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Antioxidant Activities of the Leaf Extract and Fractions of *Cola lepidota* K. Schum (sterculiaceae)

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Abstract

***Cola lepidota* (CL) K. Schum (sterculiaceae) used in Nigeria folk medicine as febrifuges, pulmonary problems and cancer related ailments. This study evaluated scientifically the *in vitro* antioxidant activity of extract and fractions with a view to validate its folkloric usage. *In vitro* antioxidant properties of extract and fractions were evaluated using the free radical scavenging activities by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) with ascorbic acid as control. The total antioxidant activity results indicated that, the inhibition percent of chloroformic extract was significantly higher than the inhibition percent of methanol in the DPPH methods. A higher IC_{50} (50 µg/ml compared to 190 µg/ml methanol extract) value for free radical scavenging was found for chloroformic fraction when compared with the methanolic extract. These findings present the extract with significant antioxidant properties which may account in part for its anti -cancer activities.**

Key words: *Cola lepidota*, antioxidant activity, leaf extract, phytochemicals,

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Introduction

There is a renewed interest in the last decade to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes which stem from the fact that plant derived products have great potential as sources of pharmaceuticals. The medicinal properties of plants have been investigated in recent scientific developments throughout the world, due to their potential antioxidant activities, with reduced side effects and economic viability (Auudy *et al*., 2003). Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants and some fruits as antioxidants in reducing such free radical induced tissue injury (Schuler, 1990). Free radicals are chemical compounds which contain an unpaired electron spinning on the peripheral layer around the nucleus (Miller *et al*., 1990). The family of free radicals generated from oxygen is called reactive oxygen species (ROS) and those generated from nitrogen are called reactive nitrogen species (RNS). They are chemically aggressive molecules which react with different types of macromolecules in the body to cause damage to vital cell constituents such as DNA, proteins and lipids (Harman, 1981). ROS are ions, atoms or molecules that have the ability to oxidize reduced molecules. ROS include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH·), as well as non-free radicals (H_2O_2) and singlet oxygen (Halliwell, 2005).

In the body, free radicals are derived from two sources: endogenous sources, e.g. nutrient metabolism, ageing process etc and exogenous sources e.g. tobacco smoke, ionizing radiation, air pollution, organic solvents, pesticides (Buyukokuroglu *et al*., 2001). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996). Cells are equipped with natural mechanisms to fight against ROS and to maintain the redox homeostasis of cell. For example, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging the free radicals and preventing cell injury (Bergendi *et al*., 1999). In addition to antioxidant enzymes,

nonenzymatic molecules, including thioredoxin, thiols, and disulfide-bonding play important roles in antioxidant defense systems. Some of the compounds are of an exogenous nature and are obtained from food, such as α-tocopherol, β-carotene, and ascorbic acid, and such micronutrient elements as zinc and selenium (Farrukh *et al*., 2006). Medicinal plants are important sources of antioxidants (Rice-Evans, 2004). It has been reported that the use of fruits and vegetables containing antioxidant agents diminish the possibility of chronic diseases such as diabetes, cancer and cardiovascular diseases (Myojin *et al*., 2008; Saha *et al*., 2004; Horax *et al*., 2005; Semiz and Sen, 2007). Plants contain many phytochemicals that are useful sources of natural antioxidants, such as phenolic diterpenes, flavonoids, tannins, phenolic acids (Lee *et al*., 2004), and Polyphenols (Bernardi *et al*., 2008). Several synthetic antioxidants are in use, but have however been reported (Ito *et al*., 1983) that they have several side effects such as risk of liver damage, and carcinogenesis in laboratory animals (Gao *et al*., 1999; Osawa and Namiki, 1981). In order to avoid these effects, more effective, less toxic and cost effective antioxidants need to be developed. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants.

The plant *Cola lepidota*, also known as monkey cola in West Cameroons, *Duala,* mbwid; oji ochicha (cockroach cola) or achicha (Iwu, 1993) amongst the Ibo speaking people of Nigeria, belongs to the sterculiaceae family. It grows wild and sometimes cultivated in the tropics especially distributed in lower Guinea, Gabon, Western Cameroon and Eastern Nigeria. The phyto-constituents found in the seed of *Cola lepidota* include falvones; glycosides, saponins, steroids (Burkill, 1985). The seed has been used as febrifuges, for pulmonary disorders, and as an anticancer (Engel *et al*., 2011).

Until now, there has not been a report on the antioxidant activity of any part of *Cola lepidota*. As part of an ongoing biological evaluation of medicinal plants for their therapeutic uses, the current study was carried out to determine the free radical scavenging activity *viz avis* the antioxidant activity.

Materials and Methods

Plant materials: Fresh leaves of *Cola lepidota* for this experiment were collected in June 2011, at Umuosu Nsulu, Abia state, south Eastern Nigeria. The plant was identified and authenticated by the Forest Research Institute, Ibadan, Nigeria and a herbarium copy deposited in the same institute.

Processing of the plant material and preparation of plant extracts: The air dried and powdered leaves (6.5 kg) were extracted exhaustively with 7.5 L methanol in a Soxhlet apparatus. The extract (150.5 g) was partitioned into different solvents in increasing order of polarity, Petroleum ether 49.6 g, Chloroform, 42 g, ethyl acetate 7.8 g, all samples kept at 4°C till use.

Screening Method Of Antioxidant Activity: 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay: The ability of methanol extract and petroleum ether, chloroform and ethyl acetate fractions of *Cola lepidota* to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated as previously described (Jain *et al*., 2008). The extracts (3 ml) with six different concentrations (12.5, 25.0, 50.0, 75.0, 100 and 150 µg/ml) were mixed with 1 ml of a 0.1 mM ethanolic solution of DPPH. The absorbance was measured by a spectrophotometer at 517 nm at 30 minutes intervals against a blank (pure ethanol). The percentage of radical scavenging activity was calculated using the formula:

DPPH radical inhibition (%) = $[1 – (A_{test}/A_{control})] \times 100$

Where $A_{control}$ is the absorbance of the control and A_{test} is the absorbance of the sample extracts. Lower absorbance values show higher free radical scavenging activity. Ascorbic acid was used as a reference standard because it is known and used as a potent antioxidant. The 50 % inhibitory concentration value (IC_{50}) is indicated as the effective concentration of the sample that is required to scavenge 50 % of the DPPH free radicals.

Statistical Analysis: Results were expressed as means ± standard deviations (SD). Statistical comparisons were made using the student t-test, one-way analysis of variance (ANOVA) using SPSS statistics 17.0 software package.

Results and Discussion

The radical scavenging properties of the extract and fractions are shown in figure 1. Statistically significant differences were observed between the extracts and the control (ascorbic acid). Concentrations at which extracts decrease DPPH radical by 50 % (IC_{50} values) are shown in Table 1. In DPPH assay, dose-dependent inhibition was evaluated in all extracts. The results showed that chloroform and ethyl acetate fractions possess significant DPPH radical scavenging activities as evidence by their low IC_{50} values. As shown in figure 1, free radical inhibition increases as concentration of extract increase, however the percentage of free radical inhibition was higher in the chloroform fraction than in others, approximately 3 fold when compared with the ethyl acetate fraction and about 4 fold when compared with petroleum ether and methanolic fractions. All the fractions of this plant contain varying degrees of antioxidants. The result of this study is in agreement with the work of Aberoumand and Deokule, 2008.

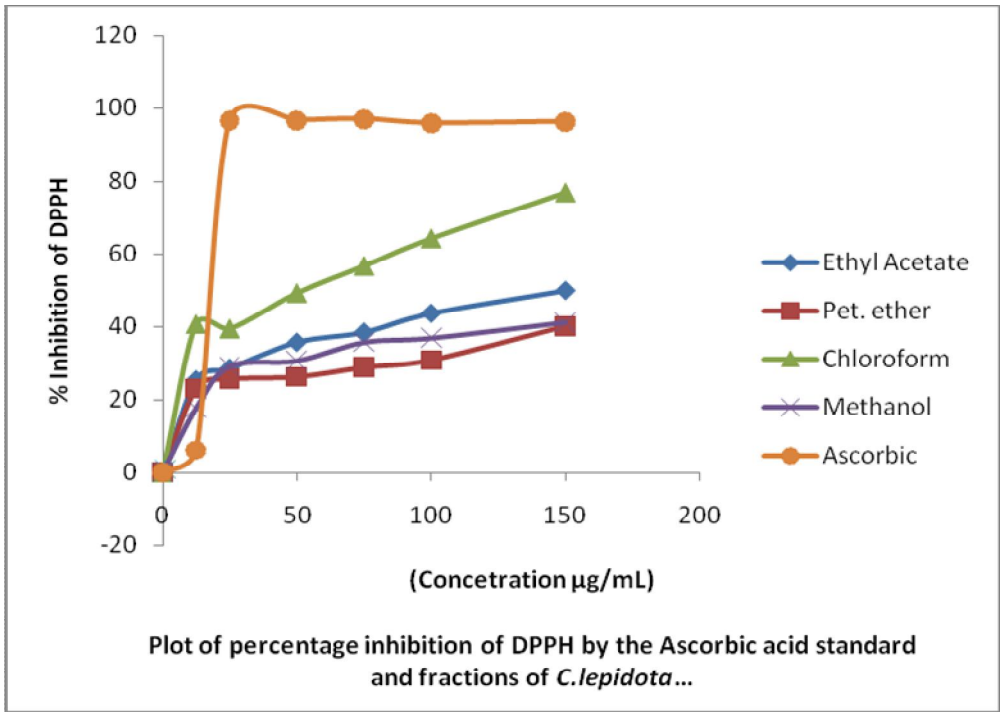

Figure 1. DPPH radical scavenging assay data represents, mean ±SD, for n = 6

Table 1: IC-50 values of extract and fractions of the leaves of *Cola lepidota* and Ascorbic acid (standard)

Sample	IC-50 values (µg/ml)
Methanol extract	210 µg/ml
Pet-Ether fraction	190 µg/ml
Chloroform fraction	50 µg/ml
Ethyl acetate fraction	150 µg/ml
Ascorbic acid	18 µg/ml

It is well recognized that free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorders, arthritis, inflammation and liver diseases (Martin *et al*., 1993). Under normal physiological conditions low concentrations of lipid peroxidation products are found in tissues and cells. In the presence of oxidative stress more lipid peroxidation products are formed due to cell damage. Cellular antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase normally challenge oxidative stress. DPPH is a stable free radical with purple color, which changes into a stable yellow compound on reacting with an antioxidant. The concentration of the antioxidant needed to decrease the initial DPPH concentration by 50 % (IC_{50}) is a parameter widely used to measure

antioxidant activity (Chun-Weng). In the present study, *in vitro* antioxidant activities of methanol extract and various fractions of the leaves of *C. lepidota* were examined. The tested leaves extract showed *in vitro* antioxidant activity against oxidative systems. The free radical scavenging activity of the crude extract (methanol), petroleum ether, chloroform and ethyl acetate fractions were evaluated based on the ability to scavenge the synthetic DPPH. DPPH shows strong absorption band at 517 nm. 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 figure 1 the plot of % inhibition by DPPH against concentration, chloroform fraction showed an increasing scavenging activity of the free radical with IC_{50} at 50 µg/ml while that of ethyl acetate 150 µg/ml. Ascorbic acid is a known and potent antioxidant agent used in medicines (Jain et al, 2008). Ascorbic acid functions as an antioxidant L-ascorbic acid, its salts (sodium-Lascorbic 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) (Jain et al, 2008).

The present results showed that standard antioxidants had stronger activity than the tested extracts, probably because the former contain more purified compounds than the latter. Chloroform fraction showed the highest and significant antioxidant activity of the four fractions. It is therefore suggested that the chloroform extract (though not as polar as methanol) with maximal inhibition of free radicals is a more potent extract when compared with the other extracts.

In conclusion, extracts and fractions of *C. lepidota* depict varying antioxidants (free radical scavenging) properties in comparison with ascorbic acid. This lends support to the ethno-medicine use of the plant in Nigerian folk medicine.

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