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Total phenolic contents and antioxidant activity of *Senna singueana, Melia azedarach, Moringa oleifera* and *Lannea discolor* herbal plants

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A B S T R A C T

Senna singueana, Melia azedarach, Moringa oleifera and *Lannea discolor* are claimed to be effective in the management of cancer by some traditional herbal practitioners in Malawi. The study evaluated methanol extracts of leaves of *S. singueana, M. azedarach, M. oleifera* and barks of *L. discolor* for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity. TPC was determined using the Folin Ciocalteau assay while TFC was determined by aluminum chloride assay. Antioxidant activity of extracts was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Barks of *L. discolor* contained the highest total phenolic content (27.64 ± 0.09 mg GAE g^{-1} DW) while leaves of *S. singueana* contained highest total flavonoid content (7.372 ± 0.01 mg QE g^{-1} DW). DPPH radical scavenging activity of all extracts ranged from 42.72 ± 0.18 to 89.09 ± 1.18% while FRAP values ranged from 68.47 ± 1.72 to 166.3 ± 1.65 mg TEACg^{-1} DW. A direct positive significant correlation ($p<0.05$) was observed between TPC and antioxidant activity. Results from the present study are comparable with literature values of well-known medicinal plants. Thus, these four medicinal plants may contain phenolic aglycones with potential anticancer activity. Future studies are needed to characterize extracts for specific phytochemicals related to anticancer activity.

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Introduction

About 80% of the world's population, particularly in developing countries, relies heavily on non-conventional medicine in their primary healthcare [1]. In developing countries, including Malawi, more people rely on herbal and traditional medicine

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for treatment of ailments mainly due to the inadequacy of modern health services and because traditional medicines are relatively cheap [2,3]. The natural phytochemicals present in the leaves, fruits, barks, stems and roots of medicinal plants include flavonoids and other phenolic compounds, which has led to their therapeutic use in the treatment of different diseases [4,5]. Phenolic compounds exhibit different biochemical and pharmacological properties and may provide health benefits through their interactions with key enzymes, signaling cascades involving cytokines and transcription factors, or antioxidant systems [6–8]. Phenolic compounds, especially flavonoids, have been investigated for their antioxidant properties and proven to be effective [7,9]. Such bioactive compounds may elicit antioxidant effects by inhibiting, delaying or preventing the oxidation of oxidizable molecules by scavenging free radicals, reducing α- tocopherol radicals, activating antioxidant enzymes, chelating metals catalysts, inducing apoptosis and diminishing oxidative stress [10,11].

Oxidative stress may lead to tissue injury in humans and cause different chronic degenerative diseases such as cancer [12–14]. The potential for bioactive compounds to provide antioxidant protection in the context of cancer depends on their ability to inactivate or inhibit and diminish the cancer-promoting action of some oxygen radicals in preventing the progression of carcinogenesis [15–17]. Cancer is one of the most common causes of morbidity and mortality worldwide and more than half of all cancer cases occur in the developing countries [18,19]. Medicinal plants have greatly contributed in the development of modern medicines that are being used to treat different ailments including cancer, of which almost 71% of new drugs that have been approved since 1981 have derived from natural products directly or indirectly [20,21]. In Malawi, some people use herbal and traditional medicines for treatment of different ailments including cancer because they are considered cheap and have less side effects [22–24]. Studies in Malawi have shown that *S. singueana, M. azedarach, M. oleifera* and *L. discolor* are claimed by some traditional herbal practitioners to be effective in the treatment of different ailments including management of cancer [25–27].

S. singueana (also known as scrambled egg, winter cassia or sticky pod) belongs to the Fabaceae family and is a shrub. Different parts of the plant are used mostly in Africa, including Malawi, for the treatment of different ailments [25]. Laboratory-based studies and information sourced from traditional herbal practitioners (THP) show that the plant may be effective in the management of skin cancer [26–28]. *M. azedarach* (chinaberry tree or pride of India) is a species of deciduous medicinal tree in the mahogany family, Meliaceae. It has been claimed that different parts of the plant (roots, barks, leaves and fruits) may be used for cancer treatment and show various *in vitro* cytotoxic activities in cancer cell lines [29,30]. *M. oleifera* (moringa or drumstick) is a tree belonging to a family of Moringaceae. Different parts of the plant, particularly the leaves, are used by traditional healers for treating various ailments and preventing malnutrition [31,32]. The leaves of the plant are also reported to be used in Malawi for treating hypertension [24]. Some THPs claim that the plant is effective in the management of breast cancer and this is supported by an *in vitro* study on the anti-cancer activity of leaf and bark extracts in breast cancer cell lines [33]. *L. discolor* (live-long) is a tree belonging to the family of Anacardiaceae. The tree grows up to 15 m in height with a neat, rounded crown. Roots, barks and leaves of the plant are used in Southern Africa, including Malawi, medicinally [26,34] and it has been shown that the bark of *L. discolor* could be a good source of natural antioxidants, which may scavenge the radicals that cause oxidative stress in the development of cancer [35].

Despite the claims and the use of *S. singueana, M. azedarach, M. oleifera* and *L. discolor* for the management of cancer in Malawi, little is known and documented regarding total phenolic content, total flavonoid content and antioxidant activity of these valuable medicinal plant species. Therefore, in the present study, we determined levels of total phenolic compounds, total flavonoids and antioxidant activity (FRAP and DPPH) in leaves of these medicinal plants. The information will be useful for further investigations to validate these claims.

Materials and methods

Chemicals and reagents

Chemicals used in this study include: Quercetin and Gallic acid from EMD Millipore Corporation (USA); Folin-Ciocalteu phenol reagent from Sigma (USA); 2,4,6-tris-2-pyridyl-s-triazine (TPTZ) and 2,2-Diphenyl-1-Picrylhydrazyl Radical (DPPH) from Sisco Research Laboratories (Maharashtra, India); 6–hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylic acid (Trolox) from Acros Organics (New Jersey, USA). All other chemicals, including sodium carbonate, aluminum chloride hexahydrate, iron (III) chloride hexahydrate and methanol, were of analytical grade and supplied by Sigma, unless otherwise stated.

Sample collection and preparation

Leaves of *S. singueana, M. oleifera, M. azedarach* and barks of *L. discolor* were collected in July 2019, just before the flowering period, from the Karonga district in northern Malawi. All plants were identified by a botanist from the National Herbarium and Botanic Gardens of Malawi. Parts were collected and air-dried in a shed for 21 days, ground to a fine powder using a domestic blender (Kenwood) and stored in black airtight bags.

Extraction of phenolic compounds was done according to the method described by several researchers [36–39] with some modification. Briefly, dry and ground materials (25 g) were dissolved in methanol (80% v/v, 300 mL) and macerated for 3 days, shaking conical flasks occasionally, and then filtered. The filtrates were concentrated by rotor evaporator (Buchi, Switzerland) at low pressure, 40 °C, and then concentrated further to dryness using a water bath at 40 °C. Then, 0.1 g of the semi-solid extracts was dissolved in methanol (80% v/v, 2.00 mL) and made up to 20 mL with distilled water and vortexed.

Exactly 1.0 mL of the dissolved extracts was transferred into falcon tubes, diluted 10-fold with distilled water, vortexed and analyzed.

Determination of total phenolic content (TPC)

Total phenolic content in the extracts was determined using Folin-Ciocalteu assay [40] and as modified by some researchers [37,41–43]. Briefly, 1.0 mL of the diluted extracts was added into falcon tubes, to which 10-fold-diluted Folin reagent (5.0 mL) was added followed by addition of sodium carbonate (1 M, 4.0 mL). This was done within 3 - 8 min and the mixtures were vortexed and left for 2 h. Absorbance of the mixtures and blank was measured using UV–Vis Spectrophotometer (Multiple plate reader, Perkin Elmer 2030 – Victor X 3) at 765 nm. Gallic acid was used as a standard solution and sample concentration was then calculated in triplicates from the standard curve (25 - 200 ug mL^{-1}). Total phenolic content was expressed as milligram gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW).

Determination of total flavonoid content (TFC)

Total flavonoid content was determined using aluminum chloride colorimetric assay [44] and as modified by some researchers [45–47]. In brief, 1.0 mL of the diluted extracts was added into falcon tubes, to which 1.0 mL of 2% aluminum chloride hexahydrate was added and vortexed. The mixture was incubated at room temperature (25 °C) for 60 min. Absorbance of the mixture and blank was measured using UV–Vis Spectrophotometer (Multiple plate reader, Perkin Elmer 2030 – Victor X 3) at 415 nm. Calibration curve with quercetin (0 - 900 µg/mL) was used for quantification in triplicates. Results were expressed as milligram quercetin equivalent per gram of dry weight (mg QE g^{-1} DW).

Ferric reducing antioxidant power (FRAP) assay

FRAP reagent was prepared freshly by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution (10:1:1 by volume) and then warmed to 37 °C before using [37,48,49]. Then 0.2 mL of diluted extracts and standard solution were added into falcon tubes, to which 6.0 mL of freshly prepared FRAP reagent was added, vortexed and incubated at RT (25^0C) for 10 min. Absorbance of the mixtures and blank was measured using UV–Vis Spectrophotometer at 593 nm. Trolox (0 to 500 ug/mL) was used for quantification and results expressed as milligram Trolox equivalent antioxidant capacity per gram of dry weight (mg TEAC g^{-1} DW).

DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) radical scavenging activity assay

Radical scavenging activity of the methanol extracts was evaluated using DPPH assay [48,50]. Briefly, 10-fold-diluted sample extracts (0.1 mL) with 80% methanol (80:20 v/v) were added to DPPH (0.1 mM, 3.9 mL). The mixtures were vortexed and incubated in the dark for 30 min at RT. Absorbance of the mixture and the control (methanol) was measured using UV–Vis Spectrophotometer at 517 nm. Trolox standards were used for quantification and results expressed as% DPPH free radical scavenging activity according to Eq. (1).

$$\%DPPH = (1 - As/Ac) \times 100 \ldots \ldots \quad (1)$$

(where As is the absorbance of the sample and Ac is the absorbance of the control.)

Statistical analysis

All the data were presented as means ± standard deviations ($n = 3$). Univariate statistical analysis was carried out using Paleontological Statistical Software Package for Education and Data Analysis –PAST-V3.26 (2019). Correlation analysis was carried out using GraphPad prism for windows, version 8.3.0 (GraphPad Software, San Diego, CA 92108, USA). Statistical significance between means was evaluated at 95% confidence level ($p < 0.05$).

Results and discussion

Total phenolic content (TPC)

Phenolic compounds have hydroxyl groups responsible for antioxidant activity important plant constituents [51,52]. Total phenolic content in herbal extracts was calculated using Gallic acid calibration curve (Table. 1).

Total phenolic content varied widely from 7.828 ± 0.05 mg GAE g^{-1} DW to 27.64 ± 0.09 mg GAE g^{-1} DW ($p < 0.05$). *L. discolor* showed the highest content of phenolic compounds while *M. azedarach* showed the lowest. The variation increased in the order of *L. discolor* > *S. singueana* > *M. oleifera* > *M. azedarach* (Fig. 1). Phenolic compounds, as a diverse group of secondary metabolites, exhibit heterogeneity in the distribution and content in plant species [9,53]. The values of phenolic content in this study differ to those in the literature [36,54]. *S. singueana* showed TPC of 15.82 mg GAE g^{-1}DW, which was lower than that reported by Alsiede et al., (3761 mg GAE g^{-1}DW) [55]. The value of TPC for *M. oleifera* (12.33 mg GAE

Table 1
The calibration curve and R^2 values of Gallic acid, Quercetin and Trolox standards.

Standard	Regression equation	R^2
Gallic acid	y = 0.0074x - 0.1065	0.9894
Quercetin	y = 0.0004x + 0.029	0.9692
Trolox	y = 0.0031x + 0.1865	0.9809

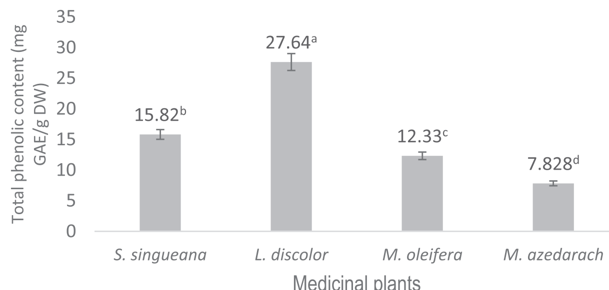

Fig. 1. Total phenolic content (mg $GAEg^{-1}$ DW) of the medicinal plants. Mean values which do not share a letter indicate significant differences (Tukey's test, $p<0.05$).

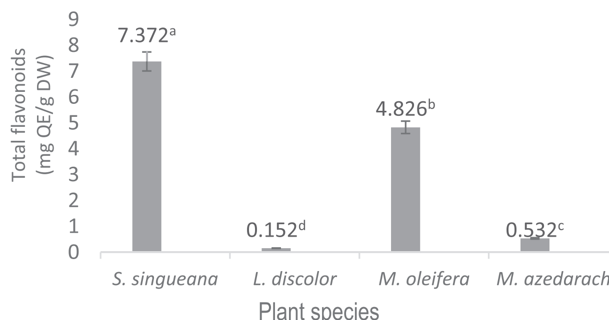

Fig. 2. Total flavonoid content (mg QE g^{-1} DW) of the medicinal plants. Mean values which do not share a letter indicate significant differences (Tukey's test, $p<0.05$).

g^{-1} DW) was lower than that reported by Charoens (216.45 mg GAE g^{-1} DW) [56], but comparable with those reported by Vyas (7.44 - 144.77 mg GAE g^{-1}DW) [57]. Munir et al., observed a TPC of 82.54 mg GAE g^{-1} DW in the extracts of *M. azedarach* [58], higher than those of the present study. Such variation in the concentrations of phenolic compounds could be due to the presence of other secondary metabolites at differing concentrations and other factors, such as plant maturity and genotypes [59,60]. The variations could also have been attributed to different methods of extraction and solvent polarities [56,57]. Different phytogeographical areas and different seasons during which the plants were harvested could also modify the contents of phenolic compounds and other phytochemicals of these medicinal plants [60–62].

Total flavonoid content (TFC)

Flavonoids play a number of functions in plants and have long been suggested to represent a secondary antioxidant defense system in plant tissues when exposed to different stresses [7,63]. A standard curve of Quercetin was used for calculating the total flavonoids content (TFC) (Table. 1). Leaves of *S. singueana* contained the highest amount of TFC (7.372 ± 0.01 mg QE g^{-1} DW), while the bark of *L. discolor* contained the lowest (0.152 ± 0.01 mg QEg^{-1} DW) (Fig. 2). The results increased in the following ranking order: *L. discolor* < *M. azedarach* < *M. oleifera* < *S. singueana.* The contents of flavonoids varied widely, as for the total phenolic content, and the ranking order was significantly correlated ($p<$ 0.05).

The bark of *L. discolor* showed the lowest amount of TFC despite showing highest level of phenolic content (27.64 ± 0.09 mg GAE/g DW). It has been reported that antioxidant flavonoids are mostly located in the nucleus of mesophyll cells and, at the primary site of reactive oxygen species (ROS) generation, in the chloroplasts [64]. The results of this study again vary to those reported in the literature. Munir et al., reported a TFC of 16.99 mg QE/g DW in the extracts of *M. azedarach* [58]. Alsiede et al., observed a TFC of 1020.64 mg QE/g DW in the methanol extract of *S. singueana* [55]. Charoens in found a TFC of 65.38 mg QE/g DW in the methanol extracts of leaves of *M. oleifera* [56], while Vyas reported that TFC varied between 2.51 and 57.64 mg QE g^{-1} DW and a significantly higher concentration was found in 20% methanol and

Table 2
Antioxidant activities of the medicinal plant species.

Parts of plant species	FRAP (mg TEAC g^{-1} DW)	DPPH (% radical scavenging activity)
Leaves of *S. singueana*	124.4 ± 0.91^b	67.2 ± 1.42^b
Leaves of L. *discolor*	166.3 ± 1.65^a	89.09 ± 1.18^a
Leaves of *M. oleifera*	85.13 ± 0.96^c	57.94 ± 2.81^c
Barks of *M. azedarach*	68.47 ± 1.72^d	42.72 ± 0.18^d

Table 3
Pearson's correlation coefficients (r) for TPC and antioxidant activity.

r	TPC	FRAP
DPPH	0.9882*	0.9790*
FRAP	0.9745*	1*

Abbreviations: Total Phenolic Content (TPC), 2, 2-diphenyl-1-picrylhydrazyl radicle (DPPH), Ferric Reducing Antioxidant Power (FRAP). *$p < 0.05$.

water extracts followed by chloroform and petroleum ether extraction [57]. The variations could be as a result of the same factors which have been suggested to affect TPC [59–62].

Antioxidant activity

DPPH and FRAP are important assays for the evaluation of the free radical-scavenging and metal ion-chelating abilities of plant extracts respectively. DPPH is a stable free radical that exhibits a deep purple color with an absorption maximum at 517 nm. This purple color generally fades when an antioxidant quenches DPPH free radicals by electron donation through redox reactions [65]. The results of DPPH ranged between 89.09 ± 1.18% and 42.72 ± 0.18%, while those of FRAP ranged between 166.3 ± 1.65 and 68.47 ± 1.72 mg TEAC g^{-1} DW (Table 2). It has been reported that the antioxidant activity of these medicinal plants might be attributed to the presence of phenolic compounds, including flavonoids, due to the presence of hydroxyl functional groups, which have redox properties [66].

Each value is expressed as mean ± standard deviation ($n = 3$), DPPH: 2, 2-diphenyl-1-picrylhydrazyl radicle, FRAP: Ferric Reducing Antioxidant Power, TEAC: Trolox Equivalent Antioxidant Capacity, DW: Dry Weight. Mean values within a column that do not share a letter indicate significant differences (Tukey's test, $p<0.05$).

The results of the antioxidant activity are within the range of those reported in the literature. Munir et al. found that the DPPH free radical scavenging activity in the extracts of *M. azedarach* was 58.87% [58]. Surveswaran reported that among 137 medicinal plant samples the total antioxidant capacity ranged from 40.00 to 31,050 mg TEAC g^{-1} DW for FRAP [36]. Alsiede et al. found that the DPPH free radical scavenging activity in the methanol extracts of *S. singueana* was 89% inhibition DPPH [55]. The results of the FRAP and DPPH assays of the present study suggest that the extracts have compounds with ability to donate protons and could serve as free radical inhibitors. Many phenolic compounds possess antioxidant effects and have the ability to inactivate lipid-free radicals or prevent decomposition of hydroperoxides into free radicals [58,67,68]. Free radicals are one of the major factors that cause DNA mutation that initiate carcinogenesis [69,70].

The present study also showed significant positive linear correlation between antioxidant activity and total phenolic content (Table 3). The correlations imply plausible contribution of phenolic compounds to the antioxidant activity of these plant extracts [71,72]. Medicinal plants with considerably high antioxidant activity have been reported to possess anticancer activities [6,51,73]. However, confirmatory investigations are needed on the medicinal plants.

Conclusion

The leaves of *S. singueana, M. azedarach, M. oleifera* and bark of *L. discolor* contained phenolic compounds that contributed to a higher antioxidant activity. Thus, these herbs can be used as potential sources of natural antioxidants in pharmaceuticals to improve the function of the endogenous antioxidant system and help to reduce levels of free radicals in the body. We recommend further experimental and clinical studies to validate the claims for their use in the management of cancer.

Declaration of Competing Interest

Authors declare that there are no conflicts of interest associated with this work and publication of this paper.

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