Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
https://www.plantsjournal.com
JMPS 2024; 12(3): 125-130

Received: 08-04-2024
Accepted: 12-05-2024

Manoharan Karuppiah Pillai
Department of Chemistry, Faculty of Science & Engineering, University of Eswatini, Kwaluseni Campus, Private Bag 4, Kwaluseni, M201, The Kingdom of Eswatini

Qhawe Bonginkhosi Mangwe
Department of Chemistry, Faculty of Science & Engineering, University of Eswatini, Kwaluseni Campus, Private Bag 4, Kwaluseni, M201, The Kingdom of Eswatini

Corresponding Author:
Manoharan Karuppiah Pillai
Department of Chemistry, Faculty of Science & Engineering, University of Eswatini, Kwaluseni Campus, Private Bag 4, Kwaluseni, M201, The Kingdom of Eswatini

Phytochemical analysis and DPPH radical scavenging activity of extracts from *Vangueria infausta*: A medicinal plant from the Kingdom of Eswatini

Manoharan Karuppiah Pillai and Qhawe Bonginkhosi Mangwe

Abstract
Vangueria infausta has been used in the traditional medicine. In this research, we focused to analyze the phytochemical constituents, to evaluate the antioxidant activity and to determine the IC_{50} values of various solvent extracts obtained from the leaves and stem-bark of *V. infausta.* Maceration technique together with hot solvent extraction approach was used for the obtainment of various solvent extracts. Phytochemical analysis, DPPH radical scavenging potential and IC_{50} values were performed as per established procedures. Several classes of phytochemicals were identified in these extracts. The radical scavenging potential of these extracts and positive control were found to be in the ranges of 2.115±0.024 - 75.668±0.001% and 53.076±0.023-86.083±0.045% at 200-3000 µg/mL. The methanol extracts from the leaves and stem-bark and positive control exhibited IC_{50} values of 732.170 and 1235.862 µg/mL and <200 µg/mL, respectively. *V. infausta* possessed various classes of phytochemicals and showed a weak to moderate DPPH radical scavenging potential.

Keywords: *Vangueria infausta*, Rubiaceae, Phytochemical analysis, DPPH assay, Radical scavenging activity, IC_{50} values

1. Introduction
Vangueria infausta, also known by its common name as Wild-Medlar, belongs to the Rubiaceae family. The Rubiaceae family consists of approximately, 600 genera and 13, 000 species [1]. *V. infausta* is a deciduous low branching tree [2] and it grows to 4.0 - 8.0 meter height [3]. *V. infausta* is a good source of diet for livestock and the fruits are eaten by humans and birds [4-7]. *V. infausta* is abundantly available in the Kingdom of Eswatini, South Africa, Angola, Tanzania, Kenya, Malawi, Mozambique, Zambia and Zimbabwe [8, 9]. Various parts *V. infausta* have been used for medications in the Kingdom of Eswatini. For example, the decoction obtained from the leaves and roots are administered to treat malaria, wounds, sores, menstrual and uterus complications and genital swellings [5, 6, 9]. The decoction obtained from the root is taken orally to alleviate chest pains and the bark has been used to treat allergies caused by red meat [10].
Phytochemicals such as flavonoids, coumarins, tannins, terpenoids, anthraquinones, saponins, saturated fatty acids, polyunsaturated fatty acids and hydrocarbons have previously been reported from *V.infausta* [8, 11, 12]. Additionally, antibacterial [14-18], antifungal [19], anti-feedant [20], prostaglandin synthesis inhibitory [21], cytotoxic [22-24] and antioxidant activities [13, 25. 26] have also previously been reported from *V. infausta*. To the best our knowledge, the phytochemical analysis and radical scavenging potential of *V. infausta* have not been studied so far. Therefore, we aimed to conduct a qualitative phytochemical analysis, to evaluate DPPH radical scavenging activity and to determine IC_{50} values of various solvent extracts obtained from leaves and stem-bark of *V. infausta* collected in the Kingdom of Eswatini. The results are discussed in this article. This is the first report of this kind, especially *V. infausta* collected in the Kingdom of Eswatini.

2. Materials and Methods
2.1 Collection of plant materials
Leaves and stem-bark of *V. infausta* were gathered in Bhunya, which is located at latitude 26 degrees 33’ 0’’ S and longitude 31 degrees 1’ 0’’ E of Manzini region of the Kingdom of

Eswatini. Dr M.N Dludlu, Botanist, Department of Biological Sciences, University of Eswatini identified the plant materials. Specimen QBMLS2023 for leaves and QBMSB2023 for stem-bark was stored at the Organic Chemistry Research Laboratory, Department of Chemistry, Kwaluseni Campus of University of Eswatini.

2.2 Processing of plant materials
The leaves and stem-bark of *V. infausta* were air-dried separately at room temperature (26-28 °C) for four weeks. A laboratory scale cutting mill (KM-1500; MRC Laboratory Instruments) was used to pulverize the dried leaves into powder. The dried stem-bark was also ground into powder using the KM-1500 laboratory scale cutting mill. A mass of 369.373 and 600.087 g of leaf and stem-bark powder were obtained, respectively.

2.3 Preparation of plant extracts
Approximately, 750 mL of hexane and 60.931 g of leaf powder were taken in a 2L RB flask and macerated two days. After filtration followed by simple distillation, hexane crude extract was obtained and it was kept in a sample vial. The marc obtained in the above process was extracted with again with hexane but this time with reflux condition for approximately 24 hrs. The hexane crude extract received in this hot solvent extraction was combined with hexane crude extract obtained from maceration technique. A total mass 2.529 g of the combined hexane crude extract was obtained. By using the same procedure, 5.761, 6.976, 8.980 and 15.168 g of chloroform, ethyl acetate, acetone and methanol crude extracts were received, respectively from 61.450, 60.607, 60.236 and 60.293 g of leaf powder. Similarly, a mass of 0.123, 3.737, 4.907, 6.857 and 10.988 g of hexane, chloroform, ethyl acetate, acetone, and methanol crude extracts were received, respectively from 101.458, 100.514, 101.067, 101.384 and 101.023 g of stem -bark powder.

2.4 Solvents, reagents and chemicals
Unless otherwise specified, Analytical Reagents (AR) grade solvents and chemicals purchased from Minema were used in this research work. 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.

2.5 Phytochemical analysis
The phytochemical analysis for the presence or absence of various classes of compounds such as alkaloids, steroids, phenols, flavonoids, saponins, glycosides, coumarins, carbohydrates, proteins, tannins, terpenoids and phlobatannins in various solvent extracts obtained from *V. infausta* were performed as per methods previously described in the literature [14] and the references therein [14].

2.6 DPPH radical scavenging assay and determination of IC_{50} values
2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was employed to evaluate various extracts obtained from the leaves and stem-bark of *V. infausta* as per the procedure given in the literature with slight modifications [27]. A stock solution for each extract, a stock solution for positive control, ascorbic acid, six different further dilutions from the stock solution of each extract and ascorbic acid and negative control were all prepared separately as per the details given in the literature [27]. A phosphate buffered saline (PBS, pH = 7.4) was prepared by mixing sodium chloride (8.0g), potassium chloride (0.2 g), sodium dihydrogen phosphate (1.44 g) of and potassium dihydrogen phosphate (0.245 g) in 1000 mL distilled water. A concentration of 0.1 mM DPPH solution (3.94 mg of DPPH in 100 mL methanol) served as an oxidant [28]. The test solution was a mixture of 0.1 mL extract solution (or positive control), 1.0 mL of 0.1 mM DPPH solution and 0.45 mL of PBS buffer. The optical density of this mixture was measured at 517 nm using a UV-spectrophotometer (Infinite M200, Tecan US, Inc.) after 30 minutes incubation. The percentage inhibition of DPPH radical scavenging potential of extract or positive control was reported from the average value of the three experiments using the equation given below [29].

$$\text{Radical Scavenged (\%)} = [(A_{cont} - A_{test})/ A_{cont}] \times 100$$

A_{test} = Absorbance of extract (or positive control). A_{cont} = Absorbance of negative control. The IC_{50} values of these extracts and positive control, ascorbic acid were calculated from graphs by plotting extract concentrations (in abscissa) *versus* percentage inhibition of DPPH radical (in ordinate) using Microsoft Excel.

2.7 Statistical analysis
Statistical analysis was conducted by using STATISTICA version 10 for DPPH radical scavenging assay. Statistical significance between means was evaluated at a 95% confidence level by applying Tukey's multiple range tests. When $p \leq 0.05$, statistically significant differences were reported.

3. Results and Discussion
3.1 Phytochemical analysis
Extracts E1-E5 were respectively hexane, chloroform, ethyl acetate, acetone and methanol crude extracts obtained from leaves of *V. infausta*. Similarly, extracts E6-E10 were respectively hexane, chloroform, ethyl acetate, acetone and methanol crude extracts obtained from stem-bark of *V. infausta*. The qualitative phytochemical analysis on these ten extracts (E1-E10) was conducted. Table 1 summarizes the presence or absence of various phytochemicals *viz.* alkaloids, steroids, terpenoids, phenolics, tannins, flavonoids, coumarins, saponins, glycosides, carbohydrates, proteins and phlobatannins in each of this extract. The distribution of various phytochemicals in these ten extracts (E1-E10) were varied (refer to Table 1). Alkaloids were present in E1-E4, E6, E7 and E9; steroids were found in E4, E7, E9 and E10; terpenoids were detected in E1-E3 and E6-E8; phenolics were present in E1, E2, E4, E5 and E7-E10; tannins were found in and E4, E5 and E9-E10; flavonoids were detected in E3-E5, E7, E9 and E10 (Table 1).

Table 1: Phytochemical screening of various extracts obtained from the leaves and stem-bark of *V. infausta*

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

Extracts E1-E5 were respectively hexane, chloroform, ethyl acetate, acetone and methanol crude extracts obtained from leaves of *V. infausta*. Similarly, extracts E6-E10 were respectively hexane, chloroform, ethyl acetate, acetone and methanol crude extracts obtained from stem-bark of *V. infausta*. The (+) and (-) signs indicate the presence and absence of the phytoconstituents, respectively.

Coumarins were present in E2, E5 and E6-E9; saponins were found in E1, E4, E9 and E10; glycosides were detected in E3-E5, E8-E10; carbohydrates were present in E3-E5, E8-E10; proteins were found in E2, E4, E5, E8-E10; phlobatannins were detected in E6, E7 and E9 (Table 1). The distribution of these phytochemicals in these ten extracts (E1-E10) is susceptible to the influence of various factors like genetic, environmental, developmental and physiological factors. In general, the phytochemicals found in the leaves are responsible for regulation of growth and development, nutrient storage activity, antioxidant protection, antimicrobial and other biological activities [4]. While, the phytochemicals found in the stem-bark are responsible for protective functions against herbivores and pathogens [4]. As stated previously that flavonoids, coumarins, tannins, terpenoids and saponins compounds have previously been reported from *V. infausta* [8, 11, 12]. Flavonoid compounds such as quercetin, daidzein, genistein, luteolin, luteolin-7-*O*-rutinoside, apigenin-7-*O*-rutinoside, luteolin-4-*O*-glucoside, quercetin-3-*O*-glucoside, dihydroquercetin-3'-O-glucoside, (-)-epicatechin and dihydrokaempferol [12, 26] have been isolated in the pure state from leaves and stem-bark of *V. infausta*. Overall, the result obtained in the current study on the qualitative phytochemical analysis of extracts obtained from the leaves and stem-bark of *V. infausta* was in good agreement with previous reports on the phytoconstituents.

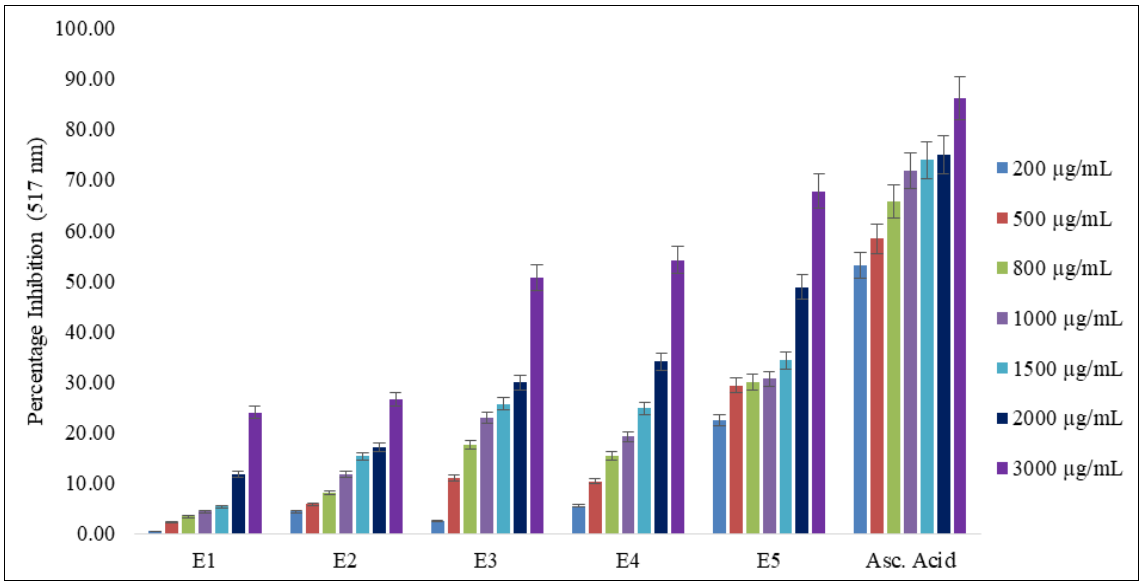
3.2 DPPH radical scavenging potential and IC_{50} values

The DPPH radical scavenging potential of E1-E10 and ascorbic acid is given in Table 2. In general, leaf extract showed relatively higher scavenging potential than stem-bark extracts. However, all extracts showed relatively lower scavenging potential than ascorbic acid. Extracts E1-E5 showed a radical scavenging potential in the ranges of 7.845±0.005 - 79.227 0.014% at concentrations 200-3000 µg/mL. Ascorbic acid showed a radical scavenging potential of 53.076±0.023 - 86.083±0.045% at the same concentrations. Extract E5 showed the highest scavenging activity at all concentrations and E1 exhibited lowest scavenging activity at all concentrations (refer to Table 2). In general, al lower concentrations, E1-E5 showed lower scavenging activity. However, at elevated concentrations, these extracts exhibited moderate to significant scavenging activity. Similarly, extracts E6-E10 showed a radical scavenging potential of 2.115±0.024-70.450±0.011% at concentrations 200-3000 µg/mL. Extract E10 showed the highest radical scavenging potential at all concentrations and E6 showed the lowest radical scavenging potential at all concentrations (refer to Table 2). Overall, E10, E9, E5 and E4 exhibited significant radical scavenging potentials; extracts E8, E7, E3 and E2 showed moderate radical scavenging potentials and E6 and E1 showed poorest radical scavenging potentials among all extracts. Among all extracts, E5 showed a highest radical scavenging potential of 79.227±0.014% at 3000 µg/mL and this radical scavenging potential of was comparable to that of ascorbic acid (86.083±0.045%) at the same concentration. Among stem-bark extracts, E10 showed a highest radical scavenging potential of 70.450±0.011% at 3000 µg/mL. The radical scavenging potential of these ten extracts (E1-F10) and ascorbic acid are shown in the bar diagrams (refer to Figure 1 and Figure 2, respectively).

Table 2: DPPH radical scavenging potential of various extracts obtained from *V. infausta*

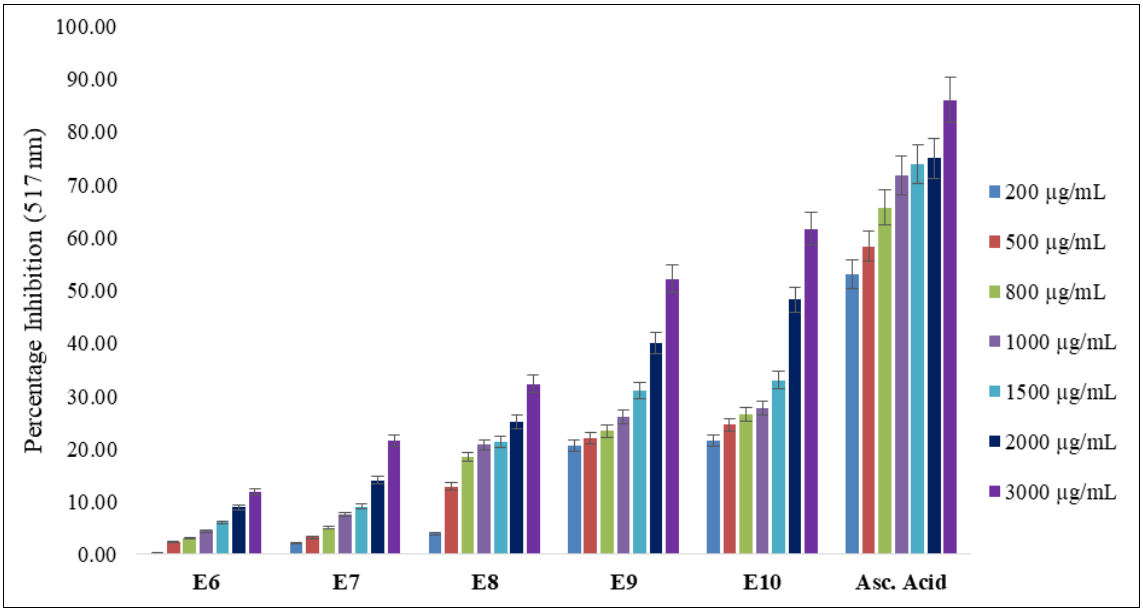
Extracts	Concentrations (µg/mL) / Inhibition (%)							IC_{50} Values
	200	500	800	1000	1500	2000	3000	
E1	7.845±0.005^{c}	10.454±0.035^{c}	15.801±0.020^{a}	18.522±0.027^{c}	22.696±0.009^{b}	26.686±0.006^{d}	36.079±0.031^{d}	>3000
E2	13.910±0.013^{b}	18.203±0.052^{b}	22.400±0.009^{a}	25.29 ±0.015^{a}	28.090±0.032^{a}	36.318±0.019^{c}	47.355±0.026^{c}	>3000
E3	15.394±0.029^{c}	19.248±0.005^{c}	24.802±0.010^{c}	33.980±0.030^{b}	39.981±0.001^{a}	44.992±0.013^{a}	55.734±0.039^{d}	2415.034
E4	25.42 ±0.009^{f}	32.535±0.028^{e}	39.997±0.018^{d}	46.381±0.011^{c}	50.339±0.009^{b}	57.529±0.006^{b}	66.10 ±0.011^{a}	1411.277
E5	34.091±0.007^{b}	41.154±0.017^{a}	52.143±0.003^{b}	60.562±0.024^{b}	67.137±0.011^{c}	75.668±0.001^{c}	79.227±0.014^{b}	732.170
E6	2.115±0.024^{a}	6.400±0.027^{a}	8.260±0.038^{a}	12.290±0.031^{a}	17.078±0.032^{a}	20.469±0.022^{a}	29.734±0.015^{a}	>3000
E7	8.698±0.006^{a}	12.872±0.018^{a}	18.450±0.014^{a}	21.539±0.008^{a}	26.957±0.013^{b}	30.652±0.031^{b}	40.276±0.027^{b}	>3000
E8	20.693±0.015^{a}	22.065±0.011^{a}	23.398±0.013^{a}	25.952±0.058^{a}	30.947±0.010^{b}	40.045±0.018^{b}	52.175±0.013^{c}	2832.662
E9	22.591±0.012^{c}	27.651±0.034^{c}	35.129±0.063^{b}	42.558±0.003^{b}	46.110±0.055^{b}	54.345±0.012^{a}	64.943±0.003^{a}	1714.887
E10	24.012±0.014^{a}	29.919±0.029^{a}	36.869±0.024^{a}	47.650±0.014^{a}	56.133±0.014^{a}	64.241±0.028^{b}	70.450±0.011^{b}	1235.862
Asc. acid	53.076±0.023^{d}	58.391±0.018^{d}	65.661±0.014^{c}	71.790±0.022^{c}	73.857±0.007^{b}	75.006±0.004^{b}	86.083±0.045^{a}	<200

E1-E10 = Refer to footnote of Table 1; Asc. acid = ascorbic acid. Values with different superscript letters are statistically different within the column



E1-E10 = Refer to footnote of Table 1; Asc. acid = ascorbic acid

Fig 1: Percentage of radical scavenging potential of various extracts from leaves of *V. infausta* and ascorbic acid at various concentrations.
E1-E10 = Refer to footnote of Table 1; Asc. acid = ascorbic acid



E1-E10 = Refer to footnote of Table 1; Asc. acid = ascorbic acid

Fig 2: Percentage of radical scavenging potential of various extracts from stem - bark of *V. infausta* and ascorbic acid at various concentrations

In a previous study, the methanolic extracts obtained from the leaves and root of *V. infausta* collected in Zimbabwe exhibited DPPH radical scavenging potential of 93.1±0.0707 and 39.7±0.212%, respectively [25]. However, in the current study, the methanolic leaf extract obtained from *V. infausta* collected in the Kingdom of Eswatini showed relatively lower scavenging activity of 79.227±0.014%. In general, geographic locations, soil compositions, seasonal variations etc. significantly contribute to the availability and their proportions of phytoconstituents in the plant materials. The above discrepancy in the radical scavenging potential of methanolic leaf extracts obtained from *V. infausta* in the previous and current studies may be due to these factors.

Extracts E1-E10 and ascorbic acid were determined for their IC_{50} values from the same DPPH assay and the values are summarized in Table 2. A lower IC_{50} value indicates higher potency of scavenging activity. Extract E5, E4, E3 showed IC_{50} values of 732.170 411.277 and 2415.034 µg/mL, respectively. Extracts E2 and E1 showed highest IC_{50} value of >3 000 µg/mL, for each (refer to Table 3). The IC_{50} value of ascorbic acid was found to be < 200 µg/mL. Similarly, E10 E9 and E8 showed IC_{50} values of 1235.862, 1714.887 and 2832.662 µg/mL, respectively. Extracts E7 and E6 showed highest IC_{50} value of >3 000 µg/mL, for each (refer to Table 2).

In a previous study, a methanolic leaf extract obtained from *V. infausta* collected in Botswana exhibited an IC_{50} value 107.88 µg/mL [13]. However, in the current study, the methanolic leaf extract obtained from *V. infausta* collected in Eswatini exhibited an IC_{50} value 732.170 µg/mL, which was much higher than previously reported IC_{50} value [13]. This discrepancy may be again due to the same factors that geographic locations, soil compositions, soil composition, seasonal variations etc. significantly contribute to the availability and their proportions of phytoconstituents in the plant materials and consequently, to their antioxidant potential. In another study, pulp, liquor and jam obtained from *V. infausta* collected in Tanzania exhibited IC_{50} values of 1.348, 13.786 and 4.485 mg/L, respectively [26]. Flavonoid compounds such as luteolin, quercetin and dihydroquercetin-

3'-O-glucoside have been isolated and reported from the leaves of *V. infausta* [30] and these compounds exhibited IC_{50} values <8.2 µg/mL [30] and other flavonoid compounds such as dihydrokaempferol, epiafzelechin, (-)-epicatechin and genistein exhibited IC_{50} values in a range of 15-146 µg/mL [30].

4. Conclusion

Hexane, chloroform, ethyl acetate, acetone and methnolic solvent extracts were obtained separately from the leaves and stem-bark of *V.infausta*. Qualitative phytochemical analysis was performed on these extracts and the phytochemical distributions were varied in these extracts. In the DPPH radical scavenging assay, the leaf and stem-bark extracts exhibited radical scavenging potential of 7.845±0.005 - 75.668±0.001% and 2.115±0.024 - 70.450±0.011%, respectively at concentrations 200 - 3000 µg/mL and the positive control, ascorbic acid exhibited radical scavenging potential at 53.076±0.023-86.083±0.045% at the same concentrations. The methanolic extracts from both leaves and stem-bark were found to be the most potent among all extracts with IC_{50} values of 732.170 and 1235.862 µg/mL, respectively. We concluded that *V. infausta* is rich in various classes of phytochemicals. Various leaf and stem-bark extracts from theof *V. infausta* exhibited weak to moderate to significant radical scavenging potential. *V. infausta* has traditionally been used in the Kingdom of Eswatini. Therefore, further studies on this plant are required to explore the medicinal properties of this plant.

5. Acknowledgements

The authors thank the University of Eswatini for its overall support. One of the authors, Qhawe Bonginkhosi Mangwe, also thanks Dr. G. S. Sibandze, The Director, Eswatini Institute for Research in Traditional Medicine, Medicinal and Indigenous Food Plants (EIRMIP) for allowing to use some of the instruments at EIRMIP and Mr. Vusi S Vilane from EIRMIP for providing the technical assistance during the use of UV-spectrophotometer at EIRMIP. This paper has been extracted from the undergraduate research project report submitted by Qhawe Bonginkhosi Mangwe to the University of Eswatini. Kwaluseni campus, The Kingdom of Eswatini.

6. Conflict of interests

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

7. References

1. Gwatidzo L, Chowe L, Mukaratirwa-Muchanyereyi N. In-vitro anti-inflammatory activity of *Vangueria infausta*: An edible wild fruit from Zimbabwe. Afr J Pharm Pharmacol. 2018;12:168-175.
2. Koche D, Shirsat R, Kawale MV. An overview of major classes of phytochemicals: their types and role in disease prevention. Hislopia J. 2018;9(1/2):1-11.
3. Kazembe T, Munyarari E, Charumbira I. Use of traditional herbal medicines to cure malaria. Environ Sci Med. 2012;1(4):63-84.
4. Kumar APN, Kumar M, Jose A, Tomer V, Oz E, Oz F. Major phytochemicals: recent advances in health benefits and extraction method. Molecules. 2023;28(2):887(1-41).
5. Gibson EL, Wardle J, Watts CJ. Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. Appetite. 1998;31(2):205-228.
6. Laboratories MB. HHS. Public Access. 2015;43(Suppl 1):101-109.
7. Lindsey K, Jäger AK, Raidoo DM, van Staden J. Screening of plants used by Southern African traditional healers in the treatment of dysmenorrhoea for prostaglandin-synthesis inhibitors and uterine relaxing activity. J Ethnopharmacol. 1999;64(1):9-14.
8. Sanad FA, International CS. Evaluation of the hypolipidemic and antioxidant activities of chloroformic extract from *Vangueria infausta* leaves in rats with hypercholesterolemia. Curr Sci Int. 2020;9(3):462-471.
9. Loffler L, Loffler P. Swaziland Tree Atlas-Including Selected shrubs and climbers. SABONET. Report No. 38; c2005.
10. Abosi AO, Mbukwa E, Majinda RRT, Raseroka BH, Yenesew A, Midiwo JO, et al. *Vangueria infausta* root bark: *in vivo* and *in vitro* antiplasmodial activity. Br J Biomed Sci. 2016;63(3):129-133.
11. Bapela MJ, Heyman H, Senejoux F, Meyer JJM. 1H-NMR-based metabolomics of antimalarial plant species traditionally used by Vha-Venda people in Limpopo Province, South Africa and isolation of antiplasmodial compounds. J Ethnopharmacol. 2019;228:148-155.
12. Mbukwa E, Chacha M, Majinda RRT. Phytochemical constituents of *Vangueria infausta*: their radical scavenging and antimicrobial activities. Arkivoc. 2007;(ix):104-112.
13. Mausse BJ, Munyemana F, Uamusse A. Determination of total phenols and evaluation of the antioxidant activity of pulps and fruit derivatives of *Vangueria infausta* and *Strychnos spinosa*. J Med Plants Stud. 2021;9(3):6-13.
14. Pillai MK, Mpopo MS, Mekbib SB. Phytochemical analysis, total phenolic, total flavonoid contents and ferric reducing power of extracts from roots and leaves of *Searsia burchellii*. Food Res. 2021;5(3):241-248.
15. Morgan ED. Bioorganic medicinal chemistry Azadirachtin, a scientific gold mine. Bioorg Med Chem. 2009;17(12):4096-4105.
16. Moshi M, Innocent E, Magadula J, Otieno D, Weisheit A, Mbabazi P, et al. Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, north western Tanzania. Tanzan J Health Res. 2010;12:63-67.
17. Mothapo MJ, Mafeo TP, Mamphiswana ND. Physico-chemical properties and selected nutritional components of wild medlar (*Vangueria infausta*) fruit harvested at two harvesting times. World J Dairy Food Sci. 2014;9:79-85.
18. Mthethwa NS, Oyedeji BAO, Obi LC, Aiyegoro OA. Anti-staphylococcal, anti-HIV and cytotoxicity studies of four South African medicinal plants and isolation of bioactive compounds from *Cassine transvaalensis* (Burtt. Davy) codd. BMC Complement Altern Med. 2014;14:512(1-9).
19. Mokgolodi NC, Yan HU, Ling-ling SHI, Yu-jun LIU. *Ziziphus mucronata*: an underutilized traditional medicinal plant in Africa. For Stud China. 2011;13(3):163-172.
20. Raice R, Sjöholm I, Wang HL, Bergenståhl B. Characterization of volatile components extracted from *Vangueria infausta* (African medlar) by using GC-MS. J Essent Oil Res. 2015;27(1):76-81.
21. Ramawat KG, Dass S, Mathur M. The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. Herbal Drugs: Ethnomedicine to Modern Medicine; c2009. p. 7-32.
22. Naskar S, Mazumder UK, Pramanik G, Bala A, Haldar

PK, Gupta M. Comparative in-vitro antioxidant activity of different parts of *Cocos Nucifera* (Linn.) on reactive oxygen and nitrogen species. Int J Pharm Pharm Sci. 2011;3:104-107.
23. Olugbami JO, Gbadegesin MA, Odunola OA. *In vitro* evaluation of the antioxidant potential, phenolic and flavonoid contents of the stem bark ethanol extract of *Anogeissus leiocarpus*. Afr J Med Med Sci. 2014;43(Suppl 1):101-109.
24. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestree database: A tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya; c2009.
25. Munodawafa T, Chagonda L, Moyo S. Antimicrobial and phytochemical screening of some Zimbabwean medicinal plants. J Biologically Active Products Nature. 2013;3(5-6):323-330.
26. Maroyi A. Medicinal uses of the Fabaceae family in Zimbabwe: A review. Plants. 2023;12(16):1-26.
27. Pillai MK, Keketso M, Matamane RP. Antioxidant activity of extracts from *Bidens pilosa* - A medicinal plant from the Kingdom of Lesotho. Fine Chem Eng. 2023;4(2):110-124.
28. Pillai MK, Matela KS, George MJ, Magama S. Antioxidant activity of extracts from *Schinus molle* L. and *Gleditsia triacanthos* L. J Med Plants Res. 2018;12(24):369-374.
29. Matamane PR, Pillai MK, Magama S. DPPH radical scavenging activity of extracts from *Urtica urens* (Urticaceae). J Med Plants Res. 2020;14(5):232-238.
30. Barton DHR, Cheung HT, Daniels PJL, Lewis KG, McGhie JF. Triterpenoids. Part XXVI. The triterpenoids of *Vangueria tomentosa*. J Chem Soc; c1962. p. 5163-5175.