

Comparative antioxidant-capacity and -content of leaves, bulbs, roots, flowers and fruit of *Gethyllis multifolia* L. Bolus and *G. villosa* Thunb. species

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Abstract

The total polyphenol, oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP), radical cation scavenging ability, flavonol and flavanone contents were measured in the leaves, bulbs, roots, flowers and fruit (dry weight) of two natural populations of *Gethyllis multifolia* (Kukumakranka) and *Gethyllis villosa*. The flowers and fruit of *G. multifolia* and *G. villosa* showed higher, and in some cases significantly ($P < 0.05$) higher antioxidant activities when compared to the leaves, bulbs and roots. This, however, was not true for the flavanone content in both species. The total polyphenol content in the fruits of *G. multifolia* (21.54 mg GAE/g) and *G. villosa* (27.64 mg GAE/g) were found to be in agreement with those of raisins (28.30 mg GAE/g), blueberries (24 mg GAE/g) and strawberries (15.40 mg GAE/g). The FRAP values of *G. multifolia* flowers (76.66 $\mu\text{mole AAE/g}$) and fruit (91.51 $\mu\text{mole AAE/g}$) were found to be significantly ($P < 0.05$) higher than those of the other plant parts (16.76 to 39.08 $\mu\text{mol AAE/g}$). On the other hand, the flowers (590.23 $\mu\text{mol TE/g}$) and fruit (741.16 $\mu\text{mol TE/g}$) of *G. villosa* revealed a significantly ($P < 0.05$) higher ORAC when compared to the other plant parts (251.25 to 410.60 $\mu\text{mol TE/g}$). A strong correlation was evident in the fruit of both species between the total polyphenols and FRAP ($r = 0.95$), ORAC ($r = 0.95$) and flavonol content ($r = 0.79$).

Keywords: Antioxidant; *Gethyllis*; Kukumakranka; Polyphenols; Flavonols; Flavanones; ORAC; FRAP; ABTS

1. Introduction

The medicinal value of plants has become more evident during the past few decades owing largely to the discovery of extracts from plants that contain a diverse array of secondary metabolites with antioxidant potential (Akinmoladun et al., 2007). Free radicals, as associated with exposure to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, may cause depletion of antioxidants which are implicated in the protection of the

immune system (Halliwell, 1994; Kuhn, 1976; Kumpulainen and Salonen, 1999; Younes, 1981). These free radicals also contribute to many disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Cook and Samman, 1996; Kumpulainen and Salonen, 1999). Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants in reducing such free radical-induced tissue injury (Schuler, 1990). Fruits and vegetables contain high levels of polyphenolic compounds with antioxidant activity and can reduce the risk of chronic inflammation in humans (Finley, 2004). Plant-based antioxidants are now preferred to the synthesized ones because of safety concerns (Akinmoladun et al., 2007). These factors have inspired the widespread screening of plants for possible medicinal and

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antioxidant properties, and the development and utilization of antioxidants of natural origin (Jayaprakasha et al., 2001).

Gethyllis multifolia L. Bolus and *Gethyllis villosa* Thunb. (Family: Amaryllidaceae) are indigenous to South Africa, winter growing, summer-blooming, deciduous and bulbous geophytes (Du Plessis and Delpierre, 1973). The genus *Gethyllis* consists of 37 currently accepted species and subspecies (Müller-Doblies, 1986). The genus *Gethyllis* is more commonly known as "Kukumakranka" in South Africa, and is one of the most extraordinary and poorly researched of all southern African amaryllids (Liltved, 1992). The medicinal uses of this genus range from cures for colic, digestive disturbances, teething problems, fatigue, boils, bruises and insect bites, to being used as an aphrodisiac (Du Plessis and Delpierre, 1973). Apart from its medicinal properties, many members of this genus (including *G. multifolia*) have a highly fragrant, edible fruit, which was used in the past to perfume rooms and linen (Lighton, 1992). *Gethyllis* grows under full sun conditions and their natural habitats range from sandy soils to mountainous rocky terrains. The genus have four distinctive growth phases: a winter (cold and wet) growing phase, leaf senescence towards spring, flowering during the hot and dry summer months (when no leaves are present) and fruit formation in autumn, which is the start of a new growing season (Du Plessis and Duncan, 1989).

In South Africa, there is a reluctance amongst traditional healers to use medicinal plants from cultivated sources because they believe that plants from the wild are more "potent" (Dold and Cocks, 2002). Besides reports on anti-inflammatory, anti-bacterial and anti-mutagenic studies, no published reports on the antioxidant capacity and chemical composition of these two *Gethyllis* species could be found (Elgorashi and Van Staden, 2003). Although according to Rood (1994), the fruit and flowers are the most medicinally useful parts of the *Gethyllis* species, there is however, to our knowledge, no published literature reporting on scientific studies based on these plant parts, nor is there any report on antioxidant studies on *Gethyllis* species. Previously, the following compounds, the dihydroxydimethylbenzopyran-4-one: isoeugenitol, its 5-*O*-glycoside and 9Z-octadec-9-enamide, had been isolated from the roots and bulbs of *G. ciliaris* (Elgorashi et al., 2007). A preliminary study on the antioxidant capacity of *G. multifolia* and *G. villosa*, had revealed high antioxidant activity in the leaves and roots (unpublished data). It is also proposed, through photographic studies of the fruit of *G. multifolia* and *G. villosa*, that the dark red colour in the fruit of *G. multifolia* may be due to the presence of colour pigments i.e. carotenoids or anthocyanins that are known to have antioxidant activity. *G. multifolia* is threatened in its natural habitat and conservation methods to prevent traditional healers from plundering the natural biodiversity have largely been unsuccessful (Dold and Cocks, 2002).

As previously mentioned, the genus *Gethyllis* is poorly researched and there are many reports of its medicinal uses in the past which have not been scientifically justified. *G. multifolia* and many other members of this genus have a highly fragrant and tasty fruit with claimed medicinal uses by traditional healers and old folk. This study reports on the

comparative investigation of antioxidant capacity and content of the leaves, bulbs, roots, flowers and fruit of natural populations of *G. multifolia* and *G. villosa*.

2. Materials and methods

2.1. Plant materials

G. multifolia and *G. villosa* bulbs were obtained in 2006 after their winter growth phase (July to mid-August), from their natural habitat (Worcester, Western Cape, South Africa). New roads and sewerage lines are planned through parts of this existing population. Mature plants of the same height and diameter were excised into leaves, bulbs and roots (n=10) and dried in a laboratory oven (Mettler, Laboratory & Scientific, Cape Town, SA) at 50 °C for one week. Individual plant parts were ground to a powder in a portable spice grinder (Krupps 75, model F203). From the same population, flowers (n=10) of both species were collected at the beginning of December 2006 (summer), while fruit (n=7) of both species were collected towards the end of March 2007 (autumn). The same drying and grinding procedures as for the leaves, bulbs and roots were followed.

2.2. Sample preparation

Crude extracts of the leaves, bulbs, roots, flowers and fruit of both species were prepared by stirring the various dried, powdered plant materials (0.05 g of each) in 80% (v/v) ethanol (50 mL) (EtOH) (Saarchem, South Africa) where after it was centrifuged at 4000 rpm for 5 min. The supernatants were used for all analyses. The same sample preparation technique was followed for all assays and all analyses were performed in triplicate.

2.3. Determination of antioxidant-capacity and -content

According to Prior et al. (2005), it was recommended, through evaluation of the literature and data presented at the 'First International Congress on Antioxidant Methods in 2004', that three methods be considered for standardization of antioxidant capacity and total polyphenol determination in food and dietary supplements. Method 1: the oxygen radical absorbance capacity (ORAC) assay which represents a hydrogen atom transfer (HAT) reaction mechanism, which is most relevant to human biology. Method 2: the trolox equivalent antioxidant capacity (TEAC/ABTS) assay represents a single electron transfer (SET)-based method which indicates reducing capacity. Method 3: the ferric reducing/antioxidant power (FRAP) assay which is also a SET-based assay and a direct test of the total antioxidant power of a biological sample. It is further suggested that experimental work on a series of assays gives a better understanding of the antioxidant capacity of a sample (Prior and Cao, 1999; Prior et al., 2005).

2.4. Total polyphenol, flavonol and flavanone content

The total polyphenol content of the various crude extracts were determined by the Folin Ciocalteu method (Singleton

et al., 1974; Swain and Hills, 1959). The method of Swain and Hills (1959) was adapted for use in a plate reader. Using a 96 well microplate, 25 μL of sample was mixed with 125 μL Folin–Ciocalteu reagent (Merck, South Africa), diluted 1:10 with distilled water. After 5 min, 100 μL (7.5%) aqueous Sodium Carbonate (Na_2CO_3) (Sigma-Aldrich, South Africa) was added to the well. The plates were incubated for 2 h at room temperature before the absorbance was read at 765 nm using a Multiskan plate reader (Thermo Electron Corporation, USA). The standard curve was prepared using 0, 20, 50, 100, 250 and 500 mg/L gallic acid in 10% EtOH and the results were expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW).

The flavonol content was determined using quercetin 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, South Africa) as standard. In the sample wells, 12.5 μL of the crude sample extracts was mixed with 12.5 μL 0.1% HCl (Merck, South Africa) in 95% ethanol, 225 μL 2% HCl and incubated for 30 min at room temperature. The absorbance was read at 360 nm, at a temperature of 25 °C (Mazza et al., 1999). The results were expressed as mg quercetin equivalent per g dry weight (mg QE/g DW).

The flavanone content was determined using the method of Kosalek et al. (2004). Briefly, 100 μL of sample was mixed with 200 μL 1% 2,4-dinitrophenylhydrazine (DNPH) (2% H_2SO_4 in methanol (MeOH)). After incubation at 50 °C for 50 min., 700 μL of 10% Potassium hydroxide (KOH) in 70% MeOH was added. The samples were centrifuged and 30 μL of the resulting supernatant mixed with 270 μL MeOH in a 96-well plate and the absorbance read at 495 nm. A linear standard curve using 0, 0.2, 0.5, 1.0, 1.5, and 2.0 mg/mL naringenin (Sigma-Aldrich, South Africa) in methanol was included. The results were expressed as mg naringenin equivalent per g dry weight (mg NE/g DW).

2.5. Antioxidant capacity

2.5.1. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed using the method of Benzie and Strain (1999). In a 96-well microplate, 10 μL of the crude sample extract was mixed with 300 μL FRAP reagent [0.3 M acetate buffer, pH 3.6 (Saarchem, South Africa), 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) in 0.1 M HCl (Sigma-Aldrich, South Africa), 20 mM Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) (Sigma-Aldrich, South Africa), 6.6 mL distilled water] and incubated for 30 min at 37 °C in the plate reader. Absorbance was measured at 593 nm. L-Ascorbic acid (Sigma-Aldrich, South Africa) was used as a standard with concentrations varying between 0 and 1000 μM . The results were expressed as μM ascorbic acid equivalent per g dry weight (μM AAE/g DW).

2.5.2. 2,2'-azino-di-3-ethylbenzthiazoline sulphonate (ABTS) assay

The ABTS assay was performed following the method of Re et al. (1999). The stock solutions included a 7 mM ABTS and 140 mM Potassium-peroxodisulphate ($\text{K}_2\text{S}_2\text{O}_8$) (Merck, South

Africa) solution. The working solution was then prepared by adding 88 μL $\text{K}_2\text{S}_2\text{O}_8$ to 5 mL ABTS solution. The two solutions were mixed well and allowed to react for 24 h at room temperature in the dark. Trolox (6-Hydrox-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard with concentrations ranging between 0 and 500 μM . Crude sample extracts (25 μL) were allowed to react with 300 μL ABTS in the dark at room temperature for 30 min before the absorbance was read at 734 nm at 25 °C in a plate reader. The results were expressed as μM trolox equivalent per g dry weight (μM TE/g DW).

2.5.3. Oxygen radical absorbance capacity (ORAC) assay

The peroxy radical was generated using 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH) (Sigma-Aldrich, South Africa), prepared fresh for each determination according to the method of Prior et al. (2003). Fluorescein was used as the substrate, with the fluorescence conditions set at 485 nm excitation and 530 nm emission. Absorbance was read using a Fluorskan Ascent plate reader (Thermo Electron Corporation, USA). The standard curve was linear between 0 and 25 μM Trolox. The results were expressed as μM trolox equivalent per g dry weight (μM TE/g DW).

2.6. Statistical analysis

The statistical significance between antioxidant activity values of the various crude plant extracts was determined by an analysis of variance (ANOVA) where $P < 0.05$ was considered to be statistically significant. The computer program employed for the statistical analysis was Medcalc version 9.4.2.0 (Medcalc, Belgium). Microsoft Office Excel 2006, version 12.0.6214.1000 (Microsoft Corporation, USA) was employed to determine the correlation between antioxidant contents and activity.

3. Results

3.1. Total polyphenol, flavonol and flavanone content

The total polyphenols in the leaves and bulbs of *G. villosa* were found to be significantly ($P < 0.05$) higher than those of *G. multifolia* (Table 1). No significant ($P > 0.05$) differences were found when the total polyphenols in the roots, flowers and fruit of *G. multifolia* were compared to those of *G. villosa*. The total polyphenols in the flowers and fruit of *G. multifolia* were found to be significantly ($P < 0.05$) higher than the other plant parts. In *G. villosa*, only the total polyphenols in the fruit were found to be significantly ($P < 0.05$) higher than the other plant parts. Apart from the roots, *G. villosa* in general, had higher ($P > 0.05$) total polyphenol values than *G. multifolia*. A strong correlation was evident in the fruit of both species between the total polyphenols and FRAP ($r = 0.95$), ORAC ($r = 0.95$) and flavonol content ($r = 0.79$). The total polyphenols in a pilot study of the fresh weights of the fruit of both species were significantly ($P < 0.05$) lower than the dry weights (1.79 GAE \pm 1.24 for *G. multifolia* and 2.33 GAE \pm 0.71 for *G. villosa*).

Table 1

The total polyphenol (mg GAE/g dry weight), flavonol (mg QE/g dry weight) and flavanone (mg NE/g dry weight) content of the leaves, bulbs, roots, flowers and fruit of *G. multifolia* and *G. villosa* plants.

Plant species	Total polyphenols	Flavonols	Flavanones
<i>G. multifolia</i>			
Leaves	12.36±0.57a	2.60±0.30a	3.27±0.19a
Bulbs	7.67±1.30c	1.20±0.58b	3.02±0.45a
Roots	14.32±1.52b	3.70±0.78c	4.12±0.87b
Flowers	20.86±2.58de	7.12±1.38d	2.09±0.36ce
Fruit	21.54±11.45e	5.70±4.32eg	2.26±1.18ce
<i>G. villosa</i>			
Leaves	20.81±1.95de	6.74±0.61dg	4.42±1.11bd
Bulbs	17.84±8.16d	5.55±3.25e	5.15±2.17d
Roots	12.04±2.44a	3.20±1.46a	4.05±0.98b
Flowers	22.23±5.46e	4.76±1.65f	2.50±0.37e
Fruit	27.64±8.57f	6.38±5.25g	2.99±0.81ae

Values represent the mean±SD (leaves, bulbs, roots and flowers n=10; fruit n=7). Means in the same column with different letters are significantly (P<0.05) different.

In *G. villosa*, the flavonol contents of the leaves and bulbs were significantly (P<0.05) higher than the leaves and the bulbs of *G. multifolia* (Table 1). On the other hand, the flavonol contents of the flowers in *G. multifolia* were found to be significantly (P<0.05) higher than the flowers of *G. villosa* (Fig. 1). The flavonol contents in the roots were 8% higher (P<0.05) for *G. multifolia* when compared to the roots of *G. villosa*. The flavonol value for the fruit of *G. villosa* was found to be 6% higher (P<0.05) than that of *G. multifolia* (Fig. 1). In *G. multifolia*, the flavonol contents were found to be higher (P<0.05) in the flowers and fruit compared to the other plant parts. In *G. villosa*, the flavonol content was higher (P<0.05) in the leaves compared to the other plant parts. Generally, the flavonol contents were higher in *G. villosa* when compared to *G. multifolia*. The flavonol contents of the fresh weights of the fruit of both species were also found to be significantly (P<0.05) lower than the dry weights (0.36 QE±0.25 for *G. multifolia* and 0.39 QE±0.23 for *G. villosa*).

In *G. villosa*, the flavanone contents of the leaves and bulbs were significantly (P<0.05) higher than those of the leaves and bulbs of *G. multifolia* (Table 1). The difference in the flavanone contents in the roots of both species was very similar (less than

1%). There were no significant (P<0.05) difference in the flavanone content of the flowers and fruit of *G. villosa* when it was compared to those of *G. multifolia* (Fig. 1). The flavanone contents in the flowers and fruit of *G. multifolia*, were significantly (P<0.05) lower compared to the other plant parts. *Gethyllis villosa* revealed a similar trend with significantly (P<0.05) lower flower and fruit flavanone contents compared to the other plant parts. Generally, the flavanone contents were higher in *G. villosa* compared to *G. multifolia*. In contrast to the other antioxidants, the flavanone content was found to be the lowest in the flower and fruit of both species.

3.2. Antioxidant capacity

The FRAP values of *G. villosa* were found to be significantly (P<0.05) higher in the leaves, bulbs and roots than the leaves, bulbs and roots of *G. multifolia* (Table 2). The flower and fruit FRAP values of *G. villosa* were also found to be higher (P<0.05) than those of *G. multifolia* (Fig. 2). The flower and fruit FRAP values of *G. multifolia* were found to be significantly (P<0.05) higher than those of the other plant parts. *Gethyllis villosa* showed a similar trend with higher (P<0.05) FRAP values in the flower and fruit compared to those of the other plant parts. In general, the FRAP values for *G. villosa* were found to be higher than those of *G. multifolia*. The FRAP values of the fresh weight of the fruit for both *G. multifolia* (8.21 AAE±4.11) and *G. villosa* (10.00 AAE±2.38) were found to be significantly (P<0.05) lower than the dry weights.

Statistically, only the ORAC of the roots of *G. multifolia* was found to be significantly (P<0.05) higher (36%) when compared to the roots of *G. villosa* (Table 2). The ORAC values of the leaves and fruit of *G. villosa* were slightly (P<0.05) higher than those of *G. multifolia*. On the other hand, the ORAC values of the bulbs and flowers of *G. multifolia* were higher (P<0.05) than those of *G. villosa*. The ORAC values of the flowers and fruit of *G. multifolia* were found to be higher (P<0.05) than the other plant parts. *Gethyllis villosa* revealed a similar trend with significantly (P<0.05) higher ORAC values in the flowers and fruit when compared to the other plant parts. In general, the ORAC values for *G. multifolia* were found to be higher (P<0.05) than those of *G. villosa*. A strong correlation

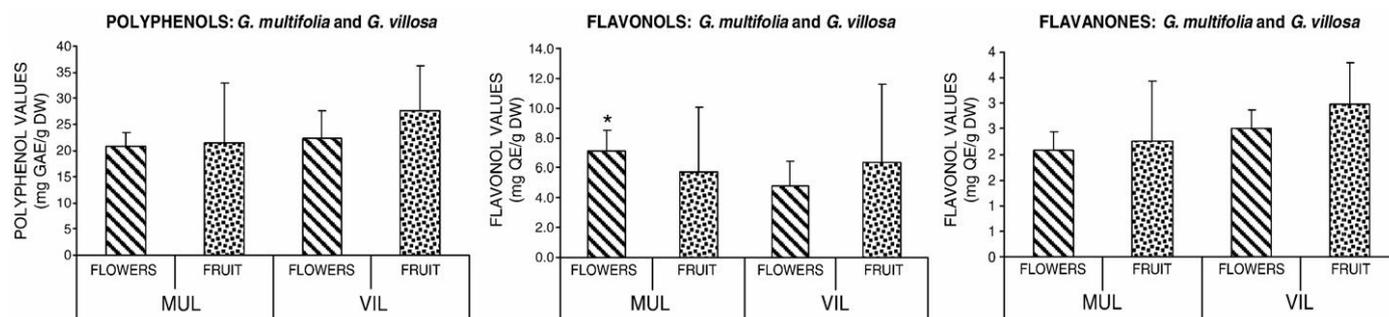


Fig. 1. The total polyphenol (mg GAE/g dry weight), flavonol (mg QE/g dry weight) and flavanone (mg NE/g dry weight) content of the flowers and fruit of *G. multifolia* and *G. villosa* plants. Bars represent the means±SD (flowers n=10; fruit n=7). An * indicates a significantly (P<0.05) higher value when comparing plant parts of the two species.

Table 2

The ferric reducing antioxidant power (FRAP) ($\mu\text{M AAE/g}$ dry weight), oxygen radical absorbance capacity (ORAC) ($\mu\text{M TE/g}$ dry weight) and ABTS radical cation scavenging ability (ABTS) ($\mu\text{M TE/g}$ dry weight) of the leaves, bulbs, roots, flowers and fruit of *G. multifolia* and *G. villosa* plants.

Plant species	ORAC	FRAP	ABTS
<i>G. multifolia</i>			
Leaves	375.29±26.99ad	38.53±2.78a	28.96±2.00a
Bulbs	322.98±54.45ae	16.76±4.08b	14.35±2.65b
Roots	525.17±75.61bg	39.08±6.60a	29.76±4.10a
Flowers	627.48±55.38cg	76.66±6.11c	105.48±6.33c
Fruit	672.67±329.00ch	91.51±36.97ce	103.09±35.51c
<i>G. villosa</i>			
Leaves	410.60±50.88d	87.90±9.68ce	76.83±3.81d
Bulbs	287.12±115.56ef	72.21±37.16cd	62.82±19.89e
Roots	251.25±39.82f	52.91±11.81da	54.53±6.92e
Flowers	590.23±156.58g	92.68±33.28ce	107.57±21.97c
Fruit	741.16±140.50h	103.45±17.81e	117.21±22.43c

Values represent the means±SD (leaves, bulbs, roots and flowers n=10; fruit n=7). Means in the same column with different letters are significantly ($P < 0.05$) different.

was also evident in the fruit of both species between the ORAC and FRAP ($r=0.94$), and ORAC and flavonol content ($r=0.77$). The ORAC values in the fresh fruit for both species (*G. multifolia* $96.85 \text{ TE} \pm 52.37$ and *G. villosa* $103.43 \text{ TE} \pm 29.14$) were also found to be significantly ($P < 0.05$) lower than the dry weights.

Statistically, the radical cation scavenging ability of the leaves, bulbs and roots of *G. villosa* were found to be significantly ($P < 0.05$) higher than those of *G. multifolia* (Table 2). Results for the flowers (less than 1%) and fruit (6%) in *G. villosa* were also higher ($P < 0.05$) than those of the flowers and fruit of *G. multifolia* (Fig. 2). The radical cation scavenging ability of the flowers and fruit of *G. multifolia* were significantly ($P < 0.05$) higher than those of the other plant parts. *G. villosa* revealed a similar trend, with significantly ($P < 0.05$) higher values for the flowers and fruit compared to the other plant parts. In general, the radical cation scavenging ability in all the plant parts of *G. villosa* was higher than those of *G. multifolia*.

4. Discussion and conclusion

According to Van Wyk et al. (2009), the ripe, aromatic, powerful sweet, edible fruits of certain *Gethyllis* species were used as an infusion in "Kukumakranka" brandy for the treatment of colic and indigestion. Watt and Breyer-Brandwijk (1962) reported that the skin of the fruit was used as a local application on boils, bruises and insect bites. It was also mentioned by Rood (1994), that the fruit was boiled by the Khoi and used as an aphrodisiac, while Louw et al. (2002) described flower decoctions of certain *Gethyllis* species were used in the past as a relief for toothache. The following compounds: pentacosane; ethyloctanoate; ethyl isovalerate; ethyl hexanoate and ethyl benzoate were found in the fruit of *G. ciliaris*, and may be the major contributors to its fruity-sweet fragrance. The compounds in the fruit of *G. afra* which may be responsible for its banana/piney/fruity fragrance were identified as α -pinene, n-butyl n-butyrate, isoamyl acetate, β -pinene and 2-methylbutyl butyrate (Kamatou et al., 2008).

Only the fruit and flowers will be discussed in this section, because of their medicinal uses. The total polyphenol and antioxidant capacities of the two *Gethyllis* species were evaluated in this study and found that, in general, the flowers and fruit of *G. multifolia* and *G. villosa* showed higher and in some cases significantly ($P < 0.05$) higher antioxidant activities than the leaves, bulbs and roots. This, however, was not true for the flavanone content in both species. The polyphenol content in the fruits of *G. multifolia* (21.54 mg GAE/g) and *G. villosa* (27.64 GAE) were found to be in line with other commercial fruits such as raisins (28.30 GAE), blueberries (24.00 GAE), strawberries (15.40 GAE), plums (9.49 GAE), oranges (7.50 GAE) and red grapes (7.39 GAE) (Brunswick Laboratories, 2005). The polyphenol content of the fruit of both *Gethyllis* species were also compared to different maturity stages of blackberry (13.47 GAE), black raspberry (15.35 GAE) and red raspberry (13.46 GAE) and found to be higher than all the maturity stages of all three berries (Wang and Lin, 2000). When compared to some commercial vegetables, the polyphenol content of the fruit of both species, was higher than that of spinach (17.70 GAE) broccoli (8.80 GAE), brussels sprouts (9.80 GAE), beetroot (8.40 GAE) and onions (4.50 GAE)

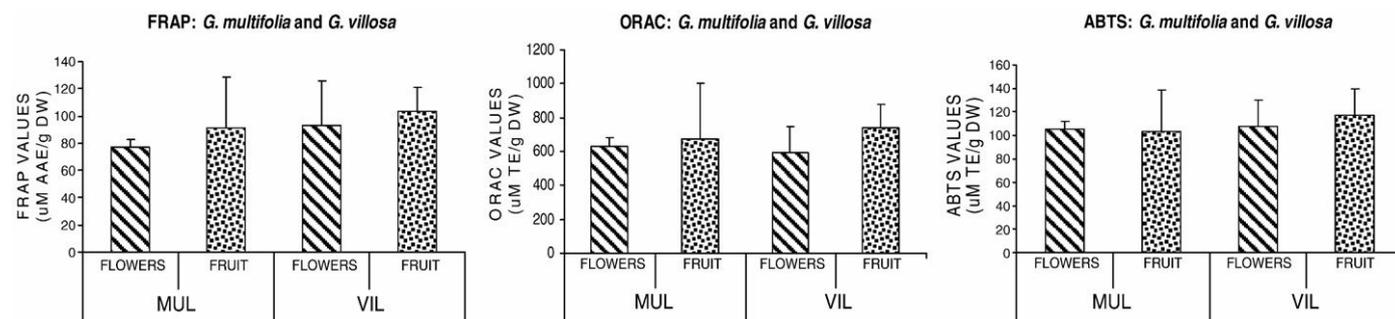


Fig. 2. The ferric reducing antioxidant power (FRAP) ($\mu\text{M AAE/g}$ dry weight), oxygen radical absorbance capacity (ORAC) ($\mu\text{M TE/g}$ dry weight) and ABTS radical cation scavenging ability (ABTS) ($\mu\text{M TE/g}$ dry weight) of the flowers and fruit of *G. multifolia* and *G. villosa* plants. Bars represent the means±SD (flowers n= 10; fruit n= 7). No significant ($P < 0.05$) differences were evident when plant parts of the two species were compared.

(Brunswick Laboratories, 2005). The polyphenol contents of certain guava cultivars were found to be similar to the fruit of *G. multifolia* and *G. villosa* when fresh weights of fruits were compared (Thaipong et al., 2006). The fruit pulp of the amarula tree (*Sclerocarya birrea*) had a higher polyphenol value than the fruit of *G. multifolia* but a lower value than the fruit of *G. villosa*, when fresh weights were compared (Ndhlala et al., 2007). The flavonol content in the fruit of *G. multifolia* and *G. villosa* was found to be higher than that of raw beehive propolis samples (Kosalek et al., 2004). The significant relationship between the antioxidant activities and total polyphenol content indicate that phenolic components such as flavonols are a major contributor to the antioxidant activity of the fruit of these plants.

The radical cation scavenging ability of the flowers and fruit of both species were significantly ($P < 0.05$) higher than those of the other plant parts. The ferric reducing antioxidant power values of *G. multifolia* flowers and fruit were also found to be significantly ($P < 0.05$) higher than those of the other plant parts. Conversely, *G. villosa* revealed significantly ($P < 0.05$) higher oxygen radical absorbance capacity values in the flowers and fruit compared to the other plant parts. The ORAC values for the flowers and fruit of *G. multifolia* were found to be higher than those of the seeds and significantly ($P < 0.05$) higher than the fruit of the caneberry range (red raspberry, black raspberry, boysenberry, Marion blackberry and evergreen blackberry) (Bushman et al., 2004; Wang and Lin, 2000). The ORAC values of the following fruits: kiwi fruit, banana, apple, tomato and orange (Wang et al., 1996) were found to be significantly lower than the fruit of these two *Gethyllis* species. ORAC values for the fresh weights of the fruit of both *Gethyllis* species were found to be higher than the fresh weights of the following herb species: *Thymus vulgaris*, *Aloe vera*, *Lavandula angustifolia*, *Petroselinum crispum* and *Rosmarinus officinalis* (Zheng and Wang, 2001).

Commercialization of southern African medicinal plants is a process that has rapidly been gaining momentum during the last 10 years (Van Wyk, 2008). This genus has not been grown commercially before and some of its species (including *G. multifolia*) appear on 'The Red Data List of Southern African Plants' as endangered, threatened or vulnerable (Hilton-Taylor, 1996). Thus, a serious need still exist for the propagation, cultivation, conservation and medicinal research on members of this genus. This *in vitro* study has shown that the flowers and fruit of both species may be used as good natural sources of dietary antioxidants and that the antioxidant capacity may be a contributing factor to the reported medicinal uses of the flowers and fruit as mentioned in earlier publications (Du Plessis and Delpierre, 1973; Lighton, 1992). Further studies are also needed to identify individual active components responsible for the antioxidant activity shown here and to investigate the powerful fragrance and taste of the fruit of *G. multifolia* for possible use in the food and beverages industry. The main objective, however, of this study was to investigate the antioxidant potential of *G. multifolia* and *G. villosa* and to create a platform for clinical studies on humans to justify claims made by traditional healers and old folk of the medicinal uses of members of the genus *Gethyllis*.

