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Research Article

Free Radical Scavenging Activity of Lupeol Isolated from the Methanol Leaf Extract of *Crateva adansonii* Oliv. (Capparidaceae)

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ABSTRACT
The study was aimed at investigating the antioxidant properties of lupeol isolated from the methanol leaf extract of *Crateva adansonii.*
In order to assess the antioxidant effect of lupeol *in vitro* (1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) spectrophotometric assay, Ferric reducing antioxidant power (FRAP) assay, Hydrogen peroxide scavenging assay, Hydroxyl radical scavenging assay, ABTS radical cation scavenging activity, Anti-lipid peroxidation assay, β -Carotene bleaching assay, Superoxide anion radical scavenging assay) and *in vivo (*Lipid peroxidation assay, Assay of catalase (CAT) activity, Assay of reduced glutathione (GSH) concentration) experimental models were used.
The effect of DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging showed that lupeol had better percentage antioxidant activities at high concentrations when compared with ascorbic acid (88.40 % and 82.37 % at 800 µg/ml respectively). The FRAP (Ferric reducing antioxidant power) results were similar to the DPPH with Lupeol at high concentration giving a FRAP value of 2.314 ± 0.06 which is slightly higher than that of ascorbic acid even at 1000 µg/ml. Lupeol efficiently scavenged hydrogen, due to its ability to donate electrons to hydrogen peroxide. The results of this work show that Lupeol possessed strong ABTS scavenging activity and a lipid peroxidation inhibitory activity in the human body. Pretreatment with the lupeol increased the activities of CAT (catalase) which may be the mechanism of action of the observed reduction in lipid peroxidation. This assay revealed that the lupeol might prevent reactive radical species from damaging biomolecules such as lipoprotein, DNA, amino acids, sugar, proteins and PUFA in biological and food systems.
The present investigation showed that lupeol has antioxidant properties by scavenging free radicals.

Keywords: Antioxidant, lupeol. Natural product**,** Free-radical.

INTRODUCTION

In the past decades, mechanisms of oxidative stress and the function of free radicals in living systems have gained increased attention. Oxygen and nitrogen uptake inherent to cell metabolism have been known to make reactive oxygen and nitrogen species (ROS and RNS) available. Reactions of these species with lipid molecules produces peroxyl radicals and their interaction with nucleic acids and proteins results to certain alterations and, therefore, functional[1]. ROS are continuously produced by the body's normal use of oxygen in metabolic respiration and some cell-mediated immune functions. ROS, which consists of free radicals in form of superoxide anion radicals $O_2^{\cdot-}$, hydroxyl radicals ($OH^{\cdot}$) and non-free-radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), are various forms of activated radical oxygen[2-4]. On the other hand, antioxidants are compounds that can retard or prevent the oxidation of lipid or other molecules by retarding the initiation or production of oxidizing chain reactions. The harmful action of the free radicals can, however, be blocked by antioxidative substances, which scavenge and reduce the free radicals, detoxifying the organism[5]. Antioxidants act mainly by removing O_2 or decreasing local O_2 concentrations, removing catalytic metal ions, removing key ROS, e.g. $O_2^{\cdot}$ and H_2O_2, scavenging initiating radicals, e.g. $OH^{\cdot}$, $RO^{\cdot}$, $RO_2^{\cdot}$, breaking the chain of an initiated sequence, quenching or scavenging singlet oxygen, enhancing endogenous antioxidant defenses by up-regulating the expression of the genes encoding the antioxidant enzymes, repairing oxidative damage caused by radicals, increasing elimination of damaged molecules and not repairing excessively damaged molecules so as to reduce the introduction of mutations[5]. Plants are the most commonly known reservoir of natural antioxidants, which includes ascorbate, tocopherols, polyphenols and terpenoids[6]. *Crateva adansonii* also known as *Crateva religiosa* or sacred garlic pear, belong to the family of

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Capparaceae and phylum Magnoliophyta. Being small a tree of forest and savanna woodland, often seen on river-banks, it is widely distributed in Nigeria, and Across Africa[7]. The leaves are applied externally to relieve joint pains, the fresh juice from the leaves is used to relief of ear ache, eye infection and anodyne in toothache. Powder of bark is used in rheumatism, itch, epilepsy, stomach troubles, and asthma[8]. Organic extract (dichloromethane & methanol, 1:1) of *C. adanosonii* DC seeds had been evaluated for their bioactivity against brine shrimp found to have very high activity. Two phytoconstituents had also been isolated and identified as oleanolic acid and 4-epi-hederagenin[8]. Lupeol is one of the identified compounds in *Crateva adansonii* which has several biological activities. Lupeol is an important lupene type of triterpene constituent present in plants. It has a wide range of therapeutic uses like antioxidant, chemoprotective, antiinflammatory, cardioprotective, antibacterial, anti-urolithasis, antiprotozoal and anti-tumor activities[9,10]. Lupeol is a common ingredient in several nutraceuticals and nutricosmetics preparations available in the market. Due to the ability of lupeol to maintain skin texture and integrity by promoting epidermal regeneration and replenishing cutaneous antioxidant enzymes it is used in anti-aging creams, lotions, gels and lip balm[11].

The objective of this study is to investigate the inhibition of lipid peroxidation, ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP), DPPH radical scavenging, ABTS radical scavenging, superoxide anion radical scavenging in the riboflavin/methionine/illuminate system, hydrogen peroxide scavenging and ferrous ions (Fe^{3+}) chelating activities of lupeol isolated from *Crateva adansonii*. In addition, the trust of this investigation is to also clarify the antioxidant and radical scavenging mechanisms of lupeol. Furthermore, an important goal of this research is to investigate the *in vivo* antioxidative effects of lupeol as compared with commercial and standard antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), α-tocopherol, Vitamin C and trolox commonly used by the food and pharmaceutical industry.

MATERIAL AND METHODS

General experimental procedures

The UV spectra were obtained with a shimadzu 3101 PC instrument and IR spectra determined with a jasco FT-IR 410 apparatus. ^{1}H (400.6MHz) and ^{13}C (100.13 MHz) nmr spectra were recorded in CDC_{l3} (with its signals at δ 7.25 and 77.0 ppm as reference) TLC was carried out on silica gel 60 GF_{254} pre-coated plates with detection by UV light or by spraying with 50% H_2SO_4 followed by heating at 100°C.

Plant material

Leaves of *Crateva adansonii* were collected from Nsukka Local Government Area, Enugu State, Nigeria. It was identified and authenticated by Mr. Alfred Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD) Nsukka, Enugu State. The voucher specimen (INTERCEDD 1047) was deposited at herbarium of InterCEDD.

Extraction and isolation

The pulverized leaves (2Kg) were extracted with methylene chloride. Methanol (1:1) for 48hours. The mixture was filtered and the filtrate concentrated using a rotary evaporator under a reduced pressure to obtain the extract (369g).

200g of the crude extract was fixed on Silica gel (60-200mesh) and subjected to column chromatography using n-hexane, ethyl acetate and methanol as eluent. The ethyl acetate fraction was then concentrated *in vacuo* and subjected to column chromatography using hexane-EtOAc mixtures as eluent. Fractions of 100ml were collected and regrouped on the basis of their TLC profile. The fractions eluted with hexane-EtOAc (8:20) (600mg) were further purified by repeated column chromatography on silica gel (70-230 mesh) to yield lupeol (200mg).

In vitro anti-oxidant analysis

Lupeol and positive standards (ascorbic acid, butylated hydroxytoluene, catechin and gallic acid) were assay for different *in vitro* anti-oxidant capacities. Of each sample 800 µg was dissolved in 1 ml analytical methanol. These solutions were further serially diluted to 400, 200, 100, 50 and 25 µg/ml. In all the different antioxidant assays, same dilutions of sample and standards were used while standard altered as per assay requirement. The sample at different concentrations was prepared in triplicates.

Evaluation of antioxidant capacity using the 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) spectrophotometric assay

The free radical scavenging activity of Lupeol was analyzed by the DPPH assay following a standard method[12]. A given volume (2 ml) of the extract at varying concentrations ranging from 800-25 µg/ml each was mixed with 1 ml of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 min of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage antioxidant activity was calculated as follows:

% Antioxidant Activity [AA] = 100 – [{(Abs sample – Abs blank) X 100}/Abs control].

Methanol (1.0 ml) plus 2.0 ml of Lupeol was used as the blank while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol was used as the negative control. Ascorbic acid was used as reference standard.

Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of the sample was determined using a ferric reducing ability of plasma (FRAP) assay of Benzie and Strain (1999)[13] as a measure of "antioxidant power". FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from colorless oxidized Fe^{III} form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100-1000 µmol/L) of $FeSO_4$ x $7H_2O$. All solutions were used on the day of preparation. In the FRAP assay, the antioxidant efficiency of the extracts under the test was calculated with reference to the reaction signal given by an Fe^{2+} solution of known

concentration, this representing a one-electron exchange reaction. Ascorbic acid was measured within 1 h after preparation. Lupeol was first adequately diluted to fit within the linearity range. All determinations were performed in triplicate.
Calculations were made by a calibration curve.
FRAP value of sample (µM) =
Changes in absorbance from 0-4 min x FRAP value of std (1000 µM)
Changes in absorbance of std 0 min-4 min

Hydrogen peroxide scavenging assay

The method of Bokhari et al. (2013)[14] was followed to investigate hydrogen peroxide scavenging capacity of samples. Hydrogen peroxide (2 mM) solution was prepared in phosphate buffer (50 mM, pH 7.4). Samples (100 µl) were pipetted into flasks and their volume made up to 400 µl with 50 mM phosphate buffer (pH 7.4). H_2O_2 solution (600 µl) was added and absorbance at 230 nm was taken 10 min after vortexing the flasks. Percent scavenging activity was determined by following formula;
H_2O_2 % scavenging activity
= (1- absorbance of sample) x 100
Absorbance of control
Ascorbic acid served as standard.

Hydroxyl radical scavenging assay

The antioxidant activity was evaluated by method reported by Halliwell et al (1987)[15]. The reaction mixture comprised of 2-deoxyribose (2.8 mM, 500 µl) in 50 mM of phosphate buffer, 100 µl of 0.2 M hydrogen peroxide solution, 200 µl of 0.1M ferric chloride, 0.1M EDTA and 100 µl of test sample. The reaction was initiated by the addition of 100 µl of ascorbate (0.3M). The mixture was incubated at 37 °C for 60 min. TCA (2.8% w/v, 1 ml) and 1 ml of thiobarbituric acid (TBA) solution in 50 mM of sodium hydroxide (1%; w/v) was added. This reaction mixture was heated for 15 min in boiling water bath and then allowed to cool. Absorbance was recorded at 532 nm.
Hydroxyl scavenging activity (%)
=1-(Absorbance of sample ×100)
Absorbance of control

ABTS radical cation scavenging activity

Re *et al*. (1999)[16] methodology with slight modification was followed for ABTS (2, 2 azobis, 3 ethylbenzothiozoline-6-sulphonic acid) radical cation scavenging activity. ABTS (7 mM) solution was reacted with 2.45 mM potassium persulfate and kept overnight in dark for generation of dark colored ABTS radicals. For the assay, the solution was diluted with 50 % ethanol for an initial absorbance of 0.7 at 745 nm. Activity was determined by adding 100 µl sample of different dilution with 1 ml of ABTS solution in glass cuvette. Decrease in absorbance was measured after one min and 6 min of mixing. The difference was calculated and compared with control. Percent inhibition was calculated by the formula:
% ABTS scavenging effect
= (control absorbance - sample absorbance) X 100
Control absorbance

Anti-lipid peroxidation assay

This assay was performed as illustrated by Dorman et al. (2003)[17]. An aliquot of egg yolk (10%, w/v) was prepared in KCl (1.15 %, w/v). The yolk was homogenized for 30 sec and subsequently subjected to centrifugation for 15 min. Each sample (100 µl) at varying concentrations (800, 400, 200, 100, 50 µg/ml in methanol) and 500 µl of yolk homogenate were pipetted into flasks and volume was made up to 1 ml with distilled water. It was mixed with 1.5 ml of acetic acid (20 %, pH 3.5) and TBA (0.8 %, w/v) in sodium dodecyl sulphate (1.1 %, w/v). The reaction mixture was vortexed and incubated for 60 min in a water bath. *n*-Butanol was added after cooling at room temperature, stirred and then centrifuged for 10 min at 3000 rpm. Butylated hydroxytoluene served as standard. The absorbance at 532 nm of supernatant was recorded.
The percent anti-lipid peroxidation was determined by the formula (1-S /C) ×100
Where, C = Absorbance of control and, S= Absorbance of test sample

β -Carotene bleaching assay

Elzaawely *et al.* (2007)[18] modified method was used for β-carotene bleaching assay. β-Carotene (2 mg) was dissolved in 10 ml of chloroform and blended with 20 mg of linoleic acid and 200 mg of Tween 20 followed by removal of chloroform under nitrogen with subsequent addition of 50 ml of distilled water with vigorous shacking to prepare β-carotene linoleic acid emulsion. An aliquot of each sample (50 µl) was mixed with 1ml of the emulsion, vortexed and absorbance was determined at 470 nm immediately against the blank solution. Capped tube was then kept in a water bath at 45 °C for 2 h and the difference between the initial readings was calculated by measuring the reading after 2 h. β-Carotene bleaching inhibition was estimated by the following equation:

$$\% \text{ bleaching inhibition} = \frac{A_{ot} - A_{120t}}{A_{oc} - A_{120}} \times 100$$

Superoxide anion radical scavenging assay

Riboflavin light NBT system assay was followed for superoxide radical scavenging activity as described by Nishikimi (1972)[19]. The reaction mixture containing 0.5 ml of phosphate buffer (50 mM, pH 7.6), 0.3 ml riboflavin (50 mM), 0.25 ml PMS (20 mM), and 0.1 ml NBT (0.5 mM), prior to the addition of 1 ml sample in methanol. Florescent lamp was used for starting the reaction. Absorbance was recorded at 560 nm after incubation for 20 min under light. The percent inhibition of superoxide anion generation was calculated using the following formula:
% Percent scavenging activity = (1- Absorbance of sample /Absorbance of control) ×100

In vivo anti-oxidants activities

Animals

Albino mice weighing 28-35 g of both sexes were used for the experiments. Animals were housed at 25 ± 5 °C under a 12-h light/12-h night conditions with free access to standard pellet feed and clean drinking water. All experiments carried out in this study were approved by the Animal Ethics Committee, University of Calabar, Nigeria. Animals were divided into groups A, B and C

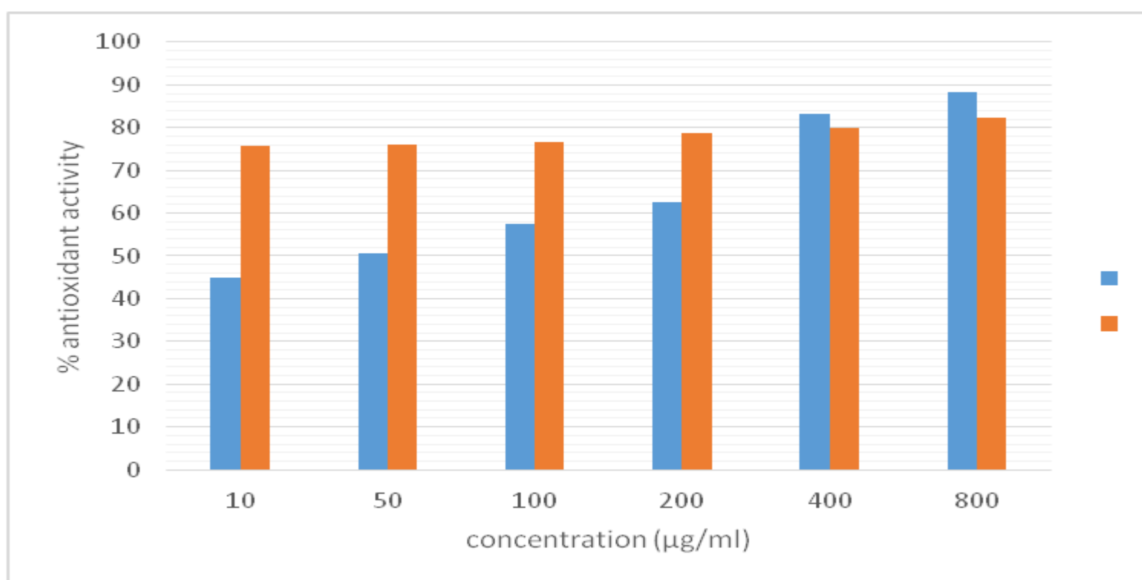


Figure 1: Antioxidant activities of Lupeol as compared with ascorbic acid using the DPPH assay method

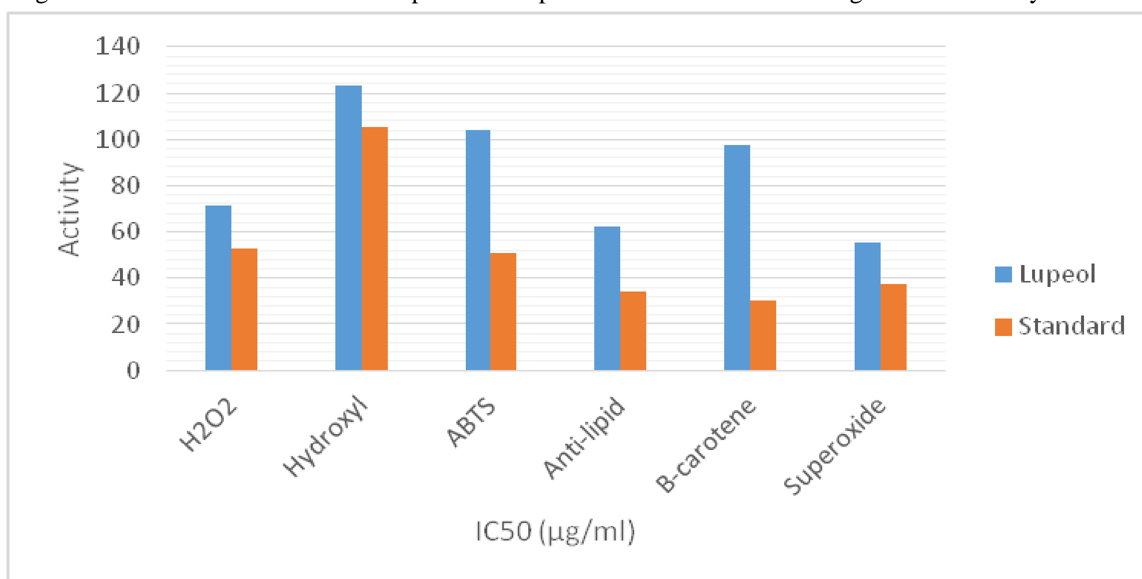


Figure 2: IC_{50} values of different antioxidant assays of Lupeol

Table 1: Antioxidant performances of Lupeol using the FRAP method

Concentration (µg/ml)	FRAP value (µM)
20	0.542±0.04
50	0.884±0.09
100	1.321±0.03
200	1.467±0.04
400	2.041±0.06
800	2.314±0.02

* $P < 0.05$ significantly different from reference compound (Ascorbic acid). FRAP value of ascorbic acid between 10 – 1000 µg/ml = 2.000

treated orally with Lupeol at 15, 30 and 60 mg/kg for 28 days. At the end of 28 days, blood samples were collected from the above challenged mice via the median canthus of the eyes from the retrobulbar plexus and used for the various antioxidant assays listed below.All authors hereby declare that principles of laboratory animals care (NIH publication No. 85-23, revised 1985) were followed.

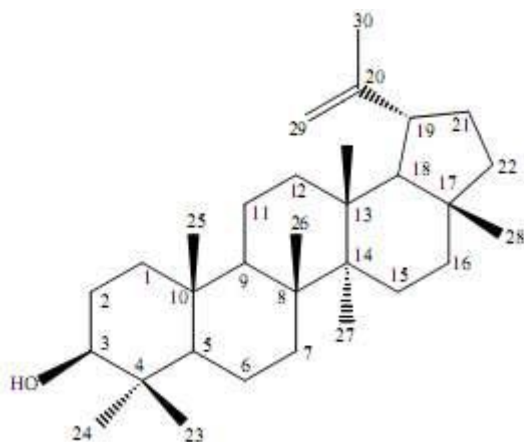
Lipid peroxidation assay

Lipid peroxidation in the serum from animals on Day 28 was estimated colorimetrically as thiobarbituric acid reactive substances (TBARS) using the method of Buege and Aust (1978)[20]. A principal component of TBARS is malondialdehyde (MDA), a product of lipid peroxidation. In brief, 0.1 ml of tissue homogenate (Tris-HCl buffer, pH 7.5) was treated with 2 ml (1:1:1 ratio) of TBA-TCA-HCl reagent (thiobarbituric acid 0.37 %, 0.25 N HCl and 15 % TCA). The mixture was placed in a water bath for 15 min; it was then allowed to cool. The absorbance of clear supernatant was measured against reference blank at 535 nm. Concentration was expressed as nmol/ml.

Table 2: Activities of Lupeol on malondialdehyde, catalase, superoxide dismutase, glutathione levels in mice

Dose (mg/kg)	Serum MDA (nmol/ml)	Serum CAT(U/ml)	Serum GSH(U/ml)
15	5.22±1.03	0.57±0.03	7.89±0.34*
30	3.32±0.72*	0.63±0.04*	9.61±0.43*
60	1.88±1.27*	0.78±0.04*	11.14±0.22*
Distilled water (0.03ml/10g)	1.28±0.79	0.80±0.04	11.54±0.38

* $p<0.05$ compared to respective negative control. Values are mean ± S.E.M. n = 6.



Lupeol

Assay of catalase (CAT) activity

Catalase activity was measured according to the method of Aebi (1984)[21]. A given volume (0.1 ml) of the serum was pipetted into cuvette containing 1.9 ml of 50 mM phosphate buffer of pH 7.0. Reaction was started by the addition of 1.0 ml of freshly prepared 30 % (v/v) hydrogen peroxide (H_2O_2). The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. The enzyme activity was expressed as units/ml protein.

Assay of reduced glutathione (GSH) concentration

Reduced glutathione was determined by the method of Ellman (1959)[22]. A volume (1.0 ml) of serum was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5, 5-dithiobisnitrobenzoic acid (DTNB) in 100 ml of 0.1 % sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). Then 0.4 ml of distilled water was added. The mixture was thoroughly mixed and absorbance was read at 412 nm and expressed as units/ml.

Statistical analysis

All data were expressed as Mean ± S.E.M. or % mean. Data were analyzed using one way analysis of variance (ANOVA) at the 5% level of significance.

RESULTS AND DISCUSSION

The result showed that the compound had better percentage antioxidant activities at high concentrations when compared with ascorbic acid. The compound showed 88.40 % activity at 800 µg/ml while ascorbic acid gave 82.37 % at the same concentration (Fig. 1).

The FRAP results were similar to the DPPH with Lupeol at 800 µg/ml giving a FRAP value of 2.314 ± 0.06 which is slightly higher than that of ascorbic acid even at 1000 µg/ml (FRAP value of ascorbic acid between 100 and 1000 µg/ml is 2) (Table 1). Lupeol appeared as white needles; mp 120-122ºC. IR *ν* max (CCl4)cm-1: 3056, 2929, 2313, 1593, 1435, 1265, 898, 741; 1H NMR(CDCl3, 400MHz): δ 4.70, 4.55(2H, *s*, H-29a, 29b), 3.2(1H, *m*, H-3), 0.77, 0.79, 0.85, 0.94, 0.97, 1.05, 1.65 (each 3H, *s*); 13C NMR(CDCl3, 100MHz): δ 151.0(C-20), 109.0(C-29), 79.0(C-3), 55.5(C-5), 50.5(C-9), 48.3(C-18), 48.0(C-19), 43.0(C-17), 42.9(C-14), 40.9(C-8), 40.0(C-22), 38.9(C-4), 38.7(C-1), 38.1(C-13), 37.2(C-10), 35.5(C-16), 34.2(C-7), 29.9(C-21), 28.0(C-23), 27.4(C-2), 27.1(C-15), 25.2(C-12), 21.0(C-11), 19.5(C-30), 18.5(C-6), 18.0(C-28), 16.1(C-25), 16.0(C-26), 15.5(C-24), 14.8(C-27). Different methods have been adopted to evaluate the antioxidant activity *in vitro* so as to allow rapid screening of substances since substances that have low antioxidant activity *in vitro*, may likely show little activity in vivo[23]. Free radicals play enormous roles in a wide variety of pathological manifestations. Antioxidants neutralize the free radicals and prevent them from causing diseases. Thin action is accomplished either by scavenging the reactive oxygen species or by protecting the antioxidant defense mechanisms[24]. The electron donation ability of natural products can be measured by 2, 20-diphenyl-1- picrylhydrazyl radical (DPPH) purple-coloured solution bleaching[25]. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolorizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test[26]. In the present study the result showed that lupeol had better percentage antioxidant activities at high concentrations when compared with ascorbic acid. The compound showed 88.40 % activity at 800 µg/ml while ascorbic acid gave 82.37 % at the same concentration (Fig. 1).

The ability of plant extracts to reduce Fe3+/Fe2+ was determined by FRAP assay. FRAP assay measures the reducing capacity by increased sample absorbance based on the formed ferrous ions, and the assay may not be complete even several hours after the reaction starts, such that a single end-point of the reaction cannot be determined[27,28]. The change in absorbance at 593 nm owing to the formation of blue coloured Fe2+ - TPTZ complex from the colourless oxidized Fe3+ form by the action of electron donating antioxidants[29]. The FRAP results were similar to the DPPH with Lupeol at 800 µg/ml giving a FRAP value of 2.314 ± 0.06 which is slightly higher than that of ascorbic acid even at 1000 µg/ml (FRAP value of ascorbic acid between 100 and 1000 µg/ml is 2) (Table 2). Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidants present, thus it can be reported that lupeol may act as a free radical scavenger, capable of

transforming reactive free radical species into stable non radical products. Hydroxyl radical, one amongst other reactive oxygen species in living systems, reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes cellular damage[30,31]. It is considered a damaging species in pathophysiological processes and often leads to mutagenesis, carcinogenesis and cytotoxicity[32]. Hydroxyl radicals were made from the reaction of H_2O_2 and the ferric compound that would react with 2-deoxyribose. The ability of an extract or compound to scavenge hydroxyl radical is directly proportional to its antioxidant property that is evident from the low intensity of red colour. Hydroxyl radicals were effectively scavenged and 2-deoxyribose was prevented from degradation by the sample lupeol when added to the mixture. The natural occurrence of hydrogen peroxide in the atmosphere, water and living organisms makes it possible to rapidly disintegrate into oxygen and water, forming hydroxyl radicals and consequently leads to lipid peroxidation and DNA damage[33,34]. Study showed that Lupeol effectively scavenged hydrogen neutralizing it into water. ABTS radical scavenging assay involves a method that generates a blue/green ABTS+ hromophore via the reaction of ABTS and potassium persulfate. The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 745 nm. This study demonstrated that Lupeol possessed strong ABTS scavenging activity. The β-carotene bleaching assay is a commonly used model to analyze the antioxidant activity of the plant extracts because β-carotene is extremely sensitive to free radical mediated oxidation of linoleic acid. β-carotene in this model system readily discolours in the absence of an antioxidant as a result of coupled oxidation of β-carotene and linoleic acid, that forms free radicals. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecules. As a result, β-carotene will be oxidized and broken down in part; subsequently the system loses its chromophore and characteristic orange colour, which can be monitored spectrophotometrically[35]. The tested compound inhibited β-carotene oxidation, suggesting that the antioxidant activity could be related to free hydroxyl groups in the compound. Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Especially, linoleic acid and arachidonic acid is the target of lipid peroxidation. Free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation and it is generally thought that the inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Radical scavengers may directly react and impede peroxide radicals to stop the peroxidation chain reaction and generally improve the food products[36]. This study demonstrated that lupeol possess a lipid peroxidation inhibitory activity in the human body. Oxidative stress contributes to some clinical disorders through the role of supeoxide and hydroxyl radicals. Such damages might be totally or partially alleviated by natural or synthetic compounds with antioxidant properties. Therefore, removing these radicals could help defend a living body against diseases[37]. Reduced activities of CAT seen in the gastric secretion and gastric mucosa homogenate of ulcerated rats might have been as a result of their utilization from the decomposition of superoxide anion generated by lipid peroxidation. Lowered activities of these enzymes may result in a number of deleterious effects. Pretreatment with the lupeol increased the activities of CAT which may be the activity procedure of the observed drop in lipid peroxidation. Lipid peroxidation, which is widely recognized as a primary toxicological event, is caused by the generation of free radicals from a variety of sources including organic hydro peroxides, redox cycling compounds and iron-containing compounds. The TBARS assay has been used to measure the degree of lipid peroxidation. TBA reacts specifically with malondialdehyde (MDA), a secondary product of lipid peroxidation to give a red chromogen, which may then be determined spectrophotometrically[38]. In this study, lupeol was capable of preventing the formation of MDA in a dose dependent manner, furthermore lupeol demonstrated highest anti –lipid peroxidation activity. This assay revealed that the lupeol might prevent reactive radical species from damaging biomolecules such as lipoprotein, DNA, amino acids, sugar, proteins and PUFA in biological and food systems.

GSH is an intracellular reductant and protects cells against free radicals, peroxides and other toxic compounds. GSH is a naturally occurring substance that is abundant in many living creatures; GSH depletion increases the sensitivity of cells to various aggressions leading to tissue disorder and injury[39]. In the present study, we demonstrated the effectiveness of lupeol by using CCl_4 induced rats and found that exogenous. TLM supplementation elevated GSH levels in rats with CCl_4 treatment and thus might provide a mean of recovering reduced GSH levels and to prevent tissue disorders and injuries. Therefore, it is valid to consider that TLM, may be because of its antioxidant property, might be capable of protecting the hepatic tissue from CCl_4-induced injury and inflammatory changes. Liver damage is very common since liver has to detoxicate a lot many toxic substances. There are several chemicals that have been known to induce hepatotoxicity by producing the reactive species which form covalent bonds with the lipids of the tissue[40,41]. Liver injury due to CCl_4 in rats was first reported in 1936[42] and has been widely and successfully used by many investigators[43,44]. Carbon tetrachloride is metabolized by cytochrome P-450 in the endoplasmic reticulum and mitochondria with the formation of CCl_3O-, a reactive oxidative free radical, which initiates lipid peroxidation[45-47]. These findings confirm the results published by Santiago et al on fraction containing lupeol of *Ficus pseudopalma* Blanco (Moraceae)[48].

CONCLUSION

The current evaluation indicated that lupeol has antioxidant properties by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous blood antioxidant enzymes levels.

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AUTHORS CONTRIBUTIONS
Michel K. Tchimene designed the study, performed the extraction isolation of compounds, structural elucidation and wrote the first draft
Chinaka O. Nwaehujor performed the in vitro and in vivo analysis and the statistical analysis
Moses Ezenwali, Ugwoke, C.E.C, Charles C. Okoli and Maurice M. Iwu managed the analysis of the study and the literature searches, All the authors read and approved the final manuscript.

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