

# Estimation of Phytochemical, Vitamins Composition and Antioxidant Activity of *Pelargonium inquinans* Leaves

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## ABSTRACT

**Aims:** To estimate the phytochemical and vitamins composition and the antioxidant activity of *Pelargonium inquinans* leaves. **Background:** Some plants that are used in the traditional setting for the management of different ailments have documented observations and scientific studies. However many other plants lack scientific reports to support their medicinal use. One of such plant is *Pelargonium inquinans* Ait that is traditionally used to treat headaches and flu by tribes in South Africa. Not much is known about its chemical composition. **Methods:** The phytochemical composition of acetone, aqueous and ethanol extract of leaves of *Pelargonium inquinans* as well as the vitamins A, C and E of its dried weight leaves and the antioxidant activities were evaluated using standard *in vitro* methods. The phytochemical composition and vitamins were determined spectrophotometrically, while the antioxidant activities were determined by DPPH, nitric oxide and hydrogen peroxide scavenging activity and phosphomolybdenum (total) antioxidant activity. **Statistical analysis:** The One-way analysis of variance (ANOVA) and the Tukey test were used to determine the differences among the means of the various samples. **Results:** The phytochemical composition differ significantly in the various extracts. The total phenolics were higher than other phytochemicals in all the extracts used. The hydrogen peroxide and DPPH scavenging activity were very high and dose-dependent. The aqueous extract had the highest antioxidant activity at 98.4 % of control (Hydrogen peroxide), while the ethanol extract had the lowest at 37.5% control (Nitric oxide). **Conclusion:** The results supports the notion that the Geraniaceae family and *Pelargonium inquinans* in particular is rich in polyphenolic compounds and has good antioxidant activity.

**Key words:** *Pelargonium inquinans*, Phytochemical, Antioxidant, Vitamins, Phenols, Cancers. **Key message:** *Pelargonium inquinans* leaves are particularly rich in polyphenolic compounds and thus have good antioxidant activity.

## INTRODUCTION

Over many centuries, plants have devised many mechanisms to repel attack by predators like insects and fungi by synthesizing different biochemical compounds. Many of these compounds have proved useful in the management of different human diseases. Phytochemicals are secondary metabolites found in plants with no nutritive value. They are known to biological activities such as antioxidation.<sup>1</sup>

Oxidative stress (OS) can be defined as a yin-yang relationship between free radicals/reactive oxygen species (ROS) generated as a result of some cellular (re)actions and antioxidants (Constitutive or endogenously) produced to either mollify or abrogate their effects. OS is important in health and disease, especially chronic diseases. It has both beneficial and deleterious effects. For example, OS has been shown to induce cell death and also enhance antimicrobial phagocytosis by the release of reactive oxygen species through the immune cells.<sup>2</sup> They do this by acting as stress signals for certain signaling pathways and intermediates that modulate physiological processes.<sup>3</sup> However, excessive

production of ROS without a counterbalance by the innate antioxidant system will cause the cell to be stressed. OS is involved in many degenerative diseases and inflammatory conditions like atherosclerosis and cancer.<sup>4</sup> ROS are produced as a result of endogenous and exogenous factors. The endogenous factors include cellular metabolites from mitochondria- catalyzed electron transport reactions and activities of leukocytes such neutrophils, eosinophil, monocytes and cellular organelle peroxisomes during inflammation (the enzymes involved in generating ROS are found in these cells and organelles, e.g., macrophages can trigger an increase in ROS during an inflammatory response). Exogenous factors include irradiation, various drugs and xenobiotic agents. ROS include superoxide anion (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (HO•) and singlet oxygen (<sup>1</sup>O<sub>2</sub>). Superoxide anion can also combine with nitrous oxide (NO) to form Reactive nitrogen species (RNS). They can cause nitrosative stress from its various species which include

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peroxynitrite (ONOO<sup>-</sup>), nitrosoperoxycarbonate (ONOOCO<sub>2</sub><sup>-</sup>), nitrogen dioxide (•NO<sub>2</sub>) and dinitrogen trioxide (N<sub>2</sub>O<sub>3</sub>).

The body has a defense network of antioxidants, which include the tripeptide glutathione and enzymes like superoxide dismutase and catalase, to nullify the harmful effects of free radicals on cells. These antioxidants are endogenous and are produced mainly in the mitochondria. Others include alpha lipoic acid (ALA), Coenzyme Q10 (CoQ10), glutathione peroxidase (Gpx), ferritin, uric acid, bilirubin, metallothionein, L-carnitine and melatonin.<sup>5</sup> Where the endogenous antioxidants do not suffice, the exogenous ones which are acquired from foods can help out. These include vitamins such as E and C which are involved in the prevention of lipid peroxidation and enhancement of OS cells respectively.<sup>6,7</sup> Other important exogenous antioxidants found in plants include phenolic compounds (phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins, etc.), nitrogen compounds (alkaloids, amines, betalains). The effect of OS can be either acute or chronic. For chronic OS, a small amount of oxidative damage can persist during physiological functioning of the cell and lead to disruption of essential cellular functions and a premonitory state of carcinogenesis through the promotion of cell survival,<sup>8</sup> cellular proliferation,<sup>9</sup> metastasis,<sup>10</sup> and even drug resistance.<sup>11</sup> ROS plays a role in many cancers including haematological cancers.<sup>12,13</sup> Acute myeloid leukaemia relapse has also been strongly associated with increased markers of oxidative stress, implying that ROS can be a pivotal factor in AML progression.<sup>1</sup> Thus the regulation of OS is important in cancer growth and management and so a better understanding of it will aid in both the definitive and supportive care of various types of leukaemia.

*Pelargonium inquinans* is a small to medium-sized woody shrub with velvety and glandular branches. Like most other *Pelargonium* species. It is indigenous to South Africa and found especially in the Eastern Cape up to southern Kwazulu- Natal. The plant's stems and leaves are crushed and used to treat headaches and flu by the local people, who also use it as a deodorant.<sup>14</sup> The crude extracts have been reported to have strong antioxidant activity.<sup>15</sup> While several species of the family Geraniaceae have been investigated, there is a paucity of information on the phytochemical composition and antioxidant activities of this species. Not much has been reported about *Pelargonium inquinans* in the literature and neither has the chemical composition of any of the parts of the plant been determined.

This present work was undertaken to estimate the total phenol, flavonoid, flavonol, proanthocyanidin, tannins, alkaloids, saponins and phytate contents in the water, acetone and ethanol extracts of *Pelargonium inquinans* leaves as well as the vitamins A, E and C content and antioxidant activity.

## MATERIALS AND METHODS

### Plant material

The leaves of *Pelargonium inquinans* were collected from the plant growing within the University of Fort Hart Campus at Alice and was authenticated by Tony Dolds at the Albany herbarium in Rhodes University, Grahamstown, South Africa.

### Preparation of extracts

The collected leaves were cleaned of extraneous materials, dried in an oven at a temperature of 40°C and later pulverized. About 300g of the pulverized samples were extracted with separate solvents (water, ethanol and acetone) on a shaker for 48h. They were later filtered with a funnel and Whatman filter paper. While the ethanol and acetone extracts of the samples were concentrated at 78°C and 56°C, respectively using a rotary evaporator, the water extracts of both samples were frozen at -40°C with

an acetone chiller, then freeze-dried. The extracts were stored away in a refrigerator at 4°C.

### Estimation of total phenol content

The total phenol was estimated spectrophotometrically by using the Folin-Ciocalteu assay method.<sup>16</sup> About 0.5ml of the extract was mixed with 2.5ml of 10% Folin-Ciocalteu reagent in tubes, vortexed for about 30s and allowed to stand for 10 min at 25°C. 2 ml of 7.5% anhydrous sodium carbonate was added and vortexed again for another 30s. The tubes were incubated in a water bath at 40°C for 30min for colour development and absorbance read at 765nm using a spectrophotometer. Gallic acid standards were prepared in the same manner as the extracts as described earlier. The total phenolic content was then expressed as mg/g gallic acid (GAE/gm) equivalent.

### Estimation of total flavonoid content

The total flavonoid content was estimated spectrophotometrically by using the aluminum chloride colorimetric assay with some modifications.<sup>16</sup> The reaction mixture was made up of 0.5ml of the plant extract, 2ml of distilled water in a tube and 0.15 ml of 5% sodium nitrite. The mixture was allowed to stand for 5 min at room temperature after which 0.15 ml of 10% aluminum chloride was added to the solution and allowed to incubate for another 5 min. After incubation, 1ml of 4% sodium hydroxide was added and the solution made up to 5 ml with distilled water. The solution was then vortexed and incubated for 15 min to observe a colour change. The absorbance was measured at 420nm. The total flavonoid content was calculated as mg/g quercetin equivalent.

### Estimation of total flavonol content

The total flavonol content was estimated using the method described by Wintola and Afolayan with some modifications.<sup>17</sup> 2 ml of the plant extract was added to 2 ml of 10% aluminum chloride prepared in ethanol. To this 3 ml of 5%, sodium acetate was added and then incubated at 20°C for 2½ h. The absorbance was measured at 440nm with a spectrophotometer. Total flavonol content was expressed as mg/g of quercetin equivalent.

### Estimation of proanthocyanidin content

The total proanthocyanidin were estimated using the method described by Caceres- Mella *et al.* with some modifications<sup>18</sup> by mixing 0.5 ml of the prepared plant extract with 3 ml of 4% vanillin-methanol. 1.5 ml of hydrochloric acid was added to the solution and vortexed. The mixture was left to stand for 15 min at room temperature. The absorbance was then read at 500 nm using a spectrophotometer. Total proanthocyanidin content was calculated as mg/g of catechin equivalents.

### Estimation of tannin content

The total tannin content was estimated using the Folin - Ciocalteu method.<sup>16</sup> 7.5 ml of distilled water was added to a tube containing 0.1ml of the plant extract. 0.5 ml of Folin-Ciocalteuphenol reagent and 1 ml of 35 % Na<sub>2</sub>CO<sub>3</sub> solution was then added. The whole solution was made up to 10 ml with distilled water. The mixture was vortexed and kept at room temperature for 30 min. The absorbance was read at 725 nm using a spectrophotometer. The total tannin content was expressed as mg/g GAE equivalent.

### Estimation of alkaloid content

The total alkaloid content was estimated using the method described by Unuofin *et al.*<sup>19</sup> 5 g of the pulverized plant was immersed in 200 mL of 10% acetic acid in ethanol. The mixture was allowed to stand for 4hr at room temperature. It was subsequently filtered and the filtrate was

concentrated using a water bath at 55°C to a quarter of its original volume. Concentrated ammonium hydroxide was added in single drops until completion of the precipitation process. The solution was then washed with dilute ammonium hydroxide and filtered again. The residue obtained was first dried and then weighed. The alkaloid content was calculated using the equation:

$$\% \text{ Alkaloid} = \frac{\text{Weight of precipitate}}{\text{Weight of original sample}} \times 100$$

### Estimation of saponin

The saponin content was determined according to the method described by Omoruyi *et al.*<sup>20</sup> with some modifications. 5 g of the pulverized plant was added to 20 ml of 20% ethanol and extracted on a shaker for 30 min. The plant sample was heated over a water bath at 55°C for four h. The mixture was filtered and the residue was re-extracted again with 20 ml of 20% aqueous ethanol. The filtrate was then reduced to 40 ml over a water bath at 90°C. The concentrate was transferred into a 250 ml separatory funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while purification process was repeated. Sixty milliliters (60 ml) of n-butanol was added and the extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated over a water bath and evaporated to dryness to a constant at 40°C. The saponin content was calculated using the following equation:

$$\% \text{ Saponins contents} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

### Estimation of phytate

The total phytate content was estimated using the method described by Unuofin *et al.*<sup>19</sup> 2g of the pulverized plant was soaked into a conical flask with 50 ml of 2% hydrochloric acid for 3h and afterward filtered. 25 ml of the filtrate was taken and 5 ml of 0.3% ammonium thiocyanate solution was added. 53.5 ml of distilled water was also added to achieve the desired acidity. Then 0.05 M of iron III chloride was titrated into it until a reddish brown colour persists for 5 min. Phytate content was calculated as:

$$\text{Phytate (\%)} = \text{titre value} \times 0.00195 \times 1.19 \times 100.$$

### DPPH radical scavenging assay

DPPH radical scavenging activity of the plant extracts was determined according to the method described by Wintola and Afolayan<sup>17</sup> with some modifications. A preparation of 1 ml of 0.135 mM DPPH in methanol was mixed with 1ml of various concentrations (0.2 – 1.0 mg/ml) of the plant extracts, vitamin C and gallic acid. The mixture was left in the dark at room temperature for 30 min after being vortexed. The absorbance of the mixture was then measured spectrophotometrically at 517 nm. Both vitamin C and gallic acid were used as standards. The DPPH radical scavenging activity was calculated from the equation:

$$\text{DPPH radical scavenging activity} = \frac{\{(\text{Abs control} - \text{Abs sample})\}}{(\text{Abs control})} \times 100$$

where Abs control was the absorbance of DPPH radical + methanol; Abs sample was the absorbance of DPPH radical + sample extract or standards (Vitamin C and gallic acid).

### Nitric oxide scavenging activity

Nitric oxide scavenging activity was determined according to the method described by Boora *et al.*<sup>21</sup> with some modifications. 2 ml of 10 mM Sodium nitroprusside was prepared in 0.5 ml phosphate buffer saline (pH 7.4) and mixed with 0.5 ml of either plant extracts, vitamin C or gallic acid, at various concentrations (0.2-1.0 mg/ml). The mixture was incubated at 25°C for 150 min. After incubation, 0.5 ml of Griess reagent (1.0 ml of 0.33% sulfanilic acid reagent prepared in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethyl-enediamine dichloride) was added to an equal volume of the incubated solution. The mixture was incubated for another 30 min at room temperature and the absorbance was then measured at 540 nm. The amount of nitric oxide radical inhibited by the extracts was calculated using the following equation:

$$\text{Nitric oxide (NO) radical scavenging activity} = \frac{\{(\text{Abs control} - \text{Abs sample})\}}{(\text{Abs control})} \times 100$$

where Abs control was the absorbance of NO radical + methanol; Abs sample was the absorbance of NO radical + sample extract or standards (Vitamin C and gallic acid).

### Hydrogen peroxide radical scavenging assay

Hydrogen peroxide scavenging activity was determined using the method described by Oyedemi *et al.*<sup>22</sup> 4 ml of plant extract, vitamin C or gallic acid was prepared in distilled water at different concentrations (0.2-1.0 mg/ml) and mixed with 0.6 ml of 4 mM Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution prepared in phosphate buffer (0.1 M, pH 7.4). The solution was incubated for 10 min at room temperature. The absorbance of the solution was then measured at 230 nm. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation:

$$\text{H}_2\text{O}_2 \text{ radical scavenging activity} = \frac{\{(\text{Abs control} - \text{Abs sample})\}}{(\text{Abs control})} \times 100$$

where Abs control was the absorbance of H<sub>2</sub>O<sub>2</sub> radical + methanol; Abs sample was the absorbance of the H<sub>2</sub>O<sub>2</sub> radical + sample extract or standard (Vitamin C and gallic acid).

### Phosphomolybdenum antioxidant assay

The total antioxidant capacity was determined by the method described by Ahmed *et al.*<sup>23</sup> with some modifications. 0.5 ml of plant extracts, vitamin C and gallic acid prepared in varying concentrations (0.1-0.5 mg/ml) were mixed with three ml of distilled water and 1mls of phosphomolybdate reagent in test tubes. The solutions were then incubated at 95°C for 90 min. After incubation, the tubes were normalized to room temperature for about 30min. The absorbance of the solution was then measured at 695 nm. The amount of phosphomolybdenate radical inhibited by the extract was calculated using the following equation:

$$\text{Phosphomolybdate antioxidant activity} = \frac{\{(\text{Abs control} - \text{Abs sample})\}}{(\text{Abs control})} \times 100$$

where Abs control was the absorbance of phosphomolybdate reagent + methanol; Abs sample was the absorbance of phosphomolybdate reagent + sample extract or standard (Vitamin C and gallic acid).

### Vitamin A estimation

Vitamin A estimation was done by the method described by Onyesife *et al.*<sup>24</sup> 20ml of petroleum ether was added to 1g of pulverized plant and



put on a shaker for about 30mins. The petroleum ether was decanted and evaporated to dryness. 0.2ml of chloroform-acetic anhydride (1:1 v/v) was added to the residue. Later on 2ml of trichloroacetic acid- chloroform (1:1 v/v) was added. The absorbance of the solution was then measured at 620 nm. The vitamin A standard was also prepared in the same way at varying concentrations and a standard curve plotted.

### Vitamin C estimation

Vitamin C estimation was done by the method described by Igwe and Okwu.<sup>25</sup> A 1g of the pulverised plant was put in 20ml of 0.4% oxalic acid. It was then filtered using a Whatman filter paper and 1ml of the filtrate was mixed with 9ml of indophenol reagent. The absorbance of the solution was measured at 520nm. The vitamin C standard was also prepared in the same way at varying concentrations and a standard curve plotted.

### Vitamin E estimation

Vitamin E estimation was done by the method described by Onyesife *et al.*<sup>24</sup> 20ml of ethanol was added to 0.5g of the pulverized sample and then left on a shaker for 20mins. It was then filtered. 1ml of the filtrate was then mixed with 1ml of 0.2% of ferric acid in ethanol and 1ml of 0.5%  $\alpha$ -dipyridine. The solution was made up to 5mls with distilled water. The absorbance of the solution was read at 520 nm. The vitamin E standard was also prepared in the same way at varying concentrations and a standard curve plotted.

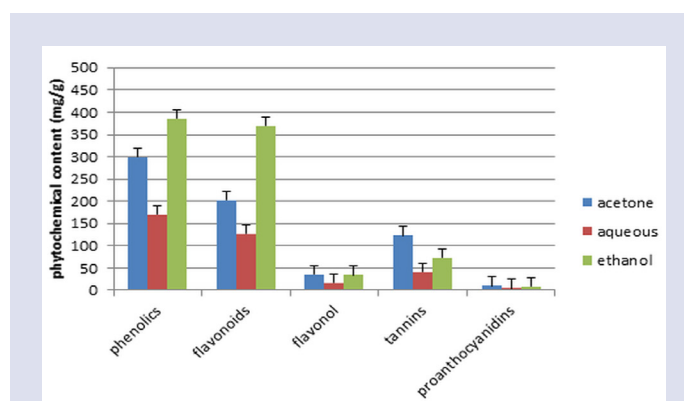
### Statistical analysis

Data obtained were presented as means  $\pm$  SD. All experiments were done in triplicates. The One-way analysis of variance (ANOVA) and the Tukey test were used to determine the differences among the means of the various samples. P values < 0.05 were regarded to be significant.

## RESULTS

### Phytochemical composition

The *Pelargonium spp* are noted for their medicinal benefits in herbal medicine, as a result of its rich composition of phytochemicals. In this study the amount of the various phytochemicals investigated differ among the extracts (Figure 1). The total phenolic content in each of the leaf extract showed varying yields with ethanolic extract showing the highest yield at 385.25mg of GAE per gram of dried extract, followed by the acetone extract at 299mg and water at 170.5mg of GAE per gram of dried extract. The ethanol extract was significantly higher ( $P < 0.05$ ) than the acetone and aqueous extract. The total flavonoid content were 369.65mg of quercetin equivalent per gram of dried extract for the ethanol extract, the acetone extract 202.3mg quercetin equivalent per gram of dried extract and aqueous extract 127.2mg quercetin equivalent per gram of dried extract. The flavonol content of the leaf extracts in this study showed acetone leaf extract (35.67mg of quercetin equivalent per gram of dried extract) was slightly higher than the ethanol leaf extract (34.52mg of quercetin equivalent per gram of dried extract). The water extract was the lowest at 16.42mg of quercetin equivalent per gram of dried extract. The acetone and ethanol extract had significantly higher levels than the aqueous extract ( $P < 0.05$ ). The proanthocyanidin level was highest in the acetone leaf extract (10.4mg of catechin equivalent per gram of dried extract). The ethanol and aqueous extracts yielded 8.8 and 5.33mg of catechin equivalent per gram of dried extract respectively. High levels of tannins were observed in the acetone leaf extract of *P. inquinans* (123.8mg/g GAE per gram of dried extract) compared to the ethanol (73.3mg/g GAE per gram of dried extract) and aqueous leaf extracts (41.3mg/g GAE per gram of dried extract). The acetone extract showed significantly higher levels than the ethanol and the aqueous extracts ( $P < 0.05$ ).



**Figure 1:** Phytochemical constituents identified in the various extracts of *Pelargonium inquinans*. Values are expressed as mean  $\pm$  standard deviation (SD) of three separate determinations (n = 3).

**Table 1:** Anti-nutrient composition of *Pelargonium inquinans* leaves.

Anti-nutrient	Content (%)
Saponin	36.2 $\pm$ 4.38
Alkaloid	0.52 $\pm$ 0.10
Phytate	0.65 $\pm$ 0.07

**Table 2:** Vitamin contents of *Pelargonium inquinans* leaves (dried weight).

Vitamin	Mean $\pm$ SD (mg/100g)
Vitamin A	1900 $\pm$ 16.4
Vitamin E	280.9 $\pm$ 26.2
Vitamin C	2.0 $\pm$ 0.02

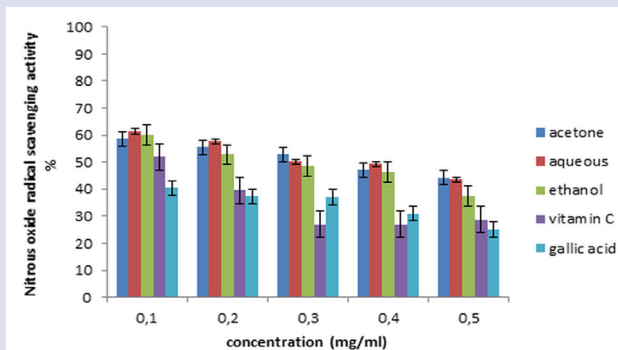
The anti-nutrient composition of *Pelargonium inquinans* leaves showed it contains saponin, alkaloid and phytate in varying percentages (Table 1). These results show that the total phenolic content in this study was higher than the other phytochemicals. Some anti-nutrient are also present in the leaves of *Pelargonium inquinans*.

### Vitamins analysis of *Pelargonium inquinans*

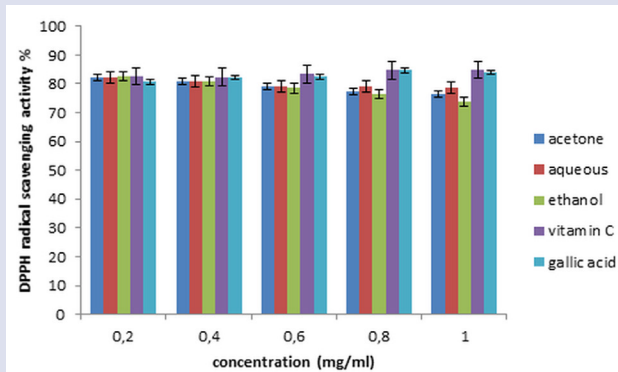
This study observed vitamins A, E and C in the dried leaves of *Pelargonium inquinans* (Table 2). The highest vitamin content was vitamin A at 1.9g/100g of dried extract. The vitamin E content was 280.9mg /100g of dried extract while the vitamin C content was 2.0mg /100g.

### Nitric oxide scavenging activity

In this study the acetone, aqueous and ethanol extracts of *Pelargonium inquinans* leaves exhibited a high capacity to scavenge nitrous oxide radicals in a dose-dependent decreasing manner, i.e. the concentration was the inverse of scavenging activity. The aqueous extracts of the plant showed the highest activity at 0.1mg/ml (61.4%  $\pm$  0.03), higher than the ethanol extract at 60%  $\pm$  0.01 and aqueous extract at 58.7%  $\pm$  0.01 (Figure 2). The acetone extract also had greater activity than gallic acid (44.5%  $\pm$  0.05) and Vitamin C (52%  $\pm$  0.03). The values were statistically significant ( $p < 0.05$ ). The  $IC_{50}$  values were 0.744, 0.741, 0.805, 1.18 and 1.18mg/ml for acetone, aqueous, ethanol, Vitamin C and gallic acid respectively.



**Figure 2:** Nitric oxide radical scavenging activity of the extracts of *Pelargonium inquinans* leaves. The values represent mean  $\pm$  S.D (n=3).  $P < 0.05$ .



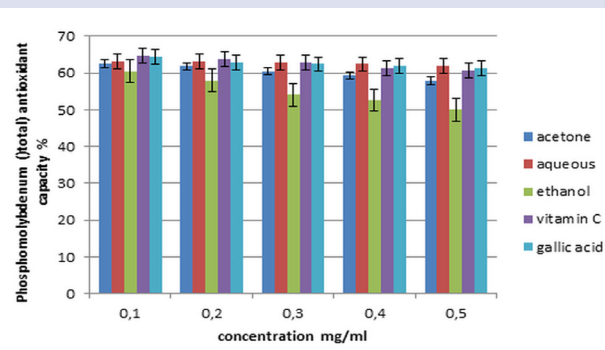
**Figure 3:** DPPH radical scavenging activity of the extracts of *Pelargonium inquinans* leaves. The values represent mean  $\pm$  S.D (n=3).  $P > 0.05$ .

### DPPH scavenging activity

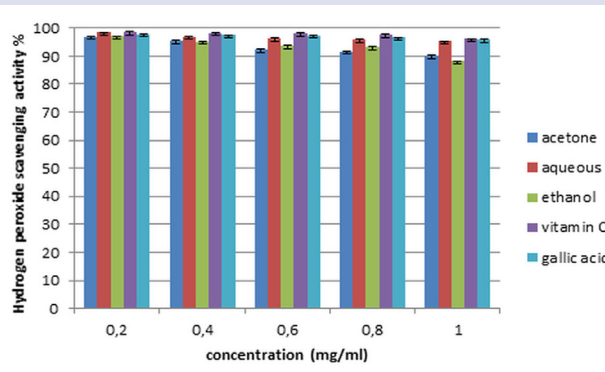
The three solvent extracts of *Pelargonium inquinans* evaluated scavenged DPPH efficiently. Their scavenging activity at optimal concentration (0.2mg/ml) was similar and not statistically different in a dose-dependent decreasing manner, i.e., the concentration was the inverse of scavenging activity. The ethanol extract exhibited the highest scavenging activity at  $82.7\% \pm 0.01$  followed by acetone and aqueous extracts at  $82.29\% \pm 0.01$  and  $82.25\% \pm 0.003\%$  respectively. All the extracts had lower activity than the standards- vitamin C ( $84.9\% \pm 0.01$ ) and gallic acid ( $84.65\% \pm 0.04$ ) (Figure 3). The  $IC_{50}$  values were 0.468, 0.461, 0.476, 0.436, 0.439mg/ml for acetone, aqueous, ethanol, vitamin C and gallic acid respectively. The DPPH scavenging activity of the acetone extract of *Pelargonium inquinans* recorded in this study was higher than that reported for the acetone extract of *Geranium macrorrhizum*, though the latter's methanolic extract was higher than *Pelargonium inquinans*'.

### Phosphomolybdenum (total) antioxidant activity

The aqueous extract of *Pelargonium inquinans* exhibited a greater antioxidant capacity than the ethanol and acetone extracts. Their antioxidant capacity was in the order aqueous > acetone > ethanol, although less than vitamin C and gallic acid. The  $IC_{50}$  values were 0.293, 0.306, 0.344,



**Figure 4:** Phosphomolybdenum (total) antioxidant activity of the extracts of *Pelargonium inquinans* leaves. The values represent mean  $\pm$  S.D (n=3).



**Figure 5:** Hydrogen peroxide scavenging activity of the extracts of *Pelargonium inquinans* leaves extracts. The values represent mean  $\pm$  S.D (n=3).

0.296, 0.298 mg/ml for aqueous, acetone, ethanol, Vitamin C and gallic acid respectively (Figure 4). This increased total antioxidant capacity still explains the solvent polarity of the fractions used. *Pelargonium spp.* are known to have the good antioxidant capacity. However a search of the literature did not reveal any phosphomolybdenum antioxidant capacity work done before and neither did it reveal any too for *Pelargonium inquinans*.

### Hydrogen peroxide scavenging activity

The results of the hydrogen scavenging activity of the extracts are as shown in Figure 5. The aqueous extracts of the leaves used had the highest scavenging activity of all the extracts used. The scavenging activity of the extracts were aqueous  $98.39\% \pm 0.009$ ; acetone  $97.11\% \pm 0.008$  and ethanol at  $96.99\% \pm 0.04$ . The differences were however negligible. There were no significant differences in the activity ( $P > 0.05$ ). The  $IC_{50}$  values were 0.381, 0.397, 0.397, 0.376 and 0.378 mg/ml for aqueous, ethanol, acetone, vitamin C and gallic acid respectively. The aqueous extract was also slightly lower than vitamin C and gallic acid. A search of the literature did not reveal any information about hydrogen peroxide scavenging ability of *Pelargonium inquinans*. However, coumarins, compounds consisting of fused benzene and alpha pyrone rings are found abundantly in *Pelargonium spp.* The hydrogen peroxide

scavenging activity of some coumarin derivatives showed that some of them have high hydrogen peroxide scavenging activity, which may explain the high activity observed in all the extracts of *Pelargonium inquinans*.

## DISCUSSION

### Phytochemical composition

Epidemiological studies have shown that food rich in fruits and vegetables can lower the risk of some chronic diseases.<sup>26</sup> This effect have been partially linked to the intake of polyphenols and potential mechanism include antioxidant and anti-inflammation. Some studies have reported that polyphenols are efficient antioxidants.<sup>27</sup> Phenolic compounds have been reported to have many biological activities including antioxidative and anti-neoplastic activities.<sup>28</sup> The presence of high phenol content may indicate that *P. inquinans* have high antioxidant activity.

Flavonoids are one of the most studied groups of phytochemicals and account for more than half of the natural polyphenols.<sup>29</sup> They are known to exhibit several biological activities including anti-inflammatory, antioxidant and immunomodulatory properties all which play a role in chronic diseases. *Pelargonium spp.* leaves are known to be rich in flavonoids and this study further confirmed. Flavanols, also known as flavan-3-ols are a subclass of flavonoids with very complex structures. Flavanols are known to demonstrate good antioxidant and anti-inflammatory activities and are readily distributed in the leaves of *Pelargonium spp.* Dietary flavanol intake has been shown to be associated with low risk of breast cancer.<sup>30</sup> Proanthocyanidins are a group of phytochemicals that are readily found in many medicinal plants and are known for their powerful antioxidant activities.<sup>31</sup> Proanthocyanidins have been reported to have higher antioxidant activity than ascorbic acid and tocopherol.<sup>32</sup> They are also known to have anti-adhesive activities.<sup>33</sup> Proanthocyanidin oligomers have been characterized in root extracts of *P. sidoides*,<sup>34</sup> but they have only moderate pharmacological effects. *Pelargonium spp.* have been observed to be a rich source of tannins. Although tannins have been reported to be anti-nutritive, they have been discovered to possess biological and pharmacological actions such as antimicrobial, anti-neoplastic, anti-plasmin inhibitory activity as well as superoxide anion scavenging activity.<sup>35</sup> Geraniin, a dehydroellagitannin found in *Pelargonium spp.*, especially *Geranium thunbergii*, has been shown to play an inhibitory role in human melanoma cells by mediating apoptosis through the cleavage of focal adhesion kinase and up-regulation of Fas ligand expression.<sup>36</sup> The results of this study were similar to what was reported by Petlevski *et al.*<sup>37</sup> for the total tannin content using water and ethanolic leaf extracts of *P. radula*, though the result of their aqueous extract was significantly lower than the one obtained in our study.

*Pelargonium inquinans* leaves is also composed of anti-nutritive factors like alkaloids, saponin and phytate. The *Pelargonium spp.* have been reported to have some alkaloids.<sup>38</sup> The indole alkaloids elaeocarpidine and its 20-H isomer epielaecarpidine were characterized in the leaves of the *Pelargonium* appleblossom.<sup>39</sup> Also recently, Igwenyi and Elekwa<sup>40</sup> were able to quantify the total alkaloid content of the fresh leaves of *Geranium robertianum* from Nigeria. From a search of the literature, this study appears to be the first to quantify the alkaloid content of *Pelargonium. inquinans*. The pharmacological importance of alkaloids cannot be understated especially as anticancer agents in the management of many malignancies.<sup>41</sup> Saponins are known to have medicinal values which include anti-inflammatory, cholesterol-lowering activity as well as anti-neoplastic activity. The antineoplastic activity is reported to be achieved via anti-angiogenesis and anti-metastasis.<sup>42</sup> Saponin was present in all the solvent extracts of *P. graveolens* leaves, in a qualitative screening done by Pradeepa *et al.*<sup>43</sup> Saponin was also observed in *P. luridum*.<sup>44</sup> To the best of our knowledge, this is the first study to report the saponin content in

*Pelargonium. inquinans* leaves. Phytate also known to be anti-nutritive, but has been discovered to have some health benefits. Some of their pharmacological activity includes lowering of blood sugar and lipids, antioxidants and prevention of nephrolithiasis.<sup>45</sup>

### Vitamins

The study showed that Vitamins A, E and C were present in the leaves of *Pelargonium inquinans*. Vitamins are well-known nutrient that contributes to health and well-being. Although needed in small amounts they play a vital role in normal body physiology and their deficiencies have been linked to some diseases.<sup>46</sup> Vitamin C and E are well-known antioxidants that play major roles in cells that display oxidative stress including cancer cells.<sup>67</sup> Vitamin A derivatives such as all-trans- retinoic acid are used in the management of acute promyelocytic leukaemia.<sup>47</sup> Some of these vitamins and their derivatives have been mooted as chemopreventive agents.<sup>48</sup> The discovery of more sources of these vitamins and their derivatives like *Pelargonium spp.* can be a way to manage chronic diseases.

### Nitric oxide scavenging activity

Nitric oxide is a well-known free radical with pleiotropic effects across some physiological processes in the body.<sup>49</sup> It is known to play a role in vasodilatation, smooth muscle relaxation, inhibition of platelet aggregation, immunity.<sup>50</sup> It is constitutively produced by the body in nanomolar concentration to maintain normal cellular function. However, nitric oxide produced in greater concentration can exacerbate inflammatory processes causing tissue damage. The nitric oxide scavenging activity of *Pelargonium inquinans* was higher than the standards confirming several reports that showed a close relationship between total phenolic content and high antioxidant activity.<sup>51</sup>

### DPPH radical scavenging activity

Alpha, alpha-diphenyl-  $\beta$ -picrylhydrazyl (DPPH) is a stable free radical that reacts slowly with most compounds. The method was developed by Blois in 1958 and is based on the ability of the odd nitrogen electron in its molecules to accept a hydrogen atom from a donor antioxidant thereby becoming reduced in the process, i.e., DPPH-H.<sup>52</sup> *Pelargonium inquinans* has been shown to exert strong DPPH scavenging with its ethyl acetate fraction showing excellent antioxidative potential.<sup>15</sup> The compound 1,2,3,4,6-penta-O-galloyl-beta-d-glucose (PGG) is taken as the active antioxidative component. The *Pelargonium spp.* are known to possess antioxidative activity. This study further confirmed it.

### Phosphomolybdenum (total) antioxidant activity

The phosphomolybdenum antioxidant assay is a quantitative method based on the reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V). This antioxidant assay evaluates the total antioxidant capacity (water-soluble and fat-soluble) of a crude extract. The assay is involved in thermally generating auto-oxidation during extended incubation periods at high temperature. This assay is different from the other reducing assays because it doesn't change form irrespective of the free metal concentration, thus maintaining its green phosphomolybdenum complex form. It is therefore an assay that gives a more direct estimation of reducing capacity of antioxidants. *Pelargonium spp.* are known to have the good antioxidant capacity. However a search of the literature did not reveal any phosphomolybdenum antioxidant capacity work done before and neither did it reveal any too for *Pelargonium inquinans*.

### Hydrogen peroxide scavenging activity

Hydrogen peroxide is an important reactive oxygen species that is formed as a result of the enzymatic conversion of superoxide radical by superoxide dismutase. It is then either converted to water by catalase



or to hydroxyl radical in the Fenton reaction. Hydrogen peroxide is cytotoxic to cells and it is used by the body to fight bacterial and also to enhance the process of inflammation.<sup>53</sup> The product of hydrogen peroxide catalysis, hydroxyl ion is regarded as the most reactive of all reactive oxygen species capable of causing lipid peroxidation, protein damage and membrane damage.<sup>54</sup> Due to its role in inflammation and cell damage, control of the cellular level of hydrogen peroxide is important.

The scavenging ability of *Pelargonium inquinans* was high and dose-dependent. This could be as a result of high levels of phenolic compounds and flavonoids; compounds with high amounts of polyphenol have good antioxidant activities.<sup>55</sup> Thus the high antioxidant activity in the different extracts of *Pelargonium inquinans* could be as a result of the high amount of phenolic compounds and flavonoids.

## CONCLUSION

The antioxidative abilities of extracts from plant sources is of great interest to researchers and industry because of their medicinal properties in the definitive and supportive care of cancer. This study investigated the phytochemical composition, vitamin content and antioxidative properties of *Pelargonium inquinans*. The study showed that different phytochemicals and anti-nutrients were present in varying amounts. The various antioxidant assays done showed that extracts of *Pelargonium inquinans* have strong antioxidant activities which makes them potential candidates in the management of cancers.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**DPPH:** Alpha, alpha-diphenyl-β-picrylhydrazyl; **OS:** Oxidative stress; **ROS:** Reactive oxygen species; **RNS:** Reactive nitrogen species; **H<sub>2</sub>O<sub>2</sub>:** Hydrogen peroxide; **PGG:** 1,2,3,4,6-penta-O-galloyl-beta-d-glucose; **NO:** Nitric oxide.

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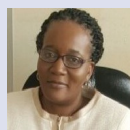
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#### SUMMARY

- This work investigated the chemical composition of a member of the Geraniaceae family, *Pelargonium inquinans* Ait. It estimated its phytochemical contents as well as the composition of vitamins A, C and E. It also investigated the antioxidant activity of the plant extracts. The extracts were found to be rich in phytochemicals especially phenolics, flavonoids and tannins. They also showed good antioxidant activities and thus justified their use in folk medicine for the management of some ailments.

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