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IN VITRO ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITES OF EXTRACTS AND FRACTIONS, ORAL ACUTE AND SUB-ACUTE TOXICITY OF AQUEOUS EXTRACT OF *URENA LOBATA* OLIV. (MALVACEAE) LEAVES GROWING IN CENTRAL KASAI-DEMOCRATIC REPUBLIC OF CONGO IN EXPRIMENTAL ANIMALS

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ABSTRACT

The present investigation reported for the first time the antioxidant activity of extracts and fractions as well as acute and sub-acute toxicity of aqueous extract from *Urena lobata* (Malvaceae) leaves from Demoratic Republic of Congo. Results revealed that all samples inhibited the activities of all selected reactive oxygen species (ROS) with inhibitory concentrations 50 (IC_{50},) µg/ml from 2.16 ± 0.00 to 6.06±0.01 µg/ml against DPPH (2,2′-diphenyl-31-picrylhydrazyl), 5.22 ± 0.00 to 13.± 0.01 µg/ml against ABTS (2, 2′ - azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid, 12.36 ±0.03 to 24.65± 0.00 µg/ml against superoxide anion ($O^{2\bullet-}$), 6.25 ± 00 to 21.50 ± 00 µg/ml against $OH^{\bullet}$ (hydroxyl) and 14.62±0.02 to 25.003 µg/ml against hydrogen peroxide (H_2O_2). The most active sample was 80% methanol extract exhibiting the antioxidant activity with IC_{50} values of 2.16±0.00, 12.56±0.02, 12.36±0.03, 6.25±0.00 and 14.68±0.02 µg/ml against DPPH, ABTS, $O^{2\bullet}$, $OH^{\bullet}$ and H_2O_2 radicals respectively, followed by aqueous extract with IC_{50} values of 3.52±0.02, 7.15±0.03, 15.06±0.02, 8.26±0.08 and 10.15±0.10 against the same ROS in same order. Among soluble fractions, ethylacetate soluble fraction was the most active exhibiting antioxidant action with IC_{50} values of 4.15±0.02, 8.62±0.00, 18.36±0.05, 1025±0.012 and 16.25±0.02 µg/ml against DPPH, APTS, $O^{2\bullet-}$, $OH^{\bullet}$ and H_2O_2 radicals respectively. Chloroform, n-butanol and residual aqueous soluble fractions showed appreciable antioxidant activity against all selected ROS with IC_{50} values ranging from 4.15±0.02 to 24.65±0.10 µg/ml. *U. lobata* leaves aqueous and 80% methanol extracts showed a content of total phenols (TP) of 25.86 and 28.62 mg of gallic acid equivalent and 7.52 and 9.06 mg flavonoids (TF) of quercetin equivalent respectively. They exhibited antioxidant activity against these selected ROS with IC_{50} values ranging from 4.01±0.03 to 8.65±0.06 for TP and 4.27±0.02 to 9.03±0.01 µ/ml for TF. In acute and sub-acute toxicity, aqueous extract did not induced mortality at the highest oral dose of 5000 m/kg body weight, i.e LD_{50} > 5000 g/kg body weight. It did not influence the levels of organ weights, haematological and biochemical parameters of treated animals compared to untreated. This finding showed that aqueous extract was found safe and well tolerated in animals. *U. batatas* was considered as a good source of phenolic and flavonoids compounds exerting significant antioxidant activity associated to that of extracts beneficials for human health in the preventing cardiovascular diseases.

KEYWORDS: *Urena lobata*, Malvaceae, leaves, extracts, fractions, antioxidant activity, acute and sub-acute toxicity.

INTRODUCTION

Free radicals which are delivered as a consequence of typical biochemical responses in the body and are involved in several diseases like atherosclerosis, cancers, inflammation, ischemic aging, heart diseases, immunosuppression, diabetes and neurodegenerative disorders.[1,2] The human body is equipped with characteristic and special barrier systems as catalase, glutathione peroxidase, proteins and superoxide dismutase to counter free radical effects. Compounds such as selenium, vitamin C, β-carotene, vitamin E, lycopene, lutein and different carotenoid systems, have been utilized as supplementary antioxidants in dietary. Hence, the secondary metabolites of the medicinal plant extracts like flavonoids, alkaloids, tannins, saponins,

anthocyanin, steroids, proantocyandins, terpenoids, polysaccharides, etc. play an important role in the defense against different reactive oxygen species or free radicals effects.[2,3]

Antioxidants are substances that at low concentrations, prevent or delay the oxidation of easily oxidizable biomolecules such as lipids, proteins, desoxyribonucleic acid (DNA) and other. They counteract free radical effects and thus prevent oxidative damages. There are two basic categories of antioxidants, namely, synthetic and natural. Natural antioxidants, found particularly in natural sources such as medicinal plants, fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risks of cardiovascular diseases and other like cancers.[4] Almost all processed foods have synthetic antioxidants incorporated, which are reported to be safe, non-toxic or have low toxicity although some studies indicate otherwise, such as the examples of BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole), which are the most widely used as chemical antioxidant products.[5,6]

Antioxidants are manufactured within the body and can also be extracted from the food humans eated such as fruits, vegetables, seeds, nuts, meats, oil and in any part of medicinal plants. Vitamin E, C and A, β-carotene and co-enzyme Q are considered as the most potent chain-breaking antioxidant within the membrane of the cell. Inside the cell, water-soluble antioxidant scavengers are also present.[7,8]

These compounds occur naturally in leafy vegetables and seeds, such as ascorbic acid, vitamin E, A, phenolic compounds and other, possess the ability to reduce the oxidative damages associated with many diseases. It is why many researchers have focused on natural antioxidants in medicinal plants kingdom. Numerous medicinal plant crude extracts, fractions, subfractions and pure natural compounds have been reported to have antioxidant properties. A large number of medicinal plants has been investigated for their antioxidant properties and reported to exhibit this activity at different extents.[4,8,9,10,11,12] Natural antioxidants either in the form of raw extracts or chemical constituents are very effective to prevent the destructive processes caused by oxidative stress.

The origin of *Urena lobata* is not certain, but it is probably of African or Asian origin. It is now widely distributed in a wild or naturalised state throughout the tropics and subtropics. In tropical Africa it occurs naturally from Cape Verde and Senegal eastward to Ethiopia and Eritrea, and southward across the continent to South Africa. It also occurs in the Indian Ocean islands. *Urena lobata* is grown as a commercial fibre crop in DR Congo, and for local use in Ghana, Nigeria and elsewhere in tropical Africa; commercial fibre production in Angola and Madagascar is mainly based on wild or naturalized plants.[13]

The traditional medical uses of *Urena lobata* are at the same time wide and numerous in tropical Africa and in other countries in the world. The plant is commonly used in traditional medicine, with the leaves and roots as the most commonly employed.

In Benin, a maceration of the leaves is taken against infectious diarrhoea. In Ivory Coast, the plant decoct (decoction) is taken as oxytocic and antipyretic, a decoction of the plant is taken as oxytocic and against fever, a preparation of the leaves with those of other plants is applied to treat menstrual problems and in Central Africa against pneoumonia. Leaves preparation mixed to those of other plants is given to treat menstrual troubles. In Benin, leaves macerate is taken to treat infectious diarrhea. In Congo-Brazzaville, leaves juice is prescribed to women which are in their first chilbirth and the decoct drunk to treat hypertension. In Democratic Republic of Congo, leaves reduced in powder and added to argil powder and diluted in water in taken to treat asthma and diarrhea. Leaves and root decoct are taken to treat diarrhea, diabetes, abdominal pains and other gastro-intestinal disorders. In Gabon, leaves macerate is prescribed in the case of diarrhea. In Guinea, flowers as aqueous macerate is taken as antiseptic. Leaves is also applied on wounds. Flowers macerate is use to treats respiratory affections. In Madagascar, leaves juice is used in drops on lesions and is applied in cataplasm, cooked leaves are used against rhumatisimal inflammations, leaves and seeds in cataplasm are applied in biliary vesicule problems and other intestinal troubles. A preparation of the root is applied to treat infected eyelids and against syphilis. A decoction of bark and root is given to children with enteritis or stomach pain. The leaves are widely used to induce labour or facilitate childbirth. A decoction of the flowers is taken against respiratory problems.

In Nigeria, root preparation is externally used against rheumatism in application as in Vietnam. The stem and root decocts are administered to children with enteritis or stomacal pains. Root decot is used in application on infected eyelids in case of syphilis. Leaves are used to launch contractions or to ease childbirth and less against sterility. In Togo, woman drink a decoction of the leaves and sometimes also from the stems and leaves of *Vernonia cinerea* (L.) Less. against infertility.

In Ouganda, leaves are used against snakebites and infusion against diarrhea in the case of diabetes. In Zanzibar and Indonesia, root decoct is prescribed against indigestion. Other uses of *Urena lobata* parts and products are diversified. Indeed, liberian fibers traditionally serve to the construction of ropes cables (strings), and crude textiles are employed in industry to substitute jute (*Corchorus* spp.), for example for the

manufacturing of bags, tapis, robes, cables and for padding. It is also used mixed to jute. The bast fibre from *Urena lobata* is widely used traditionally for making cordage and coarse textiles, and industrially as a substitute for jute (*Corchorus* spp.), for instance to make sacks, carpets, cordage and upholstery. It is often used mixed with jute. In tropical Africa and elsewhere *Urena lobata* serves for making string, twines, ropes, fishing lines and nets, and nets for hunting, while the unprocessed bark is often used as tying material. In Ghana the stripped and plaited bark is used to bind loads. In Gabon it is used to affix leaves for thatching. In Malawi the stems are woven into walls of grain stores. In Madagascar the fibre is locally used in the manufacture of bags. The fibre of *Urena lobata* can be made into strong, bank-note quality paper and whole plants can be pulped as well. Young shoots and leaves are eaten as a vegetable. In Malawi the flowers are eaten as a side-dish. The seeds contain oil and are mucilaginous and they are boiled in soups and with cereals. Cooked with rice they give it a pleasant slipperiness. Domestic animals eat the foliage. Some selections are grown as ornamental pot plants, flowering in winter.

In tropical Africa and other places, *Urena lobata* serve to make cords, stings, pieces of strings, shopping bag fishing, seto entrap, while stem non modified often serve as ligature materials. In Ghana, decorticated stem and braideds are employed for link burdens. In Gabon, it serves to fix leaves to thartch roof. In Madagascar, the fibre localy serve to make bags and solid papers having a quality of bank money and the entire plant can be used to prepare a paste. Young shoots and leaves are consumed as vegetals in many worldwide countries and particularly in Africa. The leaves are diuretic, emollient, refrigerant, styptic, vulnerary. They are used to treat intestine inflammations and bladders. The leaves are used externally as a poultice on wounds and skin disease. An leaf infusion is given to aid difficult childbirth. In Malawi, flowers are served in accompaniment. Seeds contain mucilaginous used to prepare soups and with cereals. Cooked with rice, they constitute an easy meal to consume. Domestic animals eat leaves and some selections are cultivated as ornements in pots and flower in winter. In Malawi, stems are used to weave the partition of silos to seeds.

The leaves juice and root are widely used in bowel complaints, especially stomach-aches, diarrhea, dysentery, also to treat gonorrhoea and persistent fever from malaria. A decoction for leaves and root is drunk to relieve pains all over the body due to exessive exertion. A decoction of old plant with eggs is said to induce abortion. A lotion from the plant is used to treat yaws and headache. The whole plant is macerated and used externally for treating fractures, wounds, mastitis and snake bites.

The roots are used to treat rheumatism and lumbago. A decoction of the root is used to treat colds, dysentery, enteritis, goitre, indigestion, leucorrhoea, malaria, rheumatism and tonsillitis. Used externally, they are chewed and applied to swellings caused by filariasis. The twigs are chewed to treat toothache. The bark is used to heal cuts.

The flowers are considered maturative and are taken in decoction as a pectoral and expectorant in dry cough. An infusion of the flowers is used as a gargle for aphthae and a sore throat. A decoction of the seeds is taken as a vermifuge. The roots are diuretic[13,14,15,16,17,18,19,20,21] *Urena lobata* can be seriously damaged by several fungi that form stem lesions (‘stem canker’). The most widespread of these fungi are *Botrytis cinerea* and *Macrophoma urenae*. Another disease is damping-off or seedling blight caused by *Fusarium* spp. In India *Urena lobata* is attacked by *Corynespora callicioidea*, causing scattered, yellowish-red, irregular lesions on the leaves, with a black centre that develops into a hole. The fungal diseases can be controlled by treating seed with fungicides and by crop rotation. *Urena lobata* is an alternate host for the okra mosaic virus attack.

Urena lobata is attacked by some serious pests of cotton (*Gossypium* spp.), kenaf, roselle and ramie (*Boehmeria nivea* (L.) Gaudich.), such as cotton stainers (*Dysdercus* spp.) and leaf rollers (*Sylepta* spp.). *Dysdercus superstitiosus* can strongly reduce the viability of *Urena lobata* seeds, but the fibre yield is unaffected. In Africa and Asia *Urena lobata* is attacked by spiny bollworms (*Earias* spp.). *Urena lobata* seems highly resistant to nematodes.[13] Its different biological activies in relation with its traditional uses were scientifically evaluated. Those include antioxidant activity/root.[22,23,24] antidiarrheal/leaves,[24,25] immunomodulatory,[24,26] hypoglycemic/long-term effects of root extract.[27] antidiabetic/hypolipidemic of root and leaves,[28] sperm abnormality effects leaf [29] antifertility/spermatogenic effect of leaves [30] antioxidant /cytotoxic/antiproliferative /antioxidant /leaf/root.[24,31] antioxidant/antimicrobial/leaves/root[24,32] wound healing/leaf,[24,27] liver effect and toxicity study/root,[33] antihyperglycemic/antinociceptive /leaves[34] and activities.

From the chemical composition point of vue, the leaves of this plant contain alkaloids, cardiac glycosides, tannins, terpenoids and saponins; while flavonoids, phlobatanins and steroids were not detected in this study [35]. Alkaloids, flavonoids, saponins, tannins and phenolic compounds were detected other studies.[36] **(**Flavonoids like kaempferol, quercetin, and tiliroside were isolated from the leaves.[24] Gossypetin, chrysoeriol, and mangiferin were isolated from the leaves [37]. Phytosterols like β-sistosterol, β-sistosterol-3-O-β-glucopyanoside, 2-acetylamino-3-phenylpropyl 2-benzoylamino-3-phenylpropanoate were isolated in the whole plant [38]. Tenine, flavonoids, steroids, alkaloids, saponins, cardiac glycosides, terpenoids, sugars and

phenols were detected in leaves and root aqueous extracts of *U. lobata* leaves.[39] Flavonoids, coumarins and steroids such as β-sitosterol, stigmasterol, furocoumarin, imperatorin, mangiferin and quercetin were also detected in the leaves od *U. lobata*. It also contains kaempferol, luteolin, hypolatin and gossypetin isolated form the leaves and root of *U. lobata*.[40]

The present work has been intended to assess the flavonoids and total phenol contents and their antioxidant activity, together with the antioxidant potentials of extracts and fractions, as well as acute and subacute toxicity of aqueous extract from *U. lobata* leaves collected in Central Kasai in Democratic Republic of Congo.

2. MATERIALS AND METHODS

2.1. Vegetal material and identification

Leaves of *Urena lobata* were collected in Central Kasai, one of provinces of Democratic Republic of Congo. The plant was authenticated in INERA (Institut National d'Etudes et de Recherches Agronomiques) in Departement of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen was deposited in the herbarium of this institute and in the laboratory of Pharmacognosy and Phytochemistry of the faculty of Pharmaceutical Sciences of the same university. The plant material was dried at room temperature and reduced to powder using an electronic blender and the resulting powder was kept in burn bottles.

Figure 1: ***Urena lobata*** **(Malvaceae) twigs, leaves and fruits.**

2.2. Preparation of extracts and fractionation

50 g of powdered leaves were mixed with 200 ml distilled water and boiled at 100°C on a hotplate for 15 minutes. After cooling and filtration on a filter paper Whatman N° 1 , the filtrated was evaporated in vacuum using a rotary evaporator resulting in a dried extract named Ull-1 (41.07 g). 15 g of Ull-1 extract were dissolved in 100 ml distilled water, filtered as described above and the resulting filtrate extracted successively and exhaustively with solvents with different polarities as chloroform, ethylacetate, *n*-butanol and the resulting aqueous phase. All fractions were treated as described above yielding corresponding dried extracts named as Ull-1.1 (3.05 g), Ull-1.2 (3.72 g), Ull-1.3 (2.85 g), and Ull-1.4 (5.02 g) for chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions respectively.

On the other hand, 50 g of plant material were macerated in 300 ml 80% methanol for 24 h. After filtration giving 80% methanol macerate, the marc was exhaustively percolated with the same solvent. Macerate and percolate were combined and evaporated in vacuum yielding dried extract named Ull-2 (42.85 g).[41,42]

2.2. Assessment of antioxidant activity

2.2.1. Free radical scavenging activity (FRSA) using DPPH *(*2,2' diphenyl-1-picrylhydrazyl)

The ability of extracts, fractions, polyphenols and flavonoids extracts from *Urena lobata* leaves to scavenge the radical DDPH was evaluated using the methods previously described by [43, 44]. Briefly, 2 mg of each test sample were dissolved in 2 ml methanol to have stock solutions of concentration of 1 mg/ml. These lasts were diluted in two fold-dilutions to have a series of test concentrations from 1 to 20 µg/ml. 1 ml of each test sample dilution was mixed with 1 ml DPPH 0.4 M MeOH solution and the mixture was left in obscurity for 30 minutes before the measurement of absorbance on a spectrophotometer Shimaduzu (USA) at 517 nm. DPPH 0.4 M MeOH solution was used as negative control.

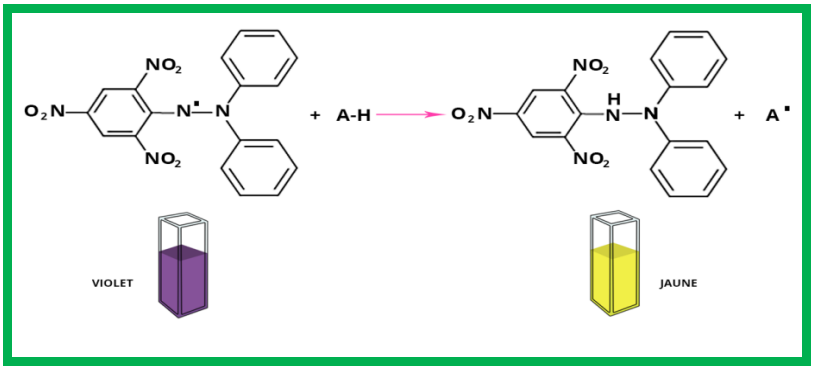


Figure 2: Transformation of DPPH in radical and its reactivity.

The effect of tested samples on DPPH activity was calculated using the following formula:

$$\% \text{ inhibition of DPPH activity} = \frac{\text{AbsNC - AbsTS}}{\text{AbsNC}} \text{ X } 100$$

Where AbsNc was the absorbance of the negative control and AbsTs was the absorbance of the tested sample. The inhibition concentration 50 (IC_{50}) of each tested sample was derived from linear courbes concentrations-responses.

2.2.2. $ABTS^{\bullet+}$ (2,2-azino-bis-3-ethylbenzthiazoline-6-sulphonate or 2,2-azino-bis-ethylbenthiasoline sulfonic acid) radical cation decolorization assay

Methods previously reported by.[4,45] based on the oxidation of ABTS were used. The oxidated ABTS solution was prepared by reaction of 2 mM ABTS in deionized water with 2.45 mM potassium persulfate ($K_2S_2O_8$) (1:1). Before use, ABTS solution was diluted with phosphate sodic tampon (0.1 M, pH 7.4) to have an absorbance of 0.750 at 734 nm. After, 1 ml of ABTS solution was mixed with 1 ml of test sample dilution (test concentrations: 1-20 µg/ml), well mixed and kept in obscurity for 4 h before to measure the absorbance. ABTS MeOH solution was taken as negative control. Absorbances were taken on the same apparatus at 734 nm.

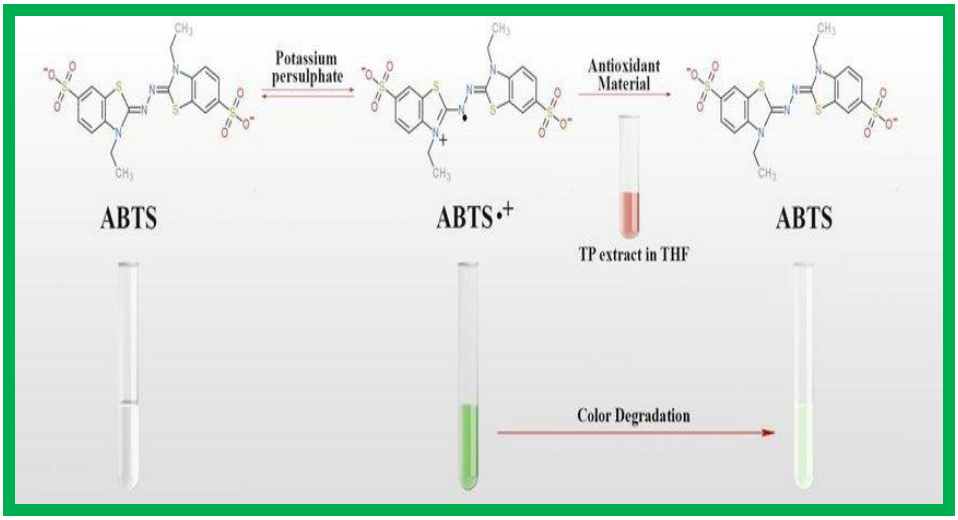


Figure 3: Transformation of the ABTS in radical and scavenging method to determine the antioxidant capacity.

The percentage inhibitions were calculated using the same formula and the inhibitory concentration 50 (IC_{50}) of each tested sample was derived in the same manner as described also above.

2.2.3: Superoxide anions ($O_2^{\bullet-}$) radical scavenging activity

For this test, 2 mg of each test sample were dissolved in the same manner as described above to have respective stock solutions (concentration: 1 mg/ml) leading to a series of test concentrations from 1 to 20 µg/ml by two-fold dilutions of each stock solution with methanol. The test was carried out in microtiter plates with 6 holes. Each hole contained 1 ml of each test sample dilution mixed with 250 mM nitrobleutetrazolium (NBT, 100 µL) and 390 µM NAD (100 µL).

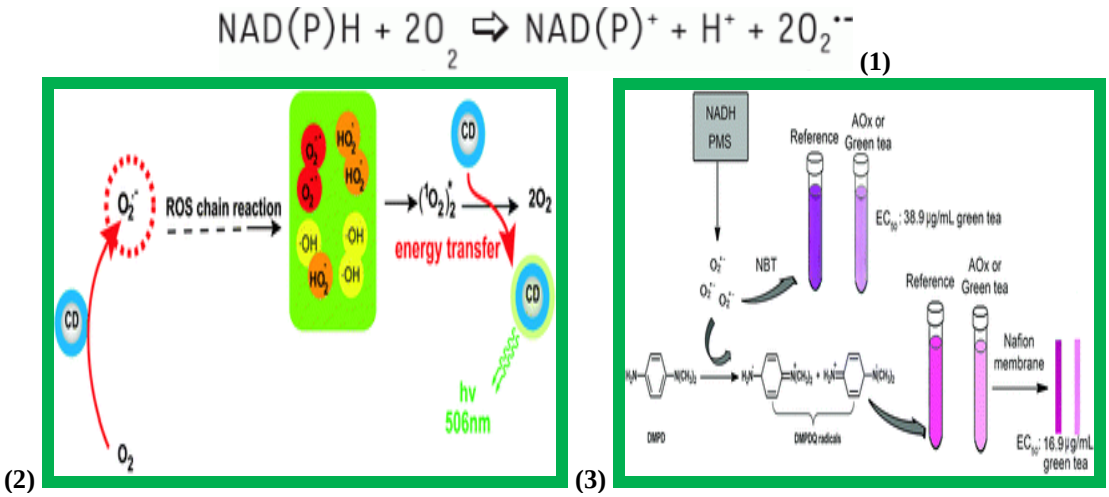


Figure 4: The production of superoxide anion $O_2^{\bullet-}$ by complex mitochondrial enzymatic of the respiratory chain (1), its production as evidence for carbon nanodots acting as electron donors by the chemiluminescence method (2) and its detection (3)

Absorbances were recorded on the same apparatus, the same formula above was used to calculated the percentage inhibitions of the production of superoxide anions (de Vargas et al. (2016) and IC_{50} values were obtained using linear courbes-responses.[46,47]

2.2.4. Hydrogen peroxide (H_2O_2) scavenging activity

The scavenging activity for hydrogen peroxide was measured according to the methods of.[48,49] Different concentrations of plant extract were added to 2 ml of H_2O_2 solution (10 mM) in phosphate buffer (50 mM, pH 7.4), and there action mixture was incubated at 25 °C for 30 min. The hydrogen peroxide can be synthetized, decomposed and detoxified as illustrated below:

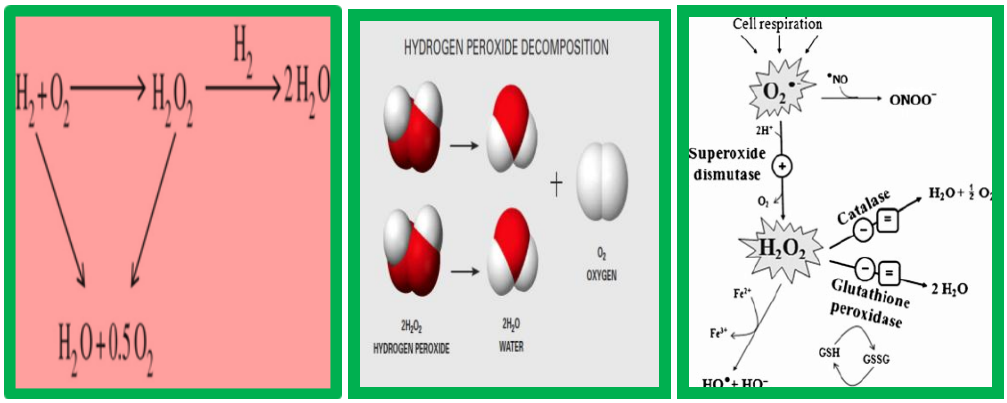


Figure 5: Reaction scheme for the direct synthesis of H_2O_2 (A), its decomposition (B) and detoxification (C) (Crole et al., 2016).

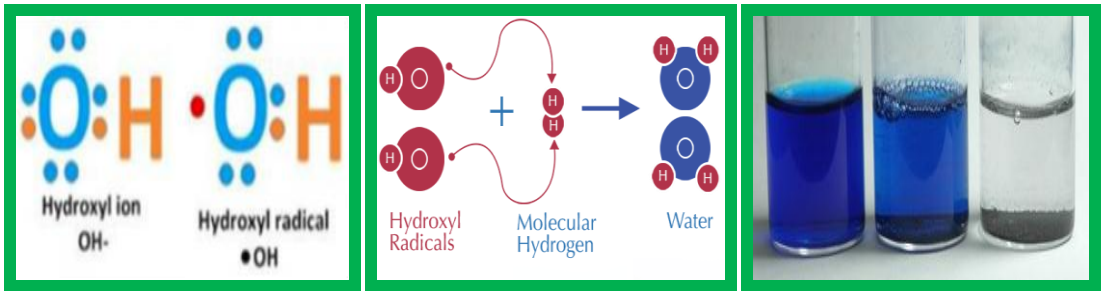
The unreacted H_2O_2 was determined by measuring the absorbance of their action mixture at 230 nm with respect to the blank solution. The percent inhibition was calculated using the following formulas.

$$\% \text{ Inhibition of superoxide anions activity} = \frac{A0 - A1}{A0} \times 100$$

Where A was the absorbance of negative control and A1 the absorbance of tested sample and IC_{50} values were derived by linear courbes concentrations-responses.

2.2.5. Hydroxyl radical ($HO^{\cdot}$) scavenging activity assay

Hydroxyl radical scavenging activity of the samples from *B. sumatrana* leaves was assessed by the methods of [50, 51]. Hydroxyl radical was generated by Fenton reaction (Fe^{3+}-ascorbate-EDTA-H_2O_2 system): The first step involved reduction of ferric into ferrous ion: $Fe^{3+} + O^{2\bullet} \longrightarrow Fe^{2+} + O_2$. The second step was the Fenton reaction: F^{2+} (aq) + $H_2O_2 \rightarrow F^{3+}$ (aq) + OH- +$OH^{\bullet}$ (pH 3-5) (aq: aqueous) and its reaction with antioxidant substance: RH + $OH^{\bullet} \rightarrow R^{\bullet} + H_2O$ where RH was an antioxidant substance.[52]



(A) (B) (C)

Figure 6: Hydroxyl OH^- ion and $OH^•$ radical (A), its reaction with hydrogen molecule (B) and its reactivity or detection (C).

The assay was based on the quantification of the 2-deoxy-D-ribose degradation product, which formed a pink chromogen upon heating with TBA (thiobarbituric acid) at low pH. The reaction mixture contained 0.8 ml of phosphate buffer solution (50 mmol/L, pH 7.4), 0.2 ml of test sample/standard at different concentrations (1-50 µg/ml), 0.2 ml of EDTA (1.04 mmol/L), 0.2 ml of $FeCl_3$ (1 mmol/L) and 0.2 ml of 2-deoxy-D-ribose (28 mmol/ L). All mixtures were kept in a water bath at 37 °C and the reaction was started by adding 0.2 ml of ascorbic acid (2 mmol/L) and 0.2 ml of H_2O_2 (10 mmol/L). After incubation at 37 °C for 1 h, 1.5 ml of cold TBA (10 g/L) was added to the reaction mixture followed by 1.5 ml of HCl (25 %). The mixture was heated at 100 °C for 15 min and then cooled down with water. The absorbances were measured at 532 nm with the same spectrophotometer. The hydroxyl radical scavenging capacity (activity) was evaluated by the calculation of percentage inhibitions of 2-deoxy-D-ribose oxidation on hydroxyl radical or percentage inhibitions of hydroxyl radical scavenging activity were calculated according to the following formula:

$$\% \text{ Inhibition of hydroxyl radical scavenging activity} = \frac{A0 - (A1 - A2)}{A0} \times 100$$

where A_0 was the absorbance of the negative control without a sample. A_1 was the absorbance after adding the sample and 2-deoxy-D-ribose. A_2 is the absorbance of the sample without 2-deoxy-D-ribose. The percentage of inhibitions were plotted against concentrations, and from the graph, IC_{50} was each tested sample was derived.

o a test tube were added 3 ml of phosphate

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2.3. Estimation of phenolic compounds content

The quantity of total phenolic compounds (TPC) in aqueous Ull-1 and 80% methanol Ull-2 extracts of *Urena lobata* leaves respectively, was determined using Folin-Ciocâlteu's reagent (FC) or Folin-Denis's reagent (FD) (phosphomolybdate and phosphotungstate composition) with the methods described by [53, 54, 55]. 1 ml of each extract dilution (1-20 µg/ml) was introduced in a tube and mixed with 1 ml of FC (1:1 dilution). 5 minutes after, 2 ml sodium carbonate 20% were added, mixed and left in obscurity for 30 minutes. Gallic acid was used as a standard (5-25 µg/ml). The absorbances were recorded at 765 nm using the same spectrophotometer apparatus. The TPC in both extracts was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dried extract.

2.4. Estimation of flavonoids content

The content of total flavonoids (TF) in aqueous Ull-1 and 80% methanol Ull-2 extract from *U. lobata* leaves was determined quantitatively with ACl_3 5% MeOH solution using methods described by [53, 54]. 2 mg of both extracts were diluted in two fold dilutions to have a series of test concentrations from 1 to 50 µg/ml. To 1 ml of test sample dilution, 1 ml of ACl_3 5% in MeOH was added, fully mixed and incubated at room temperature for 60 minutes. After, absorbances were measured at 430 nm on Shimaduzu (USA) spectrophotometer. Quercetin was used as a reference and the quantity of total flavonoids in both extracts was expressed in term of quercetin equivalent as mg/100 g dried extract.

2.5. Evaluation of acute and sub-acute toxicity

2.5.1. Toxic effects and mortality

Acute Test No 425 and sub-acute toxicity Test No 407 of aqueous extract of *U. lobata* leaves were evaluated in Wistar rats weighing 143-150 g using procedures described by OECD (Organization of Economic Cooperation and Development) [56,, 57] for the test chemical products. Animals were administered once oral doses of 500, 1000 and 5000 mg/kg bw in acute toxicity and daily in sub-acute toxicity and 500, 2000 and 5000 mg/kg bw in sub-acute toxicity for 28 days. The experimental protocol was followed:

●- Group I (2 rats) were given orally 5 ml distilled water and constituted negative control group,

●- Groups II to IV (5 rats for each oral dose) were separately and orally administered 500,1000 et 5000 mg/kg bw once in acute toxicity and 500, 2000 and 5000 mg/kg bw daily of aqueous Ull-1extract in sub-acute toxicity respectively.

Animals were placed in individual plastic cages. Clinical observations for possible occurring of side toxic effects and mortality were observed for 28 days and their weight was daily measured. All suspect movements and death of animals if occurred were recorded. Also, observations were made for behavioral, neurological, sensorial, gastro-intestinal disorders, mobility and any other abnormalities. On the last day of final measurement of

rat weights, animals that had received the highest oral dose of 5000 mg/kg bw were anesthetized under diethyl ether, sacrificed. Blood samples were collected from each animal by cardiac puncture for haematological and biochemical analysis, followed by histologic examination of liver, heart, kidney, spleen, pancreas, lungs after dissection, removing and washing.[58,59,60]

2.5.2. Evaluation of effects of *U. batata* samples on haemtological and biochemical parameters levels in treated Wistar rats

The collected blood from rats that had received the highest oral dose of 5000 mg/kg bw was placed in tubes into two groups: on group of the test tubes containing anticoagulant, ethylene diaminotetraacetic acid (EDTA) and blood, and the other group with blood, but without anticoagulant. Blood samples in the test tubes containing EDTA were used to analyse haematological parameters (WBCs, RBCs, HGB, HCT, platelets and other) using Automated Hematology Analyzer (Symex-RX, 21, Japan). Blood samples in the test tubes without anticoagulant could clot and sera were obtained by centrifuging the blood using an electrical centrifuge (HUMAX-K, HUMAN-Germany) from which blood chemistry were studied to analyse biochemical parameter levels of glucose, urea, creatinine, total protein, ALT, AST and other. Values in the sera were analysed using Automated Clinical Chemistry Analyser (AUTO LAB 18, clinical chemistry analyser, Italy). After collection of blood samples, the rats were sacrificed by cervical dislocation and parts of some vital organs like kidney, liver, heart, spleen, lungs and pancreas dissected out and gross pathological observation was performed to check for any gross lesions.[58]

2.5.3. Relative organ weight

On 29th day, all the treated animals which received oral dose of 5000 mg/kg bw, were anaesthetized with ketamine and sacrificed. Organs namely, liver, spleen, heart, pancreas, lungs and kidneys were carefully dissected out and removed, plentifully washed with distilled water, dried in hot at 50^{o}C and weighted to a constant weight. The relative organ weight of each animal was then calculated as follows:

Relative organ weight = absolute organ weight (g) × 100/body weight of rat on sacrifice day (g).[58,61]

2.6. Histological analysis

Animals that had received the highest oral dose of 5000 mg/kg bw were sacrificed by anesthesia with dietylether and dissected. Vital organs cited above were removed and plentifully washed with distilled water and dried. They were fixed in formaldehyde for analysis. They next pushed in paraffin, cuted in small pieces and spotted with haematoxylin and eosin. The spotted sections were visualized on electronic microscope Nikon eclipse e200 300-3.0 connected to camera and ordinator where pictures were transfered and analysed with a Scope tek photo x 86, 3.1.475,[61,62]

3. RESULTS AND DISCUSSION

3.1. Effects of extracts and fractions from *Urena lobata* stem baks on some reactive oxygen species (ROS)

Nowadays, much attention and researchs had been devoted to natural antioxidants from medicinal plants and their health benefits in the prevention and treatment of many complex diseases such as cardiovasculars and other. Plants were well known as a major source of natural antioxidants. They produced a wide range of secondary metabolites with antioxidant activity that had therapeutic potential mainly in the preventing of cardiovascular diseases. Polyphenolic compounds were the most abundant antioxidant compounds in plant raw material. Their antioxidant activity was based on to their redox properties, which facilitated their activity as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductants of ferryl hemoglobin. Their reducing ability was generally associated with the presence of reductants which exert antioxidant action through breaking the free radical chain by donating a hydrogen atom or preventing peroxide formation [63]. Medicinal plant organs were commonly rich in phenolic compounds such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins, some alkaloids, polysaccharides, steroids and triterpenoids. These compounds were known to exhibit multiple biological effects among which antioxidant activity with various magnitudes.[63,64,65]

●- **DPPH** was a free radical compound which had scavenging ability for antioxidants samples and showed good absorbance at 517 nm. It lost this absorption when reduced by an antioxidant substance (AH) leading to the change of color as illustrated in figure 7.

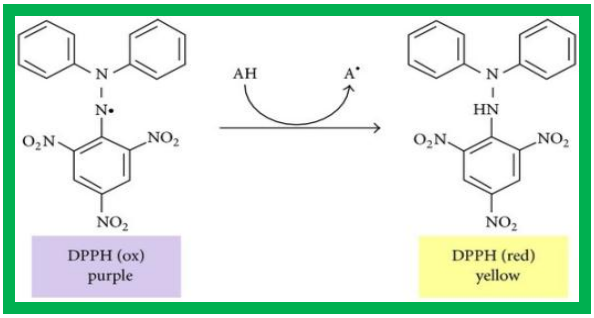

Figure 7: Mechanism of DPPH reduction and its reactivity.

Table 1: Antioxidant activity of extracts and fractions from *Urena lobata* leaves against selected reactive oxygen species (ROS), IC_{50}: µg/ml).

Sample codes	DPPH	ABTS	$O^{2\bullet}$	$OH^{\bullet}$	H_2O_2
Ull-1	3.52±0.02	7.15±0.03	15.96±0.02	8.26±0.04	16.15±0.10
Ull-1.1	6.06±0.04	13.25±0.01	24.65±0.01	21.56±0.00	25.14±0.03
Ull-1.2	4.15±0.02	8.63±0.00	18.36±0.03	10.25±0.02	16.25±0.02
Ull-1.3	5.25±0.00	10.53±0.03	22.63±0.01	18.24±0.04	21.35±0.03
Ull-1.4	4.85±0.01	9.23±0.02	20.65±0.07	15.69±0.03	18.63±0.02
Ull-2	2.16±0.01	5.28±0.03	12.36±0.03	6.85±0.00	14.68±0.02
Total phenol	4.01±0.03	5.15±0.04	4.07±0.01	6.45±0.03	8.65±0.03
Total flavonoids	4.27±0.02	6.23±0.02	4.22±0.02	7.06±0.04	9.03±0.01
Vitamin C	9.10±0.03	8.20±0.01	20.54±0.03	6.52±0.02	8.05±0.01
Gallic acid	1.35±0.02	3.65±0.04	25.68±0.00	2.58±0.02	5.65±0.03

Ull: *Urena lobata* leaves, Ull-1 and -2: aqueous and 80% methanol extracts respectively, Ull-1.1 to -1.4: chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions respectively, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS : 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), $O^{2\bullet}$: superoxide anions, $OH^{\bullet}$: hydroxyl, H_2O_2: hydrogen peroxide.

The DPPH method was widely used to determine antiradical/antioxidant activity of purified phenolic compounds as well as natural plant extracts and fractions. The most phenolic antioxidants reacted slowly with DPPH, reaching a steady state in 1-6 h or longer. This suggested that antioxidant activity using DPPH should be evaluated over time. The method also had good repeatability and was used frequently to evaluate antioxidant activity of extracts and fractions from various medicinal plants as well natural and synthetic products [65, 66, 67].

In the present study, it was observed that the **activity of DPPH** was markedly inhibited by aqueous Ull-1 extract and its soluble fractions Ull-1.1 to -1.4 as well as 80% methanol Ull-2 extract with IC_{50} values < 10 µg/ml as pronounced activity. Among these samples, 80% methanol extract Ull-2 recorded high activity with IC_{50} value of 2.16±0.10 µg/ml and was followed by aqueous extract with IC_{50} value of 3.52±0.02 µg/ml. Soluble fractions from the partition of aqueous extract Ull-1 also showed pronounced antioxidant activity with IC_{50} values ranging from 4.15 to 6.06. The most active soluble fraction was ethylacetate Ull-1.2 rich in flavonoids displaying antioxidant activity with IC_{50} value of 4.15±0.02 µ/ml. It was followed by residual aqueous fraction Ull-1.4 rich phenols other than flavonoids (IC_{50} = 4.85±0.11 µg/ml), *n*-butanol Ull-1.3 rich in saponins (IC_{50} = 5.25±0.10 µg/ml) and chloroform Ull-1.1 rich in steroids and terpenoids (IC_{50} = 6.06 ± 0.07 µg/ml). A significant difference in activity was deduced ($p < 0.05$). Total phenols and flavonoids exhibited antioxidant activity against this radical with IC_{50} values of 4.01±0.03 and 4.27±0.02 µg/ml respectively while Vitamin C and Gallic acid exerted the same activity with IC_{50} values of 9.10±0.03 and 1.35±0.02 µg/ml respectively as pronounced activity. [66] reported the antioxidant activity of aqueous and methanol extract of the whole plant against DPPH where the first extract showed very low IC_{50} of 16.51 µg/ml compared to the second one with IC_{50} > 100 µg/m. Compared to the present results, it can presume that aqueous extract (3.52±0.02 µg/ml) from the leaves exhibited high activity than aqueous extract from the whole plant (IC_{50} = 16.51 µg/ml) suggesting the possible existence of antagonism between different constituent in this extract. On the other hand 80% methanol Ull-2 extract from the leaves (IC_{50} = 2.16±0.11 µg/ml) exhibited high activity compared to methanol extract (IC_{50} = 16.50 µg/ml) from the whole plant suggesting the influence of the nature of the extractive solvent (80% methanol versus methanol) although the activity was qualitatively maintened. [67] reported the antioxidant activity of ethylacetate and *n*-butanol fraction from the partition of 50% EtOH-H_2O_2 leaves extract and their finding were in good agreement with our results indicating the non influence of the nature of the parent extract (aqueous versus EtOH-H_2O_2). [68] also described the antioxidant activity of methanol extract from *U. lobata* leaves against ABTS and its results were in good agreement with those reported in the present study.

Figure 8 put back the percentage reductions of DPPH activity by aqueous extract Ull-1, 80% methanol extract Ull-2, total phenols (TP) and flavonoids (TF) and gallic acid (Ga) used as reference antioxidant product. They exerted this effect in dose-dependent manner (Fig. 5).

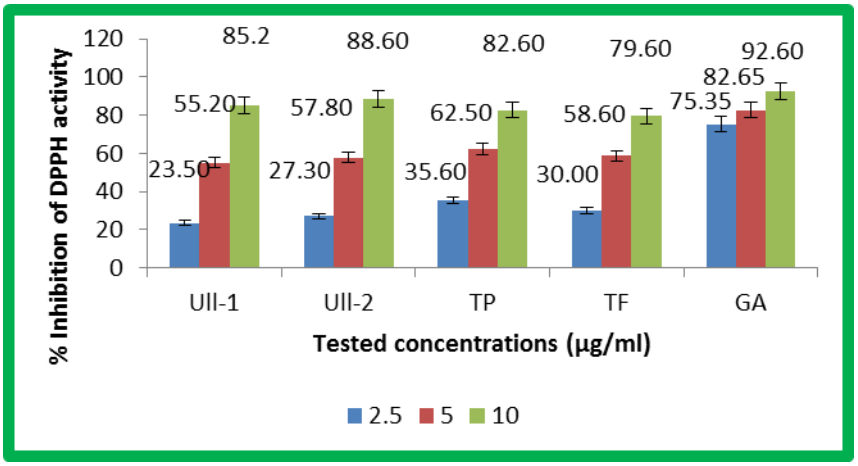

Figure 8: Percentage reductions of DPPH activity by aqueous extract Ull-1, 80% methanol extract Ull-2, Gallic acid (Ga).

Tested at the highest concentration of 10µg/ml, Ga showed 92.60 % reduction of DPPH activity. At the same tested concentration, aqueous extract Ull-1 and 80% methanol Ull-2 produced 85.20 and 88.60% reduction of the same activity. TP and TF produced 82.60 and 79.60%. The effect of Ga was high compared to UII-1 and UII-2 extracts, and TP and TF with significant difference ($p < 0.05$). The effect of Ull-2 was high compared to UII-1 and TF, but low compared to TP. At the lowest tested concentration of 2.5 µg/ml. Ga still showed 75.35%, aqueous Ull-1 extract 23.50%, 80% methanol Ull-2 extract 27.30%, TP and TF extracts 35.60 and 30.00%. At all tested concentrations, significant difference was observed by comparison the activities of these samples between them ($p < 0.05$). Our results were in good agreement with.[39,68,71] for the effect of *U. lobata* leaves extract against DPPH activity.

●- **ABTS** (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) assay was described in 1997 by.[70] The method was developed based on absorbance of the $ABTS^+$ radical cation for the evaluation of the total antioxidant capacity of body fluids and drug solutions. This method was first based on the production of ABTS radical cation by activation of methaemoglobin with hydrogen peroxide in the presence of ABTS, with or without antioxidants. Then, antioxidants arrested $ABTS^+$ radical leading to a decrease in absorbance, which was detected by the antioxidant combination of antioxidant with radicals at different times.[72]

ABTS radical was generated by reacting with a strong oxidizing agent (potassium permanganate or potassium persulfate) with the ABTS salt. The reduction of the blue-green.

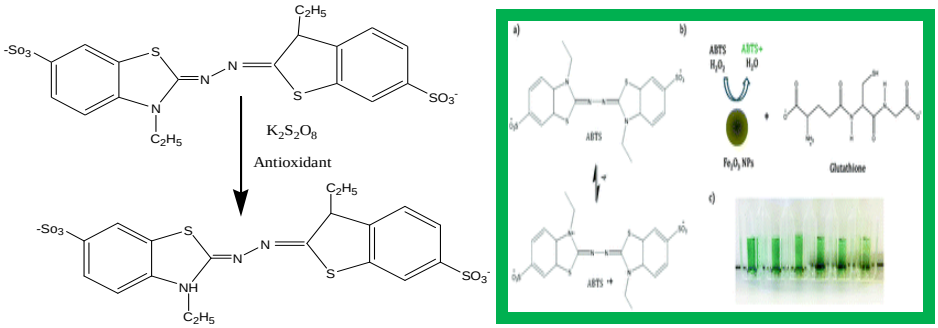

Figure 9: ABTS antioxidant capacity in the presence of antioxidant and potassium persulfate and its reactivity.

ABTS radical by hydrogen-donating antioxidants was measured by the suppression of its characteristic long wave absorption spectrum. The method was usually expressed as Trolox equivalent antioxidant capacity (TEAC). It was rapid and can be used over a wide range of pH values, in both aqueous and organic solvent systems. It also had good repeatability and is simple to perform; hence, it was widely reported.[67]

Indeed, in this test, results revealed that all samples form *U. lobata* leaves were able to inhibit ABTS radical activity with IC_{50} values from 5.22 to 13.25 µg/ml. Among these samples, 80% methanol Ull-2 extract showed high activity with IC_{50} value of 5.28±0.03 µg/ml compared aqueous Ull-1 extract with IC_{50} value of 7.15±0.03 µg/ml. Both extracts exhibited pronounced antioxidant activity regarding this ABTS radical. Following these results, it was also observed that ethylacetate Ull-1.2 and residual aqueous Ull-1.4 soluble fractions exhibited also pronounced activity with IC_{50} value of 8.63±0.00 and 9.23±0.02 µg/ml respectively. They were followed by *n*-butanol Ull-1.3 with IC_{50} value of 10.53±0.03 µg/ml and chloroform Ull-1.1 with IC_{50} value of 13.25±0.01 µg/ml as good activity. Total

phenols and flavonoids displayed pronounced antioxidant activity with IC_{50} values of 5.15±0.05 and 6.23±0.02 µg/ml respectively while Vitamin C and Gallic acid inhibited the activity of this radical with IC_{50} values of 3.65±0.04 and 8.20±0.10 µg/ml respectively. All samples from *U. lobata* samples exhibited promising scavenging effect against this radical. Our results were in good agreement with Rajagopal et al. (2019) who had reported the antioxidant activity of methanolic extract of *U. lobata* leaves against ABTS radical at the tested concentrations from 50 to 1000 µg/ml and suggesting that the nature of extract (aqueous and 80% methanol versus methanol) had no influence for the qualitative manifestation of this biological activity, but the activity of this extract was weak (IC_{50} estimated to be 50.10 µg/ml compared to that reported in the present study (IC_{50} = 7.15±0.03 and 5.28±0.05 µ/ml respectively, seven and ten times high respectively). This difference in activity level must be due to the preparation procedures (maceration versus Soxhlet).

Figure 10 reported the percentage reductions of ABTS activity by aqueous extract Ull-1, 80% methanol extract Ull-2 and Gallic acid (Ga) used as antioxidant reference products. They exerted this activity in dose-dependent manner (Fig. 10). Results indicated that, when tested at the highest concentration of 7.5 µg/ml, aqueous extract Ull-1 and 80% methanol extract Ull-2 caused a reduction of the activity of this radical to 52.44 and 71.06 % with significant difference ($p < 0.05$) in activity. Gallic acid (Ga) tested at the same concentration produced 98.67% reduction of the activity of this radical.

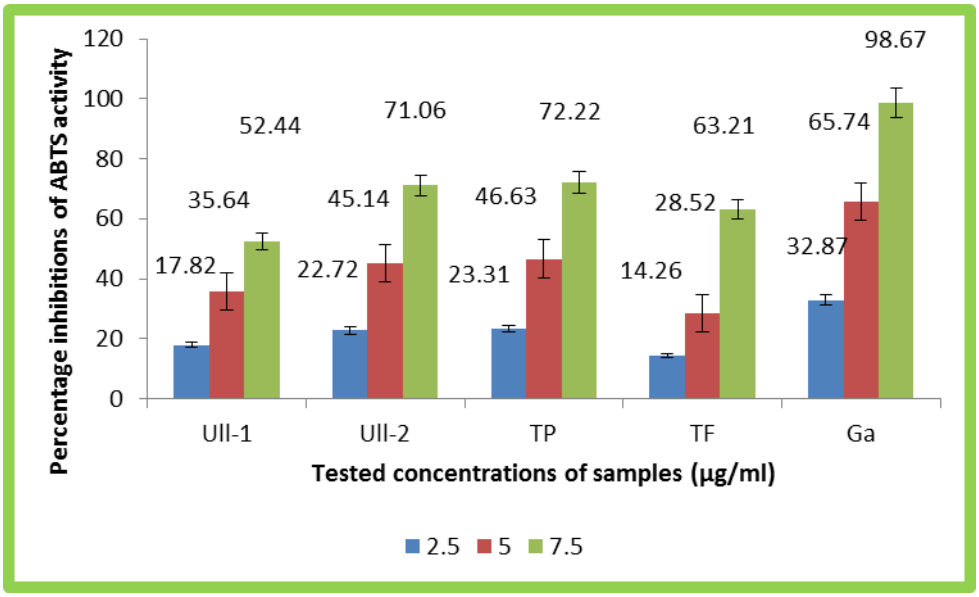

Figure 10: Percentage reductions of ABTS radical activity of by aqueous extract Ull-1, 80% methanol extract-Ull-2 and Gallic acid (Ga).

TP and TF supplied 72.22 and 63.21%. Ga showed high activity compared TP, TF, Ull-1 and Ull-2 extracts. In turn, the effect showed by Ull-2 extract was high compared to Ull-1 with significant difference ($p < 0.05$) (Fig. 10), comparable to TP with no significant difference, and high compared to TF with significant difference. Tested at the lowest concentration of 2.5 µg/ml, Ull-1 and Ull-2 extracts showed 17.82 and 22.72% with significant difference ($p < 0.05$) TP and TF 23.30 and 14.26% with significant difference, Ga gave 32.87% with significant difference compared to Ull-1 and Ull-2 , TP and TT extracts. TF did not statistically difference ($p > 0.05$) compared to Ull-2, but this was observed compared to Ull-1 (p, 0.05).

●- **Superoxide anion $O^{2\bullet-}$** was another reactive oxygen species. The reactive oxygen ion superoxide is particularly important as the product of the one-electron reduction of dioxygen O_2 [73], which occurred widely in nature. Although it was a weak oxidant, it gave rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contributed to oxidative stress [74, 75]. Superoxide anion ($O_2^{\bullet-}$) was generated from oxygen (O_2) by multiple pathways such as oxidation by NADPH oxidase, xanthine or hypoxanthine oxidase. Generally, superoxide anion was converted to hydrogen peroxide by superoxide dismutase (SOD) or reacted with nitric oxide ($NO^{\bullet}$) to form peroxynitrite. And hydrogen peroxide can be further converted to water and oxygen by catalase and glutathione peroxidase. However, superoxide anion was believed to be the cause of other ROS formations such as hydrogen peroxide, peroxynitrite, and hydroxyl radicals. It was a reduced form of molecular oxygen created by receiving one electron. These radicals had been observed to kill cells, inactivate enzymes and degrade DNA, cell membranes and polysaccharides. It was, therefore, proposed to measure the comparative interceptive ability of antioxidant extracts to scavenge the superoxide radical.[76]

In the present study, against superoxide anion ($O^{2\bullet}$), it was also pointed out that all extracts and factions from *U. lobata* leaves had capacity to inhibit the activity of this radical with different magnitudes. Indeed, aqueous Ull-1 and 80% methanol Ull-2 extract recorded the highest superoxide anion $O^{2\bullet}$ radical scavenging activity with IC_{50} values of 15.96±0.12 and 12.36±0.05 µg/ml respectively as good activity. In addition, ethylacetate soluble fraction Ull-1.2 also showed good activity with IC_{50} value of 18.36±0.05 µg/ml. Chloroform Ull-1.1, *n*-butanol Ull-1.3 and residual aqueous Ull-1.4 soluble fractions exhibited moderate activity with IC_{50} values of 24.65±0.10, 22.63±0.11 and 20.65±0.07 µg/ml respectively. The last soluble fraction showed high activity than the two first one and significant difference was observed ($p < 0.05$). Against this radical, total phenols and flavonoids acted by inhibiting its activity with IC_{50} values of 4.06±0.05 and 4.22±0.03 µg/ml respectively, Vitamin C and Gallic acid exhibited antioxidant activity with IC_{50} values of 25.68±0.10 and 20.5±0.03 µg/ml respectively as pronounced activity for all these samples.

Figure 11 reported the percentage diminutions of superoxide anion $O_2^{\bullet-}$ activity by aqueous extract Il-1, 80% methanol extract, form *U. lobata* and Gallic acid (Ga) used as antioxidant reference products. Results revealed that, when tested at the highest concentration of 7.5 µg/ml, aqueous extract Ull-1 and 80% methanol extract UIl-2 caused a reduction of the activity of $O_2^{\bullet-}$ to 23.50 and 30.34% with significant difference ($p < 0.05$) in activity.

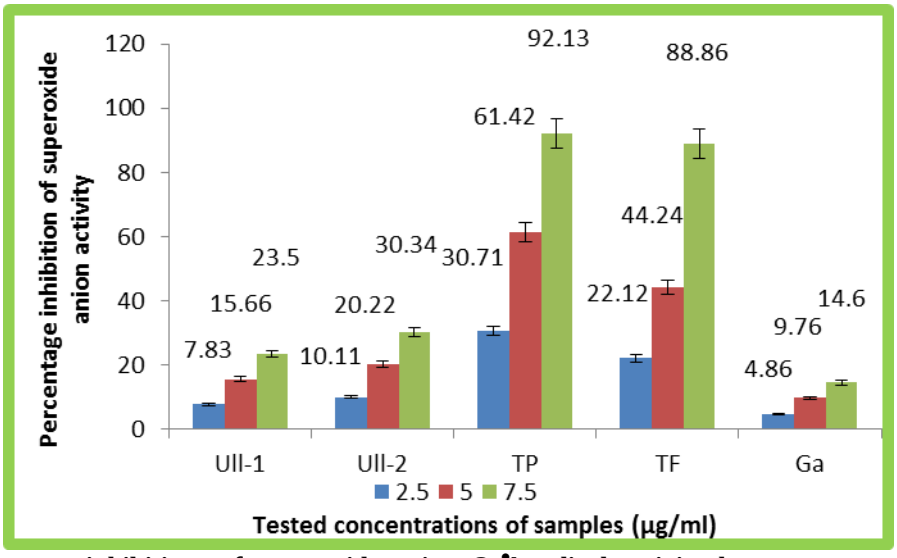

Figure 11: Percentage inhibitions of superoxide anion $O_2^{\bullet-}$ radical activity by aqueous extract Ull-1, 80% methanol extract UIl-2, Gallic acid (Ga) and Ascorbic acid (Aa).

On the other hand, total phenols (TP) and flavonoids (TF), and Gallic acid (Ga) exerted the same effect by producing 92.13, 88.86 and 14.60% reduction of the activity of this radical. The effect of Ull-2 being high compared to Ull-1 and Ga with significant difference ($p < .005$). That of Ull-1 was high compared to Ga with also marked difference ($p < 0.05$). Ga exerted low effect at all tested concentrations compared to Ull-1, Ull-2, TP and TF (Fig. 11).

Tested at the lowest concentration of 2.5 µg/ml, Ull-1 and Ull-2 still produced 7.83 and 10.11% with significant difference 9$p < 0.05$), TP and TF extracts 30.71 and 29.62% and Ga 4.86% with significant difference ($p < 0.05$) compared between them.

●- **The hydroxyl radical ($OH^{\bullet}$)** was the most reactive product of ROS formed by successive electron reductions of molecular oxygen (O_2) in cell metabolism and was primarily responsible for the cytotoxic effects observed in aerobic organisms extending from bacteria to plants and animals. It was generally assumed that $OH^{\bullet}$ radical was generated in biological system from H_20_2 by Fenton reaction $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+}$ $OH^{\bullet} + OH^{-}$ whereby Fe^{2+} was regenerated by superoxide anion O_2 giving rise to an overall Fe (II,III)-catalyzed Haber–Weiss reaction producing OH from H_2O_2 potentially available in acrobic cells.[75] Oxidative attacked of hydroxyl radicals generated from such a Fenton reaction on deoxyribose produced malondialdehyde (MDA) and similar substances that were colorimetrically or fluorometrically reactive toward thiobarbituric acid [TBARS (thiobarbituric acid reactive substance assay)] method based on the formation of colored [TBA-MDA (thiobarbituric acid-malondialdehyde)] adducts, forming the essence of $OH^{\bullet}$ detection.[76,77] Hydroxyl radicals are the major active oxygen species causing lipid oxidation and enormous biological damages.[39]

In the present study, the evaluation of samples from *U. lobata* leaves against hydroxyl radical ($OH^{\bullet}$) indicated that these tested samples inhibited its activity at different

degrees. They produced inhibitory effect with IC_{50} values ranging between 6.85 and 21.56 µg/ml. Among them, aqueous Ull-1 and 80% methanol Ull-2 extracts showed prominent antioxidant effect with IC_{50} values of 8.26±0.03 and 6.85±0.10 µg/ml respectively. Ethylacetate Ull-1.2 and residual aqueous Ull-1.4 soluble fractions displayed good activity with IC_{50} values of 10.25±0.12 and 15.69±0.08 µg/ml respectively, while chloroform Ull-1 and *n*-butanol Ull-1.3 exhibited moderate and good activity respectively with IC_{50} values of 21.56±0.00 and 18.24±0.04 µg/ml respectively.

Figure 12 contained the percentage reductions of hydroxyl $HO^{\bullet}$ activity by aqueous extract Ull-1, 80% methanol Ull-2 extract from *U. lobata* and Ascorbic acid (Aa). Tested at the highest concentration of 5 µg/ml, Aa showed 53.76% reduction of the activity of $HO^{\bullet}$. At the same time, Ull-1 and Ulb-2 produced 26.00 and 49.40% reduction of $HO^{\bullet}$ activity. TP and TF acted as more by producing 65.00 and 40.00%. The activity of TP was high compared to Ull-1 and UIl-2 with significant difference ($p < 0.05$) while that of Ull-1 was low compared to compared UIl-2 and TF with also significant difference.

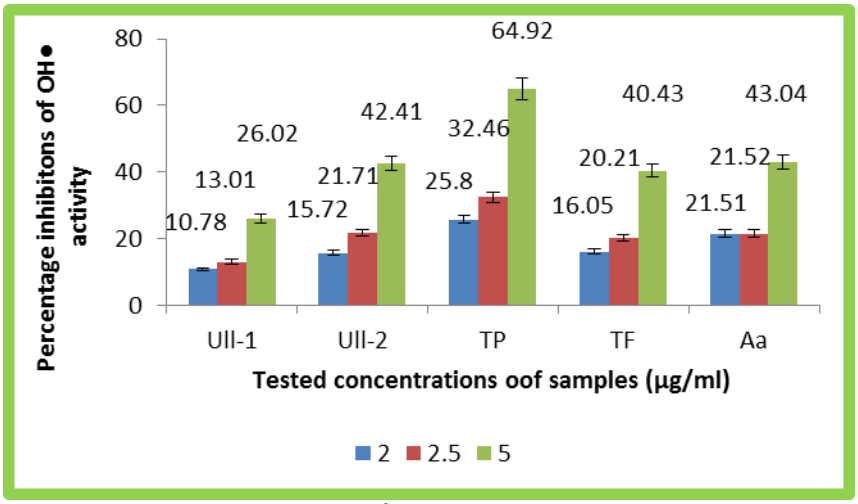

Figure 12: Percentage reductions of hydroxyl $HO^{\bullet}$ radical activity by aqueous extract Ull-1, 80% methanol extract UIl-2 and Ascorbic acid (Aa).

At last, Aa exerted his effect by supllying 53.76% high compared to Ull-1 and Ull-2, but low compared to TP and high compared to TF with in all cases, with significant difference ($p < 0.05$).

The effect Ull-1 (30.26%) was comparable to Aa (31.05%) and no significant difference ($p > 0.05$) was deduced while the effect of Ull-1 and Ull-2 extracts was low compared to Aa and significant difference ($p < 0.05$) was observed. At the lowest tested concentration of 2.5 µg/ml, Ull-1, Ull-2, TP, TF and Aa still showed 7.78, 14.80, 19.50. 12.00 and 16.12% reduction respectively of the activity of $HO^{\bullet}$ radical with significant difference ($p < 0.05$) in comparison between them.

●-The naturally occurring of H_2O_2 in the air, water, human body, plants, microorganisms and food was at low concentration levels. It was quickly decomposed into oxygen (O_2) and water (H_2O) and may create hydroxyl radicals ($OH^{\bullet}$) that can initiate lipid peroxidation and cause DNA damage (Bhatti et al., 2015). Absorbances of hydrogen peroxide at 230 nm were determined spectrophotometrically after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide.[80,81] The hydrogen-donating activity measured utilizing hydrogen peroxide radicals as the hydrogen acceptor, demonstrated that a strong association could be found between the concentration of the antioxidant molecule and the rate of inhibition [48]. Hydrogen peroxide (H_2O_2) was a reactive oxygen metabolic byproduct that served as a key regulator for a number of oxidative stress-related states. Functioning through NF-kappaB and other factors, hydroperoxide-mediated pathways had been linked to asthma, inflammatory arthritis, atherosclerosis, diabetic vasculopathy, osteoporosis, neuro-degenerative diseases, Down's syndromes and immune system diseases [82]**)**. H_2O_2 was very important radical because of its ability to penetrate biological membranes. H_2O_2 itself was not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radical in the cells. Thus, removal of H_2O_2 is very important for protection of food systems.[80]

When tested against **hydrogen peroxide (H_2O_2)**, aqueous Ull-1 and 80% methanol Ull-2 extracts, ethylacetate Ull-1.2 and residual aqueous Ull-1.4 soluble fractions recorded good activity with IC_{50} values of 16.15±0.10, 14.68±0.01, 16.25±0.12 and 18.63±0.02 µg/ml respectively. Chloroform Ull-1.1 and *n*-butanol Ull-1.3 soluble fractions exhibited moderate activity with IC_{50} values of 25.14±0.03 and 21.35±0.08 µg/ml respectively. Total phenols (TP) and flavonoids (TF) showed IC_{50} values of 8.05±0.06 and 9.03±0.01 µg/ml respectively wile Vitamin C and Gallic acid exhibited this activity with IC_{50} values of 8.05±0.01 and 5.65±0.0123µg/ml respectively. The activity of these two

reference antioxidant products being comparable or high compared to Ull-1 and Ull-2 extracts, TP and TF with or without significant difference according the case ($p < 0.05$ or $p > 0.05$).

Briefly, in all tests, it came out that 80% methanol extract Ull-2 showed high antioxidant compared to aqueous extract Ull-1. This finding suggested that this organic solvent must be the better which can be used in further for the isolation of active antioxidant constituents in *U. lobata* leaves. In addition ethylacetate Ull-1.2 soluble fraction was the most active soluble fraction compared to other Ull-1.1, 1.3 and 1.4 and this also suggested that it can selected in further for the isolation active antioxidant constituents if it was obtained in high amount. The activities displayed by all soluble fractions from aqueous Ull-1 extract were weak compared to parent extract. This observation suggested that these fractions would act in synergistic manner to restore the activity of the parent extract.

Moreover, the activity of *U. lobata* samples was compared to the reference products Vitamin C and Galllic acid. Results revealed that all extracts and fractions (IC_{50} = 2.16±0.10 to 6.06±0.07 µg/ml) from *U. lobata* leaves showed high antioxidant activity compared to Vitamin C (9.10±0.03 µg/ml) and weak activity compared to Gallic acid (IC_{50} = 1.35±0.02 µg/ml **against DPPH**. **Against ABTS**, all samples (IC_{50} = 5.28±0.03 to 9.23±0.02 µg/ml) form *U. lobata* displayed weak activity compared to Gallic acid (IC_{50} = 3.65±0.04 µg/ml) while aqueous Ull-1 and 80% methanol Ull-2 extracts (IC_{50} = 7.15±0.05 and 5.28±0.03 µg/ml respectively) exhibited high activity compared to Vitamin C (IC_{50} = 8.20±1.00 µg/ml). The activity of ethylacetate Ull-1.2 (IC_{50} = 8.63±0.02) was comparable to Vitamin C (IC_{50} = 8.20±0.10 and residual aqueous soluble fraction Ull-1.4 (IC_{50} = 9.23±0.02 µg/ml) showed weak activity compared to Vitamin C (IC_{50}= 8.20±0.10µg/ml) In comparison **against superoxide anion $O^{2\bullet}$** radical, it was pointed out that all samples form *U. lobata* leaves exhibited high antioxidant activity (IC_{50} = 12.36±0.05 to 24.65±0.10 µg/ml) compared to Gallic acid (IC_{50} = 25.68±0.10 µg/ml), while aqueous Ull-1 and 80% methanol Ull-2 extracts, and ethylacetate Ull-1.2 soluble fraction (IC_{50} = 15.96±.02, 12.36±0.05 to 18.36±0.05 µg/ml) showed high activity compared to Vitamin C (IC_{50} = 20.54±0.03 µg/ml)). Chloroform Ull-1.1 and *n*-butanol Ull-1.3 (22.63±0.01 µg/ml) presented weak activity compared to Vitamin C (IC_{50} = 20.54±0.03 µg/ml) while Ull-1.4 (IC_{50} = 20..65±0.07 µg/ml) was comparable to Vitamin C (IC_{50} =20.54±0.03 µg/ml). When tested **against hydroxyl $OH^{\bullet}$** and **hydrogen peroxide H_2O_2 radicals**, reference products Vitamin C (IC_{50} = 6.52±0.02 and 8.50±0.01 µg/ml respectively) and Gallic acid (IC_{50} = 2.58±0.02 and 5.65±0.10 µg/ml) exhibited high antioxidant activity compared to all samples of *U. lobata* leaves (IC_{50} = 6.85±0.02 to 21.56±0.00 and 14.62±0.02 to 25.14±0.03 µg/ml respectively) though the activity of 80% methanol extract Ull-2 (IC_{50} = 6.85±0.10 µg/ml was comparable to Vitamin C (IC_{50} = 6.52±0.02 µg/ml against hydroxyl radical without significant difference ($p < 0.05$).

Figure 13 showed the effects on aqueous Ull-1 and 80%methanol Ull-2 extracts, total polyphenols (TP), total flavonoids (TF) and Gallic acid (GA) as reference expressed in percentage diminution of the activity of $HO^{\bullet}$ radical. These results indicated that these samples decreased or diminished $HO^{\bullet}$ activity in dose-depend manner (Fig. 2). At the highest tested concentration of 5µg/ml, Gallic acid produced 97.00% inhibition while aqueous Ull-1 and 80% methanol extract Ull-2 supplied 30.26 and 36.5 %respectively. Total phenols (TP) and flavonoids (TF) showed 38.76 and 35.46 % inhibition respectively high compared to aqueous and 80% methanol Ull-1 and Ull-2 respectively. The effect of Gallic acid was high compared to both extracts Ull-1 and Ull-2, total phenols and flavonoids (Fig. 2).

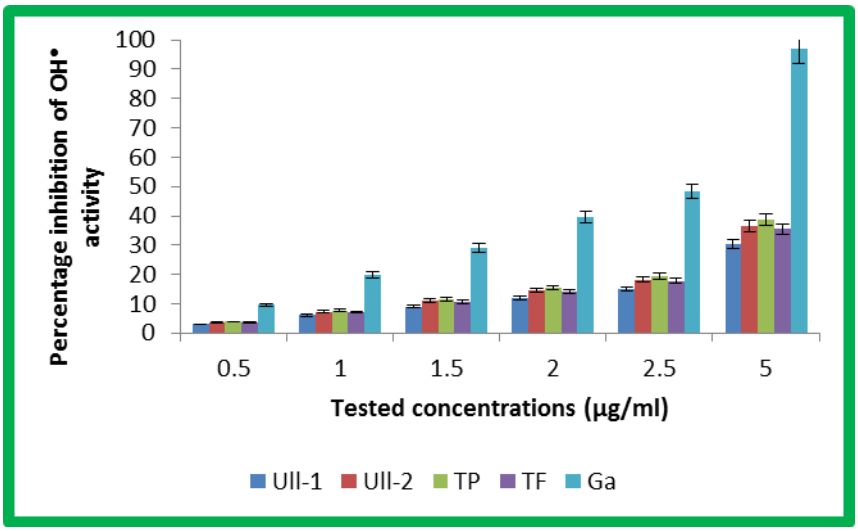

Figure 13: Percentage reductions of hydroxyl $HO^{\bullet}$ activity by aqueous extract Ull-1, 80% methanol extract Ull-2, total polyphenol (TP), total flavonoids (TF) and Gallic acid (GA).

Figure 14 showed the percentage reductions effect of aqueous Ull-1, 80% methanol Ull-2 extracts and Gallic acid as reference product on hydrogen peroxide (H_2O_2) activity. Results revealed that all samples reduced the activity of this ROS radical in dose-dependent manner (Fig. 14). At the highest tested concentration of 5 µg/ml, aqueous Ull-1 and 80% methanol Ull-2 extracts produced 15.48±0.04 and 17.03±0.07% reduction. TP and TF acted in the same manner in suppressing this activity by 29.00±0.05 and 27.68±0.01% while Gallic acid (Ga) and Ascorbic acid (Aa) produced 31.05 and 44.24% diminution at this high concentration and high compared to all samples from *U. lobata* leaves with significant difference ($p < 0.05$). The effect of TP was high compared to Ull-1 and Ull-2 extracts and TF while that of Ull-2 was high compared to Ull-1 with significant difference ($p < 0.05$) (Fig. 14).

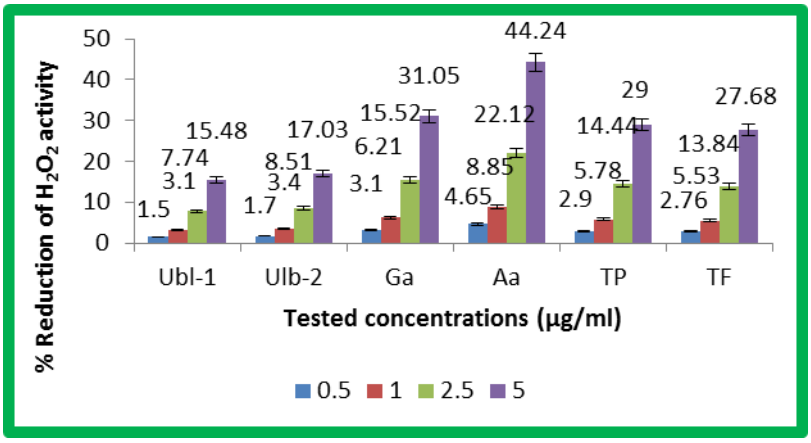

Figure 14: Percentage diminutions of H_2O_2 activity by aqueous extract Ibl-1, 80% methanol extract Ibl-2, total phenols (TP) and flavonoids (TF) and Gallic acid (Ga).

3.2. Polyphenols and flavonoids contents

The total phenols of aqueous and 80% methanol Ull-1 and Ull-2 extracts respectively of *U. lobata* leaves were measured using Folin-Ciocalteau's reagent while the total flavonoids were estimated using aluminium chloride 5% in methanol by spectrophotometric method. Total phenolic and flavonoid contents were determined and expressed in mg of gallic acid and quercetin equivalents/100g of dried extract respectively to evaluate their content in *U. lobata* leaves.

Total phenolic and flavonoid contents were commonly found in plants and had been reported to have several biological activities including antioxidant activity. The total phenolic content in aqueous Ull-1 and 80% methanol Ull-2 extracts from *Urena labata* leaves were estimated to be 25.86±0.06 and 28.62±0.04 mg expressed in gallic acid (GA) equivalent/100 g dried extract respectively while that of flavonoid content were 7.52 ± 0,02 [1] and 9.06± 0,10 mg expressed in quercetin (QE) equivalent/100 g dried extract respectively.

According to the total phenolic and total flavonoids assay, the obtained results proved that both extracts aqueous Ull-1 and 80% methanol Ull-2 for *U. lobata* leaves had the highest total phenol and flavonoid contents. This demonstrated that the phenolic compounds of this medicinal plant might consist mainly of polyphenols and flavonoids, more concentrated in polar solvents, which were more effective in extracting in these solvents. Previous studies recorded that phenolic and flavonoid compounds were associated with strong antioxidant activity by which they expressed healthy benefits.[12,49]

Phenolic and flavonoid contents in aqueous extract Ull-1 compared to 80% methanol Ull-2 extract of *U. lobata* leaves was significantly different ($p < 0.05$), i.e, total phenol and flavonoid content in aqueous extract Ull-1were significantly lower ($p < 0.05$) than in 80% methanol extract Ull-2. These results suggested that the higher level of antioxidant activity were due mainly to the presence of phenolic than flavonoid components. Phenolic compounds may be responsible for the free radical scavenging activity of *U. lobata* leaves extracts observed against all selected ROS in the present study, but, it can not be excluded that phenols and flavonoids in extracts from *U. lobata* leaves would act synergistically. Total phenolic concentrations in high amount than flavonoids, expressed as gallic acid equivalents showed good correlation with the antioxidant activity of these extracts in the present study.

According to the antioxidant activity developed by TP and TF, it can be confirmed the assertion according to which phenols had marked potential to exhibit high antioxidant activity against ROS compared to other phytochemicals present in plant extract [2, 12, 49, 81, 82]. This observation also suggested these phytochemicals TP and TF would also act in synergistic manner to restore the activity of the parents extracts.

Figure 15 presented the percentage reductions of DPPH and H_2O_2 activity caused by total phenols (TP) and flavonoids (TF), Gallic acid (Ga) and Ascorbic acid (Aa)

tested at the highest concentration of 5 µg/ml. At this test concentration, TP and TF presented 62.34 and 58.54% reduction of DPPH activity and 29 and 27.68% against H_2O_2 activity. Ga and Aa supplied 100 and 27.47%, and 44.24 and 31.05% reduction of DPPH and H_2O_2 activity respectively. From these results, it was observed that the activity of TP and TF against DPPH activity was high compared to Aa, but low compared to Ga. TP exhibited high activity compared to TF while GA showed high activity against the two selected ROS compared to TP, TF and Aa (Fig. 16).

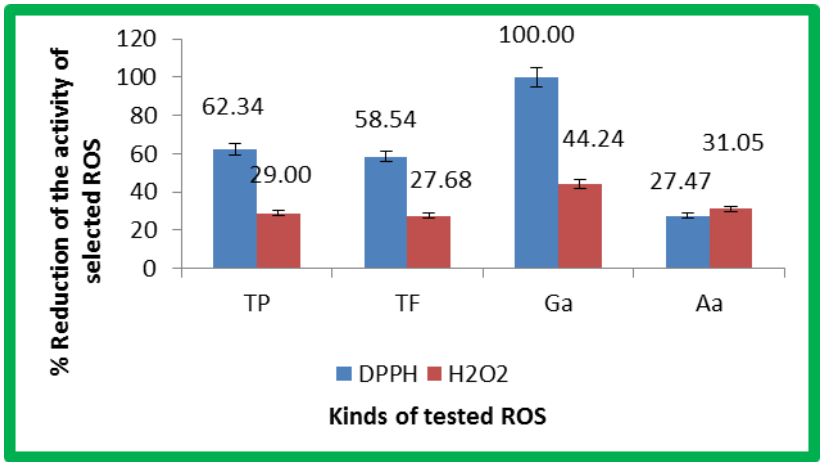

Figure 15: Percentage reductions of DPPH and H_2O_2 activity by total phenols (TP) and flavonoids (TF), Gallic acid (Ga) and Ascorbic acid (Aa).

Figure 16 brought back the percentage reductions of ABTS, superoxide anion $O_2^{\bullet-}$ and hydroxyl $HO^{\bullet}$ radicals activity produced by total phenols (TP) and flavonoids (TF), Gallic acid (Ga) and Ascorbic acid (Aa) at the highest concentration of 5 µg/ml. It was observed that at this concentration, TP and TF produced 48.54, 61.42 and 38.75%, 40.12, 59.24 and 35.41 reduction of ABTS, $O_2^{\bullet-}$ and $HO^{\bullet}$ activity respectively. Ga caused a reduction of ABTS, $O_2^{\bullet-}$ and $HO^{\bullet}$ activity by producing 68.50, 9.43 and 96.90% respectively while Aa showed 30.48, 12.17 and 38.34%. By comparison, it was observed that the effect of TP and TF on ABTS and $O_2^{\bullet-}$ activity was high compared to Aa with significant difference ($p < 0.05$). The activity of TP (38.75%) against $OH^{\bullet}$ activity was comparable to Aa (38.34) with no significant difference ($p > 0.05$), that of TF (35.41%) was low compared to Aa (38.34%) with significant difference ($p < 0.05$). The effect of Ga (9.43%) against superoxide anion was very low compared to TP (61.42%), TF (59.24) and Aa (32.17%) with prominent difference ($p < 0.05$).

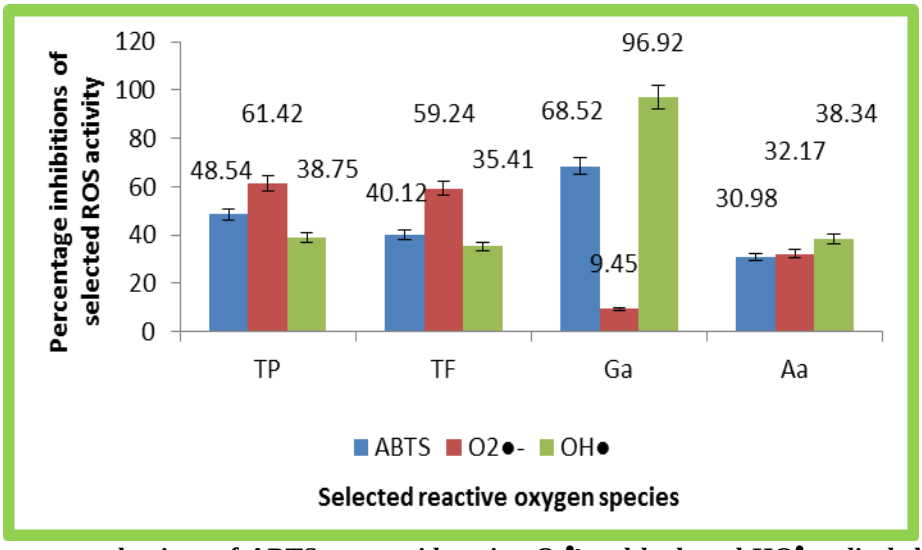

Figure 16: Percentage reductions of ABTS superoxide anion $O_2^{\bullet-}$ and hydroxyl $HO^{\bullet}$ radicals by total phenols (TP), flavonoids (TF), Gallic acid (Ga) and Ascorbic acid (Aa).

In return, Ga exhibited high (96.90%) activity against $HO^{\bullet}$ activity compared to TP (38.75%), TF (35.41) and Aa (38.34%) with marked difference ($p < 0.05$). Aa showed low effect compared to TP, TF and Ga at all tested concentrations all selected ROS. TP and TF exhibited high effect against $O_2^{\bullet-}$ compared to Ga and Aa (Fig. 16).

3.2. Effects of aqueous extracts on treated animals in acute and sub-acute toxicity

3.2.1. Acute toxicity

The acute toxicity study did not show any toxic and symptom signs with the administered oral doses of 500, 1000 and 5000 mg/kg of the aqueous extract Ull-1 of *U. lobata* leaves. It did not produce significant changes in behaviour, breathing, cutaneous effects (irritation, ulceration, caustic injuries and skin rashes), sensory nervous system responses, motility and gaestinal disorders in treated animals as also reported by [84] for the aqueous extract of *Combretum molle.*

3.2.2. Sub-acute toxicity

3.2.2.1. Effects on the behaviour, gross pathology, organ, and body weight

Throughout the study period, no sign of toxicity and mortality was observed on treated rats, which received all administered oral doses of aqueous extract Ull-1 of *U. lobate* leaves. Gross observations of the liver and kidneys of the treated rats did not show significant changes in treated rats compared with the negative control group and no significant difference ($p > 0.05$) was observed in the mean absolute organs weight between control and treated groups. Treated animals did not show signs of lethargy, nausea, vomiting, weakness, salivation, diarrhea, abnormally slow motor and reflex activities as also previously reported by [85, 86] for the methanolic extract of *Alstonia scholaris* leaves and ethanolic extract of *Marsdenia tenacissima* leaves in experimental rats respectively suggesting the indifference of the nature extracts on rats behavior. During the experimental period, no death or no apparent behavioral changes were observed in treated group compared to negative control group in both toxicity tests as also mentioned by [57] for 80% methanol **e**xtract of *Syzygium guineense* leaves. The normal behavior of animals during the observation of 28 days suggested the safety and harmless nature of aqueous Ull-1 extract even up to 5000 mg/kg bw in acute and sub-acute toxicity, no change in intake food was observed and in both tests, treated animals gained body weight compared to negative control as illustrated in figure 17. Our observations from both toxicities were in good agreement with.[24,55,58,61,89] At Day-0, the mean weight of negative control was 145.3 g and continue to slightly increase until 148.2 g on Day-14 with significant decrease on Day-21 (145.2 g) by a loss of 3 g (2.02%). It continued to significantly increase to attain 150.3 g on Day-28 by a gain of 5.1 g.

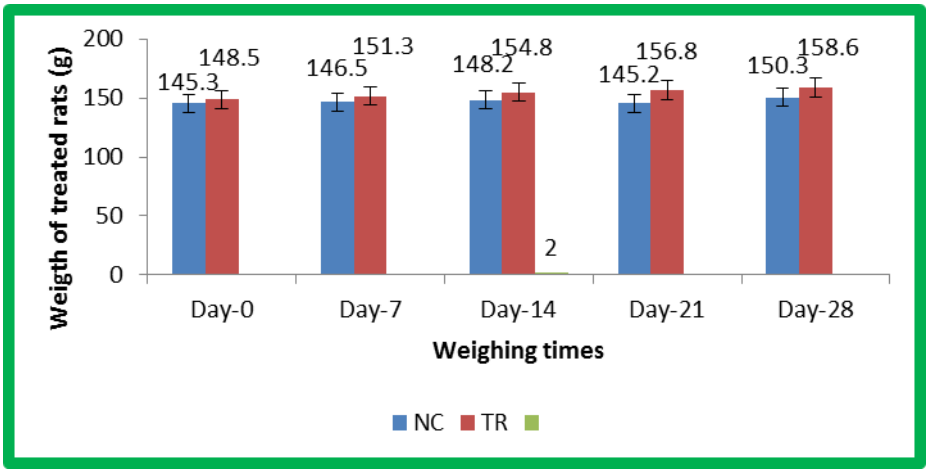

Figure 17: Variation of body weights of treated rats (TR) at oral dose of 5000 mg/kg bw with the aqueous extract Ull-1 of *U. lobata* compared to negative (NC) control in sub-acute toxicity.

On the other hand, body weight of treated rats was 148.5 on Day-0 and known gradual enhancement until 158.6 g on Day-28 by a gain of 10.1 g and statistically difference ($p < 0.05$) was deduced at the end of weighing since negative control gained 5 g (3.44%) and treated group 10 g (6.80%) at the end of weighing with significant difference ($p < 0.05$). All the animals exhibited a normal increment in body weights with significant difference ($p < 0.05$) between negative control and treated groups. In both toxicity tests, consumption of water and pellets was normal. The routine treated animal weights gained over the period of exposure may be due to the stimulating appetite by the administered extract and the presence of some phytochemicals in this extract. Tannins have been previously implicated in the increasing body mass.[88,89]

No mortality was observed in the treated groups at all doses during both toxicity studies at the highest oral dose of 5000 mg/kg bw. As a result, the LD_{50} of aqueous extract Ull-1 from *U. lobata* leaves could be estimated to be greater than 5000 mg/kg bw and the extract was considered pratically non-toxic per oral route, safe and well tolerated in treated animals.[53,58]

3.2.2.2. Effects of aqueous extract on organ weights

The effects of aqueous Ull-1 extract from *U. lobata* leaves on principal organ weights relative to body weight were presented in Table 2. There was no significant difference in average.

Table 2: Body weights of vital organs (g).

Organs	Average organ weights		
	Negative control	Ull-1: 1000 mg/kg bw	Ull-1: 5000 mg/kg bw
Liver	0.82 ± 0.016	0.84 ± 0.01	0.87 ± 0.05
Kidney	0.31 ± 0.01	0.32 ± 0.030	0.33 ± 0.02
Pancreas	0.14 ± 0.011	0.12 ± 0.013	0.16 ± 0.04
Heart	0.16 ± 0.03	0.15 ± 0.01	0.18 ± 0.02
Intestine	0.66 ± 0.01	0.65 ± 0.02	0.68 ± 0.04
Spleen	0.42 ± 0.02	0.44 ± 0.01	0.46 ± 0.04

organ weights ($p > 0.05$ between control and aqueous extract Ull-1 treated group at oral dose of 1000 mg/kg bw, but significant difference ($p < 0.05$) was observed with the highest oral dose of 5000 mg/kg bw (Table 2). The organs were found to be devoid of any sign of toxicity and no significant changes in any of the organ were observed. The results also revealed that the vital organs such as liver, kidney, heart, pancreas, spleen, small intestine and spleen were not adversely affected throughout the treatment by aqueous extract Ull-1and presented normal architecture in both groups. Thus, the administered aqueous extract Ull-1 from *U. lobata* leaves was devoid with toxicity on selected vital organs. These observations were also found and reported in other studies on the effects of some medicinal plant extracts on vital organ weights.[42,88,89] and our results were in good agreement with these studies.

3.2.2.2. Effects of aqueous extract Ull-1 on haematological parameters of treated rats at oral dose of 5000 mg/kg bw

In the sub-acute toxicity study, the haematological parameters such as haemoglobin, red cells, platelets, mean volume concentration (MVC), mean corpuscular haemoglobin concentration (MCHC), neutrophils, monocytes, platelets and segmented leucocytes of the treated rats known significant increase and showed significant difference ($p < 0.05$) compared

Table 3: Haematological parameter levels of treated animals exposed to aqueous extract Ull-1 of *U. lobata* leaves to 5000 mg/kg bw oral dose.

Parameters	Negative control	Ull-1: 5000 mg/kg bw	Reference values
Haemoglobin (g/dL)	15.8 ± 0.2	17.6 ± 0.1	15.0-18.2
Haematocrit (%)	47.5 ± 0.3	48.8 ± 0.0	40.7-50
Platelets (x $10^3\mu L^{-1}$)	1291.0 ± 0.6	1272.5 ± 0.0	995-1713
WBC (x $10^3\mu L^{-1}$	16.2 ± 0.3	17.5 ± 0.2	6.6-20.5
RBC (x 10^6 µL-1)	8.8 ± 0.4	9.2± 0.5	7.6-10.3
PVC (L/L)	41.5 ± 0.2.	43.4±2.940	42.50-49.40
MCV (fL)	46.2 ± 0.1	49.3±0.5	46.0-65.0
MCH (pg)	19.0 ± 1.1	20.5± 0.5	18.7-21.2
MCHC (g/dL)	38.5.1 ± 1.2	39.2.0±0.5	38-43
Eosinophils	0.15±0.02	0.17±0.03	0.03-0.21
Neutrophils x10^3/mm^3	21.2 ± 0.3	23.3 ± 0.3	3.0-24.7
Monocytes x10^3/mm^3	2.9 ± 1.1 2	3.2 ± 0.1	0.0-4.0
Basophils x10^3/mm^3	0.0±0.0	0.0±0.0	0.0±0.03
Lympocytes x10^3/mm^3	8.1 ± 0.3	8.7 ± 0.1	4.78-9.12
Segmented leucocytes (%)	17.6 ± 0.6.	21.7 ± 00.2	-

WBC; white blood cells, RBC: red blood cells, PVC: polyvinyl concentration, MCV: mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, fL: femotoliter, -: not found to negative control. On the other hand, haematocrit, white red blood cells WBC), red blood cells (RBC), polyvinyl chloride (PVC) and mean concentration haemoglobin known slight increase of their levels,, but did not present significant difference ($p > 0.05$) between both groups. Fortunately, all recorded values were within the reference ranges (Table 3).

The increase of platelet counts may indicate that the extract was endowed with anticoagulant activity and led to a shortening of the duration of bleeding and thus realized that the platelets were necessary for haemostasis since the decrease of platelet count was found often to carry high risk of bleeding.[666,92]

Eosinophils, basophiles, lymphocytes and monocytes also know slight increase in treated rats compared to untreated with not significant differences ($p > 0.05$). Segmented leucocytes in treated rats known significant increase compared to negative control ($p < 0.05$) (Table 3).

2.2.3. Effects of aqueous extract Ull-1 from *U. lobata* leaves on biochemical parameter levels of treated animals at oral dose of 5000 mg/kg bw

In the sub-acute toxicity study, glucose level in treated rats was low compared to negative control with significant difference ($p < 0.05$). This effect produced by aqueous extract from *U. lobata* leaves was probably due to its hypoglycemic properties as also previously reported for other medicinal plant extracts [42, 59, 93, 94] or to the effect of T3 and T4 hormones which accelerated the degradation of glycogen and glucose biosynthesis by neoglucgenesis,[95] In the current work, potential hepatotoxicity of the aqueous extract Ull-1 was assessed by measuring the enzymatic activities of aminotransferases ALAT and ASAT. An abnormal increase in these aminotransferase activities could frequently refer to hepatotoxicity [94]. Enzymatic activities of the liver function ALT, AST, and total proteins and albumins were used in this study to evaluate liver dysfunction. Serum activities of AST and ALT as well as the amount of total protein and albumin in treated rats with aqueous Ull-1 extract of *U. lobata* leaves were not significantly ($p > 0.05$) different when compared to the negative control.

Table 9: Biochemical parameters levels of treated animals exposed to aqueous extract Ull-1 of *U. lobata* leaves at oral dose 5000 mg/kg bw.

Parameters	Negative control	Ull-1: 5000 mg/kg bw	Reference values
Glucose mg/dl	86.5 ± 0.4	78.3 ± 0.3	62.4-201.8
Creatinine (mg/dL)	0.6 ± 0.3	0.7 ± 1.0	0.2-0.8
AST or SGPT (IU/L	181.6 ± 0.3	179.3 ± 0.2	0.-832.3
ALT or SGOT (UI/L)	51.2 ± 1.2	50.8 ± 0.2	1-223.3
Total cholesterol (mg/dL)	73.5 ± 1.3	72.0 ± 1.2	14.4-81.7
Triglycerides (mg/dL) 5	45.2 ± 0.	44.2 ± 0.2	2.7-47.8
Total bilirubin (mg/dL)	1 0.1 ± 1.3	11.3 ± 0.0	5.3-12.6
Direct bilirubin (mg/dL)	3.3 ± 0.0	3.5 ± 0.2	3.26-4.32
Total proteins (g/dL)	7.0 ± 1. 3	7.4 ± 1.1	5.6-7.6
Albumin (g/dL)	3.9 ± 0.2	4.3 ± 0.3	3.8-4.8
ALP (IU/L)	161.4 ± 1.0	165.3 ± 1.2	160.8-838.3
HDL-cholesterol (mg/dL)	37.8 ± 0.3	40.0 ± 0.4	7.2-42.0
LDL-cholesterol (mg/dL)	33.1 ± 1.1	32.6 ± 0.3	-20.66-49.22
Uric acid (mg/dL)	1.85 ± 0.8	1.90 ± 0.5	-
Urea (mmol/L)	6.2 ± 0.3	6.5±0.5	-

AST: aspartate transaminase, ALT: alanine transaminase, Alp, alkaline phosphate, HDL: high density lipoprotein, LDL: low density lipoprotein, SGPT: serum glutamopyruvate transaminase, SGOT: serum glutamic oxaloacetic transaminase, -: not found.

There was no deleterious effects on the activity of these aminotransferases or no significant change in parameter levels ($p > 0.05$) in treated rats compared to the negative control group suggesting good maintenance of hepatic function in the present study.

Moreover, AST and ALT had also been noted to increase in cytotoxic and cholestatic hepatic injury [42, 95]. ALT was found mainly in the liver and it's the most sensitive marker for hepatic cellular injury. Elevation of AST had been associated with hepatocellular injury in rats, while higher ALT activity has been linked to necrotic state [42, 97, 98,]. Also, reduction of albumin in serum may suggest continued loss of albumin or infection [95]. All levels of these enzymes, proteins and albumin were not modified by the administration of aqueous Ull-1 extract and did not show statistically significant difference compared to negative control. They were all within the accepted physiological ranges.[42,98,99,10,101,102]

Creatinine, SGPT and SGOT were also considered as hepatic biomarker enzymes. It was observed an insignificant difference in value levels of these biochemical parameters between the treated group compared to negative control group ($p > 0.05$), and suggested that aqueous extract of *U. lobata* leaves did not possess or not exert significant deleterious effects on hepato-renal functions or against the liver and kidney functions of treated animals, which in turn, were considered to be kept in good state during the treatment period.[103,104]

A slight decrease of the concentration levels of cholesterol and triglycerides in treated rat groups did not show significant difference compared to control negative group ($p > 0.05$) (Table 5). This effect may be due to the hypolipidimic property of the aqueous extract and to the increase secretion of thyroid hormones T3 and T4,[42,98] HDL and LDL in treated animals known also slight decreased or increased according to the case, but, it did not show significant difference ($p > 0.05$) compared to negative control group. These results suggested that the aqueous extract Ull-1 from *U. lobata* leaves, had beneficial effects by reducing some risk factors related to cardiovascular diseases [41, 42]. The remaining other biochemical parameters (Table 9) of treated groups at the highest oral dose of 5000 mg/kg bw known slight

increase or decrease according to the case, but it did not show significant difference compared to negative control ($p < 0.05$) and were in within the physiological reference ranges for rats.[42,97,98,99,100,101] or comparable to other value levels reported in other studies.[42,58,94,95,98] No significant clinical observations, haematological, biochemical and histopathological changes were observed in treated animals versus negative control in 28 days of oral toxicity study. These results clearly demonstrated that the administered aqueous extract Ull-1 from *U. lobata* leaves can not be considered as a source of any pathological state because it was taken as safe and well tolerated by treated animals without any toxic effect.

3.3. Histological analysis

The gross pathological studies on the liver and kidneys of treated rats showed no significant abnormal changes in color, size, shape and texture compared with the negative control. Histopathological evaluation showed no adverse effects of aqueous Ull-1 extract on the dissected organs as swelling, atrophy and hypertrophy were not observed as also reported by.[105] for methanolic extract of *Pistacia integerrima* leaf. Particularly, histopathological analysis of the kidneys sections of rats treated with doses of 5000 mg/kg bw showed no significant microscopic changes compared with the control group. In the treated rats of kidney sections, glomerulus (G) was found normal, Bowman's capsule lined with outer parietal layer/squamous cells (SC) and inner visceral layer/podocytes (P), distal convoluted tubules (DCTs) lined by simple cuboidal epithelium with more nuclei per cross-section and macula densa (MD) with taller cells around the vascular pole, proximal convoluted tubules (PCTs) lined by simple cuboidal epithelium with brush borde and urinary space (US), were detected in good state with normal architecture as also previously reported by,[59] for methanol extract of *Syzygium guineense* leaves. In addition, examined kidney and liver showed any morphological alterations or abnormalities under the light microscope as also previously reported by.[58] for aqueous extract of *Vernonia mesp*ilifolia whole plant.

4. CONCLUSION

Antioxidant-rich plant extracts serves as sources of nutraceuticals that alleviate the oxidative stress and therefore prevent or slow down the degenerative diseases. An effort has been made to explore the antioxidant properties of commercial available herbal extracts. This indicates the potential of

Antioxidant-rich plant extracts serves as sources of nutraceuticals that alleviate the oxidative stress and therefore prevent or slow down the degenerative diseases. An effort has been made to explore the antioxidant properties of commercial available herbal extracts. This indicates the potential Based on the results obtained in the present study, it was concluded that the aqueous and 80% methanol extracts as well as soluble fractions from the partition of aqueous extract, exhibited considerable antioxidant radical and scavenging activity on all selected tested assays as 2,2'-diphenyl-1-picrahydrazyl (DPPH), 2,2′- azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), superoxide anion ($O^{2\bullet-}$), hydroxyl ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) radicals. They possessed substantial amounts of phenolic and flavonoids compounds which exerted also this activity with different magnitudes. In acute and sub-acute toxicity tests, aqueous extract used once at oral dose of 500, 1000 and 5000 mg/kg bw or daily at oral doses of 500, 2000 and 5000 mg/k bw on 28-days, in acute and sub-acute toxicity respectively, did not induced mortality and any toxic effect in treated animals. It was thus considered as safe and well tolerated in animals. Its lethal dose 50 (LD_{50}) was estimated to be > 5000 mg/kg bw. In addition, it did not produce any significant change of organ weights, haematological and biochemical parameter levels of treated animals compared to untreated. Therefore, *Urena lobata* leaves can be considered practically non-toxic per oral route and as new good source of antioxidants which might be beneficial for combating oxidative stress and other diseases particularly cardiovascular illenesses.

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