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Total Phenolic Content and Free Radical Scavenging Activity of *Malva parviflora* L. (Malvaceae)

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Abstract: The free radical scavenging activity of the methanolic extract of *Malva parviflora* L. was examined using spectroscopic method against 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH[•]), radical cation ABTS^{•+} (2, 2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt) and the ferric reducing agent. Total phenolic, flavonoid and proanthocyanidin contents were also determined to assess their effects on the antioxidant activity of the extract. The results showed that the methanol extract of *M. parviflora* differed in its capacity to quench or inhibit DPPH[•] and ABTS^{•+}. The extract showed a greater ability to quench ABTS^{•+} by inhibiting 94.3% of the radical cation while it inhibited 9.3% of DPPH[•]. The free radical scavenging activities were compared using BHT and rutin as reference antioxidants. The plant possessed a higher flavonoid content than phenolics and proanthocyanidins and a positive linear correlation was established between these polyphenols and the free radical scavenging activities.

Key words: *Malva parviflora*, flavonoids, antioxidants, phenolics, reducing power

INTRODUCTION

Malva parviflora L. (Malvaceae) is a cosmopolitan weed found in gardens and waste places in South Africa and Namibia. Traditionally, the leaves of the plant are used in the treatment of wounds in South Africa (Shale *et al.*, 1999) and the roots for the treatment of asthma and wound in Ethiopia (Hailu *et al.*, 2005). Ethnobotanical survey carried out by Grierson and Afolayan (1999) showed that the leaves and stems of this plant, with or without the addition of heated brown sugar, is applied as hot poultice to wounds and boils by the Xhosa people of South Africa. Shale *et al.* (1999) also reported the use of its lotion to treat bruises and broken limbs and the dried powder or infusion of the leaves and roots to clean wounds and sores by the herbalists in Lesotho. The methanol extract of *Malva parviflora* also possessed appreciable activity against Gram-negative and Gram-positive bacteria as well as anti-inflammatory activity against COX-1 (Shale *et al.*, 1999).

The process of wound healing involves a series of cellular and cytokine mediated events, resulting in the contraction and closure of the wound and restoration of a functional barrier (Rasik and Shukla, 2000; Chattopadhyay *et al.*, 2002). These events include inflammation, tissue formation and skin remodeling (Wlaschek and Scharffetter-Kochanek, 2005). Inflammation results in the generation of Reactive Oxygen Species (ROS) which have been found to play both

beneficial and deleterious roles in the process of wound healing (Shukla *et al.*, 1997; McDaniel *et al.*, 1998; Sen *et al.*, 2002). The harmful effect of overproduction of free radicals on cells and tissues consequently leads to diseases such as skin cancer, psoriasis and impaired skin wound healing (Virág *et al.*, 2002). According to Thiem and Grosslinka (2003), surface application of substances with free-radical scavenging properties has shown to significantly improve wound healing and protect tissues from oxidative damage.

This study aims to justify the possible role which the polyphenolic contents and free radical scavenging property of *M. parviflora* can play in the treatment of wounds.

MATERIALS AND METHODS

Collection and extraction of plant materials:

Malva parviflora was collected from the Nkonkobe Municipality of the Eastern Cape Province, South Africa in May 2007. The plant was previously authenticated (Grierson and Afolayan, 1999) and deposited at the Giffens herbarium of the University of Fort Hare. Air dried sample (65 g) of the whole plant was extracted with methanol by shaking for 24 h in an orbital shaker. The extract was filtered using a Buchner funnel and Whatman No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40°C to yield 9.0 g of methanolic extract.

Reagents used: The following reagents were used for the analysis: Butylated hydroxytoluene (BHT), 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), gallic acid, rutin, catechin, potassium ferricyanide, 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), quercetin and FeCl₃ were purchased from Sigma Chemical Co. (St. Louis, MO, USA); folin-ciocalteu phenol reagent and sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany), while vanillin was from BDH Chemicals Ltd. (Poole, England). All chemicals used, including the solvents, were of analytical grade.

ABTS assay: The method of Re *et al.* (1999) was adopted for the ABTS radical scavenging assay. The stock solution which was allowed to stand in the dark for 16 h at room temperature contained equal volumes of 7 mM ABTS salt and 2.4 mM potassium persulfate. The resultant ABTS^{•+} solution was diluted with methanol until an absorbance of about 0.70±0.01 at 734 nm was reached. Varying concentrations of the plant extracts (1 mL) was reacted with 1 mL of the ABTS^{•+} solution and the absorbance taken at 734 nm between 3-7 min using the spectrophotometer. The ABTS^{•+} scavenging capacity of the extract was compared with that of BHT and rutin and the percentage inhibition calculated as:

$$\text{ABTS radical scavenging activity (\%)} = \left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \right] \times 100$$

where, Abs_{control} was the absorbance of ABTS radical+methanol; Abs_{sample} was the absorbance of ABTS radical+sample extract/standard.

DPPH assay: The effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). A solution of DPPH (0.135 mM) in methanol was prepared and 1 mL of this solution was mixed with 1 mL of varying concentrations of the methanolic extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm using rutin and BHT as references. The ability to scavenge DPPH radical was calculated as:

$$\text{(\%)} \text{ DPPH radical scavenging activity} = \left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \right] \times 100$$

where, Abs_{control} was the absorbance of DPPH radical+methanol; Abs_{sample} was the absorbance of DPPH radical+sample extract/standard.

Determination of total phenols: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Wolfe *et al.*, 2003). The extract (1 mg mL⁻¹) was mixed with 5 mL folin-ciocalteu reagent

(previously diluted with distilled water 1:10 v/v) and 4 mL (75 g L⁻¹) of sodium carbonate. The mixture was vortexed for 15 sec and allowed to stand for 30 min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer. Samples of extract were evaluated at a final concentration of 1 mg mL⁻¹. Total phenolic content was expressed as mg g⁻¹ gallic acid equivalent using the equation obtained from the calibration curve: $y = 5.068x + 0.3755$, $R^2 = 0.9247$.

Determination of total flavonoids: Total flavonoid was estimated using the method of Ordonez *et al.* (2006). A 2% AlCl₃ ethanol solution (0.5 mL) was added to 0.5 mL of extract. After 60 min at room temperature, the absorbance was measured at 420 nm. The extract was evaluated at a final concentration of 1 mg mL⁻¹. Total flavonoid content was calculated as quercetin equivalent (mg g⁻¹) using the equation obtained from the calibration curve: $y = 55.232x - 0.4499$, $R^2 = 0.955$.

Determination of total proanthocyanidins: The procedure reported by Sun *et al.* (1998) was used to determine the total proanthocyanidin. A volume of 0.5 mL of 0.1 mg mL⁻¹ extract solution was mixed with 3 mL of 4% vanillin-methanol solution and 1.5 mL hydrochloric acid, the mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm. Extract was evaluated at a final concentration of 0.1 mg mL⁻¹. Total proanthocyanidin content was expressed as catechin equivalents (mg g⁻¹) using the equation based on the calibration curve: $y = 1.8223x + 0.0157$, $R^2 = 0.7246$.

Determination of ferric reducing power: The ferric reducing potential of the extract was assayed as described by Duh *et al.* (1999). The different concentrations of the extract and the standards, rutin and BHT (0.02-0.10 mg mL⁻¹; 1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1% w/v). The mixture was incubated at 50°C for 20 min. 2.5 mL of TCA (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000 g. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL 0.1% w/v FeCl₃. The absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Statistical analysis: The experimental results were expressed as mean±standard deviation (SD) of three replicates.

RESULTS AND DISCUSSION

Free radicals and their scavenging systems play important roles in the healing of normal and

Table 1: ABTS radical scavenging activity of the methanolic extract of *M. parviflora*

Concentration (mg mL ⁻¹)	Inhibition (%)		
	BHT	Methanol extract	Rutin
0.025	80.72±0.00	71.13±0.03	85.43±0.06
0.05	90.05±0.01	72.14±0.01	94.95±0.03
0.1	96.18±0.02	76.97±0.02	78.93±0.01
0.2	96.76±0.02	84.00±0.01	77.01±0.05
0.5	97.57±0.01	94.32±0.03	79.45±0.00

delayed healing types of wounds (Shukla *et al.*, 1997; McDaniel *et al.*, 1998). The results of the evaluation of the antioxidant potential of *M. parviflora* and synthetic antioxidants BHT and rutin through the free radical scavenging of DPPH is shown in (Fig. 1). The dose-response curve of DPPH radical scavenging activity of the extract and standards showed that at the highest concentration (0.5 mg mL⁻¹), the scavenging effect of the methanolic extract reached 9.3% as compared to 98.2 and 97.7% for BHT and Rutin respectively. The weak DPPH radical scavenging activity of *M. parviflora* may be a result of very low concentration of phenolic compounds.

The ABTS assay is based on the inhibition by antioxidants of the absorbance of the ABTS radical cation (ABTS^{•+}) (Sanchez-Moreno, 2002). A concentration-dependent activity was observed in this assay (Table 1). Higher concentrations of the extract were more effective in quenching free radicals in the system. At a concentration of 0.5 mg mL⁻¹, the methanol extract possessed a comparable activity to BHT (94.3 and 97.6%, respectively) whereas rutin quenched all the radicals at a concentration of 0.05 mg mL⁻¹.

The radical scavenging capacity of the methanol extract of *M. parviflora* against the tested radicals (DPPH and ABTS^{•+}) may be due to the different mechanisms involved in the radical-antioxidant reactions. These assays differ from each other in terms of substrates, probes, reaction conditions and quantitation methods. Radical systems in antioxidant evaluations may influence the difference in the results obtained in an experiment (Yu *et al.*, 2002). Some compounds, though possessed ABTS^{•+} scavenging activity, did not exhibit DPPH scavenging activity (Wang *et al.*, 1998). Thus, comparison of assays is difficult and ranking of antioxidant activity is strongly dependent on the test system and on the substrate to be protected by the antioxidants (Frankel and Meyer, 2000). It is interesting to note that in this study only BHT exhibited the same activity in both radical scavenging systems.

The reducing power of the extract, BHT and rutin increased with increasing concentration (Fig. 2) in the following order, BHT > rutin > methanol extract. Antioxidant activity has been shown to be related to the development of reductones, which are terminators of free

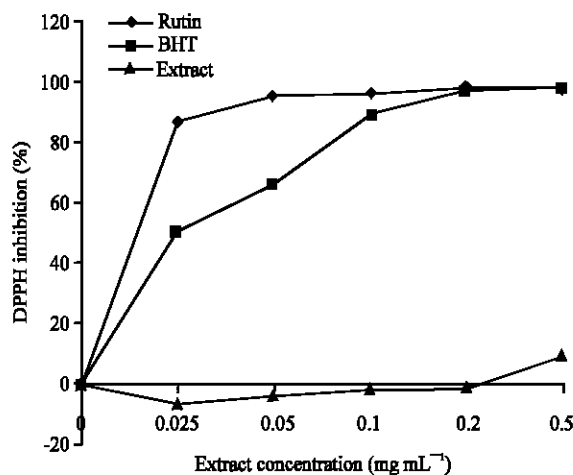


Fig. 1: DPPH radical scavenging activity of the methanolic extract of *Malva parviflora*

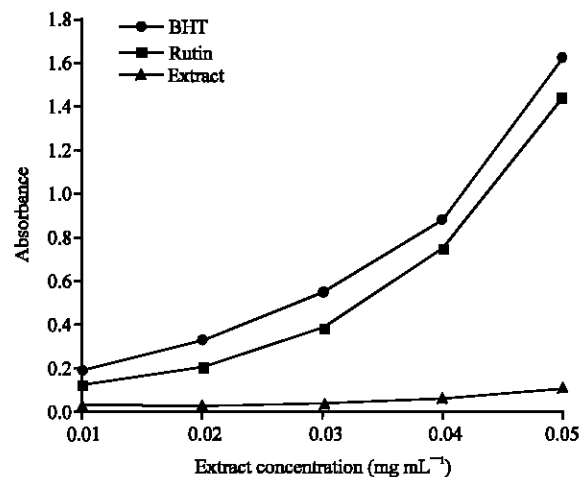


Fig. 2: Total ferric reductive potential of methanol extract of *Malva parviflora*

radical chain reactors (Gordon, 1990). The presence of reductants such as antioxidant substances in the samples causes a reduction of the Fe³⁺/ferricyanide complex to the ferrous form. Therefore, the ability of a compound to transfer electron is a significant indicator of its potential as an antioxidant (Meir *et al.*, 1995). The results showed that a good correlation exist between reducing power, DPPH radical scavenging activity and total phenolic content of the extract. This may explain the low activity observed in these assays.

Phenolic compounds are high level antioxidants (Hall and Cuppett, 1997) because they possess the ability to adsorb and neutralize free radicals, quench active oxygen species and decompose superoxide and hydroxyl radicals (Duh *et al.*, 1999). In this investigation, *M. parviflora* possessed a considerable low level of

Table 2: Polyphenolic contents and antioxidant activity of *Malva parviflora*

Extract	Total phenol ^a	Flavonoids ^b	Proanthocyanidins ^c	DPPH	ABTS*
Methanol	2.9±0.03 ^a	32±0.2 ^a	2±0.01 ^a	09.3±0.01	94.3±0.03
BHT	ND	ND	ND	98.2±0.01	97.6±0.01
Rutin	ND	ND	ND	97.7±0.00	79.4±0.00

Analyses were means of three replicates±standard deviations, ^aExpressed as mg gallic acid g⁻¹ of dry plant material, ^b expressed as mg quercetin g⁻¹ of dry plant material, ^c expressed as mg catechin g⁻¹ of dry plant material ND: Not Determined

phenols and proanthocyanidins (Table 2). The relatively low level of total phenols might account for the weak activity observed in the DPPH radical scavenging assay. The total flavonoid content of *Malva parviflora* was high compared to the phenolic and proanthocyanidin contents. A positive linear correlation was observed between the total phenol and flavonoid contents ($R^2 = 0.9299$) and also between total phenol and proanthocyanidins ($R^2 = 0.7378$), thus indicating a significant positive relationship between the total phenols, flavonoids and proanthocyanidins.

CONCLUSION

The traditional use of *Malva parviflora* as a good wound healing herb may be explained in part by the activity of the flavonoids present in its extract since a weak content of phenols was observed. Flavonoids have been reported to possess anti-inflammatory and antibacterial activities which might be responsible for the strong antioxidant activity (94.3%) observed in the ABTS radical scavenging activity.

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