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Original Article

Comparative Free Radical Scavenging Efficacy of Leaves Extract of *Moringa Oleifera* and Petals Extract of *Hibiscus Sabdariffa*

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ABSTRACT

Background: Use of molecules with antioxidant properties have evolved as effective strategy for preventing oxidative damage caused by reactive oxygen species. *Moringa oleifera* and *Hibiscus sabdariffa* are ancient plants with antioxidant properties, and have served numerous therapeutic purposes, in addition to their nutritional benefits. **Aim**: This in vitro study compared the free radical scavenging efficacy of ethylacetate leaves extract of Moringa oleifera and ethylacetate petal extract of *Hibiscus sabdariffa*. **Method**: Determinations were carried out following standard procedures for analytical experiments. The leaves of Moringa oleifera and petals of *Hibiscus sabdariffa* were extracted by cool maceration with distilled water and ethylacetate, independently for 48 hours using soxhlet extractor. The free radical scavenging activities of the extracts were determined spectrophotometrically. DPPH free radical was used to determine the free radical scavenging activities of the extracts. The reducing power efficacy of the extracts was determined by their ability to reduce Fe^{3+} to Fe^{2+} ions using FERAP. **Results**: Ethylacetate extract of *Hibiscus sabdariffa* petals had higher free radical scavenging efficacy and more reducing power with an inhibitory concentration (IC_{50}) of 1.57 mg/ml compared to the ethylacetate extract of Moringa oleifera leaves which had an IC_{50} of 2.60 mg/ml. Phytochemical profile revealed that the predominant compounds in both extracts were flavonoids, phenols, and amino acids. **Conclusion**: This study has established that ethylacetate extract of the Petals of *Hibiscus sabdariffa* has more reducing power and free radical scavenging efficacy compared to ethylacetate extract of *Moringa oleifera* leaves. These plant parts could serve as novel sources for clinically efficient antioxidants.

Key words: Oxygen-free radicals, Oxidative stress, Antioxidant, Free radical scavenger, Moringa oleifera, Hibiscus sabdariffa.

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INTRODUCTION

Antioxidants are agents or compounds that are capable of inhibiting the oxidation processes that occur under the influence of reactive nitrogen species (RNS) and reactive oxygen species (ROS) such as singlet oxygen (O.), hydroxyl (.OH), peroxide (H_2O_2) and superoxide (O_2.) radicals. Antioxidants play crucial role in the defense mechanism of living cells against the pathologies associated with the attack of ROS and RNS.[1-3] Antioxidants mediate their role of preventing oxidant-induced cell damages either through the reduction of ROS generation, scavenging ROS, or interfering with ROS-induced alterations.[1] By its role of reducing the oxidative damages in the body and inhibiting lipid peroxidation, antioxidants retard the progress of many diseases.[1,3,4] An imbalance between ROS and antioxidants defense system results to abnormal physiological condition known as oxidative stress[1,3]. During energy production in the body by oxidation process, the associated oxygen molecule has the tendency to generate free radicals[5]. These free radicals which are by-products of the oxidation attacks specific region in the body, leading to oxidative stress, and ultimately results to the damage of affected tissues and organs. Under stress, the living cell end up having more reactive oxygen species than antioxidant, this imbalance leads to deleterious damage of the cellular components, particularly lipid peroxidation, protein oxidation and deoxyribonucleic acid mutation.[3] This is considered to be the major reason for many diseases such as cancer, cardiovascular diseases, neurodegenerative diseases, rheumatoid arthritis, atherosclerosis, hypertension among others.[1,2] Even though almost all organisms possess antioxidants and several enzyme systems such superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase to protect them from oxidative damage, these systems are inadequate to prevent the damage entirely, hence, the need to supplement antioxidants for efficient and adequate scavenging of free radical.[2] Currently available synthetic antioxidants including Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), Gallic acid among others have been found to cause negative health effects,[6,7] hence, the search for antioxidants without side effects, from natural sources, with keen interest in plants due to the bioactive secondary metabolites they produce.[8,9] It has been established that anti-oxidants play important role in the prevention of many chronic diseases including cancer, cardiovascular disease, atherosclerosis, diabetes mellitus, asthma, hepatitis and arthritis.[10,11] The consumption of traditional diets prepared with spices, medicinal and aromatic

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herbs have attracted increasing interest among consumers and scientists because they exhibit antioxidant activity, attributed to a variety of bioactive phytochemicals present.[12,13] Antioxidants are thus useful in protecting cells from oxidative damage.[14] Studies have reported that polyphenols, such as flavonoids, hydroxycinnamic acids and proanthocyanidins, act as powerful antioxidants.[15,16] Phenolic antioxidants have been recognized as an important class of food ingredients and are being added to various food products in order to provide additional health benefits.[17,18] Studies have reported that natural antioxidant molecules found in the bark of certain trees can inhibit reactive oxygen species and thus contribute to the health benefits of the forest biomass.[19-21] Based on the fore-going, it has been established that plant-based system plays essential role as a source of novel and effective therapeutic agents, especially in Africa. Antioxidants from natural sources are preferred and advantageous since there is no report of toxicity or side effects associated with increased intake. More so, they are better alternative in view of the variety of their structures and chemical interactions with cells during their protective biological activities. *Moringa oleifera* is one of the medicinal plants known to possess antioxidant activities. It is considered as one of the world's most useful trees, since almost every part of the plant have been used for treatments of various ailments including ascites and rheumatism, as well as cardiac and circulatory stimulants. This is attributed to the presence of alkaloids, carotenoids, tannins, anthraquinones among other secondary metabolites.[22,23] Nutritionally, the leaves contain essential amino acids, vitamins, minerals and β-carotene, making it an invaluable commodity in the food industries.[14,21] Similarly, *Hibiscus sabdariffa;* the plant from which the Nigeria's popular local juice drink known as Zobo is made is another plant that is well known for its medicinal properties. It is rich in vitamins, natural carbohydrate, protein, tannins, gums and other antioxidants including minerals. *Hibiscus sabdariffa* has been used and are still being used to prevent oxidation by free radicals.[24] Different reports have been documented about nutritional and health benefits of *Moringa oleifera and Hibiscus sabdariffa* but there is no report on the comparative analysis of the reducing power and free radical scavenging activity of ethylacetate leaves extract of *Moringa oleifera* and ethylacetate petal extract of *Hibiscus subdariffa*, hence the first report that compared their free radical scavenging activity using 2,2-diphenyl-1 picrylhydrazyl (DPPH) free radical and ferric ion reducing antioxidant power (FRAP).

MATERIALS AND METHOD

Chemicals and reagents

Ethylacetate, 1-1-diphenyl-2-picrylhydrazyl (DPPH), methanol, hydrochloric acid, potassium ferricyanide, trichloroacetic acid, phosphate buffer, Mayers reagent, ferric chloride, NaOH, 0.2% Ninhydrin reagent, dimethylsulfoxide (DMSO), Ferric ion reducing antioxidant power (FRAP) were purchased from sigma Aldrich USA. All other chemicals were of analytical grade.

Plant materials and preparation of extracts

Leaves of *Moringa oleifera* and Petals of *Hibiscus sabdarifffa* were collected in February 2015 from their natural habitat in Sabon-Gari Local Government Area, Kaduna State, Nigeria. They were identified and authenticated at the Herbarium by Taxonomist in the department of Botany, Ahmadu Bello University Zaria with voucher number 3317 and 1056 respectively. They were washed with clean tap water and dried at room temperature for 14 days at 38 ^{0}C. The dried samples were pulverized using pestle and mortar, sieved and stored in air-tight container until used. Using a weighing balance (Contech® instruments Ltd India. Model CAC-224), 25 g of each of the powdered plant parts were weighed and extracted by cool maceration with 250 ml distilled water and ethylacetate, independently for 48 hours using soxhlet extractor. About 3 drops of chloroform was added to ensure there was no fungi growth during the extraction duration of 48 hours. The extracts were filtered using Whatman filter paper No.1 and concentrated by freeze drying using bench top freeze dryer (Labconco™) at 4 ^{0}C for about 3 hours. They were stored in the refrigerator until used. This served as the crude extract.[25,26]

Determination of extract yield (%)

The percentage yield of each extract was obtained by dividing the weight of the concentrated crude extract by the initial weight (25 g) of dry milled starting material and multiplying the ratio by 100, thus:

$$\frac{Mass\ yield\ of\ extract}{Mass\ of\ \ plant\ material} X \frac{100}{1}$$

Preliminary phytochemical analysis was carried out for the extract as per standard methods described.[27]

DPPH free radical scavenging activity

The 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay was carried out following the method described by Torres-Castillo *et al*.[28] Briefly, 2.9 mL of 60 µM DPPH' radical were added to 100 µL of each extract at different concentrations thus: (1 mg/ml, 0.75 mg/ml, 0.5 mg/ml and 0.25 mg/ml). Then, samples were kept in a dark place, and after 30 minutes the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity. The control sample was a mixture of 100 µL of DPPH and distilled water. The free radical scavenging activity of the extracts was calculated with the following equation and expressed as DPPH percent of inhibition:

$$Inhibition\,(\%) = \frac{(A_{blank} - A_{sample})}{(A_{blank} X 100)}$$

Where ^{A}blank is the absorbance of the control reaction (mixture of all the reagents except the extract), ^{A}sample is the absorbance of the sample mixture. The IC_{50} is the concentration giving 50% inhibition of DPPH. It was determined by a graph of percentage inhibition (I %) versus extract concentration.

Reducing power assay

Antioxidant activity of the leaves extract of *Moringa oleifera* and the Petal extract of *hibiscus sabdariffa* in different extracting solvents (distilled water and ethyl acetate) were determined to assess their ferric ion (Fe^{3+}) reducing ability.[29] This method is based on the ability of the extracts to reduce Fe^{3+} to Fe^{2+} ions. Different concentrations (1 mg/ml, 0.75 mg/ml, 0.5 mg/ml and 0.25 mg/ml) of each extract were prepared and 1 mL of each concentration was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.8) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 minutes. To this mixture, 2.5 mL of 10% trichloroacetic acid was added and then centrifuged at 3000 x g for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride was added. Absorbance of the Pert Prussian blue solution formed was measured at 700 nm. Ascorbic acid was used as standard reference compounds for comparison and prepared in same concentrations as the extracts.

RESULT

The free radical scavenging activity using DPPH is shown in Table 3. At 0.25 mg/ml, there was no significant difference between the ethylacetate extract of *Moringa oleifera* and aqueous extract of *Moringa oleifera* and *Hibiscus sabdariffa*. There was no significant difference between the aqueous and ethylacetate extracts of *Hibiscus sabdariffa*.

This implies that similar level of scavenging activity was exhibited by the extracts. This similarity in activity could be attributed to the presence of same bioactive compounds. However, the scavenging activity of the ethylacetate extract of the two plant parts differs significantly, indicating variation in the composition of the bioactive compounds. At 0.5 and 0.75 mg/ml, no significant difference were observed among the various extracts but at 1.0 mg/ml, the ethylacetate extract of *Hibiscus sabdariffa* exhibited a high level of scavenging activity with a value of 32.26 ± 1.04, compared to the aqueous extract of *Moringa oleifera* with a value of 13.31 ± 5.33. In the determinations, the standard (ascorbic acid) depicted the highest level of scavenging activity. There was significant difference in the reducing power potential of *Moringa oleifera* and *Hibiscus sabdariffa* at different concentrations using Ferric ion Reducing Antioxidant Power (FRAP) as shown in Table 4. At 0.25 mg/ml, there was significant difference between the ethylacetate extract of *Hibiscus sabdariffa* and the aqueous extract of *Moringa oleifera*, with the former possessing more reducing power. Interestingly, similar trends were observed for the two plant parts at increasing extract concentration from 0.50 to 1.0 mg/ml. The phytochemical profile as shown in Table 1 revealed that amino acids, phenols, and flavonoids were present in both the aqueous and ethylacetate extracts of the leaves of *Moringa oleifera* and petals of *Hibiscus sabdariffa*. Alkaloid was present in the aqueous and ethylacetate extracts of *Moringa oleifera* but were absent in the aqueous and ethylacetate extracts of *Hibiscus sabdariffa*. Interestingly, tannins were present in the aqueous and ethylacetate extracts of *Hibiscus sabdariffa* but absent in the aqueous and ethylacetate extracts of *Moringa oleifera*. Saponins were present only in the aqueous extract of *Moringa oleifera*. From the results of the successive extraction (Table 2), *Moringa oleifera* gave the highest yield of 5.9% with distilled water and low yield of 3.63% with ethylacetate, while the ethylacetate extract of *Hibiscus sabdariffa* gave the highest yield of 14.75% and a corresponding low yield of 4.55% with distilled water.

Table 1: Phytochemical profile of the extracts.

Phytochemical constituents	Moringa oleifera		Hibiscus sabdariffa	
	aqueous extract	ethylacetate extract	aqueous extract	ethylacetate extract
Alkanoid	+	+	–	–
Flavonoid	+	+	+	+
Phenols	+	+	+	+
Saponin	+	–	–	–
Tannin	–	–	+	+
Amino acids	+	+	+	+

(+) present, (-) not present.

Table 2: The yield of successive extraction of the plant parts.

Sample	Moringa oleifera		Hibiscus sabdariffa	
Solvent used in extraction	Distilled water	ethylacetate	Distilled water	Ethylacetate
% yield	5.9	3.63	4.55	14.75

Table 3. DPPH scavenging activity by extracts of *Moringa oleifera* and *H .sabdariffa*.

Extract Conc.(mg/ml)	Percentage inhibition (%I)				
	Moringa oleifera		Hibiscus sabdariffa		Standard
	aqueous extract	ethylacetate extract	aqueous extract	ethylacetate extract	ascorbic acid
0.25	6.91 ± 4.24^{ab}	5.56 ± 3.15^{a}	13.22 ± 5.03^{abc}	12.83 ± 0.89^{c}	47.47 ± 4.50^{d}
0.50	7.50 ± 6.09^{a}	12.59 ± 1.77^{ab}	13.98 ± 2.25^{abc}	19.07 ± 7.76^{abc}	50.67 ± 1.46^{d}
0.75	8.31 ± 3.77^{a}	16.12 ± 2.04^{b}	17.39 ± 12.74^{bc}	30.64 ± 0.36^{c}	52.79 ± 1.37^{d}
1.00	13.31 ± 5.33^{a}	19.75 ± 3.06^{ab}	33.00 ± 13.90^{bc}	32.26 ± 1.04^{c}	55.14 ± 2.08^{d}

Means with different superscript along the same horizontal array differ significantly ($p<0.05$) from each other. IC_{50} value for ascorbic acid, *Hibiscus sabdariffa* ethylacetate extract, *Hibiscus sabdariffa* aqueous extract, *Moringa oleifera* ethylacetate extract and *Moringa oleifera* aqueous extract were 0.47 mg/ml, 1.57 mg/ml, 1.84 mg/ml, 2.60 mg/ml and 5.80 mg/ml respectively.

Table 4: Ferric ion reducing antioxidant power (FRAP) of *Moringa oleifera* and *Hibiscus sabdariffa*.

Extract Conc. (mg/ml)	Absorbance (700 nm)				
	Moringa oleifera		Hibiscus sabdariffa		Standard
	aqueous extract	ethylacetate extract	aqueous extract	ethylacetate extract	ascorbic acid
0.25	0.65 ± 0.04^{cd}	0.62 ± 0.04^{c}	0.53 ± 0.03^{ab}	0.52 ± 0.02^{a}	1.39 ± 0.05^{e}
0.50	0.74 ± 0.08^{cd}	0.71 ± 0.01^{c}	0.64 ± 0.01^{ab}	0.61 ± 0.03^{a}	1.45 ± 0.04^{e}
0.75	0.77 ± 0.02^{cd}	0.76 ± 0.01^{c}	0.71 ± 0.02^{ab}	0.66 ± 0.06^{a}	1.66 ± 0.03^{e}
1.00	0.99 ± 0.14^{cd}	0.81 ± 0.01^{b}	0.87 ± 0.07^{bc}	0.70 ± 0.03^{a}	1.75 ± 0.01^{e}

Means with different superscript along the same horizontal array differ significantly ($p<0.05$) from each other.

DISCUSSION

Medicinal plants are regularly screened for free radical scavenging properties basically from the reports on their safety, efficacy and cost effectiveness.[30] We investigated comparative free radical scavenging efficacy of ethylacetate leaves extract of *Moringa oleifera* and ethylacetate petal extract of *Hibiscus sabdariffa*. Interestingly, we have established that the free radical scavenging agents present in the petals of *Hibiscus sabdariffa* is best extracted using ethylacetate. This is probably due to the intermittently polar nature of ethylacetate (polarity index of 4.4), thus having the ability of extracting series of saturated and unsaturated fatty acids and other bioactive agents with free radical scavenging potentials, while water is the solvent of choice for the extraction of the scavengers from the leaves of *Moringa oleifera*.

Result of the phytochemical constituents (Table 1) revealed that flavonoid, amino acid and phenol were present in all the extracts; in addition, tannin was present in both the ethylacetate and aqueous extracts of *Hibiscus sabdariffa*. The synergy of the bioactive compounds could be the most probable reason for the higher free radical scavenging effect observed with the ethylacetate extract of Petals of the plant. This is in tandem with previous report that phenols contributed more to the antioxidant activity of *Hibiscus sabdariffa* calyx compared to flavonoids.[31,32] Similarly, the presence of saponin in addition to the flavonoid, amino acid, phenol and alkanoid in the aqueous extract of *Moringa oleifera* is a possible reason for the free radical scavenging effects observed with the aqueous extract, unlike the ethylacetate extract that exhibited low scavenging effect. This implies that the viability and potency of free radical scavengers extracted is a function the type of the solvents used for extraction, similar observation were recorded previously.[33,32] A synthetic free radical 2,2-diphenyl-1picrylhydrazyl (DPPH) was used to measure the *in vitro* ability of the extracts to scavenge free radicals. The effects of phenolic compounds on scavenging DPPH radical are thought to be due to their hydrogen donating ability to the unstable DPPH free radical that accepts an electron or hydrogen to become a stable diamagnetic molecule.[30] It is most likely that the decrease in absorbance of DPPH radical caused by the phenolic compound in our result is due to reduction reaction between antioxidant molecules in the extracts and the radicals. Although the amount of DPPH scavenged by the two plant extracts in the respective extracting solvents are not equivalent to the standard; ascorbic acid. However, this efficacy is adequate to infer that the extracts; especially ethylacetate extract of *Hibiscus sabdariffa* exacts viable potential in scavenging free radicals.

The result obtained from the inhibition studies on the DPPH, ascorbic acid (standard) had lower inhibitory concentration (IC_{50}) of 0.47 mg/ml, compared to the 1.84 mg/ml of *Hibiscus sabdariffa* aqueous extract and 1.10 mg/ml of *Hibiscus sabdariffa* ethylacetate extract, which in turn is lower than the corresponding 5.8 mg/ml of *Moringa oleifera* aqueous extract and 2.6 mg/ml of *Moringa oleifera* ethylacetate extract, indicating that the free radical scavengers were more concentrated in the ethylacetate extract of *Hibiscus sabdariffa* than any other extracts. This further justifies our claim that free radical scavengers are present in the Petals of *Hibiscus sabdariffa* extracted with ethylacetate. The observed low IC_{50} value indicated high potentials to scavenge 50% of the free radicals. This finding is in agreement with the report that free radical scavengers are present in the leaves of *Moringa oleifera* and *Azadiracta indica*.[28-30]

The reducing power of the free radical scavengers in the extracts was confirmed by the ability of the scavengers in the extracts to donate electron(s), causing the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}).[34] Different concentrations of the sample extracts were charged with solutions containing Fe^{3+} and the absorbance were measured at 700 nm. The absorbance gave indication about the concentration of Fe^{2+} in solution, so the higher this absorbance, the higher the concentration of Fe^{2+} and the higher the ability of the extract of interest to donate electrons. This implies that the higher the reducing power of the extract, the greater the antioxidant activity.[35]

From Table 3, *Moringa oleifera* aqueous extract at 1.0mg/ml had high ability to donate electron due to high absorbance recorded and *Hibiscus sabdariffa* ethylacetate extract had lowest ability to donate electrons in order to facilitate the reduction of ferric ions to ferrous ions because of its low absorbance. *Moringa oleifera* extracts had higher reducing power than did the *Hibiscus sabdariffa*. The result was in agreement with previous report.[34] Having explored the free radical scavenging profile of the leaves of *Moringa oleifera* and Petals of *Hibiscus sabdariffa*, the bioactive compounds present in the extracts of these plants are promising sources of free radical scavengers.

CONCLUSION

In conclusion, the petals of *Hibiscus sabdariffa* extracted with ethylacetate had more free radical scavenging potentials compared to the ethylacetate leaves of *Moringa oleifera*. This became evident by the Ferric ion Reducing Antioxidant Power (FRAP) of the ethylacetate extract of *Hibiscus sabdariffa* which reduced Fe^{3+} to Fe^{2+}. However, irrespective of their free radical scavenging and FRAP abilities, the synergy of the two plant parts can be useful in the field of drug discovery and are better alternatives to the life-threatening synthetic sources.

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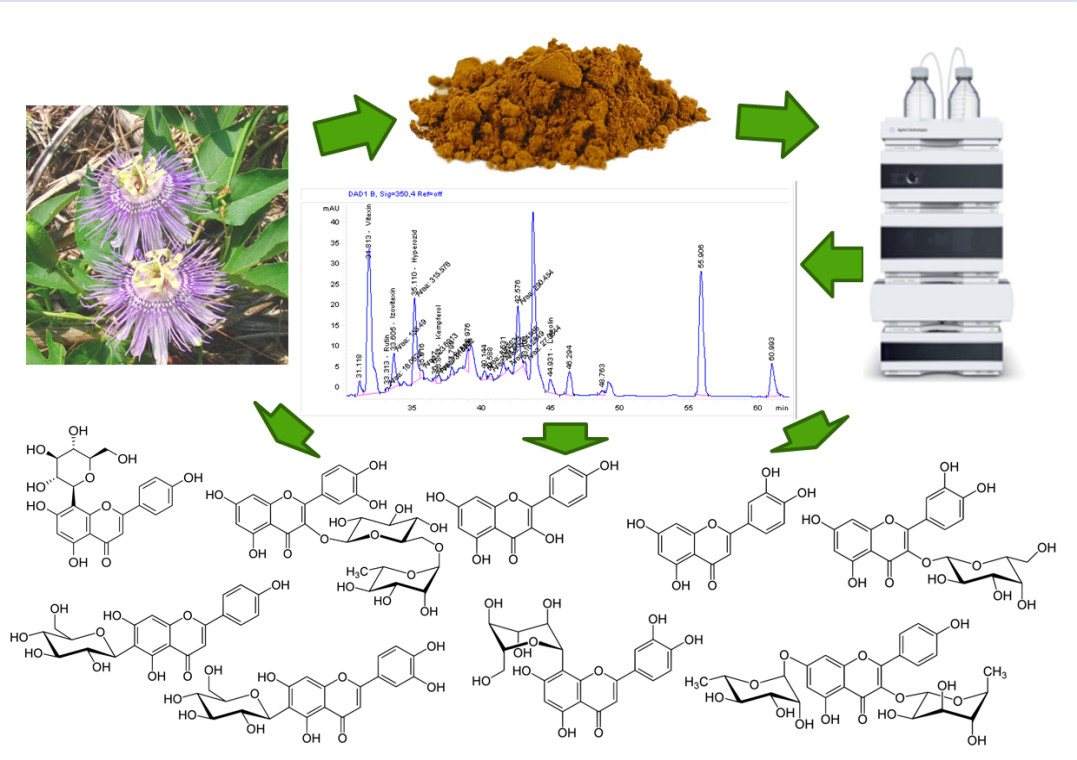
GRAPHICAL ABSTRACT



SUMMARY

Comparative free radical scavenging efficacy of ethylacetate leaves extract of *Moringa oleifera* and ethylacetate petal extract of *Hibiscus sabdariffa* was undertaken. Results established that ethylacetate extract of the Petals of *Hibiscus sabdariffa* has more reducing power and free radical scavenging efficacy compared to ethylacetate extract of *Moringa oleifera* leaves. These plant parts could serve as novel sources for clinically effective antioxidants.

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