnace incineration described by (AOAC 2003).At low temperature, the organic matter in the fruit was burnt off and the inorganic material left cooled and weighed. This was then heated in a muffle furnace to reduce the sample to ash.

Determination of crude protein

Crude protein content of the samples was determined using the Micro Kjeldah method (AOAC 2003). The nitrogen value obtained was converted to protein by multiplying it by a factor of 6.25.

Determination of crude fat

The crude fat content of the samples was determined using the soxhlet extraction method of A.O.A.C (2003). The crude fat content of the fruit consisting of neutral fats (triglycerides)

and free fatty acids was determined by extracting the samples with diethyl ether in a continuous extraction process.

Determination of carbohydrate

The carbohydrate content of the samples was determined by subtracting the summed-up percentage of moisture, protein, lipid, fiber and ash contents from 100 (Otitoju, 2009).
N.F.E= 100 - (%moisture + %ash + %crude fiber + %crude fat + %crude protein).

PHYTOCHEMICAL ANALYSIS

Determination of Tannin

Spectrophotometric method of Trease & Evans,1989) was used in the determination of tannin in the samples.

Determination of alkaloid

Alkaloids were determined by gravimetric method of Harborne,(1973).

Determination of total flavonoid

Total flavonoid content was determined according to the procedure of Chang et al., (2002), validated by Nugroho and Malik, (2013) with some modifications using rutin as reference standard.

Determination of total phenol

Total phenol was determined by folinciocaletu reagent (Malik et al., 1980). The total phenol values are expressed in terms of gallic acid equivalent (mg of dry mass), which is a common reference compound.

ANTIOXIDANT ANALYSIS

DPPH Scavenging Effect

The free radical scavenging activity of the samples was measured by 2,2-diphenyl-1-1-picryhrazyl (DPPH) according to the method described by Hasan et al., (2006) and Alam et al., (2008b).DPPH (2,2-DIPHENYL-1-1-PICRYHYRAZYL), a stable free radical, when acted upon by an antioxidant was converted into dipenyl-picryhrazylhydrazine with a color change from deep violet to light yellow color. This was quantified spectrophotometrically at 517nm to indicate the extent of DPPH scavenging activity by the plant sample.

Reducing Power Activity Determination

Various concentrations of the samples were prepared (0.2mg/ml - 1mg/ml). About 1ml of 0.2mol/dl sodium phosphate buffer (pH 6.6) and 1ml of freshly prepared 1% potassium ferric cyanide were added to the various sample concentrations.The mixtures were incubated in water bath at 50^{o}c for 20 minutes, after which1ml of 10% TCA was added to the mixture. The mixtures were centrifuged at 3000rpm for 10 minutes. About 2ml of the supernatants were mixed with 2ml of distilled water and 500µl of freshly prepared 1% ferric chloride. Rutin was used as standard. The absorbances were read at 700nm and the amount of rutin extrapolated from a standard calibration curve for reducing power activity.Higher absorbance indicated higher reducing power activity.

Statistical Analysis

Data were analyzed using the statistical package SPSS version 21.0 and Microsoft Excel to show significant difference between multiple mean variables while Paired T-test was used to show significant difference between two mean variables. Statistical significance was set at $P<0.05$.

Results

The comparative proximate (nutritional), phytochemical and antioxidant composition of the dried powder and ethanol extract of *Dennettiatripetala* fruit are as shown below.

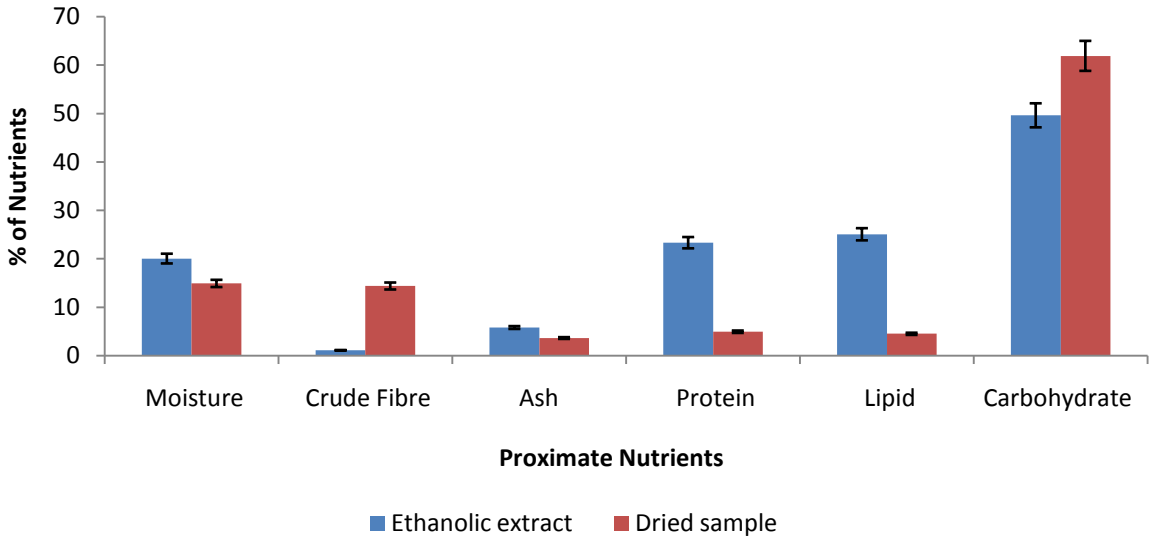

Figure 1: Comparative proximate composition of the ethanol extract and the dried powdered sample of *DennettiaTripetala* fruit.

Figure 1 showed that the percentages of the moisture, ash, protein, and lipid contents of the ethanol extract were significantly higher ($P<0.05$) as compared with those of the powdered sample. However, the percentage contents of carbohydrate and crude fiber were found to be significantly ($P<0.05$) higher in the powdered sample as compared with the ethanol extract.

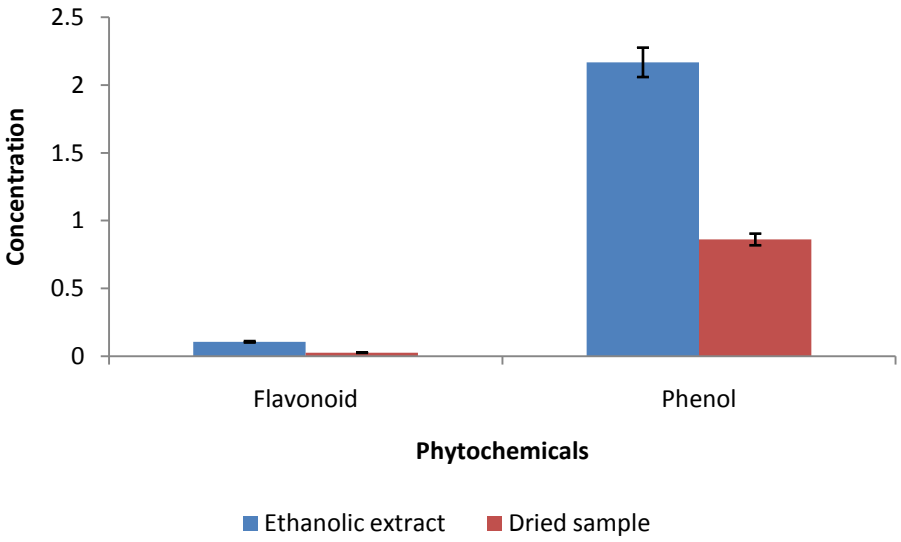

Figure 2: Comparative flavonoid and phenol compositions of the ethanol extract and the dried powdered sample of *Dennettiatripetala* fruit.

In figure 2, comparatively, the compositions of flavonoid and phenol were significantly ($P<0.05$) higher in the ethanol extract than the powdered sample.

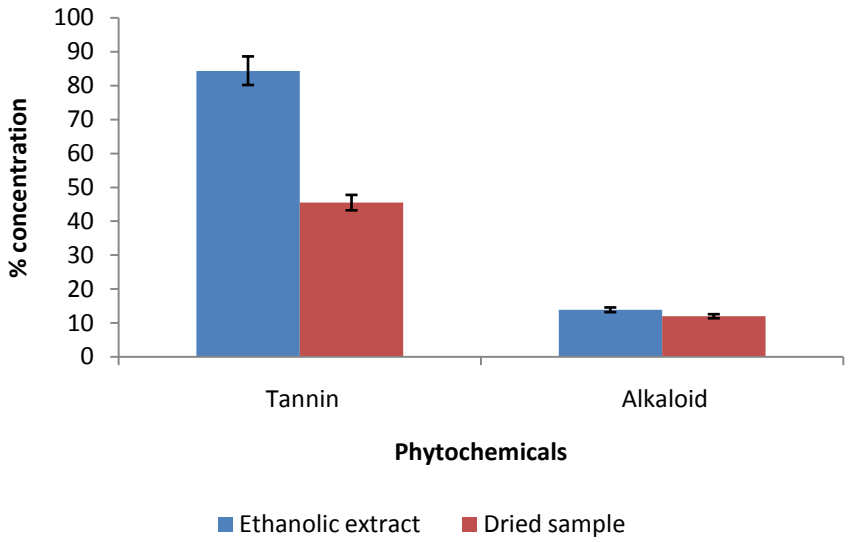

Figure 3: Comparative tannin and alkaloid compositions of the ethanol extract and the dried powdered sample of *Dennettiatripetala* fruit.

In figure 3, comparatively, the composition of tannin was shown to be significantly ($P<0.05$) higher in the ethanol extract than the powdered sample.

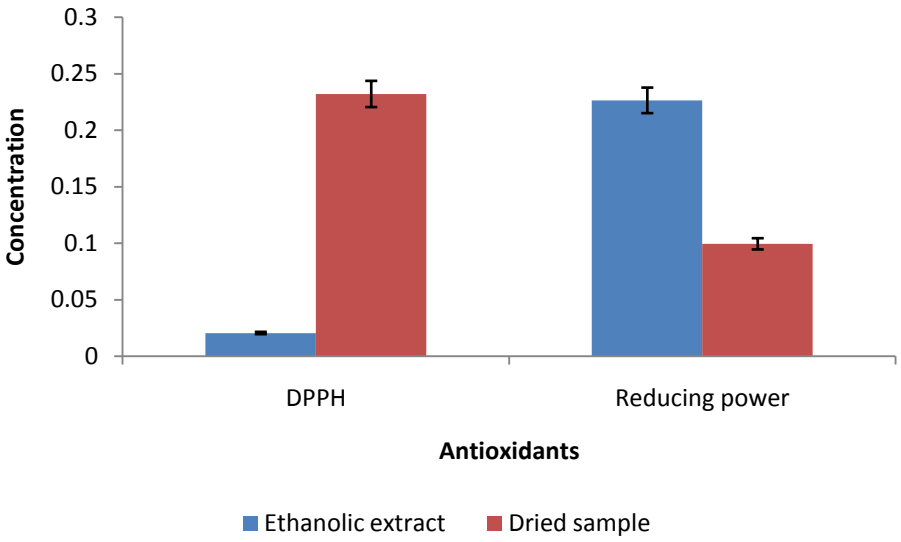

Figure 4: Comparative DPPH activity and Reducing Power of the ethanol extract and the dried powdered sample.

Figure 4 showed the DPPH radical scavenging activity as significantly ($P<0.05$) higher in the dried powdered sample as compared to that in the ethanol extract. However, the reducing power was significantly ($P<0.05$) lower in the dried powdered sample as compared to that in the ethanol extract.

Discussion

In most developing countries like Nigeria, fruits have been part of human diet and food supplements over the years. The fruit samples studied had a considerable level of moisture

content which is typical of fresh fruits at maturity. The comparatively higher moisture content of the ethanol extract is understandable considering the method of preparation of the extract. The moisture content, a factor that increases the chances of microbial contamination, may render the ethanol extract liable to contamination by microbes. Microbial contamination tends to reduce the nutrient and phytochemical constituents of the sample as they feed on them. Although the high moisture content makes the fruit a good source of hydration for the body (Ogbonna et al., 2013), it may be at the expense of relevant nutrients. Foods rich in dietary fiber contribute to the prevention of various diseases such as constipation, hemorrhoids, colon cancer, hypercholesterolemia, diabetes, cardiovascular diseases, renal disorders, and diverticulitis (Hassan et al., 2011; Iseghohi et al, 2017; Omage et al, 2019; Omage et al, 2021; Omage et al, 2022). Our results however showed that to derive these health benefits, eating the whole fruit (i.e., the dried powdered sample) is a better alternative. The ash content, a measure of mineral elements and inorganic compounds (Coimbra and Jorge, 2011) of the fruit samples compared favorably with most fruit values (Bello et al., 2008, Ekpete & Edori, 2013). The comparatively higher ash content of the ethanol extract makes it a better source of various mineral elements and inorganic compounds which are needed for optimal metabolic processes with improved growth and development. Proteins, essential components of diets needed for survival of animals and humans, supply adequate amounts of required amino acids. Our study showed that the ethanol extract of *Dennettiatripetala* fruit is a better source of useful proteins than the dried powder. For the possibility of weight reduction, the powdered sample seems better as its content of crude fat is lower. The higher content of crude fat in the ethanol sample is most probably because of the solvent used for extraction. This makes it fattening and not a good option for individuals who are conscious of their body weight. Carbohydrates are good sources of energy and fruit samples with low carbohydrate content may be ideal for diabetic and hypertensive patients who require low sugar diets (Ekpete & Edori, 2013). Thus, the ethanol extract of the fruit may be useful in the management of diabetes and other related diseases.

Phytochemicals or phytonutrients are naturally occurring substances found in plants and are beneficial to human health (Udeme et al., 2013; Ugwu et al., 2013; Iseghohi et al, 2017; Omage et al, 2019).However, some of these bioactive substances are also anti nutrients since they render some of the essential nutrients unavailable for human nutrition (Okunrobo et al., 2012). Some flavonoids have inhibitory activity against organisms which cause plant diseases e.g.,*Fusarium oxysperum.* The ethanol extract of *Dennettiatripetala* fruit may comparatively show a better inhibitory activity than the powdered sample. Phenols are a group of natural antioxidants and nutraceuticals found in fruits and are renounced for their enormous ability to combat cancer, prevent heart diseases and act as anti-inflammatory agents (Sarker et al., 2008; Omage et al, 2021; Omage et al, 2022). As compared to the powdered sample, the ethanol extract of the fruit is better as a source of phenols. Also, the ethanol extract which is a better source of tannins may act as anastringent and more potent in treating intestinal disorders such as diarrhea and dysentery. Alkaloids, also found to be higher in the ethanol extract of the fruit, have pharmacological applications as anesthetics and CNS stimulants (Madziga et al., 2010). Thus, the ethanol extract of *Dennettiatripetala* fruit may elicit better pharmacological activity than the dried powdered fruit.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions generate free radicals that can initiate dangerous chain reactions within the biological systems. The addition of antioxidants to packaged food items greatly extends their shelf lives and keeps flavors and aromas fresh for as long as possible. Several antioxidants of plant origin have been shown to be effective as protective agents against oxidative stress by

destroying various free radicals (Reddy et al.,2012; Omage and Omage, 2020; Omage et al, 2021). Our study revealed that the DPPH scavenging activity was higher in the dried powdered and probably better at maintaining freshness and preventing browning or rancidity in foods than the ethanol extract. However, the reducing power activity was shown to be higher in the ethanolic extract than the dried sample.

Conclusion

Our study showed that comparatively, the ethanol extract of *Dennettiatripetala* fruit contains higher amounts of moisture, ash, protein, crude fat, flavonoid, phenol, tannin, alkaloid and reducing power, while the dried powder contains higher amounts of crude fibre, carbohydrate, and DPPH activity. Thus, the ethanol extract is richer in nutrients, phytochemicals and highly valuable as a source of natural antioxidants which may help combat oxidative stress and inhibit degenerative disorders.

Acknowledgment

The Author is grateful to the authorities of the Department of Biochemistry, Western Delta University, Oghara Delta State, Nigeria, for providing the necessary laboratory facilities.

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