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Antioxidant Potential of Extracts from *Grewia bicolor*: A Traditional Swazi Medicinal Plant

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A B S T R A C T

Objective: *Grewia bicolor*, a plant from the Malvaceae family traditionally used in Swazi ethnomedicine, is reported to exhibit various therapeutic activities, which may be linked to its antioxidant potential. Although *G. bicolor* is widely used in ethnomedicine, the antioxidant potential of various extracts obtained from this plant has not been explored well, particularly the species collected in the Kingdom of Eswatini. It is this research gap prompted us to evaluate the antioxidant activity of this plant by determining IC50 values, total phenolic contents (TPCs) and total flavonoid contents (TFCs).

Methods: Maceration technique was used first and then hot solvent extraction technique was used subsequently to obtain various solvent extracts from the leaves and stem-bark. DPPH radical scavenging assay was used to assess the antioxidant potential and determine the IC50 values. Folin-Ciocalteu colorimetric method and aluminium chloride colorimetric method were used to determine the TPCs and TFCs, respectively.

Results: The percentage of DPPH radical scavenged by various extracts from the leaves, stem-bark and the positive control (ascorbic acid) were determined at a concentration range of 200-3000 µg/mL. The pentane and ethyl acetate extracts from leaves showed relatively higher scavenging potential among the leaf extracts. Similarly, the dichloromethane and acetone extract from stem-bark showed relatively higher scavenging potential among the stem-bark extracts. In general, the stem-bark extracts showed slightly higher scavenging potential compared to leaf extracts. However, all extracts from the leaves and stem-bark showed relatively lower scavenging activity compared to the positive control. The IC50 values of leaves and stem-bark extracts were 457.78-2425.37µg/mL and 649.29-1869.62µg/mL, respectively. The IC50 value of the positive control was <200 µg/mL. The acetone extracts from both leaves and stem-bark showed lower IC50 values compared to other extracts. The TPCs of leaves and stem-bark extracts were 0.850-9.728 and 0.813-12.259 mg GAE/g DW, respectively. The TFCs of leaves and stem-bark extracts were 20.211-46.004 and 22.054-42.128 mg QE/g DW, respectively.

Conclusion: This study demonstrated various extracts from the leaves and stem-bark of *G. bicolor* showed a moderate to significant DPPH radical scavenging potential and possessed a moderate to significant TPCs and TFCs. In general, the TFCs of these extracts were relatively higher than TPCs. These findings support the ethnomedicinal use of G. bicolor and suggest its potential in developing antioxidant-based therapeutics.

Keywords: *Grewia bicolor*, Malvaceae, antioxidant activity, DPPH radical scavenging assay, IC50 values, total phenolic contents (TPCs), total flavonoid contents (TFCs)

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Introduction

Grewia bicolor belongs to the Malvaceae family of the Grewia genus [1,2]. G. bicolor is also known by other vernacular names such as mongwane, white raisin, white-leaved raisin, false brandy bush, bastard brandy bush and donkey berry. The Grewia genus has approximately 159 plant species and these species are distributed in tropical and sub-tropical regions of Africa, Asia and Australia [3]. Various *Grewia* species have been traditionally used for treating cardiac, respiratory and hematologic disorders [4,5]. Additionally, the species belong to this Grewia genus exhibited antioxidant, anticancer, antiinflammatory, antibacterial, antimicrobial, antimalarial, antiviral, antipyretic, antiemetic, analgesic, antihyperglycaemic, enzyme inhibition, hepatoprotective and radioprotective [4-6]. Steroids, triterpenoids, alkaloids, flavonoids, glycosides, phenolics, lignans, anthocyanins, lactones, tannins and organic acids have been reported from various species belong to this Grewia genus [6-8]. Particularly, flavonoids, phenolics, anthocyanins and tannins are found at higher levels in various species of this Grewia genus [7,8].

G. bicolor is a multipurpose small tree or shrub and it can grow to 5-10 meters in height depending on the available conditions for growth [1]. *G. bicolor* has elliptical leaves, small flowers and round-shaped edible fruits [1]. *G. bicolor* is widely distributed all across the African continent [1]. *G. bicolor* is one of the widely used medicinal herbs in Swaziland (Eswatini), South Africa, Botswana, Somalia and Ethiopia [5]. G. bicolor finds several therapeutic applications in the ethnomedicine, which include in the treatment of syphilis, fever and intestinal inflammation, to maintain epilepsy and dental hygiene and as diuretic, vermifuge, laxative and antidote for common poisons [1,9-12]. In a previous report, liquid nitrogen-ethanol and liquid nitrogen-methanol extracts were obtained from the leaves of *G. bicolor* [1]. The extract obtained from the liquid nitrogen-ethanol solvent mixture has been evaluated for its antioxidant activity and the extract obtained from the liquid nitrogen-methanol solvent mixture has been subjected to determination of its total phenolic contents [1]. The presence of chlorophyll a, chlorophyll b, carotenoids and phenolic compounds has been identified as potential active metabolites for exhibiting antioxidant activity [1]. In another report, ethanol-water and deionized water extracts have been received using maceration technique from the peels of berries of G. bicolor [13]. Antioxidant activity of these two extracts was evaluated and total phenolic contents of these were also determined [13]. Despite its widespread ethnobotanical use, *G. bicolor* remains under-investigated, particularly in the Kingdom of Eswatini. Therefore, this study aimed to assess the antioxidant potential (via DPPH assay and IC_{50} values), total phenolic contents (TPCs), and total flavonoid contents (TFCs) of various solvent extracts obtained from the leaves and stem-bark of *G. bicolor* collected from Eswatini. The results are discussed in this article.

Materials and methods

Plant materials

Fresh leaves and stem-bark of *G. Bicolor* were collected on 25th November 2024 in the area of Mafutseni near Mhlathuze River in the Kingdom of Eswatini. Identification of plant materials was achieved with the help of Dr. M. N. Dludlu from the Department of Biological Sciences, University of Eswatini (UNESWA). Samples for leaves (SNLS2024) and stem-bark (SNSB2024) were kept at the Chemistry Project Laboratory (CPL).

Processing the plant materials

The plant materials spread and allowed for air-drying at CPL for three to four weeks and then ground into fine powder using a laboratory grinder (MRC Laboratory Equipment, Model KM 1500). Powdered masses of 451.688g from leaves and 225.697g from stem-bark were obtained.

Preparation of plant extracts

The following Table 1 summarizes the amount of plant materials used for extraction, solvents used

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for extraction, amount of plant materials used for extraction and amount of crude extract obtained from each solvent. The solvents were selected based on their increasing polarity to extract a broad spectrum of phytochemicals.

Table 1: Plant parts, solvents and amount of plant materials used for extraction and amount of crude extract obtained from each solvent.

S. No.	Plant parts used	Solvents used	Amount of plant materials used (g)	Amount of crude extract obtained (g)
1	Leaves	Hexane	60.412	0.758
2	Leaves	Dichloromethane	60.250	1.057
3	Leaves	Ethyl acetate	60.246	1.808
4	Leaves	Acetone	60.331	1.366
5	Leaves	Methanol	60.531	7.039
6	Stem-bark	Hexane	40.531	0.369
7	Stem-bark	Dichloromethane	40.805	0.587
8	Stem-bark	Ethyl acetate	40.010	2.098
9	Stem-bark	Acetone	40.260	0.893
10	Stem-bark	Methanol	40.568	4.393

The powdered leaves were macerated separately with each one of the above solvents for 24 hours at room temperature at 27-29°C with occasional shaking. Thereafter, the solvent was decanted, filtered and distilled. A crude extract was obtained and kept in a previously weighed china-dish. The powdered leaves were recovered from the above maceration process and then extracted again with the same solvent for 8 hours but this time under reflux conditions. The solvent was decanted, filtered and distilled as previously. The crude extract obtained in this hot solvent extraction technique was combined with previously obtained crude extract. The procedure was followed and repeated for each one of the above solvents.

Chemicals, reagents and solvents

Pentane, dichloromethane, ethyl acetate, acetone, methanol, ascorbic acid, tris- hydrochloric acid buffer and 2,2- diphenyl-1-picrylhydrazyl (all from Promark chemicals), quercetin, tannic acid, Folin-Ciocalteu reagent and sodium carbonate (all from Associated Chemical Enterprises), sodium phosphate (from Glass World), sodium nitrite (from Rochelle Chemicals) and sodium hydroxide (from MCB Laboratory and Medical Suppliers) were used in this study and they are all AR grades.

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2-2-Diphenyl-1-picrylhydrazyl radical scavenging potential and IC50 values of extracts

Free radicals are reactive and unstable species which are generated through normal metabolic processes in the human body. When present in excess, these reactive species cause several adverse effects which include the development of inflammation, cancer and cardiovascular diseases [14]. Antioxidant activity is the capability of a compound to inhibit the formation of these harmful free radicals by donating antioxidants such as hydrogen and electron. Therefore, these free radicals are reduced and the degree of reduction is proportional to the antioxidant activity of the extract or pure substance [15]. In the present study, 2-2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to assess the antioxidant activity of leaves and stem-bark extracts of G. Bicolor. In this DPPH assay, a purple-colored methanol solution of stable DPPH free radical is reacted with hydrogen donor compounds from the extracts of *G. Bicolor* and converted to yellow colored DPPH-H neutral molecule and the reduction is measured using UV-Vis. Spectrophotometer. DPPH radical scavenging assay was performed as per methods described in the literature [16-20]. Stock solution of each extract at a concentration of 3000µg/mL was prepared separately using 50% methanol (v/v). Similarly, a stock solution at the same concentration was prepared for positive control (ascorbic acid) using 50% methanol (v/v). Further dilutions such as 2000, 1500, 1000, 800, 500, and 200µg/mL were made separately from each one of the above stock solutions. A 50% methanol (v/v) served as negative control. DPPH solution served as an oxidant and it was prepared by dissolving 3.94mg of DPPH in 100mL of methanol. A phosphate-buffered saline (PBS) at a pH of 7.4 served as a buffer solution. This buffer solution was used to maintain a stable pH and to ensure the solubility of DPPH radical in the reaction mixture, which in turn maximize the absorbance of the reaction mixture [21,22]. The reaction mixture was prepared by pipetting out 20µL of test sample to a 96 well-plate and 50µL of PBS and 100µL of DPPH were added to it. The absorbance of the resulting mixture was then measured at 517nm using a UV-Vis. Spectrophotometer (Infinite M200) after 30 minutes incubation. The percentage inhibition of the radical scavenging ability was calculated by using the following equation [23-25] by averaging triplicate values.

$$\text{DPPH Scavenged (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Half-minimal concentration (IC50) values of the various extracts and the positive control were determined from graphs by plotting extract concentrations (in x-axis) against the percentage inhibition of the DPPH radical (in y-axis). A solution prepared from extract or pure compound that inhibits the formation of DPPH radical by 50% is called an IC50 value and the concentration of solution is usually expressed in µg/mL.

Determination of total phenolic contents (TPCs)

The Folin-Ciocalteu colorimetric method was used to determine the total phenolic contents (TPCs) extracts from the leaves and stem-bark of *G. Bicolor* by procedures as described in literature [26-28]. Each extract solution was prepared separately by dissolving 1000µg of extract in 1.0mL of 50% methanol (v/v) and these solutions were used as the test solutions. The reaction mixture was prepared by pipetting out 50µL of 10% Folin-Ciocalteu reagent to a 96 well-plate and 50µL of test solution was added to it. The reaction mixture was incubated for 5 minutes in a dark cabinet and then 5.0mL of 10% sodium carbonate solution was added into this reaction mixture. The resulting mixture was incubated again for 30 minutes at 40ºC and the color change was observed. The absorbance was then measured at 760nm using a UV-Vis. Spectrophotometer (Infinite M200). An average of triplicate values was used to estimate the TPCs of the individual extract. Gallic acid served

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as standard. A mass of 1.0g standard dissolved in 1.0mL of 50% methanol (v/v) served as a stock solution of standard. Thereafter, 200, 100, 80, 40, 20, and 10µg/mL dilute solutions were obtained from previously prepared stock solution. Using a similar procedure as described previously, the absorbance of various concentrations of standard was measured. A calibration curve y = 0.0119x + 0.0183; R2 = 0.9938 was obtained from the standard by plotting various concentrations taken is x-axis versus absorbance taken in y-axis. This calibration curve is shown Figure 1 and this calibration was used to estimate the TPCs of those extracts. The TPCs of various extracts were reported as milligrams of gallic acid equivalent per gram of dry weight of extract (mg of GAE/g DW of extract).

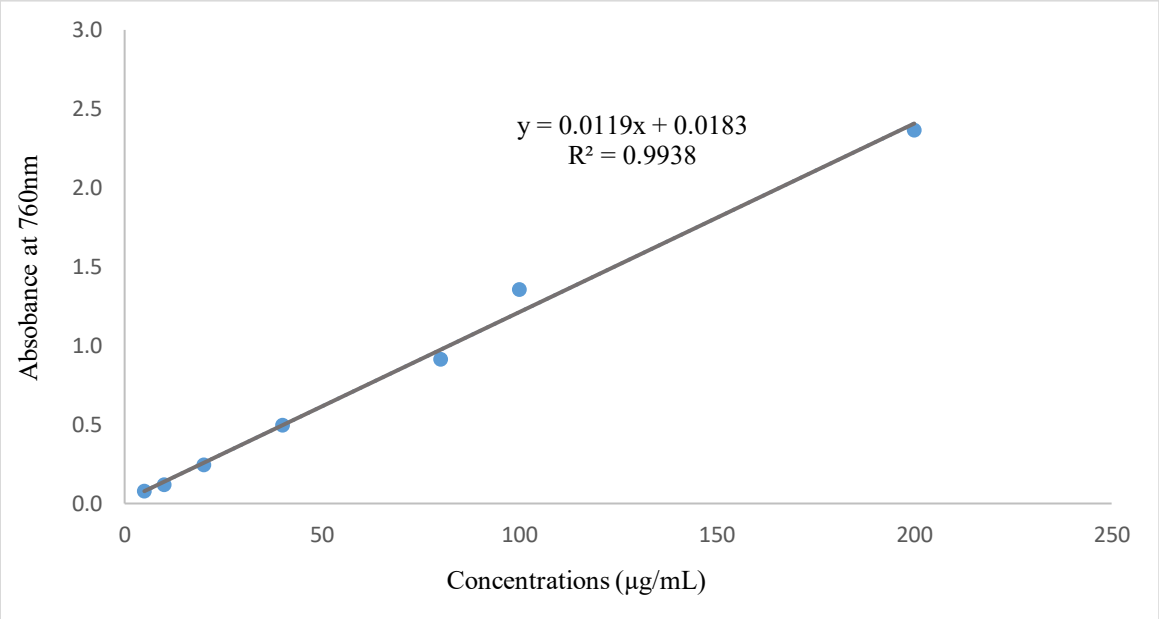

Figure 1: The calibration curve of gallic acid used to determine the TPCs of various extracts.

Determination of total flavonoid contents (TFCs)

Aluminium chloride colorimetric method was utilized to estimate the total flavonoid contents (TFCs) of various extracts obtained from the leaves and stem-bark of *G. Bicolor* by procedures as described in literature [26-28]. A solution of each extract was prepared separately at a concentration of 1000µg/mL using 50% methanol (v/v) and these solutions served as the test solutions. The reaction mixture was prepared by pipetting out 150µL of test solution to a 96 well-plate and 150µL of 5% NaNO2 solution was added to it. After a 5 minutes interval, 150µL of 10% aluminium chloride solution added to the above mixture. The resulting reaction mixture was incubated for 5 minutes in a dark cabinet and then 150mL of 1.0M sodium hydroxide solution and 6.0mL of deionized water were added to this reaction mixture. The resulting mixture was incubated again for 30 minutes in a dark cabinet. The absorbance of this reaction mixture was then measured at 510nm using a UV-Vis. Spectrophotometer (Infinite M200). An average of triplicate values was used to estimate the TFCs of the individual extract. Quercetin served as standard. A mass of 1.0g standard dissolved in 1.0mL of 50% methanol (v/v) served as a stock solution of standard. Thereafter, 100, 80, 40, 60, 20, 10, and 5µg/mL dilute solutions were obtained from previously prepared stock solution. Using a similar procedure as described previously, the absorbance of various concentrations of standard was measured. A calibration curve y = 0.0076x + 0.1675; R2 = 0.9901 was obtained from the standard by plotting various concentrations taken is x-axis versus absorbance

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taken in y-axis. This calibration curve is shown in Figure 2 and this calibration cueve was used to determine the TFCs of those extracts. The TFCs of various extracts were reported as milligrams of quercetin equivalent per gram of dry weight of extract (mg QE/g DW of extract).

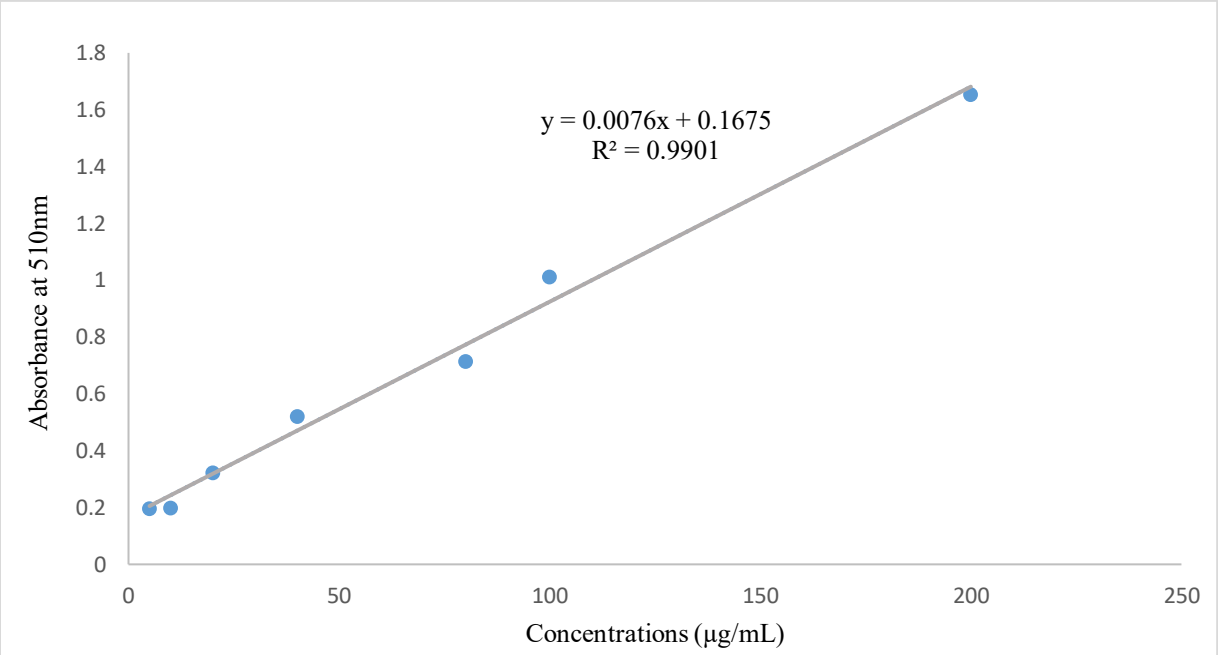

Figure 2: The calibration curve of quercetin used to determine the TFCs of various extracts.

Statistical analysis

STATISCA software version 8.0 was utilized for statistical analysis. The difference between means were statistically significant when $p \leq 0.05$. One-way ANOVA followed by Turkey's post-hoc test was used to determine significant differences among groups at $p \leq 0.05$.

Results and Discussion

The crude extracts obtained from leaves of *G. bicolor* using pentane, dichloromethane, ethyl acetate, acetone and methanol were labeled as E1, E2, E3, E4, and E5, respectively. Similarly, the crude extracts obtained from stem-bark of G. bicolor using pentane, dichloromethane, ethyl acetate, acetone and methanol were labeled as E6, E7, E8, E9 and E10, respectively. The scavenging capacity of E1-E5, E6-E10 and ascorbic acid (positive control) was assessed at various concentrations viz. 200, 500, 800, 1000, 1500, 2000, and 3000µg/mL and the results are summarized in Table 2. At 200-3000µg/mL, E1-E5 showed scavenging capacity of 28.66±0.22-74.75±0.03, 6.53±0.15-59.66±1.40, 28.42±0.16-65.07±0.54, 40.12±0.36-71.80±0.65, and 9.43±0.62-57.35±0.17%, respectively (Table 2). At 200-3000µg/mL, the positive control showed a scavenging capacity of 53.76±0.13- 83.30±0.96% (Table 2). Analysis of this result indicated that the radical scavenging capacity E1-E10 showed a linear relationship with concentrations and therefore, a maximum scavenging potential was observed at a concentration of 3000µg/mL. In addition, the scavenging potential of E1-E5 at a concentration of 3000µg/mL was 74.75±0.03, 59.66±1.40, 65.07±0.54, 71.80±0.65, and 57.35±0.17%, respectively (Table 2). Therefore, E1 (pentane extract) showed higher radical scavenging potential by followed by E4 (acetone extract). The scavenging capacity of E1 and E3 was comparable to each other. Extracts E2, E3 and E5 showed lower radical scavenging potential compared to E1 and E3. Extract E3 showed higher

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scavenging potential than E2 and E5. In other words, the scavenging capacity of E2 and E5 were comparable to each other. However, E2 and E5 showed lower scavenging potential than E3 and much lower scavenging potential compared to E1 and E4. Among the leaf extracts, E1 (pentane extract) exhibited the highest radical scavenging activity, while E5 (methanol extract) had the lowest. Furthermore, it was observed that all five extracts form the leaves (E1-E5) exhibited lower scavenging capacity at all concentrations compared to the positive control (Table 2).

Similarly, all five extracts from the stem-bark (E6-E10) also showed a linear relationship with concentrations and at concentration of 200-3000µg/mL. The scavenging capacity of these five extracts was 29.43±0.80-73.77±0.62, 35.71±0.52-80.27±0.52, 29.68±0.68-70.79±0.34, 38.81±1.38-86.04±0.29, and 17.87±0.56-66.55±0.20%, respectively (Table 2). Moreover, the scavenging potential of E6-E10 at a concentration of 3000µg/mL was 73.77±0.62, 80.27±0.52, 70.79±0.34, 86.04±0.29, and 66.55±0.20%, respectively (Table 2). Analysis of this result indicated that E9 (acetone extract) showed higher radical scavenging potential by followed by E7 (dichloromethane extract). The radical scavenging potential of E9 and E7 was comparable to each other. At higher concentrations at 1000µg/mL and above, E9 showed relatively higher scavenging potential than the positive control. On the other hand, at higher concentrations at1000µg/mL and above, E7 exhibited comparable scavenging capacity as that of the positive control. Extracts E6, E8, and E10 exhibited comparatively lower radical scavenging capacity compared to that of E9 and E7. Similarly, E6, E8, and E10 also exhibited comparatively lower radical scavenging capacity at all concentrations compared to that of the positive control. Among the extracts from stem-bark, E9 (acetone extract) exhibited the highest radical scavenging activity, while E10 (methanol extract) had the lowest. Extract E9 (acetone extract) from the stem-bark outperformed the others. In general, the extracts obtained from the stem-bark of *G. bicolor* showed higher radical scavenging potential compared to the extracts obtained from the leaves of *G. bicolor* (Table 2). For ease of comparison, the percentage of DPPH radical scavenging potential of extracts obtained from the leaves and stem-bark of G. bicolor are also shown in bar diagrams in Figure 3 and Figure 4, respectively together with radical scavenging potential of the positive control. Statistical analysis revealed significant differences between extract activities ($p < 0.05$), particularly between E4 and E5.

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Table 2: DPPH radical scavenging potential of various extracts obtained from the leaves and stem-bark of G. bicolor.

Extracts	Concentrations (µg/mL)/Inhibition (%)							IC50 (µg/mL)
	200	500	800	1000	1500	2000	3000	
E1	28.66±0.22 c	31.95±0.09 a	38.14±0.10 d	42.59±0.16 a	52.68±0.17 a	64.47±0.01 a	74.75±0.03 b	1419.89
E2	6.53±0.15b	17.83±0.17 a	28.49±0.64 b	38.52±0.01 d	48.96±0.01 c	59.52±0.36 b	59.66±1.40 a	1568.85
E3	28.42±0.16 a	38.66±0.84 a	49.17±0.60 c	52.29±0.95 b	58.20±1.26 a	62.39±0.23 a	65.07±0.54 b	867.15
E4	40.12±0.36 b	51.09±1.60 a	58.82±2.78 d	61.88±2.21 a	67.26±2.24 c	69.58±0.95 b	71.80±0.65 a	457.78
E5	9.43±0.62a	13.76±0.30 b	19.67±0.03 a	24.76±0.28 a	35.71±0.01 a	45.79±0.38 d	57.35±0.17 c	2425.37
E6	29.43±0.80 a	38.87±0.24 a	41.71±0.03 a	52.04±1.46 a	62.72±0.92 a	68.98±1.36 a	73.77±0.62 b	969.77
E7	35.71±0.52 c	39.17±2.04 b	49.64±1.25 a	55.03±0.44 b	65.17±0.03 d	70.03±0.45 b	80.27±0.52 d	846.77
E8	29.68±0.68 b	35.45±0.38 d	40.86±0.87 b	48.58±0.69 d	57.75±0.64 b	62.37±0.16 c	70.79±0.34 a	1125.23

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E9	38.81±1.38 b	42.38±1.17 c	52.99±0.12 c	65.13±0.65 b	72.15±1.42 a	78.88±0.20 d	86.04±0.29 a	649.29
E10	17.87±0.56 a	23.29±0.57 b	30.33±0.27 d	36.39±0.34 a	42.95±0.09 b	58.47±0.38 a	66.55±0.20 b	1869.62
Asc. acid	53.76±0.13 a	54.53±0.33 a	55.39±0.31 b	57.50±0.62 b	69.89±0.67 c	70.20±0.75 c	83.30±0.96 d	<200

E1-E5 = Pentane, dichloromethane, ethyl acetate, acetone and methanol crude extracts from leaves, respectively; E6-E10 = Pentane, dichloromethane, ethyl acetate, acetone and methanol crude extracts from stem-bark, respectively. Asc. acid = Ascorbic acid. Values with different superscript letters are statistically different within a column.

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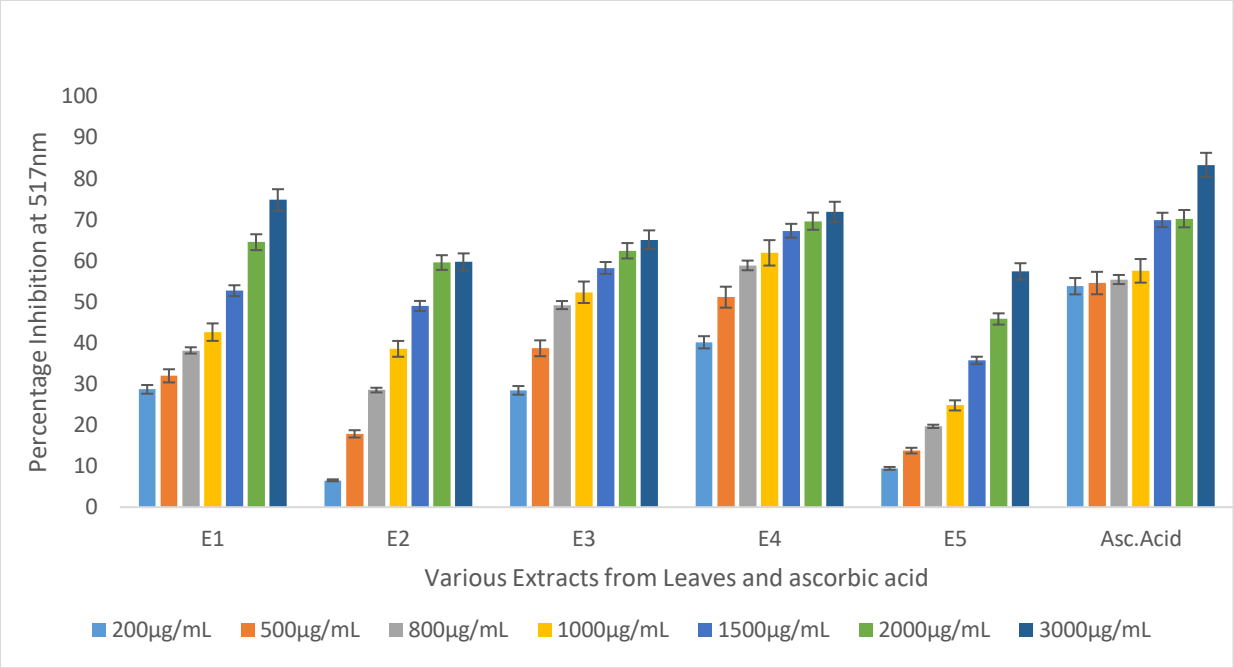
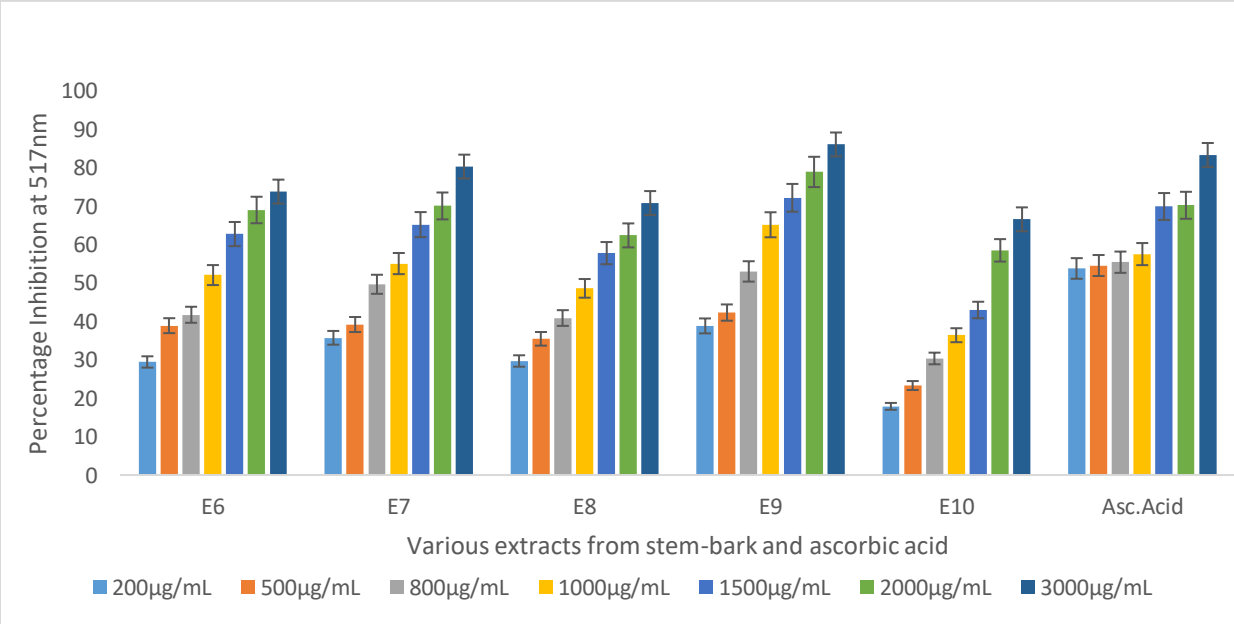


E1-E5 = refer the foot note of Table 2.

Figure 3: DPPH radical scavenging capacity of various extracts from the leaves of G. bicolor and ascorbic acid.



E6-E10 = refer the foot note of Table 2.

Figure 4: DPPH radical scavenging capacity of various extracts from the stem-bark of G. bicolor and ascorbic acid.

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The IC50 values for E1-E5, E6-E10 and ascorbic acid are also listed in Table 2. Ascorbic acid exhibited an IC50 value of >200µg/mL. The IC50 values for E1-E5 and E6-E10 were >1419.89, 1568.85, 867.15, 457.78, 2425.37µg/mL and 969.77, 846.77, 1125.23, 649.29, and 1869.62µg/mL, respectively. Analysis of the result revealed that all ten extracts (E1-E5 and E6 –E10) showed relatively higher IC50 values. In other words, all extracts exhibited relatively lower antioxidant potential. However, E4 exhibited relatively highest antioxidant potency among the extracts from leaves with an IC50 value of 457.78µg/mL followed by E3 with IC50 value of 867.15µg/mL. Similarly, E9 exhibited relatively highest antioxidant potency among the extracts stem-bark with IC50 value of 649.29µg/mL followed by E7 with an IC50 value of 846.77µg/mL. Extracts, E5 and E10 exhibited the lowest scavenging potential among all extracts with IC50 values of 2425.37 and 1869.62µg/mL. Overall, all ten extracts (E1-E5 and E6-E10) exhibited relatively higher IC50 values compared to the positive control. In other words, all ten extracts (E1-E5 and E6-E10) showed relatively lower scavenging potential compared to the positive control (refer Table 2).

The total phenolic contents (TPCs) of E1-E5 and E6-E10 are summarized in Table 3. Gallic acid served as standard. The calibration curve of this standard is given in Figure 1 and this calibration was used to determine the TPCs of those extracts. The TPCs of E1-E5 were 0.850±0.02, 0.944±0.021, 2.989±0.047, 9.444±0.42 and 9.728±0.09 mg GAE/g DW, respectively. Similarly, the TPCs of E6-E10 were 0.813±0.16, 1.082±0.08, 5.542±0.45, 11.276±0.21, and 12.259±0.03 mg GAE/g DW, respectively. Analysis of this result revealed that among the extracts from leaves, E5 (methanol extract) exhibited highest TPCs followed by E4, E3, E2 and E1 (Table 3). Additionally, the TPCs of E5 and E4 were very much comparable to each other. Extracts, E1 and E2 showed much lower TPCs compared to E4 and E5. Extract, E3 also showed lower TPCs compared to E4 and E5 but showed relatively higher TPCs than E1 and E2. Moreover, the analysis of the above result also revealed that among the extracts from stem-bark, extract E10 exhibited highest TPCs followed by E9, E8, E7 and E6 (Table 2). Extract E9 showed comparable TPCs as that of E10. Extracts E7 and E6 showed comparable TPCs with each other. However, E7 and E6 showed much lower TPCs compared to E9 and E10. Although, E8 showed relatively higher TPCs than E6 and E7, it showed relatively lower TPCs than E9 and E10 (Table 3). Overall, E10 highest TPCs whereas E6 showed lowest TPCs among all ten extracts.

Table 3: Determination of TPCs and TFCs of various extracts obtained from the leaves and stem-bark of *G. bicolor*

Extracts	TPCs (mg GAE/g DW)	TFCs (mg QE/g DW)
E1	0.850±0.02	20.211±0.54
E2	0.944±0.21	22.005±0.23
E3	2.989±0.47	34.957±0.26
E4	9.444±0.42	36.402±0.05

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E5	9.728±0.09	46.004±0.08
E6	0.813±0.16	22.054±0.25
E7	1.082±0.80	27.723±0.22
E8	5.542±0.45	38.647±0.99
E9	11.276±0.21	40.093±0.21
E10	12.259±0.03	42.128±0.18

E1-E5 and E6-E10 = Refer to the footnote of Table 2.

TFCs = Total flavonoid contents, TPCs = Total phenolic contents

The total flavonoid contents (TFCs) of E1-E5 and E6-E10 are also summarized in Table 3. Quercetin served as standard. The calibration curve of the standard is given in Figure 2 and this calibration curve was used to determine the TFCs of those extracts. The TFCs of E1-E5 were 20.211±0.0542, 22.005±0.23, 34.957±0.26, 36.402±0.05, and 46.004±0.08mg of quercetin mg QE/g DW, respectively. Similarly, the TFCs of E6-E10 were 22.054±0.025, 27.723±0.22, 38.647±0.99, 40.093±0.21, and 42.128±0.18 mg QE/g DW, respectively. Analysis of this result revealed that among the extracts from leaves, E5 (methanol extract) exhibited highest TFCs followed by E4, E3, E2, and E1 (Table 3). Extracts E4 and E3 exhibited relatively lower TFCs than E5 but they showed comparable TFCs to each other. Extracts E1 and E2 showed lower TFCs compared to E5, E4 and E3. However, E1 and E2 showed comparable TFCs to each other (Table 3). Additionally, among the extracts from stem-bark, extract E10 (methanol extract) has highest TFCs followed by E9, E8, E7, and E1 (Table 3). Extracts E9 showed comparable TFCs as that of E10. Extracts E7 and E6 showed comparable TFCs with each other. However, E7 and E6 showed much lower TFCs compared to E9 and E10. Extract E8 showed relatively higher TFCs than E6 and E7 but relatively lower TFCs than E9 and E10 (Table 3). Overall, extract E10 (methanol extract from stem-bark) showed highest TFCs among all ten extracts and extract E1 (pentane extract from leaves) showed lowest TFCs among all ten extracts. Further studies such as chromatographic profiling using HPLC or GC-MS are recommended for identification of individual bioactive compounds.

As we stated previously that leaves and peels of berries of G. bicolor have previously been evaluated for their antioxidant activity [1,13]. Fresh leaves of G. bicolor was taken in a solvent mixture of liquid nitrogen and ethanol in a ratio of 1:10 (w:v) and the mixture was homogenized [1]. This mixture was then powdered, centrifuged and the supernatant was employed to evaluate DPPH radical scavenging potential [1], ferric reducing antioxidant power (FRAP) and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity (TEAC). The DPPH radical scavenging potential, FRAP and TEAC of this supernatant liquid have been found to be ~150, ~90 and ~125µmol TE per g DW, respectively. Similarly, in another report, the deionized water and ethanol-water (60:40, v/v) extracts obtained separately by maceration technique from the peels of berries of G. bicolor [13]. The water and ethanol-water extracts showed DPPH radical scavenging potential of ~110 and ~125µM gallic acid equivalent per 0.7 g of wet samples, respectively [13]. The total phenolic contents (TPCs) of leaves and peels of berries of G. bicolor have also been reported previously [1,13]. Leaves of G. bicolor was taken in a solvent mixture

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of liquid nitrogen and methanol in a ratio of 1:5 (w: v) and the mixture was homogenized [1]. This mixture was then powdered, centrifuged and the supernatant was employed to determine the (TPCs). The TPCs of this supernatant liquid has been found to be ~32mg gallic acid equivalent per g of DW [1]. Similarly, in another report, the deionized water and ethanol-water (60:40, v/v) extracts were obtained separately by maceration technique from the peels of berries of G. bicolor [13]. These water and ethanol-water extracts showed the TPCs of ~650 and ~200mM ferulic acid equivalent per 0.7g of wet samples, respectively [13].

Conclusions

The percentage of DPPH radical scavenging capacity of extracts from the leaves and stem-bark of G. bicolor was assessed. This study demonstrated that G. bicolor extracts, particularly acetone extracts from leaves and stem-bark, pentane extracts from leaves and dichloromethane extract from stem-bark possess significant antioxidant activity. The acetone stem-bark extract (E9) showed the lowest IC50 value, surpassing ascorbic acid at higher concentrations. Methanol extracts, though weaker in activity, were richest in phenolics and flavonoids, making them promising antioxidant reservoirs. The acetone extracts obtained from both leaves and stem-bark of G. bicolor showed relatively lower IC50 values compared to other extracts. The TPCs and TFCs of these extracts were also determined. The methanol extracts from both leaves and stem-bark showed relatively higher TPCs and TFCs compared to other extracts. In general, the TFCs of various extracts were determined to be much higher than TPCs. We concluded that various extracts obtained from the leaves and stem-bark of G. bicolor showed a moderate to significant DPPH radical scavenging potential. Additionally, these extracts possessed a moderate to significant TPCs and TFCs. Further investigation into their phytochemical profiles and biological activities is warranted to explore therapeutic applications.

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Authors' contributions

Manoharan Karuppiah Pillai: Proposed idea, supervised the research work, drafted, reviewed and edited the manuscript.

Ndzinisa S Sibongalitsemba: 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.

Conflict of interests

The authors declare that there are no conflicts of interests.

Ethical approval

This study did not involve with humans or animals for experimentation and therefore, ethical approval is not applicable.

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