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Full Length Research Paper

Phytochemical and antioxidant nutrient constituents of *Carica papaya* and *Parquetina nigrescens* extracts

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Medicinal plants (a.k.a. Phytomedicines) are parts of a plant or the whole plant that possess healing properties. Folk medicine reportedly uses *Carica papaya* L. (Caricaceae) and *Parquetina nigrescens* L. (Asclepiadaceae) as a herbal remedy for the management of sickle cell anemia. This study was carried out to screen the leaf extracts of *P. nigrescens* and *C. papaya* L. (Caricaceae) for possible antioxidant phytochemicals, proximate nutrient constituents, amino acid composition and mineral content present in the samples using standard chemical and chromatographic procedures. Phytochemical screening confirmed the presence of folic acid, vitamin B_{12}, alkaloids, saponins, glycosides, tannins and anthraquinones. This study also showed that each of these plants extracts contained flavonoids and the antioxidant vitamins A and C. Some of the previously established antisickling amino acids were also present in the plants. Cyanogenic glycosides were absent from both plant extracts, indicative of the non-toxic effects of these plants when taken orally. These results indicate that the previously reported antisickling properties of these herbs may be due to their inherent antioxidant nutrient composition, thus supporting the claims of the traditional healers and suggests a possible correlation between the chemical composition of these plants and their uses in traditional medicine.

Key words: Amino acids, antioxidants, micronutrients, phytochemical, vitamins.

INTRODUCTION

Antioxidants are a special group of nutritional supplements that scavenge free radicals (Padma et al., 2006). Free radicals impair the proper functioning of the immune system leading to various disease conditions. Flavonoids are naturally occurring phenolic compounds in plants which have antioxidant effects. Alkaloids on the other hand, are known for their anti-inflammatory activity (Iyamu et al*.,* 2003). Phenylalanine and a host of amino acids have been reported to have antisickling activity (Onah et al*.,* 2002) and sources rich in amino acids and antioxidants are essential for maintaining sound health. Glutathione is the major endogenous antioxidant produced by the cell (Padma et al., 2006) which removes peroxides via the action of antioxidant enzyme glutathione peroxidase and also regulates the action of nutrient antioxidants such as vitamins C and E within the body. The inability to maintain reduced glutathione in red blood cells leads to increased accumulation of peroxides that in turn results in the weakening of the cell wall and concomitant hemolysis leading to anemia. Some plants reported to have antisickling properties are *Cajanus cajan* seed extract and *Zanthoxylum macrophylla* root extract (Iwu et al., 1984; Elekwa et al., 2005). Others used as traditional medicines by the local folks in treating Sickle Cell Anemia are *Parquetina nigrescens* leaves and stem (family: Periplocaceae) and *Carica papaya* leaves (family: Caricaceae) (Imaga et al., 2009). However, little has been reported about their antioxidant nutrient composition. This prompted us to carry out this study of assessment of the

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phytochemical antioxidant nutrient properties of these plants used ethnomedically for sickle cell management.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used were of analytical grade and obtained from Sigma Chemical Company and used without further purification.

The plant material

The leaves of *C. papaya* and *P. nigrescens* were collected from the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria in 2008 and authenticated by Usang Felix of the same Institute. Voucher specimens of the *C. papaya* leaves (FHI: 106994) and *P. nigrescens* leaves (FHI: 106998) were deposited at the Institute's herbarium.

Extraction of the leaves

The two plants were extracted by exhaustive aqueous- methanolic extraction using the soxhlet apparatus in a method described earlier (Onah et al., 2002) using aqueous – methanol (1:3, 60 - 80°C) as solvent after de-fatting with pet-ether (60 - 80°C). The extracts were then freeze-dried and used for the Phytochemical and nutrient analyses.

Phytochemical screening

Chemical tests were carried out qualitatively on the extracts and on the powdered specimens using standard procedures to identify the amino acids and phytochemical constituents as described by Edeoga et al. (2005); Sofowara (1993); Trease and Evans (1996); Harborne (1973) with little modification.

Test for alkaloids

Each plant sample (0.5 g) was dissolved in 5 ml dilute HCl in a steam bath and filtered. Three different methods were used. Turbidity or precipitation with either of the following reagents was taken as evidence for the presence of alkaloids. 1 ml of the above filtrate was treated with few drops of Mayer's reagent giving rise to a cream or pale yellow precipitate. Another 1 ml of filtrate was treated with a few drops of Dragendoff's reagent giving rise to an orange precipitate. Lastly, 1 ml of filtrate was treated with Wagner's reagent giving rise to a brown or reddish brown precipitate.

Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatinins.

Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Teat for flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample (Sofowara, 1993; Harbrone, 1973). 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H2S04. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids.

A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

Test for steriods

Two milliliters of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H2S04. The colour changed from violet to blue or green in the samples indicating the presence of steroids.

Test for cardiac glycosides (Keller-Killani test)

Five milliliters of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer, a greenish ring formed just gradually throughout thin layer.

Proximate analysis

The proximate composition of carbohydrate, lipid, protein and other nutrients were determined on the dry ground leaves of *C. papaya* and *P. nigrescens*__according to the appropriate protocol identification number (Horwitz, 2000). The AOAC Official methods for analyzing the various parameters are as listed: Crude protein 955.04 (2.4.03), crude fibre 962.09 (4.6.01), moisture 934.01 (4.1.03), ash 942.05 (4.1.10), crude fat 920.39 (4.5.01), and carbohydrate by difference.

Micronutrient determination

Test for the presence of minerals –copper, iron, magnesium, manganese and zinc were done following standard procedures via atomic absorption spectroscopy as described by Okwu and Josiah (2006) with little modification. The ground plant samples were

Table 1. Qualitative Phytochemical analyses of the plant extracts.

Test	*P. nigrescens* aqueous methanol extract	*C. papaya* aqueous methanol extract
Alkaloids	±	±
Flavonoids	±	±
Tannins	±	±
Cardiac glycosides	±	±
Anthraquinones(Free)	±	±
Anthraquinones (Bound)	±	±
Phlobatinins	±	±
Saponins	±	±
Anthocyanosides	-	-

All experiments were done in triplicate. Legend: ± Present, - Absent.

Table 2. Proximate composition of *C. papaya* and *P. nigrescens*.

Composition	Percentage of *C. papaya*	Percentage of *P. nigrescens*
Lipid	8.27 ± 0.00	11.03 ± 0.01
Protein	0.05 ± 0.00	40.96 ± 0.00
Crude fibre	16.05± 0.01	21.09 ± 0.05
Moisture	4.39 ± 0.00	2.15 ± 0.00
Ash	15.52 ± 0.05	10.66 ± 0.01
Carbohydrate	55.74 ± 0.00	14.12 ± 0.00

All experiments were done in triplicate and data presented as mean ± SEM %.

sieved with a 2 mm rubber sieve and 2 g of each of the plant samples were weighed and subjected to dry washing in a well-cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO3/HCl/H2O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100-ml volumetric flask by filtration through a whatman No 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10-cm long cell was used and concentration of each element in the sample was calculated on percentage of dry matter.

Qualitative tests for the presence of antioxidant vitamins A, C, E, B_{12} and folic acid were determined by High Performance Liquid Chromatography using standard procedures as described by Okwu and Josiah (2006) with little modification.

The amino acid composition in *C. papaya* extract was determined qualitatively by Thin Layer Chromatography using the methods of Ekeke and Shode (1990) with little modification. Prepared TLC plates were spotted with plant extracts and amino acid standards and allowed to stand in the TLC tank saturated with the solvent system- a mixture of n-butanol, acetic acid and water in the ratio of 8:2:2 v/v. The experiment was terminated when the solvent front was at a suitable distance in the tank [near the top of the plate]. The plate was allowed to air dry and sprayed with a locating agent – ninhydrin solution. The plate was then put in an oven at 110°C for 5 min after which the visible coloured spots on the plate were circled and movement of the amino acids present were calculated by its retardation factor, R_F.

RESULTS

The yield of extract was 5.78 and 10.87% for *C. papaya* and *P. nigrescens*, respectively. Qualitative phytochemical screening for *C. papaya* and *P. nigrescens* leaf extracts were found to contain alkaloids, flavonoids, glycosides, tannins, saponins and anthraquinones (Table 1). Anthrocyanosides were not detected in the samples.

Nutrient components

Proximate analysis of the plants showed that all the macronutrients were present, with carbohydrate being the most abundant in *C. papaya* and protein being the most abundant in *P. nigrescens* (Table 2). Vitamin and mineral analyses revealed the presence of important antioxidant micronutrients as shown in Table 2. Both leaves contain Vitamins A,C, B_{12} and Folic acid but are void of Vitamin E. Papaya leaf extract contained only magnesium, the other metals tested were not detected. Whereas the Parquetina extract contained copper, iron, magnesium, manganese and zinc. Histidine, glycine, arginine, threonine, cysteine, glutamic acid were the amino acids found in both plant extracts Table 3.

Table 3. Micronutrient composition of the plant extracts.

Test samples (mg/kg)	*Carica papaya* leaves	*Parquetina nigrescens* leaves
Vit. A	7873.4 ± 0.05	1063.5 ± 0.05
Folic acid	2.4 ± 0.05	3.2 ± 0.05
Vit. B12	2.8 ± 0.05	3.9 ± 0.05
Vit. C	89.0 ± 0.05	131.4 ± 0.05
Vit. E	0.00	0.00
Cu	-	4610 ± 0.05
Fe	-	6740 ± 0.01
Mg	6.76 ± 0.04	0.1 ± 0.04
Mn	-	204 ± 0.05
Zn	-	9870 ± 0.05

Legend: (n = 3) all tests were done in triplicate and values are expressed as mean ± SEM.

DISCUSSION

The constituents of the extracts of *C. papaya* (dried) leaves and *P. nigrescens* leaves contain compounds and micronutrients which may be responsible for their observed antioxidant activities. These are phenolic compounds, alkaloids, glycosides, amino acids, antioxidant vitamins and minerals. Previous studies on the antisickling activity of phytomedicines showed that aqueous –methanol forms of extraction contained the active constituents responsible for their observed activity (Elekwa et al., 2005; Onah et al., 2002; Ekeke and Shode, 1990; Sofowora et al., 1975). This is therefore, an indication that the biologically active compounds are polar and as such, are contained in the aqueous-methanol extract of the leaves.

The extracts were found to be rich in carbohydrate, some lipids and protein. This suggests that the leaves can be used as a nutraceutical because the leaves have some nutritional value when taken as food or potent medicinal properties when used as an herb. The plants can also be kept for long periods without losing flavour as evidenced by the proximate ash and moisture content.

Alkaloids have established broad-spectrum anti-bacterial activity and are also used as analgesics and narcotics for pain relief. This supports earlier findings [Sofowora, 1979] which reported the anti-inflammatory action of *Fagara*, a known antisickling phytomedicine (Elekwa et al., 2005). *C. papaya* and *P. nigrescens* leaf extracts may have anti-inflammatory property as a result of the phytochemicals that can exert that property possibly contained in the extracts, which may assist in relieving the pains associated with sickle-cell crisis and also may prevent opportunistic infections in sickle cell disease. Flavonoids, glycosides and cardiac glycosides found in the extracts are suggestive of their antioxidant property. Flavonoid glycosides are reported to be antioxidants and used as anti-inflammatories in the treatment of capillary fragility (Iwu, 1993). Their presence in the extracts is an indication of the plants' potent antioxidant and membrane-stabilizing properties. This indication of antioxidant activity is further confirmed by the presence of the antioxidant nutrient vitamins, A and C and some amino acids.

Amino acids have long been associated with the antisickling activity of compounds (Acquaye et al., 1982). Phenylalanine has been reported to be the predominant antisickling agent in *C. cajan* seed extract (Ekeke and Shode, 1990). In this study, amino acids indicated in glutathione formation [cysteine, glutamic acid] were found to be present in the leaf extracts. Glycine, cysteine and glutamic acid are known precursors of glutathione, a naturally occurring protein that protects every cell, tissue and organ from toxic free radicals and diseases. Oxidative stress ensuing from lipid peroxidation of erythrocyte membranes is a leading sign of sickle cell crisis phenomenon. Intake of these nutraceutical herbs (papaya and parquetina leaves) supplies some of the required amino acids for glutathione production, as well as the antioxidant nutrients needed to protect the red blood cell membrane from lysis and destruction. The reported antisickling activity of the leaf extracts may in part be as a result of the presence of precursors for GSH biosynthesis present in abundance in the extract. However, an *in vivo* study may be required to substantiate this claim.

The possible use of papaya and parquetina leaf extracts as a nutraceutical is further supported by the presence of vitamins B_{12} and folic acid. Folic acid and vitamin B_{12} are useful in the synthesis of red blood cells, therefore, their presence in plant extracts are suggestive of the blood - building properties of the extracts. Magnesium was found present in the extracts. This is important because earlier studies reported that oral magnesium supplements reduce erythrocyte dehydration in patients with sickle cell disease and is indicated in several transport systems (De Franceshi et al., 1997). The leaf extracts may thus be a good source for these micronutrients needed for the optimal function of the Gardos Channel and other membrane transport systems

in the cell. Cyanogenic glycosides/anthocyanides are reported to be toxic and harmful secondary metabolites when present in a plant (Iwu, 1993). These were absent from the papaya and parquetina extracts thus suggesting that the leaves might be non-toxic. The presence of the antioxidant nutrient vitamins, A and C and some amino acids is a further indication of possible antioxidant activity of the extracts. These nutrients may act singly or synergistically to potentiate the plants' antisickling action. The aqueous-methanol extracts of *C. papaya* dried leaf and *P. nigrescens* leaves are therefore, recommended for appropriate clinical trials and governmental.

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