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IN VITRO ANTIOXIDANT AND FREE RADICAL SCAVENGING EFFECTS OF EXTRACTS AND FRACTIONS, ACUTE AND SUB-ACUTE TOXIXITY OF AQUEOUS EXTRACT FROM *IPOMOEA BATATAS* (L.) LAM. (CONVOLVULACEAE) LEAVES FROM CENTRAL KASAI IN DEMOCRATIC REPUBLIC OF CONGO

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ABSTRACT

The potential antioxidant and free radical scavenging activities of aqueous extract and its soluble fractions, and 80% methanol extract from *Ipomoea batatas* leaves was measured using various *in vitro* assays. The acute and sub-acute toxicity aqueous was also evaluated. In antioxidant assays, among these tested samples, 80% methanol extract showed greater antioxidant activity measured as scavenging effects on DPPH, ABTS, superoxide anions $O_2^{\bullet-}$, hydroxyl $HO^{\bullet}$ and hydrogen peroxide H_2O_2 radicals with IC_{50} values of 2.3±0.03, 3.62±0.06, 6.52±0.10, 5.06±0.11 and 8.63±0.01 µg/ml. respectively, It was followed by aqueous extract showing IC_{50} values of 3.56±0.02, 5.62±0.03, 8.56±0.03, 9.63±0.02 and 14.63±0.01 µg/ml respectively against the same order of selected ROS. Choroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions from the partition of the aqueous extract exerted also scavenging activity against these ROS with IC_{50} values ranging from 13.25±0.08 to 23.02±0.06 µg/ml as their antioxidant activity. Total polyphenols and flavonoids inhibited the activities of these selected ROS with IC_{50} values from 1.75±0.02 to 7.65±0.02 µg/ml, showing thus their antioxidant activity. In acute and subacute toxicity, aqueous extract of *Ipomea batatas* leaves did not induced mortality of treated rats and its lethal dose 50 (LD_{50}) was estimated to be > 5000 mg/kb body weight. It had no influence on haematological, biochemical and organ weights of treated rats. The extract was considered non-toxic per oral route, safe and well tolerated. Data from the present results revealed that samples form *Ipomoea batatas* acted as antioxidant agents due to its free radical scavenging activities and the plant can be considered as new source of antioxidant constituents which remained to be isolated in future.

KEYWORDS: *Ipomoea batatas*, Convolvulaceae, leaves, extracts, fractions, total phenols and flavonoids, free scavenging and antioxidant activities.

1. INTRODUCTION

Reactive oxygens species include various free radicals such as singlet oxygen, superoxide , hydroxyl, hydroperoxyl ($HO_2^{\bullet}$), carbonate, peroxyl, alkoxyl, and carbon dioxide radical, and non-radicals, such as hydrogen peroxide, hypobromous acid, hypochlorous acid, ozone, organic peroxides, peroxynitrite, peroxynitrate, peroxynitrous acid, peroxomonocarbonate, nitric oxide), and hypochlorite, 2,2-diphenyl-1-picryhydrazyl, 2,2-azino-bis-3-ethylbenthiazoline-6-sulphonate used in different assays to assess antioxidant and free radical scavenging activities of natural and synthetic products, medicinal plant extracts and their fractions (Augusto et al, 2011, Wu et al., 2014; Dayem et al., 2017).

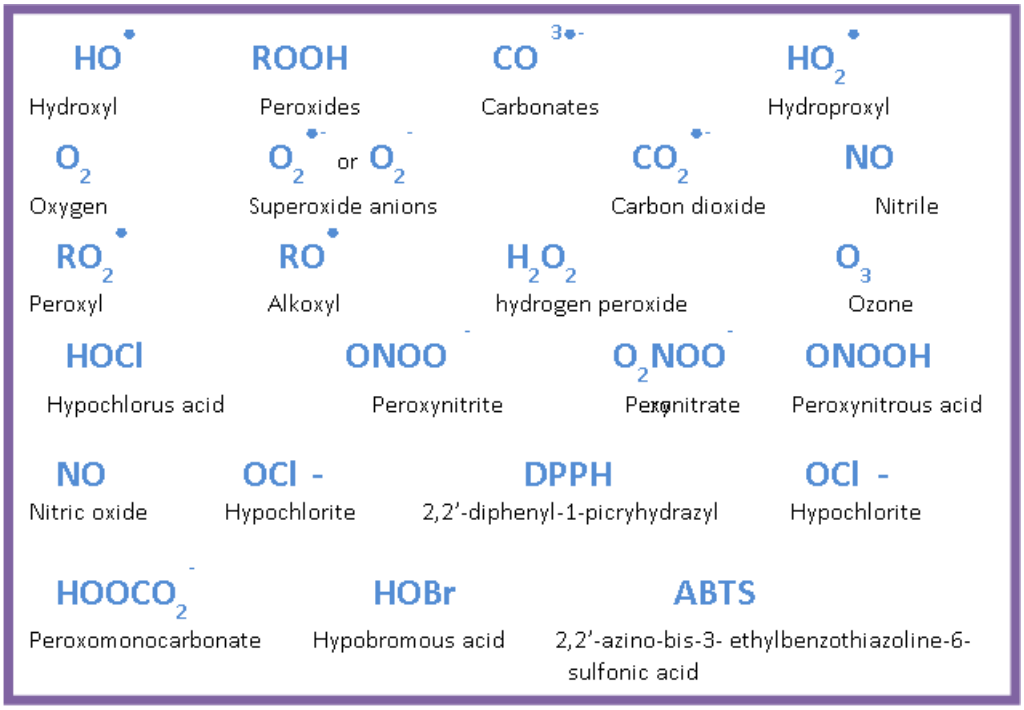


Figure 1: Chemical symbols some reactive oxygen species.

The key roles played by reactive oxygen species (ROS) and other oxidants in causing numerous disorders and diseases are well documented. The role of free radical reactions in disease pathology is known to be involved in many acute and chronic disorders in human beings such as diabetes, atherosclerosis, aging, inflammation, immunosuppression and neurodegeneration, etc. (Dayem et l., 2017). Reactive oxygen species (ROS) are natural byproducts of cellular oxidative metabolism and play important roles in the modulation of cell survival, cell death, differentiation, cell signaling, enzyme inhibition and inflammation-related factor production. An imbalance between ROS and the inherent antioxidant capacity of the body directed the use of dietary and/or medicinal supplements particularly during the disease attack are well known and documented (Maqsood et al., 2010, Zengin et al., 2011).

Studies on herbal medicinal plants, vegetables, nuts and fruits have indicated the presence of antioxidant constituents such as phenolics like catechic and gallic tannins, proanthocyanidins flavonoids, anthocyanins and tannins, with polysaccharides, alkaloids, steroids and terpenoids (Saeed et al, 2012). Their effects have appointed the attention of scientists to understand their actions for good preventing and treatment of diseases that they generate and to assure good maintenance of human health (Saeed et al., 2012). Human body is equipped with inherent antioxidative mechanisms such as **(1)** Glutathione system includes glutathione S-transferases, glutathione peroxidases, and glutathione reductase, **(2)** Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH), **(3)** Catalase (H_2O_2 oxidoreductase) (Almokhtar et al., 2018), **(4)** Catalytic removal of free radicals and reactive species by factors such as CAT, SOD, GPx and thiol-specific antioxidants, **(5)** binding of proteins (e.g., transferrin, metallothionein, haptoglobins and caeroplasmin) to pro-oxidant metal ions such as iron and copper protection against macromolecular damage by proteins such as stress or heat shock proteins; and **(6)** reduction of free radicals by electron donors, such as GSH, vitamin E (α- tocopherol), vitamin C (ascorbic acid), bilirubin, and uric acid, **(7)** Animal CAT are heme-containing enzymes that convert hydrogen peroxide (H_2O_2) to water and O_2, and they are largely localized in subcellular organelles such as peroxisomes (Praveen and Ashish (2012) having many biological functions such as the anti-aging, anti-carcinogenic and anti-mutagenic (Gocer et al., 2011; Glulcin et al., 2012,). Antioxidant substances can stabilize, deactivate or destroy free radicals often before they attack targets in biological cells (Nunes et al, 2012, Saeed et al., 2012).

The antioxidant contents of medicinal plants may contribute to the protection that they offer from various diseases. It is well known that free radicals cause cell damages through mechanisms of covalent binding and lipid peroxidation with subsequent tissue injuries. The ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Gulcin et al, 2012). They are very effective to prevent the destructive processes caused by oxidative stress (Shah et al., 2015**)** and reduce risks of degenerative diseases (Qasim et al., 2015).

The use of medicinal plants containing different substances of natural origin with high level of antioxidant properties has been proposed as an effective

and alternative therapeutic approach for hepatic damages and other illness (Govind, 2011).

Nowadays, there is a great interest in the evaluation of antioxidant of medicinal extracts and fractions, and more reported results showed that these natural materials exhibit this activity *in vitro* and *in vivo* tests at different extents (Chen et al., 2014; Rahman et al., 2015; Rezaeian et al., 2015; Mancarz et al., 2016; Al-Rifai et al., 2017; Dong-Ping et al., 2017; Xu et al., 2017; Bukuté et al., 2018; Venkatachalam et al., 2018; Alhage and Elbitar, 2019; Merghem et al., 2019, Unuofin et al., 2020).

Ipomoea batatas is a plant belonging to the Convolulaceae family originate to central America and Pacific Islands. In its originate countries, it is a perenial climbing plant that attains 1 to 3 m of length. Sweet Caroline Light Green' in a dwarf form, selected for its foliage and its bearing and lining with high ornamental value. This plant forms a ramified tuft, rounded and thick (dense), drooping or covering according to it and is cultivated in suspension. It will attain in season around 30 cm high for a diameter of 70 cm minimum. Leaves are big, palmed , decoupled in 5 lobes lengthened, green limes, finely margined with a green more dark, pointed to their extremities. This plant less flowered under small pink funnels, appears very obliging towards to soil and show good tolerance to heat and summer dryness state (https://www.promessedefleurs.com/annuelles/fleure-annuelles-en-minottes/annuelles-par-varites/ipomee/ipomee-poliseron-, 2021).

The medicinal uses of *Ipomoea batatas* are widespread and numerous in tropical Africa and in other countries of the world. The leaves and roots of *U. lobata* are mainly used. They are considered as expectorant and emollient.

In Benin, a maceration of the leaves is taken against infectious diarrhoea. In Côte d'Ivoire, a decoction of the plant is taken as oxytocic and against fever. In the Central African Republic, it used as against pneumonia. A preparation of the leaves with those of other plants is applied to treat menstrual problems. In Congo-Brazzaville, the sap expressed from the leaves is given to women giving birth to their first child and a leaf decoction is drunk against hypertension.

In Democratic Republic of Congo (DRCongo) powdered leaves with powdered clay in water is taken regularly as a treatment of asthma, diarrhea and diabetes. In Togo, women drink a decoction of the leaves and sometimes also, in combining with the stems and leaves of *Vernonia cinerea* (L.) Less. against infertility. In Gabon, a macerate of the stem bark or root is prescribed against diarrhoea. In Madagascar, a preparation of the root is applied to treat infected eyelids and against syphilis. Scrapings of the stem bark are put on wounds. A decoction of bark and root is given to children with enteritis or stomach pains. The leaves are widely used to induce labour or facilitate childbirth. In Madagascar and Nigeria, a preparation of the root is applied externally against rheumatism, a purpose for which it is also used in Vietnam. The sap from the leaves is dripped onto wounds and a poultice of boiled leaves is applied to rheumatic inflammations, while a poultice of leaves and seeds is applied against the gall bladders problems and other intestinal complaints. A decoction of the flowers is taken against respiratory problems. 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 and flowering in winter. In Zanzibar and Indonesia, a decoction of the root is taken against indigestion. In Uganda, leaves are used against snake-bites and a leaf infusion is taken against diarrhoea. In Guinea, water in which flowers are steeped is drunk as antiseptic

(http://uses.planetproject.org/en/Urena_lobata(PROTA)♯:□ :text%20bast%fibre520Urena,%C%..., 2021)

In Malaysia, Indo-China, the Philippines, Papua New Guinea, Fiji and Java, the juice of the leaves or roots is widely used for bowel complaints, especially colic, stomach-ache, diarrhoea and dysentery, and to treat gonorrhoea and persistent fever from malaria. The leaves are externally applied as a poultice on wounds and skin diseases as an emollient and refrigerant because of their styptic and healing properties. A decoction from the leaves and roots is drunk to relieve pains all over the body due to excessive exertion. An infusion of the roots is given to aid difficult childbirth. A lotion made from the plant is used to treat yaws and headache.

In Burma (Myanmar), India and Malaysia, the roots are used to treat rheumatism and lumbago, while the twigs are chewed for toothache. In India, the root is popular as a diuretic, while the leaves are prescribed for inflammation of the intestines and bladders.

In China and Fiji, the whole plant is macerated and used externally for treating fractures, wounds, mastitis and snake bites. A decoction of the root is used to treat colds, dysentery, enteritis, goitre, indigestion, leucorrhoea, malaria, rheumatism and tonsilitis. A decoction of a very old plant, boiled with eggs, is said to induce abortion. In Fiji, the roots are also chewed and applied to swellings caused by filariasis, while the bark is used to heal cuts.

In Thailand, the leaves and stems are used as a diuretic, while the roots are taken for stomach-ache.

In India and Indo-China, the flowers are considered maturative and are taken in decoction as a pectoral and expectorant in dry coughs. An infusion of the flowers is used as a gargle for aphthae and a sore throat. In Malaysia, a decoction of the seeds is taken as a vermifuge. Young shoots and leaves are eaten as a vegetable and animals eat the foliage.

The seeds are used in Africa in stews and in India for making soap, while the charcoal of the whole plant is used for blackening teeth. Like *Sida rhombifolia* L., *Ipomoea batatas* is considered in Malaysia a magic plant and is used in similar ways in healing rites, for protection and in wedding and rice ceremonies.

Ipomoea batatas L. Lam. is a functional food and belongs to Convolvulaceae family. Some of its biological activities such as antioxidant, hypoglycemic, wound healing, antiulcer, anti-inflammatory, antimutagenic, antivascular, heapatoprotective, immunomodulatory, antiproliferative, antifungal and antimicrobial (Panda and Sonkamble, 2012), antioxidant, antidiabetic, anticancer, antitumor, effect on immune system, antiulcer, cardiovascular antimicrobial and antiinflammatory (Ayeleso et al., 2016; Zengin et al., 2017), antiinflammatory, antiarthritic, antiedema, antioxidant (Majid et al., 2018, Rafu et al., 2018), antimicrobial and antioxidant (Osuntokum, 2020), effects were evaluated *in vitro* and *in vivo* models.

The literature record don't give any information on the antioxidant activity of aqueous extract and its soluble fractions, and 80% methanol extract, gastro-intestinal motility and enteropooling tests, as well as the acute and sub-acute toxicity of aqueous extract of *I. batatas* leaves. Thus, the present work was initiated to assess these biological activities in experimental rats.

2. MATERIALS AND METHODS

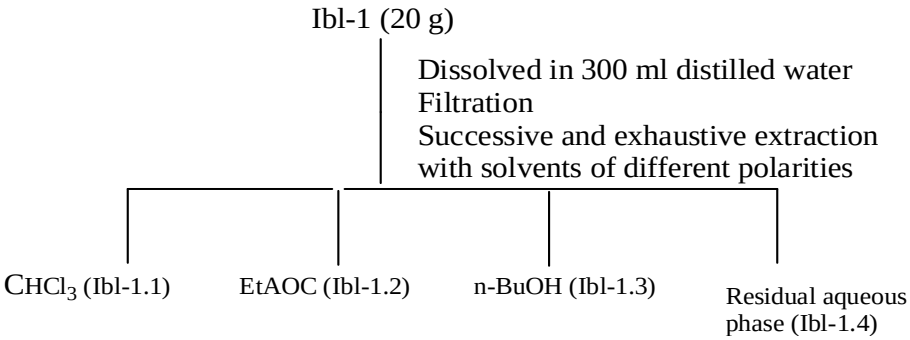
2.1. Plant material

Leaves of *Ipomoea batatas* (Ranonculaceae) were collected in Central Kasai, one of the provinces of Democratic Republic of Congo (DR-Congo). It was identified at the National Institute of Studies and Researches in Agronomy (NISRA), Departement of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen of the plant had been deposited in the herbarium of this institute and in Laboratory of Phytochemistry at the faculty of Pharmaceutical Sciences at the same university. Leaves were dried at room temperature and reduced to powder using an electronic blender and were kept in brown bottles hermetically closed before use.

Figure 1: *Ipmoea batata* (Convolvulaceae), leaves, flowers and sweet potato.

2.2. Preparation of extracts and partition of lyophilized aqueous extract

45 g of powdered leaves were mixed with 300 ml distilled water and boiled on hotplate for 15 minutes. The mixture was cooled and filtered on a filter paper F001 grade (CHLAB GROUP, 08205, Barcelona, Spain). The filtrate was evaporated in vacuum using rotary evaporator yielding dried aqueous extract named as Ibl-1 (40.84 g). 20 g of Ibl-1 were dissolved in 200.



(Ibl: *Ipomoea batatas* leaves)

Figure 2: Partition of lyophilized aqueous extract Ibl-1.

ml distilled water, filtered as described above, and fractioned according to the scheme (Fig. 2) and each fraction was treated as described above yielding corresponding dried extracts named as Ibl-1.1 (4.31 g, Ibl-1.2 (5.12 g), Ibl-1.3 (3.05 g) and Ibl-1.4 (7.35 g) for chloroform, ethylacetate, *n*-butanol and residual aqueous soluble fractions respectively (Harborne, 1998; Trease and Evans, 2000). On other hand, the same amount of plant material was macerated with 80% methanol for 24 h and after filtration in the same conditions as described above to have 80% macerate, the marc was exhaustively percolated with the same solvent. The macerate et percolate were combined and evaporated in vacuum to give a dried methanol extract denoted as Ibl-2 (41.36g).

2.3. Qualitative phytochemical screening

The identification of major phytochemical groups like alkaloids, nitroso compounds, anthocyanins, anthraquinones, coumarins, flavonoids, polysaccharides, catechic and gallic tannins, proantocyanidins, saponins, reducing sugars or carbohydrates, steroids and terpenoids in aqueous extract Ibl-1 of *I. batatas* leaves was carried out in tube solutions and by TLC (thin layer chromatography on silica gel plates, thickness layer: 0.25 mm, Merck, Germany) using different chemical reagents and mobile phases described in the literature (Harborne, 1998; Trease and Evans and, 2000).

2.5. Evaluation of antioxidant activity

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

The capacity of extracts and fractions from *I. batatas* leaves to inhibit DDPH radical activity was evaluated using the methods previously described by Ekin et al., (2017) and Manthal et al. (2019). 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 test concentrations from 1 to 20 µg/ml. 1 ml of each test sample dilution was mixed with 1 ml DDPH 0.4 M MeOH solution and the mixture was left in obscurity for 30 minutes before the measure of absorbance on a spectrophotometer Shimaduzu (USA) at 517 nm. DPPH 0.4 M MeOH solution was used as negative control. 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 (n=3). 3

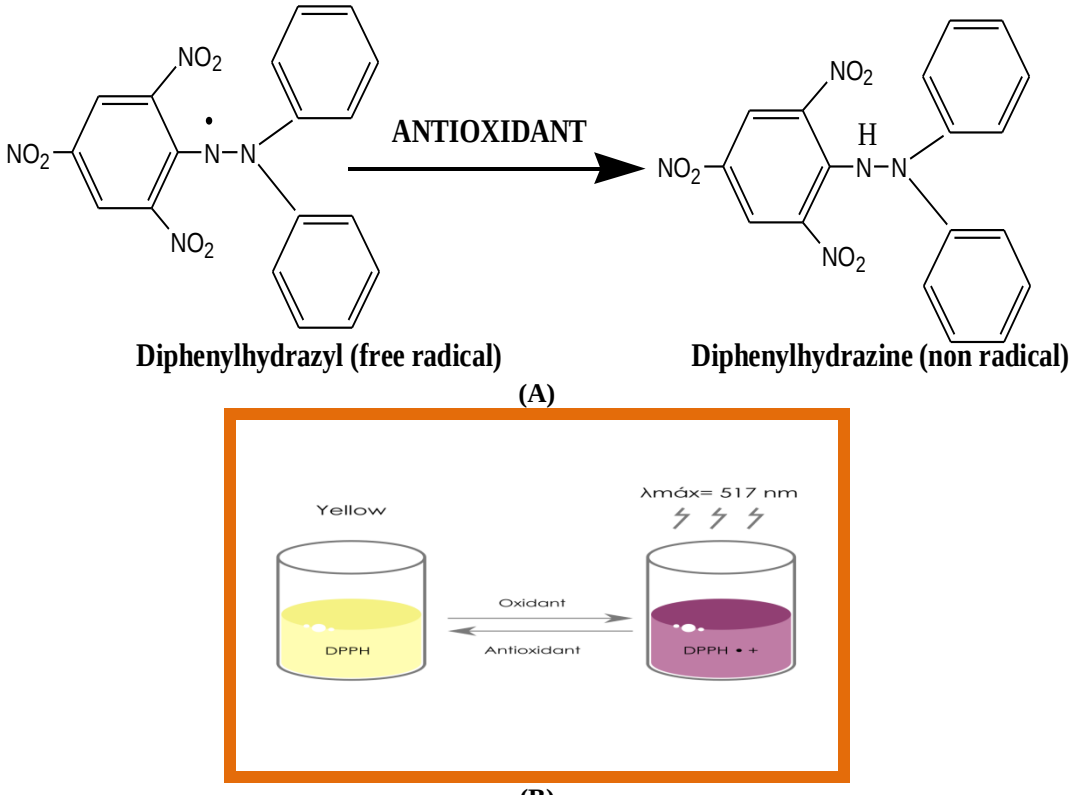


Figure 3: Reduction of DPPH in the presence of antioxidant substance (A) and its reactivity (B) (Kedare et al., 2011).

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

Methods previously reported by de Vargas et al. (2016) and Chaves et al., (2020) based on the oxidation of ABTS were used. The oxidated ABTS solution was prepared by reaction of 2 mM ABTS in deionized water with potassium persulfate ($K_2S_2O_8$). Before used, ABTS solution was diluted with phosphate sodic tampon (0.1 M, pH 7.4) to have an absorbance of 0.750 at 734 nm.

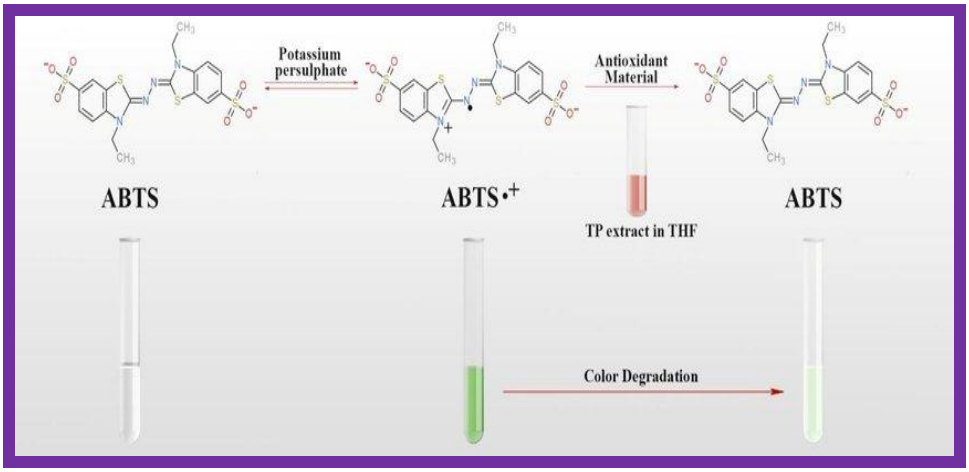


Figure 4: Reduction of ABTS and reaction of the formed radical with antioxidant material.

After, 1 ml of ABTS solution was mixed with 1 ml of test sample (test concentrations: 1-50 µg/ml), well mixed and kept in obscurity for 4 h before to measure the absorbance. ABTS solution was taken as negative control. Absorbances were taken on the same apparatus at 734 nm. The percentage inhibitions of the activity of ABTS 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.

2.5.3. Superoxide anions ($O^{2\bullet-}$) radical scavenging activity

For this test, the methods described by de Vargas et al. (2016), Keshari et al, (2018) and Chaves et al., (2020) were employed. For this test, 2 mg of each test samples were dissolved in the same manner as described above to have respective stock solutions (concentration: 1 ml/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 a test dilution sample mixed with 250 mM nitrobleuterazolium (NBT, 100 µL) and 390 µM NAD (100 µL). Absorbances were recorded at the same apparatus, the same formula was used to calculated the percentage inhibitions of the production of superoxide anions and IC_{50} values were obtained using linear courbes-responses.

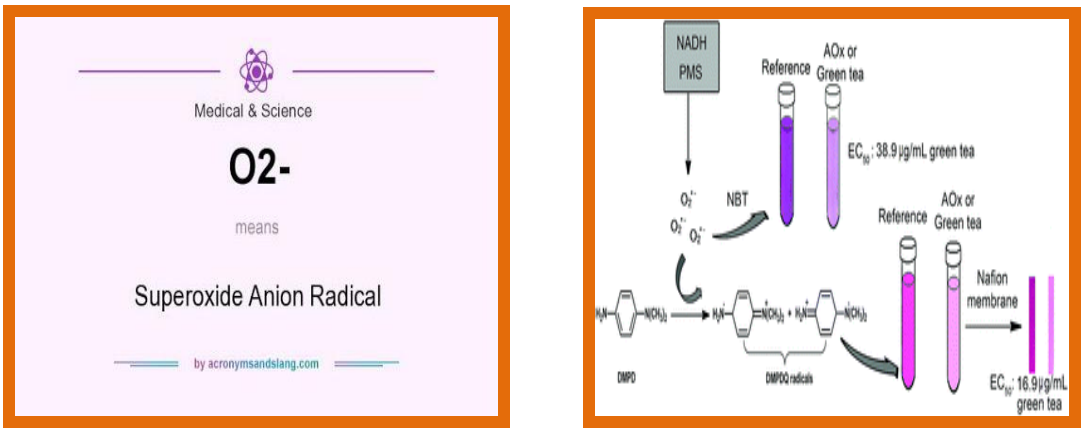


Figure 5: Superoxide anion and its reactivity.

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

The hydroxyl radical scavenging activity of *I. batatas* leaves extracts and fractions was assayed according to the earlier methods described by Keahari et al., (2018) and Lalhminghlui and Jagetia (2018) with minor modifications. The reaction mixture contained deoxyribose (2.8 mM), KH_2PO_4-NaOH buffer, pH 7.4 (0.05 M), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM) and different concentrations of *I. batatas* extracts and fractions in a final volume of 2 ml and other tested samples without deoxyribose. The mixture was incubated

at 37°C for 30 min followed by the addition of 2 ml of trichloroacetic acid (TCA) (2.8% w/v) and thiobarbituric acid (TBA). Thereafter it was kept for 30 min in a boiling water bath, and cooled. The absorbance was recorded at 532 nm in a UV–VIS spectrophotometer.

$$HO^{\cdot} \text{ scavenging effect (\%)} = \frac{A1 - A2}{A3} \times 100$$

where A1 was the absorbance of the negative control without a test sample. A2 was the absorbance after adding the sample and 2-deoxy-D-ribose, A3 is the absorbance of the sample without 2-deoxy-D-ribose. The percentage inhibitions were plotted against concentrations, from the graph and IC_{50} values for each tested sample were calculated.

Figure 6: Common reactions for hydroxyl radical and its reactivity.

2.7. Hydrogen peroxide assay

For this test, methods proposed by Al-Amiery et al., (2015) and Etkin et al,., (2017) were followed. Briefly, solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations from 1 to 20 µg/ml of samples from *I. batatas* leaves (1 ml each) were added to 1 ml hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The hydrogen peroxide percentages scavenging activity were then calculated using the following equation:

$$H_2O_2 \text{ scavenging effect (\%)} = \frac{A0 - A1}{A0} \times 100$$

Where A0 was the absorbance of the negative control and A1 the absorbance of the tested sample.

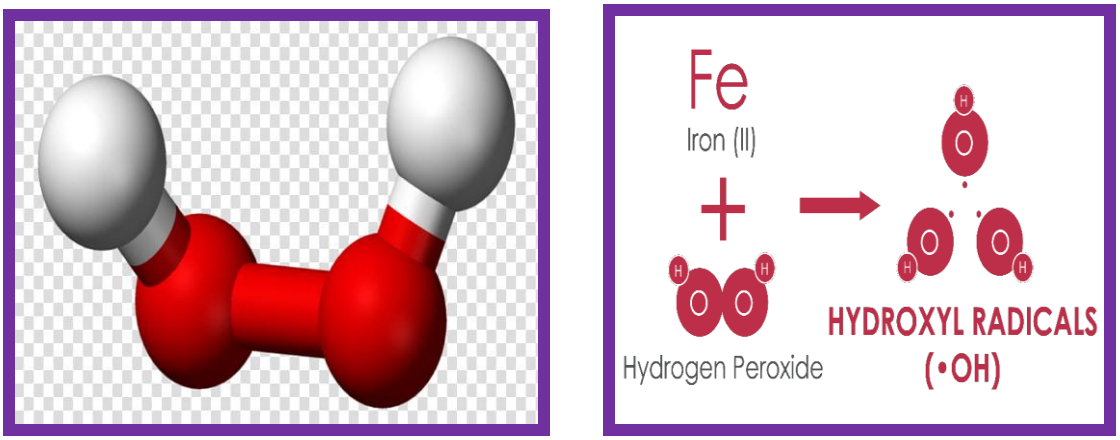


Figure 7: Hydrogen peroxide and its conversion in hydroxyl radical.

o a test tube were added 3 ml of phosphate
o a test tube were added 3 ml of phosphate

2.6. Estimation of phenolic compounds content

The quantity of total phenolic compounds in aqueous Ibl-1 and 80% methanol Ibl-2 extracts of *I. batatas* leaves was determined quantitatively using Folin-Ciocalteu's (FC) reagent with the method described by Abdollah et al, 2014; Akalin et al., 2017). 2 mg of aqueous Ibl-1and 80% methanol Ibl-2 extracts of *I. batatas* leaves were dissolved in 2 ml MeOH to have stock solutions of 1 mg/ml. It was further diluted in two-fold dilutions of have a series of test concentrations from 1 to 20 µg/ml. 1 ml of each test dilution was introduced separately in different tubes 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 (5-20 µg/ml). After this period, the absorbances were recorded at 765 nm using the same spectrophotometer apparatus. The quantity of total phenolic compounds was expressed as mg/100 g gallic acid equivalent of dried extract.

2.7. Estimation of flavonoids content

The content of total flavonoids in aqueous Ibl-1 and 80% methanol Ibl-2 extracts of *I. batatas* leaves was determined quantitatively with ACl_3 % MeOH solution using methods described by Abdollah et al, 2014; Talla et al., (2016) and Manthal et al., (2019). 2 mg of aqueous Ibl-1 and 80% methanol Ibl-2 extracts of *I. batatas* leaves were dissolved in 2 ml MeOH to have stock solutions of 1 mg/ml. They were further diluted in two-fold dilutions to have a series of test concentrations from 1 to 20 µg/ml. To 1 ml of each test dilution, 1 ml of ACl_3 5% in MeOH was added, 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 was expressed in term of quercetin equivalent as mg/100 g dried extract.

2.8. Evaluation of acute and sub-acute toxicity

2.8.1. Toxic effects and determination of lethal dose 50 (LD_{50})

The sub-acute toxicity was determined according to the methods described by Unuofin et al., (2018) and Loha et al., (2019) by the administration once in acute toxicity and daily in sub-acute toxicity oral doses of 500, 1000 and 5000 mg/kg bw of aqueous extract Ibl-1 of *I. batata*s leaves. Briefly, rats were divided in 4 groups as followed:

●- Group I (2 rats) orally received 5 ml distilled water by gavage as negative control group,

●- Groups II, III and IV were administered aqueous extract Ibl-1 at respective doses above.

Animals were placed in individual plastic cages and fed as much as water and pellets. They were continually observed for toxic effects during the first 4 hours and daily weighed. All animals were observed for a total of 28 days. All abnormal suspect movements and death of animals were recorded. The lethal dose 50 (LD_{50}) was determined if mortality was observed.

2.8.2. Estimation of haematological and biochemical parameters in treated 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 was 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 (Loha et al., 2019).

2.8.3. Relative organ weights

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, plentifully washed with distilled water, dried in hot at 50^{o}C and weighted. The relative organ weights of each animal was then calculated as follows:

$$\text{Relative organ weight (\%)} = \frac{100 \times \text{Absolute organ weight (g)}}{\text{Body weight (g)}}$$

(Unuofin et al., 2018; Ugwah-Oguejiofor, et al., 2019).

2.9. Histological study

Liver, heart, lungs, kidneys and pancreas were excised from dissected animals, plentifully washed with NaCl 0.9% (for removal of blood), dried, weighed, and stored in 10% formalin saline solution for histopathological analysis according to the techniques described by Kotué et al., (2013) and Loha et al., (2019). Briefly, the tissue sections were again plentifully washed with distilled water and stained with haematoxylin-eosin for light microscop analysis. The slides of all selected vital organs were made and then observed under light microscop at a magnification of x 20 objective for the investigations of any histological change in treated group compared to negative control group.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

Results from the phytochemical screening of aqueous extract Ibl-1 of *Ipomoea batatas* leaves revealed the presence of alkaloids, flavonoids, saponins, catechic and gallic tannins, proanthocyanidins, reducing sugars or carbohydrates, glycosides, steroids, polysaccharides and terpenoids. Anthocyanins, anthraquinones, coumarins and cardiotonic heterosides were not detected in our experimental conditions. The chemical composition of aqueous Ibl-1 extract was similar to that 80% methanol Ibl-2 extract. Our results were in good agreement with Luo and Kong (2005) for the presence of flavonoids among which some were isolated as rhamnetin, rhamnocitrin, tiliroside, astragalin and kaempferol, Ling-Yuz et al., (2009) for the presence of steroids and flavonoids among which some were isolated as tetracosane, myristic acid, beta-sitosterol, beta-carotene,

daucosterol and quercetin, Mbaeyi-Nwaoha and Onweluzo, (2013), Ayeleso et al., (2016) and Osuntokum et al., (2020) for the presence of tannins, alkaloids, steroids, glycosides, saponins, flavonoids, carbohydrates and except cardiac heterosides that were not identified in the present study and Hossain, (2019) for the presence of carbohydrates.

Table 1: Phytochemical screening.

Phytochemical groups	Results	Phytochemical groups	Results
Alkaloids	++	Saponins	+
Anthraquinones	_	Catechic tannins	++
Anthocyanins	_	Gallic tannins	++
Coumarins	_	Proanthocyanidins	++
Flavonoids	++	Polysaccharides	++
Reducing sugars	++	Carbohydrates and glycosides	++

3.2. Effects of samples from *I. batatas* on the activities of selected ROS

Extracts and fractions from *Ipomoea leaves* were tested against a series of reactive oxygen species (ROS) including 2.2'-diphenyl-1-picrylhydrazyll (DPPH), 2,2'-azino-bis(3-éthylbenzothiazoline-6-sulphonique acid (ABTS), superoxide anion ($O^{2\bullet}$), hydroxyl ($HO^{\bullet}$) and hydrogen peroxide (H_2O_2). For good understanding of the interpretation of results from this study, following criteria were adopted: $IC_{50} \leq 10$ µg/ml: strong or pronounced activity, $10 < IC_{50} \leq 20$ µg/ml: good activity, $20 < IC_{50} \leq 30$ µg/ml: moderate activity, $30 < IC_{50} \leq 40$ µg/ml: weak activity, $IC_{50} \geq 40$ µg/ml: inactive.

Results revealed that all samples including extracts and soluble fractions from *Ipomeoa batatas* leaves exhibited strong antioxidant activity **against DPPH** by producing IC_{50} values < 10 µg/ml. Among these samples, 80% methanol Ibl-2 extract exhibited high activity with IC_{50} value of 2.03±0.03 µg/ml. It was followed by aqueous extract Ibl-1, ehylacetate soluble fraction Ibl-2 rich in flavonoids, residual aqueous soluble fraction Ibl-1.4 rich in phenolics compounds other than flavonoids , *n*-butanol Ibl-13 soluble fraction rich in saponins and chloroform soluble fraction Ibl-1 rich in steroids and terpenoids showing IC_{50} values of.

Table 2: Antioxidant activity of extracts and fractions from *Ipomoea batatas* leaves against selected ROS (IC_{50}, µg/ml).

Sample codes	DPPH	ABTS	$O^{2\bullet}$	$HO^{\bullet}$	H_2O_2
Ibl-1 (aqueous)	3.56±0.02	5.62±0.03	8.56±0.03	9.63±0.02	14. 63±0.01
Ibl-2 (80% methanol)	2.03±0.03	3.62±0.04	6.52±0.04	5.06±0.03	8.63±0.04
Ibl-1.1 (chloroform)	9.62±0.02	13.65±0.01	16.25±0.01	18.52±0.05	20.03±0.05
Ibl-1.2 (ethylacetate)	5.25±0.02	7.15±0.05	13.25±0.03	12.52±0.01	16.58±0.03
Ibl-1.3 (*n*-butanol)	11.25±0.03	15.68±0.04	17.02±0.02	21.03±0.05	23.02±0.02
Ibl-1.4 (residual aqueous)	6.02±0.02	8.25±0.03	15.02±0.05	14.65±0.05	17.26±0.02
Total phenols (TP)	1.75±0.02	1.90±0.01	3.25±0.02	3.85±0.04	4.26±0.05
Total flavonoids (TF)	3.05±0.00	3.45±0.01	4.15±0.04	6.25±0.05	7.65±0.02
Ascorbic acid	1.56±0.02	1.85±0.05	3.02±0.01	4.65±0.02	6.12±0.05

3.56±0.02, 5.25±0.02, 6.02±0.02 and 9.62±0.02 µg/ml respectively. Total phenols and flavonoids showed strong antioxidant activity with IC_{50} values of 1.75±0.02 and 3.05±0.00 µg/ml respectively. Ascorbic acid used as reference antioxidant product exhibited high activity with IC_{50} value of 1.56±0.02 µg/ml compared to all samples from *I. batatas* leaves samples, total phenols and flavonoids.

Figure 8 showed the percentage reductions of DPPH activity by aqueous Ibl-1 and 80% methanol Ibl-2, total phenol and flavonoid extracts and ascorbic acid. They exerted this activity in dose-dependent manner. At the highest tested concentration of 5 µg/ml, aqueous extract Ibl-1 and 80% methanol extract Ibl-2 produced 70.22 and 100.00% inhibition of DPPH activity respectively, total phenols (TP) and flavonoids (TF) produced 100.00 and 82.00 % reduction respectively.

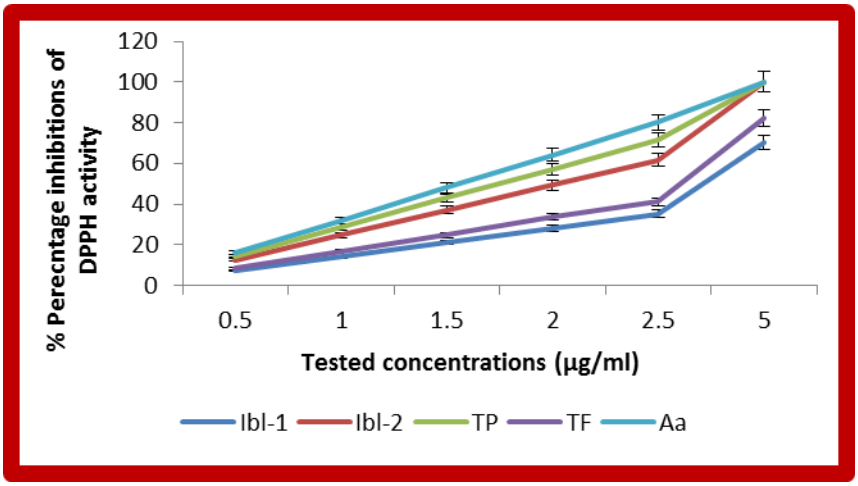


Figure 8: **Percentage reductions of DPPH radical activity by aqueous extract Ibl-1, 80% methanol extract Ibl-2, total phenols (TP) and flavonoids (TF), and Ascorbic acid (Aa)**

Ascorbic acid (Aa) gave 100% diminishing as a high activity compared to Ibl-1 and TF, but it was in the same range with TP and Ibl-2 with no significant difference ($p < 0.05$). Significant difference ($p < 0.05$) was observed in the comparison of the activity of TP, Ibl-2 and Aa to TF and Ibl-1. This last (70.22%) showed low effect compared to Ibl-2 (100.00%) and TF (82.00%) with significant difference ($p < 0.05$).

Next, when tested **against ABTS radical**, extracts and soluble fractions from this medicinal plants inhibited the activity of this radical with different magnitudes. 80% methanol extract Ibl-2 exhibited high activity with IC_{50} value of 3.62±0.06 µg/ml compared to aqueous extract Ibl-1 with IC_{50} value of 5.62±0.03. Soluble fractions also exhibited interesting antioxidant activity against this radical by producing IC_{50} values ranging from 7.15 ±0.05 to 17.02±0.02 µg/ml. Ethylacetate Ibl-1.2 with IC_{50} of 7.15±0.05 µg/ml was the most activity followed by residual aqueous Ibl-1.4 with IC_{50} value of 8.25±0.03, choroform Ibl-1.1 with IC_{50} of 16.25±0.01µg/ml and *n*-butanol Ibl-1.3 with IC_{50} value of 17.02±0.02 µg/ml. Total phenols and flavonoids exerted pronounced antioxidant activity against this radical with IC_{50} values of 1.90±0.01 and 3.45±0.01 µg/ml respectively compared to Ibl-1, Ibl-2 and soluble fractions, but low compared to Ascorbic acid. Ascorbic acid showed low IC_{50} value of 1.85±0.05 µg/ml as its high activity compared to all samples from *I. batatas* leaves samples and total flavonoids, but was comparable to total phenols (Table 2).

The effect of extracts and fractions **against superoxide anions and hydroxyl activity** was appreciable because these samples inhibited the activity of these radical with IC_{50} values ranging from 6.52±004. to17.02±0.02 µg/ml, and between 5.06±0.11 and 15.03±0.05 µg/ml respectively. Their activities were strong or good according to the case. Indeed, 80% methanol extract Ibl-2 displayed strong and high activity with IC_{50} values of 6.52±0.04 and 5.06±0.03 against $O_2^{\bullet}$-and $HO^{\bullet}$ respectively with statistically significant difference ($p < 0.05$) compared to aqueous extract Ibl-1 with respective IC_{50} values of 8.56±0.05 and 9.65±0.02 µg/ml as also a pronounced activity. Total phenols and flavonoids showed antioxidant activity against these ROS radical with IC_{50} values of 3.25±0.02 and 4.15±0.04, and 3.85±0.04 and 6.25±0.05 µg/ml respectively. Their activities were high compared to Ibl-1 and Ibl-2 extract and soluble fractions Ibl-1 to -1.4.

Soluble fractions Ibl-1.1 to -1.4 also showed good activity with IC_{50} values ranging between 13.25±0.02 to 17.02±0.02 against superoxide anion $O^{2\bullet}$ and good or moderate activity with IC_{50} values of 12.50±0.02 to 21.03±0.06 µg/ml against hydroxyl radical $HO^{\bullet}$. Ibl-1.2 soluble fraction was the most active (IC_{50} = 13.25±0.08 and 12.52±0.01 µg/ml respectively), followed by Ibl-1.4 (IC_{50} = 15.2±0.05 and 14.65±0.05 µg/ml respectively), Ibl-1.1 (IC_{50} = 16.25±0.01 and 18.25±0.05 µg/ml respectively) and Ibl-1.3 (IC_{50} = 17.020.02 and 21.03±0.05 µg/ml respectively) soluble fractions. Ibl-1.2 showing significant difference ($p < 0.05$) compared to the remaining soluble fractions. It was the same for Ibl-1.4 compared to Ibl-1.3 and no significant difference ($p > 0.05$) was deduced between Ibl-1.4 and Ibl-1.1, and between Ibl-1.1 and Ibl-3 for their activity against superoxide anion and this was observed for their activity against hydroxyl radical. They generally showed good activity against $O_2^{\bullet-}$ and good or moderated activity against $HO^{\bullet}$ according to the case (Table 2). Total phenols showed pronounced antioxidant activity against these radicals with IC_{50} values of 3.25±0.04 and 4.15±0.04 µg/ml respectively while total flavonoids exerted the same activity with IC_{50} values of 6.25±0.05 and 7.65±0.02 µg/ml respectively. Ascorbic acid displayed high activity against these two ROS compared to all samples from *I. batatas* leaves with IC_{50} values of 3.02±0.01 and 4.65±0.02 µg/ml, total phenols and flavonoids respectively Figure 9 showed the inhibitions of hydroxyl radical activity by aqueous extract Ibl-1,80% methanol extract Ibl-2, total phenols (TP) and flavonoids (TF), and Ascorbic acid exerting this effect in dose-dependent manner. At the highest tested concentration of

5 µg/ml, aqueous extract Ibl-1 and 80% methanol extract Ibl-2 produced 26 and 49.40% inhibition of the activity of this radical. Total phenols (TP) and flavonoids (TF) supplied 65 and 40% inhibition of this activity while at the same tested concentration, Ascorbic acid (Aa) exerted it to 53.76% low and high compared to TP and TF, aqueous and 80% methanol extracts Ibl-1 and Ibl-2 respectively according to the case (Fig. 3).

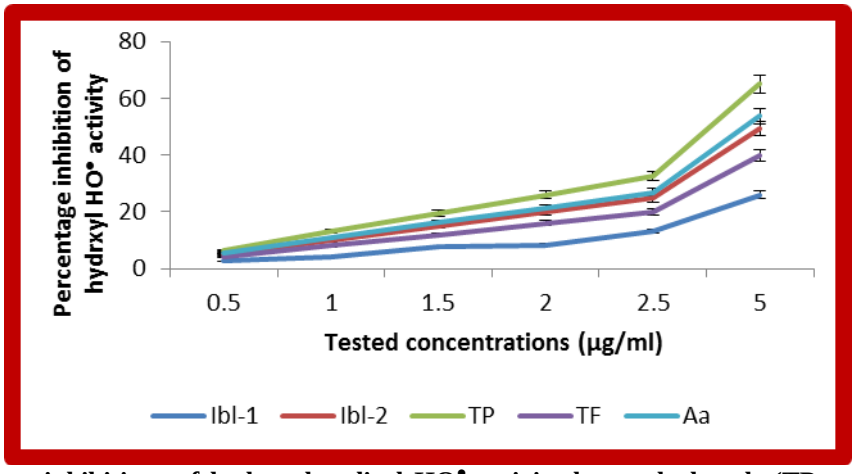


Figure 9: Percentage inhibitions of hydroxyl radical HO• activity by total phenols (TP and flavonoids (TF), aqueous extract Ibl-1, 80% methanol extract Ibl-2 and Ascorbic acid (Aa)

Tested **against hydrogen peroxide (H_2O_2)**, 80% methanol extract Ibl-2 exhibited strong activity with IC_{50} value of 8.63±0.04 µg/ml compared to aqueous extract Ibl-1 with IC_{50} of 14.63±0.03 µg/ml as good activity. Ethylacetate Ibl-1.2 and residual aqueous Ibl-1.4 soluble fractions showed also good activity with IC_{50} value of 16.52±0.06 and 17.26±0.02 µg/ml respectively while chloroform Ibl-1.1 and *n*-butanol Ibl-1.3 soluble fractions exhibited moderate or good activity with IC_{50} values of 20.03±0.03 and 17.26±0.02 µg/ml respectively with the last fraction as the most active with significant difference ($p < 0.05$). Total phenols and flavonoids strongly inhibited the activity of this radical with IC_{50} values of 4.26±0.05 and 7.65±0.02 µg/ml respectively and showed high activity compared *to I. lobata* leaves samples with significant difference ($p < 0.05$). Ascorbic acid as reference antioxidant product showed high activity with IC_{50} value of 6.12±0.05 µg/ml compared to extracts and soluble fractions from *I. batatas* leaves, comparable to TF, but low compared to TP with significant difference ($p < 0.05$).

Figure 10 put back the percentage reductions of hydrogen peroxide H_2O_2 activity by aqueous extract Ibl-1, 80% methanol Ibl-2 and Ascorbic acid (Aa). It was observed that all tested samples inhibited this activity in dose-dependent manner (Fig. 4). Ascorbic acid (Aa) tested at the highest concentration of 5 µg produced 40.85% reduction of the activity of this radical. Aqueous extract Ibl-1 and methanol extract Ibl-2 acted the same manner at the same tested concentration in causing 17.08 and 29.00% reduction of this activity and significant difference ($p < 0.05$) was observed.

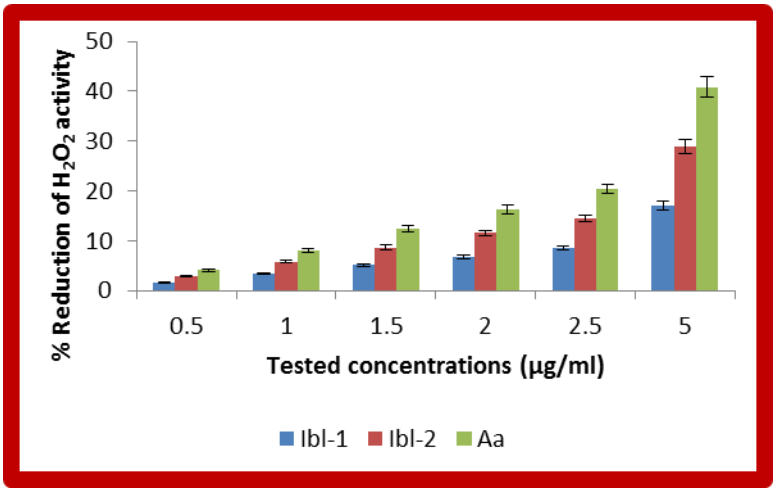


Figure 10: Percentage reductions of hydrogen peroxide H_2O_2 by Aqueous extract Ibl1 and 80% methanol extract Ibl2, and Ascorbic acid (Aa).

In all antioxidant assays, 80% methanol extract Ibl-2 was the most active, followed by aqueous extract Ibl-1, ethylacetate Ibl-1.2, residual aqueous extract Ibl-1.4, chloroform Ibl-1.1 and *n*-butanol Ibl-1.4 soluble fractions. In considering extracts and soluble fractions together, the decreasing order of activity in all antioxidant assays can be established as Ibl-2 (80% methanol extract) > Ibl-1 (aqueous extract) > Ibl-12 (ethylacetate) > Ibl-1.4 (residual aqueous) > Ibl-1.1 (chloroform) > Ibl-1.3 (*n*-butanol). Ascorbic acid used as reference antioxidant product showed high activity with IC_{50} values ranging from 1.56±0.02 to 6.12±0.05 µg/ml against all selected ROS compared to *I. batatas* samples, except against hydroxyl and hydrogen peroxide radicals on which total phenols (IC_{50} = 3.85±0.04 and 4.26±0.05 µg/ml) exhibited high activity compared to Ascorbic acid (IC_{50} = 4.65±0.02 and 6.12±0.05 µg/ml respectively). A significant correlation was found between the antioxidant activity of extracts and their total phenolic and total flavonoid content, but mainly due to TP content than on TF as reported by Esmaeili et al., (2015) for various solvent extracts of *Trifolium pratense* seeds and Kefayati et al., (2017) for extracts and subfractions of *Euphorbia splendida* aerial parts though the high activity was recognized to be attributed to high phenolic content than other constituents (Mounguengui et al., 2016; . Suleria et al., 2020).

3.2. Phenolic and flavonoid contents

Phenolics content was determinated using Folin Ciocalteau's reagent and flavonoids content by using ACl_3 5% methanol solution. Results indicated that, aqueous Ill-1 and 80% methanol Ill-2 extracts contained 40.56±0.05 and 55.12±0.03 g of total phenols (TP) expressed in mg gallic acid/100 g of dried extract respectively and 9.65 and 32.06 g of total flavonoids (TF) expressed in mg quercetin/100g of dried extract respectively. These amounts were similar to those reported by Koncic et al., (2013) and Zhang et al., (2019), but were high compared to those reported by Hue et al. (2012) and Fu et al., (2016) in the same medicinal plant. Indeed, the antioxidant activity of *I. batatas* leaves were previously reported against DPPH and reported results showed that ethanol and water extracts exhibited antioxidant activity with various IC_{50} values and contained variable total phenol and flavonoid contents depending probably to the region collection (Huang et al, 2004; Hue et al., 2012; Koncic et al, 2013; Fu et al., 2016; Dewijanti et al., 2017; Jang and Koh, 2019; Zhang et al., 2019) and that of tubers (sweet potato) (Huang et al., 2004) and petioles (Jang and Koh, 2019).

Figure 11 brought back the percentage reductions of DPPH, ABTS and superoxide anions $O_2^{\bullet-}$ induced by total phenols (TP) and flavonoids (TF), and Ascorbic acid (Aa) tested at the highest concentration of 5 µg/ml. At this concentration, TP and TF reduced the activity of DPPH, ABTS and $O_2^{\bullet-}$ by 100, 100 and 77.00%, 82.00, 72.96 and 60.24% respectively. TP exhibited high activity against all selected ROs compared to TF with significant difference ($p< 0.05$). Aa displayed this effect by showing 100, 100 and 82.78% reductions of the activity of the three selected ROS respectively. Its effect was comparable to TP against DPPH and ABTS activity, but high against $O_2^{\bullet-}$ activity, and high compared to TF effect against all selected ROs activity (Fig. 11).

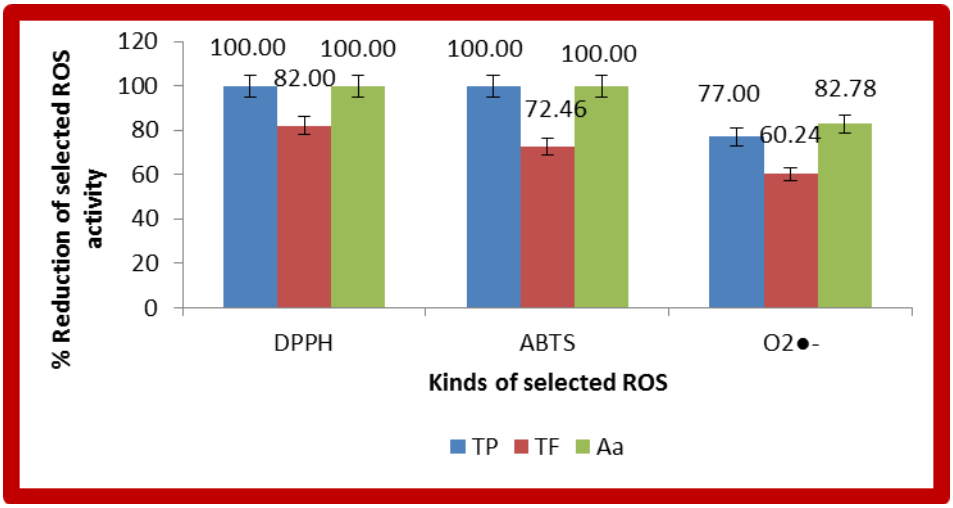


Figure 11: Percentage reductions of DDPH, ABTS and superoxide anion O2●- activity by total phenols (TP) and flavonoids (TF0, and Ascorbic acid (Aa).

Figure 12 put back the percentage reductions of hydroxyl $HO^{\bullet}$ and hydrogen peroxide H_2O_2 activity by total phenols (TP) and flavonoids (TF), and Ascorbic acid (Aa) tested at the highest concentration of 5 µg/ml. TP and TF exhibited this activity by producing 65.00 and40.00 % against hydroxyl $HO^{\bullet}$ radical and58.68 and 32.68% against hydrogen peroxide H_2O_2 radical.

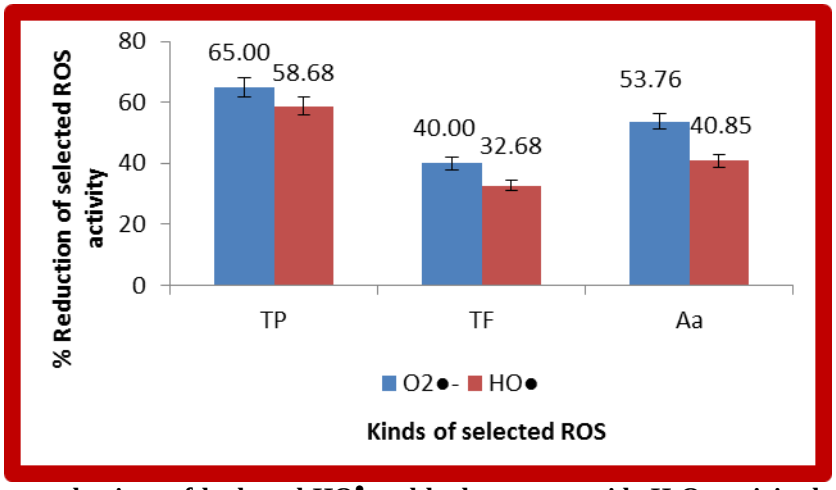


Figure 12: Percentage reductions of hydroxyl $HO^{\bullet}$ and hydrogen peroxide H_2O_2 activity by total phenols (TP) and flavonoids (TF), and Ascorbic acid (Aa).

The effect of TP against these two radicals was high compared to TF with marked difference ($p < 0.05$). On the other hand, Aa displayed this reduction of activity with 53.76% against hydroxyl $HO^{\bullet}$ and 40.85% against hydrogen peroxide H_2O_2 radical. Its effect was high against both selected radicals compared to TF and was low compared to TP with significant difference ($p < 0.05$) (Fig. 12).

3.2. Effects of aqueous extract Ibl-1 form *I. batatas* leaves in acute and sub-acute toxicity

3.2.1. Acute toxicity

The acute toxic effect of aqueous extract Ibl-1was determined at test dose of 500, 1000 and 5000 mg/kg bw administered once. No treatment-related toxic symptoms or mortality was observed after oral administration of the tested aqueous Ibl-1 plant extract at oral doses of 500, 1000 and 5000 mg/kg respectively. The general behavior of the extract-treated animals and control group was observed first for a short period of 24 h, thereafter on a daily basis until 28 days and presented no change. Furthermore, the animals did not display any drug-related changes in behavior, mobility, gastro-intestinal disorders (diarrhea, nausea, vomiting, flatulence), breathing, skin effects, mortality, salivation, lethargy, tremors, convulsions, water consumption, impairment in food intake and temperature.

3.2.2. Sub-acute toxicity

The sub-acute toxicity study of aqueous extract Ibl-1 was determined by the proposed methods mentioned above. All study animals given aqueous extract Ibl-1 daily at all studied oral doses of 500, 1000 and 5000 mg/kg survived the entire 28-day period. No signs of toxicity or mortality were observed. In both tests, treated animals gained body weight compared to negative control. Therefore, the extract was seemed to be safe at all administered doses and the median lethal dose (LD_{50}) was estimated to be > 5000 mg/kg bw. It was thus considered to be non-toxic an safe assuring its use on a long-period without significant toxicity. According to Kennedy et al. (1986), substances that present LD_{50} higher than 5000 mg/kg bw by oral route may be considered practically non-toxic. Our results were in good agreement with Imafidon et al., (2015) who reported the safety of leaves ethanol extract from *I. batatas* in rats and this suggested that the nature of extract (water versus ethanol) had not significant influence on the toxicological parameters.

3.2.3. Effects of aqueous extract Ibl-1 on body weights of treated rats at 5000 mg/kg bw

When administered at oral dose of 1000 mg/kg bw, the administration of aqueous extract Ibl-1 induced significant indifference ($p > 0.05$) in organ weights of treated animals compared to negative control at oral dose of 1000 mg/kg bw, but this was observed at the high oral dose of 50000 mg/kg bw ($p < 0.05$) (Table 4). No any sign of toxicity and significant changes in any of the organ were observed.

Table 3: Body weights of vital organs (g).

Organs	Average organ weights		
	Normal	Ibl-1: 1000 mg/kg bw	Ibl-1: 5 000 mg/kg bw
Liver	0.83 ± 0.016	0.84 ± 0.01	0.86 ± 0.05
Kidney	0.30 ± 0.01	0.32 ± 0.030	0.34 ± 0.02
Pancreas	0.12 ± 0.011	0.14 ± 0.013	0.16 ± 0.04
Heart	0.15 ± 0.03	0.16 ± 0.01	0.18 ± 0.02
Intestine	0.64 ± 0.01	0.66 ± 0.02	0.68 ± 0.04
Spleen	0.41±0.02	0.43±0.01	0.46±0.04

The results also revealed that, the vital organs such as liver, kidney, heart, pancreas, spleen and small intestine and spleen were not adversely affected throughout the treatment period by aqueous Ibl-1 extract and presented normal architecture in both groups. These observations were also found and reported in other studies on the effects of some medicinal plant extracts on vital organ weights and our finding was in good agreement of these studies (Kifayatullah et al., 2015; Cimanga et al, 2018; Unuofin et al, (2018); HemalathaLoha et al., 2019; Mary, 2019). But, the administration of leaves ethanol extract of *I. batatas* in animals caused significant reductions in the absolute weights of the heart, pancreas, liver and the kidneys. Greater reductions in weights were observed at the oral doses of 2500 and 5000 mg/kg as reported by Imafidon et al., (2015). These results suggested that the nature of extract can significantly cause some modifications in on organ weights (aqueous versus ethanol).

3.2.4. Effects of aqueous extract Ibl-1 from *I. batatas* on haematological and biochemical parameters of treated Wistar rats

The effect of aqueous extract Ibl-1 from *Ipomoea batatas* leaves on hematological parameters was examined at the end of treatment (Table 2). Variances analysis showed no significant difference on several haematological parameters haemoglobin (HB), HCT (haematocrit) WBC white blood cells), RBC (red blood cells), MCH mean corpuscular haemoglobin), PVC (polyvinyl concentration), MCHC (mean corpuscular haemoglobin concentration), neutrophils, monocytes ($p > 0.05$), and lymphocytes know slight increase in treated animals compared to untreated and this did not show significant difference ($p > 0.05$).

Only platelets (1301.2 versus 1312) and segmented lymphocytes (19.8 versus 22.30) showed significant increase in treated animals compared to untreated leading to observed significant difference ($p < 0.05$). The increase of platelet amount indicated the administrered aqueous extract Ibl-1 from *I. batatas*, was endowed with anticoagulant activity because the increased platelet count led to a shortening of the duration of bleeding and thus realized that the platelets were apparently necessary for haemostasis (Cimanga et al., 2015, 2018; De Gruyter, 2020) and in opposite sens, low patelet counts leaded to high risk of bleeding (Di Micco and Monreal, 2020). Our results did not collaborate with Rafiu and Luka (2018) on the level of some haematological parameters like RBC, HB, PVC, platelets and WBC reported to be significantly increased in their study.

Table 4: Haematological parameter levels of treated animals exposed to *I. batata* leaves aqueous extract Ibl-1 at oral dose of 5000 mg/kg bw.

Parameters	Negative control	Mms-1: 5000 mg/kg bw	Reference values
Haemoglobin (g/dL)	16.3 ± 0.2	17.5 ± 0.1	15.0-18.2
Haematocrit (%)	47.8 ± 0.3	48.3 ± 0.0	40.7-50
Platelets (x $10^3\mu L^{-1}$)	1301.0 ± 0.6	131.2 ± 0.0	995-1713
WBC (x $10^3\mu L^{-1}$	17. 8± 0.3	18.7 ± 0.2	6.6-20.5
RBC (x 10^6 µL-1)	7.8 ± 0.4	8.8± 0.5	7.6-10.3
PVC (L/L)	45.8± 0.2.	46.4±0.2	42.50-49.40
MCV (fL)	56.2 ± 0.1	59.3±0.5	46.0-65.0
MCH (pg)	19.3± 1.1	20.8± 0.5	18.7-21.2
MCHC (g/dL)	37.5 ± 1.2	38.3±0.5	38-43
Neutrophils $x10^3/mm^3$	22.6 ± 0.3	23.8 ± 0.3	3.0-24.7
Monocytes $x10^3/mm^3$	3.2 ± 1.1 2	3.5 ± 0.1	0.0-4.0
Basophils $x10^3/mm^3$	0.0±0.0	0.0±0.0	0.0±0.03
Lympocytes $x10^3/mm^3$	8.4 ± 0.3	9.0 ± 0.1	4.78-9.12
Segmented leucocytes (%)	19.8 ± 0.6.	22.3 ± 0.2	-

WBC; white blood cells, RBC: red blood cells, PVC: polyvinyl concentration, MCV: mean corpuscular volume, MCH; mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration.

The effects the administration of aqueous extract Ibl-1 form *I.batatas* leaves on biochemical parameters were presented in Table 5. Results indicted significant decrease of glucose level in treated animals compared to untreated. This effect may be due to the hypoglycemic property of the administered aqueous extract Ibl-1 from *I. batatas* leaves as also previously reported in other studies (Sumithregowda et al., 2017; Unuofin et al., 2018; Loha et al., 2019). Our results well particularly collaborated with Rafiu and Luka (2018) when the leaves ethanol extract from *I. batatas* was used and showed non-influence of the solvent nature on blood glucose level (water versus ethanol).

Table 5: Biochemical parameters levels of treated animals exposed to *I. batatas* leaves aqueous extract Ibl-1 to oral dose of 5000 mg/kg bw

Parameters	Negative control	Ibl-1: 5000 mg/kg bw	Reference values
Glucose mg/dl	84.2 ± 0.4	81.3 ± 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)	192.6 ± 0.3	196 ± 0.2	0.-832.3
ALT or SGOT(UI/L)	61.2 ± 1.2	63.8 ± 0.2	1-223.3
Total cholesterol (mg/dL)	76.5 ± 1.3	74.8 ± 1.2	14.4-81.7
Triglycerides (mg/dL) 5	43.2 ± 0.	44.8 ± 0.2	2.7-47.8
Total bilirubin (mg/dL)	10.5 ± 1.3	11.8 ± 0.0	5.3-12.6
Direct bilirubin (mg/dL)	3.6 ± 0.0	3.8 ± 0.2	3.26-4.32
Total proteins (g/dL)	7.2 ± 1. 3	7.4 ± 1.1	5.6-7.6
Albumin (g/dL)	4.1 ± 0.2	4.5 ± 0.3	3.8-4.8
ALP (IU/L)	177.4 ± 1.0	179.3 ± 1.2	160.8-838.3
HDL-cholesterol (mg/dL)	38.8 ± 0.3	41.0 ± 0.4	7.2-42.0
LDL-cholesterol (mg/dL)	43.1 ± 1.1	41.6 ± 0.3	-20.66-49.22
Uric acid (mg/dL)	3.80 ± 0.8	3.85 ± 0.5	3.2-7.5
Urea (mmol/L)	6.0 ± 0.3	6.8±0.5	5-7

AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphate, HDL: hight density lipoprotein, LDL: low density lipoprotein, SGPT: serum glutamic-pyruvic transaminase, SGOT: serum glutamic oxaloacetic transaminase.

The kidney function parameters like urea, creatinine and uric acid, did not reveal any significant changes ($p > 0.05$). There were no significant difference ($p > 0.05$) in the levels of liver function enzymes, such as ALT, AST, SGPT, SGOT and ALP of treated animals compared to untreated. An abnormal increase in aminotransferase activities (ALAT and ASAT) could frequently refer to hepatotoxicity (Fortson et al., 1985; El Moussaoui et al., 2020). In the present study, no abnormality in the levels of these two enzymes was observed and suggested good maintenance of hepatic and kidney functions. Furthermore, there was no significant difference in the levels of total protein, albumin, total and direct bilirubins of treated rats when compared to the negative control group ($p > 0.05$) (Table 4). All reported haematological and biochemical parameter levels were in within acceptable physiological ranges (Tables 3 and 4) or were in the same ranges with those reported in other studies (Kifayatullah et al., 2015; Unuofin et al., 2018; Loha et al., 2019, Moussaoui et al., 2020).

4. CONCLUSION

This study had reported for the first time the antioxidant activity of aqueous extract and its soluble fractions as well as that of 80% methanol in combination with the acute and sub-acute toxicity of the aqueous extract from *Ipomoea batatas* leaves growing in Central Kasai in Democratic Republic of Congo. Results revealed that all tested samples extracts and fractions were able to inhibit the activities of all selected ROS and to exert radical scavenging activity on DDPH, ABTS, $O^{2\bullet}$, $HO^{\bullet}$ and H_2O_2 radicals. The most active sample was 80% methanol Ibl-2 extract followed by the aqueous extract, ethylacetate Ibl-1.2, residual aqueous Ibl-1.4, *n*-butanol Ibl-1.3 and chloroform Ibl-1.1 soluble fractions. In acute and sub-acute toxicity, aqueous Ibl-1 extract tested at the highest oral dose of 5000 mg/kg body weight did not induce any sign of toxicity and no mortality in treated animals was observed. Its lethal dose 50 (LD_{50}) was estimated to be greater than 5000 mg/kg body weight. The extract was thus considered pratically non-toxic per oral route, safe and good tolerated in treated animals and possessed high security for its use on a long period.

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