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Research Article

Phytochemical Screening and *In Vitro* Evaluation of the Antioxidant Potential of Dichloromethane Extracts of *Strychnos henningsii* Gilg. and *Ficus sycomorus* L.

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Medicinal plants are a rich source of antioxidants such as flavonoids, phenols, tannins, and alkaloids among others and are currently used as alternative and complementary drugs in the management of stress-related disorders. *Strychnos henningsii* and *Ficus sycomorus* have been traditionally used by the people of Mbeere, Embu county, Kenya, as medicine for the treatment of various oxidative stress-related disorders such as diabetes and rheumatism; however, no empirical data are available to authenticate the said claim. The aim of this study was to evaluate preliminary phytochemical screening and in vitro antioxidant activity of dichloromethane (DCM) leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* using DPPH, hydrogen peroxide, and ferric reducing power assays; total flavonoids and phenolic compounds were also determined by colorimetric assay and Folin–Ciocalteu reaction, respectively. Phytochemical screening showed that both extracts possessed saponins, flavonoids, phenols, steroids, alkaloids, and cardiac glycosides; however, terpenoids were found to be absent in *S. henningsii*. The total phenolic and flavonoid content of the DCM stem bark extract of *F. sycomorus* was lower than that of the leaf extract of *S. henningsii*. These extracts significantly exhibited strong antioxidant activities at different concentrations tested. The IC_{50} values of *S. henningsii* and *F. sycomorus* were 0.325 mg/ml and 0.330 mg/ml for hydrogen peroxide and 0.068 mg/ml and 0.062 mg/ml for DPPH, respectively. Both DCM leaf and stem bark extracts of *S. henningsii* and *F. sycomorus* were found to have strong ferric reducing power. Therefore, both extracts showed significant nonenzyme-based antioxidant activities. The two plants possess phytochemicals that have significant antioxidant properties.

1. Introduction

Oxidative stress is the disparity between the production of free radicals and antioxidant defenses in the body [1–5]. Free radicals are defined as compounds with unpaired electrons, making them highly reactive molecules that can attack any stable molecules such as proteins, carbohydrates, and lipids [6, 7]. Reactive oxygen species (ROS) are the most common and widely known free radicals. They include superoxide (O_2^-), hydroxyl (HO^-), hydrogen peroxide (H_2O_2), and nitric oxide (NO^-). Most biochemical reactions in the body are known to generate ROS [8], which are potent in damaging important biomolecules such as proteins, nucleic acids, and lipids if they are not scavenged by antioxidants [9]. Free radicals are well known to be involved in aging and pathogenesis of stress-related disorders such as diabetes, nephrotoxicity, hepatotoxicity, malignancy, cardiovascular disorders, inflammation, and neurological disorders [10–12].

Human cells are well protected by antioxidant defense systems against ROS attack; however, at low concentrations of antioxidant enzymes, some cells have been shown to be sensitive to ROS [13–16]. The cellular antioxidant level is used to determine the susceptibility of tissues to oxidative damage. This level normally changes during oxidative stress [17–19]. A wide variety of antioxidants are naturally

obtained from plants that constitute our daily diet. Commonly known dietary antioxidants are vitamins C and E, carotenoids, and green tea [20, 21]. The consumption of food and fruits rich in antioxidants plays a significant role in augmenting the body's natural resistance to oxidative stress [22, 23]. Plants also have many other nonnutrient antioxidants such as phenols, flavonoids, and alkaloids. These polyphenol compounds have been extensively studied and documented as quenchers of free radicals [24, 25].

Since antioxidants hold a key in preventing oxidative stress-related disorders, many plant extracts and their secondary metabolites are being explored for their antioxidant effects [26, 27]. The use of plant-based antioxidants plays an important role in preventing the activation of the oxidation-induced signaling pathways in our bodies [28]. Therefore, the identification of the antioxidant activities of the DCM leaf extract of *S. henningsii* and stem bark of *F. sycomorus* is an important step in increasing our understanding about their usage in the treatment of various stress-related disorders.

Commercially available antioxidant drugs include butyrate hydroxyanisole, butylated hydroxytolune, propyl gallate, and fluconazole [29–31]. However, studies have shown that these synthetic antioxidants have toxic effects and show negative health influence [32, 33] and have led to some restrictions being imposed on their use [34]. Researchers now have focused their attention on plant-derived antioxidants among others [35].

Globally, several plants have been traditionally used for their antioxidant activities [36, 37]. Ethno-pharmacological surveys indicate that medicinal plants play a vital role in the management of oxidative stress-related disorders [38, 39]. Plant extracts naturally possess phytochemicals such as flavonoids, tannins, phenols, and alkaloids [40], which are well-known antioxidants and are currently pursued as alternative and complementary remedies against oxidative stress-related disorders [41]. Several efficacy studies conducted on herbal plants have shown that plant-based antioxidants are relatively safe, cost efficient, and effective in disease management [42].

The genus *Ficus* is widely known to have strong antioxidant properties due to its richness in phenols and flavonoids [43–45]. Traditionally, *Ficus sycomorus* fruits, stem barks, and roots have been used as herbal remedies for several ailments such as diarrhea, liver disease, skin infections, stomach disorders, helminthiasis, lactation disorders, epilepsy, tuberculosis, sterility, and diabetes mellitus [46–48]. *S. henningsii* is a widely distributed evergreen herb in East Africa [47] and is used in the management of rheumatism, snake bite, abdominal pain, gastrointestinal pain, gynecological complaints, malaria, and diabetes mellitus [49]. The crude extracts of *Strychnos henningsii* have been documented to possess significant therapeutic effects against stress-related disorders [50]. Based on traditional pharmacology, it has been actively and successfully employed by the Mbeere community in Embu county, Kenya, in the management of diabetes, which is an oxidative stress-related disorder.

In view of this background, the present study seeks to investigate the *in vitro* antioxidant activities of dichloromethanolic (DCM) leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus*. The study aims to explore and provide preliminary information on the in vitro antioxidant activities of S. *henningsii* and *F. sycomorus* as possible bioresources for the generation of herbal formulations used in the treatment and management of oxidative stress-related disorders. The study also aims to reveal relevant research gaps that need to be explored further.

2. Materials and Methods

2.1. Collection of Plant Materials. The authors sought authorization from The National Commission for Science, Technology and Innovation (NACOSTI/P89/6765/9816). Plant materials *of S. henningsii* leaves and stem barks of *F. sycomorus* were collected from their natural habitats from Makunguru village, Mbeere north subcounty, Embu county, Kenya, in October, 2015. The GPS locations for *S. henningsii* and *F. sycomorus* specimens were 0°34′11″S, 37°37′31″E and 0°35′28″S, 36°36′22″E, respectively. The collection of these samples was done based on ethnobotanical information availed by local herbalists in the area. The plant identification was done by an acknowledged authority from Kenyatta University authenticating their botanical identities. *S. henningsii* and *F. sycomorus* were assigned voucher specimen numbers (*S. henningsii*. (Wkw001/10/21015) and *F. sycomorus* (Wkw 002/10/2015), respectively). They were deposited in the Kenyatta University Herbarium for future reference. Sample materials were carefully sorted, packed in sealed bags, and transported to the Department of Biochemistry and Biotechnology, Kenyatta University, where further processing and subsequent study was undertaken.

2.2. Extract Preparations. The fresh plant materials were air dried at room temperature under a shade for a week. The dried leaves were milled into fine powder by use of an electric mill. The powdered plant materials were sieved using a mesh pore of 0.5 mm and packed in closed, dry sealed bags and stored awaiting extraction. Two hundred and fifty grams (250 g) of each powdered plant material was soaked in 1 litre of dichloromethane (DCM) and macerated for 24 hours. The resultant extract was poured into a clean dry conical flask and then filtered using Whatman's No. 1 filter papers. The filtrate was extracted using Soxhlet apparatus for 5-6 h and then concentrated under reduced pressure and vacuum using a rotary evaporator at a temperature of 40°C. The concentrates were placed in airtight containers weighed and stored at −4°C awaiting use in bioassays.

2.3. Qualitative Phytochemical Screening. Qualitative phytochemical screening of DCM extracts of *S. henningsii* and *F. sycomorus* was performed to determine the presence or absence of selected plant secondary metabolites using standard methods described by Harbone [51] and Kotake [52]. Secondary metabolites screened include flavonoids, cardiac glycosides, saponins, alkaloids, sterols, phenolics,

and terpenoids. These phytochemicals are associated with antioxidant activities.

2.4. Quantitative Phytochemical Determination

2.4.1. Determination of Total Phenolic Contents. Folin–Ciocalteu reagent was used to determine the total phenolic levels of the plant extracts as described by Spanos et al. [53] and modified by Lister and Wilson [54]. Briefly, 2 ml of each plant extract, 2.5 ml of 10% dilution of Folin–Ciocalteu reagent and 2 ml of Na_2CO_3 (7.5%, w/v) were mixed and left to stand for 15 minutes at 45°C. The absorbance of all the treatments was determined at 765 nm spectrophotometrically. Gallic acid was used as the reference to derive the calibration curve. The total phenolic content was determined using the linear equation based on the calibration curve and contents expressed as milligrams of gallic equivalent per gram of dry weight (mg GAE/g dw) [55].

2.4.2. Determination of Total Flavonoid Contents. The colorimetric methodology described by Lamaison and Carnet [56] and Nurcholis et al. [57] was used to determine the total flavonoid contents of the extracts. Briefly, a volume of 1.5 ml of the extracts was mixed with an equivalent volume of 2% $AlCl_3.6H_2O$ (2 g in 100 ml methanol) solution. The solution was vigorously shaken to mix and then incubated for 10 minutes, and the absorbance was read at 430 nm using a spectrophotometer. Rutin was used as the reference to generate the calibration curve. The total flavonoid content was expressed as milligrams of rutin equivalent per gram of dry weight (mg RE/g dw) based on the calibration curve [55].

2.5. Determination of In Vitro Antioxidant Activities

2.5.1. Determination of In Vitro Hydrogen Peroxide Scavenging Activity. The *in vitro* hydrogen peroxide scavenging potential of DCM extracts of *S. henningsii* and *F. sycomorus* was determined following the protocol described by Ruch et al. [58]. Briefly, 50 Mm, pH 7.4, phosphate buffer solution was used to prepare 250 ml solution of hydrogen peroxide (40 mM). Hydrogen peroxide solution at a volume of 0.6 ml was added to 1 ml of varying concentrations (0.1–0.5 mg/ml) of the plant extract and ascorbic acid (standard). The mixture was left to stand for 10 minutes, after which the absorbance was determined at 560 nm using a UV spectrophotometer. The blank solution containing phosphate buffer was used as the negative control. This was done in three replicates. The percentage radical scavenging activity is as follows:

$$\%\ \text{Hydrogen scavenging activity} = \frac{\text{Abs. of control} - \text{Abs. of sample/standard}}{\text{Abs. of control}} \times 100, \quad (1)$$

where Abs. is the absorbance.

2.5.2. Determination of In Vitro Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity. The abilities of the DCM extracts of *S. henningsii* and *F. sycomorus* to scavenge DPPH radicals *in vitro* were determined based on the method documented by Mehrotra et al. [59–61]. Following this method, 2.66 mg of DPPH was dissolved in 50 ml of ethanol to form a concentration of 0.135 mM. Various dilutions, namely, 0.2, 0.1, 0.05, 0.025, and 0.0125 mg/ml, of the plant extracts and ascorbic acid (standard) were prepared. One milliliter of the DPPH solution dissolved in methanol was mixed with 1 ml of each diluted plant extract and ascorbic acid (reference drug). The mixtures were then agitated thoroughly and left in a dark room for 30 minutes at room temperature. Three replicates of the assays were prepared. The absorbance of the mixture was then measured at 517 nm using a spectrophotometer. The actual decrease in absorbance was measured against that of the control. The negative control was a blank solution containing ethanol without H_2O_2. The percentage DPPH scavenging abilities of the plant extracts were then derived using the following equation:

$$\%\ \text{DPPH scavenging activities} = \frac{\text{Abs. of control} - \text{Abs. of sample/standard}}{\text{Abs. of control}} \times 100, \quad (2)$$

where Abs. is the absorbance.

2.5.3. Calculation of Half Maximal Inhibitory Concentrations (IC_{50}) in Hydrogen Peroxide and DPPH Radicals. The half maximal inhibitory concentration (IC_{50}) of DCM extracts of *S. henningsii*, *F. sycomorus,* and ascorbic acid (standard) were analyzed using linear regression analysis in MS Excel. The IC_{50}, which represents the concentration at which 50% of the radicals were scavenged by test samples, was determined from a graph of percentage scavenging activity against the concentration of the test sample.

2.5.4. In Vitro Ferric Reducing Power Assay. The in vitro ferric reducing power of the DCM extracts of *S. henningsii*

and *F. sycomorus* alongside ascorbic acid (positive control) was established according to the protocol described by Oyaizu [62]. In brief, various concentrations (0.2–1 mg/ml) of 1 ml of the plant extracts and ascorbic acid were added to 2.5 ml of 0.2 M phosphate buffer of pH 7. The resulting solution was then mixed with 2.5 ml of potassium ferricyanide and incubated at 50°C for 20 minutes. Afterwards, 2.5 ml of trichloroacetic acid (10%) was then added to the mixture and centrifuged for 10 minutes at 3000 rpm. Then, 2.5 ml was drawn from the upper layer of the solution and then added to 2.5 ml of distilled water and 0.5 ml freshly prepared ferric chloride ($FeCl_3$) solution (1%) was added. The assay was done in triplicates. The absorbance of the extracts and ascorbic acid was determined at 700 nm using a spectrophotometer.

2.6. Statistical Analysis. The data were subjected to descriptive statistics using Minitab Statistical Software 17.0 (State College, Pennsylvania) and expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was performed to determine the statistically significant difference among treatments. Tukey's tests were performed for pairwise comparison of means. Unpaired student's *t*-test was used for the comparison of mean total phenolic and flavonoid contents of DCM leaf extract of *S. henningsii* and stem bark of *F. sycomorus*. The values of $p \leq 0.05$ were considered to be significantly different. The data obtained were presented in a tabular and graphical form. The phytochemical screening was done qualitatively, and the result obtained (positive/negative) for each test was recorded in a table.

3. Results

3.1. Qualitative Phytochemical Screening. The phytochemistry of the leaf extract of *S. henningsii* revealed the presence of alkaloids, phenols, saponins, cardiac glycosides, flavonoids, and steroids while terpenoids were absent. On the other hand, *F. sycomorus* contained saponins, flavonoids, alkaloids, steroids, phenols, cardiac glycosides, and terpenoids (Table 1).

3.2. Quantitative Phytochemicals Screening

3.2.1. Total Phenolic Contents. The total phenolic concentration in the leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* were quantified and expressed as milligrams of garlic acid equivalent per gram of dry weight (mg GAE/g dw) (Table 2) using a standard gallic acid calibration curve ($y = 1.52x + 0.234$; $R^2 = 0.9782$). Generally, at all the concentrations tested (0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml), the two extracts had significantly different phenolic contents ($p \leq 0.05$), with *S. henningsii* extract having the highest phenolic content (Table 2).

3.2.2. Total Flavonoid Contents. The total flavonoid contents of the DCM leaf and stem bark extracts of *S. henningsii* and *F. sycomorus* were calculated from the standard rutin calibration curve ($y = 2.535x - 0.047$; $R^2 = 0.9778$). The leaf extract of *S. henningsii* had significantly higher flavonoid concentrations than the *F. sycomorus* stem bark extract ($p \leq 0.05$; Table 2). However, the rutin equivalence at the lowest concentration of 0.1 mg/ml for both extracts was not significantly different ($p > 0.05$; Table 3).

Table 1: Phytochemical screening of DCM leaf extracts of *S. henningsii* and stem bark of *F. sycomorus*.

Composition	*F. sycomorus*	*S. henningsii*
Saponins	+	+
Alkaloids	+	+
Terpenoids	+	−
Flavonoids	+	+
Steroids	+	+
Phenolics	+	+
Cardiac glycosides	+	+

+, present; −, absence.

3.3. In Vitro Hydrogen Peroxide Radical Scavenging Activities of DCM Extracts of S. henningsii and F. sycomorus. The *in vitro* hydrogen peroxide scavenging potential of the DCM leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* was analyzed, as shown in Figure 1. Generally, both extracts of *S. henningsii* and *F. sycomorus* showed remarkable *in vitro* hydrogen peroxide scavenging activity at all the concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml). The extracts showed H_2O_2 scavenging activities in a dose-related manner. The H_2O_2 scavenging activities of both extracts were significantly lower than that of the standard (ascorbic acid). However, there was no significant difference in the hydrogen peroxide scavenging activities of *S. henningsii* and *F. sycomorus* at all the tested concentrations ($p > 0.05$; Figure 1 and Table 4).

3.4. In Vitro DPPH Radical Scavenging Activities of DCM Extracts of S. henningsii and F. sycomorus. As shown in Figure 2 and Table 5, both extracts of *S. henningsii* and *F. sycomorus* as well as the ascorbic acid demonstrated dose-dependent scavenging of DPPH radicals. At all the tested doses (0.00125–0.2 mg/ml), the two extracts showed lower DPPH scavenging activities than the standard (ascorbic acid). The extract activities were found not to be significantly different at all the tested concentrations.

3.5. IC_{50} for Hydrogen Peroxide and DPPH. The half maximal percentage inhibition (IC_{50}) value is widely used as a quantitative measure of extracts, antioxidant potential. The two extracts showed a lower IC_{50}, as shown in Table 6.

3.6. In Vitro Ferric Reducing Power Activities of DCM Extracts of S. henningsii and F. sycomorus. In this assay, the extracts were tested for their ability to reduce Fe^{3+} to Fe^{2+} via electron donation. The results showed a dose-related ferric reduction by the DCM extracts of *S. henningsii* and *F. sycomorus*. The leaf extract of *S. henningsii* was

Table 2: Total phenolic contents of DCM extracts of *S. henningsii* and *F. sycomorus*.

	Mass in mg/g gallic acid equivalent				
Groups	0.1 mg/g	0.2 mg/g	0.3 mg/g	0.4 mg/g	0.5 mg/g
S. henningsii	4.3 ± 0.01^{a}	10.7 ± 0.01^{a}	16.7 ± 0.01^{a}	22.0 ± 0.01^{a}	29.0 ± 0.01^{a}
F. sycomorus	2.3 ± 0.01^{b}	5.0 ± 0.01^{b}	9.7 ± 0.01^{b}	16.0 ± 0.01^{b}	23.0 ± 0.015^{b}

Values are expressed as mean ± SEM of the three replicates. Column means followed by the same superscript letters are not significantly different ($p > 0.05$) by the unpaired Student's *t*-test.

Table 3: Total flavonoid content of DCM extracts of *S. henningsii* and *F. sycomorus*.

	Mass in mg/g rutin equivalent				
Groups	0.2 mg/g	0.4 mg/g	0.6 mg/g	0.8 mg/g	1.0 mg/g
S. henningsii	15.7 ± 0.00^{a}	22.7 ± 0.00^{a}	37.7 ± 0.00^{a}	44.3 ± 0.00^{a}	53.0 ± 0.00^{a}
F. sycomorus	10.3 ± 0.01^{a}	16.0 ± 0.01^{b}	23.7 ± 0.01^{b}	34.3 ± 0.00^{b}	38.3 ± 0.00^{b}

Values are expressed as mean ± SEM of the three replicates. Column means followed by the same superscript letters are not significantly different ($p > 0.05$) by the unpaired Student's *t*-test.

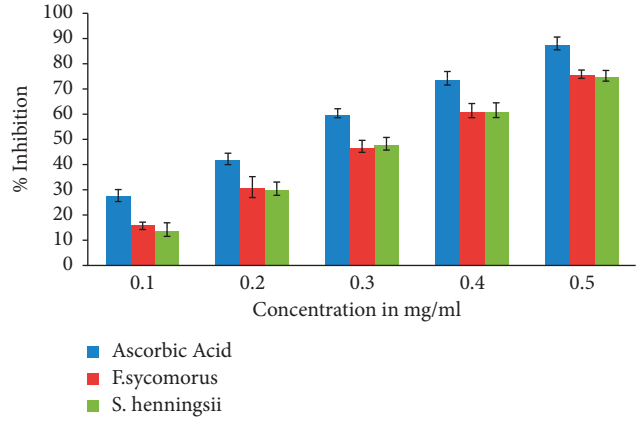


Figure 1: Percentage hydrogen peroxide inhibition of the DCM leaf extract of *S. henningsii*, the stem bark extract of *F. sycomorus*, and the standard ascorbic acid. Values are expressed as mean ± SEM of the three replicates. Column means followed by the same superscript letters were not significantly different ($p > 0.05$) by one-way ANOVA followed by Tukey's post hoc test.

Table 4: % in vitro hydrogen peroxide radical scavenging activities of DCM extract of *S. henningsii* and *F. sycomorus*.

	Concentrations					
Groups	0.1 mg/ml	0.2 mg/ml	0.3 mg/ml	0.4 mg/ml	0.5 mg/ml	IC_{50} value
Ascorbic acid %	27.72 ± 2.38^{a}	42.25 ± 2.27^{a}	60.38 ± 1.77^{a}	74.24 ± 2.68^{a}	88.05 ± 2.55^{a}	0.245 mg/ml
F. sycomorus %	15.71 ± 1.47^{b}	31.07 ± 4.15^{b}	47.24 ± 2.38^{b}	61.41 ± 2.81^{b}	75.85 ± 1.66^{b}	0.330 mg/ml
S. henningsii %	14.22 ± 2.69^{b}	30.45 ± 2.64^{b}	48.27 ± 2.50^{b}	61.57 ± 2.92^{b}	75.22 ± 2.12^{b}	0.325 mg/ml

Values are expressed as mean ± SEM for three replicates. Column means with the same superscript were not significantly different by ANOVA followed by Tukey's post hoc test ($p > 0.05$).

comparable to that of standard ascorbic acid at the lowest concentration of 0.2 mg/ml. However, at this concentration, the stem bark extract of *F. sycomorus* had significantly lower ferric reducing power than ascorbic acid. At all the other tested concentrations (0.4–1 mg/ml), the ferric reducing power activities of the two extracts had no significant difference ($p > 0.05$). However, they were significantly different from that of the standard (ascorbic acid) ($p \leq 0.05$; Figure 3 and Table 7).

4. Discussion

4.1. Qualitative and Quantitative Analysis. The search for natural antioxidants has grown rapidly amongst clinical and medical practitioners due to the interest generated by reactive oxygen species (ROS) and pathogenesis of oxidative stress-related disorders [63, 64]. Plant leaves, stems, flowers, fruits, and roots have been known for centuries to possess therapeutic value and, therefore, have been extensively

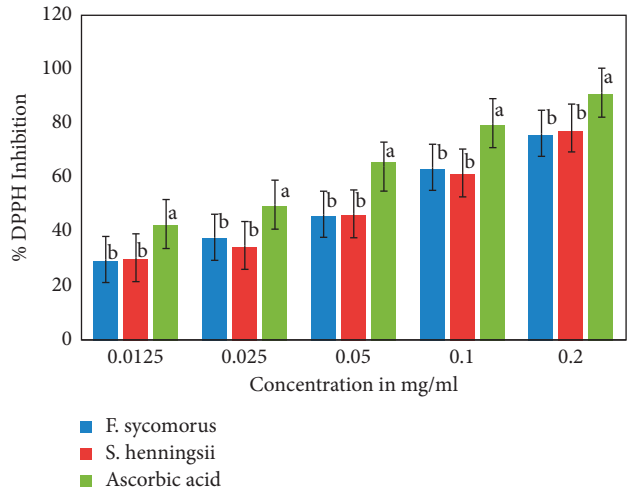


Figure 2: Percentage DPPH scavenging activities of *S. henningsii*, *F. sycomorus*, and standard ascorbic acid. Values are expressed as mean ± SEM of the three replicates. Column means followed by the same superscript letters were not significantly different ($p > 0.05$) by one-way ANOVA followed by Tukey's post hoc test.

Table 5: % in vitro DPPH radical scavenging activities of DCM extracts of *S. henningsii* and *F. sycomorus*.

	Concentrations					
Groups	0.0125 mg/ml	0.025 mg/ml	0.05 mg/ml	0.1 mg/ml	0.2 mg/ml	IC_{50} value
Ascorbic acid %	42.84 ± 1.05^a	50.00 ± 1.85^a	64.06 ± 1.75^a	80.13 ± 2.06^a	91.39 ± 1.38^a	0.025 mg/ml
S. henningsii %	30.38 ± 1.61^b	34.93 ± 2.42^b	46.60 ± 1.49^b	61.71 ± 1.69^b	78.33 ± 1.65^b	0.068 mg/ml
F. sycomorus %	29.73 ± 2.21^b	37.94 ± 1.15^b	46.45 ± 2.74^b	63.81 ± 2.27^b	76.38 ± 1.92^b	0.062 mg/ml

Values are expressed as mean ± SEM of the three replicates. Column means followed by the same superscript letters were not significantly different ($p > 0.05$) by one-way ANOVA followed by Tukey's post hoc test.

Table 6: IC_{50} values of DCM extracts of *S. henningsii* and *F. sycomorus*.

	Hydrogen peroxide	DPPH
S. henningsii	0.330 mg/ml	0.068 mg/ml
F. sycomorus	0.325 mg/ml	0.062 mg/ml
Ascorbic acid	0.245 mg/ml	0.025 mg/ml

studied to provide alternative answers to the diverse stress-related disorders. Arguably, plants tend to offer better alternatives for varied sources of medicinal remedy including antioxidants.

Based on phytochemical studies, the yield and the antioxidant activity of a plant extract depend on the selected extraction solvent [65–67]. Different solvents are employed to isolate different antioxidant compounds based on their disparities in polarities [68]. In this study, dichloromethane solvents were used as a midpolar solvent for extraction of polyphenols and other midpolar phytochemicals. Several assays (DPPH radical scavenging assay, hydroxyl radical assay, hydrogen peroxide radical scavenging assay, ferric reducing power, and total determination of flavonoid and phenolic contents, among others) have been developed for the determination of in vitro nonenzymatic antioxidant activities of medicinal plants [69, 70].

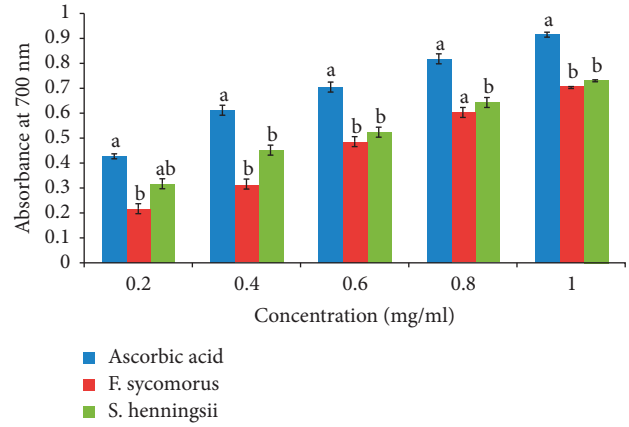


Figure 3: Analysis of ferric reducing power of *S. hennningsii*, *F. sycomorus*, and the standard ascorbic acid. Values are expressed as mean ± SEM of the three replicates. Column means followed by the same superscript letters were not significantly different ($p > 0.05$) by one-way ANOVA followed by Tukey's post hoc test.

Flavonoids and phenols naturally exhibit strong scavenging abilities for free radicals due to their hydroxyl groups [71, 72], which are attached to their aromatic ring structures and help to quench the radicals either by donating their electrons thus neutralizing them or via the electron

Table 7: In vitro ferric reducing power activities of the DCM extracts of *S. henningsii* and *F. sycomorus*.

	Concentrations				
Groups	0.2 mg/ml	0.4 mg/ml	0.6 mg/ml	0.8 mg/ml	1.0 mg/ml
Ascorbic acid (nm)	0.427 ± 0.02^{a}	0.612 ± 0.02^{a}	0.705 ± 0.04^{a}	0.864 ± 0.02^{a}	0.915 ± 0.02^{a}
S. henningsii (nm)	0.317 ± 0.04^{ab}	0.452 ± 0.02^{b}	0.524 ± 0.02^{b}	0.643 ± 0.02^{b}	0.730 ± 0.02^{b}
F. sycomorus (nm)	0.217 ± 0.04^{b}	0.316 ± 0.04^{b}	0.486 ± 0.02^{b}	0.603 ± 0.02^{b}	0.703 ± 0.02^{b}

Values are expressed as mean ± SEM of the three replicates. Column means followed by the same superscript letters were not significantly different ($p > 0.05$) by one-way ANOVA followed by Tukey's post hoc test.

delocalization over all three ring systems achieved by ortho-dihydroxy of the B-ring and 4-oxo group of the ring C of the flavonoid, which actively reduce radicals such as DPPH and Fe^{3+} to Fe^{2+} ions [73, 74]. Thus, polyphenols directly augment the antioxidant potential through the restoration of redox balance [75].

In most plant extracts, there is a positive correlation between antioxidant activity and the amount of polyphenolic compounds [76, 77]. However, some studies have reported that there is no positive relationship between the polyphenolic compounds and their antioxidant activities [78, 79]. The total phenolic and flavonoid content of the DCM stem bark extract of *F. sycomorus* was lower than that of the leaf extract of *S. henningsii* at all the tested concentrations. This partly explains why *S. henningsii* had a better result against % DPPH radical scavenging activities and ferric reducing power assay (Figures 1 and 2) than *F. sycomorus*. Nevertheless, the difference in polyphenolic content between the two extracts was not statistically significant against hydrogen peroxide scavenging abilities (Figure 3). In this study, the antioxidant activity observed against hydrogen peroxide, DPPH radicals, and FARP were due to the presence of phenolic and flavonoid components. Therefore, a positive correlation was noted between the amount of polyphenolics and the antioxidant activity.

4.2. Statistical Analysis. Hydrogen peroxide (H_2O_2) is a harmless and less reactive molecule, which becomes harmful, toxic, and reactive to the cell when it is converted to hydroxyl radical [80, 81]. Hydroxyl compounds are among the most deleterious ROS produced by mitochondria, causing oxidative damage, and are clinically linked to causes of various stress-related disorders [82]. Thus, the removal of H_2O_2 is a critical step for maintaining a functional antioxidant defense system in cells or food systems [83, 84].

DCM leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* showed significant antioxidant activity against hydrogen peroxide in a dose-associated trend. Sirisha et al. [85] also demonstrated similar in vitro antioxidant activity while working on the methanolic leaf extract of *F. carica*. The dose-dependent activities of both extracts showed that an increase in the concentration of the extract increased the levels of bioactive antioxidant compounds [86]. Similarly, studies conducted on different *Ficus* species by Ahoua et al. reported the species exhibit strong hydrogen peroxide antioxidant activity in a dose-dependent manner [87]. The strong scavenging potential of the extracts is reflected in their low IC_{50} value. A lower IC_{50} is normally associated with a higher radical scavenging activity [88].

The findings of this study showed that both *S. henningsii* and *F. sycomorus* extracts had low IC_{50} of 0.330 mg/ml and 0.325 mg/ml, respectively, against the H_2O_2 radicals. Thus, the low IC_{50} values obtained from the study indicate that the two extracts have strong H_2O_2 scavenging activities. The IC_{50} value obtained for *F. sycomorus* was similar to that obtained by Deo et al. [89] working on some selected herbal extract inhibitory properties against protein glycation and angiotensin enzyme linked to type II diabetes. Additionally, *P. amarus* and *L. pumila* var. alata medicinal plants have been shown to possess potent radical inhibiting properties with low IC_{50} values of 3.4 and 5.7 μg/ml, respectively [90].

The potential of plant extracts to inhibit the DPPH radical is strongly linked to their ability to donate electrons to the radical [46, 91]. Normally, DPPH radical is stable in various solvents including methanol, ethanol, and water. Therefore, the radical is usually prepared in a solution of either ethanol or methanol [70, 92]. The results obtained in this study showed dose-dependent DPPH scavenging activities of the two extracts. It was, however, noted that the DCM leaf extract of *S. henningsii* and *F. sycomorus* stem bark extract had lower DPPH scavenging abilities than ascorbic acid [93]. This could be due to the crude nature of extract as compared to the refined standard drug. This result corresponded to the observations of Igbinosa et al. [94] who found that *Jatropha curcas* had lower DPPH activities than ascorbic acid (standard).

The results of the stem bark extract of *F. sycomorus* agree with a study by Kambli et al. [95], who found that the DPPH scavenging activity of *F. racemosa* was considerable but not higher than that of the standard drug. The good antioxidant property of *F. sycomorus* stem bark extract against DPPH corroborates well with the findings of Santiago and Mayor [96], who noted that *F. odorata* had a good antioxidant activity against DPPH radicals. The DCM leaf extract of *S. henningsii* also showed a good antioxidant activity against DPPH. However, studies conducted by Oyedemi et al. [48], while working on the stem bark extract of *S. henningsii*, reported a weaker antioxidant activity against DPPH radicals and attributed it to low levels of flavonoids in the stem bark extract compared to that of the leaf. The potential antioxidant activities of the DCM extract of *S. henningsii* and *F. sycomorus* against DPPH radicals in this study can be positively related to their higher total phenolic ($R^2 = 0.9782$) and flavonoid ($R^2 = 0.9778$) compounds (Tables 2 and 3). This higher antioxidant activity of the two extracts was a reflection of the lower IC_{50} values obtained (0.062 mg/ml

and 0.068 mg/ml, Table 1). In DPPH assay, it has been strongly suggested that samples with lower IC_{50} values of 50 μg/ml are very strong antioxidants, while with a range of 50–100 μg/ml are strong antioxidants, and with values above 150 μg/ml are weaker antioxidants [97].

The potential of the two extract samples to reduce Fe^{3+} to Fe^{2+} via electron donation was determined by the amount of Fe^{2+} complex generated and measured in terms of the absorbance of Perl's Prussian blue colour at 700 nm wavelength [98]. The change of yellow colour of the test solution to various shades of green and blue indicated the reducing power of the extract [99]. The phytochemicals in an extract either directly bind the metal ions or indirectly suppress their chelating reactivity by occupying their coordination sites [100, 101]. The ultimate outcome of the reduction reaction in the antioxidant defense system is to terminate the radical chain reactions, which may otherwise be very detrimental to tissues.

It was evident that increased extract concentrations increased the ferric reducing power of the two DCM extracts. Basically, as a result of more Fe3+ being reduced to Fe2+ as more electrons were being donated by antioxidant components [102, 103]. High absorbance is an indicative of the increased ferric reducing ability of the extracts. Both extracts were, however, found to have a lower reducing capacity at all the tested concentrations compared to ascorbic acid, the reference compound. This observation was starkly different from the one done by Daniel and Dluya [46] in which they demonstrated that the methanolic stem bark extract of *F. sycomorus* had a higher ferric reducing power than ascorbic acid (reference drug) which could be the case of different solvents used. Nevertheless, the findings correlate with one done by Ahoua et al. [87] on eight different *Ficus* species and it was noted that the majority had significantly lower reducing power against the ascorbic acid.

5. Conclusions and Recommendations

The findings of this study demonstrate that the DCM extracts of *S. henningsii* and *F. sycomorus* possess alkaloids, phenols, saponins, cardiac glycosides, flavonoids, and steroids while *F. sycomorus* contains saponins, flavonoids, alkaloids, steroids, phenols, cardiac glycosides, and terpenoids. The total phenolic and flavonoid content of the DCM stem bark extract of *F. sycomorus* was lower than that of the leaf extract of *S. henningsii*. The said phytochemicals possess antioxidant activity. The DCM extracts of *S. henningsii* and *F. sycomorus* significantly exhibited strong radical scavenging activities against hydrogen peroxide and DPPH solution at different concentrations used. The IC_{50} values of *S. henningsii* and *F. sycomorus* were 0.325 mg/ml and 0.330 mg/ml for hydrogen peroxide and 0.068 mg/ml and 0.062 mg/ml for DPPH, respectively. Both the DCM leaf extract and stem bark extract of *S. henningsii* and *F. sycomorus* were found to have strong ferric reducing power at all the tested concentrations. Therefore, both extracts exhibited significant nonenzyme-based antioxidant activities.

Therefore, the DCM leaf extract of *S. henningsii* and stem bark extract of *F. sycomorus* studied plants can be potential antioxidant compound sources and alternatives for the management of oxidative stress. In addition, studies aimed at investigating the in vivo antioxidant efficacy of the studied plant extracts are encouraged.

Abbreviations

ANOVA: Analysis of variance
DCM: Dichloromethane
DPPH: 2,2-Diphenyl-1-picrylhydrazyl
ROS: Reactive oxidative species
SEM: Standard error of the mean
GAE: Gallic acid equivalent
RE: Rutin equivalent.

Data Availability

The data used to support the findings of this study are provided in this article. However, any additional information can be provided by the corresponding author upon request.

Ethical Approval

All the reagents used in this study were prepared, used, and disposed of according to the set laboratory guidelines and the material safety and data sheets (MSDS).

Disclosure

This article is part of the Kenedy Wanjala Wafula Thesis (Wanjala) [104], and no preprint version had been deposited in a journal in any form.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Kenedy Wanjala Wafula, Joseph Kiambi Mworia, and Mathew Piero Ngugi designed the project, collected the specimen, performed the experiments, analyzed and interpreted data, and read and approved the manuscript for publication.

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