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# Comparative Evaluation of the Secondary Metabolites Concentrations in *Prunus africana* on the Slopes of Mount Cameroon

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**ABSTRACT**
Mount Cameroon is a biodiversity hotspot and a home to diverse fauna and flora species harbouring a variety of endemic and endangered species including *Prunus africana*. This plant is a tree restricted to mountain forest in Africa at altitude of 1500 to 3000 m. The species occurs naturally in 3 regions of Cameroon: Northwest, Adamawa and Southwest Regions. Little or no investigation has been carried out to check if the plants growing at lower altitudes (in agroforestry systems) have the same secondary metabolites concentration in different plant parts as those growing at an upper elevation (wild). This work was carried out on a 1 ha plot subdivided into 16 sub-plots of 25 m x 25 m located in the upper mountain forest of Mount Cameroon and agroforestry system in Buea. Samples were collected from the wild at the upper elevations (1672 m-2094 m) and in agroforestry systems at lower elevations ranging between 563 m and 923 m to determine the concentration variations of phytochemicals, specifically phenols and triterpenes. Phenols were present in the stem bark and root bark, but absent or barely detectable in leaf samples and phenolic concentrations might not be affected by the elevation, tree size and the bark thickness. The concentration of Triterpenoids did not differ with respect to the plant part used, the size of the tree, bark thickness and elevation. This suggests that the concentration of triterpenoids in the wild *P. africana* is the same as those in agroforestry systems.
**Key words:** Mount Cameroon, *Prunus africana,* secondary metabolites, concentration, slopes.
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## 1. Introduction

Mount Cameroon is a Pleistocene refuge and a biodiversity hotspot of international reputation with the most diverse ecosystem in Cameroon (Ako *et al*., 2012). It is one of the largest mountains in Africa and the highest mountain in Central and West Africa. Mount Cameroon is an active volcano and in the last century, the mountain has erupted a number of times with the longest quiescence period of 32 years and the shortest 1year between 1999 and 2000. It is characterized by young volcanic tertiary and quaternary rock and fertile soils with poor water-retaining capacity (Vander Werf *et al.,* 2009). Its soil's nature has attracted its surrounding villages (about 41 villages) to depend only on farming as their main source of income, in addition to hunting, timber and non-timber forest products exploitation, petty trading, livestock with a few cases of salary earners (Tanjong, 2014). Its landscape supports forest of exceptional scientific, economic and social value, harbouring a variety of endemic and endangered flora and fauna species, supplying many timber and non-timber forest products as well as providing a valuable ecosystem (Ako *et al.,* 2012). It is also a refuge of diverse flora species (ranging from trees, shrubs, lianas, herbs, epiphytes and orchids) known for its exceptional plant diversity having a number of endemic and threatened plant species with over 800 genera, 210 families and 49 plant taxa which are strictly endemic to the Mountain (*Schefflera abyssinica, Canthium dunlapii, Nuxia congesta, Clausena anisata, Syzygium staudtii*) (Oates *et al.,* 2008; Böhme & Hillers, 2006).

Among the endangered and threatened plants species on Mount Cameroon includes *Prunus africana*, also known as African cherry, African plum, African prune or Bitter almond. It has a wide distribution in Africa, occurring in mountainous regions of Central and Southern Africa and on the islands of Bioko, São-Tomé, Grande Comore and Madagascar (Maloueki *et al.,* 2015). It can be found at 900-3,400 m (3,000-10,000 ft) above sea level. It is a canopy tree 30-40 m in height, and is the tallest member of *Prunus* (Hall *et al*., 2000). It is a long-lived evergreen canopy tree which grows up to 30 m tall with thick fissured bark and straight bole reaching a diameter of 1.5 m (Hall *et al.,* 2000). It is a light demanding plant, found in afromontane forests belonging to the Rosaceae family (Hall *et al.,* 2000). According to Graham (1960), the word *'Prunus'* was derived from the plum-like shape of its fruit which reflects the characteristics of the Genus of this fruit and '*africana'* as an indication that the tree is endemic to mountain forest of Africa and said to grow well in the Sub Mountain and mountain forests at an altitude of 1500 to 3000 m (Hall *et al.,* 2000). In Cameroon, the plant is largely found in 3 regions including

Adamawa, Northwest (Kilum-Ijum Reserve forest), and Southwest (on Mount Cameroon) (Fraser *et al*., 1996). This species is best known for its hard wood used in production of high quality timber that is used locally for building poles, furniture making as well as fuel wood and besides its use for timber, it is a medicinal plant with leaves, root bark and stem bark used in the production of traditional medicine in Africa because it contains various bioactive substances with anti-inflammatory, anti-cancer or antiviral effects found in different members of the genus *Prunus* (Shenouda *et al*., 2007). Extracts of the bark of this species significantly improve urologic symptoms, having anti-proliferate and apoptotic effects on the prostrate (Ishani *et al.,* 2000; Teshale, 2020). Aside from the treatment of benign prostatic hyperplasia, a disorder of the prostrate that is common in older men, extracts from this species are also said to be used for the treatment of malaria, diarrhoea, dysmenorrhea, epilepsy, impotence, infertility, irregular menstruation, kidney disease, mental illness, eye disorders, fevers, obesity, pneumonia, arthritis, haemorrhage, hemorrhoids, hypermenorrhea, hypertension, prostate gland enlargement, antigonorrheic, antihelmentic, anti-inflammatory, chest pain, anti-parasiticide, anti-rheumatic and in particular gastrointestinal disorders, ranging from abdominal pain to intestinal parasites in children (Bii *et al.,* 2010; Jimu, 2011; Bodeke *et al.,* 2014).

The main characteristic constituents are phytosterols (0.05%), e.g. beta-sitosterol, beta-sitosterol-3-glucoside and beta-sitostenone, free C, -C 24 fatty acids. Pentacyclic triterpenic acids are present (14%) (ursolic and oleanolic acid derivatives) as well as long chain aliphatic alcohols (n-docosanol, n-tetracosanol and their trans-ferulic acid esters) (Shenouda *et al*., 2007). The extraction and characterization of several phytochemicals from *Prunus africana* have been the main topics of research in the Mount Cameroon area. Ngassapa *et al.* (2018) looked into the amounts of alkaloids, flavonoids, tannins and saponins in various plant parts utilizing typical extraction techniques using solvents such as ethanol and methanol and found significant amounts of these beneficial chemicals. The antioxidant activity of *Prunus africana* extracts on Mount Cameroon was examined with emphasis on the possible health advantages of consuming products made from *P. africana* (Nguemo *et al.,* 2020). Variation in concentration was found between the Adama *Prunus* relating to those on Mount Cameroon and Mount Oku carried out by Powell (1999).
However, little or insignificant research has been carried out or documented on the investigation of the concentration of secondary metabolite relating to prostate treatment with respect to plant part, tree size, and bark thickness and different habitats around Mount Cameroon (the wild and agroforestry system).

The main objective of this study was to evaluate the concentration of secondary metabolites in samples with respect to altitude.

## 2. Materials and methods

### 2.1. The study site

#### 2.1.1. Description

The research was carried out on Mount Cameroon, located in the Southwest Region of Cameroon in the Fako Division. It lies on the coast, in the Gulf of Guinea, between 3°57'- 4°27' N and 8°58'-9°24'E. It is a huge volcanic mass with its long axis (about 45 km long and 30 km wide) running South West to North East (Scholl *et al.,* 2010).

The main peak is at 4°7'N and 10'E at an altitude of about 4,100 m and is the highest mountain in West and Central Africa with the peak just about 20 km inland from the Atlantic coastline (Bubb *et al.,* 2004). Mount Cameroon is an active volcano and covers a surface area of 58.154 ha (Toteu *et al*., 2010). It is boarded by 41 villages (Likomba, Bwassa, Boando, Ekona Lelu, Woteva, Bonakands, Bova, Bokwoango, etc) whose activities either directly or indirectly affects its management (Leat *et al.,* 2003).

The climatic conditions are characterized by two seasons: one dry season from November to mid-March and one wet season from mid-March to October with the wettest months being July and September (Ingram *et al.,* 2009). Sometimes, insignificant rains occur in the month of March, April and May, and vary depending on the year. Variation between wet and dry season rainfall is greatest at the coastal sites particularly in the west coast of Mount Cameroon (Emma and Neil, 2019). Annual rainfall ranges from over 10,000 mm at Cape Debundscha to less than 2,000 mm in the North-East of the massif around Munyenge Metombe (Fraser *et al.,* 1998). The Mean annual rainfall decreases with altitude to approximately 4,000 mm at 1,000 m and to less than 3,000 mm above 2,000 m (Payton, 1993). The mean annual temperature is about 25 ºC and this decreases by 0.6 ºC per 100 m of ascent to 4 ºC at the summit with its humidity between 75-85 % due to the marine influence and cloud formation (Bruijnzeel *et al.,* 2010).

### 2.1.2. Study plot

This work was carried out on a 1ha plot (100 m x 100 m) subdivided into 16 sub-plots of 25 m x 25 m located in the upper mountain forest of an altitude of about 2000 m (fig 1) above sea level that was setup in 2023 for tree species diversity studies with coordinates. The location of this plot was very close (about 50 m) to the grassland and the forest often covered with very low to ground level clouds (cloud forest).

The key characteristics of the vegetation at this altitude and on recent rocky lava is the numerous and small stems of trees. These tree stems are covered by abundant mosses and lichens. The understory was sparsely populated with a few herbs species (*Oreacanthus manni*, *Mimolopsis soimsii*, *Impatiens sakeriana, Impatiens burtonii*) dominated by *Oreacanthus manni* (a biannual herb) belonging to the Acanthaceae Family and which was on its first year of its growth with a height of at most 1.3 m at the time of this work.

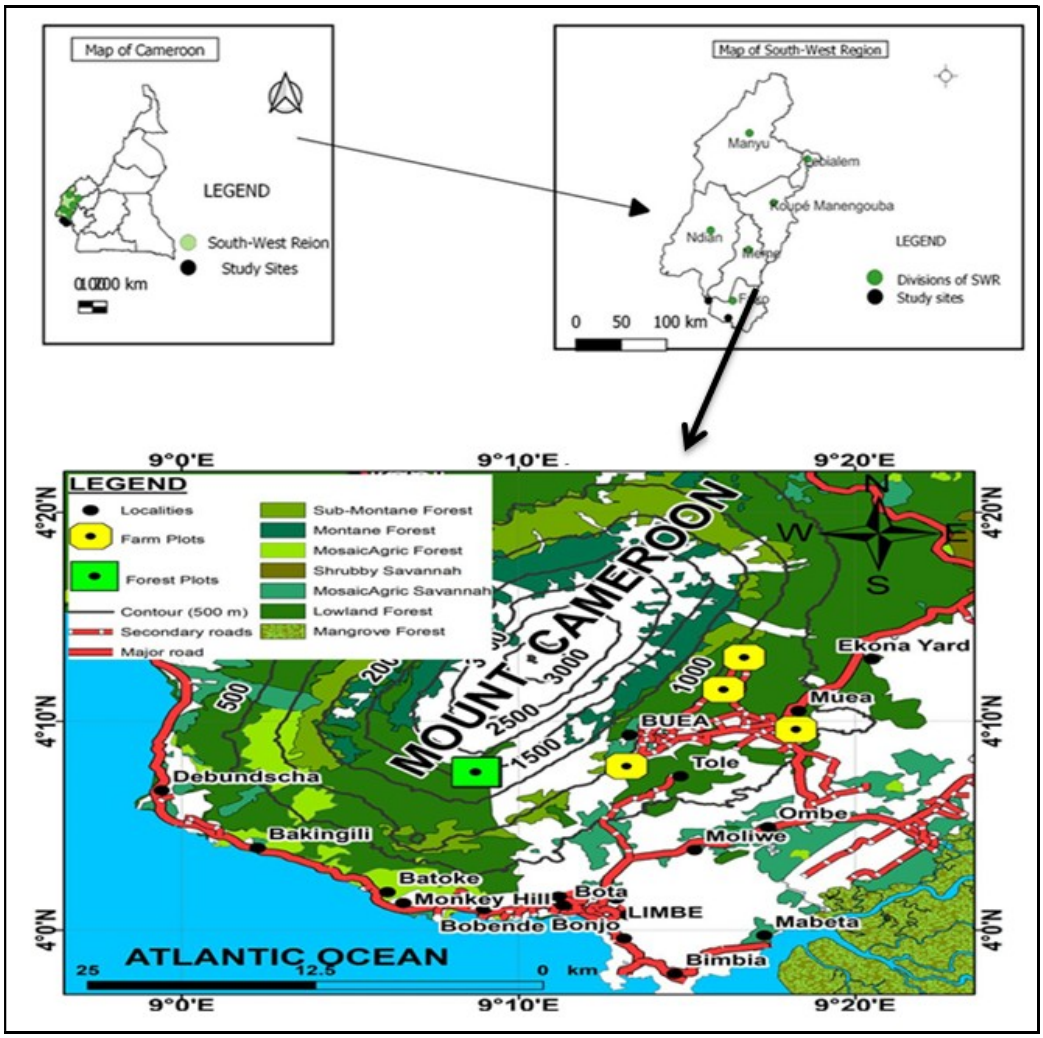



Figure1. Study site.

### 2.2. Collection of plant samples

To determine the difference in concentration of various phytochemicals found in *P. africana*, samples were collected from the wild plants at upper elevations (from 1672 m - 2094 m) and in agroforestry systems at lower elevations ranging from 563 m - 923 m for this purpose (fig 3). Generally, stem bark was obtained from small trees (within the diameter of 11-20 cm), medium-sized classes (within the diameter of 21-30 cm) and big size class (within the diameter of 40 cm and above) using an axe and machete at 4 small quotas on each main stem. Where possible, root bark and leaf samples were obtained. A total of 7 stem-bark samples, 2 root-barks and 1 leaf sample were collected from the wild; whereas, in the agroforestry systems, a total of 5 stem-barks, 1 root-bark and 2 leaves samples were collected. GPS points of every sampled tree were noted and the DBH of the tree was measured using a diameter tape.

Once the stem and root-barks were collected, the death stem-bark was piled off and bark thickness measured instantly using a Vernier calliper and recorded. The stem-bark was the most collected sample because it's the mostly used and most accessible. All the different samples were packaged in separate zip lock bags and labelled appropriately for onward transmission and storage in the Plant Science Laboratory of the University of Buea.

### 2.3. Preparation of extracts

In the Plant Science Laboratory, the stem-bark and root-bark samples were sliced into smaller pieces, air dried in an open room space for 2-3 weeks to reduce their moisture content, while leaf samples were rinsed from debris and air-dried for 1-2 weeks. The dried samples were then blended separately using an electric blender to semi fine powder. After blending a sample, the blender was cleaned properly with a brush and neat cloth so as to avoid contamination. Fifty grams, (50 g) of each powdered sample were weighed and introduced into 1000 mL of the 2 solvents (distilled water and acetone) for extraction for 24 hours in clean tight bottles. The mixtures were carefully separated to get the filtrates which were kept in clean vials under room temperature. Each crude extract obtained was used to carry out phytochemical analysis.

### 2.4. Phytochemical Screening

The concentrated residues from acetone and distilled water extracts were used to detect the secondary plant metabolites including alkaloids, flavonoids, steroids, saponins, glycosides, phenolics and tannins using standard methods by (Mac Nee, 2005; Teshale, 2020) with some few modifications.

#### 2.4.1. Test for flavonoids (cyanidine test)

Half gram (0.5 g) of the crude extracts was dissolved in methanol and 2 mL of concentrated hydrochloric acid added. A few pieces of magnesium turnings were added and the mixture observed for effervescence. A brick red coloration observed indicated the presence of flavonoids.

#### 2.4.2.Test for Saponins (frothing test)

Saponins were tested by dissolving half gram (0.5 g) of the crude extracts in a test tube containing 3 mL of hot distilled water and then the mixture was shaken vigorously for 1 minute and persistent foaming observed indicated the presence of saponins.

#### 2.4.3.Test for Tritepenoids (Lieberman-Burchard test)

About half gram (0.5 g) of the crude extracts was dissolved in dichloromethane to give a dilute solute solution and then 0.5 mL of acetic acid anhydride added, followed by three drops of concentrated sulphuric acid. A brick red or red violet colouration indicated the presence of triterpenoids.

#### 2.4.4. Test for Alkaloids (Dragendorf's test)

The samples were dissolved in dichloromethane and then spotted and a thin film layer chromatographic plate which was developed in 20 % hexane in ethylacetate. The presence of alkaloids in the developed chromatogram was detected by spraying with freshly prepared Dragendorf's reagent in a fume chamber. A positive reaction on the chromatogram indicated by an orange or darker coloured spot against a yellow background is confirmatory evidence that the plant extract contained alkaloids.

#### 2.4.5. Test for cardiac glycosides

An extract of the plant was added to 2 mL of glacial acetic acid plus one drop of ferric chloride. The setup was underplayed with 1mL of concentrated sulphuric acid. There was the appearance of violet and brownish rings below the interface, followed by the formation of a greenish ring in the acetic acid layer indicating the presence of cardiac glycosides.

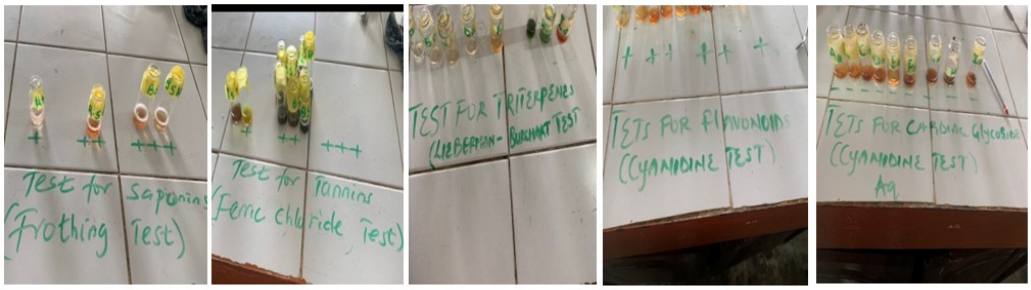
#### 2.4.6. Test for Phenols

To 1mL of either plant extract, one drop of 5 % $FeCl_3$ (w/v) was added. Formation of greenish precipitate indicated the presence of phenols.

#### 2.4.7. Test for tannins (Ferric chloride test)

Half gram (0.5 g) of either crude extract was dissolved and added to a tube containing 20 mL of boiling distilled water and then boiled for an hour. A few drops of ferric chloride were added and allowed to stand for proper colour development. A blue-black colouration indicated the presence of tannins.




a b c d e

Figure 2. Extracts from *Prunus africana* plant parts for phytochemical screening
a) Saponins b) Tannins c) Triterpenoids d) flavonoids e) cardiac glycosides

**2.5. Data analysis**

Data was keyed into Excel version 2021 and analysed using R version 4.4 R Core. Both descriptive and inferential statistics were used to summarise the data. Descriptively, data was summarised using means, standard deviation, frequency, and percentage frequency. Inferential statistics made use of the Chi Square test of association to test the relationship between variables and ordinal logistic regression to predict the outcome of ordinal dependent variables. Results were presented in tables.

## 3. Results

**Phytochemical constituents *of Prunus africana* stem bark, root bark and leaves**

The various plant parts: stem-bark, root-bark and leaf samples collected from the wild and agroforestry system showed varied concentration of phytochemicals for the various solvents used (acetone, non-polar solvent and distilled water, a highly polar solvent).

### 3.1. Results of the phytochemical screening of the various plant parts gotten from the wild

The results from the phytochemical screening of the various plant parts gotten from the wild showed that Phenolics were present in small amounts (+) in some stem-barks and moderate quantity (++) in others and was the same for some root-barks, but absent or barely detectable (-) in leaf samples.

Tannins were typically found in bark and root samples, with bark samples exhibiting higher (++) amounts of this compound but absent or barely detectable in leaf samples (-).

Flavonoids had less prevalence in distilled water extracts, but present in the majority of samples with acetone extraction. Saponins were present more in the stem-barks than root-barks and leaves comparatively. Saponins concentration showed a high quantity (+++) in smaller and very mature trees when extracted with distilled water but was present in small amount (+) for all plant parts except the stem-bark of small tree where it was present in moderate amount (++) with acetone extracts. Alkaloids and triterpenoids were said to be found in small quantity(+) in all stem-bark and root-bark samples when both solvents were used. Cardiac Glycosides were rarely found in any extract, with no detectable presence in any sample (Table 1).

Table 1. Phytochemical results of stem-bark, root-bark and leaf samples of *P. africana* from the wild.

| Solvents | Plant samples | Elevation | Tree size | Phytochemical constituents | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Distilled $H_2O$** | | | | Phenolics | Tannins | flavonoids | Saponins | Tritepenoids | Alkalloids |
| | Bark sample 1 | 2094 | 31.2 | + | ++ | - | + | + | + |
| | Bark sample 2 | 2073 | 50.9 | ++ | ++ | + | ++ | + | + |
| | Bark sample 3 | 2042 | 22.4 | + | ++ | - | ++ | + | + |
| | Bark sample 4 | 2033 | 18.9 | ++ | ++ | + | +++ | + | + |
| | Bark sample 5 | 2019 | 105 | ++ | ++ | + | +++ | + | + |
| | Bark sample 6 | 1992 | 61 | ++ | ++ | + | ++ | + | + |
| | Bark sample 7 | 1672 | 38 | + | ++ | - | ++ | + | + |
| | Root sample 2 | 2073 | 50.9 | + | ++ | - | ++ | + | + |
| | Root sample 6 | 1992 | 61 | ++ | ++ | + | ++ | + | + |
| | Root sample 7 | 1672 | 38 | ++ | + | - | + | + | + |
| | Leaf sample 2 | 2073 | 50.9 | - | - | - | + | + | + |
| **Acetone** | Bark sample 1 | 2094 | 31.2 | ++ | ++ | + | + | + | + |
| | Bark sample 2 | 2073 | 50.9 | ++ | ++ | + | + | + | + |
| | Bark sample 3 | 2042 | 22.4 | ++ | ++ | + | + | + | + |
| | Bark sample 4 | 2033 | 18.9 | ++ | ++ | + | ++ | + | + |
| | Bark sample 5 | 2019 | 105 | ++ | ++ | + | + | + | + |
| | Bark sample 6 | 1992 | 61 | ++ | ++ | + | + | + | + |
| | Bark sample 7 | 1672 | 38 | ++ | ++ | + | + | + | + |
| | Root sample 2 | 2073 | 50.9 | ++ | ++ | + | + | + | + |
| | Root sample 6 | 1992 | 61 | ++ | ++ | + | + | + | + |
| | Root sample 7 | 1672 | 38 | ++ | ++ | + | + | + | + |
| | Leaf sample 2 | 2073 | 50.9 | + | + | + | + | + | + |

Legend: (-) = absent or below detection limit; (+) = present in small quantity; (++) = present in moderate quantity; (+++) = present in high quantity

**3.2. Results of the phytochemical screening `of the various plant parts gotten from agroferestry**

The results from the phytochemical screening `of the various plant parts gotten from agroferestry systems showed that Phenolics were present in moderate quantity (++) in all stem-barks and root-barks samples and were

present in small quantities (+) when distilled water was used. When acetone was used, phenols were present in moderate quantity in all bark samples but absent or barely detectable (-) in leaf samples.

Tannins were typically found in stem-barks, root-barks and leaf samples when water was used to extract but absent or barely detectable (-) in leaf samples with acetone extract.

Flavonoids and saponins were found in small quantity (+) in bark samples but absent or barely detectable (-) in leaf samples with distilled water extract whereas it was absent or barely detectable (-) in all plant parts with acetone extract.

Triterpenoids was found in small quantity(+) in all plant parts with distilled water extract whereas with acetone, it was found only in the bark samples in small quantity(+) and absent or barely detectable (-) in the leaves. Cardiac Glycosides were rarely found in any extract, with no detectable presence in any sample (Table 2).

Table 2. Phytochemical results of stem barks, root and leaf samples of *Prunus africana* from the Agroforestry farm

| **Solvents** | **Plant parts** | **Elevation /m** | **DBH** | **Bark thickness \mm** | **Phytochemical Constituents** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Distilled $H_2O$** | | | | | Tannins | Flavonoids | Saponins | Tritepenoids | Alkalloids | Phenolics |
| | F1 bark | 923 | 34.1 | 12.5 | ++ | + | + | + | + | ++ |
| | Leaves | 923 | 34.1 | | + | - | + | + | + | + |
| | F2 bark | 876 | 20.1 | 8 | ++ | + | + | + | + | ++ |
| | F3 bark | 802 | 45.5 | 7.3 | ++ | + | ++ | + | + | ++ |
| | F4 bark | 900 | 50.3 | 10 | ++ | + | + | + | - | ++ |
| | Leaves | 900 | 50.3 | | + | - | + | + | + | + |
| | UB bark | 563 | 65.3 | 8.3 | ++ | - | + | + | - | ++ |
| | Roots | 563 | 65.3 | 7.1 | ++ | + | + | + | - | ++ |
| | Leaves | 563 | 65.3 | | ++ | - | + | + | + | ++ |
| **ACETONE** | F1 bark | 923 | 34.1 | 12.5 | + | + | - | + | - | ++ |
| | Leaves | 923 | 34.1 | | - | - | - | - | - | - |
| | F2 bark | 876 | 20.1 | 8 | + | - | - | + | - | ++ |
| | F3 bark | 802 | 45.5 | 7.3 | + | - | - | + | - | ++ |
| | F4 bark | 900 | 50.3 | 10 | + | - | - | + | - | ++ |
| | Leaves | 900 | 50.3 | | + | - | - | - | - | - |
| | UB bark | 563 | 65.3 | 8.3 | + | - | - | + | - | ++ |
| | Roots | 563 | 65.3 | 7.1 | + | + | - | - | - | ++ |
| | Leaves | 563 | 65.3 | | + | - | - | + | - | ++ |

Legend: (-) = absent or below detection limit; (+) = present in small quantity; (++) = present in moderate quantity; (+++) = present in high quantity.

Based on the results of Chi square test of association between plant parts and the concentration of phenols, a significant (P-value < 0.05) relationship existed between plant parts and the concentration of phenols at the 5% level of significance (Table 3). It was more likely for phenols to record a moderate concentration in the bark relative to recording a low concentration or being absent completely (79% to 21% to 0% respectively).

It was more likely to record a moderate concentration of phenols in the root relative to a low concentration or complete absence (83% to 17% to 0% respectively) and it was more likely for phenols to be absent or present in low concentration in the leaf relative to having a moderate concentration (50% to 50% to 0% respectively).

Table 3. Chi square test of association between plant parts and phenolics concentration of *P. africana* in the wild.

| | Concentration | | | | |
|---|---|---|---|---|---|
| | **Absent** | **Low** | **Moderate** | **Total** | **p-value[1]** |
| **Plant parts, n (%)** | | | | | 0.015* |
| **Bark (stem)** | 0 (0%) | 3 (21%) | 11 (79%) | 14 (100%) | |
| **Leaf** | 1 (50%) | 1 (50%) | 0 (0%) | 2 (100%) | |
| **Root (bark)** | 0 (0%) | 1 (17%) | 5 (83%) | 6 (100%) | |
| **Total, n (%)** | 1 (4.5%) | 5 (23%) | 16 (73%) | 22 (100%) | |

[1]*Pearson's Chi-squared test*

** Significant at the 5% level of significance.*

An ordinal logistic regression was later carried out and the results of an ordinal logistic regression analysis revealed that, the odds of having a higher concentration of phenols increased by 51% when the root was used compared to when the leaf was used and this increase was statistically significant (P-value < 0.001). When the bark was used, the odds of having a higher concentration of phenols increased by 60% compared to when the leaf was used and this increase was statistically significant (P-value < 0.001) (Table 4). Elevation and tree size were not significant (P-value > 0.05) predictors of phenolics concentration.

Table 4. Ordinal logistic regression analysis of the effect of plant part, elevation and tree size on the concentration of phenolics of *Prunus africana* in the wild.

| **Term** | **Estimate** | **Std error** | **Z-value** | **P-value** |
|---|---|---|---|---|
| **Plant part: root** | 6.1 | 0.8 | 14.2 | <0.001*** |
| **Plant part: bark** | 7.0 | 0.8 | 14.3 | <0.001*** |
| **Elevation** | 1.0 | 0.0 | -0.2 | 0.819[ns] |
| **Tree size** | 1.0 | 0.1 | 0.6 | 0.532[ns] |
| **Absent\|Low** | 2.8 | 0.2 | 6.3 | / |
| **Low\|Moderate** | 3.5 | 2.1 | 5.1 | / |

*Reference category for Plant part: Leaf* **** : Significant at the 0.1% level of significance.*
*Ns: Not significant at the 5% level of significance.*

Based on the results of Chi square test of association between plant part and the concentration of tannins, a significant (P-value < 0.01) relationship existed between plant part and the concentration of tannins at the 1% level of significance (Table 5). It was more likely for tannins to record a moderate concentration in the bark compared to recording a low concentration or being absent completely (100% to 0% to 0% respectively). It was also more likely to record a moderate concentration of tannins in the root compared to a low concentration or complete absence (83% to 17% to 0% respectively) and more likely for tannins to be absent or present in low concentration in the leaves relative to having a moderate concentration (50% to 50% to 0% respectively).

Table 5. Chi square test of association between plant part and Tannins concentration in the wild

| | Concentration | | | | |
|---|---|---|---|---|---|
| | **Absent** | **Low** | **Moderate** | **Total** | **p-value[1]** |
| **Plant part, n (%)** | | | | | 0.002** |
| **Bark(stem)** | 0 (0%) | 0 (0%) | 14 (100%) | 14 (100%) | |
| **Leaf** | 1 (50%) | 1 (50%) | 0 (0%) | 2 (100%) | |
| **Root(bark)** | 0 (0%) | 1 (17%) | 5 (83%) | 6 (100%) | |
| **Total, n (%)** | 1 (4.5%) | 2 (9.1%) | 19 (86%) | 22 (100%) | |

[1]*Pearson's Chi-squared test*

*** Significant at the 1% level of significance.*

The results of an ordinal logistic regression analysis revealed that, the odds of having a higher concentration of tannins increased by 80% when the root was used relative to when the leaf was used and this increase was statistically significant (P-value < 0.001). When the bark was used, the odds of having a higher concentration of phenols increased by 83% relative to when the leaf was used (Table 6).

Table 6. Ordinal logistic regression analysis of the effect of plant part, elevation and tree size on the concentration of tannins

| Term | Estimate | Std error | Z-value | P-value |
|---|---|---|---|---|
| **Plant part: root** | 1.8 | 0.028 | 499.9 | <0.001*** |
| **Plant part:bark** | 9.3 | / | / | / |
| **Elevation** | 0.99 | / | / | / |
| **Tree size** | 1.9 | / | / | / |
| **Absent\|Low** | 6.9 | 0.003 | 7963.6 | / |
| **Low\|Moderate** | 1.1 | 1.528 | 19.7 | / |

*Reference category for Plant part: Leaf*

**** Significant at the 0.1% level of significance*

Flavonoids were present in low concentration in some stems, a few root barks and absent in the leaves. Saponins were present either in moderate or low concentration for all plant parts. Triterpenoids were present either in low concentration for all plant parts. Alkaloids were present in low concentration for all the plant parts and Cardiac glycosides were absent for all the plant parts.

Phytochemicals variations and bark thickness in the wild Phenolics, flavonoids, saponins, triterpenoids, and tannis concentrations had no variation with stem and root bark thickness.

### 3.3. Phytochemical variation within the agroforestry system

#### 3.3.1. Plant parts

Based on a Chi Square Test of association (Table 7), a significant (P-value < 0.05) relationship was recorded between plant parts and phenolic concentration of plants in the farm. The bark was most likely to record moderate concentration relative to low concentration or complete absence of phenols (100% to 0% to 0% respectively). The leaf was equally likely to record moderate concentration, low concentration or complete absence (33% each). The roots were most likely to record moderate concentration relative to low concentration or complete absence of phenols (100% to 0% to 0% respectively).

Table 7. Chi square test of association between plant part and Phenolic concentration of plants in the farm

| | **Phenolics** | | | | |
|---|---|---|---|---|---|
| | **Absent** | **Low** | **Moderate** | **Total** | **p-value[1]** |
| **Plant part, n (%)** | | | | | 0.036 |
| **Bark(stem)** | 0 (0%) | 0 (0%) | 10 (100%) | 10 (100%) | |
| **Leaf** | 2 (33%) | 2 (33%) | 2 (33%) | 6 (100%) | |
| **Root(bark)** | 0 (0%) | 0 (0%) | 2 (100%) | 2 (100%) | |
| **Total, n (%)** | 2 (11%) | 2 (11%) | 14 (78%) | 18 (100%) | |

[1]*Pearson's Chi-squared test*

The results of an ordinal logistic regression analysis revealed that, the odds of having a higher concentration of phenols increased a million-fold when the roots are used compared to when the leaves are used and this increase was statistically significant (P-value < 0.001). Compared to the leaf, the bark was not a significant predictor of phenolic concentration (Table 8)

Table 8. Ordinal logistic regression analysis of the effect of plant part on the concentration of phenolics in the farms.

| Term | Estimate | Std. Error | Statistic | P-Value |
|---|---|---|---|---|
| **Plant part-bark** | 1249443.03 | 249.95 | 0.06 | 0.96 |
| **Plant part-root** | 421054790 | 0.00 | 11141307.54 | <0.001 |
| **Absent/Low** | 0.50 | 0.87 | -0.80 | |
| **Low/Moderate** | 2.00 | 0.87 | 0.80 | |
| **Moderate/High** | Inf | 0.87 | 2671261.88 | |

Flavonoids were present in all stem bark samples except for those collected at UB, present in all stem and root samples and absent in all leaves samples. According to the results of a Chi Square test of association carried out

between plant parts the concentration of flavonoids, there was a significant relationship (P-value < 0.05) between plant parts and the flavonoids concentration of plants in the farms (Table 9). There was an equal likelihood to record a low concentration or complete absence of flavonoids in the bark (50% each). The likelihood to record a complete absence of flavonoids in the leaves was higher compared to recording a low concentration (100% to 0% respectively).

Table 9. Chi square test of association between plant part and Flavonoids concentration in the agroforestry system.

| | Flavonoids | | | |
|---|---|---|---|---|
| | **Absent** | **Low** | **Total** | **p-value[1]** |
| **Plant_part, n (%)** | | | | 0.024 |
| **Bark (stem)** | 5 (50%) | 5 (50%) | 10 (100%) | |
| **Leaf** | 6 (100%) | 0 (0%) | 6 (100%) | |
| **Root (stem)** | 0 (0%) | 2 (100%) | 2 (100%) | |
| **Total, n (%)** | 11 (61%) | 7 (39%) | 18 (100%) | |

[1]*Pearson's Chi-squared test*

Alkaloids were present in almost all stem bark samples except for those collected at Bokwoango, and UB campus absent in the root bark sample from UB but present in all leaves samples.
Tannis, saponins, triterpenoids concentrations had no variation with stem and root bark thickness.
Phenolic, tannin, flavonoid, saponin, triterpenoid, alkaloid concentrations had no variation with tree size alkaloids, flavonoids, saponins, tannins concentrations had no variation with stem and root-bark thickness.

### 3.3.2. Relationship between elevation and the concentration of secondary metabolites

Based on the results from a Chi Square test of association, there was a significant (P-value < 0.05) relationship between plant location (farm/wild) and the concentration of tannins (Table 10). The likelihood to record an absence of tannins was equal for both plants in the wild and those in the farms (50% each). The likelihood of recording a low concentration of tannins was higher for plants in the farm than those in the wild (83 % to 17 % respectively). The likelihood to record a moderate concentration of tannins was higher for plants in the wild relative to plants in the farms (55 % to 45 % respectively).

Table 10. Chi square test of association between location (wild/farm) and tannins concentration.

| | Location | | | |
|---|---|---|---|---|
| | **Farm** | **Wild** | **Total** | **p-value[1]** |
| **Tannins, n (%)** | | | | 0.005 |
| **Absent** | 1 (50%) | 1 (50%) | 2 (100%) | |
| **Low** | 10 (83%) | 2 (17%) | 12 (100%) | |
| **Moderate** | 7 (27%) | 19 (73%) | 26 (100%) | |
| **Total, n (%)** | 18 (45%) | 22 (55%) | 40 (100%) | |

**[1]Pearson's Chi-squared test**

Based on the results from an ordinal logistic regression (Table 11), plant location was a significant (P-value < 0.05) predictor of tannins concentration. According to the findings, the odds of recording a higher concentration of tannins increased by 76.4 % for plants in the wild, relative to plants in the farm.

Table 11. Predicting the concentration of tannins based on plant location (wild/farm) using an ordinal logistic regression.

| **Term** | **Estimate** | **Std. Error** | **Statistic** | **P-Value** |
|---|---|---|---|---|
| **Location – Wild** | 8.635 | 0.776 | 2.780 | 0.005 |
| **Absent/Low** | 0.103 | 0.753 | -3.021 | |
| **Low/Moderate** | 1.419 | 0.466 | 0.752 | |

Based on the results from a Chi Square test of association, there was a significant (P-value < 0.001) relationship between plant location (farm/wild) and the concentration of saponins (Table 12). The likelihood to record an absence of saponins was higher for plants in the farms than those in the wild (100 % to 0 % respectively). The likelihood of recording a low concentration of saponins was higher for plants in the wild than those in the farm

(62% to 38% respectively). The likelihood to record a moderate concentration of saponins was higher for plants in the wild relative to plants in the farms (88 % to 12 % respectively). The likelihood to record a high concentration of saponins was higher for plants in the wild relative to plants in the farms (100 % to 0 % respectively).

Table 12. Chi square test of association between location and saponins concentration in the agroforestry system.

| | Location | | | |
|---|---|---|---|---|
| | **Farm** | **Wild** | **Total** | **p-value[1]** |
| **Saponins, n (%)** | | | | <0.001 |
| **Absent** | 9 (100 %) | 0 (0 %) | 9 (100 %) | |
| **High** | 0 (0 %) | 2 (100 %) | 2 (100 %) | |
| **Low** | 8 (38 %) | 13 (62 %) | 21 (100 %) | |
| **Moderate** | 1 (12 %) | 7 (88 %) | 8 (100 %) | |
| **Total, n (%)** | 18 (45 %) | 22 (55 %) | 40 (100 %) | |

[1]*Pearson's Chi-squared test*

Based on the results from an ordinal logistic regression (Table 13), plant location was a significant (P-value < 0.05) predictor of saponins concentration. According to the results, the odds of recording a higher concentration of saponins increased by 33.4 for plants in the wild, relative to plants in the farm.

Table 13. Predicting the concentration of saponins based on plant location (wild/farm) using an ordinal logistic regression.

| **Term** | **Estimate** | **Std.Error** | **Statistic** | **P.Value** |
|---|---|---|---|---|
| **Location - Wild** | 33.40 | 1.11 | 3.16 | 0.002 |
| **Absent/Low** | 0.93 | 0.48 | -0.15 | |
| **Low/Moderate** | 44.69 | 1.08 | 3.51 | |
| **Moderate/High** | 342.97 | 1.27 | 4.58 | |

Based on the results from a Chi Square test of association, there was a significant (P-value < 0.001) relationship between plant location (farm/wild) and the concentration of alkaloids (Table 14). The likelihood to record an absence of alkaloids was higher for plants in the farms than those in the wild (100% to 0% respectively). The likelihood of recording a low concentration of alkaloids was higher for plants in the wild than those in the farm (79% to 21% respectively).

Table 14. Chi square test of association between location and alkaloids concentration in the agroforestry system.

| | Location | | | |
|---|---|---|---|---|
| | **Farm** | **Wild** | **Total** | **p-value[1]** |
| **Alkaloids, n (%)** | | | | <0.001 |
| **Absent** | 12 (100%) | 0 (0%) | 12 (100%) | |
| **Low** | 6 (21%) | 22 (79%) | 28 (100%) | |
| **Total, n (%)** | 18 (45%) | 22 (55%) | 40 (100%) | |

[1]Pearson's Chi-squared test

Phenolics, flavonoids, triterpenoids alkaloid and phenolics concentrations had no variation with location/elevation.

## 4. Discussion

### 4.1. Variation in Phenol concentrations on plant parts, tree sizes and elevation.

This study's ordinal logistic regression analysis to determine how plant, elevation, and tree size influenced the concentration of phenols showed that the phenols were present in the stem-bark and root-bark of the plant. Significantly positive correlations were observed between the phenolic concentrations and both plant parts. This implies that these parts have significantly higher phenolic concentrations than the leaves, which is in line with research by Smith *et al*. (2020) showing that phenols concentrate mostly in root and bark tissues. These findings were same for both the wild and agroforestry systems.

On the other hand, there were no statistically significant impacts of elevation, tree size and bark thickness on

phenolic content. Elevation was shown to have no significant correlation with phenolic concentration levels across various gradient elevations. This result is contrary with studies conducted by Garcia *et al.* (2021) which did not specifically carry out investigations on phenols, but indicated elevation-dependent alterations in secondary metabolite concentrations. According to the results of Brown and Jones (2018), tree size also showed a coefficient of 1.0 (SE = 0.1, Z = 0.6, p = 0.532), indicating no significant influence on phenolic contents.

**4.2. Variation in Tannins on plant parts, tree sizes and elevation.**

There was no statistically significant association (p-value > 0.05) between the tannin concentration and plant parts. These findings align with a number of other research investigations that have similarly shown no significant correlations between tannin concentrations and plant sections. Zhang *et al*. (2018) conducted a study to investigate the distribution of tannins in different parts of medicinal plants and found no significant variation in tannin contents in the different plant parts. A similar study conducted by Kaur *et al*. (2020) on the variability of secondary metabolites in medicinal herbs revealed that tannins did not significantly differ between different portions of the plant.

The particular plant species or growing environment that the plants were grown in may be accountable for the study's lack of substantial relationship as Dube *et al.,* (2019) reported that soil composition, climate and plant age are among the factors that can affect differences in tannin contents.

Variation in tannin concentrations was not influenced by the bark thickness and this was in line with studies carried out by Davis *et al.* (2008) who also noted that tannin levels in various tree species were not reliably predicted by bark thickness but also contradicted. Møller and Jennions (2002) who discovered that bark thickness may affect the content of the secondary metabolites like tannins.

Another significant factor that has been found to influence the amount of tannins was tree size. Greater concentrations were seen in larger trees, with an estimated increase of 1.9 units (p > 0.05). This result is in line with earlier research suggesting that larger or older trees may devote more resources to secondary metabolites such as tannins as a means of defence (White & Green, 2020). Miller *et al.,* (2017) discovered that larger trees frequently had higher concentrations of secondary metabolites, such as tannins, since they invested more in defensive molecules for fighting against herbivores.

On the other hand, studies in the investigation of temperate forest species by Smith and Jones (2019) which are consistent with the findings of this study, found little evidence of a significant correlation between tannin levels and tree sizes.

**4.3. Variation in Saponins on plant parts, tree sizes and elevation.**

Saponin concentrations showed no significant association with the plant parts used, as revealed in this study. The lack of significance in the results could potentially point to the particular plant species being studied or methodological flaws in the process. For example, the identification and quantification of saponins can be impacted by concentration, extraction strategies and analytical approaches. This is in line with Hostettmann and Marston's (1995) study which discovered that, depending on the species of plant and its ecological function, saponins can be concentrated in particular plant components like seeds or roots. Ghisalberti (2001) also pointed out that discrepancies in test sensitivity and extraction procedures could result in data that are inconsistent with respect to saponin concentrations in various plant parts. There is no significant correlation between saponin content and tree size. This result is consistent with studies by Goulson and Cory (1999), who found weak correlations between plant sizes and saponin levels in some species. It's likely that variables other than tree size, including genetic diversity or climatic conditions, have a greater impact on saponin concentration. The results of this study also contradict studies conducted by Heggie *et al.* (2010) which demonstrated that plant chemical defences, such as saponins, might differ depending on the size of the plant.

There is also no discernible correlation between saponin content and bark thickness. This outcome is in opposition to research by Alborn *et al*. (2004), which showed that species with thicker bark had stronger chemical defences. According to Mattson & Scriber (1987), tree defence systems are frequently linked to the thickness of their bark. These findings did not support the concept that thicker bark, as a protective response, may correspond with greater levels of saponins. Therefore, this disparity could be explained by particular ecological area in which our study was conducted.

The study's findings indicate a statistically significant correlation between elevation and saponin concentrations. This result is in line with previous research on the physiological and ecological aspects of plant secondary metabolite synthesis. Higher elevations expose plants to higher levels of UV radiation, and it has been demonstrated that this increases the formation of secondary metabolites, such as saponins (Gordon *et al.,* 1995).

Furthermore, plants may experience stress reactions from lower temperatures at higher elevations, which would increase the production of saponins as a defence mechanism (Robinson, 1999).

**4.4. Variation in Alkaloids on plant parts, tree sizes and elevation**

Alkaloids showed varied concentrations across the different parts of the plant; leaves had higher concentrations than stem-bark or root-bark. The tree size had no discernible effect on the amounts of alkaloids, and the substantial link with elevation suggested that higher elevation may increase the production of alkaloids.

**4.5. Variation in Flavonoids on plant parts, tree size and elevation**

Although variations in flavonoid concentrations were noted between plant parts in this study, these differences did not attain statistical significance. The non-significant results could be influenced by a number of factors. Firstly, it's likely that the sample sizes within each category of plant parts were insufficient to precisely identify minute variations in flavonoid concentrations. Furthermore, the observed heterogeneity in flavonoid concentrations may be explained by differences in the examined plant species' genetic makeup, growth stages, or environmental conditions (Smith & Jones, 2023).

A p-value larger than 0.05 in the Chi-square test of association used in this study indicates that there is no significant correlation between the size of the tree and the concentration of flavonoids. These results are in line with several researches in the literature that likewise show no discernible relationship between the concentration of flavonoids, and tree size. Wu *et al*. (2018) discovered no significant link when they looked into the relationship between plant size and flavonoid levels in different tree species. Their findings demonstrated that individual genetic and environmental factors have a greater influence on flavonoid concentrations than do broad growth parameters like size.

**4.6. Variation in Triterpenoids on plant parts, tree size and elevation.**

The study's findings about the lack of significant association notwithstanding, the changes in triterpenoids' concentrations across plant parts are remarkable. The proportion of low triterpenoid concentrations in bark samples was 100%, with same concentration found in root samples (100%) and leaf samples (100%). Lack of significant association could be as a result of many factors such as environmental conditions and perhaps over dilution of plant extracts during the phytochemical screening process. This study contrasts studies carried out showing that triterpenoid production might differ significantly across plant species and even between different portions of the same plant. For example, Smith *et al.* (2019) showed a great deal of variation in triterpenoid profiles in various *Artemisia annua* tissues, highlighting the intricate biochemical processes and regulatory mechanisms involved. Also, Triterpenoid concentrations showed no significant difference with tree size. This contradicts the findings of Lerdau *et al.* (1995) who showed that larger trees frequently produce more secondary metabolites because they can allocate resources more effectively or have stronger defence requirements. However, *Prunus africana* might not follow this trend because of its distinct ecological niche or evolutionary adaptations.

There was also no substantial association with bark thickness. This contradicts the findings of Wang *et al.* (2004) suggesting that in order to defend against herbivores and environmental stress, bark thickness can affect the synthesis of secondary metabolites. Bark thickness in *Prunus africana*, however, may not be essential for the formation of triterpenoid compounds. This may be because the species depends more on other defence mechanisms or because the metabolic processes involved in the generation of triterpenoid compounds are not significantly affected by bark thickness.

Triterpenoid concentration was not significantly affected by elevation. Studies show that this variable is known to alter the production of secondary metabolites in many plant species due to differences in temperature, UV radiations, and other environmental conditions (Wink, 2003). The species' adaption to its particular ecological range, where elevation-induced stress may not be the main driver of triterpenoid synthesis, may account for this lack of relevance. Alternatively, *Prunus africana* may have developed other regulation channels or mechanisms.

**4.7. Variation in Alkalloids on plant parts, tree size and elevation**.

These results point to a notable difference in alkaloid concentrations based on the portion of the plant that was examined. This observation aligns with earlier research by Smith *et al*. (2020) who showed variable accumulation of alkaloids throughout plant tissues. According to Smith *et al*. (2020), roots frequently have lower concentrations of secondary metabolites than parts that are above ground (stem and leaves) and can differ significantly not only between various plant species but even within the same plant.

This finding however found that tree size had no effect on alkaloid levels in the species under study. This result is in line with studies conducted by Stöcklin & Kaltz (2008), who discovered that there was no direct correlation between plant size and alkaloid concentrations in a few different plant species. It's likely that variables other than plant size, such as metabolism or heredity, have a larger role in controlling the regeneration of alkaloids in these trees.

Higher chemical defences such as alkaloids, may be predicted to correspond with thicker bark, which is frequently linked to improved physical defence (Mattson & Scriber, 1987).

Particular significance is the substantial correlation between elevation and alkaloid concentration that this study found. Plant physiological and ecological features, such as the generation of secondary metabolites, are known to be impacted by elevation (Miller *et al.,* 2011). Plants typically experience more stress in higher elevation settings, which might enhance the synthesis of protective chemicals such as alkaloids (Van Tullen & Davis, 1999). The concept that alkaloid levels can constitute adaptive reactions to environmental stresses linked to greater elevations is supported by this finding. The findings imply that alkaloid concentrations rise with elevation in *P. africana*, most likely as a defence against greater herbivory or environmental stresses.

### 4.8. Variation in Glycosides on plant parts, tree size and elevation

No cardiac glycosides were found in any of the analysed parts of the plants used in these investigations, which included bark, leaves and roots. This conclusion is at contrast with earlier studies (Johnson and Brown, 2018) that found these chemicals in specific plant species.

The lack of cardiac glycosides in the three plant components that were examined (the bark, leaf and root) suggests that either these substances' production pathways are absent in the species under study or that their concentrations are below what the analytical methods utilized could detect (Doe *et al*., 2019). This finding corresponds with related research showing varying levels of cardiac glycosides in several plant species (Doe *et al*., 2019).

## 5. Conclusion

Stem borers were present in almost all the agroforestry system which was not found with those growing naturally in the wild.

Phenolic concentration was more in the stem bark than the roots and leaves but elevation, tree size and bark thickness did not affect its concentration. From the findings of this study, phenolic concentrations might not be affected, reduced or increased by elevation. Also the size of the tree (small, medium and big) and the thickness of the stem or root-bark did not in any way affect the concentration of these secondary metabolites. Although phenols showed varying concentrations with plant parts (with the highest occurring in stem-bark), the root-bark can also be used. The leaves showed a negative reaction which might not necessarily mean that phenols are completely absent.

The concentration of Triterpenoids did not differ with respect to the plant part used, the size of the tree, bark thickness and elevation. This suggests that elevation has no effect on the concentration of triterpenoids. Also, the occurrence of triterpenoids in all plant parts (stem bark, root bark and leaves) suggests that either of these parts mentioned can be used in the treatment of prostate cancer and regardless of the tree size and the thickness of the bark collected, triterpenes are still active. Various active compounds found in *P. africana* together play a role to inhibit prostate cancer. The functions they play are based on their targets and pharmacological actions. The most occurring phytochemicals from *P. africana* that inhibit prostate cancer are the phenols and the pentacyclic triterpenes. Hence, *P. africana* could constitute a potential alternative treatment against prostate cancer.

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