CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

MORPHOLOGY, GROWTH VARIABILITY AND CHEMICAL COMPOSITION OF INDIAN AND NIGERIAN ACCESSION OF OCIMUM SPECIES GROWN IN INDIA

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https://doi.org/10.34302/2019.11.4.6

Article history:
Received:
17 June 2019
Accepted:
25 October 2019

Keywords:
Ocimum sanctum;
Ocimum gratissimum;
Proximate;
Mineral;
Phytochemical.

ABSTRACT
Demand for medicinal plants is increasing by the day, due to their health benefits. In this study, the morphological attributes, proximate, mineral and phytochemical compositions of Indian accessions of *Ocimum sanctum* and *Ocimum gratissimum* as well as that of *Ocimum gratissimum* (Nigerian accession) grown in India under greenhouse conditions were investigated. *Ocimum* leaves showed significant variations in their morphometric attributes and these attributes increased with increasing days after planting. The leaves were good sources of fibre and relatively low in protein. *O. sanctum* showed superior iron content than the other species. Total phenolic and cardiac glycoside contents of the three leaves were very similar but they showed significant variations in their saponins, tannins, flavonoids and alkaloid contents. PCA revealed that Indian accessions of *O. sanctum* and *O. gratissimum* are distinctly separated and different from Nigerian accession of *O. gratissimum* in morphometric data and phytochemical constituents. Nigerian accession had superior phytochemical contents than the Indian accessions and may be further explored for breeding purposes to complement the Indian accessions for enhanced applications in the pharmaceutical industry in India.

1. Introduction

Medicinal plants including the genus *Ocimum*, are well-known to have health-promoting benefits and nutraceutical functions. These functions have been associated with the presence of plant components such as proteins, vitamins, fibres, several kinds of secondary metabolites (Bhattacharya et al., 2014), essential oils and phenolic compounds (Joshi et al., 2011). The genus *Ocimum* belongs to the family Lamiaceae and consist of about 30-35 species indigenous to tropical regions of Asia, Africa, central and south America (Okunlola et al., 2017; Paton, 1992). These plant species have been reported to possess antidiabetic (Gholap and Kar, 2004), antioxidant (Akinmoladun et al., 2007; Bhattacharya et al., 2014; Joshi et al., 2011; Siti et al., 2018) anti-asthmatic (Singh and Agrawal, 1991), anti-inflammatory (Mequanint et al., 2011; Singh and Agrawal, 1991; Singh et al., 1996), antimicrobial (Dambolena et al., 2010; Joshi et al., 2011; Nakamura et al., 1999), anti-stress (Gupta et al., 2007) and anticancer (Prashar et al., 1998) activities.

The nutritional value of genus the *Ocimum* may vary with species, growing location and processing method. For example, differences in the protein content (7 and 25%) (Dry weight basis) of these species have been reported in different parts of the world including Nigeria (Okunlola *et al.*, 2017) and India (Barua *et al.*, 2015; Siti *et al.*, 2018). In India, *Ocimum sanctum* Linn also known as Tulsi or Holy Basil is an Ayurvedic herb of Southeast Asia with a long history of traditional uses (Singh and Chaudhuri, 2018). It is a common specie that is widely grown in many parts of India. Besides the afore-mentioned therapeutic uses of the species, *Ocimum sanctum* can also be consumed as herbal tea, to treat cough, cold and malaria (Prakash and Gupta, 2005). However, In Nigeria and many African countries, *Ocimum americanum*, *Ocimum basilicum* and *Ocimum gratissimum* are the most important and popular members of these species (Okunlola *et al.*, 2017). Due to the growing demand of medicinal plants in many parts of the world, researchers are now focusing on the possibility of increasing their production either through micro-propagation (Okunlola et al., 2017; Saha et al., 2010; Saha et al., 2012) or macro-propagation (Ehiagbonare, 2007). *Ocimum* species are generally propagated using both seeds and stem cutting, but farmers have problems with cultivating plants from seeds due to their low viability (Okunlola et al., 2017). Many factors such as the method of propagation, specie type, as well as growing conditions may influence the growth pattern and phytochemical constituents of *Ocimum* plants. Okunlola et al. (2017), recently studied the growth and nutritional qualities of *Ocimum basilicum, Ocimum gratissimum,* and *Ocimum americanum*, propagated by stem cutting and seed. According to their report, *Ocimum* species propagated by stem cutting performed better in terms of morphometric attributes than those propagated through the seed. For instance, *Ocimum gratissimum* propagated through the stem had higher number (8 times) of branches and were taller (4 times) than the same specie propagated by seed (Okunlola et al., 2017). However, seed propagated samples contained more phytochemicals than those propagated by stem cutting, suggesting that seed propagation may be better in growing *Ocimum* species for pharmaceutical applications. Hence, in this study, the morphological and growth variability as well as the proximate, mineral and phytochemical composition of *Ocimum sanctum* and *Ocimum gratissimum* were investigated.

2. Materials and methods

2.1. Plant materials

Seeds of three *Ocimum* species were used in this study. Indian accessions of *Ocimum gratissimum* and *Ocimum sanctum* were collected from Maliba Pharmacy College, Uka Tarsadia University (UTU), Gujarat, India, while seeds of one Nigerian accession of *Ocimum gratissimum* was obtained from the botanical garden of University of Ilorin, Nigeria. Body text TNR 12 normal, ident first line 0.66 cm , line spacing Single)

2.2. Seed propagation and processing

Ocimum seeds were sown in the greenhouse of UTU and growth was monitored for 3, 60 and 90 days after planting. Fresh plant leaves of the Indian and Nigerian accessions were collected and washed thoroughly using distilled water. Leaves were separated for different analysis including proximate mineral and photochemical screening as described below. Samples for phytochemical screening were air-dried under shaded condition at room temperature. The dried leaves were crushed into powder and stored in sealed bottles until needed.

2.3. Qualitative and quantitative screening of phytochemicals

Alkaloids, tannins, cardiac glycosides, flavonoids and saponins were determined as previously described (Sofowora, 1993), while the presence of steroids was determined using the method of Khandelwal (2007).

2.4. Proximate composition

Moisture, fat and ash contents of the samples were determined using AOAC (2000) methods. Dry matter contents of the samples were calculated by deducting percentage moisture content from 100. Protein content was determined by the Kjeldahl method (6.25×N) and carbohydrate was calculated by difference. Fibre contents were determined by digestion in sulfuric acid and sodium hydroxide (Kirk and Sawyer, 1991).

2.5. Mineral composition

Mineral content of the samples was determined as described by Amonsou et al. (2014) using Inductively Coupled Plasma (ICP) spectroscopy. Samples were acid-digested by the addition of 1 mL of 55% (v/v) HNO3.

2.6. Statistical analysis

Duplicate samples were prepared and analyses done in triplicate. Data was analysed using one way analysis of variance (ANOVA) and means were compared using the Fisher Least Significant Difference (LSD) test ($p \leq 0.05$) using the Statistical Package for the Social Sciences (SPSS) Version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was used to determine the similarity and differences in the three *Ocimum* accessions based on morphometric data and phytochemical constituents.

3. Results and discussions

3.1. Morphometric characteristics

With the exception of the colour of the leaves and stem, which were generally green and white respectively, the three *Ocimum* species investigated showed significant ($p<0.05$) variations in their morphometric characteristics (Table 1). Regardless of the specie, height, number of leaves, leaf length, leaf width leaf area and number of branches increased with increasing days after planting. Nigerian accession of *O. gratissimum* generally had higher height, number of leaves, leaf length, leaf width and leaf area compared with the Indian accessions (*O. sanctum* and *O. gratissimum*). The height, leaf length, leaf area and number of branches of Indian accession of *O. sanctum* were significantly ($p<0.05$) different from those of *O. gratissimum* throughout the period of planting (30-90 days). Previous research similarly found that *O. gratissimum* performed better in morphometric attributes compared to *O. basilicum* and *O. americanum* (Okunlola et al., 2017). The height (approx. 26-38), number of leaves (approx. 21-24), and number of branches (0-4) observed for *O. gratissimum* (both Nigerian and Indian accessions) examined after 60 days (approx. 9 weeks) of planting in this study, were much higher (2-6 times) than values reported for *O. gratissimum* examined after 8 weeks of planting (Okunlola et al., 2017). Variation in the morphometric data could be due to the differences in days after planting as well as the green house growing conditions such as soil type and seed sowing depth. For example, *O. gratissimum* seed sown at 1 cm depth in humus rich topsoil was reported to have higher germination rate (80%) compared to seed sown on river sand (26.3%) from of the same sowing depth (Ehiagbonare, 2007).

Table 1. Morphometric characteristics of three *Ocimum* species grown by seed propagation

Species	DAP	Height (cm)	NL	LL (cm)	LW (cm)	LA (cm^2)	NB	LC	SC
**Sanctum*	30	$12.19^{g}\pm0.05$	$6.00^{f}\pm1.00$	$3.20^{e}\pm0.26$	$1.67^{e}\pm0.06$	$5.13^{f}\pm0.06$	$0.00^{d}\pm0.00$	Green	White
**Gratissimum*	30	$9.27^{h}\pm0.16$	$8.33^{f}\pm0.58$	$2.46^{f}\pm0.35$	$1.61^{e}\pm0.01$	$4.00^{a}\pm010$	$0.00^{d}\pm0.00$	Green	White
***Gratissimum*	30	$12.31^{g}\pm0.12$	$11.33^{e}\pm0.58$	$3.03^{ef}\pm0.32$	$2.13^{d}\pm0.06$	$6.27^{fg}\pm0.31$	$0.00^{d}\pm0.00$	Green	White
**Sanctum*	60	$34.53^{e}\pm0.36$	$23.33^{cd}\pm1.52$	$5.33^{d}\pm0.40$	$3.90^{c}\pm0.10$	$20.67^{e}\pm0.05$	$6.67^{b}\pm0.57$	Green	White
**Gratissimum*	60	$26.02^{f}\pm0.12$	$21.33^{d}\pm1.52$	$7.53^{bc}\pm0.40$	$3.80^{c}\pm0.17$	$28.50^{d}\pm0.50$	$0.00^{d}\pm0.00$	Green	White
***Gratissimum*	60	$37.95^{d}\pm0.12$	$24.33^{c}\pm0.58$	$8.93^{a}\pm0.31$	$5.20^{b}\pm0.20$	$46.67^{b}\pm1.52$	$4.33^{c}\pm1.52$	Green	White
**Sanctum*	90	$69.36^{b}\pm0.66$	$58.67^{a}\pm3.06$	$7.20^{a}\pm0.20$	$3.90^{c}\pm0.10$	$27.37^{d}\pm0.40$	$10.67^{a}\pm1.52$	Green	White
**Gratissimum*	90	$48.05^{c}\pm0.14$	$42.67^{b}\pm0.58$	$7.90^{b}\pm0.30$	$3.90^{c}\pm0.10$	$30.93^{c}\pm0.12$	$5.33^{bc}\pm0.58$	Green	White
***Gratissimum*	90	$74.87^{a}\pm0.37$	$58.33^{a}\pm2.08$	$9.10^{a}\pm0.50$	$5.47^{a}\pm0.31$	$50.93^{a}\pm2.08$	$10.33^{a}\pm0.57$	Green	White

Mean ± S.D. Means with same superscript within the same column are not significantly ($p<0.05$) different.
DAP: Days after planting; LN: Number of leaves; L: Leaf length; LW: Leaf width
LA: Leaf Area; LC: Leaf Colour; SC: Stem colour; NB: Number of branches *Indian accession** Nigerian Accession

3.2. Proximate composition

The proximate composition data of the three Ocimum species are presented in Table 2. Expectedly, the moisture content of the three *Ocimum* leaves were generally high (average 77%). *O. gratissimum* (both Nigerian and Indian accessions) had significantly ($p<0.05$) lower moisture values (approx. 74-78%) than O. sanctum (approx. 80%). Beside the moisture content, which was the major component of the leaves, ash (average 11%), followed by fibre (4.72-8.47%) were present in fairly good quantities. Protein (0.13-0.21%), carbohydrate (1.63-2.53%) and fat (average 3.31%) were found in relatively small quantities (Table 2). Nigerian accession and Indian accession of *O. gratissimum* had almost similar composition except in their protein and fibre contents, which could be attributed to inherent differences in the plant species. This seems plausible, since both plants were grown under the same conditions. Although the protein content of the leaves were generally low, Nigerian accession of *O. gratissimum* had slightly higher protein content than other *Ocimum* species. Similarly low levels (0.20-1.21%) of protein have been reported for different *Ocimum* leaves (Idris et al., 2011; Oboh et al., 2009). Some authors, however, reported higher protein values (2.88-9.10) for different *Ocimum* leaves (Emeka and Chimaobi, 2012; Mlitan et al., 2014; Okunlola et al., 2017; Shuaib et al., 2015). Generally, fresh leafy vegetables have been reported to have low levels of protein, which are mostly in the form of enzymes, rather than acting as a storage pool, as in grains and nuts (Oboh et al., 2009).

3.3. Mineral composition

There were significant ($p<0.05$) differences in the mineral composition of the three *Ocimum* leaves (Table 3). Nitrogen (4.28-5.58%), followed by calcium (2.92-3.58%), phosphorus (0.38-4.83%) and potassium (2.05-3.51%) were the major mineral elements in the leaves. Sodium (0.02-0.92%) and magnesium (1.17-1.49%) were found in relatively small quantities. High levels of potassium in human diet is important for the protection against life-threatening diseases such as hypertension, cardiac dysfunctions and osteoporosis (Demigne et al., 2004; Lewu et al., 2010). Among the microelements, iron (263.01-863.00 ppm) was the major mineral in the leaves. Iron content of *O. sanctum* was substantially higher (about 3 times) than values recorded for the *O. gratissimum* species. The consumption of such foods rich in micronutrients such as iron helps to build a strong immune system and facilitate nutrient absorption, utilisation and digestion (Njoku and Ohia, 2007). Furthermore, iron is known to play a vital role in haemopoiesis, control of infection and cell mediated immunity (Barua et al., 2015; Bhaskaram, 2001). Thus, in addition to the well-known medicinal properties of these leaves, they can also be explored in haematinic applications.

The leaves were fairly good sources of manganese (16.00-40.67 ppm), zinc (19.60-39.33 ppm) and copper (25.96-36.27 ppm). The zinc, copper and iron values obtained in this study are in agreement with the literature (Idris et al., 2011; Kashif and Ullah, 2013; Vidhani et al., 2016). Nigerian accession of *O. gratissimum* was lower in nitrogen phosphorus, sodium, magnesium and iron, but higher in manganese and copper compared with the Indian accessions. Nitrogen is important for amino acid and protein production and plays a pivotal role in many critical functions such as photosynthesis in plant. Thus, the lower amount of nitrogen in the *O. gratissimum* (Nigerian accession) may explain why it protein content was higher than the Indian accessions (Table 2).

3.4. Phytochemical composition

The three *Ocimum* leaves showed significant ($p<0.05$) variations in their phytochemical composition, except in their total phenolic and cardiac glycoside contents, which were very similar (Fig. 1). In general, *O. gratissimum* (Nigerian and Indian accessions) displayed significantly ($p<0.05$) higher alkaloids, tannin, flavonoids and saponin contents compared with *O. sanctum* (Indian accession). However, Nigerian accession of *O. gratissimum* was higher in tannins, flavonoids and saponin contents than the other two species. Pachkore and Dhale (2012), working with three *Ocimum* species reported lower tannin values for *Ocimum gratissimum* (0.12 mg/100 g) and *Ocimum sanctum* (0.42 mg/100 g), when compared to values in this study. However, other authors reported substantially higher tannins (3.29-4.60 mg/100 g) and saponins (4.50-6.86 mg/100 g) for *Ocimum gratissimum* cultivated by seed and stem cutting (Okunlola et al., 2017). Variation in the phytochemical constituents may be associated with inherent genetic differences among the species studied in the respective studies as well as the environmental condition of the various growth locations (Okunlola et al., 2017). The phenolic and flavonoid contents recorded in this study were almost 3-6 times higher than values reported for *Ocimum* leaves in earlier studies (Okunlola et al., 2017; Pachkore and Dhale, 2012).

The relatively higher phenols and flavonoids of the leaves suggest that these species will have high antioxidant activities. Phenols and flavonoids are important groups of secondary metabolites, synthesized by plants and have been previously associated with antioxidative activities (Akinmoladun et al., 2007; Bhattacharya et al., 2014; Joshi et al., 2011; Siti et al., 2018).

3.5. Principal component analysis

Morphometric data and phytochemical constituents of the three accessions were analysed using principal component analysis (PCA). PCA revealed that Indian accessions of *O. sanctum* and *O. gratissimum* are distinctly separated and different from Nigerian accession of *O. gratissimum* in morphometric data and phytochemical constituents, despite growing the three plant under the same greenhouse conditions (Figure not shown). The *O. sanctum* and *O. gratissimum* are also different from each other based on these parameters. The first two principal components (PC) accounted for approximately 92% of the total variability of the data (Fig. 2). PC1 accounted for approximately 59% of the total variation and was predominantly a function of total phenolic content, tannin, cardiac glycoside, flavonoids, saponins, plant height, leaf length, leaf width and leaf area. The PC2, which only accounted for 32% of the total variation was made up of alkaloids, number of leaves and number of branches.

Table 2. Proximate composition of leaves of three *Ocimum* species at 90 days after planting (%)

Parameters	**Sanctum*	**Gratissimum*	***Gratissimum*
Dry matter	19.77^{b}±0.21	27.85^{a}±0.55	26.07^{a}±1.33
Moisture	80.23^{a}±0.21	78.15^{b}±0.55	73.93^{b}±1.33
Protein	0.17^{ab}±0.04	0.13^{b}±0.02	0.21^{a}±0.04
Ash	10.07^{b}±0.05	11.27^{a}±0.31	11.40^{a}±0.53
Fibre	4.72^{b}±0.10	4.88^{b}±0.24	8.47^{a}±0.40
Fat	3.18^{b}±0.07	3.3^{ab}±0.13	3.46^{a}±0.11
Carbohydrate	1.63^{b}±0.30	2.25^{a}±0.07	2.53^{a}±0.39

Mean ± S.D. Means with same superscript within the same row are not significantly (p<0.05) different.
*Indian accession ** Nigerian Accession

Table 3. Mineral composition of leaves of three *Ocimum* species at 90 days after planting

Minerals	**Sanctum*	**Gratissimum*	***Gratissimum*
Nitrogen (%)	5.58^{a}±0.39	4.84^{b}±0.07	4.28^{c}±0.02
Phosphorus (%)	2.05^{b}±0.03	4.83^{a}±0.02	0.38^{c}±0.02
Potassium (%)	2.04^{c}±0.04	3.51^{a}±0.02	2.61^{b}±0.03
Sodium (%)	0.92^{a}±0.06	0.08^{b}±0.02	0.02^{c}±0.01
Calcium (%)	3.58^{a}±0.03	2.92^{c}±0.07	3.13^{b}±0.06

Magnesium (%)	1.49ᵃ±0.03	1.40ᵇ±0.03	1.17ᶜ±0.03
Iron (ppm)	863.00ᵃ±1.15	285.00ᵇ±1.53	263.01ᶜ±0.58
Manganese (ppm)	16.00ᶜ±2.00	29.00ᵇ±1.00	40.67ᵃ±1.53
Zinc (ppm)	39.33ᵃ±0.58	19.60ᶜ±1.22	25.63ᵇ±0.67
Copper (ppm)	25.96ᵇ±0.97	26.57ᵇ±0.58	36.27ᵈ±1.18

Mean ± S.D. Means with same superscript within the same row are not significantly ($p<0.05$) different.
*Indian accession
** Nigerian Accession
***Values are expressed in %

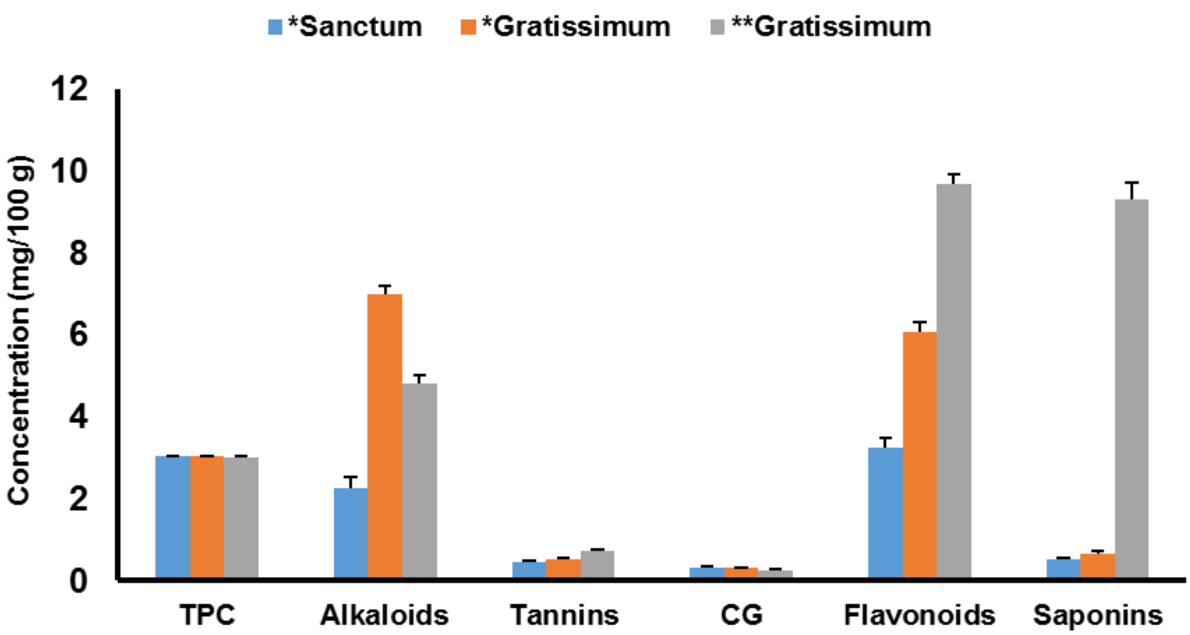

Figure 1. Phytochemical contents of three *Ocimum* species at 90 days after planting
Error bars indicate standard deviation (N= 3)
TPC: Total phenolic content
CG: Cardiac glycosides
*Indian accession
** Nigerian Accession

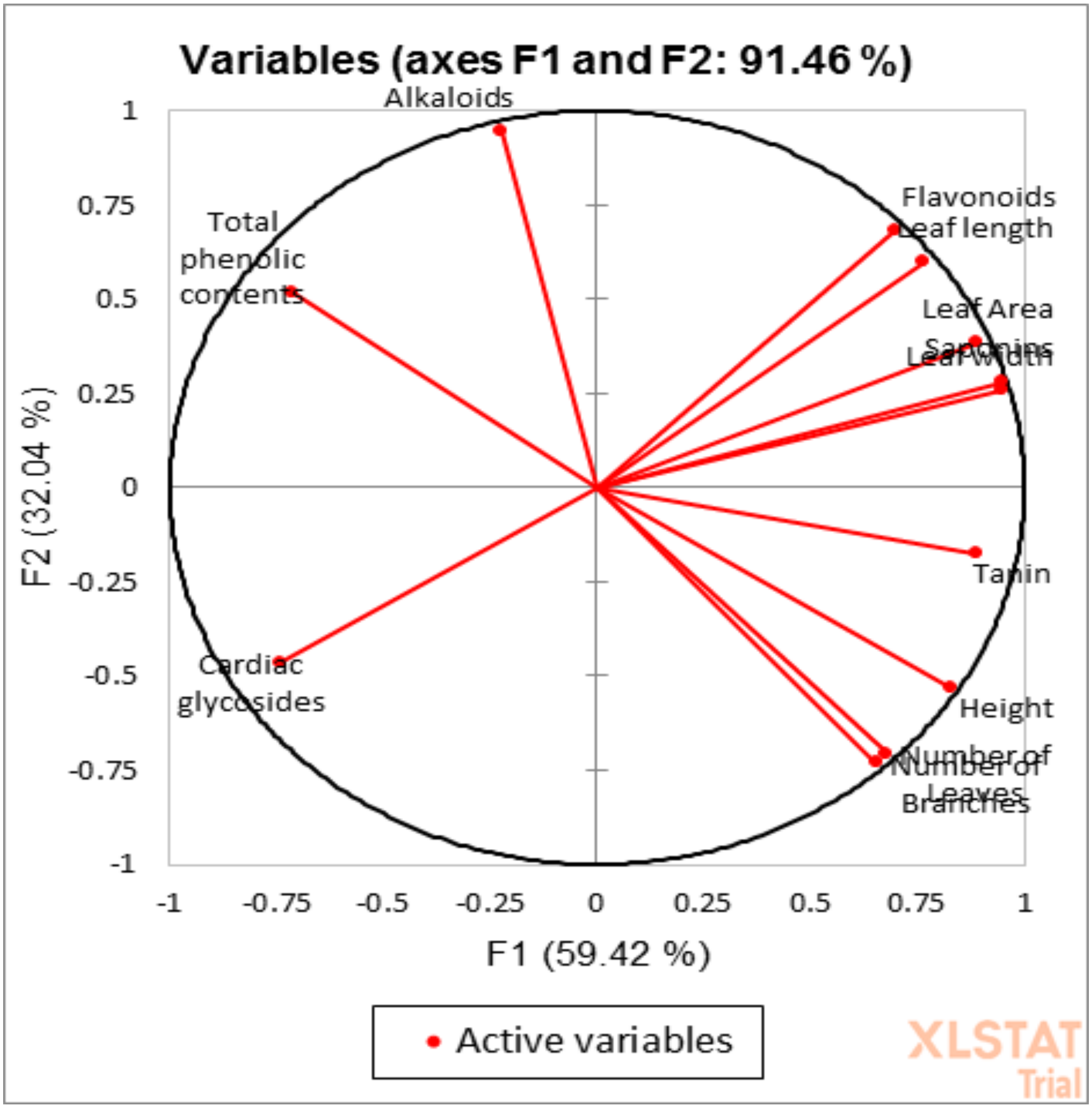

Figure 2. Principal component analysis of morphometric data and phytochemical constituents of *Ocimum* species

4. Conclusions

Nigerian accession of *O. gratissimum* had different morphometric attributes compared with the Indian accessions (*O. sanctum* and *O. gratissimum*), though they were grown under the same greenhouse conditions. All the leaves had relatively low protein content and are fairly good sources of iron, but *O. sanctum* showed superior iron content than the other species. Nigerian accession had superior phytochemical contents than the Indian accessions and may be further explored for breeding purposes through plant tissue culture, to complement the Indian accessions for enhanced applications in the pharmaceutical industry in India.

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