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# EVALUATION OF SECONDARY METABOLITE PRODUCTION IN NATURAL AND WILD POPULATIONS OF *PRUNUS AFRICANA* (HOOK.f.) KALMAN ON MOUNT CAMEROON

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**Abstract**
Mount Cameroon, 4,095 m, is a biodiversity hotspot with over 2,300 plant species, including 49 strict endemics, and critical habitats for endangered species. Among its important plants, is *Prunus africana*, vital medicinal tree primarily harvested for its bark, used to produce pharmaceuticals for treating benign prostatic hyperplasia (BPH) and prostate cancer. High bark demand in international markets has led over exploitation and its listing as a vulnerable, endangered species, necessitating sustainable management and cultivation. *Prunus africana* is extensively cultivated in the Western, North-West, and South-West regions of Cameroon as a critical agroforestry cash crop for its medicinal bark. The narrow natural altitudinal range of *Prunus africana* in the wild raises questions on whether the phytochemicals produced by this species in agroforestry systems matches those produced by wild populations. The current study was designed to test this hypothesis by collecting root, stem barks and leaf samples for phytochemical analysis from the wild (1672-2094 m altitude) and agroforestry (563-923 m) farms in Mt Cameroon for test for flavonoids, saponins, phenols, tannins, triterpenoids, alkaloids and cardiac glycosides using standard procedures. Results indicate that dbh of sampled trees ranged from 18.9 to 105 cm and 20.1 to 65.3 cm in the wild and agroforestry systems respectively. *Prunus* bark thickness increased with dbh, but not significantly, both in the wild and in cultivation. Acetone was a better extractant than water. Root and stem bark samples systematically showed higher concentration of phytochemicals than leaf samples. Phenolics, tannins and triterpenoids showed little variations in the wild and cultivated samples. Flavonoids, saponins and alkaloids showed lower concentrations in agroforestry systems than from the wild. A borer pest observed in farms was not seen in the wild. We suggest further testing of samples from farms to suit purpose for which they are needed.
**Key words:** Mount Cameroon, *Prunus africana* wild populations, *Prunus* agoforestry, secondary metabolites concentration.
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## INTRODUCTION

Mount Cameroon (4100 m above sea level), is a biodiversity hotspot of international reputation that has developed on one of the most active volcanoes in Africa, a record seven eruptions in the last century (Tajeukem *et al.,* 2025; Fitton *et al.,* 1983; Suh *et al*., 2003; Manga *et al*., 2008). It is a massive basalt with very fertile volcanic soils of varying ages along its slopes, supporting huge plantations of oil palms, rubber, tea, banana and cocoa at low altitude, aided by a conducive climate of two seasons (Gil Yaron, 2001).

The vegetation of Mount Cameroon showcases a dramatic altitudinal zonation, transitioning from dense lowland rainforest at sea level (0 -800 m) with evergreen, species-rich forests with tall canopies, buttressed trees, and abundant lianas, part of the Cross-Sanaga-Bioko coastal forests ecoregion. This is followed by the Submontane Forest and Montane Forest (800-2400 m), also known as "Cloud Forest," characterized by high humidity, dense mist, and profuse epiphytes like mosses, ferns, orchids and denser, shorter canopy trees with species like *Schefflera, Nuxia* and *Prunus*. The Montane Grassland (2000-3000m) is dominated by Festuca pilgeri grasses and dwarf shrubs. Sub-alpine/Alpine Grassland (3000–4100m) occur at higher elevation with Helichrysum scrub, extending to the summit (Hall, 1973; Proctor *et al*., 2007). This rich and diverse vegetation contains high level of endemism and a great deal of species new to science (Cheek *et al*., 1996). The faunal diversity is also great, ranging from the forest elephants, chimpanzees, gorillas, mandrils and monkeys through reptiles like snakes, chameleons, lizards to birds like the and birds like; the Mt Cameroon Spurfowl (*Pternistis camerunensis*),

Speirops (*Speirops melanocephalus*), Turaco (*Tauraco bannermani*), Green Longtail (*Urolais epichlorus*) to mention but a few, (Dowsett-Lemaire and Dowsett, 2001; Onwouo, 2007).

Among the important plants of Mt Cameroon is *Prunus africana* (Hook.f.) Kalman**,** commonly called Pygeum or African cherry, originally described by Sir Joseph Dalton Hooker and later revised by Cornelis Kalman. It is a species of the Rosaceae family, thriving in Afromontane and montane forests at elevations of above 1,000 m, particularly along Eastern Africa (Ethiopia to South Africa) and West Africa and Central Africa, as well as Madagascar and the Comoros, in humid, middle-elevation forests, requiring light gaps for sapling survival (Cunningham and Mbenkum, 1993). It is the only *Prunus* species indigenous to mainland tropical Africa (Cunningham *et al*., 1997).

In Cameroon, *Prunus africana* is concentrated in montane forests, primarily in the North-West and South-West Regions, with significant populations around Mount Cameroon, Mount Oku, and the Adamawa Plateau, inhabiting altitudes typically between 1200-2800m, though also found in other highland areas like the Centre and Littoral, with management efforts focusing on sustainable harvesting and cultivation (Amougou *et al*., 2011).

*P. africana,* is a major component biodiversity of Afromontane forests ecosystems, an important food source for various bird species and rare primates, including monkeys, providing shelter for biodiversity and local climate support (Fashing, 2004). Its presence and health are crucial indicators of the stability of this high-altitude habitat. Locally, its durable wood serves for furniture, construction, tools, and fuelwood (Melle *et al*., 2016; Komakech *et al*., 2017).

*Prunus* is a highly valued medicinal tree, renowned for its stem bark, which is used in both traditional African medicine and modern phytotherapy to treat various ailments (Koros, 2016; Godfrey *et al*., 2020). Its bark extracts possess antimicrobial, anti-inflammatory, and antioxidant properties, used to treat fever, malaria, kidney disease, and stomach pain (Nambooze *et al*., 2022; Rubegeta *et al*., 2023). Its primary medicinal importance lies in the treatment of prostate disorders, particularly benign prostatic hyperplasia (BPH) and reducing associated inflammation, which helps improve urine flow (Yablonsky, 1997; Jena *et al*., 2016; Begeno, 2020; Nambooze, 2022).

International trade in *Prunus* bark generates significant international income (over US$220 million annually in trade) acting as a vital source of livelihood for local communities, particularly in Cameroon, which exports 38-48% of the global volume, ahead of Democratic Republic of Congo, Madagascar, and Tanzania among others (Cunningham and Mbenkum, 1993; Stewart, 2003a; Ingram *et al*., 2008). High demand of *Prunus* from European pharmaceutical companies has driven massive unsustainable harvesting, leading to drastic population decline (Popoola, 2002; Ingram, 2007). It is currently listed as Vulnerable on the IUCN Red List and in CITES Appendix II (requiring trade regulation) because it is under significant threat across its range (Stewart, 2003b; CITES, 2006; Hills & Cheek, 2021).

The threats to *Prunus* wild populations include unsustainable bark harvest, habitat loss and degradation as major threats, while poor regeneration, climate change, wild bush fires, invasive species and competition (Stewart, 2003a; Jimu, 2011). Conservation strategies for *Prunus africana* focus on sustainable harvesting, community-led management, and ex-situ cultivation to counteract overexploitation for medicinal bark (Tchouto, 1996; Dibi and Kay, 1997; Beti, 2013). Key measures include enforcing CITES Appendix II regulations, promoting 5-year bark rotation, training harvesters, tagging trees, and establishing nurseries (Wyk and Prinsloo, 2018). Enrichment planting has been carried out in the Kilum-Ijim mountain forest (Mount Oku) and within the Etinde community forest of Mt Cameroon (Tajeukem *et al.,* 2025; Ndam, 1996; Stewart, 2009; Abensperg-Traun, 2009).

In Cameroon, ex-situ conservation is common place in the some of the *Prunus* hot spots. *Prunus* agroforestry in Cameroon, is primarily concentrated in the North West, South West, and Adamaoua regions, often located on the slopes of mountainous areas (1500m-2500m) (Loden, 2009). It is a critical, profitable, and sustainable alternative to over-exploiting wild populations for medicinal bark. This system integrates the trees into farms, to increase incomes, improve livelihoods, and reduce pressure on natural resources (Tchoundjeu *et al*., 2002; Ingram *et al*., 2009; Cunningham *et al*., 2016; Melle *et al*., 2016). Major centers include Mount Oku/Kilum-Ijim forest (NW), Mount Cameroon (SW), and the Adamawa Plateau (Tchabal Gang Daba and Tchabal Mbabo), driven by high medicinal demand for the bark.

The economic value of *Prunus* lies in the bark trade and to a lesser extent its high-density wood. Environmental conditions, particularly altitude play key role in the distribution of natural populations of the species throughout

its natural range (Wyk and Prinsloo, 2018; Ingram, 2014a; Cunningham *et al*.,2016; Ndedy *et al*., 2022; Rubegeta *et al*., 2023). Therefore, it may be critical to locate agroforestry systems of prunus within the altitudinal range of its natural populations (Kadu, 2012; Ingram, 2014b).

*Prunus Africana* (African cherry) cultivation in the North West Region of Cameroon is a critical agroforestry and conservation initiative aimed at reducing pressure on wild populations, particularly in the Kilum-Ijim mountain forest. The region, specifically areas within the Bui, Boyo, and Donga Mantung divisions, has seen significant domestication efforts, with over 100,000 trees estimated to be planted in some surveys (Amougou *et al.,* 2011; Ingram, 2015).

In the Northwest Region, agroforestry farms are located within the natural altitudinal range of *Prunus* (Amougou *et al.,* 2011; Ingram, 2015). However, on Mt Cameroon, particularly in Buea, *Prunus* farms are located well below its natural altitudinal range, raising questions of whether the cultivated population contain the same phytochemicals at similar concentrations as the wild counterparts.

Current guidelines for *Prunus* exploitation puts minimum harvest diameter is set at 30 cm dbh. It would appear that this was arbitrarily set because existing data does not explain if this is the diameter at which tree matures, or has the acceptable minimum concentration of the desired phytochemicals for which a price tag is given to the bark. Previous studies have linked bark thickness to the tree size but failed to show a relationship between thickness and concentration of secondary metabolites (Betti and Ambara, 2011; Betti and Ambara 2013, Ndedy, 2022).

The question is also asked about the focus on stem bark only, given the implications of debarking on the health and survival of the species (Clemente *et al*., 2006; Delvaux *et al*., 2009; Stewart 2009; Betti *et al*., 2019). *Prunus africana* exhibits complete bark regrowth, particularly when the vascular cambium remains intact and the debarking does not encircle the entire tree. Studies suggest a, 7-year, or sometimes longer, rotation period is necessary to allow for effective bark regeneration on the same stem for limited harvest (Momo *et al.,* 2016; Betti *et al*., 2019). This means that yield decline between first bark harvest and the regenerated harvest even with the best techniques that protect Cambium and respect of CITES guidelines (Betti *et al*., 2019; Ndedy 2022). However, in cultivation, *Prunus africana* is a relatively fast-growing species that can reach a 30 cm diameter at breast height (dbh), the minimum exploitable size for bark harvesting, in approximately 13 to 20 year**s (**Ingram, 2014a, Ingram, 2015).

This current study was therefore designed to qualitatively assess the presence of important secondary metabolites (Phenolics, Tannins, flavonoids, Saponins, Triterpenoids, Alkaloids) in the stem bark, root bark and leaves of wild and cultivated prunus on the slopes of Mt. Cameroon.

## Materials and Methods

### Site 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 (Tajeukem *et al.,* 2025; 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 (Tchameni *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 (Fraser *et al*., 1998; 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, 2020). 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 $^{o}$C and this decreases by 0.6 $^{o}$C per 100 m of ascent to 4 $^{o}$C at the summit with its humidity between 75-85 % due to the marine

influence and cloud formation (Bruijnzeel *et al.,* 2010).

**Study plot**

This work was carried out within two distinct locations on the eastern slope of Mt Cameroon. Samples in the wild were taken from the montane cloud forest within the Etinde Community Forest. This community forest serves as a buffer between the farm lands of surrounding villages and the Mt. Cameroon National Park. The altitudinal range of sampling was 1672 - 2094 m above sea level, coinciding with the zone of abondance of wild prunus populations on Mt Cameroon.

The key characteristics of the vegetation at this altitude is their occurrence on rocky undulating steep slopes of recent lavas. The tree stems are covered by abundant mosses and lichens. The understory was sparsely populated with few herbaceous species (*Oreacanthus manni*, *Mimolopsis soimsii*, *Impatiens sakeriana, Impatiens burtonii*) dominated by *Oreacanthus manni* (a biannual herb) belonging to the Acanthaceae family.

Samples from cultivated *Prunus* were taken at much lower altitudes ranging from 563 on the campus of the University of Buea to 923 in the villages of Bova, Bokuva and Bokwoango within the Buea municipality, an area often affected by low level cloud cover especially in the rainy season. The locations of sampling points both in the forest and in farms are displayed on **Fig (1).**

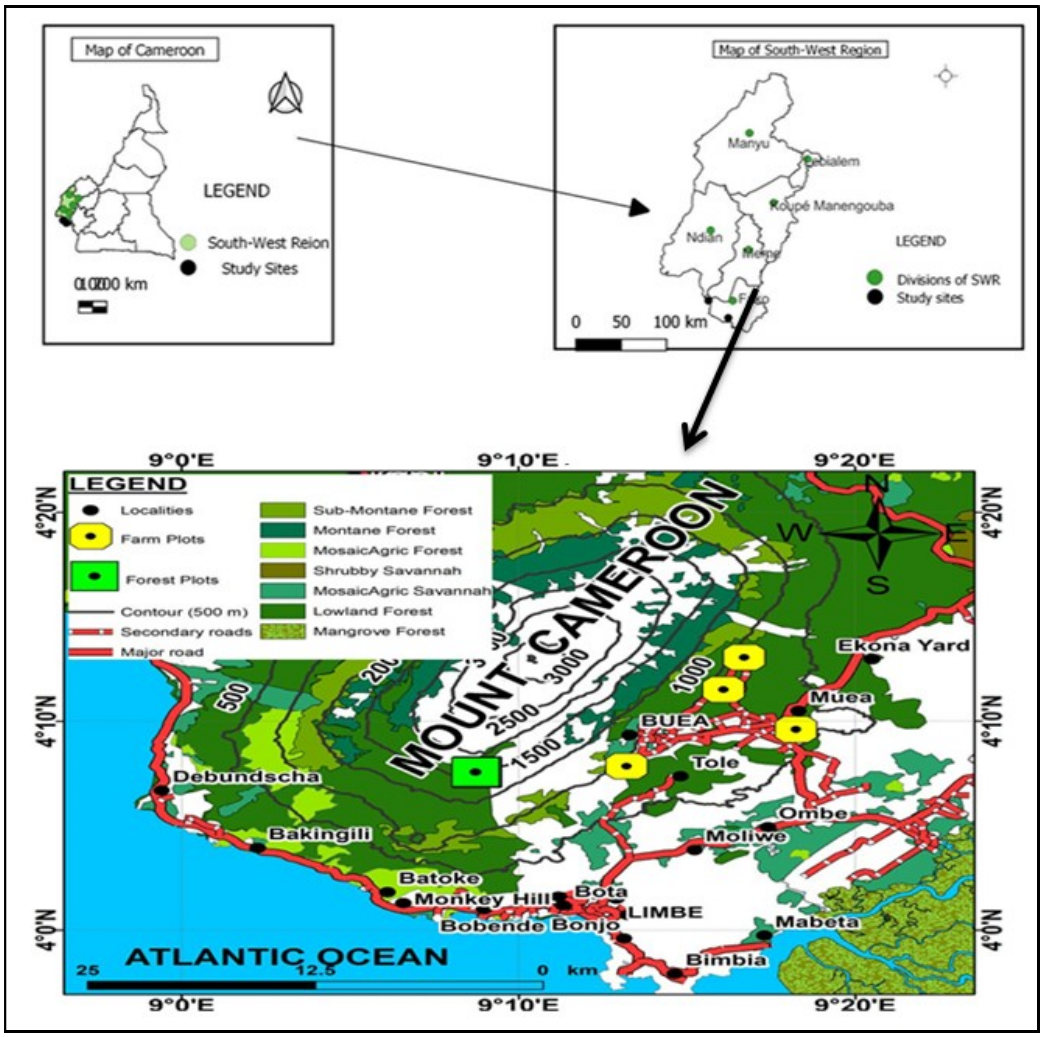


**Figure1**. Study site

**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. Trees were randomly selected at accessible locations in the forest for the collection of samples. The dbh of each selected tree was measured using a diameter tape and its location noted using a GPS Gamin 64Sx. With the aid of a machete, four small strips of stem bark were collected measuring 10 cm by 10 cm from the four opposing sides of the tree. The strips were made small and short to facilitate bark recovery. Where it was possible, leave samples and root bark samples were also taken on the same trees. Each bark sample taken was measured for thickness using a vernier caliper.

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. Once the stem and root-barks were collected, the death bark was piled off. All the samples were packaged in separate zip lock bags and labeled appropriately for onward transportation and storage in the Plant Science Laboratory of the University of Buea.

**Preparation of samples for extraction**

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.

**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; Begeno, 2020) with some few modifications.

**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 and observed for effervescence. A brick red coloration observed indicated the presence of flavonoids.

**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.

**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.

**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.

**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.

**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.

 

**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.

**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 and chats. 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

**Results**
**DBH and bark thickness**
The diameter at breast height of sampled trees ranged from 18.9 to 105 cm in the wild and from 20.1 to 65.3 cm in agroforestry systems. Bark thickness of *Prunus* trees increased with dbh, but not significantly, both in the wild and in cultivation **(Fig 2)**.

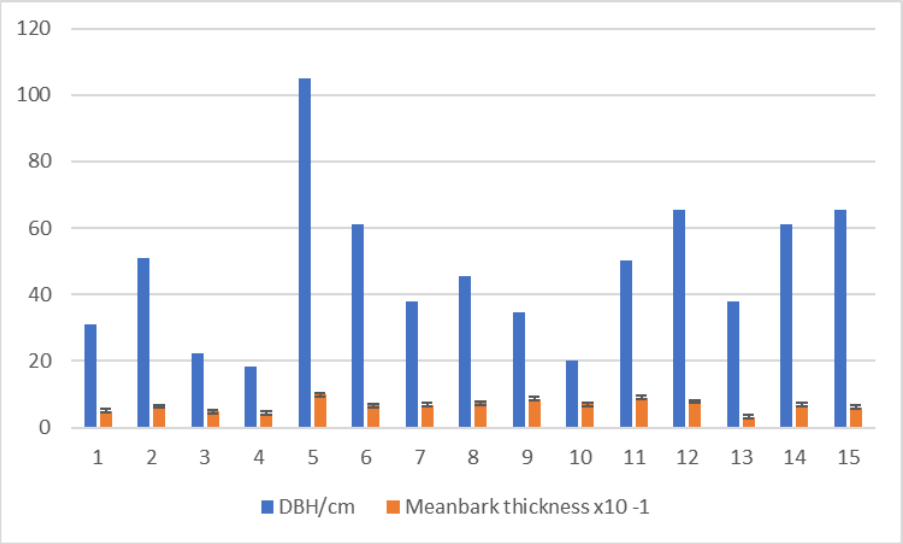


**Fig. 2:** Mean bark thickness compared to dbh of prunus Africa from Mt Cameroon

The mean dbh of trees sampled in the wild was 46.7 cm with a mean stem bark thickness of 6.6 mm while in cultivated samples, mean dbh of sampled trees was 43.2 cm with a mean bark thickness of 7.4 mm. Root bark thickness was generally smaller than stem bark thickness both in the wild and in cultivation.

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).

Acetone was a better solvent for extracting phytochemicals from *Prunus* samples except for saponins that extracted better with water. For example, while acetone extracts showed higher levels for phenolics (++) for all stem and root barks for wild and agroforestry samples, aqueous extracts showed a mix of ++ and + for the same samples. Leaf sample extracts for acetone recorded + for phenolics while aqueous extracts for same leaf samples showed -, an indication of low extraction rates by water. Phenols and tannins occurred at very similar levels in *Prunus* stem bark and root bark in the wild and in cultivation but leaves showed slightly lower concentrations. Akalloides, triterpenoides and saponnins were found to be more concentrated in samples from the wild than in those from cultivated samples. Root and stem barks all had similar concentrations while leaf samples consistently showed lower concentrations.

While phenolics were present in the stem and root barks gotten from the wild (++ or + in a few, it was noticed that Phenolics were present in smaller amounts (+) or barely detectable (-) in leaf samples. Stem-barks and root-barks showed moderate quantity (++) in most 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)**.

The results from the phytochemical screening of the various plant parts gotten from agroforestry 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 were 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 1).**

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

| SOLVENTS | PART | ELEVATION | DBH | PHYTOCHEMICAL CONSTITUENTS | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Phenolics | Tannins | Flavonoids | Saponins | Tritepenoids | Alkalloids |
| WATER | W Bark 1 | 2094 | 31.2 | + | ++ | - | + | + | + |
| | W Bark 2 | 2073 | 50.9 | ++ | ++ | + | ++ | + | + |
| | W Bark 3 | 2042 | 22.4 | + | ++ | - | ++ | + | + |
| | W Bark 4 | 2033 | 18.9 | ++ | ++ | + | +++ | + | + |
| | W Bark 5 | 2019 | 105 | ++ | ++ | + | +++ | + | + |
| | W Bark 6 | 1992 | 61 | ++ | ++ | + | ++ | + | + |
| | W Bark 7 | 1672 | 38 | + | ++ | - | ++ | + | + |
| | W Root 2 | 2073 | 50.9 | + | ++ | - | ++ | + | + |
| | W Root 6 | 1992 | 61 | ++ | ++ | + | ++ | + | + |
| | W Root 7 | 1672 | 38 | ++ | + | - | + | + | + |
| | W Leaf 2 | 2073 | 50.9 | - | - | - | + | + | + |
| ACETONE | W Bark 1 | 2094 | 31.2 | ++ | ++ | + | + | + | + |
| | W Bark 2 | 2073 | 50.9 | ++ | ++ | + | + | + | + |
| | W Bark 3 | 2042 | 22.4 | ++ | ++ | + | + | + | + |
| | W Bark 4 | 2033 | 18.9 | ++ | ++ | + | ++ | + | + |
| | W Bark5 | 2019 | 105 | ++ | ++ | + | + | + | + |
| | W Bark 6 | 1992 | 61 | ++ | ++ | + | + | + | + |
| | W Bark 7 | 1672 | 38 | ++ | ++ | + | + | + | + |
| | W Root 2 | 2073 | 50.9 | ++ | ++ | + | + | + | + |
| | W Root 6 | 1992 | 61 | ++ | ++ | + | + | + | + |
| | W Root 7 | 1672 | 38 | ++ | ++ | + | + | + | + |
| | W Leaf 2 | 2073 | 50.9 | + | + | + | + | + | + |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | W Leaf 2 | 2073 | 50.9 | + | + | + | + | + | + |
| **$H_2O$** | C bark 1 | 923 | 34.1 | ++ | ++ | + | + | + | + |
| | C Leaf 1 | 923 | 34.1 | + | + | - | + | + | + |
| | C bark 2 | 876 | 20.1 | ++ | ++ | + | + | + | + |
| | Cbark 3 | 802 | 45.5 | ++ | ++ | + | ++ | + | + |
| | C bark 4 | 900 | 50.3 | ++ | ++ | + | + | + | - |
| | C Leaf 4 | 900 | 50.3 | + | + | - | + | + | + |
| | C bark 5 | 563 | 65.3 | ++ | ++ | - | + | + | - |
| | C Root 5 | 563 | 65.3 | ++ | ++ | + | + | + | - |
| | C Leaf 5 | 563 | 65.3 | ++ | ++ | - | + | + | + |
| **ACETONE** | C bark 1 | 923 | 34.1 | ++ | + | + | - | + | - |
| | C Leaf 1 | 923 | 34.1 | - | - | - | - | - | - |
| | C bark 2 | 876 | 20.1 | ++ | + | - | - | + | - |
| | C bark 3 | 802 | 45.5 | ++ | + | - | - | + | - |
| | C bark 4 | 900 | 50.3 | ++ | + | - | - | + | - |
| | C Leaf4 | 900 | 50.3 | - | + | - | - | - | - |
| | C bark 5 | 563 | 65.3 | ++ | + | - | - | + | - |
| | C Root 5 | 563 | 65.3 | ++ | + | + | - | - | - |
| | C Leaf 5 | 563 | 65.3 | ++ | + | - | - | + | - |

**Legend: (-) =** absent or below detection limit; (+) = present in small quantity; (++) = present in moderate quantity; (+++) = present in high quantity. Also, W and C = wild and cultivated respectively

Based on the results of Chi square test of association between plant parts and the concentration of phenols, a significant (P-value < 0.015) 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 2. 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%) | |

*Pearson's Chi-squared test*

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

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 stem 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 predictors of phenolics concentration.

Table 3. 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 |
|---|---|---|---|---|
| **Root bark** | 6.1 | 0.8 | 14.2 | <0.001*** |
| **Stem 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 4). It was more likely for tannins to record a moderate concentration in the stem bark compared to recording a low concentration or being undetectable (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 4.** 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 bark was used relative to when the leaf was used and this increase was statistically significant (P-value < 0.001). When the stem bark was used, the odds of having a higher concentration of phenols increased by 83% relative to when the leaf was used (Table 5).

Table 5. 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 |
|---|---|---|---|---|
| **Root bark.** | 1.8 | 0.028 | 499.9 | <0.001*** |
| **Stem 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.

Based on a Chi Square Test of association (Table 6), 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.

Table 6. 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 7)

Table 7. 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** |
|---|---|---|---|---|
| **Stem bark** | 1249443.03 | 249.95 | 0.06 | 0.96 |
| **Root bark** | 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 leaf's 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 8). 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 8. 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 leaf's samples.
Tannins, 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.

**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 9). 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 9. 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 10), 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 10. 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 11). 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 11. 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 12), 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 12. 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 13). 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 13. 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.

One important observation during field work was the occurrence of stem borers on the stems of prunus in all agroforestry systems that could, in severe cases lead to stem breakage and death, a phenomenon not very evident in the wild populations.

## Discussion

### Phenol Variation concentrations inn 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), who showed 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.* (2023) 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.

### Tannins Variation in 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. Yang *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 (Li *et al*., 2020; Aghaei *et al*., 2022). Huang *et al.,* (2020) 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 Das *et al*., (2020) which are consistent with the findings of this study, found little evidence of a significant correlation between tannin levels and tree sizes.

**Saponins Variation in 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) who stated 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.

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. 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).

**Alkaloids Variation in 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 Ogwu *et al*. (2025) who showed variable accumulation of alkaloids throughout plant tissues. According to Bhambhani *et al*. (2021), 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 Aldulaimi *et al*. (2018), 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. 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.

**Glycosides Variation in 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. This finding corresponds with related research showing varying levels of cardiac glycosides in several plant species (Doe *et al*., 2019).

**Conclusion**

This study reveals that *Prunus* could be cultivated at altitudes different from those with a concentration of wild populations as it grows and also produce phytochemicals, especially phenolics and tannins that did not show variation with tree size and altitude. The important groups of phytochemicals were present at detectable level in root bark, stem bark and leaves, an indication of the need for a broader base exploitation of the resource to limit pressure on the stem bark currently exploited.

We therefore propose a crop rotation scheme whereby farmers can grow trees for a period of 10 to 20 years to enable maximum bark yield by debarking the entire stem and branches and possibly the major roots. At the same time the wood could be used for timber and fuelwood further maximising profits. This rotation scheme by-passes losses due to death of trees from poor harvesting. If treated as a crop, the leaves, though with lower concentrations, add another layer of gain. Of course, the mature wood from the stems and branches is also an asset. However, flavonoids, saponins and alkaloids were significantly low in most samples from low altitudes (agroforestry systems). A more detailed study of *Prunus* secondary metabolites production is recommended, in which actual concentrations be determined and their ratio in the bark of stems and roots compared to leaves, while checking the marketability of these parts from *Prunus* cultivated at low altitude.

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