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Phytochemical Analysis and Antioxidant Activity of Extracts from Berchemia zeyheri — A Swazi Medicinal Plant

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Abstract

Berchemia zeyheri belongs to the Rhamnaceae family. *B. zeyheri* has been used in the traditional medicine to treat various ailments, which include backache, cough, diarrhea, dysentery, headache, rectal ulcers, gastrointestinal issues and vomiting. The objectives of the present study were to analyze phytochemical constituents, to evaluate the antioxidant activity and to determine IC_{50} values of hexane, chloroform, ethyl acetate, acetone, and methanol crude extracts obtained separately from the leaves and stem-bark of *B. zeyheri.* Qualitative of phytochemical analysis was performed by using established methods and procedures. Various solvent extracts were obtained by means of maceration and hot solvent extraction techniques. The assessment of antioxidant activity and the determination of half-maximal inhibitory concentration (IC_{50}) values were achieved by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The presence of alkaloids, steroids, terpenoids, phenolics, tannins, flavonoids, coumarins, saponins, glycosides, carbohydrates, proteins and phlobatannins were identified in these extracts. In the DPPH assay, the positive control (ascorbic acid) showed a radical scavenging activity of 87.84±0.01 at a concentration of 3000 µg/mL. The ethyl acetate extracts obtained from leaves and stem-bark exhibited highest radical scavenging activity of 67.91±0.01% and 70.22±0.01%, respectively at a concentration of 3000 µg/mL, whilst hexane extracts obtained from leaves and stem-bark showed least radical scavenging activity of 48.88±0.04 % and 49.19±0.01%, respectively at the same concentration. The IC_{50} value of ascorbic was found to be < 200 µg/mL in the DPPH assay. On the other hand, the methanol and hexane extracts obtained from leaves showed IC_{50} values of 1513.30 and 2759.00 µg/mL µg/mL, respectively which were the lowest and highest IC_{50} values among the extracts from leaves. Similarly, the ethyl acetate and hexane extracts obtained from stem-bark showed IC_{50} values of extracts 1228.59 and 2647.28 µg/mL, respectively which were the lowest and highest IC_{50} values among the extracts from stem-bark. From this study, we conclude that various extracts obtained from the leaves of stem-bark of *B. zeyheri* possessed various classes of phytochemicals and these extracts showed a weak to moderate radical scavenging activity. Further studies on this plant are required to explore its therapeutic applications.

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1. Introduction

Known by several vernacular names such as buckthorn, buffalothorn, grubov, red ivory umNini, moye, umNeyi and umgoloty (Simpson, 2010), *Berchemia zeyheri* belongs to the Rhamnaceae family. Approximately, 52 genera and 925 plants species of are available in this Rhamnaceae family and these plant species grow mostly in temperate and tropical parts of East to Southeast Asia, Southern Africa and North America (Medan & Schirarend, 2004; Huang et al., 2005; Cheon et al., 2018). *B. zeyheri* is distributed throughout in Southern African countries (Dlamini & Geldenhuys, 2009; Dlamini & Geldenhuys, 2011; Beyene, 2015; Ulian et al., 2019), which include Botswana, Mozambique, South Africa, Swaziland and Zimbabwe. *B. zeyheri* is a deciduous small to medium sized tree which grows to 13 meter height with a stem diameter of approximately 36 cm (Maroyi, 2029). The leaves of *B. zeyheri* are opposite to sub-opposite, elliptic to ovate in shape and appeared as greyish green and lighter green above and below the leaves, respectively. *B. zeyheri* has little, unnoticeable, actinomorphic (regular and symmetric), bisexual flowers that are greenish yellow in colour and arranged in axillary clusters with a few flowers. The ovoid fruits are yellow to brownish-red in colour and juicy drupe with a solitary stone (Van Wyk, 2011). Fresh or dried fruits of *B. Zeyheri* are used to make jam and candies and as food additives (Van Wyk, 2011). *B. Zeyheri* is hardwood tree and its stems are used as fuel wood, charcoal, building materials, fences and commercial sources of timber and are used to make products such as billiard cues and knife handles. Additionally, *B. Zeyheri* is used as sources of dye, ornamental plants and herbal medicines. The foods, crafts and decorations derived from *B. zeyheri* have been a significant source of revenue for Southern African indigenous communities (Ulian et al., 2019).

Various classes of phytochemicals have been reported from various parts of *B. zeyheri,* which include alkaloids, flavonoids, glycosides, polyphenols and steroids classes of compounds (Bekker et al., 2001; Blunden et al., 2004; Blunden et al., 2006; Amusan et al., 2007; Mkhize et al., 2018). Variety of biological and pharmacological activities have been reported from various parts of *B. zeyheri,* which include anthelmintic (Blunden et al., 2004; McGaw et al., 2000; McGaw et al., 2007), antibacterial (Blunden et al., 2004; McGaw et al., 2007), cytotoxic (McGaw et al., 2000; McGaw et al., 2007) and antioxidant activities (Grace et al., 2003; Ndlala et al., 2006). In the traditional medications, the bark and roots of *B. zeyheri* have been used as ethno-veterinary medication for viral disorders in cattle (Grace et al., 2003; Masarirambi et al., 2019; Ulian et al., 2019) and as herbal remedies for anemia, backache, baby's navel issues, cough, diarrhea, dysentery, headache, rectal ulcers, gastrointestinal issues and vomiting in humans (Grace et al., 2003;

Masarirambi et al., 2019; Ulian et al., 2019). Our literature search showed that the phytochemical analysis and radical scavenging potential of *B. zeyheri* have not been explored well so far and particularly, from the species collected in the Kingdom of Eswatini. Therefore, we aimed in this current study to conduct qualitative phytochemical analysis, to evaluate DPPH radical scavenging activity and to determine IC_{50} values of various extracts obtained from leaves and stem-bark of *B. zeyheri* collected in the Kingdom of Eswatini. The results obtained from this study are summarized in this article. To the best of our knowledge, this is the first report of this kind, especially *B. zeyheri* collected in the Kingdom of Eswatini.

2. Materials and Methods

2.1. *Plant material*

Fresh leaves and stem-bark of *B. zeyheri* were gathered in May 2023 from a forest located at Dvokolwako in the Hhohho region of the Kingdom of Eswatini. Dr Dludlu, Lecturer, Department of Biology, Faculty of Science & Engineering, University of Eswatini, Kwaluseni Campus, Private Bag 4, Kwaluseni, M201, The Kingdom of Eswatini, identified the plant materials. The specimen for leaves (KHS-LS052023) and stem-bark (KHS- SB052023) were deposited at the Organic Chemistry Research Laboratory (S2.8). Department of Chemistry, University of Eswatini, The Kingdom of Eswatini.

2.2. *Processing of plant material*

The plant materials were air dried in the Organic Chemistry Research Laboratory (S2.8) at room temperature (26-27) °C for four weeks. The plant materials were then ground into powder using a laboratory scale cutting mill (KM-1500; MRC Laboratory Instruments). A mass of 296.74 and 290.19g powders of leaves and stem-bark, respectively were obtained.

2.3. *Preparation of plant extracts*

A mass of 75.28g leaf powder was macerated with 300 mL of hexane for 24 hours at room temperature and the solution was filtered using suction filtration. The resulting solution was concentrated under vacuum distillation. The hexane leaf crude extract obtained was kept in a sample vial. The procedure was repeated once again and the hexane leaf crude extract thus obtained was combined. Finally, the plant material recovered from the above maceration process was refluxed with hexane under reflux condition for 24 hours. Hexane crude leaf extract obtained in this hot extraction technique was combined with the previously obtained hexane crude leaf extract from the maceration technique. A total mass of 9.4 g of combined hexane crude leaf extract was obtained. Using the above extraction techniques and procedures, 2.25, 2.85, 3.27, 2.94 g of chloroform, ethyl acetate, acetone and methanol leaf crude extracts were obtained respectively from 55.25, 53.24, 50.25, 61.20 g of powdered leaves. Similarly, a mass of 0.22, 1.96, 2.07, 4.23, 5.53 g hexane, chloroform, ethyl acetate, acetone and methanol stem-bark crude extracts were obtained respectively from 90.43, 61.46, 51.41, 44.46, 41.44 g of powdered stem-bark.

2.4. *Solvents, reagents and chemicals*

Unless otherwise specified, all solvents and chemical were purchased from Minema. Hexane, chloroform, ethyl acetate (Promark Chemicals), acetone (Sigma-Aldrich), methanol, mercuric chloride, sodium chloride, ferric chloride, disodium hydrogen phosphate, potassium chloride, potassium iodide, potassium dihydrogen phosphate, soidium carbonate ((Promark Chemicals), disodium hydrogen carbonate, sodium dihydrogen phosphate, glacial acetic acid, sulphuric acid, hydrochloric acid, ascorbic acid (Rochelle Chemicals) and 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich) were purchased. Analytical Reagents (AR) grade solvents and chemicals were used in this study.

2.5. *Phytochemical analysis*

The qualitative phytochemical analysis on various extracts obtained from the leaves and stem-bark of*B. zeyheri* were performed as per method previously described in literature (Pillai et al., 2021) and the references therein. The qualitative phytochemical analysis such as alkaloids, steroids, terpenoids, phenols, tannins, flavonoids, coumarins, saponins, glycosides, carbohydrates, proteins and phlobatannins were conducted for each one of the extracts obtained from the leaves and stem-bark of *B. zeyheri*.

2.6. *DPPH radical scavenging activity and IC_{50} value*

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was employed to evaluate various extracts obtained from the leaves and stem-bark of *B.* zeyheri. Various solutions were prepared as per the details given in the literature with slight modifications (Pillai et al., 2018; Pillai et al., 2023). A phosphate buffered saline (PBS) was prepared by adding 8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of sodium phosphate dibasic and 0.245 g of potassium dihydrogen phosphate in 800 mL of distilled water. The pH of this buffer was adjusted to 7.4 and more distilled water was added to make up the volume as 1000 mL. A volume of 0.1 mL of each extract solution or positive control was mixed separately with 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of PBS buffer. Each of this mixture was incubated for 30 minutes and their absorbance (optical density) was measured at 517 nm using a UV-spectrophotometer (Infinite M200, Tecan US, Inc.). The percentage inhibition of radical scavenging potential of each extract and positive control was reported from the average value of the three experiments using the equation given below (Matamane et al., 2020).

$$\text{DPPH Scavenged (\%)} = [(\text{OD of Control} - \text{OD of Test})/\text{OD of Control}] \times 100$$

OD of Control = Absorbance of negative control and OD of Test = Absorbance of extract solution or positive control. The IC_{50} values of these extracts and positive control were calculated by plotting extract concentrations (in abscissa)*versus* percentage inhibition of DPPH radical (in ordinate) using Microsoft Excel.

2.7. *Statistical analysis*

Statistical analysis was performed using SPSS software versions 28.0.0.0 for DPPH radical scavenging assay. Statistical significance between means was evaluated at a 95% confidence level and the differences were statistically significant when the threshold is $p \leq 0.05$.

3. Results and Discussions

3.1. *Phytochemical analysis*

The following ten extracts (E1-E10) were prepared from the leaves and stem-bark of*B. zeyheri*: Hexane leaf crude extract (E1), chloroform leaf crude extract (E2), ethyl acetate leaf crude extract (E3), acetone leaf crude extract (E4), methanol leaf crude extract (E5), hexane stem-bark crude extract (E6), chloroform stem-bark crude extract (E7), ethyl acetate stem-bark crude extract (E8), acetone stem-bark crude extract (E9) and methanol stem-bark crude extract (E10). The qualitative phytochemical analysis on all these ten extracts (E1-E10) was performed for the presence or absence of phytochemicals such as alkaloids, steroids, terpenoids, phenolics, tannins, flavonoids, coumarins, saponins, glycosides, carbohydrates, proteins and phlobatannins. The results are summarized in Table 1.

Table 1. Phytochemical screening of various extracts obtained from leaves and stem-bark of *B. zeyheri.*

	Extracts									
Phytoconstituents	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**	**E8**	**E9**	**E10**
Alkaloids	+	+	+	+	+	+	-	+	+	+
Steroids	+	+	+	+	+	+	-	+	-	+
Terpenoids	+	+	-	+	+	-	+	+	+	+
Phenolics	+	+	+	+	-	+	+	+	-	+
Tannins	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	-	+	+	+	+	+	+
Coumarins	+	+	+	+	+	-	-	+	+	-
Saponins	+	+	+	+	-	+	-	+	+	+
Glycoside	+	+	+	-	+	-	+	-	+	+
Carbohydrates	-	+	+	+	+	-	-	+	-	-
Proteins	+	-	-	+	+	+	+	-	+	-
Phlobatannins	+	-	-	+	+	+	-	-	+	+

E1 = Hexane leaf crude extract, E2 = chloroform leaf crude extract, E3 = ethyl acetate leaf crude extract, E4 = acetone leaf crude extract, E5 = methanol leaf crude extract, E6 = hexane stem-bark crude extract, E7 = chloroform stem-bark crude extract, E8 = ethyl acetate stem-bark crude extract, E9 = acetone stem-bark crude extract and E10 = methanol stem-bark crude extract. The (+) and (-) signs indicate the presence and absence of the phytoconstituents, respectively.

The presence of alkaloids was detected in E1-E6 and E8-E1, while steroids were identified in E1-E6, E8 and E9 and terpenoids were found in E1, E2, E4, E5 and E7-E10. The presence of phenolics was found in E1-E4, E6-E8 and E10, while tannins were detected in all extracts (E1-E10) and flavonoids were found in E1-E3 and E5-E10. Similarly, presence

of coumarins was observed in E1-E5, E8 and-E9, while saponins were detected in E1-E4, E6 and E8-E10. Additionally, glycosides were found in E1-E3, E5, E6, E9 and E10, while carbohydrates were identified in E2-E5 and E8. Proteins were found in E1, E4-E6 and E9, while phlobatannins were detected in E1, E4-E6, E9 and E10. Our study showed that the distribution of these phytochemicals in E1-E10 are varied (refer to Table 1). In general, the distribution of various phytochemicals in leaves have been responsible for regulation of growth and development, nutrient storage activity and biological activities (Kumar et al., 2023), while the distribution of various phytochemicals in leaves have been responsible for protective functions against herbivores and pathogens (Kumar et al., 2023). As stated previously that alkaloids, flavonoids, glycosides, polyphenols, steroids, tannins and other classes of compounds have previously been reported from various parts of *B. zeyheri* (Bekker et al., 2001; Blunden et al., 2004; Blunden et al., 2006; Amusan et al., 2007; Mkhize et al., 2018). Overall, the results obtained in the present study on the qualitative phytochemical analysis of various extracts from the leaves and stem-bark of *V. infausta* was in good agreement with previous reports (Bekker et al., 2001; Blunden et al., 2004; Blunden et al., 2006; Amusan et al., 2007; Mkhize et al., 2018). In a previous study, anthelmintic activity of *B. zeyheri* has been reported (McGaw et al., 2007). The tannins present in *B. zeyheri* have been responsible for this anthelmintic activity since tannins have the ability to damage the protective outer layer of worms and interfere with nutrient absorption (McGaw et al., 2007).

3.2. *DPPH radical scavenging activity and IC_{50} values*

The results of DPPH free radical scavenging potential of these ten extract (E1-E10) and positive control, ascorbic acid are summarized in Table 2. The order of radical scavenging activity of leaf extracts was E3 > E5 > E4 > E2 > E1. Among the leaf extracts, E3 showed highest scavenging activity and E1 showed lowest scavenging activity at all concentrations (refer to Table 2). The order of radical scavenging activity of leaf extracts was E8 > E10 > E9 > E7 > E6 (Table 2). Among all stem-bark extracts, E8, showed highest scavenging activity followed by E9 and E1 showed lowest scavenging activity at all concentrations. It was noticed that E9 exhibited comparable activity as that of E8 at all concentrations. The positive control, ascorbic acid showed the highest radical scavenging activity at all concentrations. In other words, all extracts (E1-E10) showed relatively lower scavenging activity than positive control at all concentrations. The percentage radical scavenging potential of these ten extracts (E1-E10) and ascorbic acid are also shown in bar diagrams (refer to Figure 1 and Figure 2).

Table 2. DPPH radical scavenging activity of various extracts obtained from leaves and stem-bark of *B. zeyheri*.

Extracts	Concentrations (µg/mL) / Inhibition (%)							IC_{50} Values (µg/mL)
	200	500	800	1000	1500	2000	3000	
E1	22.05 ± 0.01^{a}	25.30 ± 0.01^{a}	30.62 ± 0.02^{a}	34.46 ± 0.02^{b}	40.50 ± 0.03^{c}	44.80 ± 0.02^{c}	48.88 ± 0.04^{c}	2759.00
E2	25.58 ± 0.03^{a}	30.55 ± 0.01^{a}	37.02 ± 0.05^{b}	40.32 ± 0.05^{b}	48.26 ± 0.01^{c}	54.26 ± 0.01^{d}	58.91 ± 0.01^{d}	1896.67
E3	29.20 ± 0.02^{c}	32.11 ± 0.02^{c}	37.06 ± 0.02^{b}	47.52 ± 0.01^{b}	52.94 ± 0.02^{b}	58.47 ± 0.01^{a}	67.91 ± 0.01^{a}	1670.89
E4	31.65 ± 0.03^{a}	33.50 ± 0.02^{a}	37.33 ± 0.00^{a}	40.98 ± 0.02^{b}	45.39 ± 0.03^{b}	50.54 ± 0.02^{b}	56.39 ± 0.03^{c}	2113.76
E5	34.7 ± 0.04^{c}	38.70 ± 0.01^{c}	41.71 ± 0.01^{b}	45.56 ± 0.06^{b}	50.53 ± 0.02^{a}	56.06 ± 0.02^{a}	64.98 ± 0.02^{d}	1513.30
E6	13.88 ± 0.01^{c}	20.44 ± 0.00^{c}	29.32 ± 0.02^{b}	33.25 ± 0.03^{b}	39.7 6± 0.03^{b}	44.73 ± 0.04^{b}	49.19 ± 0.00^{a}	2647.28
E7	25.11 ± 0.02^{a}	20.78 ± 0.04^{a}	35.99 ± 0.04^{b}	41.05 ± 0.04^{a}	49.74 ± 0.17^{a}	55.31 ± 0.01^{c}	64.19 ± 0.03^{d}	1765.49
E8	31.32 ± 0.01^{c}	39.48 ± 0.01^{c}	44.71 ± 0.01^{c}	51.03 ± 0.02^{b}	56.12 ± 0.02^{a}	62.37 ± 0.02^{a}	70.22 ± 0.01^{d}	1228.59
E9	38.11 ± 0.01^{a}	38.31 ± 0.00^{a}	41.13 ± 0.04^{b}	44.25 ± 0.04^{b}	50.82 ± 0.02^{d}	58.18 ± 0.02^{d}	65.64 ± 0.03^{c}	1512.97
E10	36.58 ± 0.03^{a}	39.88 ± 0.00^{a}	42.95 ± 0.01^{b}	45.74 ± 0.01^{d}	51.38 ± 0.03^{d}	56.04 ± 0.03^{e}	67.18 ± 0.02^{f}	1422.02
Asc. acid	58.44 ± 0.01^{a}	62.06 ± 0.01^{a}	68.77 ± 0.01^{b}	70.86 ± 0.02^{b}	75.37 ± 0.03^{c}	83.83 ± 0.03^{d}	87.84 ± 0.01^{e}	<200

E1-E10 = refer to Table 1. Asc. acid = Ascorbic acid. Values with different superscript letters are statistically different within column.

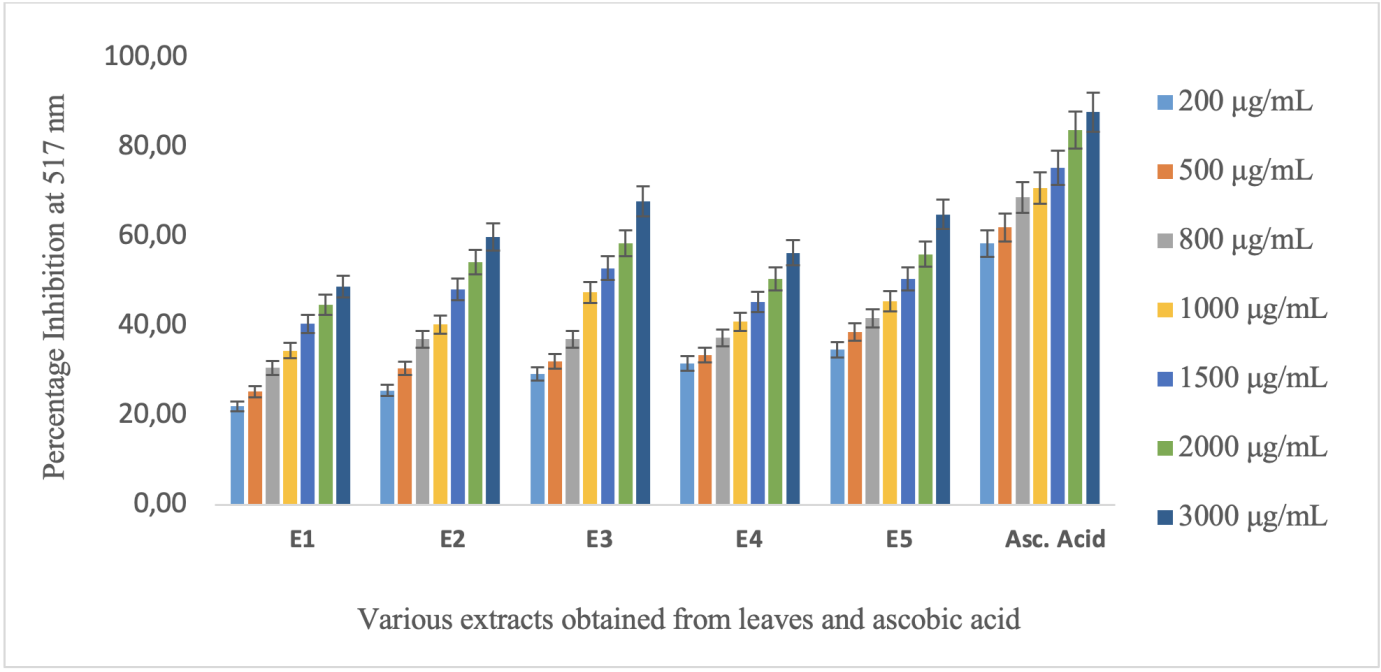

Figure 1. DPPH radical scavenging activity of various extracts from leaves of *B. zeyheri.*

E1-E5 = refer to the footnotes of Table 1.

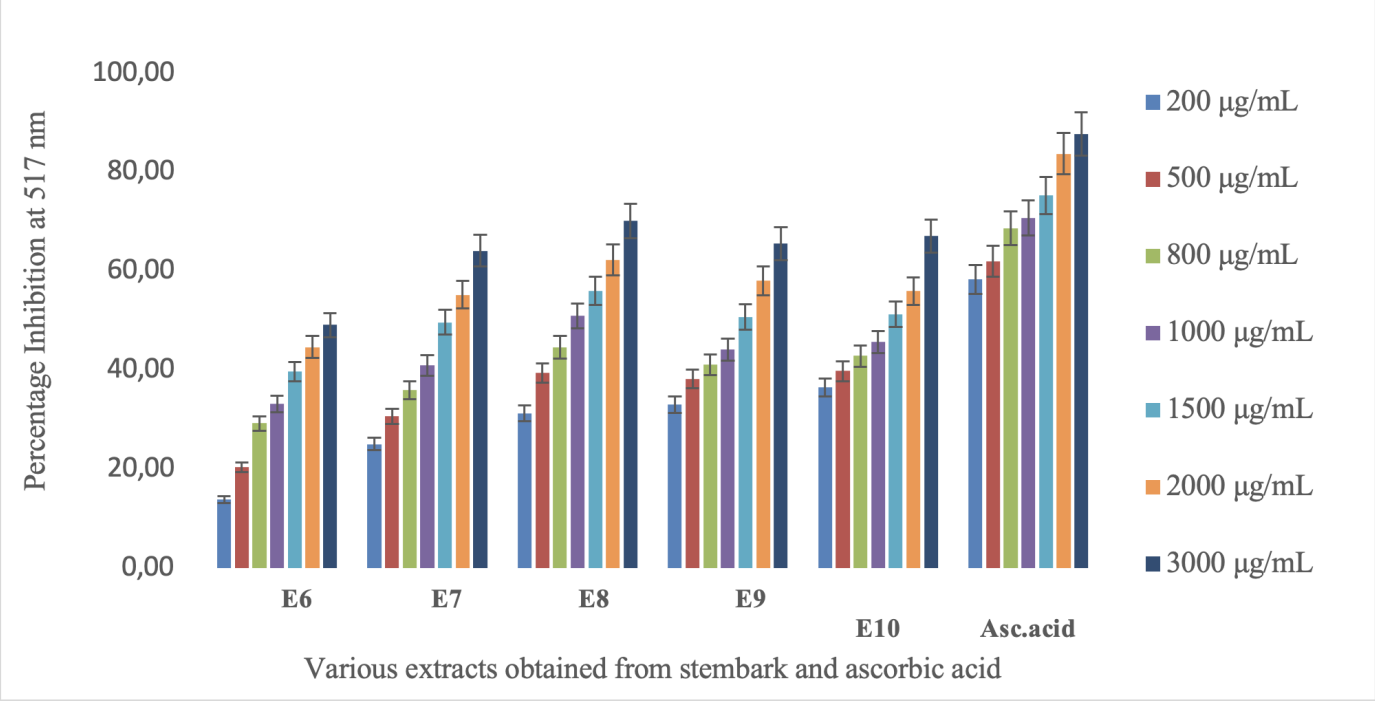

Figure 2. DPPH radical scavenging activity of various extracts from stem-bark of *B. zeyheri.*

E6-E7 = refer to the footnote of Table 1.

In a previous study, an aqueous methanolic extract obtained from wild fruitsof *B. zeyheri* collected in Zimbabwe has been investigated for its antioxidant activity in DPPH radical scavenging assay and superoxide anion scavenging assay (Ndlala et al., 2006). This methanolic extract showed approximately, 12, 40, 63 and 65% of radical scavenging activity at concentrations of 20, 40, 60 and 80 mg sample equivalent/µL, respectively in the DPPH radical scavenging assay (Ndlala et al., 2006**)** and showed approximately, 10, 60, 65 and 63% of scavenging activity at concentrations of 20, 40, 60 and 80 mg sample equivalent/µL, respectively in the superoxide anion scavenging assay (Ndlala et al., 2006). To the best of our knowledge, DPPH radical scavenging activity of other parts of this plant materials have not been reported.

The half-maximal inhibitory concentration (IC_{50}) value is a concentration of the compound or extract that is required to inhibit antioxidant activity by 50%. The IC_{50} values of E1-E10 and ascorbic acid were determined and the results obtained are listed in Table 2. The IC_{50} value of positive control, ascorbic acid was found to be <200 µg/mL. Extracts E1-E10 showed IC_{50} values of 2759.00, 1896.67, 1670.89, 2113.76, 1513.30, 2647.28, 1765.49, 1228.59, 1512.97 and 1422.02 µg/mL, respectively (refer to Table 2). This result indicated that among the leaf extracts, E5 was found to be the most potent with IC_{50} values of 1513.30 µg/mL followed by E3, which showed IC_{50} value of1670.89 µg/mL. Similarly, among stem-bark extracts, E8 was found to be the most potent with IC_{50} value of 1228.59, µg/mL followed by E10, which showed IC_{50} value of 1422.02, µg/mL. Extract E1 showed the lowest scavenging activity among all extracts with an IC_{50} value of 2759 µg/mL followed by E6 and E4 with IC_{50} values of 2647.28 and 2113.76 µg/mL, respectively (refer to Table 2). It was noticed that hexane extracts from both leaves and stem-bark (E1 and E6) exhibited highest IC_{50} values, which indicated

the lowest scavenging potency of these extracts. Overall, all extracts showed much higher IC_{50} values than positive control, ascorbic acid. In other words, all extracts showed relatively lower scavenging potency than positive control, ascorbic acid.

4. Conclusions

Various solvent extracts obtained from the leaves and stem bark of*B. zeyheri* were investigated for the presence of phytochemicals and were evaluated for their antioxidant activity by DPPH radical scavenging activity. Various classes of phytochemicals were detected in these extracts. Additionally, these extracts showed a weak to moderate radical scavenging activity. The ethyl acetate extracts obtained from both leaves and stem-bark showed higher radical scavenging activity among all extracts. Similarly, the methanol extract from leaves and the ethyl acetate extract from stem-bark showed a lowest IC_{50} value of 1513.30 and 1228.59µg/mL, respectively. In general, all extracts showed relatively lower scavenging potency than positive control, ascorbic acid. From this study, therefore, we concluded that various extracts obtained from the leaves of stem-bark of *B. zeyheri* possessed various classes of phytochemicals and these extracts showed a weak to moderate radical scavenging activity. Further studies on this plant are required to explore its therapeutic applications.

Statements and Declarations

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Authors' Contributions

Manoharan Karuppiah Pillai involved in designing the study, conceptualization, supervision, investigation, data analysis, compilation and writing the original draft. Khombisile H Simelane involved in cconceptualization, investigation, data analysis and writing a draft.

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Conflict of interests

The authors declare that there has been no conflict of interests.

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