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Full Length Research Paper

Assessment of antioxidation potential of selected plants with antisickling property

Ngozi O. A. Imaga*, S. O. Adenekan, G. A. Yussuph, T. I. Nwoyimi, O. O. Balogun and T. A. Eguntola

Department of Biochemistry, College of Medicine, University of Lagos, P. M. B.12003, Idi-Araba, Lagos State.

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Methanol extracts of herbs hitherto reported to have antisickling activity namely, *Carica papaya* leaf extract, *Fagara zanthoxyloides* root extract, *Cajanus cajan* seed extract and *Parquetina nigrescens* leaf extract, were evaluated in this study. An assessment of their antioxidation potential was determined by assaying for their phytochemical constituents, total phenol content, scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total antioxidant status through the ferric thiocyanate method. The extracts have similar phytochemical constituents and exhibited high scavenging activity compared to gallic acid and ascorbic acid standards due to their relatively high total phenol content. These findings suggest that *C. papaya* leaf extract, *F. zanthoxyloides* root extract, *C. cajan* seed extract and *P. nigrescens* leaf extract are endowed with antioxidant phytochemicals which may act singly or synergistically to potentiate the antisickling action of the plants.

Key words: Antioxidant activity, antisickling plants, phytochemicals, scavenging, sickle cell anemia.

INTRODUCTION

Medicinal plants, otherwise known as phytomedicines, are plants that produce appreciable therapeutic effects when taken by an individual. Numerous investigators have shown that some phytomedicines contain phytochemicals with antioxidation potential which are responsible for their therapeutic effects (Iwu et al., 1984). Research into medicinal plants that can be used in the management of sickle cell anemia is ongoing in Nigeria. Some of the plants reported to have antisickling effects include *Fagara zanthoxyloides* roots, *Cajanus cajan* seed extract, *Parquetina nigrescens* leaf and root extract and *Carica papaya* leaf and unripe fruit extracts (Sofowora et al., 1975; Ekeke and Shode 1985; Oduola et al., 2006; Ogunyemi et al., 2008; Imaga et al., 2009, 2010).

Phytochemical screening of plants can reveal the presence or otherwise, of polyphenolic compounds, flavonoids and other relevant phytochemicals (Edeoga et al., 2005). The antioxidant activity of phenolics is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. The antioxidant properties of phenolics are important in scavenging and neutralization of free radicals which can cause damage to cells if left in the system (Aiyegoro and Okoh, 2009). Synthetic antioxidants which are commercially accessible have been reported to be toxic whereas plants reported to exhibit antioxidant activity are relatively safe for consumption (Iwu, 1993). Prior to this study, there is no report on the comparison of the inherent antioxidation potential of the four plants being studied here. This present study, therefore, investigated the *in vitro* antioxidant and free radical scavenging potential of these plants and a comparative assessment of their antioxidant efficacy.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade, purchased from Sigma Chemical Company.

Plant materials

Dried leaves of *C. papaya, F. zanthoxyloides* roots, *C. cajan* seed extract and *P. nigrescens* leaf were collected from the Forestry

*Corresponding author. E-mail: ngoziawaimaga@yahoo.com.

Table 1. Phytochemical analyses of the plant extracts.

Phytochemical constituent	*P. nigrescens methanol extract*	*C. papaya Methanol extract*	*C. cajan seed methanol extract*	*F. zanthoxyloides methanol extract*
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Cardiac glycosides	+	+	+	+
Anthraquinones (Free)	+	+	+	+
Anthraquinones (Bound)	+	+	+	+
Phlobatannins	+	+	+	+
Saponins	+	+	+	+
Cyanogenic glycosides	-	-	-	-

+ = Present; - = Absent.

Research Institute of Nigeria (FRIN) at Ibadan, Nigeria in December 2009. Specimen of each plant was deposited at the FRIN's herbarium. The plants were identified by the curator of the herbarium. The different plant samples were then washed with water, air-dried at 30°C, pulverized and stored in a sterile air-tight container for further experimental use.

Extraction of plant samples

The methanol extract of each plant sample was obtained by soaking 50 g of dried powdered samples in 500 ml of methanol for 24 h. The extracts were filtered using Whatman filter paper (125 mm), concentrated through rotary evaporation and stored at 4°C for further use. The resulting extracts were reconstituted with sterile distilled water to give concentrations used in this study.

Phytochemical screening

Qualitative chemical tests were carried out on the extracts and on the powdered specimens using standard procedures to identify the phytochemical constituents, namely, alkaloids, flavonoids, tannins and glycosides, as described by Edeoga et al. (2005).

Determination of total phenolic composition

The amount of phenolic compound present in the methanol extracts of the plants was determined with Folin-Ciocalteau reagent using the method of Aiyegoro and Okoh (2009) with little modification. To 0.25, 0.5, 0.75 and 1 ml of each sample of plant extract solution was added 5 ml of 10% Folin- Ciocalteau reagent. After 5 mins, 4 ml of Sodium Carbonate (0.7 M) was added. The resulting mixture was incubated at 45°C with shaking for 30 mins. The absorbance of the blue-coloured sample was then measured at 765 nm using UV/visible light (UV-VIS Genesys 8 Spectrophotometer). Results were expressed as percentage milligrams of gallic acid (0 - 0.5 mg/ml) dissolved in distilled water. Gallic acid was used as a standard and the equivalents were determined from a calibration concentration curve.

Determination of free radical scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was used to measure the free radical scavenging activity of the plant samples using the methods of Yen and Chen (1995); Aiyegoro and Okoh (2009); Liyana-Pathiana and Shahidi (2005) with little modifications. 1 ml of 0.1 mM DPPH prepared in methyl alcohol was mixed with 3 ml each of the methanol plant extracts ranging from 0.2 - 1 mg/ml. The reaction mixture was thoroughly vortexed and left in the dark at room temperature for 30 min. The absorbance of the resulting yellow coloured solution was then measured spectrophotometrically at 517 nm. 0.5 ml ascorbic acid and 0.5 ml gallic acid were used as positive controls/standards and deionized water used as blank. A decrease in absorbance of the reaction mixture indicates higher free radical scavenging activity. The scavenging ability of the extract was calculated using the standard equation (Liyana-Pathiana and Shahidi, 2005).

Total antioxidant assay

The antioxidant activity of the methanol extracts was determined using the ferric thiocynate (FTC) standard method as described by (Aiyegoro and Okoh, 2009) with some modification. Extracts with varying concentrations (0.2 -1 mg/ml) were mixed with 2.5 ml of linoleic acid emulsion and 2.5 ml of phosphate buffer, the resultant mixture was incubated at 60°C in the dark for 12 h to accelerate oxidation. To 0.1 ml of each sample solution was added 4.5 ml of ethanol, 0.2 ml ammonium thiocynate solution and 0.2 ml ferrous chloride. After 3 min, the absorbance of the resulting red-coloured mixture was measured at 500 nm for each concentration. 1 ml ascorbic acid was used as positive control, while the mixture without the extracts was used as the negative control. All experiments were done in triplicate.

RESULTS

The yield of extract was 40, 13.6, 37 and 11% for *C. papaya, F. zanthoxyloides, C. cajan* and *P. nigrescens* respectively. The plant extracts were screened qualitatively for phytochemical constituents and the results are shown in Table 1. Results showed the presence of alkaloids, flavonoids, glycosides and cardiac glycosides in all the plant extracts. Cyanogenic

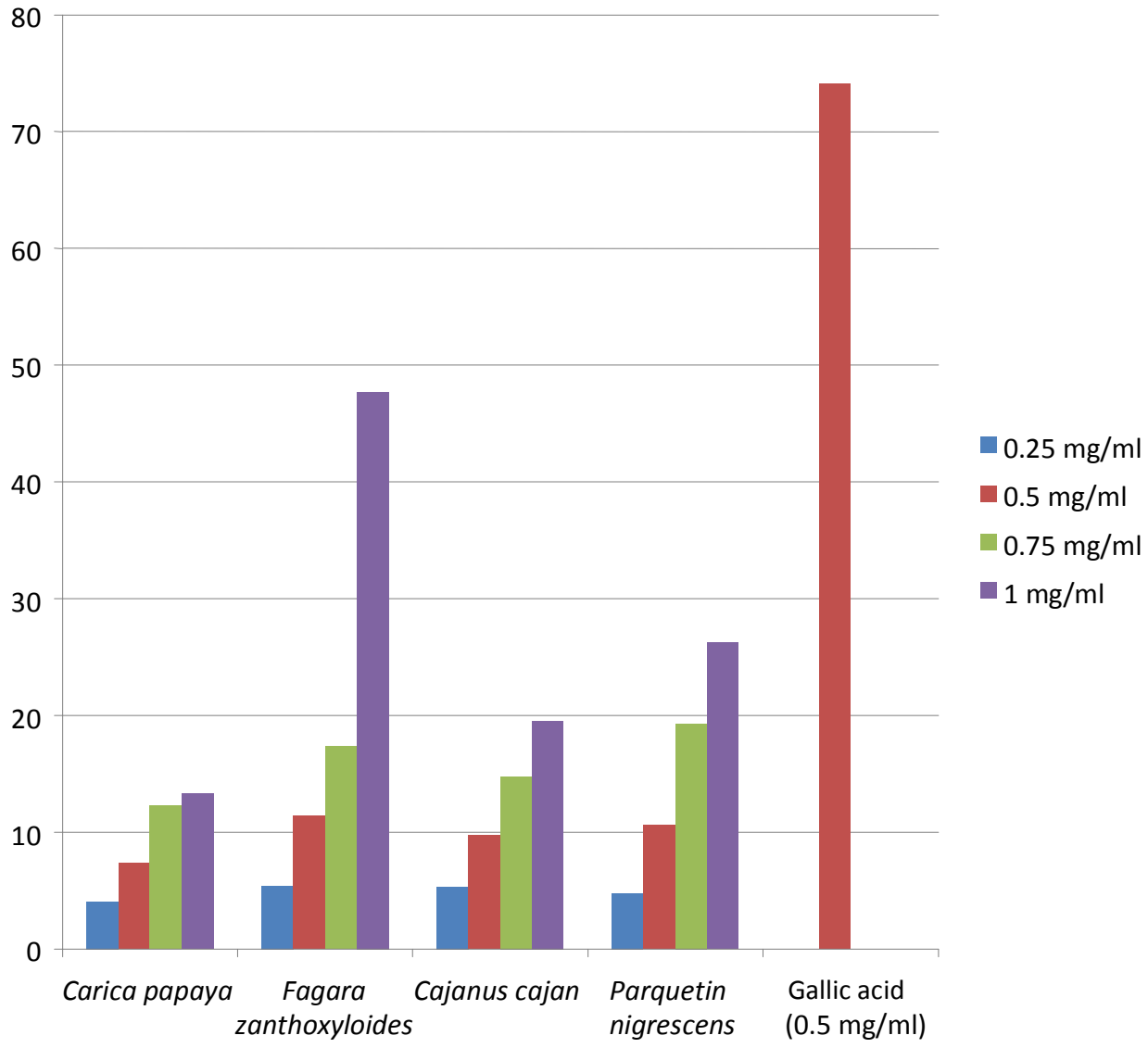

Figure 1. Total phenolic content of extracts.

glycosides/anthocyanides were absent from all the samples.

The total phenolic content of the methanol extracts of *C. papaya* leaf was 4.02, 7.35, 12.26 and 13.30% inhibition for 0.25, 0.5, 0.75 and 1 mg/ml concentrations respectively. For *F. zanthoxyloides* it was 5.41, 11.44, 17.33 and 47.74% inhibition for the same range of concentrations. For *C. cajan* the percentage inhibition was 5.33, 9.74, 14.73 and 19.52% for 0.25 - 1 mg/ml concentrations respectively. While that of *P. nigrescens* was 4.72, 10.63, 19.26 and 26.24% inhibition for the same range of concentrations. The total phenol content of the plant extracts reveal that the 1 mg/ml concentration of the plant extracts had the highest total phenolic content in this order: *F. zanthoxyloides* > *P. nigrescens* > *C. cajanus* > *C. papaya* (Figure 1).

The extracts showed appreciable free radical scavenging activities at the highest concentrations of 1 mg/ml on DPPH, (Figure 2) compared to ascorbic and gallic acid. The percentage inhibition of scavenging activities of the methanol extracts for DPPH was 14.27, 50.92, 67.95 and 87.28% respectively, for 0.25 – 1 mg/ml *C. papaya* extract; 18.53, 44.87, 56.66 and 89.43% respectively, for *F. zanthoxyloides* extract; 16.15, 38.57, 50.08 and 58.91% respectively, for *C. cajanus* extract; 14.86, 41.06, 62.75 and 78.23%, respectively, for *P. nigrescens* extract. All activities were concentration-dependent, comparing favourably well with the standards at all concentrations.

The *in vitro* total antioxidant assay of the plant extracts (Figure 3) revealed appreciable antioxidant potential compared with the standard ascorbic acid, as determined

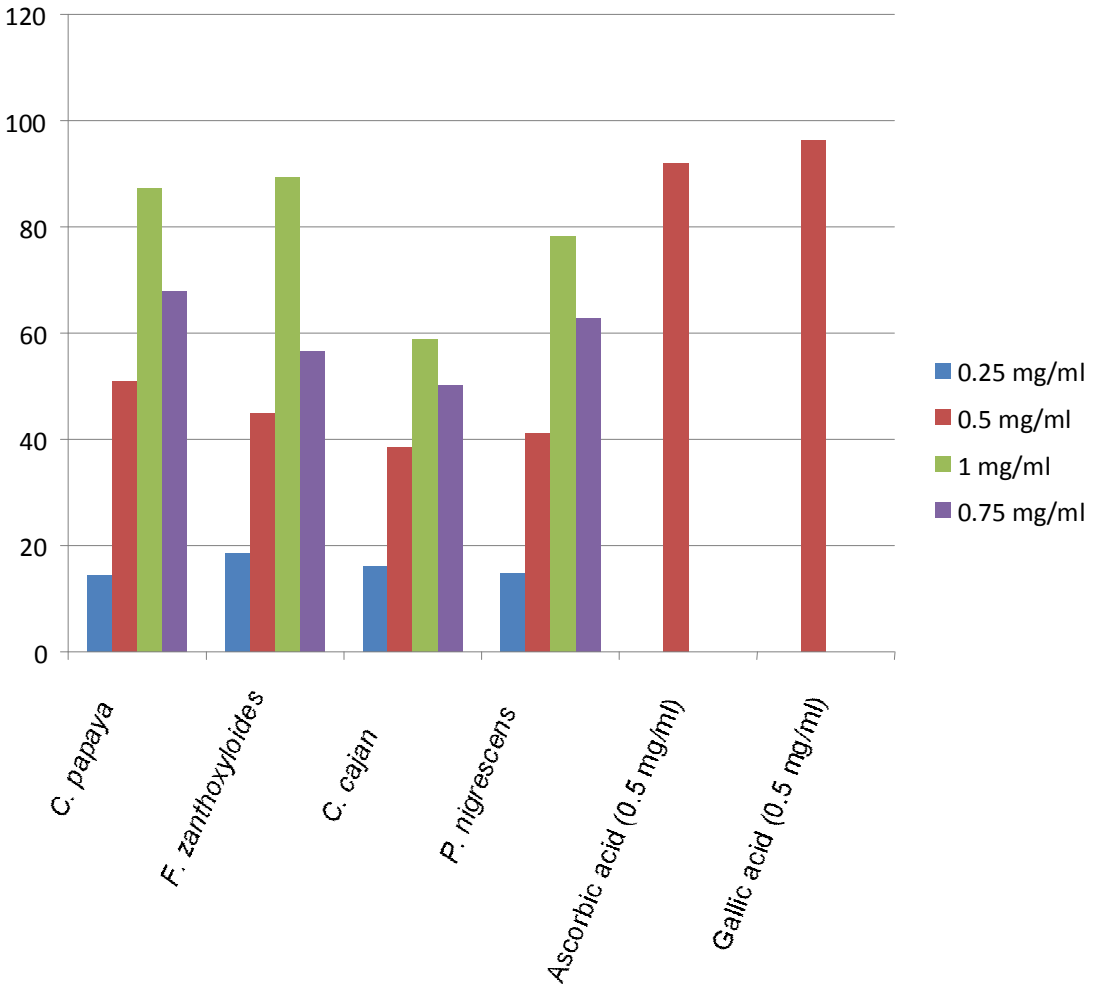

Figure 2. Percentage Inhibition (%I) of free radical scavenging by the plant extracts.

with the FTC method. *F. zanthoxyloides* extract showed the highest total antioxidant activity, followed by *C. papaya* extract, *C. cajanus* extract and *P. nigrescens* extract.

DISCUSSION

The phytochemical analyses of the extracts of dried leaves of *C. papaya, F. zanthoxyloides* roots, *C. cajan* seed extract and *P. nigrescens* leaf indicated the presence of phenolics, glycosides, flavonoids, tannins and saponins. This confirms earlier findings (Imaga et al., 2009, 2010). Phenol and phenolic compounds like flavonoids have been reported to possess significant antioxidant activities (Aiyegoro and Okoh, 2009). The four phytomedicines used in this study have high phenolic contents. These compounds are known to be biologically active through different mechanisms and may be the possible explanation of the reported antisickling effects of these four plants. Plants with antioxidant activities have been reported to possess free radical scavenging activity, which is a major contributor to severe diseases and disorders such as cancer, diabetes, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism (Aiyegoro and Okoh, 2009; Das and Pereira, 1990; Parr and Bolwell, 2009). This is further corroborated by the result of our total antioxidant assay using FTC, which indicates the ability of the phytomedicines to minimize oxidative damage to vital organs and tissues *in vivo.* The result of the DPPH scavenging activity assay in this study indicates that the extracts were potently active. The four extracts vary in their ability to scavenge free radicals, with *F. zanthoxyloides* being the most potent and *C. cajan* the least potent. These results imply that the plant extracts may be useful for treating radical-related pathological damage especially at higher concentration. Since the sickle cell anemic individual undergoes oxidative stress at the onset of crises, these plants can be used to mop up any free radical released and so contribute to the effective management of the disorder. These findings

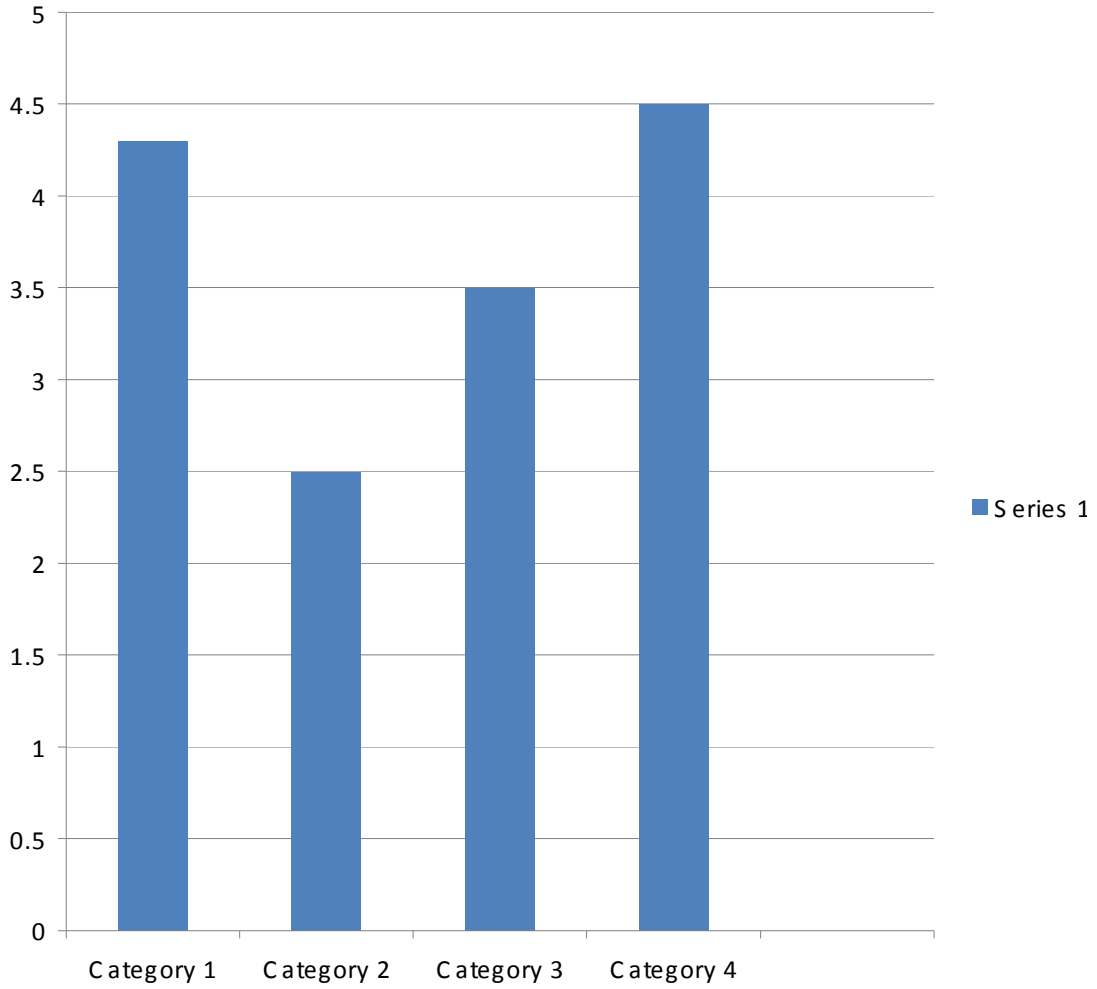

Figure 3. Comparison of total antioxidant property of peak concentrations (1 mg/ml) of each extract as determined by the FTC (500 nm) method.

may explicate the folklore use of these phytomedicines in the treatment, management and control of crises in individuals with sickle cell anemia.

REFERENCES

Aiyegoro OA, Okoh AI (2009). Phytochemical Screening and Polyphenolic Antioxidant Activity of Aqueous Crude Leaf Extract of *Helichrysum pedunculatum*. Int. J. Mol. Sci., 10: 4990-5001.

Das NP, Pereira TA (1990). Effect of flavonoids on thermal auto-oxidation of palm oil: Structure activity relationship. J. Am. Oil Chem. Soc., 67: 255–258.

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4 (7): 685-688.

Ekeke GI, Shode FO (1985). The reversion of sickled cells by *Cajanus cajan*. Planta Medica, 6: 504-507.

Imaga NO A, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, Kehinde MO, Bamiro SB (2009). Antisickling property of *Carica papaya* leaf extract. Afr. J. Biochem. Res., 3(4): 102-106.

Imaga NOA, Gbenle GO, Okochi VI, Adenekan SO, Edeoghon SO, Kehinde MO, Bamiro SB, Ajiboye A, Obinna A (2010). Antisickling and toxicological profile of *Parquetina nigrescens*. J. Med. Plants Res., 4(8): 639-643.

Iwu MM (1993). Handbook of African Medicinal Plants. CRC Press, USA, pp. 141-142.

Iwu MM, Igboko AO, Onwubiko H, Ndu UE (1984) Antisickling properties of *Cajanus cajan*: Effect on hemoglobin gelation and oxygen affinity. Planta Medica, 24: 431-432.

Liyana-Pathiana CM, Shahidi F (2005). Antioxidant activity of commercial soft and hard wheat *(Triticum aestivum* L) as affected by gastric pH conditions. J. Agric. Food Chem., 53: 2433–2440.

Oduola T, Adeniyi FAA, Ogunyemi EO, Bello IS, Idowu TO (2006) Antisickling agent in an extract of unripe pawpaw (*Carica papaya*): Is it real? Afr. J. Biotechnol., 5(20): 1947-1949.

Ogunyemi CM, Elujoba AA, Durosinmi MA (2008). Antisickling properties of *Carica papaya*, Linn, J. Nat. Prod., 1: 56-66.

Parr A, Bolwell GP (2009). Phenols in the plant and in man: The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. J. Sci. Food Agric., 80: 985–1012.

Sofowora EA, Issacs-Sodeye NA, Ogunkoya LO (1975). Antisickling properties of *Fagara*. Lloydia, 38: 169-171.

Yen G, Chen H (1995). Antioxidant activity of various tea extract in relation to their antimutagenicity. J. Agric. Food Chem., 43: 7–32.