

Determination of the Antioxidant, Phytochemical and Reducing Properties of Leaves of Some Commonly Consumed Fruit Trees

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ABSTRACT

The medicinal plants serve as an important component of the health care system in Nigeria. A large number of medicinal plants and purified constituents have shown beneficial pharmaceutical potentials. The quantity of phytochemicals present in different plants differs from one another. Therefore, the present study is to focus on the antioxidants properties of the leaves of some commonly consumed fruit tree in Nigeria which include Mango and Cashew leaves. The antioxidants potential of the leaf extract was examined by determining the Tannin content, total flavonoid content, total phenolic content, hydrogen peroxide scavenging activity and reducing power activity. The result from this study shows that Cashew leaves has the highest phytochemical contents (24.50mg/100g) flavonoid (25.80 mg/100g) and phenolic (29.58mg/100g) content respectively compared to Mango leaves with phytochemical contents tannin (23.89 mg/100g) flavonoid (20.75 mg/100g) and phenolic (11.44 mg/100g) content respectively. Also the result shows that Cashew leaves have the highest reducing power activity and hydrogen peroxide scavenging activity compared to Mango leaves. The order of decrease in reducing power is Cashew > mango. Owing to the antioxidants activities exhibited by these leaves extracts they could be considered as a natural herbal source of antioxidant materials that could be utilized in the food and pharmaceutical industries.

Keywords: Phytochemicals, flavonoids, tannins, phenolic, cashew, mango.

INTRODUCTION

The importance of medicinal plants to man cannot be over emphasized. Vast diversity of plants provides various kinds of medicinal values both in traditional and modern pharmaceutical industries. The medicinal value of plants has authenticated important dimension in the past few decades owing mainly to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential (Akinmoladun *et al.*, 2007). An antioxidant is a molecule which when in small quantity are able to inhibit or prevent the oxidation of other molecules. In nutrition, one of group of vitamin that act against the effect of free radical. The reactions of oxidants can produce free radicals and these free radicals can initiate a chain reaction in cells and destroying the infected cells.

Antioxidants terminate free radical chain reactions by removing free radicals, thereby controlling oxidation reaction (Sun *et al.*, 2007). A free radicals chain reaction is the major cause of lipid oxidation which is associated with cardiovascular disease and cancer. It is also the cause of rancidity of food (Grant, 2008; Fortunato *et al.*, 2007). Antioxidant can come from natural products or be made commercially. In the past, lipid oxidation has been controlled with the use of synthetic antioxidants such as butylated hydroxyl toluene, propylgallate and tertbutylhydroquinone (Fortunato, 2007). These synthetic antioxidants are suspected to be responsible for liver damage and carcinogenesis in laboratory animals (Van-Burden *et al.*, 1981). It is therefore necessary to shift interest from the use of synthetic antioxidants which are

harmful to the use of natural antioxidants from fruit and vegetables for the prevention of lipid oxidation.

Antioxidants prevent the human system by neutralizing the free radicals interactively and synergistically. Plants are rich source of free radical scavenging molecules such as, vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavanoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity.

There also exists a good antioxidant activity associated with constituent of plants flavonoid and phenol contents antioxidant activity. In the longer term, plant species (or their active constituents) identified as having high levels of antioxidant activity *in vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage.

Nigeria is blessed with fruit which are grown all over the thirty-six (36) states of the country including the Federal Capital Territory (FCT). It is common observation that these fruit are under-utilized with regard to their antioxidant properties and should therefore be studied thoroughly to determine their antioxidant capacity.

MATERIALS AND METHODS

Fresh leaves from mango and cashew trees were collected from local farms in Kalgo town of Kebbi State, Nigeria. They were taken into Biology laboratory, Federal University Birnin Kebbi for identification.

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The aqueous infusion of the plants was prepared (bearing in mind that people usually used water for the herbal preparation, same as native method of preparation) by cold softening maceration of 200 g of crushed plant material each in 1000cm³ of distilled water. It was kept in the laboratory at room temperature for 48 hours after which it was filtered using muslin cloth and filter paper. The filtrate was collected in a beaker and the water was allowed to evaporate over a water bath giving 19.5 g and 15.5 g extract concentrate of mango and cashew leaves. The concentrates were dissolved in normal saline for the following analysis.

Determination of Reducing Power

Reducing power of the extracts was determined according to the method reported in the previously (Jayanthi *et al.*, 2011). Various concentrations of the plant extracts (2.5 cm³) in corresponding solvent were mixed with phosphate buffer (2.5 cm³, 0.2 M, pH 6.6) and potassium ferricyanide (K₃Fe (CN)₆) (2.5 cm³, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 cm³) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 cm³) was mixed with distilled water (2.5 cm³) and FeCl₃ (0.5 cm³, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase reducing power. Ascorbic acid was used as standard.

Determination of H₂O₂ Scavenging Activity

The free radical scavenging activity of the extract, based on scavenging of hydrogen peroxide (H₂O₂) was estimated according to the procedure described previously (Ruch *et al.*, 1989). A solution of H₂O₂ (40mM) was prepared in phosphate buffer (pH 7.4). Extracts (50 µg/cm³) in distilled water at different concentration were added to the H₂O₂ solution (0.6 cm³, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution contained the phosphate buffer without H₂O₂. The scavenging effect was calculated using the expression:

$$\text{H}_2\text{O}_2 \text{ scavenging (\%)} = \frac{A^0 - A^1 \times 100}{A^0}$$

Where A⁰ is the absorbance of the blank sample and A¹ is the absorbance of the extract.

Determination of Phenolic Content

The amount of total phenolic compound contents was determined by the Folin-Ciocalteu method as reported previously (Gryglewski *et al.*, 1987). The extract samples of 0.5 cm³ and 0.1 cm³ of Folin-ciocalteu reagents (0.5N) were mixed and incubated for 30minute at room temperature. The absorbance of reaction was measured at 760 nm. The concentration of total phenol was expressed as Gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

Determination of Flavonoid Content

Total soluble flavonoid was determined with aluminium chloride using quercetine as standard (Pourmorad *et al.*, 2006). A 1cm³ of sample solution (1000µg/cm³) was mixed with 3cm³ of methanol, 0.2cm³ of 10% aluminium chloride, 0.2cm³ of potassium acetate and 5.6 cm³ of distilled water. The resulting mixture was incubated at room temperature for 30 minute and the absorbance of the reaction was measured at 415nm. The calibration curve was prepared by preparing quercetine solution at various concentrations in methanol.

Determination of Tannin Content

This was based on the method described (Moulisha *et al.*, 2010). 500mg sample of concentrate was added to 50cm³ of distilled water, and shaken for one hour. 5cm³ aliquot of the filtrate was mixed with 2cm³ of 0.1M FeCl₃ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 720nm within 10 minutes.

RESULT AND DISCUSSION

Reducing Power

Fig. 1 shows the reducing abilities of Mango and Cashew leaves at different concentrations of 20, 40, 60, 80 and 100 µg/cm³ respectively. At concentration of 20 µg the reducing abilities of Mango and Cashew are 0.5 and 0.28, at 40 µg/cm³, the reducing abilities increases to 0.51 and 0.47, at 60 µg/cm³ the reducing abilities are 0.66 and 0.67, at 80 µg/cm³ the reducing abilities are 0.66 and 1.14 and at 100 µg the reducing abilities are 0.99 and 1.27 respectively.

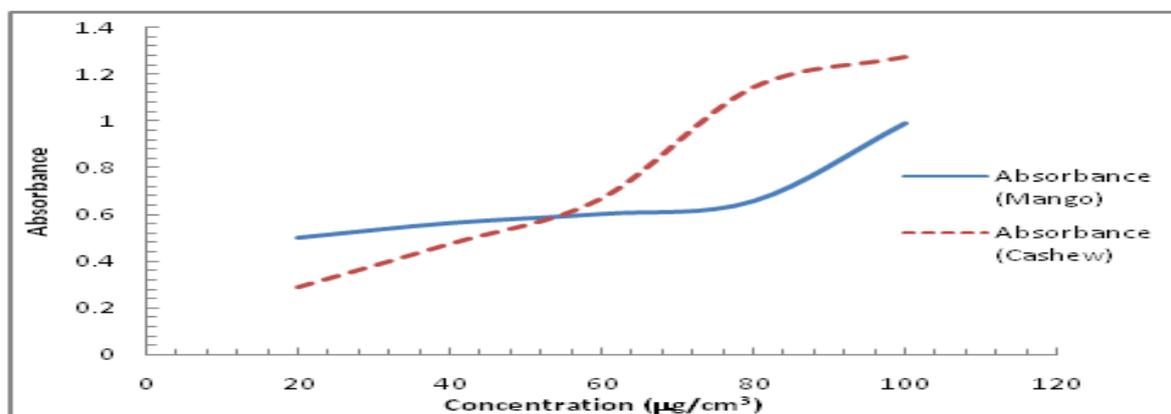


Fig. 1: The Reducing ability of Mango and Cashew leaves

This goes to show that at low concentrations (20-40 $\mu\text{g}/\text{cm}^3$) the Mango leaves exhibits higher reducing abilities, while at higher concentrations (60-100 $\mu\text{g}/\text{cm}^3$) the Cashew leaves overtakes the Mango leaves by exhibiting higher reducing ability.

The reducing powers of a compound are related to its electron transfer ability and therefore, serve as a significant indicator of its antioxidant activity (Chang *et al.*, 2002). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipids peroxidation processes, so that they can act as primary and secondary antioxidants (Jayanthi *et al.*, 2011).

Antioxidant activities and reducing power are related to one other. From Fig. 1, it can be deduced that there are variations in the antioxidant properties of the leaves, with increases in concentrations of the extracts. As can be seen from the graph there is interconnectivity between the reducing powers of the leaves, where at a concentration of 54 $\mu\text{g}/\text{cm}^3$ the reducing powers of the two leaves equate with one another at a value of 0.59. At this junction, the reducing powers of Cashew leaves thereafter move to higher values compared with the reducing powers of the Mango leaves.

From the results it was observed that the cashew leaves have the highest reducing power activities at the concentration of 60, 80 and 100 $\mu\text{g}/\text{cm}^3$, while the mango leaves have the highest reducing powers at lower concentration of 20 and 40 $\mu\text{g}/\text{cm}^3$.

Statistical analysis of variance for the means of the reducing powers of the leaves shows that there is a significant difference between the two extracts.

Statistical analysis of variance for the means of the reducing powers of the two leaves shows that there is a significant difference across the various concentrations of the extracts.

Statistical analysis on the effect of the concentrations on the reducing powers of the extracts shows that strong dependence of the reducing power on the leaves extracts.

Fig. 2 is a plot of the reducing powers of ascorbic acid, used as standard, Mango and Cashew leaves at a concentration of 50 $\mu\text{g}/\text{cm}^3$. From the plots it can be seen that the reducing powers of the two extracts by far exceed the standard reducing value of the ascorbic acid. As such, we may deduce that the two extracts exhibit reducing property that is more powerful than that of the standard solvent. Observation has also indicated that at the concentration of 50 $\mu\text{g}/\text{cm}^3$ Mango and Cashew leaves have equal reducing powers.

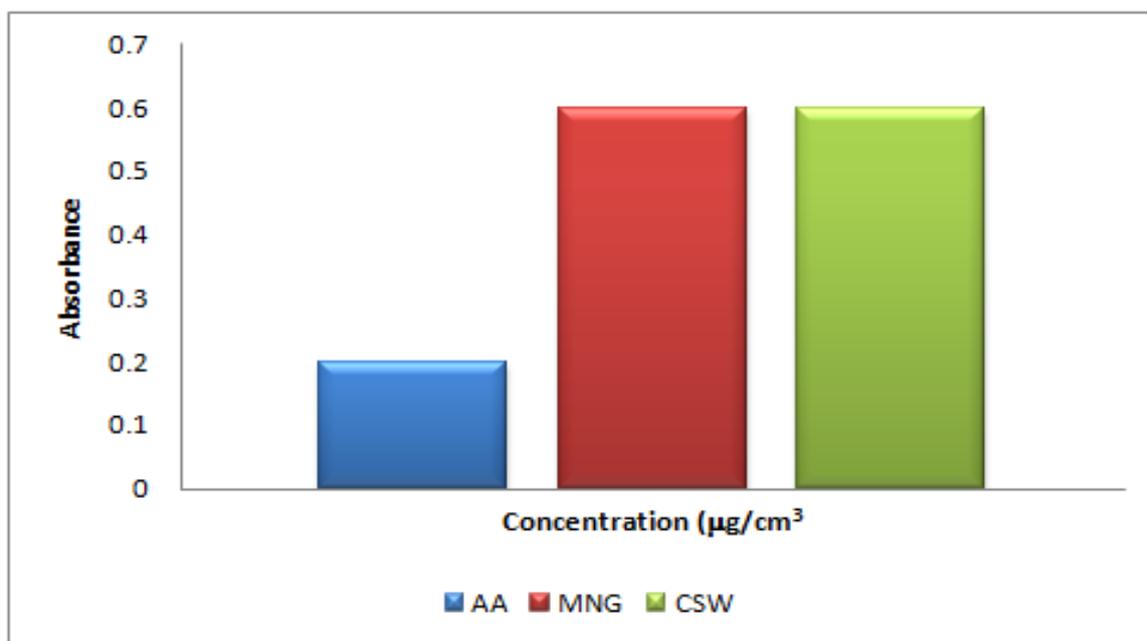


Fig. 2: The Standard Reducing Power of Ascorbic acid (AA), Mango Leaves and Cashew Leaves at Concentration of 50 $\mu\text{g}/\text{cm}^3$.

Scavenging Activity

With regards to the scavenging activities of the leaf extracts the result is depicted in fig. 3. The result shows that at concentrations of 20 $\mu\text{g}/\text{cm}^3$ the scavenging activity of Mango is 50.2%, while that of Cashew is 37.9%, at concentration of 40 $\mu\text{g}/\text{cm}^3$ the scavenging activity of Mango is 53%, while that of Cashew is

48.7%, at concentration of 60 $\mu\text{g}/\text{cm}^3$ the scavenging activity of Mango is 54.8%, while that of Cashew is 57.8%, at concentration of 80 $\mu\text{g}/\text{cm}^3$ the scavenging activity of Mango is 57.4, while that of Cashew is 75.5% and at concentration of 100 $\mu\text{g}/\text{cm}^3$ scavenging activity of Mango is 70.4%, while that of Cashew is 79.7% respectively.

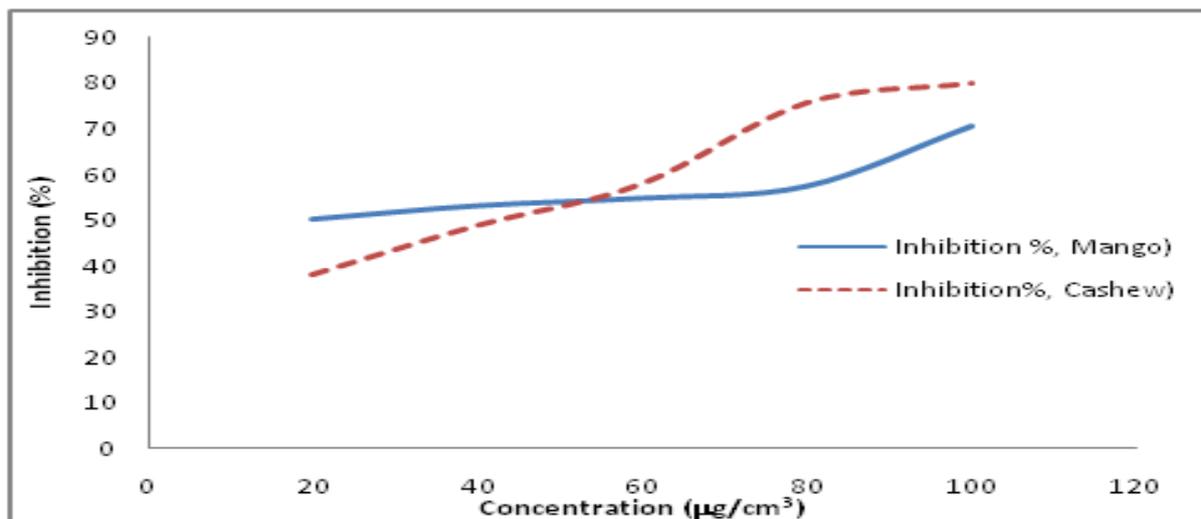


Fig. 3: The Scavenging activity of Mango and Cashew leaves

From the graph in Fig. 3 shows crossover concentration of 54µg/cm³ the scavenging activities of the two leaves equate at a value of 54.3%. After this point, the scavenging activities of Cashew leaves move to higher values compared with those of the Mango leaves. We can deduce, therefore, that at lower concentrations (20 and 40 µg/cm³) the scavenging activities of the leaves decreases in the following order: Mango > Cashew and at higher concentrations the scavenging activities of the leaves increases in the following order: Mango < Cashew.

Statistical analysis of variance for the mean of the scavenging activities shows that there is a significant difference between the two leaf sample extracts.

Statistical analysis of variance for the means of the scavenging activities of the two leaf extracts shows that there is a significant difference across the various concentrations of the extracts.

Statistical analysis on the effect of the concentrations on the scavenging activities of the extracts shows that there is a strong dependence of the scavenging activities on the leaf extracts.

Table 1: Phytochemical Contents of Mango and Cashew Leaves

Samples	Flavonoid (mg/100g)	Phenolic (mg/100g)	Tannin (mg/100g)
Mango Leaves	23.89 ± 0.03	20.75 ± 0.03	11.44 ± 0.04
Cashew Leaves	25.80 ± 0.03	29.58 ± 0.03	24.50 ± 0.03

Phytochemical Contents

Flavonoid contents

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Prenzler *et al.*, 2001). The flavonoid content of mango and cashew leaf as shown in table are 23.89 and 25.80 mg/100g respectively. Antioxidant property of flavonoids is believed to play a significant role in the lower cardiovascular mortality associated with higher flavonoid intake. The flavonoid content of the leaves decreases in the following order: Cashew > Mango.

Phenolic contents

Some evidence suggests that the antioxidant activities of plant species are thought to be due to their phenolic compounds contained in them (Poumorad *et al.*, 2006). From Table 1 the results show that the Cashew leaves contain the highest amount of phenolic content (29.58 mg/100g) compared to Mango leaves (20.75 mg/100g) at 760 nm. Perhaps, this could have explained the reason why the reducing powers of Cashew leaves are higher than that of the Mango leaves at higher solvents concentrations as mentioned in the aforementioned paragraph. The order of decrease in the phenolic content is as follows: Cashew > Mango.

Tannin content

Tannins are complex polyphenolic substances found in plants. Their antioxidant properties of the plant are associated with the presence of the phenolic compounds contained in the plant. From Table 1, the results show that the tannin contents of Cashew leave (24.50 mg/100g) exceeds by far, the tannin contents of the Mango leave (11.44 mg/100g) at wavelength of 720nm. The tannin contents in the extracts decreased in the following order: Cashew > Mango.

CONCLUSION

Antioxidant properties of various extracts of Mango (*Mangifera indica*) leaves and Cashew (*Anacardium occidentale L*) leaves were analysed in this study. The results from this work show that Cashew leaves are the best source of antioxidant materials as against the Mango leaves. In addition, from the content of the reducing power of the extract it can be deduced that there is a huge potential in the use of the extracts as replacements of the synthetic antioxidant materials in the food and

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pharmaceutical industries. As a natural source, these extracts when used as antioxidants could prevent lipid oxidation, cell damage and other associated fatal diseases that could cause death. However, further studies are needed to establish the actual compounds that may be responsible for the activities observed from the tested leaves.

Conflict of Interest

Authors wishes to pledge that there was no conflict of interest between the two parties during the course of the conceptualization and execution of this research work.

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