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IN VITRO ANTIOXIDANT AND FREE SCAVENGING ACTIVITIES OF EXTRACTS AND FRACTIONS, ACUTE AND SUB-ACUTE TOXICITY OF AQUEOUS EXTRACT FROM *MORINDA NORINDOIDES* (BAKER) MILNE-REDHEAD (RUBIACEAE) LEAVES IN EXPERIMENTAL WISTAR RATS

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ABSTRACT

Aqueous extract, its soluble fractions chloroform, ethylacetate, *n*-butanol and residual aqueous, as well as 80% methanol extract and polysaccharides from *Morinda morindoides* leaves were submitted to the evaluation of their potential antioxidant activity against some selected reactive oxygen species (ROS) including DPPH (2,2' diphenyl-1-picrylhydrazyl), ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonate or 2,2-azino-bis-ethylbenzothiasoline sulfonic acid), superoxide anion $O^{2\bullet-}$, hydroxyl $HO^{\bullet}$ and hydrogen peroxide H_2O_2. Acute and sub-acute toxicity of aqueous extract was also evaluated in experimental Wistar rats. Results showed that aqueous extract and its soluble fractions, and 80% methanol extract exerted pronounced antioxidant activity by inhibiting the activity of selected reactive oxygen species with IC_{50} values < 10 µg/ml. 80% methanol extract exhibited high activity with IC_{50} values ranging from 2.05±0.00 to 3.52±0.01 µg/ml compared to aqueous extract and its soluble fractions with IC_{50} values from 3.25±0.01 to 9.81±0.02 µg/ml. All soluble fractions displayed this activity with IC_{50} values ranging from 4.16±0.02 to 9.81±0.02 µg/ml. Polysaccharides exhibited this activity with IC_{50} values from 15.05±0.03 to 52.06±0.02 µg/ml. Flavonoids quercetin, rutin and quercetrin exhibited antioxidant activity with IC_{50} values from 3.15±0.03 to 25.60±0.01 µg/ml while kaempferol, kaempferol-3-O-rutinoside and kaempferol-7-O-rhamnosylsophoroside displayed this activity with IC_{50} values ranging from 7.82±0.01 to 25.06±0.03 µg/ml. In acute and sub-acute toxicity, aqueous extract did not induce mortality in treated animals at the highest oral dose of 5000 mg/kg body weight [(LD_{50}) > 5000 mg/kg body weight)]. It was devoid with considerable influence on vital organ weights, serum electrolytes, haematological and biochemical parameters of treated rats. Thus, it was found to be practically non-toxic and safe by oral route. Thus, the aqueous extract from *M. morindoides* leaves can be used for the prevention of the occurring of some diseases mainly cardiovascular in humans.

KEYWORDS: *Morinda morindoides*, leaves, extracts, fractions, polysaccharides, antioxidant activity, acute and sub-acute toxicity.

1. INTRODUCTION

Reactive oxygen species are highly free radicals formed by exogenous chemicals or endogenous metabolic processes in the human body. They are capable of oxidizing macrobio-molecules viz nucleic acids (DNA), proteins, lipids and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, diabetes, asthma, rheumatism, cirrhosis, atherosclerosis, arthritis, etc. Antioxidants are responsible for the defense mechanism of the organism against the pathologies associated to the attack of free radicals, and thus, the intake of plant-derived antioxidants is involved in the prevention of degenerative diseases caused by oxidative stress, such as cancer, Parkinson, Alzheimer, atherosclerosis and other (Lakshmibai and Amirtham, (2018). They are compounds that stop or reduce the oxidation of other. Free radicals are associated with the development of a number of chaos in human body such as several diseases cited above. To get rid of such diseases of free radicals, antioxidant substances are needed to protect the individual life (Ayoub and Metha, 2018).

To study the antioxidant activity, several chemical and biochemical protocols had been used in the evaluation of antioxidant activity of plant extracts, fractions, synthetic constituents and other. They include chemiluminescence (CL), croton bleaching, low density lipoprotein (LDL) oxidation, total oxidant scavenging capacity (TOSC), the oxygen radical absorbance capacity (ORAC), cupric-

reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), total radical-trapping antioxidant potential (TRAP), phytomolybdenum, potassium ferricyanide, chelating metal, peroxynitrite scavenging assays, against 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′ azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), superoxide anion ($O^{2\bullet-}$), nitric oxide (NO), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) among others. Biochemical protocols are based on animal models for *in vitro* and *in vivo* evaluations of oxidative stress biomarkers based on hydrogen atom transfer or single electron transfer mechanisms (Aliyu et al., 2019) for various medicinal plant extracts, their fractions, natural and synthetic products. Reactive oxygen species, produce as products of metabolic reactions, are widely intercorrelated in biological system controlling processes such as growth, regulation of environmental stress, development, and defense mechanisms. Due to the toxicity of these reactive molecules, the ameliorative effect of radical scavengers has become a targeted research area related to many disease preventions (Ayoub and Metha, 2018). Uncontrolled generation of ROS was known to cause redox imbalance and oxidative stress which was harmful and responsible for various diseases cited above, etc. Conversely, live organisms can develop complex system of enzymatic and non-enzymatic anti-oxidant defences which can counteract the harmful effects of free radicals (Mukhopodhay et al., 2016).

Antioxidants can either be natural or synthetic products. Natural antioxidants can be obtained through diet in the form of fruits, spices, vegetables, etc. Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole also diminish oxidation, but they have been proved to be dangerous to humankind. Therefore, the investigation for non-toxic antioxidant substances has increased in the recent years (Ayoub and Metha, 2018).

Medicinal plants have been regarded as primary source of secondary metabolites showing fascinating differents biological actions among which antioxidant activity. Normally, their chemical constituents are the chief sources of a number of structural preparations endowed with different biological properties. Well-known examples of these secondary metabolites involve alkaloids, polyphenols, flavonoids, glycosides, polysaccharides, anthraquinones, iridoids, tannins, steroids, terpenes, reducing sugars, carbohydrates, cyanogenic glycosides, and saponins, etc. exerting interesting antioxidant activity *in vitro* and *in vivo* tests (Embuscado, 2015; Cardoso, 2019; Sofia et al, 2019). Recently, it has been reported that the natural antioxidants from therapeutic plants defend from lethal and detrimental consequences of free radicals. According to the World Health Organization (WHO), it has been estimated that 80% of the world's population depend on folklore medicine for their chief health-care necessities and the remedy mostly involves medicinal plant extracts and their bioactive constituents (Ayouba and Metha, 2018).

Morinda morindoides (Baker) Milne-Redhead (Rubiaceae) is one of the most popular medicinal plants currently used in Democratic Republic of Congo (DRCongo) and other African countries to treat various diseases including amoebisis, fever and malaria, rheumatism, diabetes, abdominal pains, tiredness, diarrhea, intestinal worms, haemorrhoids, cutaneous infections (mycosis, gale and scabies), urogenital infections (gonorrhoea) and other. The aqueous decoct of the leaves is used as a tonic and stimulant of appetite, mixed to other plants such *Mangifera indica* bark and *Tetracera poggei* leaves to treat diabetes, etc. Leaves are the main part used since the preparation of an aqueous decoct which is the principal preparation used to treat various ailments and diseases, is known by all people and need not the presence of a practitioner (Kambu, 1990a, 2009, Newinger, 2000).

Moreover, the plant from DRCongo was also submitted to many scientific studies to evaluate in part some of the biological activities of extracts and isolated compounds in relation of its traditional uses as well as the isolation of constituents. Biological activities include antispasmodic (Kambu et al., 1990b; Tona et al., 2000; Cimanga et al., 2010), antiamoebic (Tona et al., 1998, 2009; Cimanga et al., 2006a,b, 2018), antidiarrheal (Mbamu et al., 2019a), antibacterial, antiviral, and cytotoxic (Cimanga, 1997a; Mbamu et al., 2019b), antifungal (Walo , 1985), antiplasmodial (Tona et al., 2001, 2004; Cimanga et al., 2008) and anticomplement (Lasure et al., 1994 ; Cimanga et al., 1995b, 1997a,b, 2003) activities *in vitro* and/or *in vivo*. The isolated constituents concerne anthraquinones (Cimanga, 1997a, Cimanga et al., 2006a), flavonoids (Cimanga et al., 1995a,; Cimanga. 1997a,b) and iridoid aglycones and iridoid glycosides (Cimanga et al., 2003) isolated from *M. morindoides* leaves.

The present investigation was undertaken to assess *in vitro* antioxidant activity of extracts, fractions, flavonoids, iridoids and polysaccharides from *Morinda morindoides* leaves and to evaluate acute and sub-acute toxicity of aqueous extract from the leaves of this medicinal plant.

2. MATERIALS AND METHODS

2.1. Plant material

Leaves of *Morinda morindoides* (Baker) Milne-Redhead (Rubiaceae) were collected in Kimwenza, Kinshasa, capital of DRCongo. The plant was identified for the first time in Ocober1990 at the Institut National d'Etudes et de Recherche en Agronomie (INERA), Faculty of Sciences, Department of Biology, University of Kinshasa.

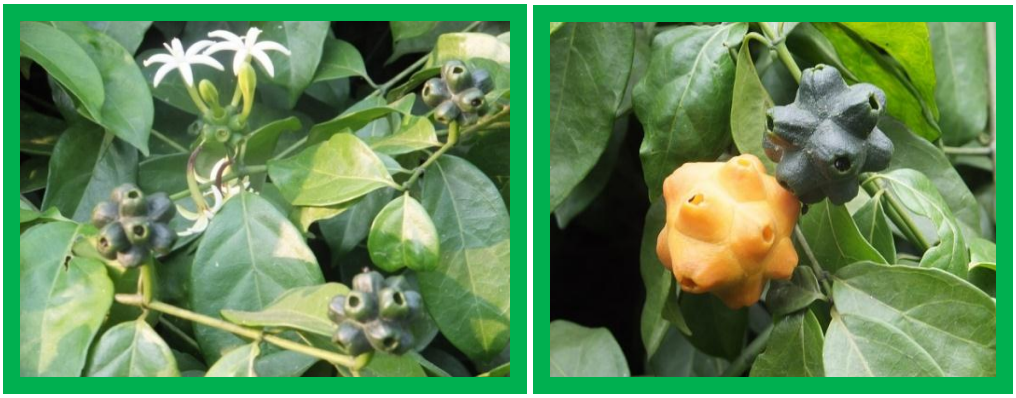

Figure 1: ***Morinda morindoides*** **leaves, flowers and fruits.**

A voucher specimen of the plant was deposed in the herbarium of this institute and in the laboratory of Phytochemistry and Pharmacognosy at the faculty of Pharmaceutical Sciences of the same university. A new batch of plant material was collected in October 2019 for the present study. Leaves were dried at room temperature and reduced to powder with an electronic blender and the obtained powder were kept in brun bottles before use to avoid contamination and deterioration.

2.2. Preparation of extracts and partition

60 g of powdered leaves were soaked with 400 ml distilled water and boiled on a hotplate at 100°C for 15 minutes. Next, the mixture was cooled and filtrated on a paper filter Watman N° 1. The filtrate was evaporated in vacuum using rotary evaporator yielding dried extract named as Mms-1 (53.87 g). An amount of 20 g of Mms-1 extract were dissolved in 200 ml distilled water, filtrated and the filtrate extracted successively and exhaustively with solvents of different polarities as chloroform, ethylacetate, *n*-butanol. All fractions including residual aqueous phase were evaporated in vacuum as described above yielding corresponding dried extracts denoted as Mms-1.1 (4.75 g), Mms-1.2 (5.05 g), Mms-1.3 (3.25 g) and Mms-1.4 (6.15 g). On the other hand, 30 g of the plant material were macerated with 80% methanol for 24h and filtrated giving a methanol macerate. After, the marc was exhaustively percolated with the same solvent giving a percolate. Macerate and percolate were combined and treated as described above yielding dried methanolic extract named as Mms-2 (25.71 g).

2.3. Extraction and purification of polysaccharides

2.3.1. Water soluble polysaccharides

The method proposed by Liu et al., (2016) was used for extraction, separation and purification of polysaccharides. About 20 g of aqueous Mms-1 extract, was dissolved in 100 ml distilled water, filtered, and the volume of the filtrate was reduced to 10 ml with rotary evaporator. After, about a five-fold volume of ethanol 95% was added into the filtrate for polysaccharides precipitation. The solution was then placed in a refrigerator at 4°C for 24 hours giving a white precipitate after this period. The precipitate was filtrated and dried in hot at 50°C to give a dried white extract denoted as CP-Mms (crude polysaccharides: 16.63 g). This extract gave positive test with phenol/H_2SO_4 (red-violet colour) for polysaccharides. The polysaccharide concentration was determined with the phenol-sulfuric acid method at 481 nm (Liu et al., 2016).

2.3.2. Purification of polysaccharides

The crude polysaccharides sample CP-Mms (15 g) was purified by gel column chromatography on DEAE-cellulose put in deionized water and dumped the clarity supernatant liquid. After, 500 ml of NaOH 0.5 mmol/L were added for 30 minutes, bathing the cellulose with water until neutral. It was then soaked in 500 ml of HCl 0.5 mol/L for 30 minutes and treated as described above for bathing, and the process was repeated three times. Now, the DEAE-cellulose was ready for use. The crude polysaccharides CP-Mms was dissolved in deionized water and centrifuged, and the supernatant was loaded onto a new DEAE-cellulose column (40g, 60 × 2.5 cm, internal diameter), which was eluted with deionized water and NaCl 0.1 M solution in order. The elution (3 ml) was collected and carbohydrates content determined based on the phenol-sulfuric acid method at 481 nm absorbance (Liu et al, 2016). The crude polysaccharides CP-Mms was separated on Sephadex LH-20 (40g, 60 x 2.5 cm) into chromatographically pure 4 fractions, which were then dried at hot at 50°C until constant weight and coded as PF-Mms- -1 (4.75 g), PF-Mms-2 (2.50 g), PF-Mms- 3 (3.38 g) and PF-Mms-4 (4.67 g) (Zhao et al., 2015; Liu et al., 2016).

2.4. Isolation of flavonoids and iridoids

Flavonoids were isolated from 80% methanol extract (maceration) by different chromatograhic methods including column on silicagel (60 Å, 70-230 mesh, Merck, Germany) eluted with $CHCl_3$/MeOH in different proportions and by prepative on silcagel plates (thickness layer 1 mm, Merck, Germany) using BAW: 4/1/5 (upper phase) as mobile phase resultting in the isolation of eleven flavonoids quercetin, quercetin 7,4′-dimethylether, luteolin-7-glucoside, apigenin-7-glucoside, quercetin-3-O-rhamnoside, kaempferol-3-O-rhamnoside, quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, chrysoeriol, chrysoeriol 7-neohesperidoside,

kaempferol-7-O-rhamnosylsophoroside (morindaoside) (Cimanga et al., 1995; 1997; Cimanga et al., 2003a) and nine iridoids isolated from 80% methanol extract (Soxhlet extraction) by HPLC using MeOH/H_2O as eluant on RP18 column. This resulted in the isolation of iridoids as gaertneric acid, and iridoids glycosides named as gaertneroside, acetylgaertneroside, dehydromethoxygaertenroside, 6-acethylgaertneroside, 6-acethylmethoxygaretneroside, methylepoxygaertenroside, epoxygaertenoroside, epoxymethoxygaertneroside. The structures of all isolated compounds were elucidated by NMR (^{1}H-NMR and ^{13}C-NMR) spectra (Cimanga et al., 2003b).

2.4. Evaluation of antioxidant activity

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

The ability of extracts, fractions and polysaccharides from *M. morindoides* leaves against the radical DDPH was evaluated using the methods previously described by Ulewicz-Magulska and Wesolowki, (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 a series of test concentrations from 1 to 20 µg/ml. 1 ml of each test sample dilution with known concentration 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 inhibitory 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.

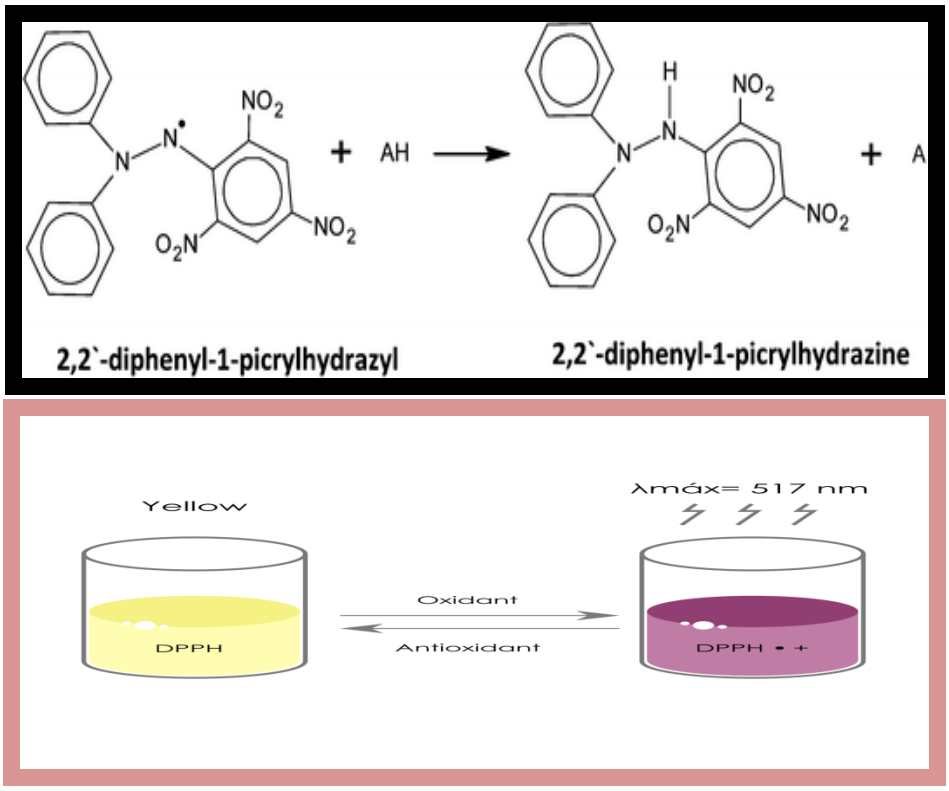

Figure 2: Transformation of DPPH in DPPH$^{\bullet+}$ (A) and change of the coloration after reaction with an oxidant or antioxidant substance (B).

2.4.2. 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 Labiad et al., (2017) and Al-Laith et al., (2019) 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 (test concentrations: 1-20 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 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.

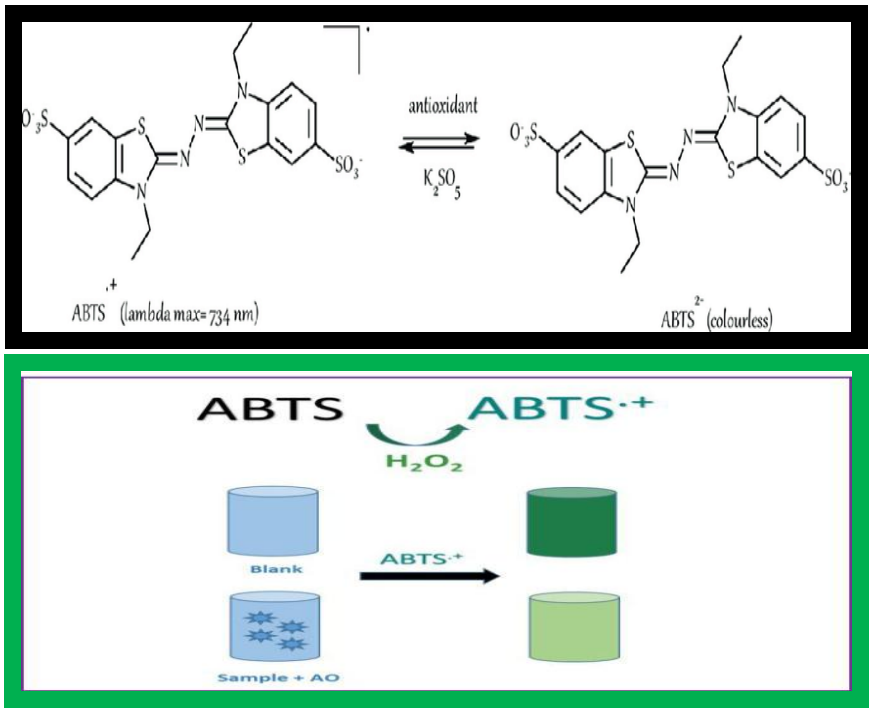

Figure 3: Transformation of ABTS in the presence of antioxidant substance (A) and potassium persulphate and its reactivity in change of color (B).

2.4.3. 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 nitrobleuterazolium (NBT, 100 µL) and 390 µM NAD (100 µL). Absorbances were recorded on the same apparatus, the same formula 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 (Esmaeli et al., (2015); Chavan et al., (2018).

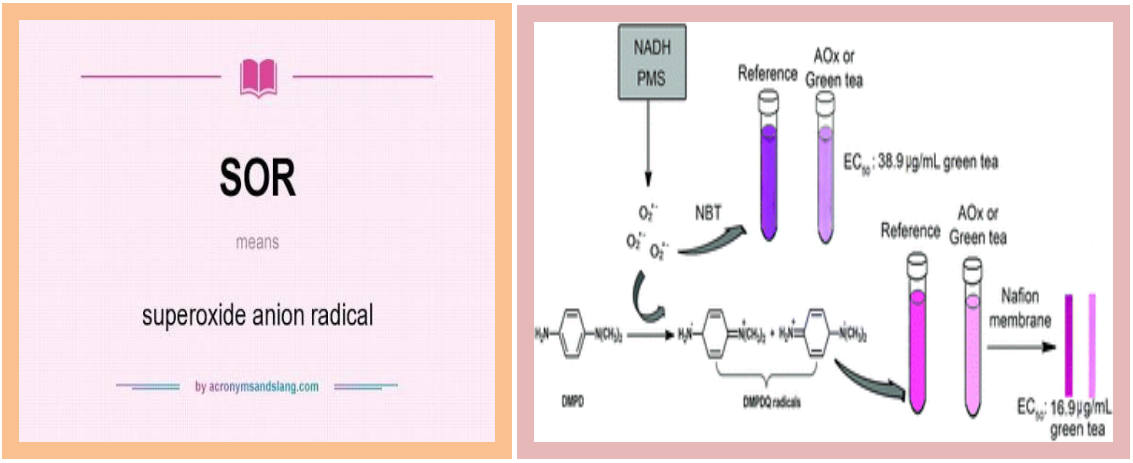

Figure 4: Simultaneous detection of superoxide anion radical.

2.4.4. Hydrogen peroxide (H_2O_2) scavenging activity

The scavenging activity for hydrogen peroxide was measured according to the methods of Leyton et al., (2015); Bayramoglu et al., (2017) and Ekin et al., (2017). Different concentrations of plant extract (1-20 µg/ml) 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 unreacted H_2O_2 was determined by measuring the absorbance of their action mixture at 230 nm with respect to the blank solution. The activity inhibition of the radical was calculated and IC_{50} values were derived in the same manner as described above.

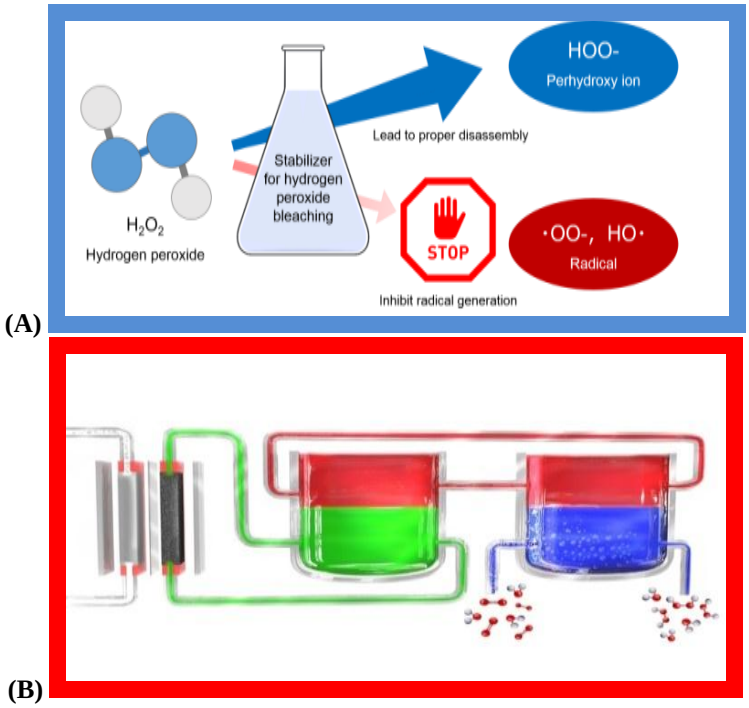

Figure 5: Disassembly of hydrogen peroxide (A) and its reactivity (B).

2.4.5. Hydroxyl radical ($HO^{\bullet}$) scavenging activity assay

Hydroxyl radical scavenging activity of samples from *M. morindoides* leaves was assessed by the method of Olugbami et al., (2015). Hydroxyl radical was generated by Fenton reaction (Fe^{3+}-ascorbate-EDTA-H_2O_2 system). 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 (1-20 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 inhibition of hydroxyl radical scavenging activity was 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 calculated.

o a test tube were added 3 ml of phosphate

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2.6. Estimation of phenolic compounds content

The quantity of total phenolic compounds in aqueous extract Mms-1 of *M. morindoides* leaves was determined using Folin-Ciocalteu's reagent (FC) or Folin-Denis's reagent (FD) (phosphomolybdate and phosphotungstate) with the methods described by Samar et al, (2018) and Sushant et al., (2019). 1 ml of lyophilized aqueous extract with known concentration (5-50 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 (5-20 µg/ml). After this period, the absorbances were recorded at 765 nm using the same spectrophotometer apparatus. The quantity 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 lyophilized aqueous Mms-1 extract of *M. morindoides* leaves was determined quantitatively with ACl_3 % MeOH solution using methods described by Samar et al., (2018) and Sushant et al., (2019). 2 mg of aqueous extract Mms-1 of *M. morindoides* leaves 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 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 determination of lethal dose 50 (LD_{50})

The acute and sub-acute toxicity were determined according to the method described by Loha et al., (2019) by the administration orally once high oral dose of 1000, 2000 and 5000 mg/kg bw of aqueous extract in acute toxicity and daily doses of 500, 1000 and 5000 mg/kg body weight (bw) of aqueous extract Mms-1 *M morindoides* leaves in sub-acute toxicity respectively. Briefly, in both toxicity tests, rats were divided in 7 groups and were orally administered respective oral doses with 5 rats for each oral dose as followed:

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

●- Groups IIa and b, IIIa and b and IVa and b were separately and orally administered by gavage aqueous extract Mms-1 once (a) and daily (b) in acute and sub-acute toxicity at respectively.

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

2.5.2. Determination of serum electrolytes

After the sub-acute toxicity experiment, all animals having received aqueous extract of *M. morindoides* at oral dose of 5000 mg/kg bw, were sacrificed. The blood from each animal was collected by cardiac puncture with sterile seringes and emptied into pre-labeled centrifuge tubes for serum separation. They were left standing at room temperature for 30 min before centrifuging at 4000 rpm for 5 min in an 80-1 electric centrifuge (B-Bram Scientific and Instrument Co., England).

Potassium, calcium and chloride determination was carried out by colorimetric methods using Bioxer reagent kits (UK)- BXCO141A for K, BXCO291A for Ca and BXCO281A for Cl-, carrefuly following the instructions and specifications of the manufacturer. The determination of bicarbonate (HCO^{3-}) and sulphate (SO^{4-}) level was done by simple titration while that of sodium (Na) was done by flame photometer (IL 943). Other eletrolytes were determinated using an electrolyte auto-analyser Landwind LW E60B used also by Imo et al., (2019) and by flame photometer (AOAC, 1984).

2.5.3. Evaluation of vital organs body weight ratio

The kidneys, liver, pancreas, lungs, spleen, ovaries, testicules and heart of the rats were removed on the 29th day after scarification of all animals that had received the highest oral dose of 5000 mg/kg bw of aqueous extract Mms-1 of *M. morindoides* leaves. The blood collection was carried out by tail and was cropped to remove other tissues. The organs were plentifully washed with distilled water an then placed on a saline soaked gauze. They were dried at 50^{o}C in hot and then weighed until constant weight (Abubakar et al., 2019).

2.5.4. Biochemical and haematological parameters analysis

Blood from rats having received 5000 mg/kg bw of aqueous extract Mms-1 in sub-acute toxicity test was collected from tail vein on Day 28 for analysis. For biochemical parameters, blood was centrifuged at 4000g for 5 min to obtain plasma, which was stored at –20°C: glucose, creatinine, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), serum glutamopyruvate transferase (SGPT), serum glutamooxalate transferase (SGOT), uric acid, total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), total and direct bilirubin were quantified using Architect (Abottâ) automation with Boehringen Ingelheimâ biochemical kits. Total proteins were estimated using Biuret's method (Saha et al., 2011). Haematological parameters were analyzed using an automatic haematological analyzer (Coulter STK, Beckam) with appropriated kits. The differential leucocytes count was performed with an optical microscopy after staining and, in each case, 100 cells were counted (Akpanabiattu et al., 2013).

2.6. Histological study

At the end of sub-acute toxicity, all animals that had received the highest oral dose of 5000 mg/kg bw were sacrificed. Liver, heart, lungs, spleen, kidneys and pancreas were excised from dissected animals, plentifully washed with NaCl 0.9% for removal of blood. They were next dried, weighed, and stored in 10% formalin saline solution for histopathological analysis according to the technique described by Loha et al., (2019). Briefly, the tissue sections were plentifully washed with distilled water and stained with haematoxylin-eosin for light microscope analysis. The slides of all selected vital organs were made and then observed under light microscope at a magnification of x20 objective for the investigations of any histological change in treated group compared to negative control group.

3. RESULTATS AND DISCUSSION

3.1. Antioxidant activity of extracts, fractions and polysaccharides from *M. morindoides* leaves

The antioxidant activity of extracts, fractions and polysaccharides was assessed *in vitro* against some reactive oxygen species (ROS) including DPPH, ABTS, superoxide anion, hydroxyl and hydrogen peroxide. For good understanding and interpretation of obtained results, following criteria were taken in account: $IC_{50} \leq 10$ µg/ml: 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, $40 < IC_{50} \leq 50$ µg/ml: very weak activity, $IC_{50} > 50$ µg/ml: inactive.

DPPH was a common method used to estimate the antioxidant activity of medicinal plant extracts, fractions, natural or synthetic compounds. DPPH was a stable free radical and accepted electron or hydrogen atom to become a stable diamagnetic molecule. This method was based on the reaction of DPPH with hydrogen donors of antioxidant compounds of medicinal plant extracts and fractions, which resulted in stable product. The reduction of DPPH in the presence of antioxidant substance became visible when its color changed from purple to yellow under antioxidant substance action (Musa et al., 2016; Shabi et al., 2017). The intensity of yellow color was directly proportional to scavenging capacity of medicinal plant extract and standard compound (Silva and Sirasa, 2018; Dehariya et al., 2020)

Proton radical scavenging was an important attribute of antioxidants. **ABTS**, a protonated radical, had characteristic absorbance maxima at 734 nm that decreased with the scavenging of the proton radicals. ABTS radical scavenging assay comprised a process that generated a blue/green/blues green $ABTS^{\bullet+}$ chromophore through the reaction of ABTS and oxidation with potassium persulfate. The ABTS radical cation as a stable form, was produced and the reduction capability of the radical was determined by the decrease of its absorbance since hydrogen donating at 734 nm, was induced by antioxidants. It was visible as a change in color from dark bluish green to colorless (Mital et al., 2017). ABTS test provided also informations on the reactivity of test compounds with a stable free radical. When the odd electrons were paired off in the presence of free radical scavenging, the absorption was reduced and the ABTS solution was decolorized as the color changed from deep violet to light yellow (Jethinlalkhosh et al., 2016). The ABTS assay was an elegant method for investigating the antioxidant activity of chain-breaking antioxidants and of hydrogen-donating antioxidants (Liu et al., 2016). After getting the stable absorbance, the antioxidant of medicinal plant extracts and fractions were added to the reaction medium and the antioxidant power was measured by studying decolorization (Vigneswaran et al., 2018).

In the PMS/NADH/NBT (phenazinemethosulphate/ nicotinamide adenine dinucleotide phosphate/ nitrobleutetrazolium) system, superoxide radicals were generated by the coupling of PMS/NADH. The oxidation reaction of NADH resulted in the superoxide radical production. This was measured by the NBT reduction. The generation of PMS-NADH took place non enzymatically. Superoxide anion was generated from the dissolved oxygen. At 560 nm, the activity was measured and a decrease in the concentration of superoxide anion was recorded because the increase in the concentration of medicinal plant extracts and fractions caused a decrease in the consumption of superoxide anion. Superoxide decomposition was evident to forms a powerful oxidative species like singlet oxygen and hydroxyl radicals. Superoxide radical scavenging assay was based on the reduction potential of medicinal plant extracts and fractions due to yellow dye nitrobluetetrazolium (NBT) reduced into formazan. Formazan was a free radical which was generated when phenazinemethosulphate (PMS) reacted with nicotinamide adenine dinucleotide (NAD). These generated ions reduced NBT into tetrazolium which was determined at 560 nm (Dehariya et al, 2020).

Against DPPH, ABTS and superoxide anion activities, results revealed that all tested extracts and fractions from *M. morindoides* leaves gave maximum free scavenging activity with IC_{50} values < 10 µg/ml. 80% methanol extract Mms-2 with IC_{50} values of 2.05±0.01, 2.52±000 and 3.12±0.03 µg/ml respectively showed high activity compared to aqueous extract Mms-1 presenting IC_{50} values of 3.25±0.01, 5.23±0.003 and 6.45±0.00 µg/ml against DPPH, ABTS and superoxide anion $O^{2\bullet-}$ respectively and significant difference was ($p < 0.05$) observed. This observation suggested that 80% MeOH was the best solvent that can be used to extract high amount of antioxidant constituents in *M. morindoides* leaves.

All soluble fractions also showed marked antioxidant activity with IC_{50} values < 10 µg/ml. Among them, ethylacetate fraction Mms-1.2 displayed high activity with IC_{50} values ranging from 4.12±0.02 to 5.12±0.00 against DPPH, ABTS and superoxide anion with significant difference ($p < 0.05$) compared to other remaining fractions. It was followed by residual aqueous Mms-1.4 soluble fraction showing antioxidant activity with IC_{50} value of 6.06 to 6.12±0.02 µg/ml against the same ROS. Chloroform Mms-1.1, *n*-butanol Mms-1.3 and aqueous residual Mms-1.4 soluble fractions exerted also pronounced antioxidant effects against these selected ROS with IC_{50} values ranging from 7.11±0.03 to 9.65±0.03 µg/ml.

Superoxide anion radical ($O^{2\bullet-}$) was generated by four-electron reduction of molecular oxygen into water. This radical was also formed in aerobic cells due to electron leakage from the electron transport chain. $O^{2\bullet-}$ were also formed by activated phagocytes such as monocytes, macrophages, eosinophils and neutrophils and the production of $O^{2\bullet-}$ was an important factor in the killing of bacteria by phagocytes. In living organisms, $O^{2\bullet-}$ was

Table 1: Effects of extracts and fractions form *M. morindoides* against the activity of some selected reactive oxygen species.

Extracts and fractions/radicals	DDPH	ABTS	Superoxide anion $O^{2\bullet-}$	Hydroxyl $HO^{\bullet}$	Hydrogen peroxide H_2O_2
Mms-1	3.25±0.01	5.23±0.02	6.45±0.00	7.52±0.01	8.05±0.03
Mms-1.1	8.25±0.00	7.11±0.03	7.25±0.02	8.35±0.02	9.02±0.01
Mms-1.2	4.12±0.02	4.56±0.01	5.12±0.00	6.85±0.03	7.06±0.01
Mms-1.3	9.65±0.03	8.43±0.02	9.25±001	9.03±0.02	9.81±0.02
Mms-1.4	6.12±0.02	5.85±0.03	6.06±0.01	7.06±0.02	8.32±0.02
Mms-2	2.05±0.00	2.52±0.01	3.12±0.03	3.23±0.02	3.52±0.01
CP-Mms	15.05±0.03	20.12±0.1	25.14±0.03	17.58±0.01	22.35±0.02
PF-Mms -1	25.25±0.02	51.22±0.02	35.46±0.02	45.05±0.03	33.25±0.01
PF-Mms-2	32.15±0.01	45.26±0.01	37.65±0.01	42.21±0.02	37.02±0.03
PF-Mms-3	37.54±0.03	41.18±0.02	43.25±0.03	39.58±0.03	45.50±0.01
PF-Mms-4	42.15±0.01	47.35±0.03	51.25±0.01	41.62±0.01	52.36±0.02
Apigenin-7-O-glucoside	59.52±0.01	62.36±0.03	82.65±0.02	76.25±0.02	72.56±0.04
Luteolin-7-O-glucoside	41.71±0.04	56.25±0.03	72.41±0.04	68.56±0.02	65.05±0.03
Chrysoeriol	> 50	> 50	> 50	> 50	> 50
Chrysoeriol-7-O-neohesperidoside	12.35±0.03	25..62±0.01	22.02±0.04	18.75±0.03	15.63±0.02
Quercetin	3.15±0.03	3.60±0.01	7.75±0.00	8.02±0.03	15.02±0.02
Rutin	8.45±0.01	6.25±0.02	15.15±0.01	18.36±0.02	25.60±0.01
Quercetrin	4.25±0.02	5.45±0.03	12.85±0.02	14.05±0.01	22.01±0.03
Kaempferol	7.82±0.01	3.81±0.02	6.15±0.02	18.24±0.02	12.06±0.01
Kaempferol-3-O-rutinoside	25.06±0.00	15.14±0.03	17.03±0.02	20.06±0.03	23.54±0.02
Kaempferol -7-O-rhamnosylsophoroside	27.25±0.02	17.62±0.00	21.02±0.03	24.56±0.01	30.25±0.01
Gallic acid	2.06±0.02	3.12±0.02	4.65±0.00	8.47±0.00	7.82±0.03
Vitamin C	3.53±0.00	2.67±0.01	7.15±0.02	7.55±0.02	8.02±0.01

Mms-1 and -2: aqueous and 80% methanol extracts, Mms-1 to -4: chloroform, ethylacetate, n-butanol and residual aqueous soluble fraction form the partition of Mms-1 extract, CP-Mms: crude polysaccharide, PF-Mms-1 to PF-4: pure polysaccharides.

removed by the enzymes called superoxide dismutase (SOD) (Shareef et al., 2014). Superoxide anion radical ($O^{2\bullet-}$) was one of the strongest reactive oxygen species among the free radicals that were generated first after oxygen was taken into living cells. $O^{2\bullet-}$ changed to other harmful reactive oxygen species and free radicals such as hydrogen peroxide and hydroxyl radicals (Shareef et al., 2014).

Hydroxyl radical was one of the potent reactive oxygen species in the biological system. It reacted with polyunsaturated fatty acid moieties of cell membrane phospholipids and caused damage to cell. The hydroxyl radical was regarded as a detrimental species in pathophysiological processes and capable of damaging almost every molecule of biological system and contributed to carcinogenesis, mutagenesis and cytotoxicity. They were produced by the reaction of H_2O_2 and the ferrous that would react with 2-deoxyribose. The reaction was stopped by adding TBA reagent that would give a red colour if the malondialdehyde was formed as the result of the reaction between the radical and 2-deoxyribose. The scavenging capacity of an extract was directly proportional to its antioxidant activity which was depicted by the low intensity of red colour (Saeed et al., 2012). It was the most reactive radical among all oxygen radicals. It can induce severe damage bio-molecules in the body, which resulted in cell damages that caused ageing, cancer and several other diseases. The removal of hydroxyl radicals was probably one of the most effective ways to defense several diseases (Yang et al., 2014).

Hydrogen peroxide: FRSA was another useful method for determination of antioxidant activity. It was itself not very reactive, but sometimes it can be toxic due to the increased hydroxyl radicals in the cells. It was a week oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects. The scavenging of hydrogen peroxide by medicinal plant extracts may be attributed to active secondary metabolites, phenolics which neutralized hydrogen peroxide by donating electrons thereby neutralizing it to water (Shabi et al., 2017). On the other hand, H_2O_2 was a biologically important, non-radical reactive oxygen species (ROS) that can influence several cellular processes. It was an important molecule although it was not toxic by itself, but can be converted to other even

more toxic radicals such as $HO^{\bullet}$ by Fenton reaction or hypochlorous acid by the enzyme myeloperoxidase (MPO, EC 1.11.2.2). It was itself not very reactive, but it can sometimes to be toxic to cells because of it may give rise to hydroxyl radical in the cells (Esmaeili et al., 2015). It was a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly, once inside the cells, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects (Ngonda, 2013). H_2O_2 and $HO^{\bullet}$ radicals were inevitably produced as byproducts of normal aerobic metabolisms and were increased during infections, exercises, stress conditions, radiations, etc. (Mukhopadhyay et al., 2016).

When tested against hydroxyl $HO^{\bullet}$ and hydrogen peroxide H_2O_2 radicals, aqueous and 80% methanol extracts Mms-1 and Mms-2 respectively, and soluble fractions Mms-1.1 to -1.4 form *M. morindoides* leaves exhibited concentration-dependent inhibition of deoxyribose oxidation, showing thus, their inhibition effect on the activity of hydroxyl and hydrogen peroxide radicals respectively. 80% MeOH Mms-2 extract exhibited pronounced and high antioxidant effect with IC_{50} values of 3.23±0.02 and 3.52±0.01 µg/ml respectively compared to aqueous Mms-1 extract exhibiting this activity with IC_{50} values of 7.52±0.01 and 8.05±0.03µg/ml respectively and significant difference ($p < 0.05$) was deduced.

Chloroform Mms-1.1, ethylacetate Mms-1.2, *n*-butanol Mms-1.3 and residual aqueous Mms-1.4 soluble fractions showed pronounced activity against hydroxyl $HO^{\bullet}$ and hydrogen peroxide H_2O_2 with $IC_{50} < 10$ µg/ml (Table 1). Ethylacetate Mms-1.2 was the most active with IC_{50} values of 6.85±0.03 and 7.06±0.01 against these two ROS respectively, followed by residual aqueous Mms-1.4 (7.06±0.02 and 8.32±0.02 µg/ml respectively) and chloroform Mms-1.1 (8.35±0.02 and 9.02±0.01 µg/ml respectively) soluble fractions. *n*-butanol Mms-1.3 exhibited good activity against these two ROS (IC_{50} = 9.03±0.02 and 9.81±0.02 µg/ml respectively). All soluble fractions showed interesting free scavenging activity against all selected ROS related to the nature of their constituents content. Considering their IC_{50} value levels, aqueous extract Mms-1 and its soluble fractions as well as 80% methanol extract Mms-2 exerted pronounced activity against these ROS (Table 2).

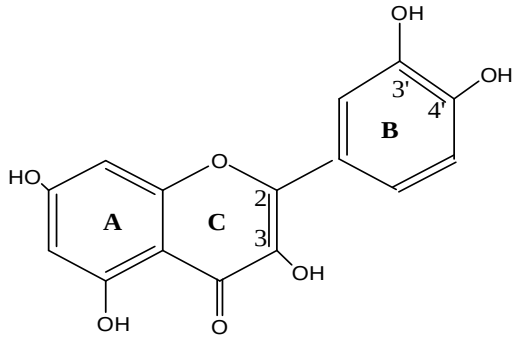
For tested flavonoids, quercetin, rutin and quercetrin exhibited pronounced activity **against DPPH and ABTS** radicals with IC_{50} values of 3.15±0.03 and 3.60±0.00 µg/ml, 8.45±0.01 and 6.25±0.02 µg/ml, and 4.25±0.2 and 5.45±0.03 µg/ml respectively, with significant different ($p < 0.05$). The reported C_{50} values of quercetin against DPPH and ABTS radicals were in the same rank (< 10 µg/ml) with those reported by Lee et al., (2015) and with Rusmana et al. (2017). **Against hydroxyl radical**, quercetin displayed pronounced activity with IC_{50} value of 8.02±0.03 µg/ml while rutin and quercetrin showed good activity with IC_{50} values of 18.36±0.02 and 14.03±0.01 µg/ml respectively. **Against superoxide anion $O^{2\cdot}$**, quercetin inhibited its activity with IC_{50} value of 7.75.±0.03 µg/ml while rutin and quercetrin and rutin acted the same manner by producing IC_{50} values of 15.15±0.01 and 12.85±0.03 µg/ml µg/ml respectively. The antioxidant activity of quercetin **against hydrogen peroxide** was good and high (IC_{50} = 15.02±0.02 µg/ml compared to its two glycosides rutin (25.60±0.01 µg/ml) and quercetrin (22.01±0.03 µg/ml) with moderate activity and notable difference ($p < 0.05$) was noted. The antioxidant activity of glycoside quecertin derivatives was weak compared to aglycone quercetin against all selected ROS with significant difference ($p < 0.05$). It is reported that the presence of sugar moiety on the basic ring skeleton attenuated the *in vitro* antioxidant activity of rutin and quercetrin, and this sugar substitution at three positions on the C ring was a major detrimental factor of the antioxidant activities of quercetin glucose derivatives (Zeng et al., 2020).

Consequently, the high antioxidant character of quercetin was associated to its chemical structure, especially to the presence and location of the hydroxyl (-OH) substitution in C-3 position, the catechol-type B ring and hydroxyl groups in B ring in C-3' and C-4' position and absence of sugar moiety in C-3 position in ring C. The presence of an O-dihydroxy structure in B-ring confered a higher degree of stability to the flavonoid phenoxyl radicals by participating in electron delocalization and was therefore, an important determinant for antioxidant potential. On the other hand, quercetrin showed high activity compared to rutin due to the substitution of a monoglucoside group in C-3 position in ring C instead of a diglucoside in this same position (Pu et al., 2015; Sarian et al., 2017).

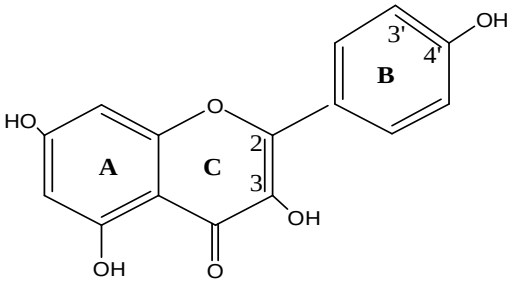
With kaempferol and its two derivatives 3-O-rutinoside and 7-O-rhamnosylsophoroside, it was observed that the aglycone kaempferol exhibited high and pronounced antioxidant activity against **DDPH and ABTS radicals** by producing IC_{50} values of 7.82± 0.01 and 3.81±0.02 µg/ml compared to kaempferol-3-*O*-rutinoside and kaempferol-7-O-rhamnosylsophoroside with good activity (IC_{50} = 25.06±0.00 and 15.14±0.03 µg/ml, and 27.15±0.05 and 17.62±0.00 µg/ml respectively). **Against superoxide anion**, kaempferol showed pronounced activity (IC_{50} = 6.15±0.02 µg/ml) while, the two diglucosides exhibited good and moderated activity with IC_{50} values of 17.03±0.02 and 21.02±0.03µg/ml respectively, with significant difference ($p < 0.05$). **When tested against hydroxyl and hydrogen peroxide radicals**, kaempferol and its glycoside derivatives exhibited good and moderate activity with IC_{50} values ranging between 18.24±0.02 and 12.06±0.01 µ/ml, 20.06±0.03 and 23.5±0.02, and 24.56±0.01 and 30.25±0.01 µg/ml against $HO^{\bullet}$ and $O_2^{\bullet-}$ respectively. The antioxidant activity of the aglycone kaempferol was

high compared to its glycoside derivatives against all selected ROS in the present study.

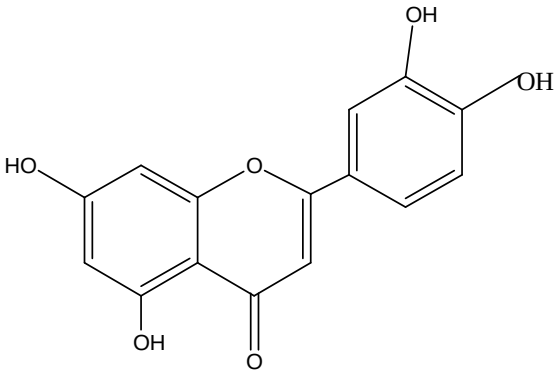
With regards to the structure-activity relationship, kaempferol exhibited high activity due to the absence of a sugar moiety either in C-3 or C-7 position in A ring respectively and the presence of hydroxyl group in C-3 and C-4' position in C and B ring respectively and C-7 position in A ring (kaempferol compared of kaempferol 3-*O*-rutinoside and kaempferol-7-*O*-rhamnosylsophoroside) while kaempferol-7-*O*-rhamonosylsophoride showed weak activity compared to its homologous kaempferol-3-*O*-rutinoside follow-up to the presence of a triglucoside part in C-7 position instead of a diglucoside in C-3 position.



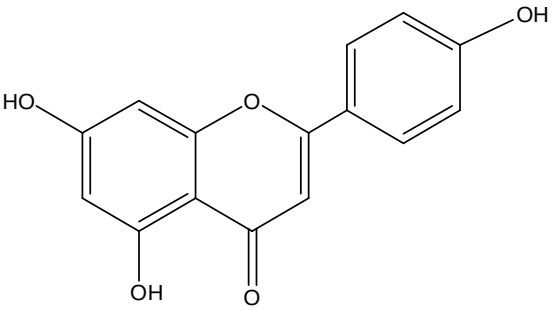
Quercetin



Kaempferrol



Luteolin



Apigenin

Figure 6: Structures of quercetin and kaempferol.

The structural elements of the flavonoid molecules, most important for hydroxyl radical scavenging, were hydroxylation of ring B and C-2-C-3 double bond connected with a C-3 hydroxyl group, C-4 carbonyl group and hydroxyl group on B ring in position C-3' and C-4' or in position C-4' alone. Hydroxylation of ring A also enhanced the activity (Tremi and Karel, 2016). In general, it was not easy to appreciate the antioxidant activity of these flavonoids due to considerable difference in their reported IC_{50} values against various ROS in divers study. Indeed, some studies had reported their antioxidant activity with $IC_{50} < 10$ µg/ml (Sarian et al., 2018, Giuxing et al., 2018) as reported in the present study or $10 < IC_{50} < 50$ µg/ml (Wang et al., 2018) and other with $50 < IC_{50} < 100$ µg/ml (Wang et al., 2018; Cao et al., 2020). For other flavonoids such as quercetin and its glucosides, their IC_{50} values were also reported in mg/ml (Srimathi et al., 2017) contrary to those reported in the present study. It was also the same for kaempferol and its glucosides in literature data.

Chrysoeriol was found to be devoid with antioxidant effect against all selected ROS ($IC_{50} > 50$ µg/ml). Our results were in good agreement with Mishra et al., (2003) and Rivière et al., (2009), but in contradiction with Bai et al., (2005), Khan et al., (2010) and Tofighi et al., 2014) who reported the antioxidant activity of this flavonoid with a percent inhibition of 53% for Khan et al., (2010) and observed at 1 mg/ml for Tofighi et al., (2014) against DPPH. Its glucoside chryoeriol-7-O-neoheperidoside showed good or moderate activity against all selected ROs with IC_{50} values ranging between 12.35±0.03 and 25.02±0.01 µg/ml (Table 1). Our finding good agreed with Mishra et al., (2003) who reported also the antioxidant activity of another chrysoeriol glucoside named chrysoeriol-6-O-acetyl-4-β-D-glucoside.

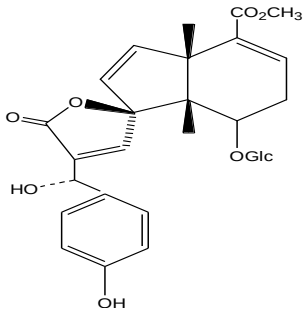
Apigenin-7-O-glucoside and luteolin-7-O-glucoside showed weak antioxidant activity against all selected ROS with IC_{50} values from 59.52±0.01 to 82.50±0.02 µg/ml and 41.71±0.04± to 72.41±0.04 µg/ml respectively. Their weak activity must be due mainly to

the absence of hydroxyl group in C-3 position in C ring although the configuration in A and B rings was similar to quercetin (flavonol) compared to luteolin (flavone) and kaempherol (flavonol) compared to apigenin (flavone), and also to the presence of a glucoside in C-7 position in ring A.

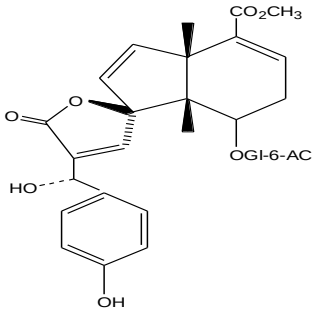
The mechanisms which followed by antioxidant defense were: **1)** Blocking of free radicals production **2)** of) oxidants Scavenging, **3)** The converting toxic free radicals into less toxic substances, **4)** Blocking the production of secondary toxic metabolites and mediators of inflammation, **5)** Blocking of the chain propagation of the secondary oxidants **6)** Repairing the injured molecules and **7)** Initiation and enhancing the endogenous antioxidant defense systems (Adwas et al, 2018). Thus, flavonoids from medicinal plants cannot only be considered purely as antioxidants, since under certain reaction conditions, they can also display prooxidant activity (Eghbaliferiz and Iranshahi, 2016; Sotler, et al., 2019).

All tested iridoids and iridoids glycosides were devoid of antioxidant activity although their chemical structures contain one hydroxyl phenolic group (Cimanga et al., 2003).

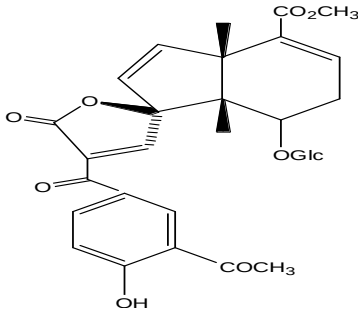
Examples of iridoid structures isolated from *M. morindoides* leaves (Cimanga et al., 2003).



Gaertneroside



Acetylgaertneroside



Dehydromethoxygaertneride

Crude polysaccharides CP-Mms and pure polysaccharide fractions PF-Mms-1 to -4 showed moderated or weak antioxidant activity with IC_{50} values ranging from 25.25±0.02 to 51.25±0.01 µg/ml. For these natural compounds, it was reported that some of them exerted antioxidant activity with IC_{50} values < 100 µg/ml (Haddad et al., 2017; Qiwu et al., 2019;) as it was found in the present study, and other with IC_{50} values in the hunder micrograms or in milligrams order (Sila et al, 2014; Zou et al., 2015; Xia et al., 2016, Zizhong et al., 2020). They can be considered as promising antioxidant compounds (Zou et al., 2015; Li-Hong et al., 2018; Rivathi et al., 2018; Zizhong et al., 2020). Their activity was related to their structure. Indeed, water-soluble crude polysaccharides obtained from the external mucilage of some plants *in vivo* were confirmed to have high content of sulfuric and uronic acids and showed strong antioxidant activity against some ROS like DPPH, hydroxyl, superoxide anion, ABTS or on reducing power assay (Liu, 2016; Xia et al., 2016) and should be explored as a potentially novel and effective natural antioxidants. The monosaccharide composition, molecular weight with sulphate content, possessed antioxidant capacity as reported for algal polysaccharides (Zhong et al., 2019). Cereal polysaccharides were reported to be associated with a certain amount of phenolic compounds and tea polysaccharides were mostly glycoconjugates in which a protein carried carbohydrate chain covalently linked with a polypeptide backbone, exhibited antioxidant activity (Wang et al., 2016). Several studies had postulated that protein or peptide moiety in polysaccharides was responsible for part of radical scavenging effect. The content of proteins in polysaccharide extracts appeared to contribute a direct scavenging effect on superoxide and hydroxyl radicals. Lentinan and *Schizophyllum commune* with only trace amount of proteins exhibited negligible scavenging effect towards superoxide radicals, whereas polysaccharide-protein complexes extracted from mushrooms such as *Ganoderma lucidum* and *Grifola frondosa* with lower polysaccharide/protein ratios, were more favorable for the scavenging function (Wang et al., 2016). Molecular weight was one of the most important structural features of polysaccharides for antioxidant activity. It was reported that polysaccharides with low molecular weights would have more reductive hydroxyl group terminals (on per unit mass basis) to accept and eliminate the free radicals (Liu et al., 2016). Some studies had indicated that polysaccharides played an important role in the prevention of oxidative damage in living organisms (Zizhong et al., 2020) and acted as dietary free radical scavengers among which *Eucalyptus* and other several medicinal plants were considered as important natural sources of antioxidant compounds (polysaccharides) with potential benefits in medicine and health care (Haddad et al., 2017). Unlike protein and nucleic acid, the structure of polysaccharides was far more complicated based on the differences found in composition of monosaccharide residues, glycosidic linkages, sequence of sugar units, degrees of polymerization and branching point. Apart from those, other factors, such as differences of cultivars, origins, and batches or even extraction methods and fraction procedures, were evidenced to have significant influence

on the physicochemical and structural properties of polysaccharides (Wang et al., 2016). Studies concerning the antioxidant and/or scavenger activities of crude plant polysaccharide extracts and purified polysaccharides from various medicinal plants have indicated that these plant carbohydrates show significant antioxidant activities and may be explored as potential antioxidants. Based on the published literature data, the antioxidant activity of natural polysaccharides might be related to their composition, water solubility, polarity, molecular weight, monosaccharide component, structure of chain conformation and intramolecular hydrogen bonds (Raza et al., 2016; Shi et al., 2017). Therefore, the antioxidant activity of polysaccharides is not attributable to a single factor, but to a combination of many factors.

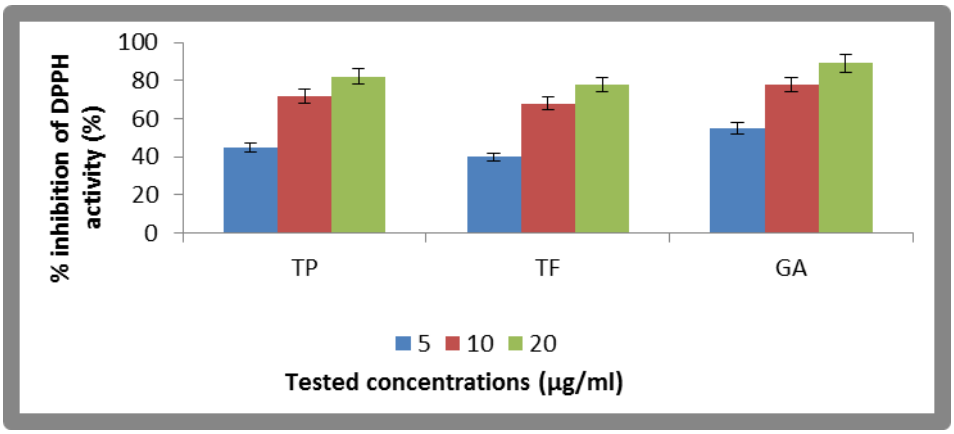
3.2. Phenol and flavonoid contents

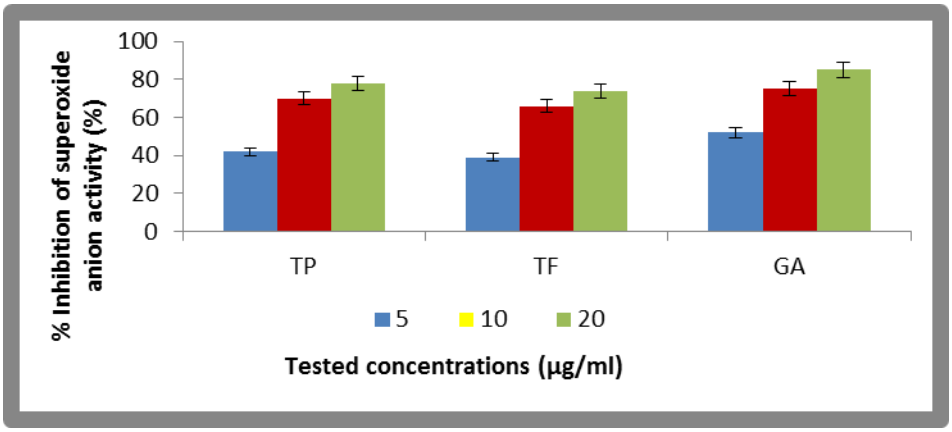
Results from the evaluation of phenolic and flavonoid contents revealed that aqueous Mms-1 extract of *M. morindoides* leaves contained 81.65 mg of phenols and 7.62 mg of flavonoids while the 80% methanol Mms-2 extract holded 92.13 mg of phenols and 11.03 mg of flavonoids expressed in gallic acid equivalent and quercetin equivalent/100 g of dried extract respectively. Phenols and flavonoids found in Mms-2 extract were high than those in Mms-1 extract with phenols in high amount compared to flavonoids. A relationship between the amount of these phytochemicals and the level of antioxidant activity was established and showed good relation between the activity of extracts and their phenolic content compared to flavonoid content. But, the high activity showed by both Mms-1 and Mms-2 extracts was mainly correlated to the high amount of phenolic content than flavonoid content. In general, extreme variations in antioxidant activity and total phenolics and flavonoids were previously found between various tested plant extracts. It can be observed that high amount of phenolic and flavonoid contents in plant extracts highly correlated with their antioxidant activity, confirming the assertion according to which the antioxidant activity of medicinal plant extracts was particularly and mainly related to the high polyphenol content (Ekin et la., 2017; Unuofin et al., 2018; Sushant et al., 2019; Chaves et al., 2020). The large variation in medicinal plant extracts for their antioxidant activity, may also resulted from differences in total phenolic contents and other phytochemicals. Such observations agreed with several previous reported findings (Unuofin et al, 2018; Sushant et al., 2019, Chaves et al., 2020).

Previous studies suggested that the flavonoids found in the plants exerted the antioxidant action by donating of a hydrogen atom to break the free radical chain. Moreover, functional hydroxyl groups in flavonoids mediated their antioxidant effects by scavenging free radicals and/or by chelating metal ions (Sofna et al., 2014). Our findings were not an isolated case, as they indicated that *M. morinoides* leaves extracts consisted of high quantity of phenolic compounds and showed promising antioxidant activity with IC_{50} value < 10 µg/ml. In general, from these results, it was observed that all samples from *M. morindoides* leaves had capacity of free scavenging activity of all selected ROS radicals and which was correlated mainly with the total phenol content suggesting thus their antioxidant activities.

Figure 3 showed the effects of total phenols (TP) and flavonoids (TF) as well as gallic acid (GA) on the activity of DPPH and superoxide anion radicals. I was observed that these phytochemicals inhibited the activity of these ROS in dose-dependent manner. When tested at highest concentration of 20 µg/ml, TP and TF produced 80 and 78% inhibition of the activity of DPPH respectively (A), and 78 and 74% against superoxide anion activity (B). Gallic acid acted in the same manner by inhibiting the activity of DPPH (A) and superoxide anion (B) by 89 and 85% respectively. It exerted high activity than TP and TF extracts while TP showed high activity compared to TF (Fig.7, A and B).

(A)





(B)

Figure 7: Inhibition of DPPH (A) and superoxide anion $O_2^{\bullet-}$ (B) activity by total phenols (TP) and flavonoids (TF), and Gallic acid (GA)

3.3. Acute and sub-acute toxicity of aqueous extract of *M. morindoides* leaves

Results from the acute and sub-acute toxicity studies did not reveal any toxicity and toxic symptom signs at the once administered oral doses of 500, 2000 and 5000 mg/kg bw in acute toxicity, and daily the doses of 500, 1000 mg/kg and 5000 mg/kg in sub-acute toxicity respectively, or again no observable toxicity signs were noticed in the aqueous extract Mms-1 from *M. morindodides* leaves treated rats compared to the negative control. No morbidity or mortality was observed in the treated groups at all administered doses during acute and sub-acute toxicity studies. No difference was observed in intake water and food in both groups. But, the weight of the treated rats increased normally in the course of treatment compared to untreated with significant difference ($p < 0.05$) (Fig. 2).

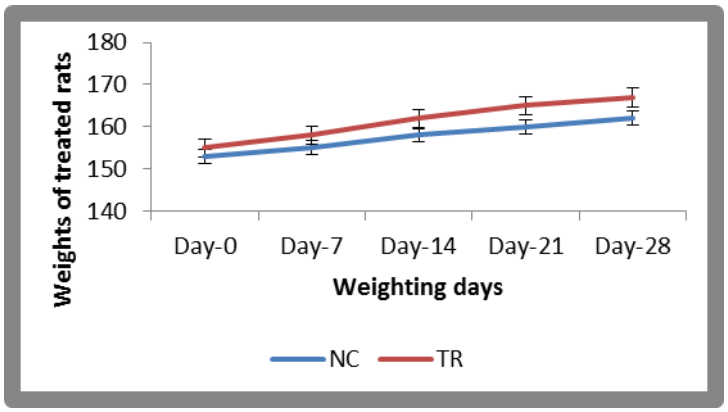


Figure 8: Variation of body weights of treated Wistar rats (TR) compared to negative control (NC).

The initial mean body of negative control rats at Day-0 was 153 g and at the end at Day-28 of the experiment, their final mean body weight was 162 g with a gain body weight of 9 g (5.88%), while for the treated rats, the body weight at Day-0 was 155 g and at Day-28 became 167g showing a gain of 12 g (7.74%) in sub-acute toxicity as illustrated in figure 2. Similar results were also recorded in acute toxicity, without results presented in this study. According to Kennedy et al., (1986), the studied extract can be considered as practically non-toxic and safe by oral route. Its LD_{50} (lethal dose 50) was estimated > 5000 mg/kg bw.

3.4. Histology study

Results from the histological investigation revealed that gross observation of the liver and kidneys of the treated rats showed no significant changes compared to control group and no significant difference ($p > 0.05$) was observed. The mean absolute weights of the liver were 7.20 ± 0.95 g (at 2000 mg/kg bw) and 8.50 ± 1.02 g (at 5000 mg/kg bw) for treated rats compared to negative control (7.17 ± 1.05 and 8.42±0.11 g) at the same times. Similarly, the mean absolute weights of the kidneys of rats in the negative control (8.15±0.04 g) and extract treated groups (8.21±0.01 g) did not show different difference ($p > 0.05$) and no toxic effects were observed as in acute and sub-acute toxicity.

3.2.2. Effects on haematological parameters by aqueous Mms-1 of *M. morindoides* leaves in treated Wistar rats

Results presented in Table 2 revealed that the haemoglobin level of treated rat groups was significantly ($p < 0.05$) increased compared to untreated group. This would be due to the absorption of iron and copper in high amounts by treated animals from the administered extract

or to the immunopotentiating effect of the extract as also previously reported for some medicinal plant extracts (Tamwar et al., 2019) and the administered Mms-1 extract from *M. morindoides* leaves can not induce anemia.

No significant change was observed in the haematocrit level of treated rats compared to negative control group ($p > 0.05$) while it was observed significant increase of WBC of treated animals compared to untreated group ($p < 0.001$), but it remained in the normal physiological limits (Table 2). WBC were essential for the protection of the animal against foreign invaders. Elevation in its level was indicative of response to an immunological challenge (Aprioku and Igbe, 2017).

A significant decrease of platelets level was observed in treated animals compared to untreated suggesting that *M. morindoides* aqueous extract Mms-1 could precipitate thrombocytopenia that was one of the most common haematologic disorders, characterized by an abnormally high number of platelets from multiple causes (Izak and Brussel, 2014).

Table 2: Effects of aqueous extract Mms-1 from *M. morindoides* on haematological parameters of treated Wistar rats.

Parameters	Negative control	Mms-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 10^3µL-1)	1291.0 ± 0.6	1272.5 ± 0.0	995-1713
WBC (x 10^3µL-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	7.6-10.29
Eosinophils	0.12±0.02	0.14±0.04	0.01-0.16
Neutrophils (%)	21.2 ± 0.3	23.3 ± 0.3	3.0-24.7
Monocytes (%)	2.9 ± 1.1 2	3.2 ± 0.1	0.0-4.0
Basophils (%)	0.0±0.0	0.0±0.0	0.0±0.05
Lympocytes (%)	89.8 ± 1.6	90.4 ± 0.1	58.8-94
Segmented leucocytes (%)	17.6 ± 0.6.	21.7 ± 00.2	-

RBC; red blood cells, WBC: white blood cells, Mms-1: aqueous extract of *M. morindoides*

In addition, the decrease of platelets level in circulatory system of treated animals caused by *M. morindoides* aqueous extract Mms-1 supported its anticoagulant property also reported for other medicinal plant extracts (Duric et al., 2015; Chegu et al., 2019) and was a high bleeding risk in thrombocytopenic patients (Slichter, 2004; Piel-Julian et al., 2018).

Neutrophils, eosinophils, lymphocytes and segmented leucocytes levels showed slight increase in treated animals compared to negative control group, with no significant difference ($p > 0.05$). Neutrophils (neutrophilia) were important phagocytic cells normally elevated in the early inflammatory response, while lymphocytes were subtypes of leucocytes critically essential for providing cell mediated immunity (Aprioku and Igbe, 2017).

Eosinophils (eosinophilia) had a role as phagocytes and also more specific functions that included providing a defense against metazoan parasites and modulating the inflammatory processes. They responded chemotactically to histamine, immune complexes and eosinophil chemotactic factor of anaphylaxis, a substance released by degranulating mast cells. Basophils (basophilia) were not true phagocytes, but contained large amounts of histamine and other inflammation mediators. Eosinophils and basophils may be seen in response to systemic allergic reactions and invasion of tissues by parasites (Cotter, 2019). Slight fluctuation was observed in the levels of these parameters without no significant difference in both treated and untreated rat groups (Table 2).

3.2.3. Effects of aqueous extract Mms-1 from *M. morindoides* leaves on biochemical parameters of treated Wistar rats

Table 3 showed the effects of the oral administration of the aqueous extract Mms-1 of *M. morindoides* leaves on biochemical parameter levels of treated compared to untreated Wistar rats. Results indicated that the oral administration of aqueous extract at the highest oral dose of 5000 mg/kg bw in sub-acute toxicity induced remarkably significant decrease of plasma glucose level in treated animals compared to untreated animals ($p < 0.05$). This decrease may be due probably to its hypoglycemic properties as also previously reported for other medicinal plant extracts (Nsaka et al., 2013; Cimanga et al., 2015) and gave credence to the use of this extract as a hypoglycemic agent for the treating of diabetes mellitus type II.

Alanine transferase (ALAT) and aspartate transferase (ASAT) were two known liver enzymes as good indicators of this organ as well as ALP and considered as biomarkers predicting possible toxicity of liver (Unuofin et al., 2018). On the other hand, the elevation of both transaminase levels in the blood can indicate the damage to parenchymal liver cells and perturbation of the function of this organ or frequently refer to hepatotoxicity (Moussaoui et al., 2020). The levels of the two enzymes were not different in treated compared to

untreated Wistar rats. In addition, no negative or deleterious effect on the activity of both transferases in treated rats compared to untreated group was observed as also reported by Moussaoui et al., (2020) for *Withania frutescens* hydro-ethanolic extract leaves.

Table 3: Effects of the aqueous extract concentrations of some biochemical parameters at oral dose 5000 mg/kg body weight.

Parameters	Negative control	Mms-1: 5000 mg/kg bw	Reference values
Glucose mg/dl	87.5 ± 0.4	76.3 ± 0.3	50-135
Creatinine (mg/dL)	0.5 ± 0.9	0.7 ± 1.0	0.2-0.8
AST (IU/L	126.3 ± 0.3	128.5 ± 0.2	45.5-143
ALT (UI/L)	38.2 ± 1.2	39.7 ± 0.2	17.5-45
Total cholesterol (mg/dL)	104.5 ± 1.3	103.9 ± 1.2	40-130
Triglycerides (mg/dL) 5	43.2 ± 0.	44.0 ± 0.2	20-144
Total bilirubin (mg/dL)	0.3 ± 0.3	0.5 ± 0.0	0.2-0.55
Direct bilirubin (mg/dL)	0.02 ± 0.0	0.04 ± 0.0	0.03-0.05
Total proteins (g/dL)	7.0 ± 0. 3	7.4 ± 1.1	5.7-7.6
Albumin (g/dL)	3.1 ± 0.2	3.4 ± 0.3	3.8-4.8
ALP (IU/L)	122.4 ± 1.0	125.3 ± 1.2	50.8-128
HDL-cholesterol (mg/dL)	60.0 ± 0.3	62.2 ± 0.4	> 35
LDL-cholesterol (mg/dL)	34.1 ± 1.1	32.7 ± 1.3	< 130
Uric acid (mg/dL)	2.88 ± 0.8	2.91 ± 0.5	2.6-7.2
SGPT (UI/L)	28.2 ± 1.3	27.3 ± 1.2	-
SGOT (UI/L)	117.3 ± 1.1	120.4 ± 1.2	-
Urea (mmol/L)	16.1 ± 0.3	16.9±0.4	11-23

AST: aspartate transferase, ALT: alanine transferase, ALP: alkaline phosphate, HDH: high density lipoproteins, LDL: low density lipoproteins, SGPT: serum glutamopyruvate transferase, SGOT: serum glutamooxalate transferase, -: not found.

Results reported here indicated that there was slight increase of both enzyme levels, but this did not show significant difference compared to the negative control ($p > 0.05$). It is also the same for the level of ALP (Table 3). Serum ALP (alkalin phosphate) was a sensitive detector for intrahepatic and extrahepatic bile obstruction. Based on this finding, it was suggested that hepatocytes of the treated rats were not damaged. The hepatic function of the treated animals was good keept or maintened and safe because the administered extract at this high oral dose of 5000 mg/kg bw did not cause significant deleterious effects in treated animals as also previously reported for other medicinal plant extracts (Nsaka et al., 2013; Cimanga et al., 2015; Unuofin et al., 2018).

SGPT and SGOT were also considered as liver biomarker enzymes Their measuring was a simple test, and was the most commonly used as indicators of hepatic functions (Imo et al., 2019). In the present study, their levels were comparable in both groups comfirming more the good state of the liver.

The normal values of renal biomarkers including creatinine, urea and uric acid (Unuofin et al., 2018), suggested that aqueous extract of *M. morindoides* did not produce any sort of disturbance in renal functions which was good maintained for the same reasons evoked above. In addition, the aqueous extract did not interfere with the hepatic and renal capacities to excrete the metabolites. As any damage was observed, it may be concluded that the administered aqueous extract did not induce significant observable toxicity on these two vital organs an no significant difference was observed in both groups ($p > 0.05$).

Cholesterol, LDL and triglycerides ($p < 0.05$) in treated rat groups showed slight decrease compared in untreated rat groups which might be attributed to the hypolidemic properties of Mms-1 extract as also previously reported by Cimanga et al., (2015), and in some times, to the increase of the secretion of thyroid hormones T3 and T4 which degraded cholesterol (Cimanga et al., 2015; https://en.wikipedia.org/wiki/Thyroid_hormones, 2021). Mms-1 extract caused also slight increase of HDL (anti-antherogenic agent). Thus, the decrease of total cholesterol and LDL, and increase of HDL concentrations were of great significance in cardiovascular diseases management. From this, aqueous extract of *M. morindoides* leaves had beneficial effects in the prevention and reducing of cardiovascular diseases and was correlated with risk factors contributing to the death of mainly diabetic patients (Akpanabiattu et al., 2013).

Albumins was proteins produced in the liver and had high concentration in plasma. Its decrease in serum may arise from liver diseases mainly cirrhosis (Wiedemann et al., 2017; Carvaldho and Machado, 2018; Lang et al., 2018; Bai et al., 2019). Fortunately, its level in treated animals compared to untreated rat groups did not show statistically significant difference ($p > 0.05$). In addition,

no significant change was observed in the level of the total and direct bilirubin in treated animals compared to control groups ($p > 0.05$) suggesting that jaundice could not be resulted in the intake of this aqueous extract Mms-1 from *M. morindoides* leaves as also reported by Tewari et al., (2017) and Raghuvanshi et al., (2021) for many other medicinal plant extracts and mentioned above based on the haemoglobin level.

The total proteins level significantly increased in treated rats compared to untreated group ($p < 0.05$) suggesting a supplement apport of an exterior supply of this element. A significant increase of urea concentration at in treated groups compared to untreated groups ($p < 0.01$) was observed, but this was not found as a sign of insufficiency renal because its concentration level remained within the normal physiological ranges (11-23 mmol/L). As urea production in mammals occurred specially in liver. Its concentration level could also be used as an indicator of hepatic and renal functions according to the case (Salih et al, 2013). Thus, our results more confirmed good maintenance of both organ functions of treated animals as already demonstrated above with the levels of their other biomarkers. Hence, the administered aqueous extract Mms-1 form *M. morindoides* leaves will be safe on its chronic use in treatment of various diseases as also previously reported for various medicinal plant extracts (Saha et al., 2011; Rajasekaran and Kannabiran, 2012; Akpanabiattu et al, 2013; Gandhare et al., 2013).

In general, our results from experimental tests were also in good agreement with other reported in other studies about the antioxidant activity of many many medicinal plant extracts and their respective fractions as well as polysaccharides (Nsaka et al., 2013, Rani et al., 2013; Cimanga et al., 2015; Wang et al., 2016; Haddad et al, 2017; Gosh et al., 2019; Ugwah-Oguejohor et al., 2019;Zhong et al., 2019; Da Fillepière et al., 2020; Zizhong et al., 2020) and remained in physiological ranks throughout the treatment period of 28 days (Tables 2 and 3). But, the levels of these parameters depended on various factors including age, nutrition, environment, gender, origin, breeding system, feeding and lineage, and genetic factors, and changes in any of these conditions would affect the reference values of the above-mentioned parameters (Shehani et al., 2018). In both evaluations, the rest of the parameters showed minor fluctuations mostly non-significant when compared to negative control. In general, our reported haematological and biochemical parameters levels in treated rats were in acceptable limits or were comparable to other reported in other studies (Cimanga et al., 2015; Badole and Kotwal., 2015; Santos et al., 2016; He et al., 2017, Elikok and Cirak., 2018).

3.2.4. Effects of the aqueous extract Mms-1 from *M. morindoides* on the levels on some serum electrolytes (mg/dL) in Wistar rats

Table 4 showed the effects of aqueous extract Mms-1 from *M. morindoides* leaves on some electrolytes level. Results revealed that the administration of the extract at all administered oral doses of 1000 and 5000 mg/kg bw induced significant increase ($p < 0.05$) of calcium, inorganic phosphorus, iron, bicarbonate and sulphate levels compared to untreated animals ($p < 0.05$) with significant difference ($p > 0.05$) mainly at the highest oral dose of 5000 mg/kg bw (Table 4). This increase may be due to the administration of aqueous extract Mms-1 of *M. morindoides* leaves that brought these elements as supplement apport. The present data particularly agreed with these previous observations of serum calcium elevation after calcium supplementation of drinking water and administered extract. This element was essential for the bone growth. On the other hand, no significant change in levels ($p > 0.05$) of in chloride, sodium and potassium levels compared to negative control was observed (Table 4).

Table 4: Effects of the administration of aqueous extract Mms-1 on some serum electrolyte levels.

Electrolytes	Negative control	Mms-1:1000 mg/kg bw	Mms-1:5000 mg/kg bw	Reference values
Calcium	9.8. ± 0.2	10.6 ± 0.	11.4. ±0.1	5.3-13
Chloride	102.5 ± 0.4	104.7 ±0.2	107.4 ± 0.4	95-110
Inorganic phosphorus	4.2 ±0.7	4.6 ± 0.2	7.7 ± 0.3	3.4-11
Iron	7.7 ±0.1	8.6 ± 0.4	9.4± 0.5	-
Potassium	4.1 ± 0.1	4.7 ± 0.3	5.1 ± 0.4	3.5-5.5
Sodium	74.3 ± 0.4	74.2 ± 1.2	74.6 ± 0.1	43-156
Sulphate	60.5±0.2	63.5±0.2	65.6±0.1	-
Bicarbonate	25.25±0.02	27.50±0.01	29.78±0.02	24-31

-: not found in literature

Sodium was regulated by the kidneys and adrenal glands. Sodium was the major cation of extracellular fluid (Imo et al., 2019). It was the major extracellular electrolyte implicated in hypertension. Extracellular sodium electrolyte level was responsible for the extent to which vessel walls contract. When the sodium level was high, there was increased contraction of the blood vessels (especially in the kidney), and hence a greater force was required to pump blood, with as consequence the occurring of hypertension. Moreover, high levels of sodium in the blood caused the cells to be dehydrated leading to hypernatrema which can cause coma or death.

Increase or decrease in sodium concentration may contribute to fluctuations of blood pressure (Enemor et al., 2013). Too much or too little sodium (hypernatremia or hyponatremia) can cause malfunction cells, and extremes in the blood sodium levels (too much or too little) can be fatal It played a central and important role in the maintenance of the normal distribution of water and osmotic pressure in the various fluid compartments (Kebe et al, 2013; Imo et al., 2019).

Chloride was important in the maintenance of the cation/anion balance between intra and extra-cellular fluids. This electrolyte was essential to the control of proper hydration, osmotic pressure, and acid/base equilibrium. Low serum chloride values were found with extensive burns, excessive vomiting, intestinal obstruction, nephritis, metabolic acidosis, and in addisonian crisis. Elevated serum chloride values may be seen in dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary obstruction (Imo et al., 2019).

Potassium ion was a major cation of the intracellular fluid and only about 10% of the total body potassium was extracellular. Fluctuations in serum potassium level were known to have serious health implications, particularly excess potassium in the blood, can occur in cases of renal failure that loosed the ability to excrete this mineral. Severe dehydration will also produce hyperkalaemia having as consequences muscle weakness and cardiac arhythmias that can lead to heart failure. Hypokalemia can lead to muscular weakness, hypotonia and cardiac arrhythmias while hyperkalemia can predispose to cardiac arrest (Enemor et al., 2013). Elevated potassium levels (hyperkalemia) were often associated with renal failure, dehydration shock or adrenal insufficiency. Decreased potassium levels (hypokalemia) were associated with malnutrition, negative nitrogen balance, gastrointestinal fluid losses and hyperactivity of the adrenal cortex and other fluids in the body (Imo et al., 2019).

Bicarbonate levels were measured to monitor the acidity of the blood and body fluids, and these results indicated that the acidity of this medium was maintained. The serum or plasma bicarbonate content was a significant indicator of electrolyte dispersion and anion deficit. Alteration of bicarbonate and CO_2 dissolved in plasma were characteristics of acid-base imbalance, which may be due to renal tubular acidosis, hyperkaliemic acidosis, renal failure or keto acidosis (Imo et al., 2019). Fortunately, in the present study, the oral administration of aqueous extract of *M. morindodides* leaves had not induce significant increase or decrease of these serum electrolytes and suggested that these electrolytes were maintained in good physiological levels (Table 4).

Electrolytes such as sodium, potassium, chloride and bicarbonate ions were among the parameters that were useful in the determination of kidney function. The elevation or depletion of the level of any of them may be an indicator for a kidney problem (Amagon et al., 2020). These reported electrolyte levels were comparable to other described in previous study (Abubakar and Sule, 2010; Enemor et al., 2013; Amagon et al, 2020).

On the organ weights, the administration of aqueous extract Mms-1 of *M. morindoides* leaves at all oral doses induced significant increase of organ weights at all administered oral doses. Moreover, Wistar rats treated at oral doses at 1000 and 5000 mg/kg bw with the plant extract showed significant increase ($p < 0.05$)) in organ weights of treated animals compared to negative control (Table 4), except pancreas for which its weight at 1000 mg/kg bw slightly increased and did not show significant difference compared to negative control (1.58 g against 1.57 g). The presence of particularly tannins which had astringent properties (pore-closing substance) could account for the observed increase in organ weights of treated animals as also reported by Maina et al.. (2013) for the aqueous extract of *Leptadenia hastata*. The administered aqueous extract Mms-1 of *M. morindoides* leaves did not detrimentally affect the organ weights, organ-to-body weight ratio and there was no change in

Table 5: Effects of aqueous extract Mms-1 from *M. morindoides* on weights of some selected vital organs.

Organs	Negative control	Mms-1: 1000 mg/kg bw	Mms-1: 5000 mg/kg bw
Heart	0.94 ± 0.00	0.97 ± 0.02	0.99 ± 0.02
Kidneys	2.34 ± 0.02	2.36 ± 0.04	2.38 ± 0.01
Spleen	0.71 ± 0.02	0.73 ±0.02	0.75 ± 0.03
Brain	3.71 ± 0.01	3.72 ± 0.04	3.75 ± 0.04
Pancreas	1.57 ± 0.03	1.58 ±0.02	1.61 ± 0.03
Testicules	9.12 ± 0.02	9.24 ± 0.03	9.26 ± 0.03
Ovaries	0.24 ± 0.03	0.26 ±0.01	0.29 ± 0.04

See Table 4.

the colour and form of various organs of treated animals compared to negative control in both toxicity tests (Table 4). Our results were in good agreement with other studies for the effects of some administered medicinal plant extracts on organ weights of treated animals (Unuofin et al., 2018; Moussaoui et al, 2020).

3.2.4. Effects of aqueous extract Mms-1 from M. *morindoides* on some vital organ weights in histological study

Histopathological study of the liver sections in the control group showed normal appearance of central vein (CV) and hepatic sinusoids (S) lined by endothelial cells

(EC) with normal radiating hepatocytes of treated animals compared to untreated group. There was also normal appearance of the portal triad including hepatic portal vein, interlobular bile duct and branches of hepatic artery. The macroscopic and histological analysis of the organs of treated animals with the highest oral dose of 5000 mg/kg bw of the aqueous extract Mms-1 from *M. morindoides* leaves did not show any changes in colour and form of selected vital organs compared to control group rat organs. They revealed no anatomical and/or structural changes in major vital organs as also reported for other medicinal plant extracts (Saha et al., 2011). No detectable abnormalities such as hypertrophy of organs were also observed by pathological examination of the tissues. No alterations in cell culture or unfavourable effects were seen in the microscopic examination of the internal organs using multiple magnification powers. No pathologie was recorded in the histological sections of the vital organs such as heart, spleen, kidney, liver and lung. Histopathological analysis showed absence of any gross pathological lesions. Heart, lung, kidney, liver, pancreas, brain and spleen of the treated rats also did not demonstrate significant changes in morphology comparable to control group, indicating the protective effect of the administered extract on these tissues. In addition, stomach parts of treated rats did not show the development of ulcerative spots. All vital organs in both groups showed similar architecture. These results more confirmed those observed in acute and sub-acute toxicity tests. The present study provided evidence for the total safety and tolerability profile of the aqueous extract Mms-1 from *M. morindoides* leaves suggesting its safe use in single oral dose treatment as well as for long term use for the treatment of various ailments without production any significant toxic effects.

4. CONCLUSION

This study consisted the first report of the antioxidant and free scavenging activities of extracts, fractions and polysaccharides from *M. morindoides* leaves on a large game of reactive oxygen species as well as acute and sub-acute toxicity of aqueous extract with its closely related effects on haematological and biochemical parameters, organ weights and some serum electrolytes of treated Wistar rats. Results indicated aqueous extract and its soluble fractions chlorofom, ethylacetate, *n*-butanol and residual phase, 80% methanol extract and polysaccharides exhibited interesting and promising antioxidant activity with different magnitudes evaluated against some selected reactive oxygen species including 2,2'dipheyl-1-pycrihydrazyl, (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). This study provided valuable scientific data on the antioxidant properties of samples from this medicinal plant and toxicity of aqueous extract which was used as principal preparation in traditional medicine. On the basis of the reported results, aqueous extract from *M. morindoides* leaves can be used as raw material for the remedy preparation to prevent the occurring of some diseases particularly cardiovascular diseases. It was considered as safe and was well tolerated in animals without visible toxic effects and did not induce mortality in animals. It was found to have no significant influence on the levels of biochemical and haematological parameters and can be considered as a non-source of any pathologic disease. It caused significant increase or decrease of some electrolytes in treated animals and did not significantly modify the weight of some vital organs of treated animals. These reported results constituted a irrefutable scientific base supporting and justifying the traditional use of the studied plant part of *M. morindoides* in traditional medicine in Democratic Republic of Congo and other African countries to treat various ailments and diseases without significant side effects.

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