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**Poloko Stephen Kheoane**
Department of Pharmacy,
National University of Lesotho,
P.O. Box Roma, Lesotho

**Mokonyana Mohale**
Department of Pharmacy,
National University of Lesotho,
P.O. Box Roma, Lesotho

**Corresponding Author:**
**Poloko Stephen Kheoane**
Department of Pharmacy,
National University of Lesotho,
P.O. Box Roma, Lesotho

# Antioxidant capacity, phenolic, flavonoid, and ascorbic acid contents of *Gunnera perpensa* from Lesotho, Southern Africa

**Poloko Stephen Kheoane and Mokonyana Mohale**

**Abstract**
**Background:** Antioxidants play a vital role in oxidizing some harmful reactive oxygen species (ROS) that develop in human bodies due to several body physiological functions and the materials which are ingested into the body. Plant materials provide a good source of natural antioxidants. The known antioxidants from plant sources include phenolics, flavonoids, and vitamin C. *Gunnera perpensa* had been traditionally used for several decades in the Southern African region to cure several diseases. Its use as an antioxidant could alleviate disease burdens that are caused by the presence of ROS in the human body.
**Methods:** *Gunnera perpensa* was extracted in methanol and the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity of extracts was investigated against the ascorbic acid positive control. The estimation of phenolic content was determined using the Folin-Ciocalteu reagent colorimetric method against garlic acid positive control while the flavonoid content was investigated using the aluminium chloride colorimetric method against quercetin as a positive control. The amount of ascorbic acid in the aqueous extract was determined using the iodine titration colorimetric method.
**Results:** *Gunnera perpensa* extracts showed a significant free radical scavenging effect ($p< 0.05$) with an inhibitory concentration ($IC_{50}$) of 396.82 ± 1.05 µg/mL. The total phenolic content was 146.40 ± 1.94 mg/g gallic acid equivalent (GAE), and its antioxidant activity was mainly due to the presence of phenolics in large amounts as compared to the contents of flavonoids and ascorbic acid. The total flavonoid content was 26.54 ± 2.07 mg/g quercetin equivalent (QE) while ascorbic acid content was 1.56 ± 1.54 mg/100 g of crude plant sample.
**Conclusion:** *Gunnera perpensa* could be an alternative natural source of phenols which could be used as antioxidant for eradicating ROS in the human body.

**Keywords:** Reactive oxygen species, antioxidants, phenols, flavonoids, ascorbic acid, *Gunnera perpensa*

## 1. Introduction
In the human body, reactive oxygen species (ROS) are free radicals that are constantly formed either due to several body physiological functions or due to chemicals such as drug metabolites that are taken into the body. For example, leukocytes like neutrophils and macrophages as well as immature spermatozoa are the core endogenous sources of ROS. Excessive alcohol intake and smoking along with other environmental factors, such as radiation and toxins, may increase ROS levels in the seminal plasma leading to infertility in men (Sanaa *et al*., 2018) [18]. Despite of being harmful to the body, many ROS play a meaningful physiological functions, but they can be harmful to the body when produced in excessive amounts. The ROS toxicity is normally intensified by the introduction of heavy metals like copper and iron in the body. Excessive generation of ROS can cause several problems within human tissues such as destruction to the cellular DNA and proteins, carbohydrates as well as lipids (Aruoma, Kaur and Halliwell, 1991) [3]. This damage to cellular components could give rise to the development of some degenerative diseases such as cardiovascular diseases like atherosclerosis, cancer, diabetes mellitus, insulin resistance, as well as ageing (Alfadda and Sallam, 2012) [1]. Halliwell and Gutteridge (1995) and Halliwell (2007) [7-8] described an antioxidant as any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate, i.e., an antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule. Plants sources such as fruits and vegetables provide some natural antioxidants such as polyphenols, flavonoids, and ascorbic acid (vitamin C) (Haseeb, Ghulam

and Imtiaz, 2018) [9]. Medicinal plants could also provide substantial amounts of antioxidants.

*Gunnera perpensa* is one of the fifty species belonging to the Gunneraceae family which is found only in Africa, and is widely distributed in Southern African region where its English name is locally known as the river pumpkin and wild rhubarb. Other local names include: Qobo (Sotho), Ugobhe and Ugobho (Zulu); rivierpampoen and wilde ramenas (Afr.); Uqobho (Swati); Iphuzi lomlambo and Ighobo (Xhosa); rambola-vhadzimu and shambola-vhadzimu (Venda). *Gunnera* species are naturally distributed in Central and Southern America, New Zealand, Mexico, Hawaii, Philippines, Indonesia, Madagascar, Tasmania, and Central and Southern Africa. *Gunnera perpensa* is widely distributed in Lesotho, Swaziland, Namibia, South Africa (Western and Northern Cape), Botswana, Zimbabwe, Uganda, Sudan, Zaire, Ethiopia, Rwanda, and Kenya (Balogun, Tshabalala and Ashafa, 2016) [4]. The medicinal uses of *Gunnera perpensa* are quite diverse; the potential medicinal uses and other pharmacological activities of this plant are summarized in Table 1. *Gunnera perpensa* has a rich phytochemical profile, for example, the presence of tannins, alkaloids, flavonoids, benzoquinones, proanthocyanidins, phenols, ellagic acids, and minerals (Maroyi, 2016) [15]. However, many reports on this plant are mostly on its antimicrobial activity (Steenkamp *et al*., 2004; Buwa and Staden, 2006; Mabona *et al*., 2013; Drewes *et al*., 2005) [20, 5, 14, 6]. *Gunnera perpensa* could provide a good source of natural antioxidants of plant origin and is found in abundance in Southern Africa. The present study investigated the antioxidant activity and phytochemical profile of *Gunnera perpensa* from Lesotho.

**Table 1:** *In vitro* and *in vivo* scientific screening and possible medicinal uses of *Gunnera perpensa*

| Part of the plant used | *In vitro*/*in vivo*/*ex vivo* scientific screening | Model | Main compounds identified | Possible medicinal use | Reference |
|---|---|---|---|---|---|
| Roots | *Ex vivo* | Rats isolated smooth muscles | Z-venusol, pyrogallol, the trimethyl ether, lactic acid, and succinic acid. | Labour | Khan *et al*., 2004 |
| Roots | *In vitro* | Human breast (the MCF-7) cancer cells and human mammary epithelial cells (HMECs) | Z-venusol | Anti-cancer | Mathibe, Botha and Naidoo, 2016 |
| Roots | *In vitro* | Human breast cancer cells (MCF-7) (IL-6 and cAMP activity) | Z-venusol | Anti-cancer | Mathibe, Botha and Naidoo, 2017 |
| Roots | *In vitro* | *Streptococcus pyogenes* and *Staphylococcus aureus*. | Aqueous and methanol extracts | Antibacterial, wound healing | Steenkamp *et al*., 2004 [20] |
| Roots | *In vitro* | *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. | Ethanol and ethyl acetate extracts | Antibacterial, antifungal | Buwa and Staden, 2006 [5] |
| Leaf | *In vitro* | Methicillin- resistant *Staphylococcus aureus*, *Staphylococcus aureus*, and gentamycin– methicillin-resistant *Staphylococcus aureus* | Dichloromethane and methanol mixture extracts, aqueous extracts | Antibacterial, wound healing | Mabona *et al*., 2013 [14] |
| Part of the plant used | *In vitro*/*in vivo*/*ex vivo* scientific screening | Model | Main compounds identified | Possible medicinal use | Reference |
| | | *Taphylococcus epidermidis*, *Brevibacillus agri*, *Pseudomonas aeruginosa*, *Propionibacterium acnes* *Trichophyton mentagrophytes*, *Microsporum canis*, and *Candida albicans* | | | |
| Leaves and stems | *In vitro* | *Staphylococcus epidermidis*, *Cryptococcus neoformans*, *Candida albicans* | benzoquinones, 2-methyl-6-(-3-methyl-2-butenyl)benzo-1,4-quinon; 6-hydroxy-8-methyl-2,2-dimethyl-2H-benzopyran | Antibacterial | Drewes *et al*., 2005 [6] |

## 2. Materials and Methods

**2.1. Materials:** The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical, quercetin, garlic acid, ascorbic acid and the Folin-Ciocalteau reagent were procured from Merck (South Africa). Other reagents were of analytical grade and were used as required.

### 2.1.1 Plant sample collection and preparation

*Gunnera perpensa* leaves, stems and roots were purchased from a local herbalist in Maseru and were sent to a Botanist in the Department of Biology, the National University of Lesotho for identification. The roots of the plant were air-dried for three weeks at ambient temperature. The dried sample was grounded into a fine powder and was passed through an 800-micron mesh sieve.

### 2.1.2 Extraction of compounds

The previously air-dried, ground, and micronized plant materials were extracted with HPLC grade methanol (Chemoquip, South Africa) at room temperature. A 10 g mass of dried and ground plant material was soaked in a 100 mL volume of the HPLC grade methanol (98% v/v) for 5 days with frequent agitation. The final extracts were filtered through the Whatman No.1 filter paper (Whatman Ltd., England). The filtrates were further concentrated under vacuum in a rotary evaporator (Model R-200 Buchi Rotavapor, Switzerland) at 50 $^{o}$C and stored at 8 $^{o}$C for further use.

## 2.2. Methods

### 2.2.1 DPPH free radical scavenging activity

The DPPH free radical scavenging activity of *Gunnera perpensa* methanolic extract was investigated following the procedure used by Khalaf *et al*. (2008) [11]. A 5 mg mass of a dry refined plant extract was dissolved in a 1 mL volume of methanol (98% v/v) to prepare a stock solution. From the stock solution, the working solutions (10, 50, 100, 250, 500 µg/mL) of the extracts were prepared using appropriate dilution factors. The ascorbic acid standard was prepared across a concentration range of 10 µg/mL to 500 µg/mL. A 0.002% w/v DPPH free radical solution was prepared in methanol. A 1.5 mL aliquot of refined plant extract solution and 1.5 mL volume of the standard solution were separately poured in a test tube and this was followed by the addition of a 1.5 mL volume of the DPPH solution into each test tube. The test tubes with their contents were kept in the dark at room temperature for 30 minutes. The optical density of each sample was measured at 517 nm. The blank was composed of methanol (1.5 mL) and the DPPH solution (0.002%, 1.5 mL). The percentage DPPH inhibition was calculated using equation (1):

$$Percentage\ (\%)\ inhibition\ of\ DPPH\ activity = \frac{A-B}{A} \times 100$$

[Equation 1]

Where A = optical density of the standard and B = optical density of the sample (Kheoane *et al*., 2017) [13].

### 2.2.2 Determination of the phenolic content

The total phenolic content of plant extract was estimated using a Folin-Ciocalteu reagent method which was used previously by Al-Owaisi, Al-Hadiwi and Khan (2014) [2]. Gallic acid reference standard was used for plotting a standard curve at the concentration ranges of 20 – 200 µg/mL. The Folin-Ciocalteu reagent was diluted 10 times with distilled water and a 1.5 mL volume of this reagent was mixed with a 0.5 mL volume of the plant extract (100 µg/mL). A 3 mL volume of sodium carbonate solution (7.5% w/v) was added to the mixture to bring it to the neutral pH. The sample mixture was placed in the dark at ambient temperature for 30 minutes with occasional agitation for colour development. The optical density of the resultant blue colour was measured at 765 nm wavelength using a UV-Vis spectrophotometer. The linear regression equation obtained from the gallic acid standard curve was utilized to estimate the total phenolic content of plant extract.

### 2.2.3 Determination of the flavonoid content

The flavonoid content of the test sample was investigated according to the procedure reported by Al-Owaisi, Al-Hadiwi and Khan (2014) [2]. Quercetin was used as a reference standard to construct a standard curve. Briefly, a 1 mg/mL stock solution of quercetin was prepared in ethanol (80% v/v) and diluted to 25, 50, 75, 100, 150 and 200 µg/mL working solutions. In a separate test tube, a 0.5 mL of either quercetin or plant sample was mixed with a 1.5 mL volume of 95% ethanol, a 0.1 mL volume of aluminium chloride (10% w/v), a 0.1 mL volume of potassium acetate (1 M) and a 2.8 mL volume of distilled water. The contents of the test tube were incubated for 30 minutes at ambient temperature. The blank was prepared by dissolving all the reagents excluding aluminium chloride which was substituted by a 0.1 mL volume of distilled water. The optical density of the reaction mixture was measured at a wavelength of 415 nm using a UV-Vis spectrophotometer. The flavonoid content of plant extract was estimated using the linear regression equation of quercetin standard curve.

### 2.2.4 Determination of the ascorbic acid content

The iodometric colorimetric methods described by Kheoane *et al*. (2017) and Ikewuchi and Ikewuchi (2011) [13, 10] were used to determine the ascorbic acid content of plant extract. Briefly, a 5 g mass of corn starch was dissolved in a 250 mL volume of distilled water in a beaker. The mixture was heated with continuous stirring until dissolved. A 5 mL volume of corn starch solution was further diluted with a 250 mL volume of distilled water, and about four drops of a 0.05 M iodine solution was added to the mixture and mixed thoroughly. The resultant iodine-corn starch dye solution was blue in color and was further standardized by volume using the known mass of the ascorbic acid standard. The ascorbic acid standard was prepared by mixing a 5 mg mass of the ascorbic acid with a 10 mL volume of distilled water. The ascorbic acid solution was titrated into the blue iodine-corn to the endpoint whereby the dye turned from the blue color to the persisted colorless state. The amount of ascorbic acid stock solution that turned a known volume of the blue iodine-corn starch dye to colourless was calculated by using equation (2):

$$M_1=VM/V_1 \qquad \text{[Equation 2]}$$

Where $M_1$ (mg) is the amount of ascorbic acid in a titrated volume $V_1$ (mL), M (mg) is the known amount of ascorbic acid in a stock solution volume V (mL).

*Gunnera perpensa* test sample for the determination of ascorbic acid was prepared by macerating a 1 g mass of a ground crude plant extract in 20 mL volume of distilled water (i.e., 50 mg/mL before filtration) for 30 minutes at room temperature. The mixture was filter through a Whatman No. 1 filter paper. The aqueous plant extract filtrate was titrated into the previously standardized iodine-corn starch dye until the colorless change of this blue dye was observed. The quantitative determination of ascorbic acid content was achieved by using equation (3):

$$M_2=V_1M_1/V_2 \qquad \text{[Equation 3]}$$

Where $M_2$ is the amount of ascorbic acid (mg) in the aliquot of crude plant extract $V_2$ (mL). $M_1$ is the mass of ascorbic acid in the volume $V_1$ of ascorbic acid solution used to reduce a known volume of the dye from blue to colourless from equation (2).

The total amount of ascorbic acid contained in the total volume of crude plant extract was calculated using equation (4):

$$M_t=V_2M_2/V_t \qquad \text{[Equation 4]}$$

Where $M_t$ (mg) is the total amount of ascorbic acid in the total stock volume ($V_t$, mL) of crude plant extract. $M_2$ (mg) is the amount of ascorbic acid in an aliquot ($V_2$, mL) of crude plant extract titrated from equation (3). The ascorbic acid content of each plant extract was determined using equation (5) and was expressed as milligrams of ascorbic acid per 100 grams of plant material:

$$\text{Ascorbic acid content} = (M_t \times 100)/M_s \qquad \text{[Equation 5]}$$

Where $M_t$ (g) is the total amount of ascorbic acid in a known

amount ($M_s$, g) of crude plant extract used to prepare the plant extract stock solution.

## 3. Results and Discussions

*Gunnera perpensa* scavenged the DPPH free radical in a concentration-dependant manner, figure 1. This plant showed the strong DPPH free radical scavenging activity of 63% at a maximum concentration of 500 µg/mL ($IC_{50}$ = 396.82 ± 1.05 µg/mL) used in this study. All the concentrations below 500 µg/mL exhibited poor antioxidant activity, indication low potency of this plant as an antioxidant. However, to achieve 50% of the inhibitory effects of free radicals, a 396.82 ± 1.05 µg/mL concentration of crude extract of this plant is required, which is fairly a low concentration for crude plant extracts. The results for the total phenolic content, the flavonoid content and the ascorbic acid content are summarized in Table 2. *Gunnera perpensa* extract had the higher content of phenolics, 146.40 ± 1.94 mg/g of GAE, followed by the flavonoids, 26.54 ± 2.07 mg/g of QE, with the lowest content of ascorbic acid.

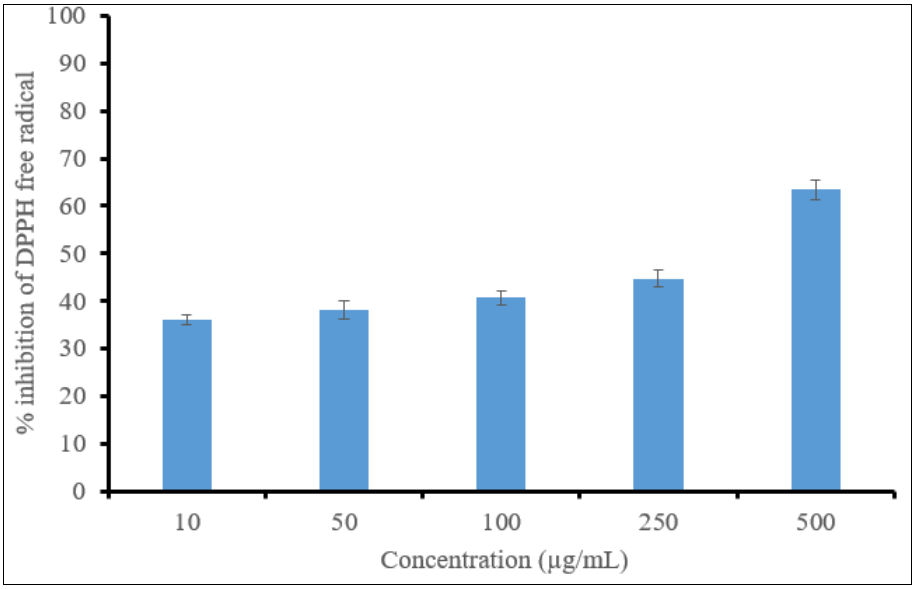


**Fig 1:** The DPPH free radical scavenging activity of *Gunnera perpensa* methanolic extract (*Values are mean ± SD, n = 3*)

**Table 2:** Summary of the antioxidant activity, phenolic, flavonoid, and ascorbic acid contents of *Gunnera perpensa plant extracts*

| Crude plant extract | Amount |
|---|---|
| Antioxidant activity ($IC_{50}$) | 396.82 ± 1.05 |
| Total phenolic content | 146.40 ± 1.94 |
| Total flavonoids content | 26.54 ± 2.07 |
| Ascorbic acid content | 1.56 ± 1.54 |

Antioxidant activity: $IC_{50}$ value µg/mL; Total phenolic content: mg of GAE/g of dry extract; Total flavonoid content: mg of QE/g of dry extract; Ascorbic acid content: mg/100 g of dry extract; Values are mean ± SD, n = 3.

The observed antioxidant activity of *Gunnera perpensa* was mainly due to the high content of phenolics. This is in agreement with the findings of Simelane *et al*. (2010) [19] who reported the total phenolic content of 248.45 mg/g of GAE for *Gunnera perpensa*. However, both flavonoids and ascorbic acid are known antioxidants, although their contents were found to be lower than the phenolics, they provided an additive effect towards the observed antioxidant activity of this plant.

## 4. Conclusion

Apart from the known traditional uses of *Gunnera perpensa* and its well-researched antimicrobial and anti-cancer properties, this plant could be an alternative source of antioxidants due to its rich phenolic content. The antioxidant activity of *Gunnera perpensa* is mainly due to the higher content of phenolics than flavonoids and ascorbic acid.

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