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RESEARCH ARTICLE

Qualitative phytochemical screening and *in vitro* antioxidant activities of aqueous extracts of *Rhaphiolepis bibas (Lour.) Galasso & Banfi*

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Reactive oxygen species causes harm to cell membranes and biomolecules, wherefore chronic diseases develop. Antioxidants scavenge such free radicals combating oxidative stress. This research aimed to determine the antioxidant potential of the aqueous stem bark, root and leaf extracts of Rhaphiolepis bibas against standards. DPPH radical scavenging activity was high from th stem bark extract at 72.33% with root extract at 65.85% and leaf extract at 55.91%, while ascorbic acid scavenged 89.53% of DPPH radicals. The leaf extract had the highest H_2O_2 scavenging activity of 91.92% with stem bark at 91.17% and the root extracts at 89.12%. The aqueous root extract of R. bibas had a significantly higher FRAP capacity in comparison to the leaf extracts and the stem bark. The abilities to chelate iron by the leaf extract were statistically higher compared to the root and stem bark extracts. Stem bark extracts had the highest phenol content of about 149.44 followed by the root extract at 141.14 and the least amount of phenol was found in the aqueous leaf extract having 73.012 Gallic acid equivalent/g. The root extracts had the highest total flavonoid 377.66-milligram quercetin equivalent/gram dry weight followed by stem bark extract at 255.72 and the least amount was found in aqueous leaf extract having 164.52 mgQE/g of sample dry weight. The existence of secondary metabolites linked to antioxidant action was shown by the qualitative phytochemical screening.

Keywords: antioxidant, Rhaphiolepis bibas, phytochemicals, oxidative stress, free radicals

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Introduction

In recent years, the use of traditional medicinal herbs for basic health care has increased steadily over the world. The search for novel phytochemicals that can behave as beneficial antimicrobials has piqued the curiosity of many scientific experts [1]. Primitive man used plants to treat various human ailments. To date, 85% of Indians make use of higher plants as effective antimicrobials to cure various diseases. The human body physiologically produces reactive oxygen species causing harm to cell membranes and biomolecules, wherefore chronic diseases develop. Antioxidants scavenge such free radicals combating oxidative stress. Plant-based therapies are becoming more popular despite advancements in modern medicine because of their lower negative effects. Oxidative stress is linked to a variety of diseases in today's world. Too much release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and/or insufficient removal of these species are both signs of oxidative stress. Radicals in the likes of superoxide, hydroxyl, and Hydrogen peroxide are examples of ROS. RNS includes peroxynitrite, nitrous oxide, and others. Oxidative stress has been attributed to cancer, neurological illnesses, arteriosclerosis, and other diseases [2]. The focus of the current research is on finding the antioxidant potentials naturally inherent in plants.

A wide range of bioactive compounds are produced by plants, which gives them an abundant supply of chemical entities for the synthesis of synthetic drugs, food supplements, nutraceuticals, and traditional medicine. The plant's strong antioxidant properties, lack of adverse effects, and economic feasibility have led to an increase in interest in ethnopharmacology around the world [2].

The best protection against reactive oxygen species (ROS) is provided by antioxidants, which slow or arrest the oxidation of important biomolecules such proteins, lipids, nucleic acids and carbohydrates. This delays oxidation process and prevents it from starting and spreading. Naturally occurring antioxidant sources and the application of different compounds with antioxidant activity by humans are therefore highly desirable. The most common antioxidants include phenols, flavonoids, vitamin C, and carotenoids [3].

R. bibas (Lour) is a large, subtropical evergreen tree belonging to the Rosacea family Maloideae, which has its origins in China, where it has been cultivated for over 2,000 years. The plant was introduced to farmers in over 30 countries globally, including Kenya, Japan, South Afria, India, Mediterranean nations in Europe, Madagascar, Australia and New Zealand. However, only a few of these countries engage in commercial cultivation of the plant [4] R. bibas can grow to be either a shrub or a tree, with a height range of 3 to 4 m. Its distinctive feature is a rounded crown on a very short stem. The leaves on the stem are organized in an alternating pattern, with the underside of the leaf being yellow-brown in colour and the upper side coated in hair. The tree bears yellow to orange-colored fruits that are round, 3–5 cm in diameterr. The fruits are edible, with a juicy flesh that leaves a faintly acidic aftertaste. In most

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varieties the fruit gives 47 kcal per 100 g, a rich source of vitamins, minerals, and organic acids. Furthermore, it is a good source of numerous bioactive substances, such as triterpenoids, flavonoids, carotenoids, and phenolic acids, which have various biological functions, such as anti-inflammatory, hypoglycemic, antiviral, anticancer, cytotoxic, and hypolipidemic effects. As a result, R. bibas serves as a therapeutic herb. Traditionally, the Chinese medicine utilized the leaves to manage several conditions such as diabetes, skin inflammations, chronic gastrointestinal irritation and respiratory system illnesses. Several studies demonstrated that the leaves of R. bibas have cytotoxic effects [3].

The study was done to evaluate *Rhaphiolepis bibas* aqueous stem bark, leaf, and root extracts for their antioxidant capacity.. This plant formulation's antioxidant activity was measured by evaluating ferric-reducing ability (FRAP), free radical (DPPH) scavenging activity and hydrogen peroxide, metal chelating, total phenol and total flavonoids.

Methodology

Collection of the study Plant Material

R.bibas roots, stem barks, and fresh leaves were gathered from Kiambu County at latitudes -0.91 and longitudes 36.78, at a height of 2128 meters above sea level. A taxonomist from the National Museums did taxonomic identity of the plant materials, and a reference specimen (I.W.K-001) was preserved. To ensure equal drying, the collected materials were cleaned with tap water, rinsed with distilled water, and then shade-dried with periodic rotation. The samples were ground into powder using a mechanical mill once they were totally dry. The powdered samples were kept at room temperature after being carefully labeled and sealed in khaki bags.

Preparation of Plant Extracts

A liter of distilled water was used to soak 100 grams of the ground samples. The samples were kept in a water bath at 60 degrees Celsius for 24 hours. Afterward decanting was done, with the remaining portion filtered through muslin cloth. Lyophilization was used to concentrate the extracts after they had been vacuum-filtered.

Determination of DPPH Scavenging Activity

Principle. DPPH is a steady chromogenic radical that gives a deep purple-colored solution when prepared in methanol or ethanol. This assay relies on the molecules' ability to scavenge radicals or donate hydrogen giving rise to 2, 2-Diphenyl-1-picrylhydrazine from DPPH, hence a pale-yellow colored reaction. Therefore, as a result of this reduction, the color of DPPH faded accounting for reduced Optical Densities at 517 nm.

Procedure. From each aqueous extract, seven concentrations (5 µg/ml- 150 µg/mlMl) were prepared in methanol. The assay mixture constituted of 3 mls sample and 1 ml DPPH solution (24mg of DPPH in 100 ml methanol). Following vigorous shaking, the test tubes were incubated at 25° C for 30 minutes. Optical densities of each extract dilution were measured spectrophotometrically at 517nm. The procedure was done in triplicate with vitamin C as the standard. The aqueous extracts' potential to scavenge DPPH was worked out and expressed as percentages using the formula described by Kim *et al.,* [5].

$$\% \text{ DPPH Radical scavenging activity} = (AC-AS)/AC \times 100$$

AC = Optical density DPPH+Methanol;
AS= Optical density DPPH + plant extract.

The Log dosage inhibition curve was used to compute the sample's IC_{50} value.

Hydrogen Peroxide Scavenging Activity

Principle. A decrease in optical density of Hydrogen peroxide following its reduction by the antioxidant compounds is monitored [6].

Procedure. Sample extract dilutions (1 µg/ml-50 µg/ml) were made where 5 mls of the aqueous sample extract in phosphate buffer were mixed with 0.5 mls of H_2O_2 (5 mM in phosphate buffer (0.1M) pH 7.4). The tubes were later incubated at 25° C for 10 minutes. Absorbance readings were then taken at 230 nm with phosphate buffer as the blank. The H_2O_2 scavenging activity of aqueous *R.bibas* extracts (stem bark, root and leaf) was calculated as per the formula described by Kim *et al.*, [5].

$$\text{Hydrogen peroxide scavenging activity (\%)} = (AC-AS)/AC \times 100$$

AC = Optical density H_2O_2+ Phosphate buffer;
AS= Optical density DPPH + plant extract/standard.

Determination of Ferric-Reducing Antioxidant Power

Principle. Potassium Ferricyanide (K3 (Fe (CN6)) Fe3+) is reduced to potassium Ferrocyanide (K4 (Fe (CN6)) Fe2+ by the antioxidants. Antioxidants in plant extracts have the ability to reduce yellow color of the test reagent to different tones of blue and green. The ultimate compound exhibits a Prussian blue hue, reaching its maximum absorption at 700 nm.

Procedure. This was perfomed using the method illustrated by Santos-Sánchez *et al.,* [7]. To 1 ml of each prepared dilution (5 µg/ml- 150 µg/ml), addition of 2 mlPhosphate buffer pH 6.6 (0.2 M) and 2 ml 60 mM Potassium Ferricyanide was done. This blend was placed in a 50 °C water bath for 20 minutes. After cooling 2 ml of Trichloro-acetic acid was put. This reaction mixture was then spun at 3000 rpm for ten minutes. Two mililiters of supernatant, 2 ml Distilled water and 0.5 ml freshly prepared $FeCl_3$ (0.1%) were added sequentially. After 10 minutes of incubation absorbance values were measured at 700 nm using a Vis spectrophotometer. Triplicates assays were done with ascorbic acid as the positive control.

Ferrous Chelating Activity

Principle. Using metal ions to form a coordination complex, this assay gauges an antioxidant's capacity to halt oxidation and the generation of free radicals. Ferrozine can bind Fe++ and produce a measurable red complex. The reaction is inhibited by additional chelating compounds in the sample, and as a result, the red tint of the ferrozine-Fe++ complex is less intense. The iron (II)-ferrozine complex loses its red color as a result of other chelators competing for ferrous ions [3]. A reduction of absorbance at 562 nm was used to measure chelating of ferrous ions by the plant extracts.

Procedure. To 1 ml ferrous sulfate (0.125 mM), 1 ml of varied quantities of the aqueous leaf, stem bark and root extracts (15.625-500 µg/ml) were added. To initiate the reaction, 1 ml of ferrozine was added, vortexed and allowed to stand at 25°C for 10 minutes. Absorbance values were read spectrophotometrically at 562 nm with EDTA as the positive control. Percentage ferrous chelating effect by the plant extracts was deduced from the formula described by Nabavi *et al*., [8].

% iron chelating effect = (AC-AS)/AC×100

Where AC= absorbance of control and AS= absorbance of the sample/standard.

Determination of Total Phenols

Principle. Phenolic compounds reduce Folin–Ciocalteu reagent (FCR) with subsequent formation of molybdenum–tungsten blue. The spectrophotometric measurement of the color intensity at 765 nm shows a linear rise with the quantity of phenolics in the aqueous *R. bibas* extracts.

Procedure. The protocol described by Juan *et al.,* [9] was used, where 0.2 ml of the test samples (aqueous leaf, stem bark and root extract) were added to Distilled water (0.6 Ml). Then 0.2 Ml of FCR diluted with distilled water at a ratio of 1: 1 was added. After thorough mixing, the setup was left undisturbed for 5 minutes at 25°C. To the mixture, 1 ml of saturated Na_2CO_3 and distilled water were used to give a final volume of 3 Ml. The reactions were allowed to stand in a light controlled place for 30 minutes after which absorbance readings were read at 765nm. The total phenol content present in the samples was computed and then reported as Gallic acid equivalents GAE/g from the log curve prepared (7.8–250 µg/ml, Y =0.004021x + 0.01024, R^2= 0.014). All the determinations for the three samples were done in triplicate.

Determination of Total Flavonoid Content

Principle. Any compound having and aromatic ring with a catechol group experiences nitration. This happens with its third or fourth loci unsubstituted or not sterically blocked during a complexation reaction that takes place in presence of $NaNO_2$. When Al(III) is added, a yellow-colored complex forms. When NaOH is added, the complex instantly becomes red, and its optical densities can be measured at 510 nm.

Procedure. The Aluminium chloride protocol described by Sawadogo *et al.,* [10] using quercetin as a standard was used in determining the flavonoid content in *R. bibas* aqueous stem bark, leaf and root extracts. One milliliter *R. bibas* crude extracts was put in a volumetric flask, and distilled water (4 ml) added. Incubation for 5 minutes at room temperature was subsequently followed by pippeting into it 0.3 mls NaNO2 (5%) and Aluminium chloride (10%). At room temperature, this mixture was incubated for 6 minutes after which 1ml of NaOH (1M)was added. Immediately distilled H_2O was used to make a final volume of 10mls. A spectrophotometer at 510 nm ws used to read the sample absorbances against the blank. The flavonoid content (Quercetin equivalent, QE) per gram) was expressed as mean ± standard error of mean. Concentration of total flavonoids in the aqueous extracts was worked out from the regression equation, (Y = 0.00493x + 0.000355, R^2= 0.0433).

Qualitative Phytochemical Screening of the Extracts

The phytochemicals linked to the antioxidant activity were tested for in the lyophilized extracts of *R. bibas*. Standardized modified methods as reported by Joshi *et al.*,(2010) were employed for the detection of various secondary metabolites.

Statistical analysis

The data on absorbance readings were fed in to a Microsoft Excel spreadsheet from where it was ordered and then exported to Minitab statistical software for analysis. This data was found to agree with the assumptions of parametric data and was thus expressed as means + standard deviations (mean + SD). Inferential statistics were carried out using One-Way ANOVA after which Tukey's post hoc tests was done for pairwise separation and comparison of means at a significance level of 5%. All the statistical analyses were executed using Minitab (Minitab, version 17.1) and the analyzed data were presented in tables and graphs.

Results

In Vitro DPPH Scavenging Activities of Aqueous Extracts of *R. bibas*

A dose-dependent DPPH scavenging activity was demonstrated from the seven tested concentrations of aqueous extracts of *R. bibas*. A similar trend was seen in the standard (P>0.05; Figure 1). It was furthers shown that, at all assayed concentrations, stem bark extract scavenged more of the DPPH radical followed by the root extract, whereas the least scavenging activity was seen in the leaf extract. As Figure 1 indicates, all concentrations across the three extracts had significantly different DPPH scavenging outcomes. The scavenging power of ascorbic acid at each concentration was significantly better than that of the plant extracts. The IC_{50} of *R. bibas* stem bark extract, root extract, and leaf

extract were 34.56, 44.76 , and 48.48 µg/ml respectively, whereas IC_{50} for ascorbic acid was 9.58 µg/ml.

Hydrogen Peroxide (H_2O_2) Scavenging by Aqueous Extracts of *R. bibas*

The capability of aqueous extracts of *R. bibas* to scavenge hydrogen peroxide was concentration-dependent. In all the tested concentrations, a comparable H_2O_2 and ascorbic acid scavenging trend was observed (p<0.05; Figure 2).

Of the three extracts, findings alluded notable differences in H_2O_2 scavenging activities from the tested doses of the three aqueous extracts. When using dilutions of 2.5, 5 and 10 µg/mlthe root extract demonstrated a higher percentage of scavenging activities compared to the potency of stem bark and leaf extracts. The leaf extract had higher scavenging of H_2O_2 at ranges of 20 and 25 µg/ml. The IC_{50} for the leaf extract was comparably greator than those of the root and the stem bark extracts whereas ascorbic acid (standard) had the lowest IC_{50} value (Figure 2).

In vitro Ferric Reducing Antioxidant Power of *R. bibas* Aqueous Extracts

As observed in this experiment, the three extracts portrayed a dose-dependent FRAP. The ferric-reducing antioxidant potential of the tested extracts concentrations had a parallel trend to that of ascorbic acidas seen in figure 3. Additionally, the FRAP of the various extract studied concentrations, varied greatly, with the high-level extract concentration proving to be the most efficacious. In comparison, the ferric reducing activity of ascorbic acid (standard) was significantly higher than that of the three extracts. Generally, the aqueous root extract of *R. bibas* had substantially higher FRAP compared to the stem bark and leaf extracts. *R. bibas* aqueous leaf extract had statistically lower FRAP activity compared to the stem and the root extracts at all tested concentrations. However, 10µg/ml of extract concentration, the stem bark, and root were noted to have no significantly different FRAP activities.

In Vitro Ferrous Chelating Activity of Aqueous Extracts of *R. bibas*

The study revealed a concentration-dependent iron-chelating activity in the extracts of *R.bibas*. All tested concentrations of the aqueous stem bark, leaf, and root extracts had significantly different iron-chelating activities. The standard (Ascorbic acid) had a similar trend to that of the extracts across each studied concentration (p>0.05; Figure 4). Comparing the ability to suppress the forming of ferrous–ferrozine complexes by the aqueous extracts at the tested concentrations, remarkable differences were observed. Results further indicated that across all tested extracts concentrations, the aqueous leaf extract had more iron-chelating power afterwords the stem bark extract, whereas the least chelating activity was seen in the root extract. The IC_{50} values of the standard, stem bark extract, root extract, and leaf extract were 88.04±5.33 µg/ml, 50.98±0.90 µg/ml, 124.5±0.20 µg/ml, and 16.28±0.29 µg/ml respectively. A better metal-chelating potential was recorded from the aqueous leaf extract than the stem bark, root extracts and the standard as well.

Total Phenols in *R. bibas* Aqueous Extracts

As figure 5. reveals, the aqueous stem extract of *R. bibas* had the highest phenol content of about 149.44±0.228 Gallic acid equivalents/g, followed by the root extract at 141.14±0.163 GAeq/g and the least amount of phenols

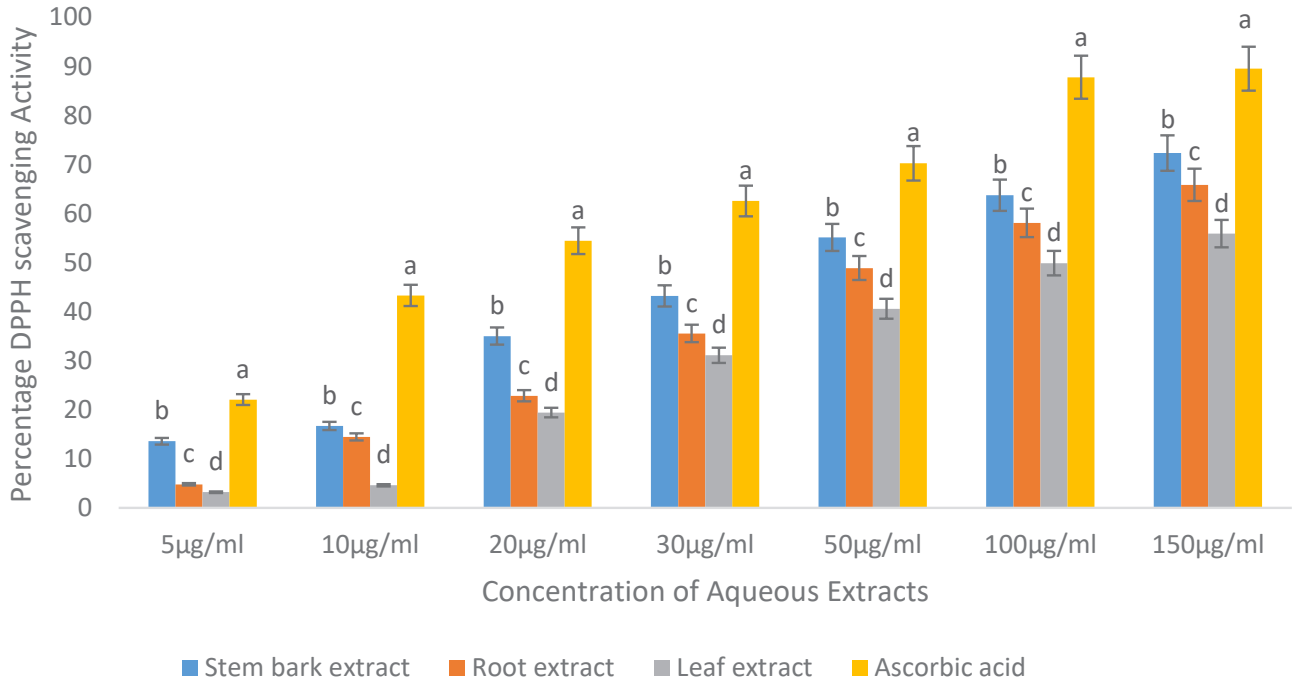

Fig. 1. *In vitro* DPPH Scavenging by *R. bibas* Aqueous Stem bark, Leaf and Root Extracts. Bar graphs with disparate letters statistically vary by One-Way ANOVA and Tukey's post hoc test (p>0.05)

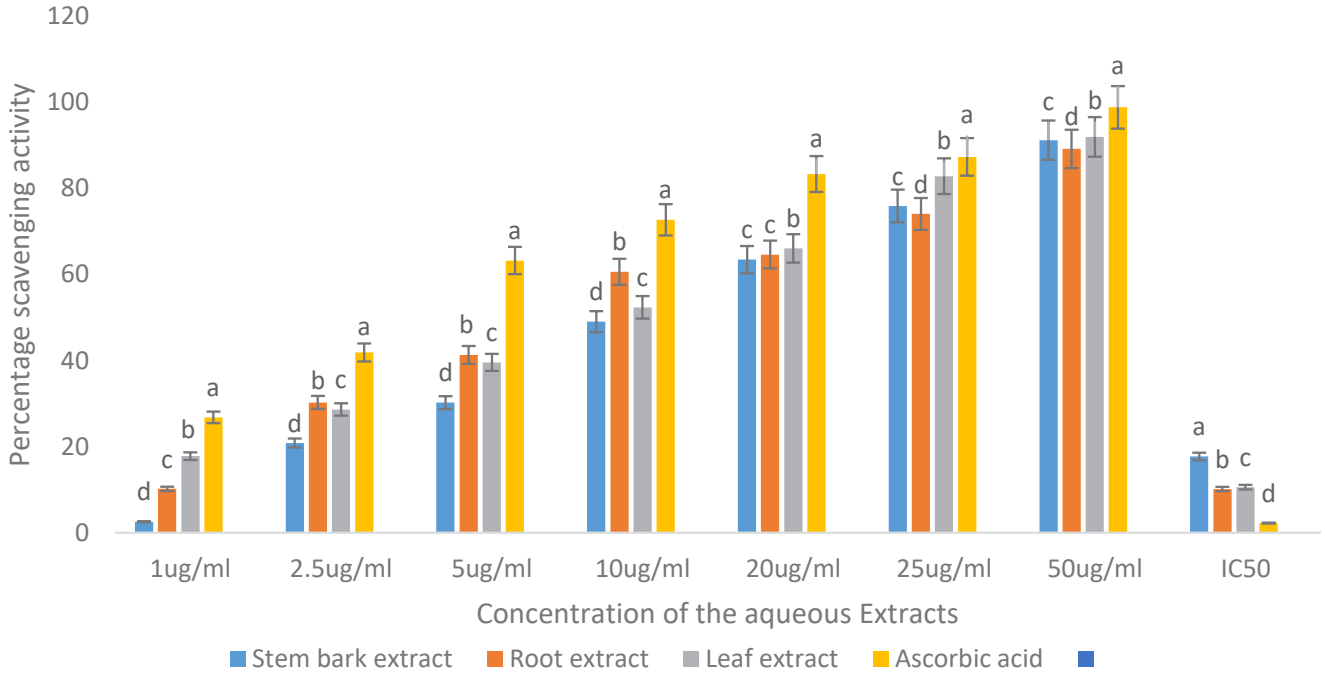

Fig. 2. *In vitro* hydrogen peroxide scavenging power of *R. bibas* aqueous extracts. Bar graphs with disparate letters statistically vary by One-Way ANOVA and Tukey's post hoc test (p>0.05).

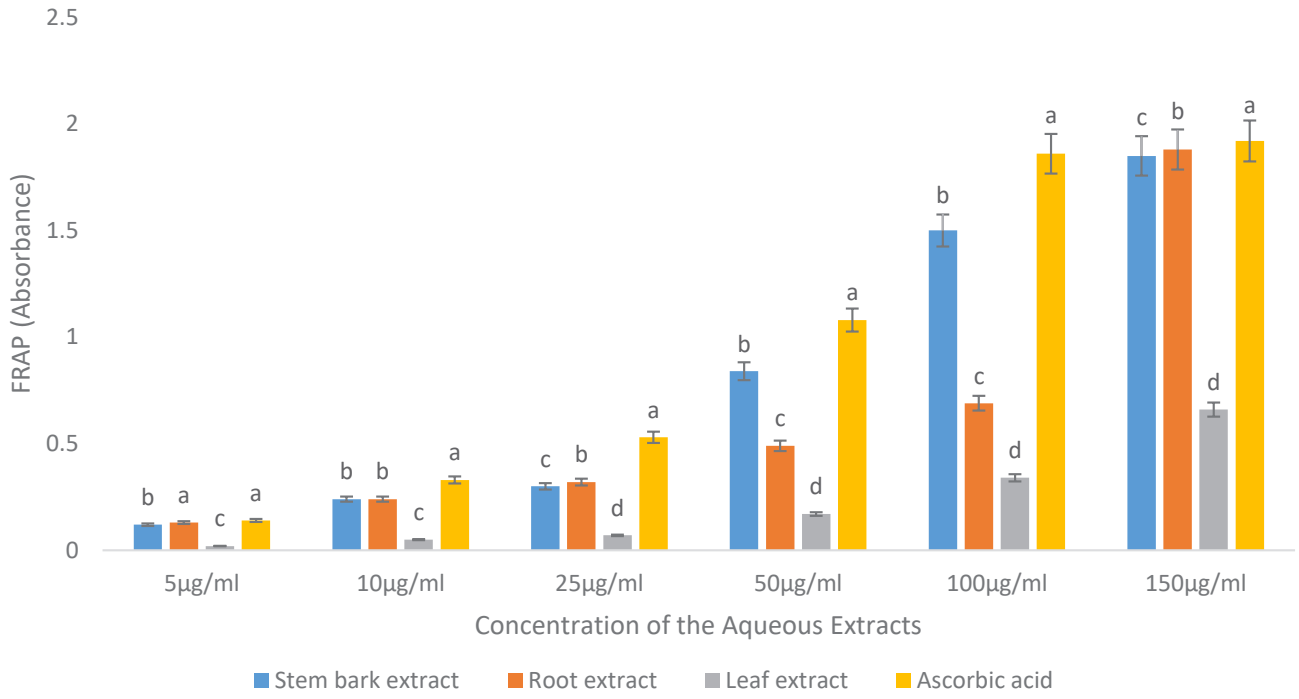

Fig. 3. *In vitro* ferric reducing antioxidant power of *R. bibas* aqueous extracts. Bar graphs with disparate letters statistically vary by One-Way ANOVA and Tukey's post hoc test (p>0.05).

was found in the aqueous leaf extract with 73.012±0.235 GAeq/g. Statistical analysis showed that the total phenolic contents differed significantly among the three studied extracts (p>0.05; Figure 5).

Total Flavonoids Content of *R. bibas* Aqueous Extracts

The aqueous *R. bibas* root extracts had a substantially higher total flavonoid level of about 377.66±4.30 milligram Quercetin equivalent/gram of the dry weight followed by the stem bark extract at 253.72±4.88 and the aqueous leaf extract (164.52±3.39 mgQE/g) of the sample dry weight. Upon statistical analysis, it was established that the total flavonoids of the three studied extracts of *R.bibas* differed substantially (p>0.05; Figure 6).

The current study also compared the total phenols and total flavonoids levels in each of the studied *R.bibas* ex-

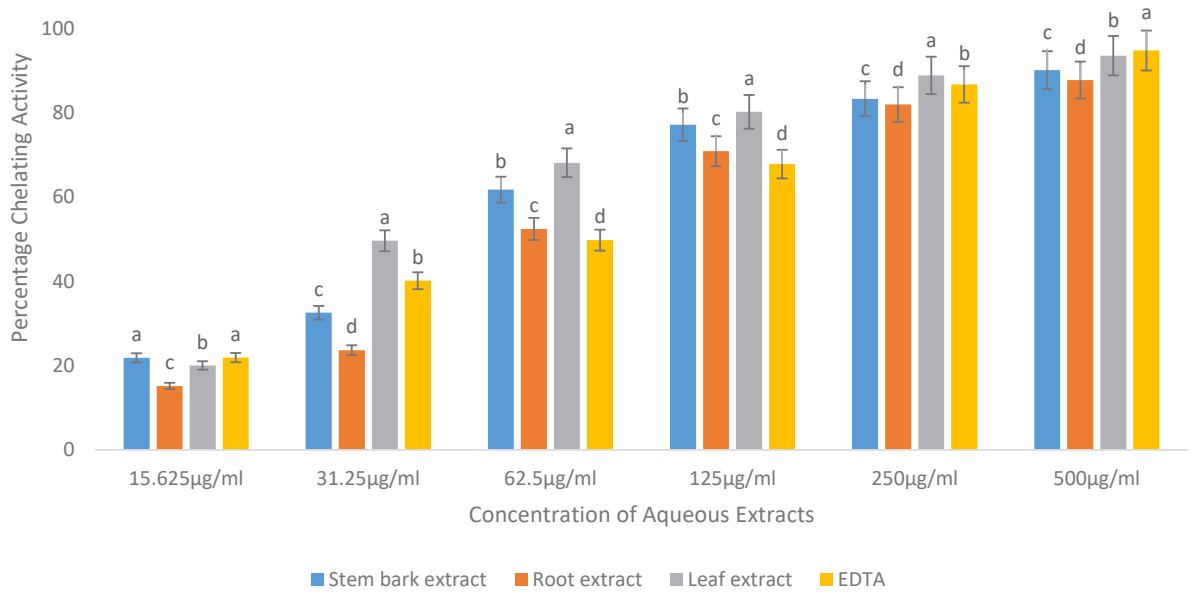

Fig. 4. *In vitro* ferrous chelating activity of *R. bibas* aqueous Extracts. Bar graphs with disparate letters statistically vary by One-Way ANOVA and Tukey's post hoc test (p>0.05).

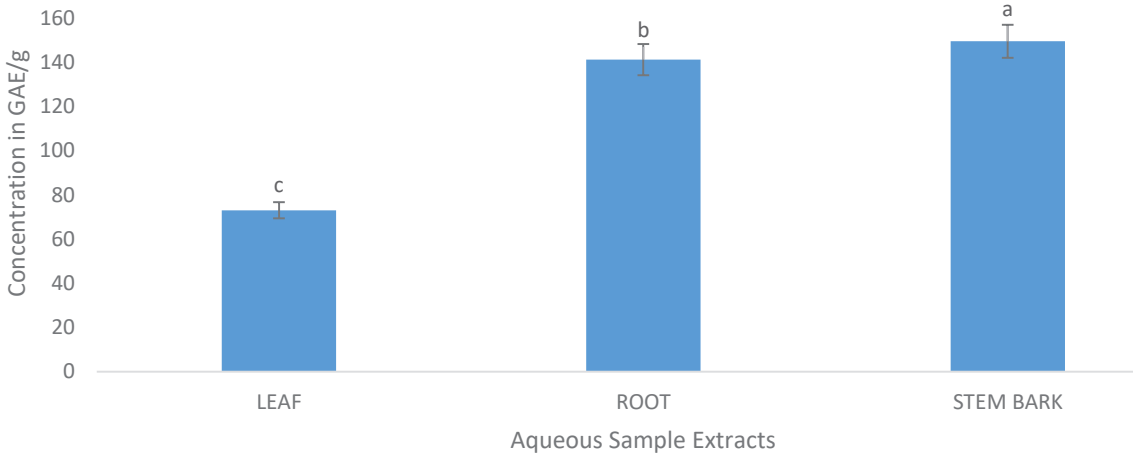

Fig. 5. Total phenolic content of *R. bibas* aqueous extracts. Bar graphs with disparate letters statistically vary by One-Way ANOVA and Tukey's post hoc test (p>0.05).

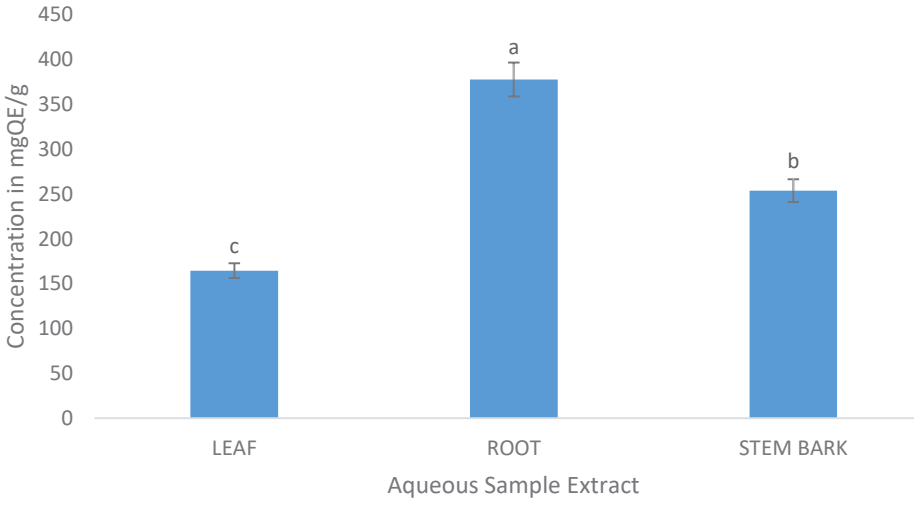

Fig. 6. Total flavonoid content of aqueous extracts of *R. bibas*. Bar graphs with disparate letters statistically vary by One-Way ANOVA and Tukey's post hoc test (p>0.05).

tracts. Results showed that there are more of the flavonoids in each of the three extracts than the phenols (p>0.05; Figure 7).

Qualitative Phytochemical Screening of Aqueous Extracts of *R. bibas*

The current study looked into the phytochemical content of aqueous root, stem bark and leaf extracts. As indicated by Table 1, the findings demonstrated presence of terpenoids, alkaloids, tannins, saponins, fixed oils, quinones, glycosides and steroids in root and stem bark extracts. Tests for fixed oils and steroids on the aqueous leaf extract came out negative.

Discussion

Antioxidants work by neutralizing, competing with, or interacting with a substance that has molecular oxygen as its terminal recipient of electron (O_2) to directly counteract the effects of free radicals. Thus molecular oxygen functions as a thermodynamic drain. Because synthetic antioxidants are regarded as hazardous and carcinogenic, native antioxidants derived from therapeutic plants are a better option in suppressing oxidative damage. The use of herbal remedies can help treat and prevent degenerative illnesses caused by oxidative stress [3]. Different solvents extract antioxidant molecules in different ways due to the varying polarity of plant phytochemicals. The extraction solvent utilized mostly determines the yield and ROS scavenging ability of a plant extract. [5]. Moreover, antioxidant activity should never be determined using only one antioxidant study design. In practice, a variety of *in vitro* testing procedures are used to determine antioxidant activity. Furthermore, antioxidant test models are not the same, making the comparison of one protocol to another difficult [11].

Generally, *in vitro* antioxidant assays aimed at free radical scavengers are simple to conduct [3]. Most of the common *in vitro* antioxidant investigations performed on crude plant extracts include DPPH, Nitric Oxide, Superoxide radical, Hydrogen peroxide scavenging, Ferric Reducing Antioxidant Power, Reducing power, Trolox equivalent antioxidant capacity, Oxygen radical absorbance capacity and Metal chelating besides the determination of the levels of Phenols and flavonoids [12].

Diphenylpicrylhydrazyl (DPPH), a stout free radical, is most often employed to elucidate the radical scavenging capability of different elements [13]. DPPH is a nonreactive free radical on account of the delocalization of extra electrons attached to the molecule, such that it does not form dimers with similar molecules. The delocalization of the lone electron produces a deep violet coloration which has an absorption peak at 517 nm [14]. Upon reacting with a molecule donating hydrogen, it converts to 2, 2-diphenyl-1-hydrazine (DPPH-H) or it undergoes substitution with analogous hydrazine (DPPH-R) resulting in the loss of violet tinge due to the picryl group [13]. In the current examination, the interaction between DPPH and

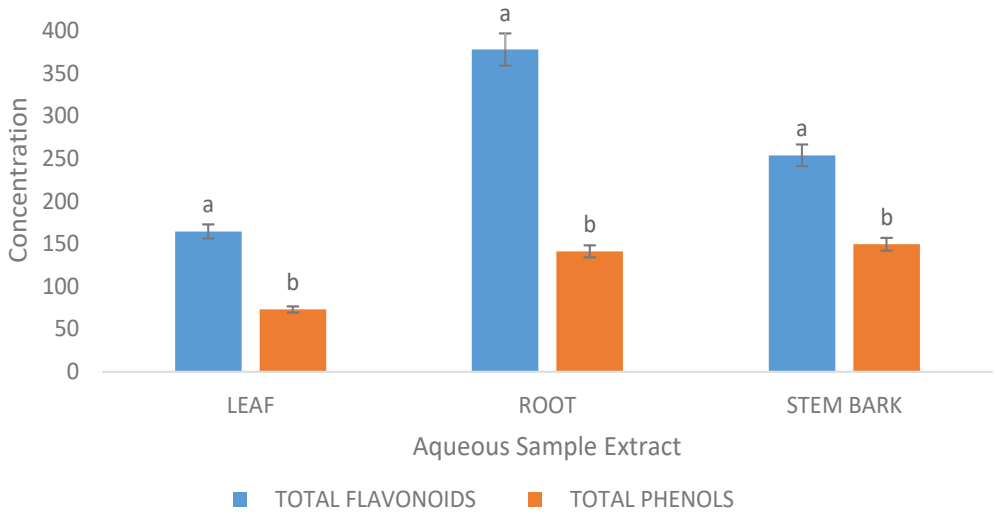

Fig. 7. Comparing total flavonoids and total phenol contents of aqueous extracts of *R. bibas*. Different letters in the bar graphs indicate significant differences in phenol and flavonoid contents by One-Way ANOVA and Tukey's post hoc test (p>0.05).

Table 1. Phytochemicals present in the aqueous extracts

Phytochemical	Stem bark extract	Root extract	Leaves extract
Alkaloids	+	+	+
Quinones	+	+	+
Glycosides	+	+	+
Tannins	+	+	+
Terpenoids	+	+	+
Saponins	+	+	+
Fixed oils	+	+	-
Steroids	+	+	-

Key: +present; - absent

the aqueous stem bark, leaf, and root extract might have occurred via the movement of hydrogen ions and electrons to DPPH, resulting in the formation of a stable DPPH-H. As the reaction continued, a drop in the absorbance was observed, where the tone changes from purple to pale yellow are directly proportional to the antiradical potential of the aqueous *R.bibas* extract. This method has widespread use for *in vitro* antioxidant assays since it is simple, stable, inexpensive, rapid, convenient, and accurate [11].

The current study results revealed that the aqueous extracts of *R. bibas* had concentration-dependent scavenging activities against DPPH radical (Figure 1). This type of relationship has previously been demonstrated by Moriasi *et al*. [13] in extracts of *Piliostigma thonningii* (Schum), Mwihia *et al*., [11] in seed extracts of *Annona squamosa* and Arika *et al*., [3] in *Gnidia glauca* (Fresen) leaf extract. In addition, phytochemical profiling of *Vernonia lasiopus*, *Caesalpinia volkensii* and *Acacia hockii* by Guchu *et al*., [14] as well as studies on pods, flowers, and leaves of *Acacia* species by Abdel-Farid *et al*.,[15] gave findings similar to those of this study. IC_{50} of aqueous leaf, stem bark and root extracts of *R. bibas* were lower than 50µg/Ml. A lower IC_{50} value, according to Kandhasamy *et al*., [16] indicates a high DPPH scavenging activity.

The current study also assessed the ability to scavenge hydrogen peroxide (H_2O_2) radical by aqueous *R.bibas* extracts. H_2O_2 possesses oxidizing powers such that through thiol (-SH) groups, it directly inactivates enzymes [17]. It penetrates bio membranes rapidly reacting with redox-active transitional metals such as ferrous ion (Fe^{2+}), a catalyst for iron utilized in the Fenton pathway and Cu^{2+} ions through the Harber Weiss reaction forming an extremely reactive hydroxyl radical that initiates an oxidative assault[3,11]. In a normal situation, therefore, body cells should effectively maintain low levels of intracellular hydrogen peroxide. Antioxidants fix the free radicals formed through the Fenton process and block the oxidizing effect of hydrogen peroxide via electrons donation.

In the current study, the aqueous *R.bibas* stem bark, root, and leaf extracts showed concentration-dependent inhibition of hydrogen peroxide radical (Figure 2). This activity can be due to phenols present in the aqueous plant extracts, which probably donated a free electron to H_2O_2, which neutralized it to water [18]. The antioxidants in the extract's ability to mediate the degradation of H_2O_2 to form water and oxygen could potentially be linked with the observed activity [17]. Previous study findings reported that aqueous extracts of *Portulaca oleracea* demonstrated dose-dependent hydrogen peroxide inhibition activity [19]. In addition, the current study findings correlate with the results demonstrated with M. *parviflora* leaf extracts [20]. A study on aqueous Peppermint extract demonstrated dose-dependent scavenging of hydrogen peroxide [21]. The half-maximal (IC_{50}) values for *R. bibas* aqueous root, stem bark, and leaf extracts were 10.16, 17.71, and 10.6 µg/ml respectively compared to ascorbic acid (2.25µg/ml), which indicated potent hydrogen peroxide inhibition at low concentration of the aqueous leaf extract [22].

The current study determined FRAP in the aqueous extracts of *R. bibas*. The reducing potential of a given molecule depends on its reducing ability in a Fe^{3+}-Fe^{2+} system [23]., where the iron-forming ferric chloride (Fe^{3+}) is changed to ferrous ions (Fe^{2+}) in the presence of antioxidants, which donates an electron. Under normal circumstances, physiologically active compounds that are able to reduce ferric ions are electron donors. These substances reduce oxidized byproducts similar to those produced by lipid peroxidation [24]. Therefore, FRAP may be employed to quantitatively indicate the antioxidant power of a molecule. The free radical antioxidant power observed revealed that the ferric reducing capacity of *R. bibas* root, stem bark and aqueous leaf extracts at different concentrations (5-150 µg/ml) obeyed Beer Lambert's law at 700 nm [25].

When Fe^{3+} is reduced to Fe^{2+} by the antioxidants present in the aqueous extracts, it indicates the extract's ability to donate electrons. The molecules of the Fe^{2+} complex generated were proportionate to the absorbance readings at 700 nm, indicating an increased aqueous extract's reductive power [3]. Previous research on FRAP activity by the aqueous extracts of three mushroom species (*Lentinus edodes, Ganoderma lucidum,* and *Agaricus bisporus*) indicated a concentration-dependent increase in FRAP [26]. Furthermore, the reducing potential of the hot water extract of commonly ingested cucurbits revealed dose dependence comparable to that of ascorbic acid, the reference compound [27]. Other studies on aqueous extracts of *Juniperus thurifera*, *J. oxycedrus, J. phoenicea* and *Tetraclinis articulata* also showed increased reducing power that was concentration-dependent [28].

Iron and copper, which are transition metal ions, have long been recognized as critical catalysts in generating the first free radicals that start the free radical cascade or facilitate lipid peroxidation [12]. Metal chelators inhibit the generation of free radicals through the stabilization of transition metals, thereby suppressing free radical-induced tissue damage. Metal chelators compete for ferrous ions with ferrozine to form a red-colored iron-ferrozine complex with a maximal optical density at 562 nm. When a metal chelator is present in a reaction, it restricts the formation of iron-ferrozine composite, resulting in a reduced intensity of the red hue formed as the chelating agent concentration rises [29].

It was observed that, aqueous leaf extract of *R. bibas* had high chelating power, whereas root extracts had moderately low chelating power. The current study also revealed a dose-dependent reduction in the color intensity of the iron-ferrozine complex due to the extracts competing for the ferrous ions with ferrozine. The existence of iron in two oxidation states gives room for the acceptance or donation of electrons via redox reactions [11]. The Haber-Weiss reaction, which occurs when iron reacts with hydrogen peroxide and superoxide anion, produces a reactive hydroxyl

radical (OH.) that aggravates cell membrane, nucleic acid, and protein damage [3]. Through the Fenton reaction, ferrous ions in the cell could stimulate lipid peroxidation. They can also speed up peroxidation via the decomposition of lipid hydro peroxides forming alkoxyl and peroxyl radicals and sustaining the lipid peroxidation reactions [30]. Metal chelators play the role of antioxidants when they minimize the redox potency, thereby resulting in the stabilization of the oxidized metal ions. Therefore, moderately low levels of ferrous ions chelation by phytochemicals, as evident in the aqueous extracts of *R.bibas,* protect the body against oxidative damage [31]. A comparable study demonstrated inhibition of iron-ferrozine complex formation by aqueous extract of *Coleus aromaticus* and potato peels which was dose-dependent [32]. *Melilotus arvensis* extract has been shown to inhibit the formation of iron-ferrozine composites, indicating the ability to chelate and competitively capture ferrous ions ahead of ferrozine [33].

The amount of phenol in *R. bibas* aqueous stem bark, root, and leaf extracts was also measured. As indicated, higher amount of phenols was scored in the stem bark followed by root and leaf extracts (Figure 5). Research by Sylvie *et al*. [34] on *Garcinia lucida*, *Acalypha racemosa,* and *Hymenocardia lyrata* were coherent with the findings of this study. Phenols are aromatic elements with hydroxyl groups and are involved in varied physiological functions. The antioxidant power of phenolic compounds differs depending on their nature and amount in samples [35]. In addition, phenolic elements such as flavonoids, are the reason for a majority of radical scavenging effects, which occur through a donation of hydrogen atoms, thereby neutralizing free radicals [36]. Early studies by Atere *et al.,* [37], established that phenols are the primary antioxidants acting as free radical delimiters. In addition, Dvorackova *et al.,* [38] observed that polyphenols in *Cinnamomum verum* leaf extract exhibit antioxidant activity through an *in vitro* free radical scavenging assay. Furthermore, phenol neutralizes H_2O_2 by emitting electrons, thereby converting it to water [39].

The total flavonoid contents of *R. bibas* aqueous stem bark, root, and leaf extracts were also determined in the current study, with results showing that aqueous root extract had the highest amounts of flavonoids followed by stem bark and leaf extracts (Figure 6). The current study results were congruent with the results reported by Ojwang *et al*., [40] while studying methanolic leaf, bark, and root extracts of jackfruit (*Artocapus heterophyllus*). Furthermore, flavonoids have been proved to scavenge reactive species and limit the generation of lipid peroxides. Further, they are essential components in the expression of natural products' antioxidant capacity [41]. Flavonoids are compounds found naturally in plants that have beneficial impacts on man's health. Flavonoid derivatives were shown to possess antibacterial, antiviral, cancer-fighting, anti-inflammatory, and ant allergic qualities in studies by Roy and Datta [42]. An array of oxidizing chemicals, such as singlet oxygen and other reactive species linked to illnesses, are scavenged by flavonoids. Compared to literature and other studies on plant extracts, the current study findings suggest that flavonoids and phenolic acids are probably the significant reason behind the antioxidant activities observed [43].

In the Folin Ciocalteu method, distinct phenolic molecules have different reactions. Similarly, depending on their chemical structure, phenolic compounds vary substantially. Other chemical elements or compounds contained in the crude extract, such as sugar and ascorbic acid may cause interferences [44]. The antioxidant activities of *R.bibas* aqueous extracts can be attributed to other phytochemicals, which singly or synergistically neutralize ROS and RNS. Many phytochemicals can act as reducing agents, such as flavonoids, phenols and their derivatives, ascorbic acid, phospholipids, phytic acid and pigments among others [3].

There have been reports of antioxidant effects of alkaloids in vitro. Furthermore, it's possible that the oils found in *R. bibas* aqueous extracts enhanced the extract's ability to capture free radicals. Fatty oils have been shown to exhibit DPPH radical scavenging action by Ismail *et al.*, [45]. Crude saponin extracted from *A. indicum l*eaves exhibit encouraging antioxidant effects, according to Arabski *et al.,* [46]. Additionally, the aqueous extracts of *R. bibas* tested positive for tannins, that have been indicated to possess antioxidant properties [47].

Conclusion

The results from this investigation showed that aqueous stem bark, root, and leaf extract of *R. bibas* had noteworthy reducing power, DPPH radical scavenging activity, hydrogen peroxide scavenging capability, phenolic content, flavonoid content, and iron (Fe^{2+}) chelating ability. These behaviors were proportionate to those of the used chemical standards. It's interesting to notice that the plant extract's IC_{50} value was within the range of the standards, suggesting a potential source of free radical scavengers**.** The antioxidant and free radical-scavenging activities of R. bibas could be attributed to the presence of phytochemicals that have been associated with antioxidant effects. Overall, the findings of this research indicate that R. bibas can be a useful therapeutic agent in the management of oxidative stress. Therefore, the aqueous extracts of R. bibas may be exploited as an alternative and complementary source of antioxidants. However, there is a need to conduct activity guided studies to isolate individual biologically active compound from R. bibas in order to establish their precise mode of action in various preclinical models.

Authors Contribution

IWK (Conceptualization; Investigation; Data curation; Project administration; Resources; Analysis of the data and Authored the manuscript)

MPN (Conceptualization; Data curation; Validation; Supervision; Resources; Visualization; Review and editing)

MJ (Formal analysis; Methodology; Project Administration; Supervision)

Conflict of interest

None to declare.

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