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Total Phenolic Content, Antioxidant and Antibacterial Properties of *Scurrula ferruginea* Extracts

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Graphical abstract

Abstract

Discovery of new therapeutic agents from nature, especially plants is one of the promising approaches for treatment of various diseases. In traditional medicine *Scurrula ferruginea* is applied to treat some disorders. To the best of our knowledge, there are no investigations on antioxidant capacity and antimicrobial activities of *S.ferruginea* in Malaysia. The present study was conducted to determine total phenolic content, Fe_2^+ chelating activity, antioxidative and antimicrobial potential of flowers, leaves and stems of *S.ferruginea* extracts. Antioxidant capacity, and total phenolic content of extracts were evaluated using DPPH free radical scavenging and Folin-Ciocalteu assays. Antibacterial properties were evaluated by disc diffusion, minimum inhibitory concentration and minimum bactericidal concentration methods. Results indicated the highest total phenolic content for stem extract (309.069). All *S. ferruginea* extracts exhibited antioxidant activity in a dose dependent manner. Stem extract showed capacity to scavenge free radicals and it was also found to chelate Fe_2^+ better than others. All extracts presented moderate inhibition ability against selected bacteria. The most significant values of MIC and MBC were belonged to the stem extract. These findings suggest that acetone extracts of *S. ferruginea,* particularly stem extract, are potentially sources of antioxidant compounds.

Keywords: *Scurrula.ferruginea*; antioxidant and antimicrobial activities; DPPH; total phenolic; chelate Fe_2^+; MIC; MBC

Abstrak

Penemuan agen terapeutik baru dari alam semulajadi terutamanya tumbuhan adalah satu pendekatan yang menjanjikan rawatan untuk pelbagai penyakit. Dalam perubatan tradisional, *Scurrula ferruginea* telah digunakan untuk merawat sesetengah gangguan. Setakat pengetahuan kami, tiada siasatan ke atas kapasiti antioksidan dan aktiviti antimikrob ke atas *S. ferruginea* di Malaysia. Kajian yang dilakukan kini telah dijalankan untuk menentukan jumlah kandungan fenolik, aktiviti penggumpalan Fe_2^+, potensi sebagai antioksidan dan antimikrob ekstrak bunga, daun dan batang S.ferruginea. Kapasiti antioksidan dan jumlah kandungan fenolik ekstrak telah dinilai menggunakan pemerangkapan radikal bebas DPPH dan ujian Folin-Ciocalteu. Ciri-ciri antibakterial telah dinilai menggunakan kaedah pembauran cakera, nilai kepekatan rencatan minimum dan kepekatan bakterisida minimum. Keputusan menunjukkan jumlah kandungan fenolik tertinggi untuk ekstrak batang (309.069). Kesemua ekstrak *S. ferruginea* mempunyai aktiviti antioksidan yang boleh dalam cara penggantungan dos. Ekstrak stem menunjukkan kapasiti untuk memerangkap radikal bebas dan didapati turut menggumpal Fe_2^+ dengan lebih baik daripada yang lain. Kesemua ekstrak mempunyai kebolehan rencatan sederhana terhadap sesetengah bakteria. Nilai ketara untuk MIC dan MBC ditunjukkan oleh ekstrak stem. Penemuan ini mencadangkan ekstrak aseton *S. ferruginea*, terutamanya ekstrak batang adalah sumber sebatian antioksidan yang berpotensi.

Kata kunci: *Scurrula.ferruginea*; aktiviti antioksidan dan antimikrob; DPPH; jumlah fenolik; gumpalan Fe_2^+; MIC; MBC

1.0 INTRODUCTION

Nowadays,various methods of disease treatment had been established using chemical drugs. Some of these therapies are less effective due to side effects and toxicity of drugs [1]. Traditional herbs as main source of medicinal products have attracted special interest of people due to survive a healthy life. Use of herbal medicine amongst patients, particularly cancer patients, have been dramatically increased and being popular because of safety and mode of action [2]. Medicinal plants play an important role in the development of new potential drugs. Recently, researchers have shown an increased interest in the investigation of new phytochemical agents, in particular anticancer agents from plant derived natural products [3]. Therefore, the discovery of new therapeutic agents from natural products is one of the promising approaches for various disease treatments. As Malaysia is a biodiversity rich country, numerous species of medicinal plants have been used for treatment of illnesses by folklore for decades [4].

It has been reported that there are approximately 1800 species of higher plants locate in diverse rainforest of Malaysia in which around 10% possess medicinal properties [5]. Mistletoe is one of the herbal medicines, with a long history in the treatment of many illnesses such as skin infection, smallpox cough and diabetes [6]. Also, as an anthroposophical medicine, it is one of the most important herbal drug which is potentially efficacious against cancer and it has been used as a complementary medicine by folklores of south Asia and Europe for centuries to treat cancer [7,8,9]. Mistletoe is an evergreen semi parasitic shrub form the family of *Loranthaceae* with various species that grows on branches of different deciduous trees (Figure 1).

Approximately 1500 species of mistletoe have been identified in the world [10]. Most of the studies on mistletoe as a candidate complementary drug for cancer therapy have been performed by European researchers, especially scientists in Germany which they carried out their preclinical and clinical experiments by using different commercial and standardized product of *Viscum album* [11]. *The Viscum album extract* has been shown to induce tumor regression by cell cycle apoptosis and activating cell death pathways. Furthermore, it can induce secretion of pro-inflammatory cytokines such as Tumor necrosis factor α, IL 1 and IL6 [7, 12].

Figure 1 Mistletoe (*S.ferruginea*) on host tree

So far, however, there has been little research about other species of mistletoe especially Southeast mistletoe. In comparison to other types of mistletoe, not much attention has been paid to *Scurrula ferruginea* which belong to the family of *Loranthaceae* [13]. Mistletoe species are locally called dedalu and mainly distributed in tropical countries like Singapore, Malaysia, India and Indonesia [14]. *S. ferruginea* is known as one of medicinal plants which has been widely used in traditional medicine as therapeutic herbs. Preparation from leaves, stems and flowers of *S. ferruginea* were used by local people for treatment of high blood pressure, hypertension and gastrointestinal malfunction [15].

A study on *S. ferruginea* was undertaken by O.Z.Ameer *et al*., investigated the effect of different extracts of plant on blood pressure reduction. It has been demonstrated that methanol extract of *S. ferruginea* show the most lowering blood pressure activity. Their findings provide direct evidence on blood pressure lowering activity of *S. ferruginea*. They also found open new possibilities to isolate and analyze the biologically active substances of this plant, which can be used for treatment of high blood pressure in human [14].

Another phytochemical study has been conducted on *S. ferruginea* by Francoise L.D *et al*. Natural flavonols compounds, including quercetin, quercitrin, and glycoside 4-O acetylquercitrin were isolated from the ethyl acetate extract. In addition, cytotoxic evaluation on different cancer cell lines was carried out. It was shown that quercetin possess the most potent cytotoxic activity on U251 (human glioblastoma cells cancer cell line) with IC_{50} of 35μm. The findings demonstrated quercetin as an active flavonol which could be developed as an anticancer agent [15].

Although there have been some reports on therapeutic potential of members of *Loranthaceae* family (mistletoe) particularly *S. ferruginea* elsewhere but not many studies regarding the antioxidant and antibacterial activities of this species in Malaysia have been reported. Therefore, the present study was carried out to examine antioxidant capacity, total phenolic content and iron chelating ability of S. *ferruginea* extracts and compare different parts of plant (leaf, flower and stem) in terms of its potential antioxidant activity and phenolic content that are beneficial for medicinal purpose. Furthermore, the acetone extracts obtained from different parts of the plant were evaluated for antibacterial preliminary screening. To our knowledge, far too little scientific evidence and no exploratory research has been done to investigate the antioxidant properties and antibacterial activity of *S. ferruginea*.

2.0 EXPERIMENTAL

2.1 Material and Methods

2.1.1 Plant Materials

Fresh aerial parts including leaves, stems and flowers of *S. ferruginea* were collected from campus of Universiti Teknologi Malaysia (UTM), Skudai, Malaysia. Plant materials were taxonomically authenticated by a qualified botanist in Institute of Bioproduct Development (IBD), UTM.

2.1.2 Crude Extracts Preparation

The plant materials were cleaned of any extraneous material and were cut and dried in oven at 50ºC. The dried samples were ground into powdered form at room temperature. The powdered materials were soaked and suspended in solution of acetone and water (8:2 v/v) for 3 days at room temperature with vigorous shaking. The aqueous extracts were then filtered by filter papers. The extraction of samples was further repeated 3 times under the same condition. All filtrated extracts were mixed and the excess solvent was vaporized using a rotary evaporator (BUCHI, SWETZERLAND, and R210) in a water bath at 50ºC. Dried

extracts colors were between dark green and dark brown. The crude extract was weighed and kept at refrigerator (4ºC).

2.1.3 DPPH Free Radical Scavenging Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity method was obtained from Poh-Hwa.T, *et al*. with some modifications [16]. DPPH refers to market accessible organic nitrogen radicals showing gloomy purple color at 515-517 nm wavelength absorbances. Yellow color will be produced after reducing DPPH due to scavenging of free radicals while progression of process. 200 µL of different concentrations of mistletoe extracts (range of 1000 µg/mL to 7.81µg/mL) in methanol were mixed with 3 mL of DPPH methanolic solution resulted to 0.2 mM concentration of DPPH. The mixture was shaken thoroughly and left in dark place for 30 minutes in room temperature. Ascorbic acid (Vitamin C) and suitable extracting buffer have been used as a positive and negative control respectively.

The absorbance determined using spectrophotometer at 517 nm. The entire measurement was done in triplicates. The percentage of DPPH scavenging concentration was calculated using equation (1). Ac refers to absorbance of control and As refers to absorbance of sample at 517 nm. IC_{50} value provides the concentration of sample which is required to scavenge 50% of 2,2-diphenyl-1-picrylhydrazyl free radicals.

$$Ac - As \times 100 / Ac \quad (1)$$

2.1.4 Determination of Total Phenolic Content (TPC)

Total phenolic content was measured using Folin–Ciocalteu or Gallic acid equivalence (GAE) method referring to Poh-Hwa.T, *et al.* [16]. Folin-Ciocalteu reagent is the combination of phosphotungstate and phosphomolybdate applied for measurement of total phenolic antioxidants in colorimetric *in vitro* assay. 1 mL of Folin-Ciocalteu reagent (dilution 1:10 with deionized distilled water) was added to 200 µL of mistletoe extracts and after 5 minutes 800 µL 7.5% Na_2CO_3 (sodium carbonate) was included into the mixture followed by gentle vortexing. The mixture was then incubated for 30 minutes at room temperature in the dark. The absorbance of solvents then measured using UV/VIS spectrophotometer at 760 nm wavelength. Some procedures were applied to prepare standard solution of Gallic acid in order to preparation of standard curve. All determinations were applied at triplicates and the outcomes were shown as means ± SD.

2.1.5 Fe_2^+ Chelation Activity

The ferrous ions (Fe_2^+) chelating ability of *S.ferruginea* extracts was determined using modified method of Carter (1971) [17, 18]. Briefly, 25 µL 1 mM iron (II) chloride ($Fecl_2$) were added to different concentrations of samples. 75 µL of 3 mM ferrozine was added to mixture after 30 minutes of incubation at room temperature. EDTA was used as control in the assay. The absorbance of samples was then measured at 570 nm using the spectrophotometer. The Fe_2^+ chelation capacity of the extracts was determined based on equation (2), where Ac indicated control absorbance and as indicated sample absorbance.

$$\%\ Fe_2^+ \text{ chelation} = Ac - As \times 100 / Ac \quad (2)$$

2.1.6 Growth and Maintenance of Bacteria

Four bacteria species including two Gram positive (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 29737) and two Gram negative (*Escherichia coli* ATCC 10536 and *Pseudomonas putida* ATCC 49128) were employed as test microorganisms to evaluate potential antibacterial activities of all extracts. All strains were maintained at the Bioassay Laboratory, Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia (UTM). All bacterial isolates were kept on agar slant at 4°C prior to analysis.

2.1.7 Antimicrobial Preliminary Screening; Disc Diffusion Method

The antibacterial activity screening of plant extracts was carried out using disc diffusion assay. Selected bacterial strains were cultured using nutrient agar plate prior to inoculating in nutrient broth medium. The culture suspensions of each bacterium (400 µL) were swabbed gently on the surface of Muller-Hinton agar medium using glass rod. Standard streptomycin sulphate disc as positive control and DMSO disc as negative control were placed on nutrient agar medium surface. A 10 µL of each crude extracts were pipetted into whatman paper disc. Sterilized paper discs containing sample were pressed on agar. All plates were then incubated for one day at 37°C in the inverted position. The diameters of zones of inhibition of growth were measured in order to assess antibacterial activities of crude extracts. This test was performed three times.

2.1.8 Determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC values were measured using method described by Poh-Hwa *et al.* [16] with minor modifications. To perform this experiment, 14.5 mg of leaf, stem and flower crude extracts were separately dissolved in 2.0 mL of DMSO (with sample concentration 1800 µg/mL). Serial dilution of samples with concentrations range from 1800 to 14.07 µg/mL were carried out. Analysis were performed using the 96 well-microtiter plate where 100 µL of sample stock was added in first two rows and 100 µL nutrient broth which used as diluents followed by addition of bacteria into all rows.

The plates of different samples were then incubated overnight at 37°C. The last clear well was determined as MIC value which is the lowest concentration that had no microscopic visible growth. In order to determine the MBC valve, 10 µL of solution from last clear well containing mixture of extracts and inoculums were distributed on the surface of nutrient agar in petri dish and were spread gently. MBC was determined as the lowest concentration at which more than 90% of bacteria were killed.

3.0 RESULTS AND DISCUSSION

3.1 DPPH Free Radicals Scavenging Assay

DPPH free radicals scavenging was used to measure the antioxidant capability of leaf, stem and flower crude extracts *of S. ferruginea*. 2,2-Diphenyl-1-picrylhydrazyl is the major chemical used in this antioxidant assay which is able to be reduced to diphenylpicrylhydrazine after exposing to plant extracts having antioxidant compounds. The process can be measured using UV-VIS spectrophotometer at absorption 517 nm.

IC_{50} value shows the amount of sample required to reduce 50% of the initial amount of DPPH and it is the main parameter to

determine the antioxidant activity [19]. Higher antioxidant activity is related to lower IC_{50} value. The comparative antioxidant activity of stem, leaf and flower acetone extracts of *S. ferruginea* assessed by DPPH method is shown in Figure 2. All samples were active as antioxidants based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. There were a small difference in amount of antioxidant activity in stem, leaf and flower extracts of *S.ferruginea*. The best IC_{50} value was gained from stem followed by leaf and flower crude extracts.

Based on DPPH radical scavenging activity analysis, all crude extracts of *S.ferruginea* represented antioxidant activity. The results of study indicated that increased concentrations resulted in enhancing the scavenging capacity of samples. Broad variation of antioxidant activity may be possibly attributable to the presence of wide range of biologically active components like tannins, phenols, carotenoids, flavonols and some other compounds [20]. Numerous studies carried out on several species of mistletoe from different geographical locations reported various degrees of scavenging capacity due to differences in harvesting time of the plants and nature of host trees [22]. The antioxidant effect of ethanolic extract of European mistletoe (*Viscum album*) has been evaluated by scientists in Romania. The value obtained by the DPPH method was 7.2% [23]. In other experiment, using DPPH free radical scavenging activity assay, methanol extracts of *Viscum* leaves exhibited considerable scavenging capacity. Methanolic extract of mistletoe grown on lime tree in summer exerted highest value of DPPH inhibition (95.12%). This experiment have shown that the antioxidant properties are varied according to harvesting time [22]. Similar finding were obtained in Romania, where researchers examined the antioxidant properties of aqueous extracts of European mistletoe (*V. album*) using DPPH method and reported various amounts of scavenging capacity with respect to the host tree and harvesting time. They observed the best antioxidant activity with aqueous extract of *Robinia pseudocacia* [24]. Further *in vitro* study using FRAP method have demonstrated antioxidant capacity of acetone and methanol extracts of European *Viscum album* stems and leaves from various host trees. Highest scavenging effect was reported for methanolic extract of *V.album* leaves from *Malus domestica* host tree. Their results proved that antioxidant potential of *V. album* extracts differ depending on host trees [25]. Water and ethanol extracts of *V. album* leaves and stems from different host trees harvested at various season time were subjected to free radical scavenging capacity examination. The ethanol extracts recorded highest value as scavenger for free radical (77.19%). Mistletoe growing on *R. pseudoacacia* showed highest activity against free radicals in different seasons (July: 11.49, May: 10.97, December: 2.72). Leaves extracts exhibited higher antioxidant activity than stems extracts [26]. In recent years, African scientists have investigated the influence of several host trees on African mistletoe. In a study performed in Nigeria, effect of cocoa and cashew host trees on antioxidant properties of *V.album* were studied using many methods. The results analysis represented a significant free radical scavenging activity of *V. album* in a dose dependent manner (0-10mg/mL). *V.album* collected from cocoa exerted better antioxidant capacity than mistletoe from cashew tree [27].

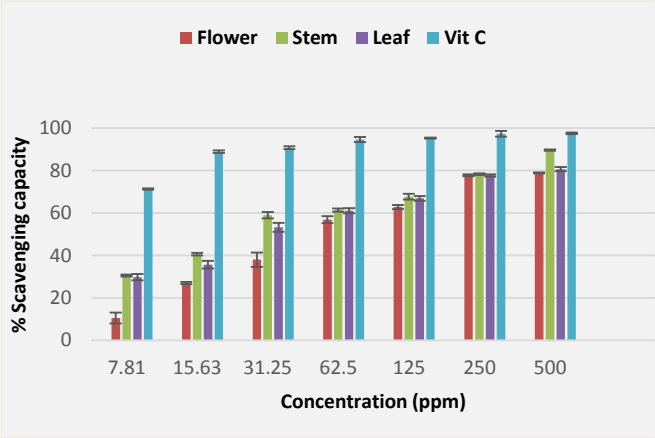

Figure 2 Antioxidant activity of acetone extracts from different parts of *S. ferruginea*

Further *in vitro* study assessed antioxidant properties of *V. album* growing on cola (*cola nitida*) tree. Dose dependent pattern (0.1-100 mg/mL) scavenging capacity of mistletoe extracts were recorded with experiment. Their results suggested that leaves of Nigerian mistletoe could served as a good natural source of antioxidants [28].

Stem extracts of *Dendrophthoe falcate* (known as Indian mistletoe) were examined for total antioxidant capacity using different *in vitro* models. Results analysis indicated that free radical scavenging ability of methanolic extract (IC_{50} 18 µg/mL) is better than aqueous extract (IC_{50} 26µg/mL). The study finding demonstrated that the antioxidant properties of *D.falcare* might be due to presence of phenolic compounds [29]. The comparative antioxidant properties of *Loranthus europaeus* hosted by oak tree evaluated by DPPH method reported significant antioxidant activity of methanolic extracts of mistletoe. Higher DPPH scavenging effect was observed in twigs and stems methanol extracts with values of 92% and 88% DPPH inhibition [30].In another study investigators examined Korean mistletoe for its antioxidant properties. The hot water and ethanol extracts of *Viscum album L* exerted DPPH scavenging ability assayed by DPPH *invitro* model.(% DPPH inhibition: 62.55% and 72% for hot water and ethanol extracts respectively) [33].

Presence of flavonol compounds may therefore contribute to the high antioxidant activity. Various antioxidant properties of mistletoe species have been reported which depends on host trees, geographical location and time of harvesting [21]. Based on our knowledge there is no report on antioxidant activity of different parts of *S. Ferruginea*.

3.2 Total Phenolic Content

Total phenolic content is an assay commonly applied to evaluate the quantity of phenolic compounds which are present in plant extracts. Gallic acid is mostly used as a standard for measurement of total phenolic compounds of various plants since this compound stands for the simplest form of phenolic acid. The results of assay will be presented using gallic acid equivalent. Color changes will then be shown depends on the various forms of phenolic compounds presents in the plant material. The changes in the color of the solution containing extracts in the presence of Folin-ciocalteu were measured using spectrophotometer. As shown in Figure 3, the amount of total

phenolic content was calculated according to equation from Gallic acid standard curve.

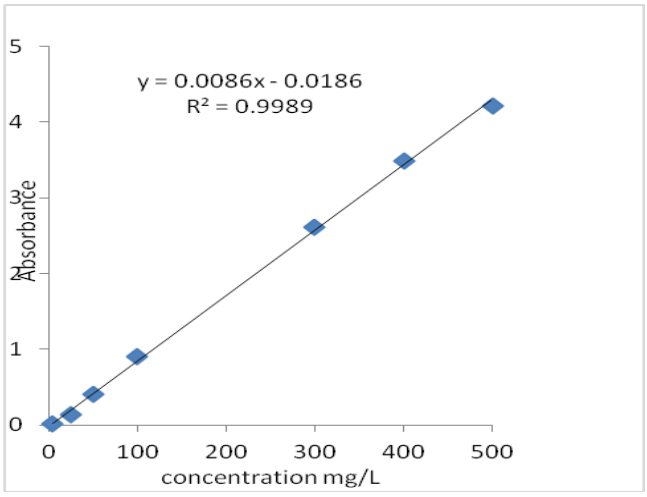

Figure 3 Gallic acid standard curve

The results expressed as mg of Gallic acid equivalents per gram [31]. The results of TPC assay (Table 1) exhibited that all leaf, stem and flower extracts of *S.ferruginea* contains considerable amounts of phenolic compounds which among these three extracts, stem crude extract possess higher levels of phenolic content followed by leaf and flower crude extracts respectively. The similar orders of ranking were also obtained for DPPH radical scavenging assay. Recently, numerous researchers reported a high relation between potential antioxidant capacity and total phenolic content of several species of mistletoe plants [20]. As overall, according to results obtained by DPPH scavenging activity and total phenolic content methods, stem crude extract showed higher antioxidant activity compared to other crude extracts. In a study carried out in Greece, using aerial tissues of *L.europeaus*, total phenolic content of ethyl acetate and methanol extracts were measured according to Folin-Ciocalteu method. Various amounts of phenolic content was observed in extracts ranged from 61 to 320 mg galic acid g^{-1} tissue.

Twigs methanol and ethyl acetate extracts showed highest value of phenolic content while methanol and ethyl acetate flower extracts presented lowest phenolic content [30]. Assessment of total content of phenol of Indian mistletoe using Folin-ciocalteu assay was performed by Indian researchers. Gallic acid was used as a standard phenolic compound. The results obtained indicated that methanolic extract of *Dendrophthoe falcata* possessed higher phenolic content (1.5 mg/g) comparative with aqueous extract (1.1 mg/g) [29]. African investigators examined total phenolic content of *Viscum album* isolated from cocoa and cashew using tannic acid as standard phenolic compound. Content of total phenolic was higher in cocoa *V. album* (182 mg/100 g) extract than cashew mistletoe (160mg/100g) [27]. Further study evaluated influence of batch reactor extraction on total phenolic content of Indonesian mistletoe (*Scurulla atropurpurea*) was conducted in Indonesia.

A better results of total phenolic content was recorded with batch reactor extraction system than conventional method. Best value of phenol compound reported for 30% ethanol solvent extraction (15.2 mg equivalent gallic acid/1 g mistletoe) [31].

Table 1 Total phenolic content of *S.ferruginea* stem, leaf and flower acetone extracts

Plant material	Total phenolic content
Leaf	144.217±0.66
Stem	309.069±1.15
Flower	126.379±0.26

Other study also investigated content of phenolic compound in stems and leaves of European mistletoe hosted by 5 different host trees. Lowest and highest values of phenolic concentration was recorded for acetone and methanol extracts respectively. *V. album* leaves extracts collected from *Acer campostre* exerted highest total phenolic content (0.55 mg GAE/g fresh weight). The study finding demonstrated that total phenolic content of mistletoe extract have a great contribution to antioxidant activity [25].

Recently, researchers in Romania performed a comparative study to examine total phenolic content of European mistletoe from various host trees harvested in 3 different months (May, July, and December). Concentration of phenolic compound were higher in aqueous extracts of *V.album*. Leaves extracts presented higher level of phenols compared to stems extracts. Best value of phenolic compound was reported for *V. album* hosted by *R. pseudoacacia* harvesting in May. *V. album* extract hosted by *Fraxinus excelsior* showed lowest amount of phenolic compound [26].

3.3 Iron Chelating Activity

The metal chelating capacity of extracts from *S.ferruginea* stems, leaves and flowers were estimated by assessment of their ability to compete with ferrozine for ferrous ions. Red color complex can be formed upon reaction of ferrozine and Fe_2^+. Red color formation can be reduced resulted by disruption of complex due to presence of other chelating agents in samples. In order to evaluate the chelating activity, rate of color reduction need to be measured. As shown in Figure 4, all extracts exhibited ability to chelate Fe_2^+ (Iron binding). Stem and flower extracts indicated higher (94.27%) and lower (16.29%) chelating activity at concentrations 2000 ppm and 125 ppm respectively. EDTA as control presented 94.27% Fe_2^+ chelation activity at concentration 10 ppm.

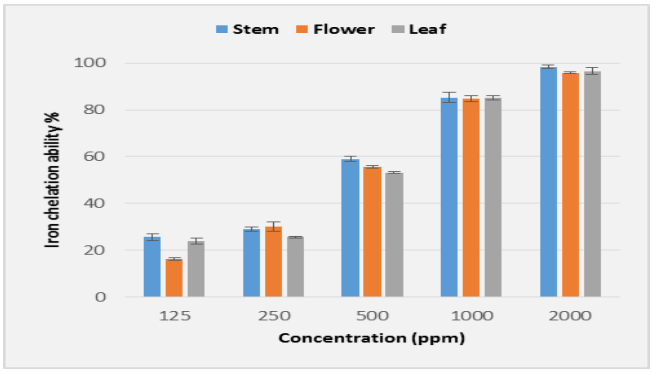

Figure 4 Fe_2^+ chelation capacity of *S.ferruginea* extracts from stem, leaf and flower

African scientists evaluated Fe_2^+ chelating ability of Nigerian mistletoe using *V. album* from two different host trees. Their results indicated that Fe_2^+ chelating capacity was enhanced with increasing extracts concentrations. The metal chelating activity was followed a dose dependent pattern. *V. album* extracts from cashew recorded greater metal chelating activity than cocoa *V.album* [27]. In other research, ethanol extracts of *V. album* collected from Europe exerted strong metal chelation capability [23].

Table 2 Antibacterial activity and diameter of inhibition zone of *S.ferruginea* leaf, stem and flower acetone extracts

	Gram negative bacteria		Gram positive bacteria	
	Zone of inhibition (mm ± SD)			
Plant crude extract	*E.coli*	*P.putida*	*S.aureus*	*B.subtilis*
Leaf	7.2±0.50	8.5±0.70	9.5±0.50	9.0±0.00
Stem	8.0±0.00	9.7±0.95	7.5±0.57	9.0 ± 0.81
Flower	7.5±1.0	9.0±0.00	8.0±0.00	8.0 ± 0.00

Table 3 Minimum inhibition concentration (MIC µg/mL) values of *S.ferruginea* extracts against Gram positive and Gram negative bacteria

Selected bacteria	Flower crude extract		Leaf crude extract		Stem crude extract	
	1	2	1	2	1	2
Staphylococcus aureus	900	450	450	450	450	450
Bacillus subtilis	900	450	450	450	450	450
Escherichia coli	900	900	900	900	450	900
Pseudomonas Putida	225	450	225	450	225	225

Table 4 Minimum bactericidal concentration (MBC µg/mL**)** values of *S.ferruginea* extracts against Gram positive and Gram negative bacteria

Selected bacteria	Flower crude extract		Leaf crude extract		Stem crude extract	
	1	2	1	2	1	2
Staphylococcus aureus	900	900	450	900	450	450
Bacillus subtilis	900	450	450	450	900	450
Escherichia coli	1800	1800	900	900	900	900
Pseudomonas Putida	450	900	450	900	225	225

3.4 Antibacterial Screening

Table 2 shows the results of the preliminary antibacterial screening. The results showed that all samples including stem, leaf and flower crude extracts possess limited range of antimicrobial activities against all tested Gram positive and Gram negative bacteria. The leaf crude extract showed the highest inhibitory zone against Gram positive bacteria whereas stem and0 flower crude extracts were observed to be more effective against *P. putida*. As overall, stem and leaf crude extracts represented similar antimicrobial activities of the selected organisms although stem crude extract recorded slightly higher antibacterial properties than leaf extract. However, the least antibacterial activity of all mistletoe extracts was observed with *E. coli*.

3.5 Determination of MIC and MBC Values

MIC values of acetonic extracts of stem, leaf and flower of *S.ferruginea* showed various results towards selected Gram negative and Gram positive bacteria. Generally, lowest MIC values observed against *P. putida* while highest values were

shown with *E.coli*. MIC values of leaf extract were approximately 450 µL against *S.aureus* and *B.subtilis*. MBC values were also observed to be similar to MIC results for these organisms. Stem extract of *S.ferruginea* recorded remarkable MIC values (approximately 225 µL) towards *P. putida*. Same MBC values were also obtained. Flower crude extract showed lowest MIC and MBC values compared to other extracts.

According to MIC and MBC results, Table 3 and 4, it was clearly observed that stem extract possessed higher antibacterial properties among all extracts. Based on obtained results of this study it could be seen that all crude extracts of *S.ferruginea* consist of high content of potential antibacterial compounds to inhibit *P. putida* while leaf and stem extracts are also capable of inhibiting the growth of selected Gram positive bacteria. Gram negative bacteria can be differentiated from Gram positive by having specific outer membrane include phospholipids and lipopolysaccharides instead of having cell wall peptidoglycan layer. This unique membrane acts as a protector against particular antibiotics [16].

Lee *et al.* (2013) carried out an antibacterial assay on hot water extract of Korean mistletoe against 5 bacterial strains (*S.aureus, E. coli, K. pneumonia, P. aeruginosa and S. typhimurium*). Their results represented that hot water extract of Korean *V.album* is more effective on Gram negative microbial strains than Gram positive with antimicrobial activity of higher than 300 µg/mL. Antimicrobial assay were also performed for ethanol extract of Korean *V.album* by same researchers. No antibacterial activities were demonstrated by any of the 5 bacterial strains [33].

In another experiment Costa *et al.* (2010), observed the antibacterial activity of *Phthinus pyrifolia* leaf lectin (PpyLL) on *S.aureus, S.epidermidis, S.faecalis,* and *B.subtilis* as Gram positive bacterial species and *P.aeruginosa* and *K.pneumoniae* as Gram negative strains. PpyLL was reported to be more active against Gram positive bacterial strains than Gram negative. Indeed most experienced pathogenic bacterial species were reported to be affected by PpyLL antimicrobial properties [34].

Methanolic, ethanolic and *n*-hexane extracts of the leaves of Nigerian mistletoe were also be studied for the measurement of antimicrobial properties against 12 microbial strains (*E. coli, K. pneumonia, P. aeruginosa, Proteus mirabilis, S. typhi, Shigella dysenteriae, Bacillus cereus, E. faecalis, S. aureus, S.faecium* and *Strep. Pyogenes*). The results of study demonstrated that Nigerian *V.album* can be considered as a capable source of antimicrobial agents with the highest activity on *S.faecium* [28].

Moreover, antibacterial activity analysis of present study makes it reasonable to further explore for existence of cytotoxic biologically active compounds in *S.ferruginea*. Numerous types of anticancer agents have been found among several mistletoe species which belong to *Loranthaceae* family [15].

■4.0 CONCLUSION

According to the best of our knowledge this is the first report on the antioxidant properties and antibacterial activity of *S. ferruginea*. The obtained results suggest that antioxidant capacity of *S. ferruginea* slightly differ depending on the plant part used. Stem extract exhibited higher phenolic content and antioxidant and metal chelation activities based on Folin–Ciocalteu, free radical scavenging and metal chelation assays compare to other extracts. The overall free radical scavenging activity might be attributed to the presence of phenolic compounds in the extracts. *S.ferruginea* crude extracts showed moderate antibacterial activity against selected Gram positive and Gram negative bacterial cultures**.** Preliminary findings of the study suggests that *S.ferruginea* extract can be considered as a new source of antioxidant agents. Currently work is under way to confirm the antioxidant activity of various parts of *S. ferruginea*, using other types of solvents. In addition, more *in vitro* study investigating the effect of *S. ferruginea* extracts on breast cancer cell lines is under progress. Further detailed investigations on isolation and identification of biologically active compounds and anticancer agents are required for using *S.ferruginea* as a new therapeutic medicinal plant in cancer care.

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