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Antioxidant potential of extracts from *Ehretia amoena* - A Swazi medicinal plant

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ABSTRACT

Ehretia amoena belongs to the Boraginaceae family. *E. amoena* finds several therapeutic applications in traditional medicine. Nonetheless, its usage in treating human trypanosomiasis (sleeping sickness) and diabetes mellitus is astonishing. The objectives of the present study were to evaluate the antioxidant potential, determine the IC_{50} value, determine total phenolic contents (TPCs), and determine total flavonoid contents (TFCs) of various solvent extracts from the leaves and stem-bark of *E. amoena.* Various solvent extracts were obtained by combining maceration and hot solvent extraction techniques. DPPH radical scavenging assay was used to evaluate the antioxidant potential and to determine the IC_{50} values. Folin-Ciocalteu and aluminium chloride colorimetric methods were employed to estimate the TPCs and TFCs, respectively. At a concentration range of 200-3000 µg/mL, the DPPH radical scavenged by various extracts from leaves and stem-bark and the positive control (ascorbic acid) was in the ranges of 22.00±0.20-65.00±0.10%, 20.85±0.20-63.04±0.30% and 55.50±0.20-85.20±0.20%, respectively. Similarly, the IC_{50} values of various extracts from leaves and stem-bark were in the ranges of 691.80- >3000µg/mL and 560.45- >3000µg/mL, respectively. The IC_{50} value of the positive control was <200 µg/mL. The ethyl acetate extract showed lowest IC_{50} value of 691.80µg/mL among the extracts from leaves and the acetone extract showed lowest IC_{50} value of 560.45µg/mL among extracts from stem-bark. The TPCs of various extracts from leaves and stem-bark were in the ranges of 1.22±0.02-6.61±0.04 and 1.48±0.01-7.45±0.01mg GAE/g DW, respectively. The TFCs of various extracts from leaves and stem-bark were in the ranges of 17.7±0.02-90.0±0.02 and 31.2±0.05-81.6±0.08mg QE/g DW, respectively. We concluded that various leaves and stem-bark extracts from *E. amoena* showed a moderate to significant radical scavenging activity and possessed a moderate to higher TPCs and TFCs. The ethnomedicinal use of *E. amoena* is supported from this findings and antioxidant-based therapeutics can be developed.

Keywords: *Ehretia amoena*, Boraginaceae, antioxidant activity, DPPH radical scavenging assay, IC_{50} values, total phenolic contents (TPCs), total flavonoid contents (TFCs).

1. INTRODUCTION

Ehretia amoena belongs to the Boraginaceae (Ehretiaceae) family of the genus *Ehretia* (Rabaey et al., 2010; Maroyi, 2021; Gottschling and Hilger, 2024a; Prem and Rosalin, 2024). This *Ehretia* genus has approximately 150 species and these species are distributed primarily in Africa, Asia, Australia and North America (Retief and Van Wyk, 2001; Mainen and Zakaria, 2002; Gottschling and Hilger, 2003; Gottschling and Hilger, 2004b; Hester, 2006; Kaur, 2024; Mandal and Joshi, 2014; Sultana, 2014; Welcome and Van Wyk, 2019). *E. amoena* is known by its vernacular names such as Mutepe, Sandpaper Bush, Shombe, Skurweblaarbos, Stamper Wood, Stamperhout, and Umklele-omkhule Mkirika (Mainen and Zakaria, 2002). *E. amoena* is mainly distributed in the southern part of the African countries which include Eswatini (Swaziland), Mozambique, South Africa, Namibia, Zambia, Zimbabwe, Kenya, Malawi, Tanzania, and Uganda (Maroyi, 2021). *E. amoena* is a deciduous shrub and it grows to 5 meters in height (Maroyi, 2021). *E. amoena* is widely distributed in alluvial and sandy soils, forests, bushveld, thickets, along the watercourses, woodland, termite mounds, and at medium and low altitudes (Maroyi, 2021). *E. amoena* has grey-brown to dark grey coloured bark, ovate or elliptical-shaped leaves, white to pale mauve coloured flowers and ovoid-shaped edible fruits (Maroyi, 2021; Welcome and Van Wyk, 2019). All parts of *E. amoena* possess medicinal properties (Freiburghaus et al., 1996; Sempompe, et al., 2014; Maroyi, 2021) and therefore, this plant finds several applications in traditional medicine to treat various diseases (Freiburghaus et al., 1996; Sempompe, et al., 2014; Maroyi, 2021). Nevertheless, the usage of *E. amoena* in treating human trypanosomiasis (sleeping sickness) and diabetes mellitus is widespread in southern African countries (Freiburghaus et al., 1996; Mainen and Zakaria, 2002; Maroyi, 2021). For example, in Uganda, the leaves and the aqueous concoctions obtained from the roots are used in the management of Human African Trypanosomiasis (HAT) (Freiburghaus et al., 1996; Mainen and Zakaria, 2002). In Tanzania, the roots of *E. amoena* have been used by traditional healers for the treatment of overt non-insulin dependent diabetes mellitus (NIDDM) symptoms such as polydypsia, excessive thirst, urine that is attracted by insects, and polyuria (Mainen and Zakaria, 2002). Additionally, the leaves of *E. amoena* are sold in the Tanzanian herbal markets as traditional herbal medicine due to their striking medicinal properties (Posthouwer et al. 2018; Maroyi, 2021).

In general, decoctions, concoctions or infusions are obtained from leaves, bark, root, root-bark, stem, stem-bark and fruits of *E. amoena* and are employed in the traditional medicine to treat various diseases (Chhabra, et al., 1984; Chhabra et al., 1987; Nahashon, 2013; Maroyi, 2021) which include anthelmintic diseases, venereal diseases, skin diseases, malaria, typhoid, diabetes mellitus, pneumonia, fever, bilharzia, wounds, genital ulcers, sleeping sickness, muscle pain, abdominal pain, menstrual and gastro-intestinal problems, rheumatism, sexually transmitted infections, menorrhagia, polymenorrhoea, gonorrhea, vomiting, dysentery, hypertension, hernia, convulsions and epilepsy (Chhabra, et al., 1984; Chhabra et al., 1987; Nahashon, 2013; Maroyi, 2021). Various classes of compounds were reported previously from the leaves and roots of *E. amoena*, which include flavonoids, saponins, steroids, tannins, terpenoids, anthoquinones, polysaccharides, polyuronoids, and volatile oils (Raz et al., 1996; Chhabra et al., 1984; Maroyi, 2021). Additionally, a few flavonoid compounds were also isolated in the pure state from the leaves and roots of *E. amoena* (Sara et al., 2004; Maroyi, 2021). Our literature search revealed that *E. amoena* has not investigated for DPPH radical scavenging activity. Particularly, *E. amoena* collected from the Kingdom of Eswatini has not investigated so far. Therefore, in the present study, we aimed to evaluate the DPPH radical scavenging potential, and to determine the IC_{50} values of pentane, dichloromethane, ethyl acetate, acetone, and methanol crude extracts obtained separately from the leaves and stem-bark of *E. amoena* collected in the Kingdom of Eswatini. Additionally, we also determined the total phenolic contents (TPCs) and total flavonoid contents (TFCs) of the above extracts. The results are discussed in this article. To the best of our knowledge, this is the first report of this kind, particularly, the *E. amoena* species collected in the Kingdom of Eswatini.

2. MATERIALS AND METHODS

Collection of plant materials

Plant materials of *E. amoena* (leaves and stem-bark) were collected in November 2024 from the forest at Mhlangatane in the area of Hhohho, Kingdom of Eswatini. Dr. M. N. Dludlu from the Department of Biological Sciences, University of Eswatini (UNESWA) identified the plant materials. Samples for leaves (NSLS2024) and stem-bark (NSSB2024) were kept separately at Chemistry Project Laboratory (CPL).

Processing of plant materials

The plant materials were air-dried for four weeks at CPL and then ground into fine powder using a laboratory grinder (MRC Laboratory Equipment, Model KM 1500). The masses of the powder obtained were 444.68 and 493.09g for leaves and stem-bark, respectively.

Preparation of plant extracts

A mass of 60.34g of powdered leaves was macerated with pentane for 24 hours at room temperature at 27-29°C. The solvent was decanted, filtered, and vacuum distilled. The pentane crude extract thus obtained in this maceration technique was kept aside in a previously weighed clean beaker. The marc recovered from the maceration process was extracted again with pentane under reflux conditions for 8 hours. The solvent was decanted, filtered, and vacuum distilled as previously. The pentane crude extract thus obtained in this hot solvent extraction was combined with the previously obtained crude extract from the maceration technique. A total mass of 0.67g of combined pentane crude extract was obtained from the powdered leaves. The aforementioned process was repeated with the other solvents such as dichloromethane, ethyl acetate, acetone, and methanol. From the masses of 60.19, 60.135, 60.320, and 60.325g of powdered leaves, respectively, 1.27, 1.50, 1.29, and 2.81g of dichloromethane, ethyl acetate, acetone, and methanol crude extracts were obtained. Similarly, from the masses of 60.32, 60.63, 60.41, 60.34, and 60.34g of powdered stem-bark, respectively, 0.39, 0.56, 0.69, 0.52, and 2.96g of pentane, dichloromethane, ethyl acetate, acetone, and methanol stem-bark crude extracts were obtained.

Chemicals, reagents and solvents

Unless specified otherwise, chemicals, solvents, and reagents used in this study were all AR grade. Solvents such as pentane, dichloromethane, ethyl acetate, acetone, and methanol were obtained from Promark chemicals. Ascorbic acid, tris- hydrochloric acid buffer, and 2,2- diphenyl-1-picrylhydrazyl were also purchased from Promark chemicals. Quercetin, tannic acid, Folin-Ciocalteu reagent, and sodium carbonate were purchased from Associated Chemical Enterprises (ACE). Sodium phosphate was obtained from Glass World. Sodium nitrite was purchased from Rochelle Chemicals. Sodium hydroxide was purchased from MCB Laboratory and Medical Suppliers.

Free radical scavenging activity of extracts

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is one of the commonly employed methods for evaluating the antioxidant activity of plant extracts. DPPH is a stable free radical, and its methanol solution is purple. When DPPH radical encounters hydrogen donors from a plant extract, the purple colored methanol solution reduced to yellow colored DPPH-H neutral molecule. In other words, DPPH acts as oxidant and the plant extract acts as antioxidant. DPPH assay was performed as per methods described in literature (Pillai et al., 2019; Mpopo et al., 2021; Pillai and Mthimkhulu, 2024; Pillai and Samkelisiwe, 2024; Pillai and Simelane, 2025). Stock solutions of each extract and positive control (ascorbic acid) were prepared separately by dissolving 3.0mg of extract or ascorbic acid in 1.0mL of 50% methanol. Further dilutions such as 3000, 2000, 1500, 1000, 800, 500, and 20µg/mL were also prepared separately from each one of the above stock solutions. A volume of 100mL of 0.1mM DPPH solution was prepared by dissolving 3.94mg of DPPH in methanol, which served as an oxidant. A solution of 50mM phosphate-buffered saline (PBS) served as a buffer with pH 7.4. The buffer has been used to maintain a stable pH and to ensure the solubility of DPPH radical in the reaction mixture, which in turn causes maximum absorbance (Sharma and Bhat, 2009; Maura et al., 2013; Sascha and Herbert, 2014). The reaction mixture consisted of 20µL of extract solution (or positive control), 200µL of 0.1 mM DPPH solution, and 90µL of 50mM PBS buffer at pH 7.40. The absorbance of the resulting reaction mixture was measured using a UV-Vis spectrometer (Infinite M200) at 517nm after 30 minute incubation in a dark cabinet. The afore-mentioned procedure was utilized to measure the absorbance of negative control but without extract solution or positive control. As such, the negative control exhibited maximum absorbance due to the presence of a large number of DPPH radicals. In the presence of extract or positive control, the DPPH radicals are reduced to DPPH-H neutral molecules due to the availability of hydrogen donors from the extract or positive control. For the same reason, lower values of absorbance are observed in the presence of extract solutions or positive control and it depends on the concentrations. Lower values of absorbance indicate higher concentrations of extract solutions or positive control and *vice versa*. In other words, a lower value of absorbance indicates the higher free radical scavenging (antioxidant) activity of an extract or positive control and *vice versa*. In the present study, the measurement of absorbance values were conducted in triplicates

and the average of three values were used to calculate the percentage inhibition of radical scavenging ability which was done by the equation given below (Matamane et al., 2020; Mokoroane et al., 2020).

DPPH Radical Scavenged (%) = [(A of control – A of test)/A of control] × 100
A of test = Absorbance of extract solution or positive control.
A of control = Absorbance of negative control.

The half-minimal inhibition concentration (IC_{50}) value (i. e. the concentration in µg/mL of an extract or pure compound that inhibits the formation of DPPH radical by fifty percent) can be determined from a graph by plotting extract concentrations (in x-axis) against the percentage inhibition of DPPH radical (in y-axis).

Determination of total phenolic contents (TPCs)

The Folin-Ciocalteu colorimetric method was used to determine the TPCs of various extracts from leaves and stem-bark of *E. amoena* as per details given in the literature (Selepe and Pillai, 2022; Pillai et al., 2023a; Pillai et al., 2023b). Gallic acid served as a standard to get a calibration curve. A stock solution of gallic acid was prepared by dissolving 1.0g of gallic acid and 1.0mL of 50% methanol *(v/v)*. Further dilutions such as 5, 10, 20, 40, 80, 100, and 200µg/mL were prepared from the stock solution. A 50% methanol (*v/v*) served as a negative control. Similarly, the test solutions of each extract were prepared by dissolving 1.0mg of sample in 1.0mL of 50% methanol (*v/v*). The Folin-Ciocalteu reagent was prepared in water at a ratio of 1:4 (*v/v*). A reaction mixture consisted of 50µL of sample and 50µL of Folin-Ciocalteu reagent. The reaction mixture was then incubated for 5 minute, and then 5.0mL of 7.5% sodium carbonate solution was added to this reaction mixture. The resulting reaction mixture was then incubated for 30 minutes at 40°C in the dark. The absorbance value of the resulting mixture was measured against 50% methanol solution at 760nm. Three experiments were conducted for each extract and the average of the three absorbance values was used for the calculation of the TPCs of each extract. . Similarly, the absorbance of the standard was measured by the procedure as mentioned above. A calibration curve y = 0.0117xx - 0.029; R^2 = 0.9925 was obtained for the standard by plotting various concentrations versus their absorbance (Figure 1). This calibration curve was used to estimate the total phenolic contents of the extracts and was expressed as milligrams of gallic acid equivalence per gram dry weight of the extract (mg GAE/g DW).

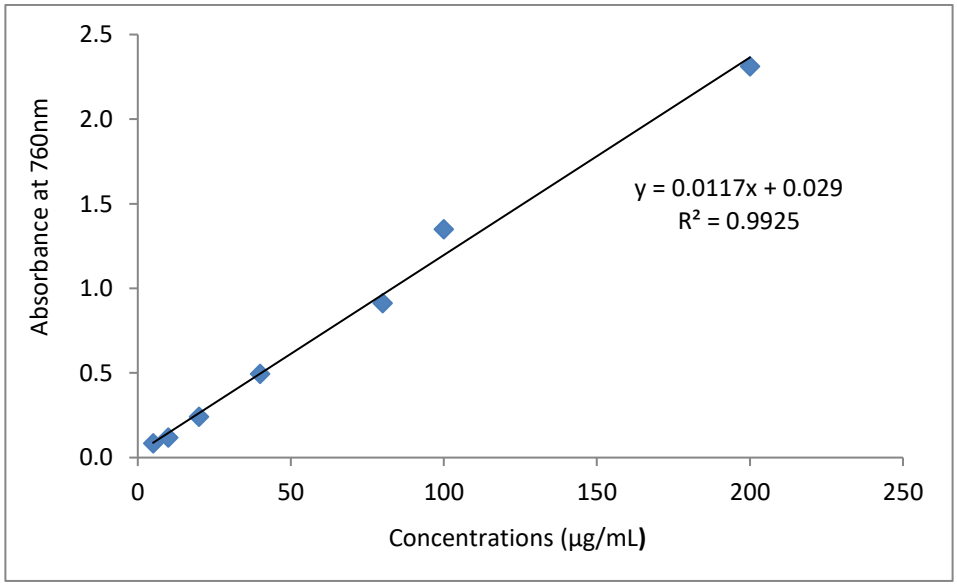

Figure 1: The calibration curve of gallic acid used to estimate the TPCs of various extracts.

Determination of total flavonoid contents (TFCs)

The Total flavonoid contents (TFCs) of various extracts obtained from the leaves and stem-bark *E. amoena* were determined using the aluminium chloride colorimetric method (Selepe and Pillai, 2022; Pillai et al., 2023a; Pillai and Thebe, 2023b). Quercetin was used as a standard and a calibration curve of the standard was obtained as described in the literature (Sharma and Bhat, 2009). A stock solution of standard was prepared by dissolving 1.0mg of quercetin in 1.0mL of 50% of methanol. Further dilutions such as 5, 10, 40, 80 and 100µg/mL were prepared from this stock solution. Similarly, stock solutions of each extract were prepared separately by dissolving 1.0mg of sample in 1.0mL of 50% methanol. A 50% methanol (*v*/*v*) solution without extract solution served as a negative control. A reaction mixture containing 150µL of extract sample and 150µL of sodium nitrite was allowed to react for five minutes. To this mixture, 150µL of 10% aluminium chloride, 200µL of 1.0M sodium hydroxide, and 600µL of water were added. The resulting mixture was then incubated for 30 minute in the dark at room temperature. The absorbance value of each one of the above reaction mixtures was then measured against 50% methanol solution at 510nm using a UV-Vis spectrometer (Infinite M200).

Three experiments were conducted for each extract and the average of the three absorbance values was used for the calculation of the TFCs of each extract. Similarly, the absorbance of the standard was measured by the procedure as mentioned above. A calibration curve y = 0.0003x+0.051; R^2 = 0.9909for standard was obtained for the standard by plotting the various concentrations versus their absorbance (Figure 2). This calibration curve was used to determine the TFCs of multiple extracts and was expressed in milligrams of quercetin equivalence per gram dry weight of the extract (mg QE/g DW).

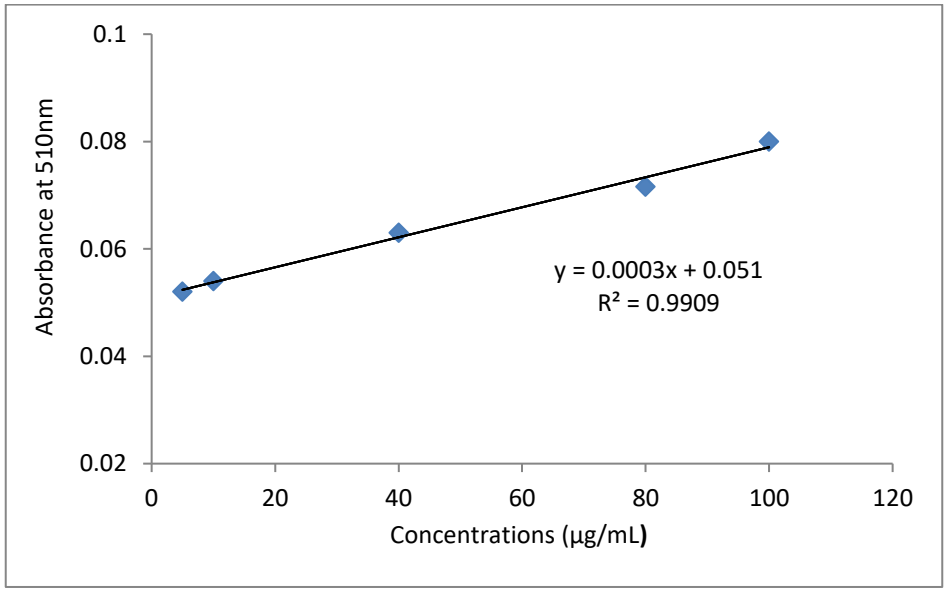

Figure 2: The calibration curve of quercetin used to estimate the TFCs of various extracts.

Statistical analysis

STATISCA software version 8.0 was employed for statistical analysis. The difference between means was statistically significant when $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Pentane, dichloromethane, ethyl acetate, acetone, and methanol crude extracts obtained from leaves of *E. amoena* were labeled as L1, L2, L3, L4, and L5, respectively. Similarly, pentane, dichloromethane, ethyl acetate, acetone, and methanol crude extracts obtained from the stem-bark of *E. amoena* were labeled as S1, S2, S3, S4, and S5, respectively. The percentage of radical scavenging capacity of these ten extracts and ascorbic acid (positive control) was evaluated at concentration of 200, 500, 800, 1000, 1500, 2000, and 3000µg/mL and the results are listed in Table 1. In general, the percentage of radical scavenging potential of these extracts showed a linear relationship with concentrations. In other words, the percentage of radical scavenging potential increased with an increase in the concentrations of the extracts. At 3000µg/mL, L1-L5 showed scavenging potential of 33.00±0.20, 36.80±0.30, 63.0±0.11, 65.00±0.10 and 61.00±0.20%,

respectively. The positive control showed a scavenging potential of 85.20±0.20% at 3000µg/mL (Table 1). Therefore, L4 (acetone extract) showed highest scavenging potential followed by L3, L5, L2, and L1 at 3000µg/mL.The scavenging potential of L4, L3, and L5 were also comparable to one another. Extracts L1 and L2 showed lower scavenging activity compared to L4, L3 and L5. Extract L1 showed the lowest scavenging activity among the extracts obtained from the leaves (Table 1).

Table 1: Percentage inhibition of DPPH radical scavenging activity of various extracts obtained from the leaves and stem-bark of *E. amoena*.

Extracts	Concentrations (µg/mL)/Inhibition (%)							IC_{50} (µg/mL)
	200	500	800	1000	1500	2000	3000	
L1	22.00±0.20^{a}	24.00±0.40^{a}	25.30±0.30^{a}	27.40±0.10^{a}	29.10±0.20^{a}	32.10±0.30^{a}	33.00±0.20^{a}	>3000
L2	22.00±0.70^{b}	24.00±0.30^{b}	26.00±0.40^{ab}	29.00±0.40^{ab}	31.21±0.10^{a}	34.90±0.40^{a}	36.80±0.30^{a}	>3000
L3	39.03±0.60^{a}	49.93±0.18^{ab}	53.39±0.10^{ab}	55.42±0.10^{ab}	58.16±0.10^{bc}	61.20±0.10^{c}	63.00±0.11^{d}	691.8
L4	40.00±0.20^{ab}	46.15±0.30^{ab}	52.14±0.10^{a}	54.00±0.20^{a}	56.50±0.10^{b}	60.00±0.20^{c}	65.00±0.10^{d}	775.5
L5	45.00±0.20^{a}	47.00±0.10^{a}	49.20±0.30^{a}	52.00±0.20^{a}	55.45±0.20^{a}	59.00±0.90^{a}	61.00±0.20^{a}	870.1
S1	20.85±0.20^{a}	25.39±0.20^{a}	28.37±0.30^{a}	33.16±0.30^{ab}	35.06±0.10^{b}	39.12±0.20^{ab}	45.63±0.70^{ab}	>3000
S2	33.20±0.11^{ab}	35.03±0.10^{a}	37.10±0.30^{ab}	39.52±0.10^{bc}	42.74±0.40^{c}	45.00±0.30^{ab}	49.00±0.20^{c}	>3000
S3	34.48±0.50^{c}	45.30±0.70^{b}	47.20±0.10^{ab}	53.10±0.40^{ab}	55.20±0.40^{a}	57.90±0.60^{d}	62.00±0.10^{e}	905.36
S4	45.70±0.40^{b}	49.30±0.14^{ab}	52.50±0.13^{ab}	55.90±0.18^{ab}	58.90±0.20^{ab}	60.02±0.10^{a}	63.04±0.30^{c}	560.45
S5	45.30±0.70^{d}	46.00±0.10^{bc}	48.00±0.50^{ab}	50.00±0.10^{bc}	52.50±0.30^{cd}	55.30±0.50^{ab}	61.60±0.30^{a}	1000
Asc. acid	55.50±0.20^{d}	60.40±0.80^{b}	67.60±0.14^{ab}	70.30±0.12^{ab}	75.30±0.60^{ac}	78.80±0.90^{ac}	85.20±0.20^{c}	<200

L1 = Pentane crude extract from leaves, L2 = dichloromethane crude extract from leaves, L3 = ethyl acetate crude extract from leaves, L4 = acetone crude extract from leaves, L5 = methanol crude extract from leaves, S1 = pentane crude extract from stem-bark, S2 = dichloromethane crude extract from stem-bark, S3 = ethyl acetate crude extract from stem-bark, S4 = acetone crude extract from stem-bark and S5 = methanol crude extract from stem-bark. Asc. Acid = Ascorbic acid. Values with different superscript letters are statistically different within a column.

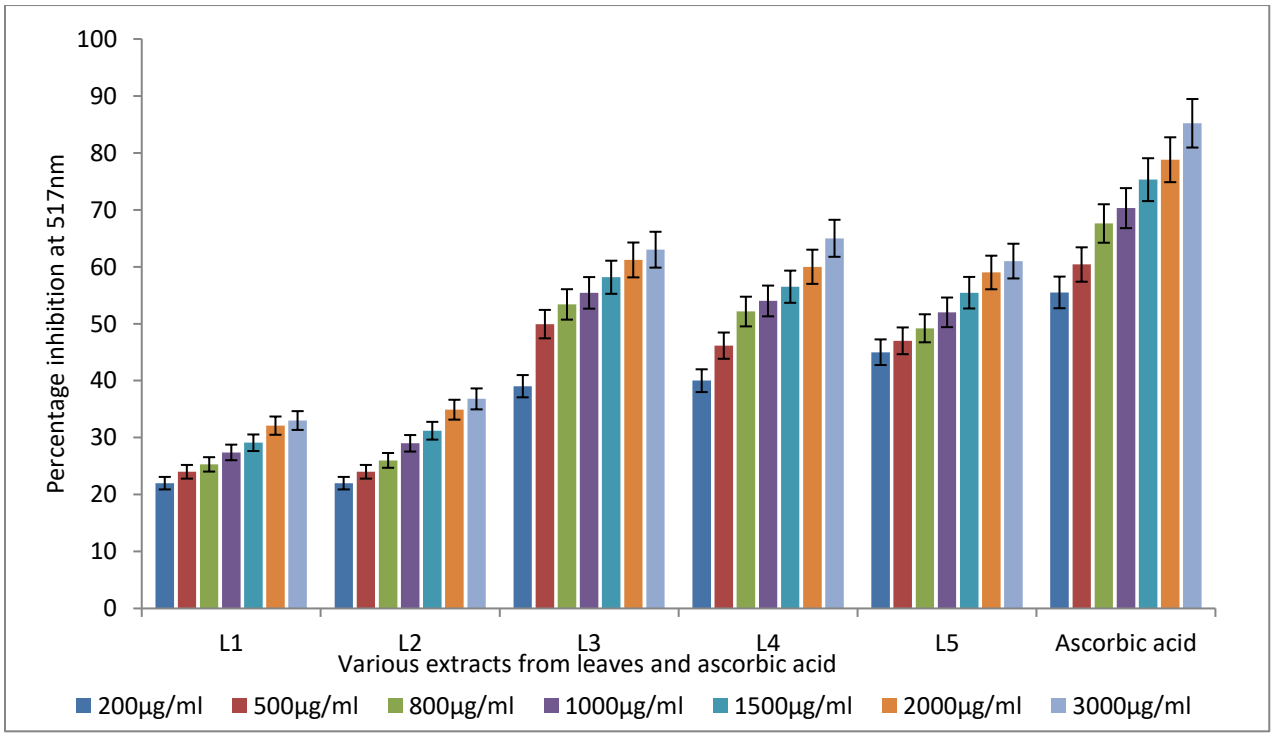

Figure 3: DPPH radical scavenging potential of various extracts from leaves of *E. amoena.* L1-L5 = Refer to the footnote of Table 1.

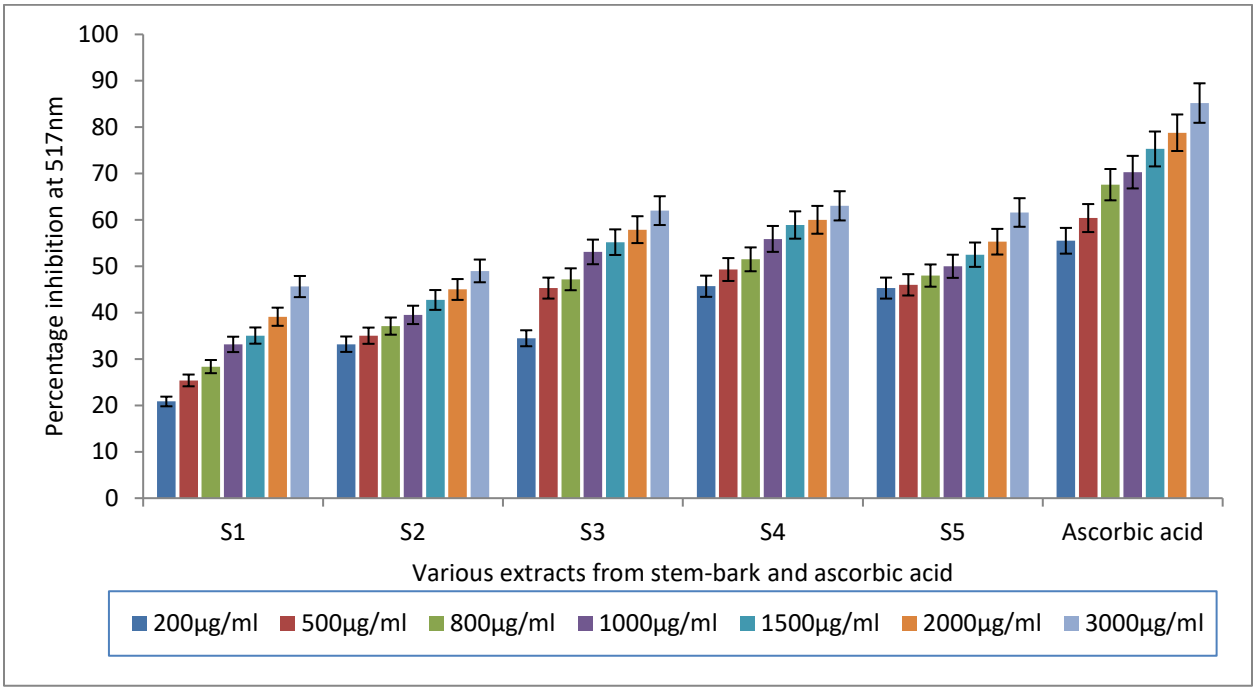

Figure 4: DPPH radical scavenging potential of various extracts from stem-bark of *E. amoena*. S1-S5 = Refer to the footnote of Table 1.

The radical scavenging potential of S1-S5 was 45.63±0.70, 49.00±0.20, 62.0±0.10, 63.04±0.30, and 61.6±0.30%, respectively at 3000µg/mL (Table 1). Therefore, at a concentration of 3000µg/mL, S4 showed the highest radical scavenging potential followed by S3, S5, S2, and S1. The radical scavenging potential of S4, S3, and S5 was comparable to one another. Extracts, S1 and S2 showed relatively lower scavenging activity compared to S4, S3, and S5. At a concentration of 3000µg/mL, S1 showed the lowest scavenging activity among stem-bark extract. In general, all ten extracts (L1-L5 and S1-S5) showed lower scavenging potential relative to the positive control at all concentrations (Table 1). However, at a concentration of 3000µg/mL, L3, L4, L5, S3, S4, and S5 showed significant scavenging potential, while S1 and S exhibited a moderate scavenging potential. Extracts L1 and L2 showed relatively lower scavenging potential. For ease of comparison, the bar graphs for scavenging potential of L1–L5 and S1–S5 together with ascorbic acid are shown in Figures 3 and 4, respectively.

The IC_{50} values for L1-L5, S1-S5, and ascorbic acid are also listed in Table 1. Ascorbic acid exhibited an IC_{50} value of <200µg/mL. The IC_{50} values for L1-L5 and S1-S5 were >3000, >3000, 691.80, 775.50, 870.10 and >3000, >3000, 905.36, 560.45, 1000.00µg/mL, respectively. The result revealed that all ten extracts (L1-L5 and S1-S5) showed relatively much higher IC_{50} values. Among the extracts from leaves, L3 showed the lowest IC_{50} value of 691.80µg/mL. In other words, L3 showed the highest scavenging potency among the extracts obtained from the leaves. Extracts L4 and L5 showed moderate scavenging potential with IC_{50} values of 775.50 and 870.10 µg/mL, respectively. Extracts L1 and L2 showed the lowest scavenging potential with IC_{50} values of >3000µg/mL for each extract. Among the extracts from stem-bark, S4 showed the lowest IC_{50} value of 560.45µg/mL. In other words, S3 showed the highest scavenging potency among the extracts obtained from the stem-bark. Extracts S4 and S5 showed moderate scavenging potential and their IC_{50} values were 905.36 and 1000.00µg/mL µg/mL, respectively. On the other hand, extracts S1 and S2 showed the lowest scavenging potential with IC_{50} values of >3000µg/mL for each extract. Overall, the IC_{50} values of all ten extracts were relatively higher compared to positive control (Table 1).

The results total phenolic contents (TPCs) of these ten extracts (L1-L5 and S1-S5) are summarized in Table 2. The calibration curve of standard (gallic acid) is given in Figure 1 and this calibration was used as standard to estimate the TPCs of various extracts. The TPCs of L1-L5 were determined to be 1.22±0.02, 2.32±0.05, 2.92±0.02, 5.80±0.06, and 6.61±0.04mg of gallic acid equivalence per gram dry weight of the extract (mg GAE/g DW), respectively. This result revealed that among the extracts from leaves, extract L5 exhibited highest TPCs followed by L4, L3, L2, and L1 (Table 2). Extracts L3, L2, and L1 showed comparable TPCs among one another. However,

these three extracts (L3, L2, and L1) showed much lower TPCs compared to L4 and L5. Extracts L4 and L5 showed comparable TPCs to each other (Table 2). The TPCs of S1-S5 were 1.48±0.01, 2.14±0.02, 2.83±0.01, 3.03±0.03 and 7.45±0.01mg of gallic acid equivalence per gram dry weight of the extract (mg GAE/g DW), respectively (Table 2). Analysis of this result revealed that among the extracts from stem-bark, extract S5 showed highest TPCs followed by S4, S3, S2, and S1 (Table 2). Extracts S4, S3, S2, and S1 showed much lower TPCs values compared to S5. However, extracts S4, S3 and S2 showed comparable TPCs among one another and S1 showed lowest TPCs among all extracts from stem-bark (Table 2).

Table 2: Determination of TPCs and TFCs of various extracts obtained from the leaves and the stem-bark of *E. amoena*.

Extracts	TPCs (mg GAE/g DW)	TFCs (mg QE/g DW)
L1	1.22±0.02	17.7±0.02
L2	2.32±0.05	67.1±0.13
L3	2.92±0.02	70.1±0.02
L4	5.80±0.06	82.1±0.02
L5	6.61±0.04	90.0±0.02
S1	1.48±0.01	31.2±0.05
S2	2.14±0.02	34.5±0.08
S3	2.83±0.01	34.9±0.05
S4	3.03±0.03	53.1±0.09
S5	7.45±0.01	81.6±0.08

L1-L5 and S1-S5 = Refer to the footnote of Table 1.

TFCs = Total flavonoid contents, TPCs = Total phenolic contents.

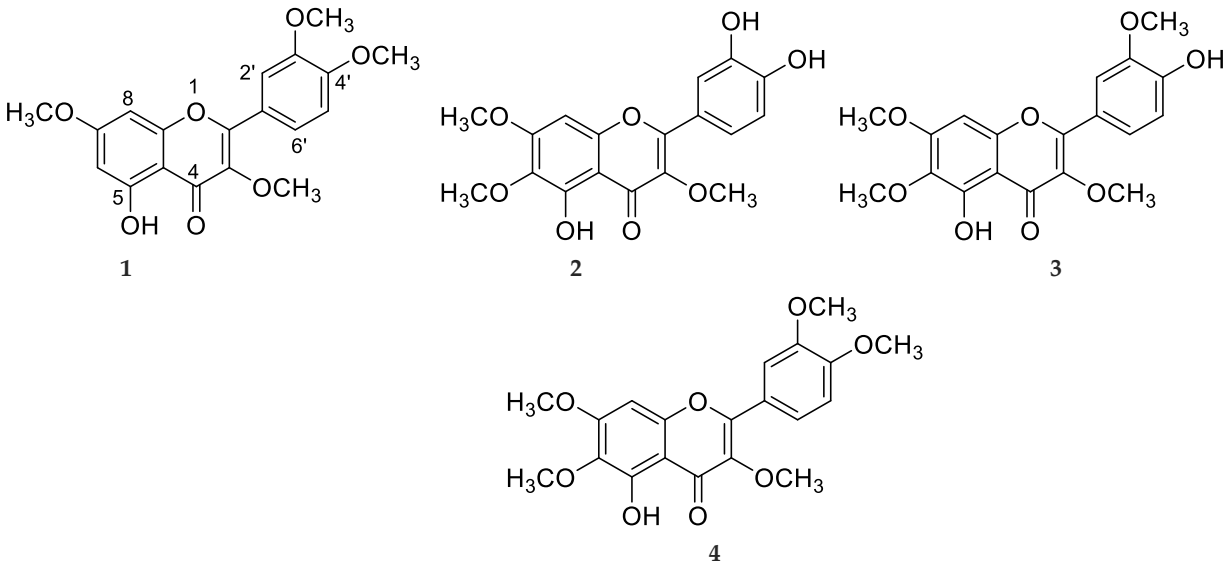

Figure 5: Structures of flavonoid compounds (**1-4**) reported previously from the leaves and roots of *E. amoena*.

Similarly, the results of total flavonoid contents (TFCs) of L1-L5 and S1-S5 are summarized in Table 2. The calibration curve of standard (quercetin) is given in Figure 2 and this calibration curve was used estimate the TFCs of various extracts. The TFCs of L1-L5 were 17.7±0.02, 67.1±0.13, 70.1±0.02, 82.1±0.02, and 90.0±0.02mg of quercetin equivalence per gram dry weight of the extract (mg QE/g DW), respectively. Analysis of this result revealed that among the extracts from leaves, extract L5 exhibited highest TFCs followed by

L4, L3, L2, and L1 (Table 2). The TFCs of L2 and L3 were comparable to each other (Table 2). Furthermore, the TFCs of S1-S5 were 31.2±0.05, 34.5±0.08, 34.9±0.05, 53.1±0.09, and 81.6±0.08 mg of quercetin equivalence per gram dry weight of the extract (mg QE/g DW), respectively. Analysis of this result also showed that among the extracts from stem-bark, extract S5 showed highest TFCs followed by S4, S3, S2 and S1 (Table 2). It was observed that extracts S3, S2, and S1 showed comparable TFCs among one another (Table 2). Extract S4 showed higher TFCs compared to S3, S2 and S1 but showed lower TFCs than S5.

As mentioned previously that *E. amoena* has not investigated for DPPH radical scavenging activity. However, the presence of various classes of compounds, including flavonoid compounds, has previously been identified from different parts of *E. amoena* **(**Raz et al., 1996; Chhabra et al., 1984; Maroyi, 2021). Additionally, flavonoid compounds such as retusin (5-hydroxy-3,7,3′,4′-tetramethoxyflavone) (**1**), chrysosplenol D (5,3′,4′--trihydroxy-3,6,7-trimethoxyflavone) (**2**), chrysosplenetin (5,4′-dihydroxy-3,6,7,3′-tetramethoxyflavone) (**3**) and artemetin (5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone) (**4**) have also been reported from the leaves and roots of *E. amoena* **(**Sara et al., 2004; Maroyi, 2021). The structures of these four flavonoid compounds (**1-4**) are given in Figure 5.

Literature search revealed that these four flavonoid compounds (**1-4**) have also been isolated and reported previously from other plants (Azizuddin et al., 2010; Gonzalez-Colomaa et al., 2012; Utkina and Kulesh, 2012; Tzy-Ming et al., 2015; Jun et al., 2017; Hyeon-Seon, et al., 2019; Dlamini et al., 2020; Minh et al., 2020). Additionally, the DPPH radical scavenging potential of these four compounds (**1-4**) has also been reported **(**Azizuddin at al., 2010; Gonzalez-Colomaa at al., 2012; Utkina and Kulesh, 2012; Tzy-Ming et al., 2015; Jun et al., 2017; Hyeon-Seon, et al., 2019; Dlamini et al., 2020; Minh et al., 2020). For example, compound **1** has been isolated from the methanolic extract of fresh fruits and rhizomes of *Amomum* **(**Minh et al., 2020) and compound **1** showed DPPH radical scavenging potential of 30.03% at a concentration of 100 µg/mL **(**Minh et al., 2020). In another report, the isolation of compound **1** from the heartwood of *Maackia amurensis* has been reported (Utkina and Kulesh, 2012) and compound **1** has been identified as the most active compound in the DPPH radical scavenging assay with an IC_{50} value of 10.9µM (Utkina and Kulesh, 2012). In another report, the radical scavenging potential of compound **1** has been assessed at two time intervals of 30 and 60 minutes and compound **1** showed a fifty percent scavenging concentration of 12.3±0.6, and 11.8±0.8 µM, respectively, in the DPPH radical scavenging assay (Tzy-Ming et al., 2015). Similarly, compound **2** has been isolated from an 85% ethanol extract obtained from the dried flowers of *Chrysanthemum morifolium* and compound **2** exhibited a fifty percent scavenging concentration of 16.22±1.80µM in the DPPH radical scavenging assay (Jun et al., 2017). In another report, the isolation of compound **2** from the chloroform fraction of 95% ethanol extract obtained from the aerial parts of *Plectranthus hadiensis* var. *tomentosus* has been reported and **2** exhibited a fifty percent scavenging concentration of 48.3±0.1µM in the DPPH radical scavenging assay (Hyeon-Seon, et al., 2019)**.** On the other hand, compound **3** has been reported from the ethyl acetate fraction of methanol extract obtained from the dried stems of *Tithonia diversifolia* (Dlamini et al., 2020) and compound **3** exhibited antiradical scavenging activity with its fifty percent inhibition concentration of 472.5µM in the DPPH radical scavenging assay (Dlamini et al., 2020)**.** Whereas, compound **4** has been reported from the chloroform fraction of methanolic extract obtained from the air-dried *Vitex agnus-castus* **(**Azizuddin et al., 2010) and compound **4** did not exhibit any scavenging activity in the DPPH radical scavenging (Azizuddin et al., 2010). Compound **4** has also been isolated from an 85% ethanol extract obtained from the dried flowers of *Chrysanthemum morifolium* and compound **4** did not show any appreciable radical scavenging potential and its fifty percent scavenging concentration has been determined to be > 500µM (Jun et al., 2017). In another report, compound **4** has been isolated from the aerial parts and roots of cultivated *Artemisia absinthium* and compound **4** exhibited a very weak DPPH radical consumption of approximately 5.0% only (Gonzalez-Colomaa et al., 2012).

4. CONCLUSION

Pentane, dichloromethane, ethyl acetate, acetone, and methanol extracts were obtained separately from leaves and stem-bark of *E. amoena*. The radical scavenging potential of extracts from the leaves and stem-bark of *E. amoena* were in the ranges of 22.00±0.02-61.00±0.02%, 20.85±0.02-63.04±0.30%, and 55.50±0.02-85.20±0.02%, respectively at a concentration range of 200-3000 µg/mL. The DPPH radical scavenging potential of positive control (ascorbic acid) was in the range of 55.50±0.02-85.20±0.02% at the same concentration range of 200-3000 µg/mL. The IC_{50} values of various extracts obtained from leaves and stem-bark were determined to be in the ranges of 691.80 **-** >3000µg/mL and 560.45**-** >3000µg/mL, respectively. The IC_{50} value of the positive control was <200 µg/mL. The ethyl acetate extract from the leaves and acetone extract from stem-bark of *E. amoena* showed lowest IC_{50} values of 691.80µg/mL and 560.45µg/mL, respectively. Additionally, the TPCs of various extracts from leaves and stem-bark of *E. amoena* were in the ranges of 1.22±0.02-6.61±0.04

and 1.48±0.01-7.45±0.01mg of gallic acid equivalent per gram dry weight of the extract (mg GAE/g DW), respectively. Moreover, the TFCs of various extracts from leaves and stem-bark were in the ranges of 17.7±0.02-90.0±0.02 and 31.2±0.05-81.6±0.08 mg of quercetin equivalent per gram dry weight of the extract (mg QE/g DW), respectively. From this study, we concluded that *E. amoena* showed a weak to moderate DPPH radical scavenging potential and possessed various classes of phytochemicals. Since *E. amoena* has been used in traditional Swazi medicine, further studies on this plant are required. Particularly, the isolation, characterization and evaluation of active compounds that are responsible for antioxidant and other therapeutic activities are recommended as future perspectives from this plant.

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Authors' Contributions
Manoharan Karuppiah Pillai: Proposed idea, supervised the research work, drafted, reviewed and edited the manuscript.
Noncedo Lomini Sithole: Conceived the idea, collected the data, performed the experiment and drafted the manuscript.
Justice Mandlenkhosi Thwala: Oversaw the research work, reviewed and edited the manuscript.

Ethical Approval
In this article, as per the plant regulations followed in the Department of Chemistry, Faculty of Science & Engineering, University of Eswatini, Kwaluseni Campus, Private Bag 4, Kwaluseni, M201, The Kingdom of Eswatini, Southern Africa; the authors observed the antioxidant potential of extracts from *Ehretia amoena*. The ethical guidelines for plants & plant materials are followed in the study for observation, identification & experimentation.

Informed Consent
Not applicable.

Conflicts of interests
The authors declare that there are no conflicts of interests.

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Data and materials availability
All data associated with this study are present in the paper.

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