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PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF PERICAP OF *GARCINIA KOLA* EXTRACT

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ABSTRACT
The powdered pericap of the plant was extracted with methanol at room temperature of 25^{O}C, partitioned with n-hexane, ethylacetate and butanol respectively. The extract and fractions were subjected to phytochemical analysis using established standard procedures. Antioxidant screening using DPPH (2, 2−diphenyl−1−picryl hydroxyl radical) procedure was carried out on the fractions and compared with ascorbic acid as standard. The result of the screening revealed that the pericalp contained alkaloids, flavonoids, tannins and saponins as chemical constituents. The (DPPH) radical scavenging assay of the plant revealed high antioxidant activity.There was significant activity among the extract and fractions, with the petroleum ether fraction showing the highest activity. The study demonstrated that the pericap of the plant is a good source of antioxidants.

Keywords: Antioxidants, phytochemical, extracts, pulp, Garcinia kola

INTRODUCTION

The term antioxidant originally was used to refer specifically to a chemical that prevented the consumption of oxygen. In the late 19th and early 20th century, extensive study were devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines (Valko et al., 2007).

Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity (German, 1999). Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamins A, C and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms (Matil, 1947; Vertuani *et al*., 2004).

The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized. Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells (Valko et al., 2007).

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Matil, 1947). Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells (Iwu, 1993). Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation

Garcinia kola is an ethnomedicinal plant used in the tropics for the treatment of some disease conditions. *Garcinia kola* is a widespread tree of evergreen forests and is found from the Democratic Republic of Congo to Ghana, where it occurs in the wet and moist semi-deciduous forest zones, in savannah as well as forest. It is cultivated and distributed throughout West and Central Africa. Medicinal uses include purgative, antiparasitic and anti-microbial (Iwu, 1993). The seeds are used in the treatment of bronchitis and throat infections (Adegbehingbe et al., 2008). They are also used to prevent and relieve colic, cure head or chest colds and relieve cough. The plant is also used for the treatment of liver disorders and as a chewing stick (Iwu, 1993; Akintonwa and Essien, 1990).

It is most effective against *Corynebacterium diphtheriae*, *Streptobacillis* sp., *Streptococcus* sp., *Neisseria* sp., *Pseudomonas aeruginosa*, *Salmonella* sp. The seeds are used in the treatment of bronchitis and throat infections. This plant has shown both anti-inflammatory and analgesic activities (Olaleye *et al.,* 2000). *G. kola* nut by the natives is used as an aphrodisiac. *Garcinia cambogia* is postulated to be a potential antiobesity agent (Valko *et al*., 2007).

No literature exists on the phytochemistry and antioxidant activity of the pericap of *G. kola*. The study was therefore aimed at investigating the phytochemical components and the antioxidant property of the pericap of *G. kola* using DPPH model.

MATERIALS AND METHOD

Collection of plant material

The ripped fruits of *Garcinia kola* were freshly plugged from the tree (not picked) in Nnewi South Local Government Area, Anambra State, Nigeria. The fruit was identified and authenticated by Mr. A. Sunny of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City.

Preparation of plant sample

The fresh riped *Garcinia kola* fruits were cut open with the aid of a kitchen knife and the seed removed. The pericarps were size reduced to a reasonable small size with the knife to enhance crushing. In order to increase the surface area, the size reduced pericarps were then crushed to a moderately fine size with the aid of a local wooden mortar.

Extraction of plant powder

The plant powder (1kg) was exhaustively extracted with 7litres of methanol by maceration process for 48 hours. The extract was filtered using Whatman filter paper No 11 and the filtrate was concentrated to dryness using a rotary evaporator at reduced pressure and temperature (30^{o}C). The dried extract was weighed and kept in the refrigerator at a temperature of -4^{o}C for a period of 48 hours.

Pre -fractionation

he crude extracts obtained from the mathanolic extraction of the pericarp of *Garcinia kola* was fractionated using petroleum ether, ethyl acetate and n-hexane and butanol.

Phytochemical screening

Phytochemical screening was carried out on the crushed *Garcinia kola* pericarp sample using standard procedure to identify the secondary metabolites (alkaloids, tannins, saponins, glycosides etc) using standard experimental procedures (Harbone, 1973; Sofowora, 1980;1993 and Trease and Evans, 1989).

Thin layer chromatography

The crude methanolic extract was partitioned into hexane, ethylacetate and butanol fractions. The ethylacetate fraction was subjected to preliminary thin layer chromatographic analysis using different solvent systems. The spots were visualized by UV lamp, concentrated sulphuric acid and ferric chloride.

Determination of antioxidant activity

The antioxidant activity of *G. kola* pericap was measured using the modified method of (Falodun *et al.,* 2009). The radical scavenging activity of the methanolic extract of the pericarp of *Garcinia kola* and its ethyl acetate and petroleum ether fractions against 2, 2−diphenyl−1−picryl hydroxyl radical (SIGMA ALDRICH®) were determined by ultraviolet (UV) spectrophotometry at 517nm. Vitamin C (ascorbic acid) was used as a standard. The following concentrations of the crude extracts ethyl acetate, petroleum ether fraction and Vitamin C were prepared, 0.02mg\ml, 0.04mg\ml, 0.06mg\ml, 0.08mg\ml, and 0.10mg\ml in methanol. 5ml of each of the concentrations was placed in a clean test tube and 0.5ml of 1mM of DPPH in methanol was added to each of the test tube. A blank solution was prepared to contain 5ml of methanol and 0.5ml 1mM of DPPH in a test tube.

On addition of the 0.5ml of 1mM DPPH, the solution was shaken and allowed for 15mins incubation before measuring the UV absorbance. The measurements were triplicated.

The radical scavenging activity was calculated using the formula:

$$\%\ inhibition = \left\{\frac{Ab - Aa}{Ab}\right\} \times 100$$

Where Aa = absorbance of the test samples; Ab = absorbance of blank

Statistical analysis

Data were expressed as mean ± standard deviations (SD) of three replicate determinations and then analyzed by SPSS V.13 (SPSS Inc., Chicago, USA). One way analysis of variance (ANOVA) and the Duncan's New Multiple-range test were used to determine the differences among these means. *P* values < 0.05 were regarded to be significant.

RESULTS AND DISCUSSION

Organoleptic evaluation showed that the fresh *Garcinia kola* pericarp is orange-red in colour, slightly bitter with a fruity smell while the crude extract is a thick syrupy black semi-solid mass.

The result of the phytochemical screening of the pulp extract is shown in Table 1. The phytochemical analysis of the extract of fresh *Garcinia kola* pericarp showed the presence of secondary metabolites such as anthraquinones, flavonoids, cardiac glycosides, reducing sugars, terpenoids and tannins. Saponins and alkaloids were found to be absent. These chemical components are also present in the seeds of the plant (Iwu, 1993).

The thin layer chromatography (TLC) carried out for the ethyl acetate fraction showed the best resolution with four (4) spots after development in a solvent mixture of petroleum ether: ethyl acetate in the ratio of 9:1. On non destructive analysis using UV lamp at 254 nm, the four spots fluorescenced. Destructive analysis using reagents like concentrated sulfuric acid (H_2SO_4), ferric chloride and Dragendorff's reagent showed no reaction.

The TLC for the petroleum fraction, showed only two (2) spots on development in a solvent mixture of methanol: ethyl acetate in the ratio of 1:3, which on non destructive analysis using a UV lamp at 254 nm revealed the two spots as dark, non-florescencing.

No reaction was seen on destructive analysis using the solvents; concentrated sulfuric acid (H_2SO_4), ferric chloride ($FeCl_3$) and Dragendorff's reagent.

The radical scavenging activity of the plant is shown in Figures 1- 4. The measurement of antioxidants is not a straight forward process as this is a diverse group of compounds with different reactivity to different reactive oxygen species. The free radical scavenging activity of the crude extract and petroleum ether and ethyl acetate fraction were evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the extract and its fractions with stable free radicals initiated by the DPPH, because of the odd number of electrons which could be due to the flavonoidal content. DPPH shows strong absorption band at 517nm. As the electrons become paired off in the presence of the extract and its fractions (free radical scavengers), the absorption varnishes or reduces.

The percentage inhibition of the free radical was dose dependent. Increase in concentration gave corresponding increased % inhibition. From the spectrum of % inhibition by DPPH against concentration of petroleum ether fraction (fig 4) showed an increasing scavenging of the free radical with maximum scavenging at 0.08mg/ml and a sharp decrease at 0.1mg/ml (minimal scavenging). The spectrum of ethyl acetate fraction(fig 3) showed decrease in scavenging from 0.02 mg/ml (maximum) to the lowest at 0.08 mg/ml while that of the crude methanolic extract (fig 4) showed decrease from 0.02mg/ml (highest) to 0.10mg/ml (lowest scavenging).

The radical scavenging property of petroleum ether fraction (fig 4) showed a close resemblance to that of ascorbic acid (fig 2). Ascorbic acid is a known and potent antioxidant agent used in medicines (Padayalty *et al.*, 2003). Ascorbic acid functions as an antioxidant L-ascorbic acid, its salts (sodium-L-ascorbic and calcium-L-ascorbate), and its isomers (D- and L-iso ascorbic acid) are classified and generally recognized as safe substances by Food Drug and Administration (FDA). The presence of the secondary metabolites in the pulp extract of this plant could be responsible for its antioxidant activity. For example, flavonoids and other phenolic constituents have been shown to play a preventive role in the development of cancer and heart diseases, potential sources of antioxidant compounds have been searched in several types of plant material such as vegetables, fruits, leaves, oil seeds, cereal crops, bark and roots, spices and herbs and crude plant drugs (Wang *et al.,* 2000; Pourmorad *et al.*, 2006; Kumaran and Karunakaran, 2007). Flavonoid and other plant phenolic such as phenolic acids, stillbenes, tannins, lignins e.t.c. are especially common in leaves, flowering tissue and woody parts such as stems and barks.

Table 1: Phytochemical screening of pericap of *G. kola* seeds

Phytochemicals	Components
Tannins	+
Saponin	+
Anthraquinone	-
Flavonoid	+
Cardiac glycosides	-
Reducing sugar	+
Terpenoid	+

- = Negative; + = positive

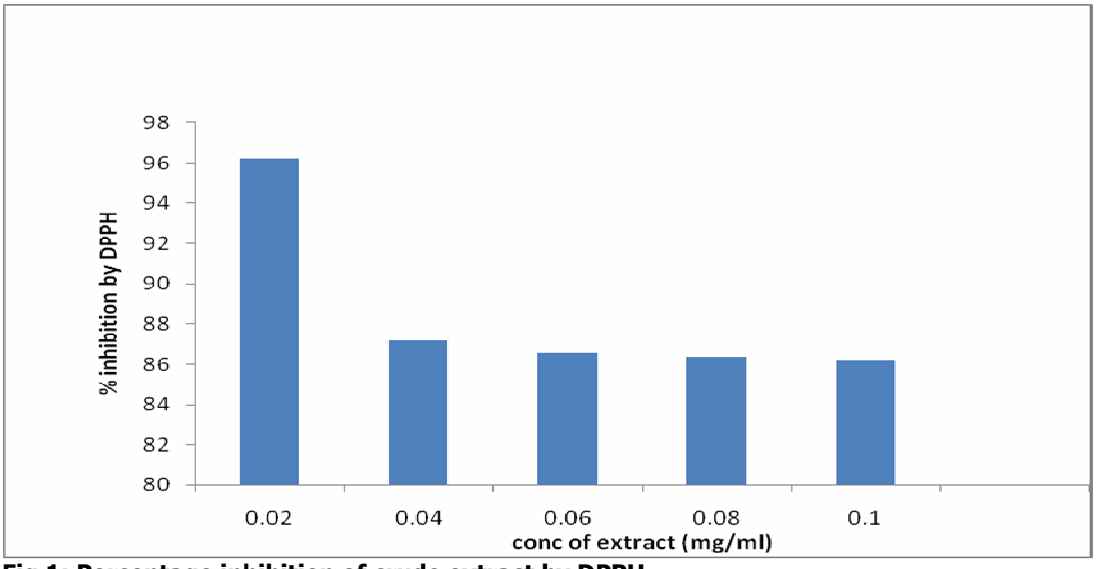

Fig 1: Percentage inhibition of crude extract by DPPH

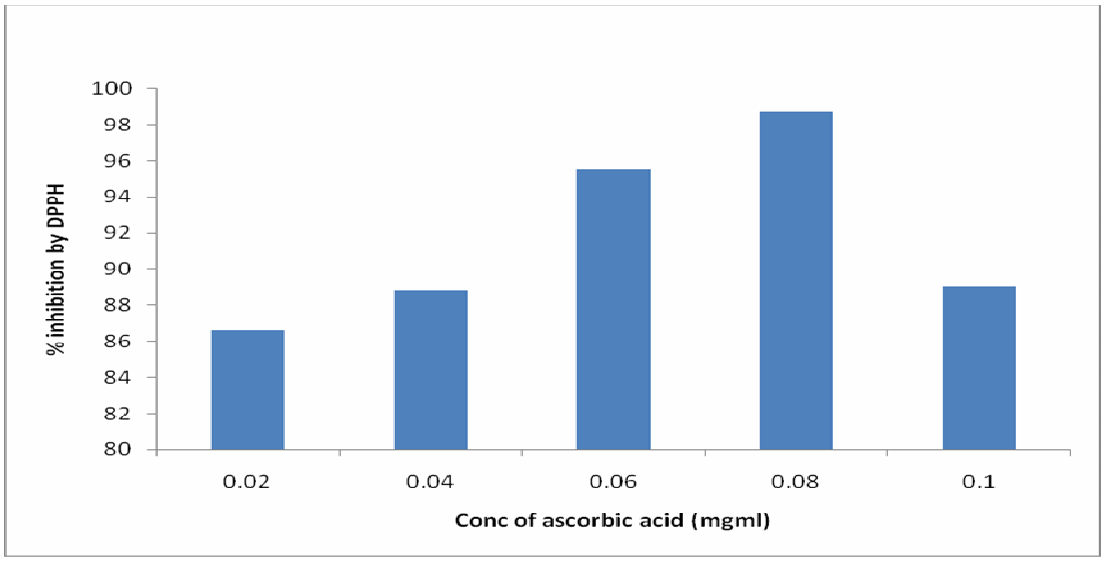

Fig. 2: Percentage inhibition of ascorbic acid by DPPH

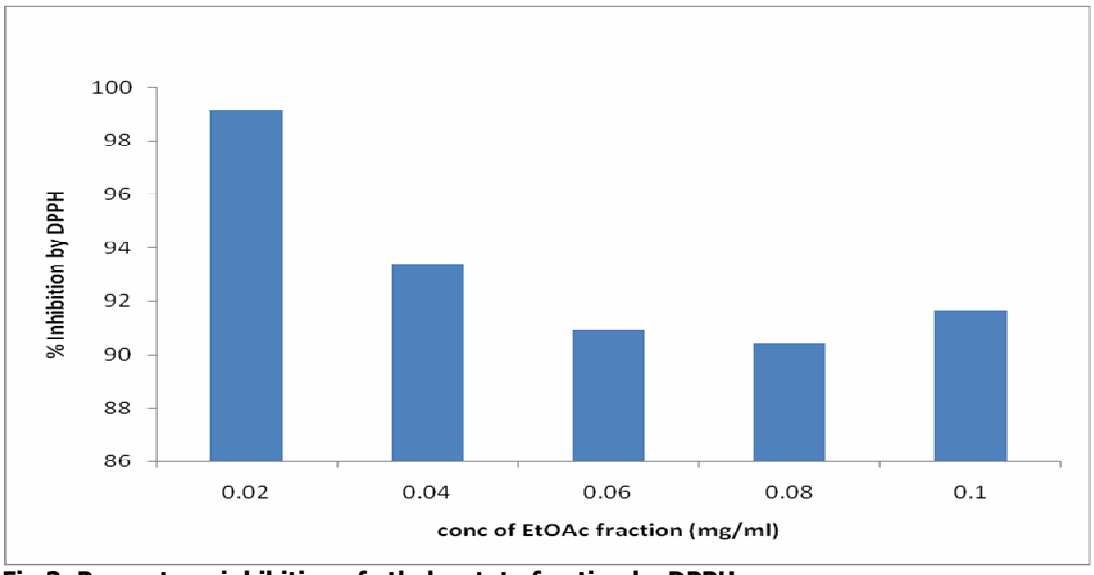

Fig.3: Percentage inhibition of ethylacetate fraction by DPPH

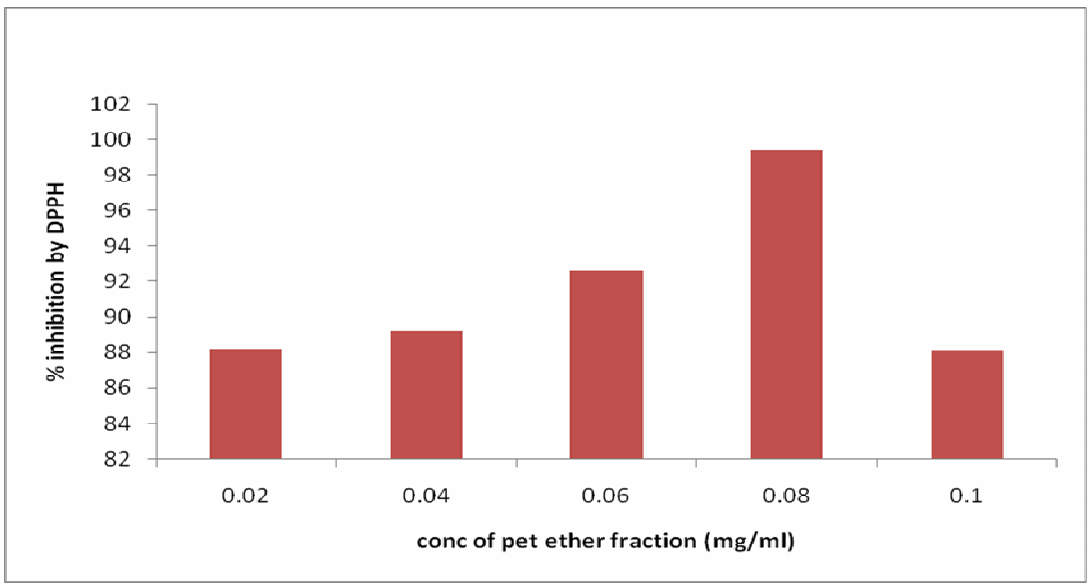

Fig. 4: Percentage inhibition of petroleum ether fraction by DPPH

CONCLUSION

The fresh pericarp of *Garcinia kola* has a lot of antioxidant property, this could justify its claims as antimicrobial, anti-inflammatory, anti-HIV, anti-obesity, anticancer, anti-ulcer, hepatoprotective, hypoglycemic, spermatogenic and aphrodisiac and its folklore use by elderly to prolong age (anti-aging). The fresh pericarp of *Garcinia kola* showed significant antioxidant property, hence, is a potent antioxidant agent. Future studies will involve the isolation and characterization of the chemical principles responsible for its antioxidant activity.

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