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Detection of Phytochemicals in Five Medicinal Species Grown in a Xerophytic Environment

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Abstract
Medicinal species grown in xerophytic conditions are a valuable source of bioactive compounds. The present study was designed to explore ten phytochemicals in the leaves and fruits of *Kigelia africana*, *Physalis minima*, *Solanum nigrum*, *Withania somnifera* and *Datura innoxia* grown in the Cholistan desert, Pakistan. Quantification was done using UV-VIS spectrophotometry and results revealed highest concentration of phenols (14.05 ± 0.63 mg/ 100 mL), proline (9.03 ± 0.31 mg/ 100 mL), curcumin (28.24 ± 0.93 mg/ 100 mL), chlorophyll a (32.89 ± 0.68 mg/ mL) and chlorophyll b (32.99 ± 0.58 mg/ mL) in *S. nigrum* leaves while saponins (12.08 ± 0.81 mg/ 100 mL) were detected highest in fruits of *S. nigrum*. Carotenoids (2004.4 ± 15.21 mg/ mL) and alkaloids (10.99 ± 0.27 mg/ 100 mL) were found abundantly in fruits of *W. somnifera*. Moreover, the highest concentration of lycopene (0.75 ± 0.09 mg/ 100 mL) and flavonoids (17.61 ± 0.68 mg/ 100 mL) were recorded in *D. innoxia* leaves and fruits. Comparatively, *S. nigrum* showed the highest phytochemical contents followed by P. minima and W. somnifera; hence, their utilization as a health beverage should be considered.
Keywords: *Bignoniaceae,* fruits, leaves, phytochemicals, *Solanaceae*.

1. INTRODUCTION

Phytochemicals are the non-nutrient compounds produced naturally in fruits, vegetables, and whole grains. They are responsible for curing several diseases due to their interference in the enzyme system, cellular processes, and energy production [1]. These compounds warrant very effective dietary and medicinal components that open new drug development horizons. The most critical phytochemicals include alkaloids, flavonoids, saponins, phenols, curcumin, proline, and chlorophyll pigments [2]. Alkaloids naturally exist in the aerial parts of most plants. They can inhibit enzymes, which ultimately decreases the incidence of diabetes [3], while flavonoids and curcumin are phenolic compounds that act as antioxidants [4].

Curcumin gives a yellow colour to the plants and is mainly extracted from the rhizome of *Curcuma longa* [5]. However, saponins are triterpene glycosides, bitter, and are produced by plants, animals and some bacteria. Besides these, saponins also possess foaming properties and are used as preservatives to control microbial spoilage [6]. These unique properties are growing interest among researchers to ascertain compounds from novel species by using analytical tools. Hitherto, numerous phytochemicals have been identified in different medicinal plants that are associated with health benefits [5-6]. *K. africana* is a unique tree due to the presence of long, sausage-like fruits hanging on its branches. The fruits of this plant are effectively utilized as traditional medicines to cure ulcers, acne, and septic sores and possess antibacterial and antioxidant capabilities [7]. *S. nigrum*, commonly known as 'black nightshade' is widely used in traditional medicines to cure skin diseases, fever, liver disorders, and hydrophobia, and its leaves and fruits possess numerous biological activities [8]. Similarly, different parts of *W. somnifera* are used to treat malaria fever, hyperglycemia, breast cancer, and wounds [9].

Furthermore, *P. minima* and *D. innoxia* are also known to possess antifungal, cytotoxic, and anticancer activities [9-10]. These species are widely distributed in the Cholistan desert, having arid conditions such as extreme seasonal temperature, moisture fluctuation, and various edaphic conditions. Cholistan desert extends over an area of 26,000 sq. km in the southern part of Punjab (Pakistan) and is divided into two geomorphic regions, *i.e., the* northern region, which comprises 7770 km^2, and the southern region, which covers about 18130 km^2 [11]. Based on the immense biological spectrum of the flora of Cholistan desert, as reported in previous literature [8, 12], the present study was designed to ascertain the phytochemical profile of leaves and ripe fruits of five species that are associated with these biological activities.

2. MATERIALS AND METHODS

2.1. Plant Collection

The fresh leaves and ripe fruits of *Kigelia africana* Lam., *Physalis minima* L., *Solanum nigrum* L., *Withania somnifera* (L.) Dunal and *Datura innoxia* Miller belonging to the family *Solanaceae* and *Bignoniaceae* were collected from the Cholistan desert, Bahawalpur (Pakistan). The collected plant parts were washed, shade-dried, powdered, and then extracted in methanol (10 g in 100 mL) for two days. Subsequently, the samples were filtered and evaporated using a rotary evaporator (Buchi-Rotavapor, R-220) to obtain the crude extract.

2.2. Detection of Alkaloids

About 5 g of plant extract was added to 200 mL of acetic acid, and the solution was filtered after 4 hours. A few drops of ammonium hydroxide (NH_4OH) were added to the solution. After some time, the precipitated solution was washed with diluted NH_4OH, followed by filtration. The obtained residue was dried and weighed [13].

2.3. Detection of Saponins

Plant extracts (0.5 g), vanillin solution (0.5 mL), and sulphuric acid (5 mL) were added to the samples. The samples were vortexed and heated for 10 min. After 5 min, absorbance was measured (520 nm) using methanol as a control and the results were interpreted as saponins mg/100 mL of samples [14].

2.4. Detection of Phenols

About 100 mg of each extract was added to triple-distilled water (10 mL). 1 mL of this solution was dissolved in 0.5 mL of the Folin-Ciocalteu reagent (5 mg in 90 mL distilled water) and 1.5 mL of Na_2CO_3 solution. Total volume was made up to 8 mL with DW, and then absorbance was taken at 765 nm after 2 hours using a spectrophotometer. Phenolic contents were determined using a standard curve made from gallic acid [15].

2.5. Detection of Flavonoids

About 10 µL of aluminum chloride (10 g in 100 mL distilled water) was added to 20 µL of plant extract and 10 µL of potassium acetate (98.15 g in 1.0 L distilled water). Almost 160 µL of distilled water was added to each microplate well to obtain a final volume of 200 µL. The resultant mixture was then incubated, and absorbance (415 nm) was measured via a spectrophotometer [16].

2.6. Detection of Proline

Plant extract (0.5 g) was added in 5 mL of sulphosalicylic acid and then centrifuged (1000 g). The supernatant was collected in which glacial acetic acid and ninhydrin reagent (2 mL each) were added. The mixture was heated (100 °C) to acquire a brick red colour and then 4 mL of toluene was added. Finally, absorbance (520 nm) was measured, and results were expressed as proline mg. 100 mL^{-1} extract [17].

2.7. Detection of Curcumin

About 0.1 g of dried extract was dissolved in 25 mL of ethanol. The solution was filtered, and the volume was made up to 100 mL. Then 10 mL of solution was transferred to a volumetric flask, and 90 mL of ethanol was added. In the end, absorbance was measured at 425 nm [18].

2.8. Detection of Lycopene

1 g of sample was added in hexane: ethanol: acetone (2:1:1), and a blank was prepared. The absorbance of the decanted upper phase was determined at 503 nm, and the lycopene content was assessed [19]:

$$\text{Lycopene } (\mu g/g) = A_{503}/(17.2 \times 10^4 \text{ L.mole}^{-1}.\text{cm}^{-1}) \times 1/b \text{ cm} \times 536.9 \text{ g/mole} \times 1 \text{ L}/10^3 \text{ mL} \times 10^6 \text{ µg/1g} \times V \text{ mL/kg tissue} \times \text{kg}/10^3 \text{ g}$$

Where; b is the cuvette's optical path length and 536.9 is the molar mass of lycopene.

2.9. Detection of Pigments (Chlorophyll a, b, and Carotenoids)

The plant extract (0.5 g) was added to 40 mL of acetone to extract the chlorophyll and carotenoid from the species. Absorbance was measured at 470, 663 and 645 nm and the pigment concentration was calculated using the following equations [20]:

$$\text{Chlorophyll a} = [(12.7\ D_{663}) - (2.69\ D_{645})]\ V/W/1000$$

$$\text{Chlorophyll b} = [(22.91\ D_{645}) - (4.68\ D_{663})]\ V/W/1000$$

$$\text{Carotenoids} = [(1000\ D_{652}) - (2.27 \text{ chl a}) - (81.4 \text{ chl b})] / (V \times 2.27/W/1000)$$

2.10. Statistical Analysis

All experiments were performed three times, mean values were calculated, and the standard deviation (SD) was recorded as a measure of dispersion. Principal component analysis (PCA) was carried out using XLSTAT software, while a heat map was produced to perceive correlation and variability among different species in terms of phytochemical contents.

3. RESULTS AND DISCUSSION

3.1. Alkaloids and Saponins

Alkaloids are mainly involved in cytotoxic, antimicrobial, and anti-proliferative activities [3]. However, saponins possess emulsifying properties, antimicrobial effect [6], insecticidal activity [21], and therapeutic potential to control obesity [22]. In the present study, highest alkaloid contents were observed in the fruits of *W. somnifera* (10.99 ± 0.27 mg 100 mL^{-1}) and *P. minima* (10.78 ± 0.74 mg 100 mL^{-1}) followed by the leaves and fruits of *S. nigrum* (10.12 ± 0.23 mg 100 mL^{-1} and 10.05 ± 0.82 mg 100 mL^{-1}). Overall, fruits exhibited highest concentration of alkaloids as compared to the leaves of these species (Fig. 1a). Saponins were found in highest concentration in the fruits of *S. nigrum* (12.08 ± 0.81 mg 100 mL^{-1}) and *P. minima* (11.08 ± 0.74 mg 100 mL^{-1}) (Fig. 1b). Our study is coherent with the previous findings [8] showing the presence of alkaloids and saponins in examined species. It can be inferred that selected species are potent sources of active compounds associated with numerous biological activities.

3.2. Phenols and Flavonoids

Phenols comprise of hydroxyl components and glycosyl linkages that scavenge free radicals. Phenolic compounds possess a wide spectrum of cosmetic, nutraceutical and pharmaceutical applications because they demonstrate anti-carcinogenic, antimicrobial, anti-oxidative and anti-mutagenic properties. Similarly, flavonoids help remove free radicals from the body and prevent damage to DNA [4, 23]. Results revealed that *S. nigrum* leaves possess the highest concentration (14.05 ± 0.63 mg 100 mL^{-1}) of phenol and *W. somnifera* fruits exhibited the lowest (10.925 ± 0.72 mg 100 mL^{-1}) phenolic content (Fig. 1c).

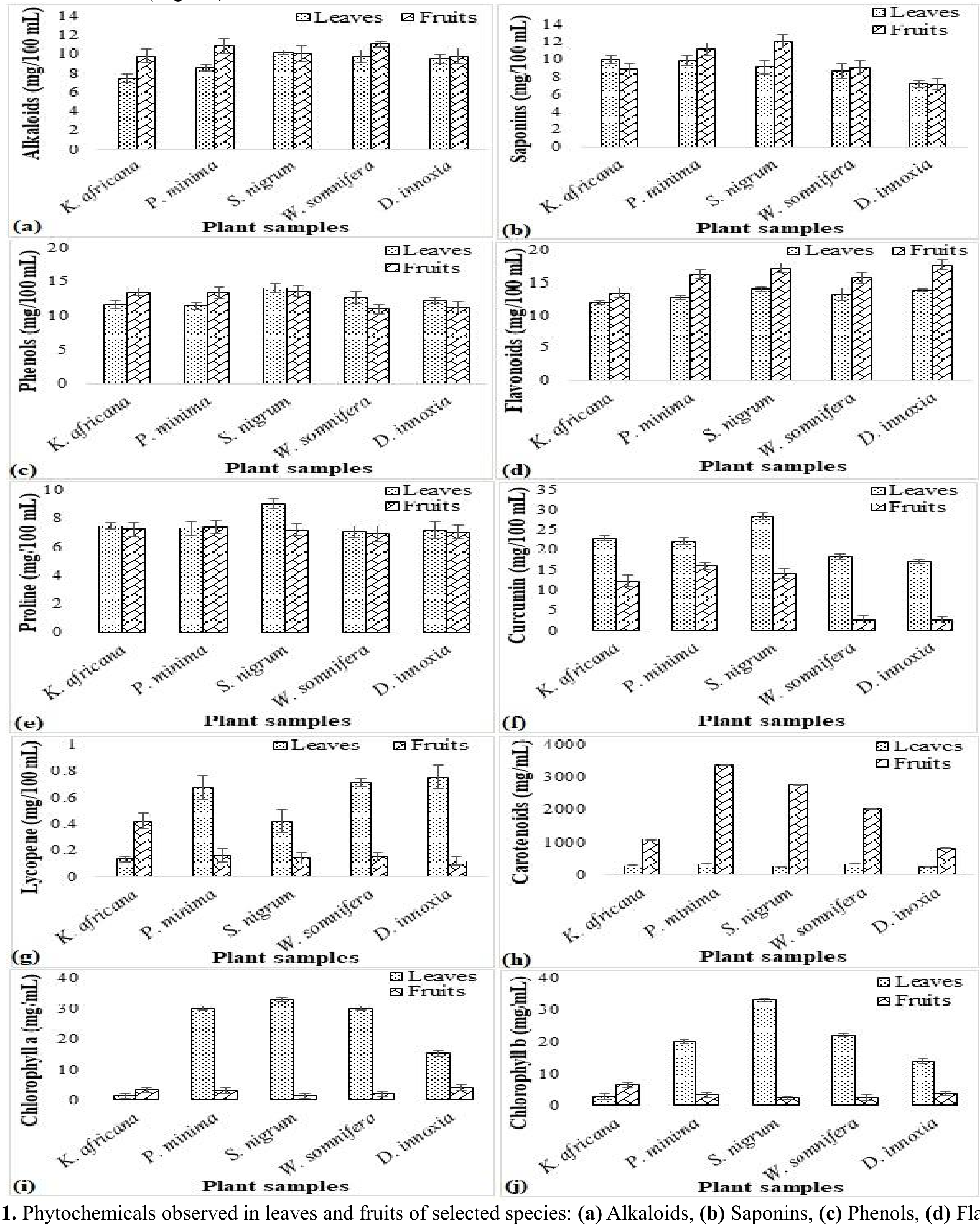

Figure 1. Phytochemicals observed in leaves and fruits of selected species: **(a)** Alkaloids, **(b)** Saponins, **(c)** Phenols, **(d)** Flavonoids, **(e)** Proline, **(f)** Curcumin, **(g)** Lycopene, **(h)** Carotenoids, **(i)** Chlorophyll a **(j)** Chlorophyll b. Data represents mean ± SD of three replicates.

Similarly, flavonoids were detected at the highest in the fruits of *D. innoxia* (17.61 ± 0.68 mg 100 mL^{-1}), *S. nigrum* (17.14 ± 0.70 mg 100 mL^{-1}), and *P. minima* (16.18 ± 0.72 mg 100 mL^{-1}). However, all other species exhibited less than 16 mg 100 mL^{-1} flavonoid contents. Altogether, fruits showed the highest phenolic contents as compared to the leaves (Fig. 1d).

Our results correlate with the earlier findings of Fagbohun *et al.* [7], Benabderrahim *et al.* [24], and Fernando *et al.* [25] indicating significant phenols and flavonoids in different organs of *K. africana, D. innoxia* and *W. somnifera*. Thus, biological properties of leaves and ripe fruits of selected species can be attributed to the presence of phenol and flavonoid contents.

3.3. Proline and Curcumin

In our study, proline content was detected highest (9.03 ± 0.31 mg 100 mL^{-1}) in the *S. nigrum* leaves and lowest in *K. africana* leaves (7.45 ± 0.22 mg 100 mL^{-1}) and *P. minima* fruits (7.40 ± 0.43 mg 100 mL^{-1}) (Fig. 1e). Fruits of *W. somnifera* and *D. innoxia* showed lowest concentration (2.60 ± 0.92 mg 100 mL^{-1} and 2.51 ± 0.76 mg 100 mL^{-1}) of curcumin. In contrast, leaves and fruits of all other species demonstrated significant concentration of curcumin, ranging from 12.23 mg 100 mL^{-1} to 28.24 mg 100 mL^{-1} (Fig. 1f).

Previously, Lin *et al.* [26] reported the existence of curcumin in *S. nigrum*. However, the quantitative estimation of proline and curcumin in other species was ascertained for the first time in our study. Curcumin is chiefly available in the form of turmeric for domestic use, serving as a coloring, flavoring, and preservative agent in different foods [5]. However, proline is the main amino acid that acts as a radical scavenger and inhibitor of the central nervous system [27,3]

3.4. Lycopene and Carotenoids

Lycopene is a fat-soluble carotenoid used as a main dietary component. It regulates many cellular processes such as inflammation, apoptosis, oxidative stress, and other biological activities [28]. Carotenoids are associated with a relatively low incidence of cancer and cardiovascular disease, and help prevent Alzheimer's disease [29]. In our study, lycopene was present in higher concentration in leaves as compared to the fruits, except *K. africana* leaves, which possess 0.13 ± 0.02 mg/100 mL lycopene content. Overall, lycopene was detected up to 0.71 mg/100 mL in the leaves and fruits of the examined species (Fig. 1g). Contrarily, the fruits' highest carotenoid contents were determined. Amongst all fruits, *P. minima* (3348.90 ± 16.11 mg/mL) exhibited the highest concentration. Likewise, leaf extracts depicted the highest carotenoids in *P. minima* (343.91 ± 12.22 mg mL^{-1}) and lowest in *D. innoxia* (246.52 ± 13.32 mg mL^{-1}) (Fig. 1h).

Previously, Dappah *et al.* [30] and Takshak & Agarwal [31] also observed lycopene and carotenoid contents in *S. nigrum* and *W. somnifera,* which have been confirmed in our study. Thus, active compounds should be isolated from examined species and investigated in detail using chromatography techniques.

3.5. Chlorophyll a and Chlorophyll b

Results displayed highest chlorophyll a and chlorophyll b content in *S. nigrum* leaves (32.89 ± 0.58 mg mL^{-1} and 32.99 ± 0.58 mg mL^{-1}) followed by *W. somnifera* leaves (30.07 ± 0.83 mg mL^{-1} and 22.05 ± 0.92 mg mL^{-1}) and *P. minima* leaves (30.07 ± 0.92 mg mL^{-1} and 20.06 ± 0.82 mg mL^{-1}). Comparatively, *K. africana* showed the lowest chlorophyll contents, while among plant organs, fruits exhibited the lowest chlorophyll as compared to the leaves (Fig. 1i and j).

The plant leaves are associated with a high rate of metabolism due to their role in photosynthesis and thus contain chlorophyll pigments to facilitate the photosynthetic activity. Although there are different photosynthetic pigments, chlorophyll is a crucial bioactive compound, and its presence is strongly correlated with other bioactive compounds. It is the primary pigment responsible for photosynthesis that converts solar energy into chemical energy used to build carbohydrate molecules (glucose) and ultimately fuels the synthesis of various other bioactive compounds within the plant. Chlorophyll and its derivatives, like chlorophyllin, are also potent antioxidants, capable of scavenging free radicals and reducing oxidative stress in cells [32, 33]. Chlorophyll is often present along with other beneficial compounds in plants such as flavonoids and polyphenols, and they work synergistically, enhancing the overall antioxidant and anti-inflammatory effects. Chlorophyll can even influence the bioavailability and activity of other bioactive compounds within a plant. Thus, chlorophyll is not just a pigment essential for photosynthesis; its presence and other bioactive compounds are directly interconnected, impacting plant and human health by consuming chlorophyll-rich foods [34]. Chlorophyll a is usually present abundantly in the youngest leaves, while chlorophyll b is highest in mature leaves. As the fruit ripens, chlorophyll degradation occurs, followed by the accumulation of carotenoid pigment [35]. In general, it can be predicted that these compounds are directly or indirectly interlinked with the biological potential of plants and thus, can be used at an industrial level.

3.6. Statistical Interpretation

PCA was performed to reduce a large number of variables into a small number that can be concisely interpreted data. In our study, biplot axes were made in which phytochemicals were taken as active variables and plants as active observations. PCA of plant leaves showed 79.23 % variability by the first two principal components (49.05 % and 30.18 %) while PCA of plant fruits displayed 79.17 % in total variance (41.93 % and 37.24 %) (Fig. 2a and b).

The position of each phytochemical symbolizes its correlation with the species. Variables lying close to each other have a high correlation with each other. Variables on the same side of the origin (O, O) are positively correlated, while those on the left side of the origin are negatively correlated. The phytochemicals on the right side of the axes are characterized by high concentration; however, phytochemicals on the left side are characterized by the lowest phytochemical contents. Only saponins and carotenoids exhibited the lowest concentration in leaves, as they lie on the left side of axes; however, all phytochemicals were detected on the right side in the fruits, except chlorophyll a and chlorophyll b. Moreover, phytochemicals at the top of the right axis represent comparatively higher concentrations than those below the axes (Fig. 2a and b).

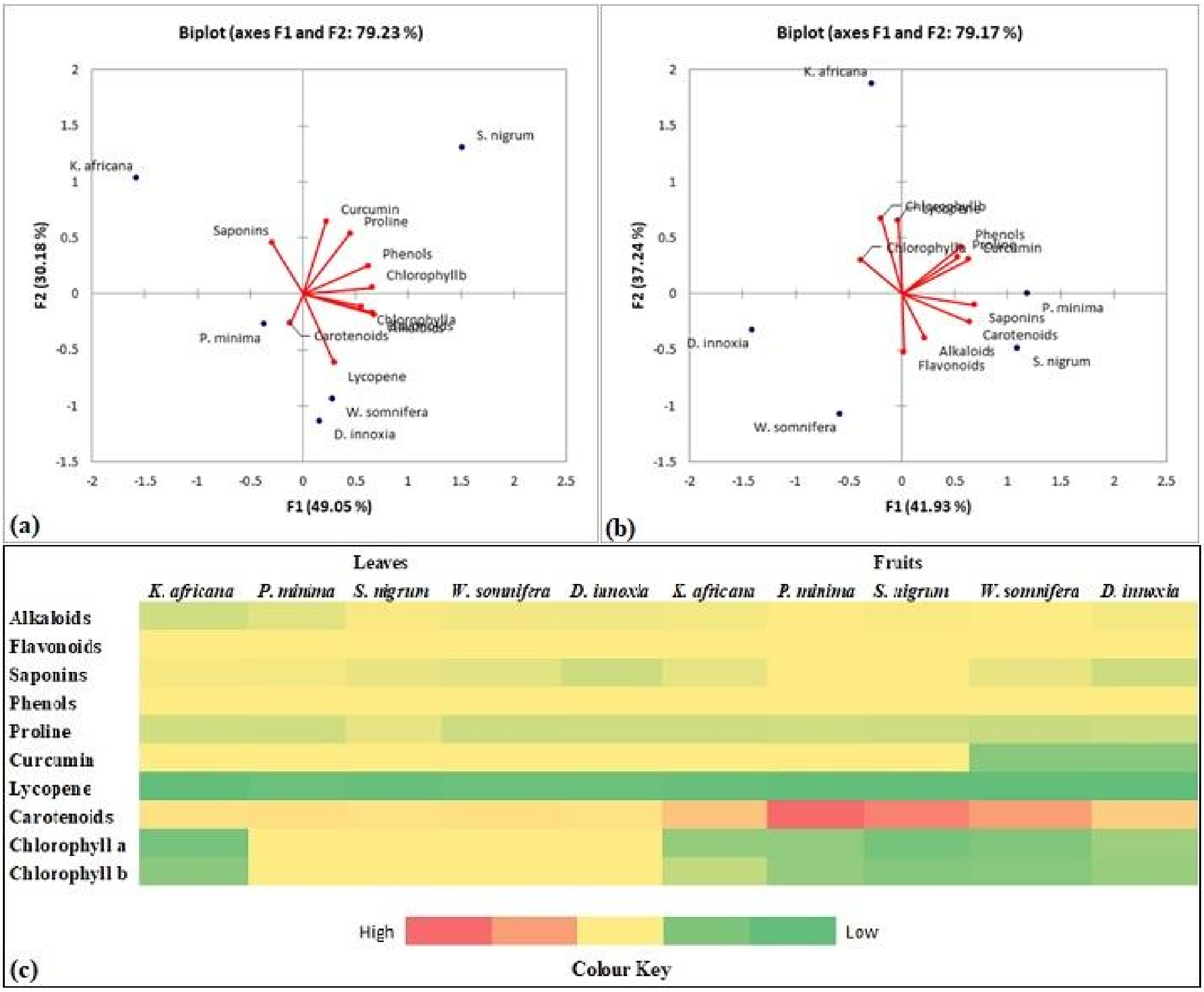

Figure 2. Statistical evaluation of phytochemicals observed in selected species (a) PCA biplot showing correlation between leaves of plants and phytochemicals (b) PCA biplot showing correlation between fruits of plants and phytochemicals (c) Heat map showing comparison among different species by displaying mean values in colors from lowest (green) to highest (red) concentration.

Moreover, the heat map showed that chlorophyll a and chlorophyll b are present in the lowest concentration in fruits as compared to the leaves. Amongst all phytochemicals, lycopene was detected in the lowest concentration in all species. Fruits revealed highest concentration of carotenoids followed by phenols, flavonoids and alkaloids contents (Fig. 2c). All-inclusive, statistical analysis showed that leaves, exhibited highest concentration of curcumin, proline, phenols and chlorophyll b. In contrast, in examined species, fruits possess higher concentrations of phenols, proline, curcumin, lycopene, chlorophyll a and chlorophyll b. Previously, researchers have previously used PCA to determine correlation between phytochemicals and other variables. Thus, it can be inferred that PCA biplots can be used to assess the magnitude of importance of each variable by the distance from the origin.

CONCLUSION

Overall, *S. nigrum, P. minima,* and *W. somnifera* possess higher phytochemical contents than *D. innoxia* and *K. africana*. Fruits exhibited the highest alkaloids, flavonoids, saponins, and carotenoids contents, while leaves showed the highest concentration of proline, phenols, curcumin, lycopene, and chlorophyll pigments. Therefore, examined species can be exploited as a potent source of bioactive compounds that could be exploited for food and medicinal purposes. In the future, advanced tools should be applied to isolate these compounds from various plant organs.

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