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Phytochemical Constituents, Antinutritional Factors and *In Vitro* Antioxidant Potential of *Synclisia scabrida* Root Extracts

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ABSTRACT: *Synclisia scabrida* is a medicinal plant commonly found in different parts of Africa. The root of this plant has not enjoyed profound investigation as more attention is paid to its leaves. Hence, this study was designed to evaluate the phyto-constituents and *in vitro* antioxidant activity of aqueous and ethanol extracts of *S. scabrida* root. The findings of this study revealed higher amounts of phenolics, saponins and flavonoids in the aqueous extract when compared to the ethanol extract. However, the ethanol extract had higher amounts of phytosterol, tannins, terpenes and alkaloids. Both extracts had excellent free radical scavenging activities. Both extracts also had higher amounts of phytate relative to oxalate content. The aqueous extract was a better scavenger of free radicals as shown by its 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with IC_{50} of 66.31 µg/mL as against 85.95 µg/mL that was recorded for ethanol extract. Similarly, the aqueous extract had higher superoxide anion and hydroxyl radical scavenging properties when compared to the ethanol extract. However, the ethanol extract had a higher metal chelating power relative to the aqueous extract. The result shows that *S. scabrida* root extracts possess excellent radical scavenging properties and suggests that it may play useful roles in nutraceutical and pharmaceutical industries.

Keywords: *Synclisia scabrida,* phytochemicals, antioxidant, antinutrients, DPPH

Introduction

Synclisia scabrida is a wild shrub belonging to the family of *Menispermaceae* which is commonly found in different parts of Africa where it occurs chiefly in rainforests at medium and low altitude. The plant is found as a slender dioecious liana with twining stems that could be up to 40m long. In Nigeria, the plant has several local names such as *nzie-nzie-nwu* (Ika), *oluku* (Bini), *onukwu* (Esan), *ezizo/eziza/uziza* (Igbo), *úrúk ìsòng* (Efik).

The plant is collected from the wild mainly for medicinal purposes although the leaves also serve as protein-rich fodder for ruminants. *Synclisia scabrida* has ethnomedicinal uses in many parts of Africa,

especially Nigeria, Cameroon, Gabon and Congo. The root, leaf, stem and liana are variously used to treat gastric ulcer, malaria, mental disorders, venereal diseases, prostate problems, asthma, hernia, upper abdominal pains, dyspepsia, menstrual pains, prevent spontaneous abortion and as an aphrodisiac.[1,2] *Synclisia scabrida* has been reported to possess anticoagulant properties,[3,4,5] antimicrobial property [2] as well as antiulcer and antispasmodic properties.[1]

There is increasing use of Mass Spectroscopy coupled with Gas Chromatography (GC-MS) technique since it is a valuable method for the identification and quantification of non-polar components and volatile essential oils, fatty acids, lipids and alkaloids that are present in plants. Most medicinal plants are now used in the formulation of herbal drugs. Therefore, identification and quantification of their active constituents using recent analytical techniques such as GC-MS is necessary for proper standardization of such herbal drugs.[6,7]

Phytochemicals are naturally occurring non-nutritive chemicals with protective or disease preventive property present in plants.[8] Several of these phytochemicals such as alkaloids, tannins, flavonoids, polyphenols are known to mediate medicinal properties. The presence and abundance of these phytochemicals in medicinal plants form the basis for their usage in local therapy. The presence of cardiac glycosides (+++), tannins (+++), saponins (+), flavonoids (++), carbohydrates (++) and alkaloids (+++) has been reported in crude extracts and fractions of *Synclisia scabrida*.[1]

This plant is suspected to be rich in phytochemicals and antioxidants due to its wide applications in folkloric medicine. However, there is a huge knowledge gap on the antioxidant activity of this plant. Furthermore, available scientific reports on the phytochemistry of this plant are few and largely focused on a qualitative analysis of phytochemicals present in the plant. Therefore, this present study will identify and quantify the phytochemicals and antinutritional factors present in the root of *Synclisia scabrida* using GC-MS as well as evaluate its antioxidant activity.

Materials and Methods

Collection and Identification of Plant

Synclisia scabrida was collected from the wild in selected forests in Umunede, Delta state, Nigeria. The plant, *Synclisia scabrida* (family: *Menispermaceae*) was identified and authenticated by a Botanist, Dr. H.A. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Thereafter, a voucher number (UBH-S469) was issued and a voucher specimen of the plant was deposited at the herbarium of the same Department.

Preparation of Plant Extracts

The root of the plant was cleaned, chopped into small bits and air dried in the absence of sun light at room temperature. The plant was then pulverised and kept in air tight containers. 100 g each of the pulverized samples of *Synclisia scabrida* was soaked in 400 mL of water and ethanol respectively. The mixture was stirred at regular intervals and kept at room temperature for 72 hours. The resultant extract was then filtered with a muslin cloth. The filtrate was concentrated using a rotary evaporator, freeze-dried and stored at 4^0C in an air tight container until needed.

Determination of Yield of Extract

The percentage yield of the aqueous and ethanol extracts of *Synclisia scabrida* was determined using the formula:

$$Percentage\ Yield\ (\%) = \frac{Weight\ (g)\ of\ the\ concentrated\ extract}{Weight\ (g)\ of\ the\ pulverised\ sample} \times 100$$

Phytochemical Analysis

The qualitative and quantitative phytochemical analyses of the aqueous and ethanol extracts of the root of *Synclisia scabrida* was carried out using GC-MS analysis.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The aqueous and ethanol root extracts of *Synclisia scabrida* were analyzed for their chemical constituents by GC-MS (GC 7890A Agilent Technology). The column (DB5) was fused silica 30 m x 0.25 mm ID × 0.25 µm film thickness. The oven temperature was programmed from 80°C @10°C/min to 200 °C @12°C/min to 260°C (30 min). Helium gas (99.999 %) was used as the carrier gas at a constant flow rate of 1ml/min and an injection volume of 1 µl was employed (split ratio of 10:1) at an injector temperature of 250°C; the ion-source temperature was set at 280°C. The compounds were detected in the range 50-550 amu.

Antioxidant Assay

Determination of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging ability of the aqueous and ethanol root extracts of *Synclisia scabrida* against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.*[9] The assay is based on the ability of the antioxidant compounds to reduce DPPH by donation of hydrogen resulting in colour change from deep violet to golden yellow. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2 mL of various concentrations (0.2 – 1.0 mg/mL) of the extracts. The reaction tubes were shaken and incubated for 15 min at room temperature in the dark; absorbance read at 517 nm. All tests were performed in triplicate. Ascorbic acid was used as standard control, with similar concentrations as the test samples prepared. A blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was prepared and treated as the test samples. The radical scavenging activity was calculated using the following formula:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \left[\frac{(A_0 - A_1)}{(A_0)}\right] \times 100$$

Where: A_0 is the absorbance of DPPH radical + methanol;
A_1 is the absorbance of DPPH radical + sample extract or standard.

The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of the extracts was determined according to the method of Klein *et al.*[10] The reaction mixture contained 1.0 ml of different concentration of extracts (2-10 mg/ml), 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate 0.26% EDTA), 0.5 ml of 0.018% EDTA, 1.0 ml of DMSO (0.85 % in 0.1 M phosphate buffer pH7.4) and 0.5 ml of 0.22% ascorbic acid. The tubes were capped tightly and heated in a water bath at 80-90ºC for 15 minutes, the reaction was terminated by adding 1.0 ml of ice-cold TCA (17.5%). To the above reaction mixture 3.0 ml of Nash reagent (75.0 g of ammonium acetate, 3.0 ml of glacial acetic acid and 2.0 ml of acetyl acetone were mixed and distilled water was added to a total volume of 1 L) was added and incubated at room temperature for 15 minutes for color development. The intensity of the yellow color formed was measured at 412 nm against a reagent blank. Ascorbic acid and gallic acid were used as standards.

$$\%\ Inhibiton = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Abs control is the absorbance of control and Abs sample is the absorbance of the extract.

Superoxide Anion Radical Scavenging Assay

The superoxide anion radical scavenging activity of *S. scabrida* root extracts were assessed using the method described by Fontana *et al.* [11] with slight modification. To various concentrations of the extracts (2-10 mg/ml), 1.0 ml of phosphate buffer (0.1 M, pH 7.2), 1.0 ml of NADH (2 mM), 1.0 ml of NBT (0.5 mM) and 0.1 ml of PMS (0.03 mM) were added. After 5 minutes incubation at room temperature, the absorbance was read at 562 nm against a reagent blank to determine the quantity of formazan generated. BHT and gallic acid was used as the standards. The % inhibition was determined as already indicated above.

Metal Chelating Power

The ferrous ion chelating potential of the extracts was evaluated by the method of Dinis *et al.*[12] The reaction mixture contained 1.0 ml of various concentrations of the extracts (2-10 mg/ml) and 0.05 ml of 2 mM $FeCl_3$. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. The reaction mixture was shaken vigorously and left standing at room temperature for 10 min and the absorbance of the reaction mixture was measured at 562 nm against a reagent blank. A lower absorbance of the reaction mixture indicated a higher ferrous ion chelating ability. The control contained all the reagents except sample. Gallic acid and ascorbic acid were used as standards for comparison. The % inhibition was determined as already indicated above.

Results

Percentage Yield of *Synclisia scabrida* Root Extracted with Aqueous and Ethanol Solvent:

The percentage yield from the root of *Synclisia scabrida* is presented in Table 1. The aqueous extract had more yield than the ethanol extract.

Phytochemical Constituents of *Synclisia scabrida* Root Extracted with Aqueous and Ethanol Solvent:

The phytochemical constituent of aqueous and ethanol root extracts of *Synclisia scabrida* is presented in Table 2. The ethanol extract appears to have higher amount of phytochemicals in comparison to the aqueous extract. Alkaloids and phenolics were the most abundant phytochemicals in the ethanol and aqueous extract when compared to other phytoconstituents. Both extracts have relatively high amounts of flavonoid, phytate, terpenes and tannins. However, they appears to be low in oxalate, steroids, saponins, coumarin and anthocyanins.

Table 1: The percentage yield of the aqueous and ethanol extracts of the root of *Synclisia scabrida*

Sample	Percentage Yield (%)
Aqueous extract	30.8
Ethanolic extract	27.9

***In Vitro* Antioxidant Activity of *Synclisia scabrida* Root Extracts:**

DPPH Radical Scavenging Activity:

In the presence of antioxidants, the deep purple colour of DPPH decolorizes and eventually becomes golden yellow. As seen in figure 1, both extracts of *S. scabrida* root scavenged DPPH radical in a

concentration-dependent manner. However, the aqueous extract of *S. scabrida* had a higher ability to scavenge DPPH radical with an IC_{50} value of 66.31 µg/mL in comparison to the ethanol extract (IC_{50} = 85.95 µg/mL).

Superoxide Anion Scavenging Activity:

The superoxide anion scavenging potential of *S. scabrida* root extracts is shown in figure 2. Both extracts scavenged superoxide anion in a concentration-dependent manner. The aqueous extract had a higher potential to scavenge superoxide anion when compared to the ethanol extract. The highest superoxide anion radical scavenging potential of both extracts was observed at 100 µg/mL.

Table 2: The phytochemicals present in aqueous and ethanol root extracts of *Synclisia scabrida*

Phytochemicals	Concentration (mg/100g) (Aqueous extract)	Concentration (mg/100g) (Ethanol extract)
Terpenes	2.81	3.28
Phytosterol	1.25	9.76
Oxalate	1.46	0.43
Steroid	0.14	0.27
Tannin	3.71	5.19
Phenolics	21.53	13.86
Saponin	2.24	1.59
Alkaloid	28.01	35.26
Coumarin	1.15	1.73
Anthocyanins	0.67	2.06
Flavonoid	7.08	3.51
Phytate	6.15	9.18
Cardiac Glycoside	2.84	2.45
Cyanogenic Glycoside	0.59	0.13

Hydroxyl Radical Scavenging Activity:

Figure 3 shows the hydroxyl radical scavenging activity of aqueous and ethanol extracts of *S. scabrida* root. The aqueous extract had a better potential to scavenge hydroxyl radical when compared to the ethanol extract. Similarly, the highest scavenging activity for both extracts was at the 100 µg/mL concentration.

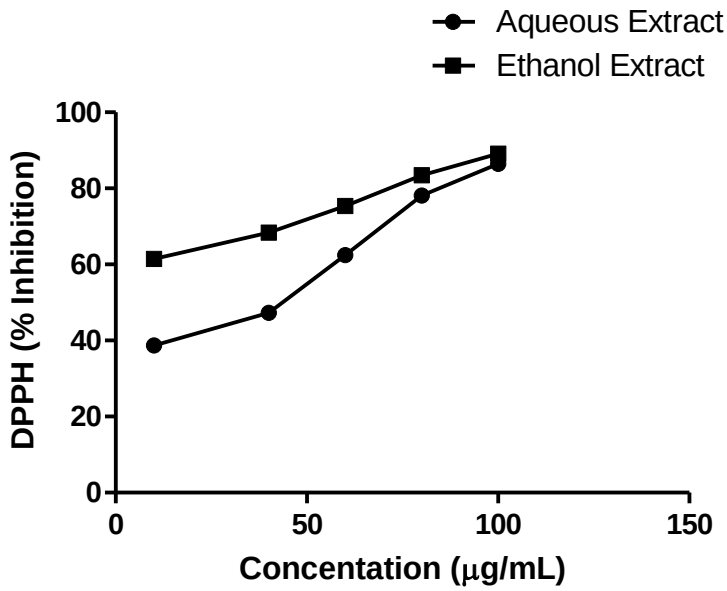

Figure 1: DPPH radical scavenging activity of aqueous and ethanol extracts of *S. scabrida* root. Experiments were performed in triplicate and data represents Mean ± SEM.

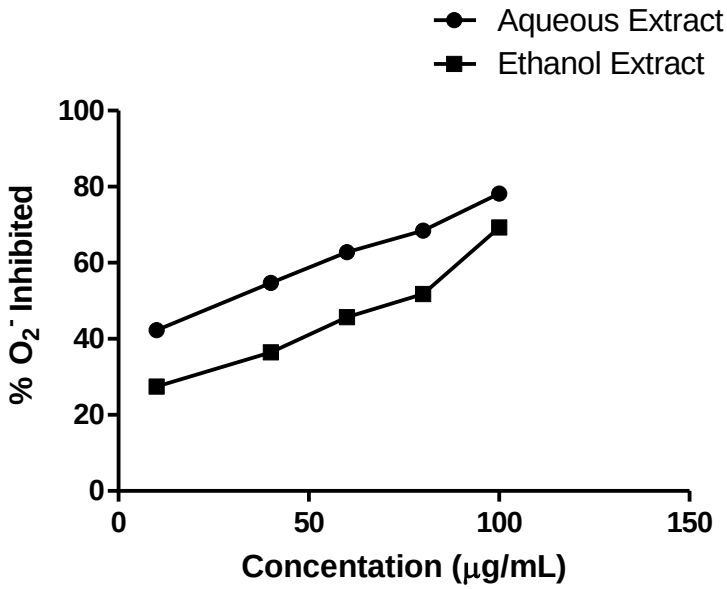

Figure 2: Percentage superoxide anion radical scavenging activity of aqueous and ethanol extracts of *S. scabrida* root. Experiments were performed in triplicate and data represents Mean ± SEM.

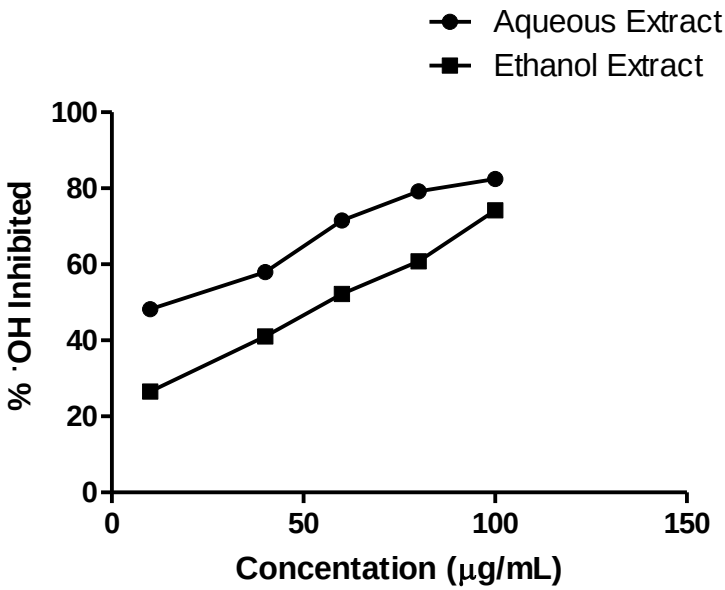

Figure 3: Percentage hydroxyl radical scavenging activity of aqueous and ethanol extracts of *S. scabrida* root. Experiments were performed in triplicate and data represents Mean ± SEM.

Metal Chelating Power:

The metal chelating power of *S. scabrida* root extracts is shown in figure 4. The present result showed that the ethanol extract had higher metal chelating properties in comparison to the aqueous extract. The activity of both extracts were concentration-dependent.

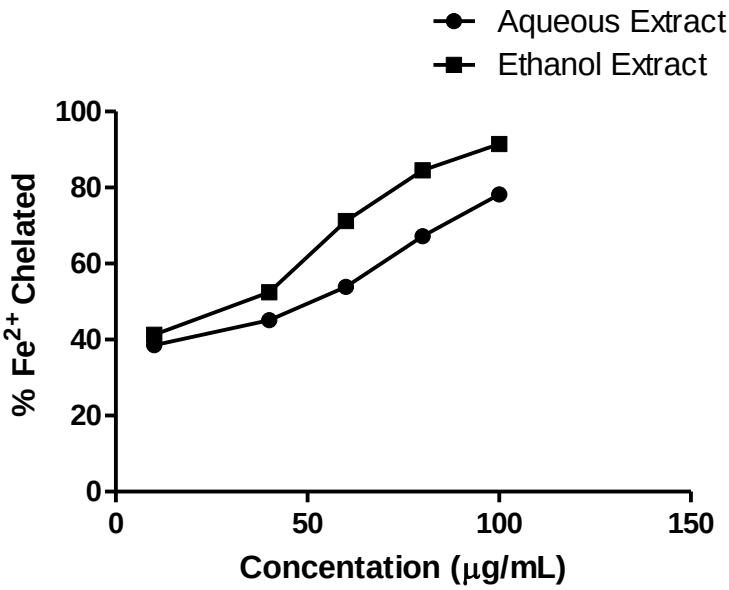

Figure 4: Percentage metal chelating power of aqueous and ethanol extracts of *S. scabrida* root. Experiments were performed in triplicate and data represents Mean ± SEM.

Discussion

Medicinal plants are one of the main sources of antioxidants and this properties has been linked to the presence of bioactive phytoconstituents. These antioxidants may act as reducing agents, eliminate free radicals, quenchers of singlet oxygen etc.[13] It is advisable to use different solvents for extraction process as antioxidants are multifunctional and each solvent may preferentially extract different antioxidant compound.[14] Correspondingly, the result from this study demonstrated that wide arrays of phytochemicals were distributed in varying concentrations in the different solvent (aqueous and ethanol) used.

The result shows both aqueous and ethanol extracts of *Synclisia scabrida* root to be very rich in phenolics and alkaloids when compared to other phytoconstituents. Steroids and cardiac glycosides were in trace amounts in both extracts while anthocyanins were in trace amounts in the aqueous extract. Generally, phytochemicals are known to confer certain health benefits such as antihypertensive, anti-inflammatory, antidiabetic and antimicrobial effects.[15] The bioactive compounds identified in extracts of *Synclisia scabrida* root may be responsible for its diverse ethnomedicinal uses. Polyphenols are known to have strong antioxidant activities. This is because they donate electrons or hydrogen atoms to terminate chain reactions initiated by radicals by converting free radicals to a more stable product, therefore exhibiting strong antioxidant activity.[15] A direct relationship between total phenolic content and antioxidant activities in different plants have been demonstrated in several studies.[16] High phenolic content-containing plant materials have high radical scavenging abilities.[17] Hence, the higher amount of phenolics present in the aqueous extract compared to the ethanol extract may be responsible for its excellent DPPH, superoxide anion and hydroxyl radical scavenging activities. Similarly, the aqueous extract showed high flavonoid content compared to the ethanol extract. Flavonoids are water soluble bioactive compounds that inhibits lipid peroxidation, scavenge free radicals and chelate transition metals.[18,19] The major flavonoid groups in the root of *Synclisia scabrida* are water soluble. Hence, water may be a better solvent to harness the flavonoid related benefits of *Synclisia scabrida* root.

Alkaloids are widely used as curative drugs in medical fields due to their ability to create intense physiological action.[20] Quinine, an alkaloid, at one time was the main drug used in the treatment of malaria infection. The alkaloid content of *Synclisia scabrida* root seems to be more soluble in ethanol than the aqueous solvent. The high amount of alkaloids widely distributed in the root of *Synclisia scabrida* indicates that this medicinal plant may possess activity against diseases such as malaria.

Coumarins have been shown to have the ability to stimulate macrophages, activate cells of the immune system and stabilize swellings.[21] The presence of coumarin in both extracts shows that *Synclisia scabrida* root may have anti-inflammatory and immune stimulating properties. Tannins are polyphenolic compounds present in many plants with a higher molecular weight and are known to have antimicrobial properties.[22] They have been reported to possess anticancer, anti-tumour, antiulcer and antimicrobial agents.[23] Tannins were abundant in the ethanol extract relative to the aqueous extract. Both extracts may therefore play useful roles in managing diseases such as ulcer, cancer, tumor etc. Interestingly, the antibacterial activity,[24] antimicrobial activity [2] and antiulcerogenic activity [25,1] of *Synclisia scabrida* have been reported in previous studies. Saponins are emulsifying agents, which in addition to being implicated for use as expectorants and cough suppressants,[26,27] have also been shown to have strong hemolytic effect.[28] Although *Synclisia scabrida* root may be useful as cough suppressant due to the presence of saponins, there is need for caution because of the hemolytic effect associated with saponins. Cardiac glycosides are useful to treat congestive cardiac arrhythmia leading to cardiac arrests/failures.[29] They have also been shown to promote the activation of tumor-specific immune responses.[30] The presence of cardiac glycosides in *Synclisia scabrida* root shows that it may be useful as an anticancer agent as well as in managing cardiac arrhythmia.

Some plant chemicals have been shown to be deleterious to health or evidently advantageous to human and animal health if consumed at appropriate amounts.[31] Phytate and oxalate (anti-nutrients) are some of these compounds which results in unwanted effects when in high amount. Anti-nutrients are compounds which act to reduce nutrient utilization and or food intake.[32] Phytate content was higher in the ethanol extracts when compared to the aqueous extract. Phytate has been suggested to serve as a store of cations, of high energy phosphoryl groups, and, by chelating free iron, acts as natural antioxidant.[33] However, in

diet, they have negative impact on the bioavailability of divalent, and trivalent mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+}.[34] Insoluble calcium oxalate can precipitate in the kidney or urinary tract [34] and therefore, they play a role in the formation of kidney stones.[35] In this study, oxalate was generally low in the root of *Synclisia scabrida*. The low level of cyanogenic glycosides in the extracts of *S. scabrida* root sheds more light on its safety level. The knowledge of the cyanogenic glycoside content of food is vital because cyanide being an effective cytochrome oxidase inhibitor, interferes with aerobic respiratory system.[36]

Phytochemicals have a very complex nature and hence, a single assay may not provide a full picture of the antioxidant potential of medicinal plant extracts.[37] Therefore, in this study, the antioxidant potential of aqueous and ethanol extracts of *Synclisia scabrida* against free radicals was evaluated using four antioxidant assays. Free radicals are highly unstable species that require electrons to be stable and hence, in the presence of antioxidants, they attain stability. At high concentrations, these free radicals disrupt antioxidant defense of the body and results in oxidative stress.[38]

DPPH assay is one of the quickest, easiest and reliable methods that is used to assess the antioxidant potential of compounds. This assay combines both Hydrogen Atom Transfer (HAT) and Electron Transfer (ET) mechanisms to deactivate radicals.[38] The decolorization of the DPPH radical reflects the ability of the plant extract to donate either electron or hydrogen atom needed to stabilize or deactivate this radical. Aqueous extract of *Synclisia scabrida* root had a lower IC_{50} value and hence a higher antioxidant potential in comparison to the ethanol extract. IC_{50} is defined as the concentration of the extract that is needed to scavenge 50% of free radicals. The lower the IC_{50} value, the higher the antioxidant potential of the extract. The higher phytochemicals in the aqueous extract may have accounted for its better DPPH radical scavenging activity as higher amounts of polyphenols and flavonoids was reported in the aqueous extract when compared to the ethanol extract. The antioxidant activity of plant extracts have been linked to polyphenols and flavonoids.[15,39] The harmful effect of superoxide anion to cells have been previously reported.[40] As shown in figure 2, the aqueous extract had a higher ability to scavenge superoxide anion radicals relative to the ethanol extract. Similarly, the presence of higher amounts of flavonoids in the aqueous extract may have accounted for this activity since flavonoids have been reported to be effective antioxidants mainly because they scavenge superoxide anions.[41]

The capacity of free radicals to cause mutation has been linked to direct interaction of hydroxyl radicals with DNA and therefore, they play an important role in causing cancer.[42] Hydroxyl radical can be generated *in* vivo; the enzyme superoxide dismutase converts superoxide radicals to hydrogen peroxide that would subsequently produce hydroxyl radicals in the presence of divalent metal ions such as iron and copper.[43] The result of this study revealed that both extracts of *Synclisia scabrida* possess moderate hydroxyl radical scavenging activity. However, the aqueous root extract of *Synclisia scabrida* had a higher hydroxyl radical scavenging property when compared with the ethanol extract. This implies that the extracts may have anticancer properties and may prevent the interaction of hydroxyl radical with DNA. Similarly, the extract may also have the ability to prevent lipid peroxidation.

Iron plays a very crucial role in the body. It is present in the blood pigment, hemoglobin where it reversibly binds molecular oxygen thereby enabling hemoglobin to transport oxygen to various tissues of the body.[44] However, free iron (Fe^{2+}) can result in lipid peroxidation in the presence of hydrogen peroxide via the Fenton reaction. Hence, medicinal plants with bioactive constituents that can chelate free iron may have the ability to manage oxidative stress. In this study, both extracts of *Synclisia scabrida* root had excellent ability to chelate Fe^{2+}. However, the ethanol extract had a higher metal chelating potential relative to the aqueous extract.

Conclusion

The result of this study revealed a wide array of phytochemicals in aqueous and ethanol extracts of *Synclisia scabrida* root. The aqueous extract had a higher free radical scavenging potential when compared to the ethanol extract. The antioxidant activity of the extracts may be attributed to the presence of polyphenolics, tannins and flavonoids. Therefore, *Synclisia scabrida* root may serve as a natural source of

antioxidants which may be useful in developing nutraceuticals for the management of oxidative stress related diseases.

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