Comparative Antioxidant Analysis of *Moringa oleifera* Leaf Extracts from South Western States in Nigeria

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Abstract

Background: *Moringa oleifera* is a medicinal plant that ethnobotanical studies have shown its inclusion in treatment of many ailments such as diarrhea, diabetes, epilepsy, wound healing and arthritis. It is a plant that was believed to originate from India but could now be found in both the tropics and the sub-topics. Earlier reports have not addressed the association between the location of plant collection and its antioxidant contents. In this study, *Moringa oleifera* leaves were collected from 21 locations within the south western states of Nigeria. Leaves from each of the locations were dried and evaluated for total phenols, tannin, saponin and flavonoid. DPPH scavenging activity, nitric oxide scavenging activity and inhibition of lipid peroxidation were also evaluated.

Results: All the parameters analysed showed significant within- and between-group differences. Some locations had greater DPPH scavenging ability than the standard (quercetin).

Conclusion: Environmental parameters like annual precipitation, minimum temperature and maximum temperature, and soil type of the location of the plants showed influence on the level of antioxidant, while further analysis using metaboanalyst showed a notable effect of soil type on the antioxidant activity.

Keywords: *Moringa oleifera*, Antioxidant, Phenolic contents, Phytochemicals

Background

The production of free radicals is basic to any biochemical process and this constitutes a pivotal part of life processes. Production of reactive oxygen species such as hydroxyl radical ($\cdot$OH), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) is associated with several degenerative diseases such as diabetes, cancer, cardiovascular diseases, Alzheimer's disease and inflammation [1, 2]. There must be balance between the generation of these free radicals and scavenging activities of antioxidants. However, when there is an alteration in this balance, oxidative stress occurs [3, 4].

Antioxidants are well known for their role in deactivating free radicals that have damaging roles to biological cells [5]. The most prevalent antioxidants seen in plants are polyphenolic compounds, which are the secondary plant metabolites that arise from a common intermediate, phenylalanine, or its close precursor, shikimic acid [6]. Polyphenolic compounds are categorized into four main types namely flavonoids, phenolic acids, stilbenes and lignans [6]. Evidences from epidemiologic studies have revealed that consumption of leafy vegetables was associated with reduced risk of diseases due to the presence of their antioxidant properties. Previous studies have demonstrated that the antioxidant activity of plant materials is positively correlated to their phenolic components which thus indicate its antioxidant activity [7, 8].

Studies to date have demonstrated that phytochemicals in fruits and leafy vegetables can have biologic

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effects including scavenging of oxidative agents, boosting of the immune system, regulation of gene expression in cell proliferation and apoptosis, hormone metabolism and antibacterial and antiviral properties [9, 10]. Therefore, in recent years, considerable focus has been geared towards identification of plants with antioxidant ability that may be used for human consumption [11].

Moringa oleifera belongs to the family Moringaceae and it is indigenous to South Asia, mainly in foothills of Himalayas, India. It is grown and naturalized in other countries such as Afghanistan, Nepal, Bangladesh, Sri Lanka, South and Central America, West Indies, Philippines and Cambodia [12, 13]. Some of the reported pharmacological activities of *Moringa oleifera* plant parts comprises of antioxidant [14], hepatoprotective [15, 16], anthelmintic [17], wound healing [18], antimicrobial [19] and immunomodulatory activities [20]. These reported pharmacological activities have been associated with the presence of various bioactive secondary metabolites [21]. Research have suggested that environmental conditions that are connected with climate change may cause up to 50% both increase and decrease in secondary metabolites [22].

Considering earlier reports that showed different levels of antioxidant/phenolic content among plants of similar species [23], and the famous antioxidant capacity of *Moringa oleifera* and its usage, this study was designed to compare the antioxidant level in *Moringa oleifera* leaf from different locations in South Western Nigeria

Methods

Sample collection
Leaves of *Moringa oleifera* were collected from four different locations each from six states in South Western Nigeria. These plants were identified and deposited in the herbariums and assigned voucher numbers. The exact location of these was measured using Geographic Information System (GIS) (GPD map 64 s) (Table 1). The leaves were air dried separately and ground into powder for further analysis.

Phytochemical screening/in vitro antioxidant activities
Phytochemical tests (flavonoid, total phenol, saponin and tannins) were performed on the powdered samples by adopting standard procedures to identify the constituents as depicted by Edeoga, and Trease and Evans [24, 25].

Test for flavonoid
To deduce the presence of flavonoids, three methods were used [24, 26]: To a portion of aqueous filtrate of each leaf sample, 5 mL of dilute ammonia solution were added and then concentrated H_2SO_4. The detection of a yellow colouration in each sample indicates the presence

of flavonoids. This yellow colouration disappears on standing.

A few drops of 1% aluminium solution was added to a portion of each filtrate, and observation of a yellow colouration also indicated the presence of flavonoid.

A portion of the powdered leaf of each samples were heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate shaken with 1 mL of dilute ammonia solution. The detection of a yellow colouration also indicates a positive test for flavonoids.

The total flavonoid content was estimated by repeated extraction of 10 g of the plant sample at room temperature with 100 ml of 80% aqueous methanol. The solution was pooled and filtered through Whatman filter paper No 42 (125 mm). After filtration, it was evaporated to dryness over a water bath using a crucible until a constant weight was recorded.

Test for total phenol
Folin-Ciocalteau reagent was used for estimating the total phenolic content of *M. oleifera* as depicted by Siddhuraju and Becker [27]. About 20 µg of the powdered leaf sample was taken separately into a test tube and made up to 1 mL with distilled water. To this, diluted Folins-phenol reagent in a ratio of 1:1 with water and 20% of 2.5 mL of sodium carbonate Na_2CO_3 were added. The mixture was properly mixed by adequate shaking and then incubated in the dark to allow the development of colour for 40 min. After the incubation, the absorbance of the mixture was measured at 725 nm. To obtain the total phenolic content, a calibration curve using gallic acid with linearity obtained within 10–50 µg/mL was used. Therefore, the total phenolic content of the samples were expressed as mg GAE/g extract (mg of gallic acid equivalent).

Test for saponin
About 2 g of the powdered leaf sample was boiled in 20 ml of distilled water in a water bath and filtered. A stable persistent froth after vigorous shaking of 10 mL of the filtrate mixed with 5 mL of distilled water was mixed with 3 drops of olive oil. This was also vigorously shaken and, then, examined for the emulsion formation.

Total saponin content was estimated using the method of Obadoni and Ochuko [28]. To 20 g of each of the samples, 100 cm^3 of 20% aqueous ethanol were added in a conical flask. This was then heated at about 55 °C for 4 h over a hot water bath with continuous stirring. After this, the mixture was filtered and the process repeated for the residue. The solutions were combined after the extractions and were reduced to 40 mL at about 90 °C

over water bath. Then, 20 mL of diethyl ether was added to the concentrate in a 250 mL separating funnel and vigorously shaken. The aqueous layer was recovered and the purification process repeated. To this, 60 mL of n-butanol was added and the combined n-butanol solution was washed two times with 10 mL of 5% aqueous sodium chloride while the remaining solution was heated in a water bath. The samples were made to dry in the oven till a constant weight was obtained after evaporation. Saponin content was then expressed as percentage of the sample.

Test for tannins
An amount of 0.5 g of the dried powdered leaf sample was added to 20 ml of water in test tube and boiled after which it was filtered. Observation of a brownish-green or blue-black colouration after adding few drops of 0.1% ferric chloride indicates the presence of tannins.

The tannin content of *M. oleifera* was estimated by weighing 500 mg of the sample into a 50-mL plastic bottle. To this, 50 mL of distilled water was added and shaken with a mechanical shaker for 1 h. This was then filtered directly into a 50-ml volumetric flask and with distilled water made up to the mark. Then 5 ml from the filtrate was mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide in a test tube. Within 10 min, the absorbance at 120 nm was measured.

Inhibition of lipid peroxidation
Using egg yolk homogenate as lipid-rich media, a modified thiobarbituric acid (TBA) reactive substances assay was used to measure the lipid peroxide formed [29]. To egg homogenate (0.5 ml, 10 % v/v) in a test tube, 0.1 mg of leaves powder was added. This was made up to 1 ml with distilled water. To induce lipid peroxidation, 0.05 ml of FeSO4 (0.07 M) was added and incubated for 30 min. A volume of 1.5 ml of 20% acetic acid that have been adjusted to pH 3.5 with NaOH together with 1.5 ml of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 20% TCA (trichloroacetic acid) were added. The mixtures were firstly vortexed and then heated for 60 min at 95 °C. This was left to cool and after the 60 min, and 5.0 ml of butan-1-ol was added to each tube and centrifuged for 10 min at 3000 rpm. At 532 nm, the absorbance of the organic layer was measured. Inhibition of lipid peroxide formed by each sample was calculated in percentage with the formula $(1 - E/C) \times 100$ (C = absorbance of the fully oxidized control while E = absorbance in the presence of extract). $(Abs_{532 + TBA} - Abs_{532 - TBA})$.

DPPH radical scavenging activity
The method of Mensor et al. [30] of the 2,2- diphenyl-2-picrylhydrazyl (DPPH) assay system was used for the DPPH radical scavenging activity. One millilitre of a 0.3 mM DPPH methanol solution was added to different concentration of 2.5 ml solution of the powdered leaf sample (50 µg/ml, 250 µg/ml, 500 µg/ml) and allowed to react at room temperature for 30 min. The control was prepared in similar manner but without the sample. Methanol was used for the baseline correction. The absorbance of resulting mixture was measured at 518 nm and calculated as percentage antioxidant activity (AA %), using the formula: $AA\% = [(Abs_{blank} - Abs_{sample}) \times 100]/Abs_{blank}$, Abs = absorbance.

Nitric oxide radical scavenging activity
Using the method of Sreejayan and Rao [31], the nitric oxide radical scavenging activity was evaluated. In this assay, the nitric oxide radicals produced from sodium nitroprusside solution were measured by the Griess reagent. When sodium nitroprusside is in aqueous solution, it spontaneously generates nitric oxide radicals when at physiological pH and thus interferes with oxygen to produce nitrite ions. There is reduction in the production of nitrite ions when scavengers of nitric oxide act against oxygen. About 3 mL of sodium nitroprusside (10 mMol/L) in phosphate buffer saline (0.2 mMol/L, pH 7.4) was added to various concentration of the powdered leaf solution (50–250 µg/mL). This was incubated at 25 °C for 150 min. After this, 500 µL of Griess reagent (2% orthophosphoric acid, 1% sulphailamide, 0.1% N-1-napthylethylenediamine dihydrochloride) was added. The absorbance at 546 nm was measured and using the same formula as DPPH, percentage of inhibition was calculated. The low optical density values indicate high nitric oxide radical scavenging activity.

Statistical analysis
All data were shown as means ±standard deviation of means. The analysis was carried out using a one-way analysis of variance (ANOVA) and the means of the groups were compared using the Duncan test. The statistical significance was evaluated at $p < 0.05$ level. In addition, the results were analysed using MetaboAnalyst 4.0. The data were normalized by log transformation and mean centered to obtain a bell shape. PCA (principal component analysis) and PLSD-DA (partial least squares-discriminant analysis) were used to determine differences between the groups and major sources of variation are shown on heat maps. Samples were grouped according to 5-year average annual precipitation, states of the location, type of soil and temperature (both maximum and minimum) of the locations.

Results
The details of samples from each location is shown in Table 1 while Figs. 1, 2, 3, 4, 5, 6 and 7 present the
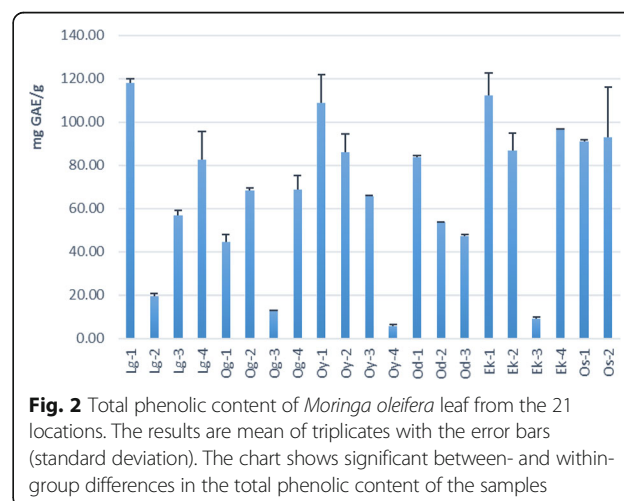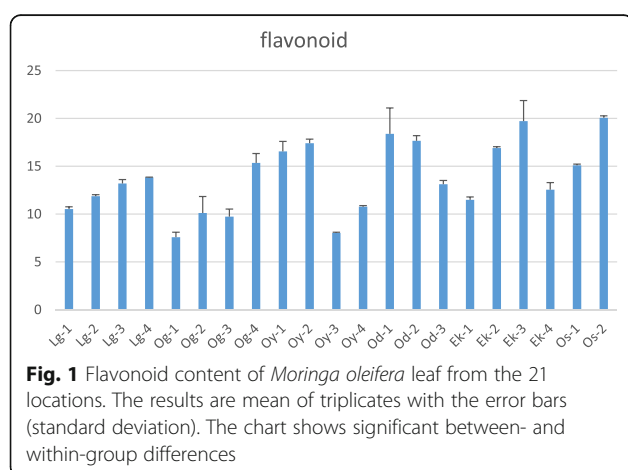
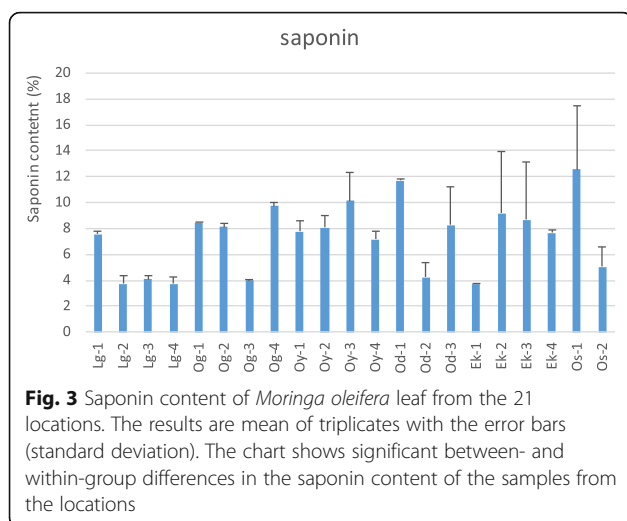**Table 1** The table showing the details of the samples collected from the 6 different states in Nigeria

S/no	Sample state	Sample city/town	Local govt area	Sample acronym	Geographical location		Herbarium number
					Latitude	Longitude	
1	Ekiti	Ado-Ekiti	Ado-Ekiti	Ek-2	07° 36′ 10.0″ N	005° 12′ 17.6″ E	FUTA/BIO/506
2	Ekiti	Ayegbaju-Ekiti	Oye	Ek-2	07° 47′ 41.4″ N	005° 17′ 37.7″ E	FUTA/BIO/505
3	Ekiti	Ikere-Ekiti	Ikere	Ek-3	07° 31′ 20.0″ N	005° 13′ 26.2″ E	UHAE2018019A
4	Ekiti	Iworoko-Ekiti	Ifelodun/Irepodun	Ek-4	07° 43′ 56.1″ N	005° 15′ 39.4″ E	UHAE2018109B
5	Lagos	Badagry	Badagry Central	Lg-1	06° 26′ 08.5″ N	002° 53′ 31.5″ E	FPI2184
6	Lagos	Ikola	Agbado/Oke-Odo	Lg-2	06° 37′ 24.7″ N	003° 15′ 15.3″ E	FUTA/BIO/511
7	Lagos	Ikorodu	Ijede	Lg-3	06° 36′ 35.3″ N	003° 33′ 54.7″ E	FUTA/BIO/525
8	Lagos	Isheri Olofin	Alimosho	Lg-4	06° 34′ 32.5″ N	003° 15′ 49.2″ E	FUTA/BIO/507
9	Ogun	Abeokuta	Abeokuta South	Og-1	07° 00′ 21.9″ N	003° 21′ 55.0″ E	FUTA/BIO/517
10	Ogun	Arepo	Obafemi-Owode	Og-2	06° 41′ 29.0″ N	003° 25′ 36.5″ E	FUTA/BIO/508
11	Ogun	Ibefun	Odogbo-Olu	Og-3	06° 43′ 00.3″ N	003° 40′ 10.7″ E	FUTA/BIO/509
12	Ogun	Sango Ota	Ado-Odo/Ota	Og-4	06° 42′ 42.5″ N	003° 16′ 27.7″ E	FUTA/BIO/513
13	Ondo	Akure	Akure South	Od-1	07° 17′ 37.0″ N	005° 09′ 39.3″ E	FUTA/BIO/523
14	Ondo	Igbara Oke	Ifedore	Od-2	07° 24′ 20.9″ N	005° 02′ 44.7″ E	FUTA/BIO/516
15	Ondo	Owo	Owo	Od-3	07° 12′ 50.4″ N	005° 33′ 06.3″ E	FUTA/BIO/522
16	Osun	Ipetu-Ijesa	Oriade	Os-1	07° 20′ 36.7″ N	004° 53′ 54.1″ E	IFE17731
17	Osun	Osogbo	Osogbo	Os-2	07° 46′ 20.3″ N	004° 31′ 52.0″ E	IFE17730
18	Oyo	Eleso	Ido	Oy-1	07° 23′ 43.2″ N	003° 42′ 46.0″ E	FUTA/BIO/521
19	Oyo	Ogbomoso	Ogbomoso North	Oy-2	00° 00′ 57.0″ N	004° 15′ 00.7″ E	IFE17734
20	Oyo	Oyo	Oyo West	Oy-3	07° 50′ 03.1″ N	003° 54′ 52.7″ E	FUTA/BIO/520
21	Oyo	Tapa	Ogo Oluwa	Oy-4	00° 03′ 19.5″ N	004° 09′ 12.5″ E	FUTA/BIO/519

flavonoid content, phenolic content, saponin content, tannin content, lipid peroxidation, DPPH radical scavenging activity and nitric oxide radical scavenging activities respectively.

The flavonoid content was presented on the chart in Fig. 1. The percentage flavonoids of the samples ranged from 8.04 ± 0.08 (Oy-3) to 20.07 ± 0.21 (Os-2) with jus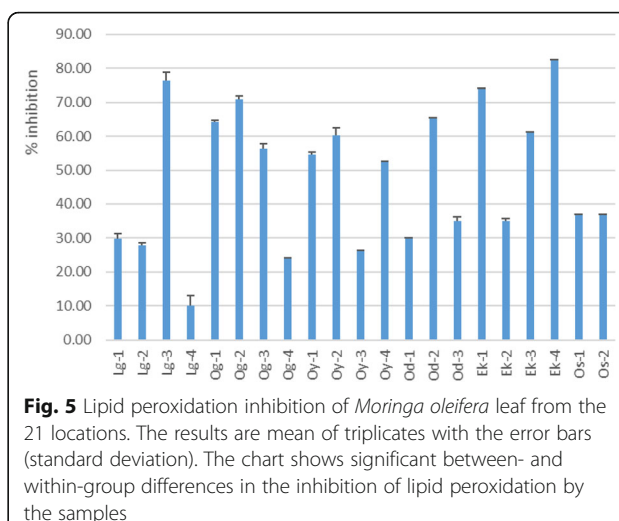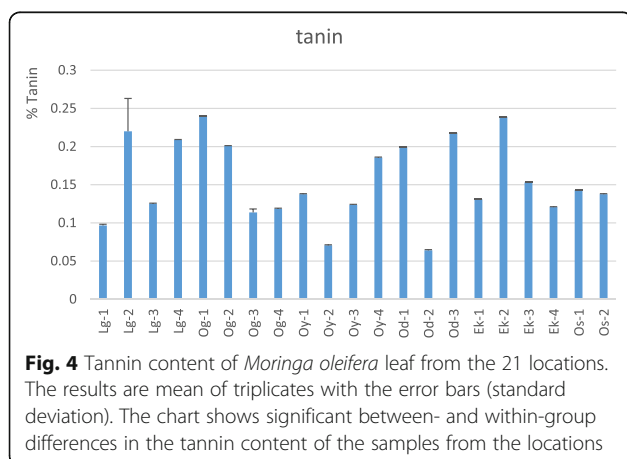t 2 samples having a value smaller than 10%. It showed between- and within-group significant differences at $p < 0.05$. The phenol content of the samples ranged from 5.78 ± 0.76 (Oy-4) to 117.83 ± 2.10 (Lag-1) (Fig. 2). Phenol content of ten of the twenty one samples was above 80 mg GAE/g but four of them had under 20 mg GAE/g. The ANOVA also showed both within- and between-group significant differences.

Fig. 1 Flavonoid content of *Moringa oleifera* leaf from the 21 locations. The results are mean of triplicates with the error bars (standard deviation). The chart shows significant between- and within-group differences

Fig. 2 Total phenolic content of *Moringa oleifera* leaf from the 21 locations. The results are mean of triplicates with the error bars (standard deviation). The chart shows significant between- and within-group differences in the total phenolic content of the samples

Fig. 3 Saponin content of *Moringa oleifera* leaf from the 21 locations. The results are mean of triplicates with the error bars (standard deviation). The chart shows significant between- and within-group differences in the saponin content of the samples from the locations

Fig. 5 Lipid peroxidation inhibition of *Moringa oleifera* leaf from the 21 locations. The results are mean of triplicates with the error bars (standard deviation). The chart shows significant between- and within-group differences in the inhibition of lipid peroxidation by the samples

The saponin content of the samples also showed significant between- and within-group differences (Fig. 3). Percentage saponin content of samples ranged from 3.72 ± 0.06 (Ek-1) to 12.56 ± 4.96 (Os-1). Majority of the samples had less than 10 % saponin content. Also, the tannin content of the samples do not look too different from the trend earlier found in the samples, with significant between- and within-group differences (Fig. 4). The lowest content was 0.06 ± 0.00 (Od-2) and the highest 0.24 ± 0.00 (Og-1).
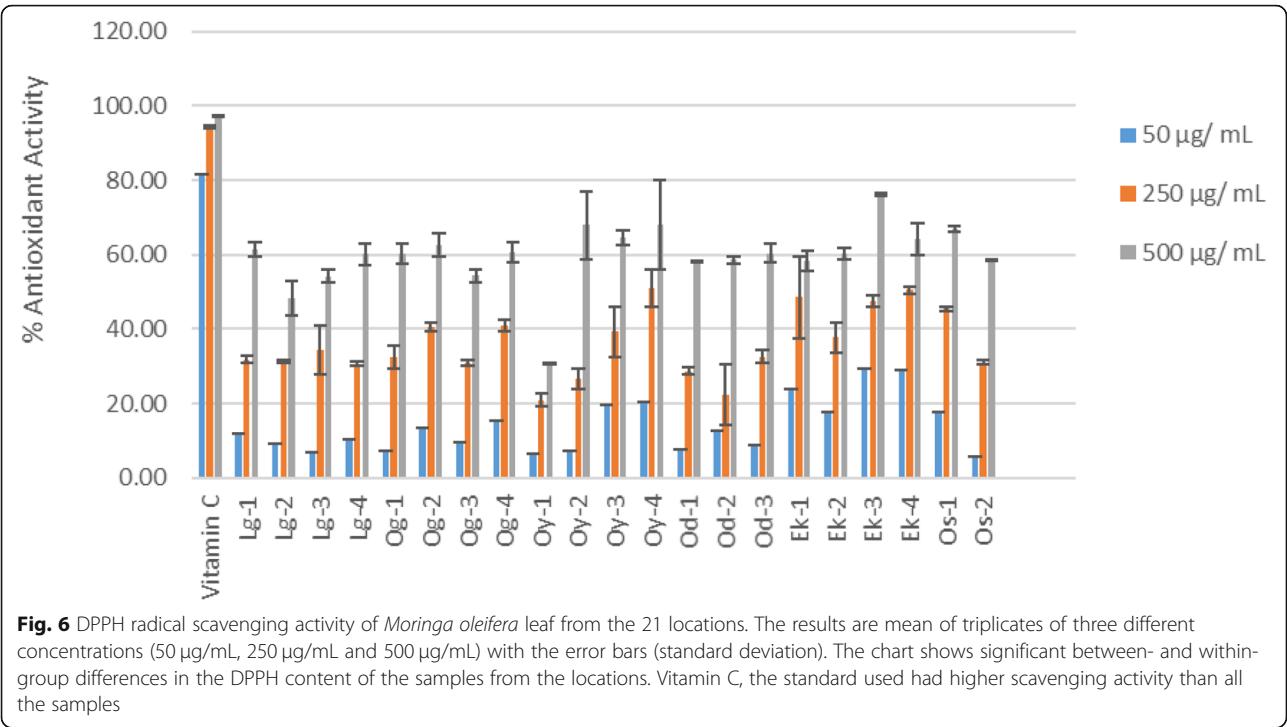
Inhibition of lipid peroxidation of eleven samples was above 50% (Fig. 5). As one sample had over 80% lipid peroxidation inhibition (Ek-4), another had as little as 10% inhibition of lipid peroxidation (Lg-4). The percentage DPPH radical scavenging activity of the standard was more than those of the samples with the ANOVA showing significant between- and within-group differences (Fig. 6). The sample with the lowest nitric acid scavenging activity (Oy-2) had
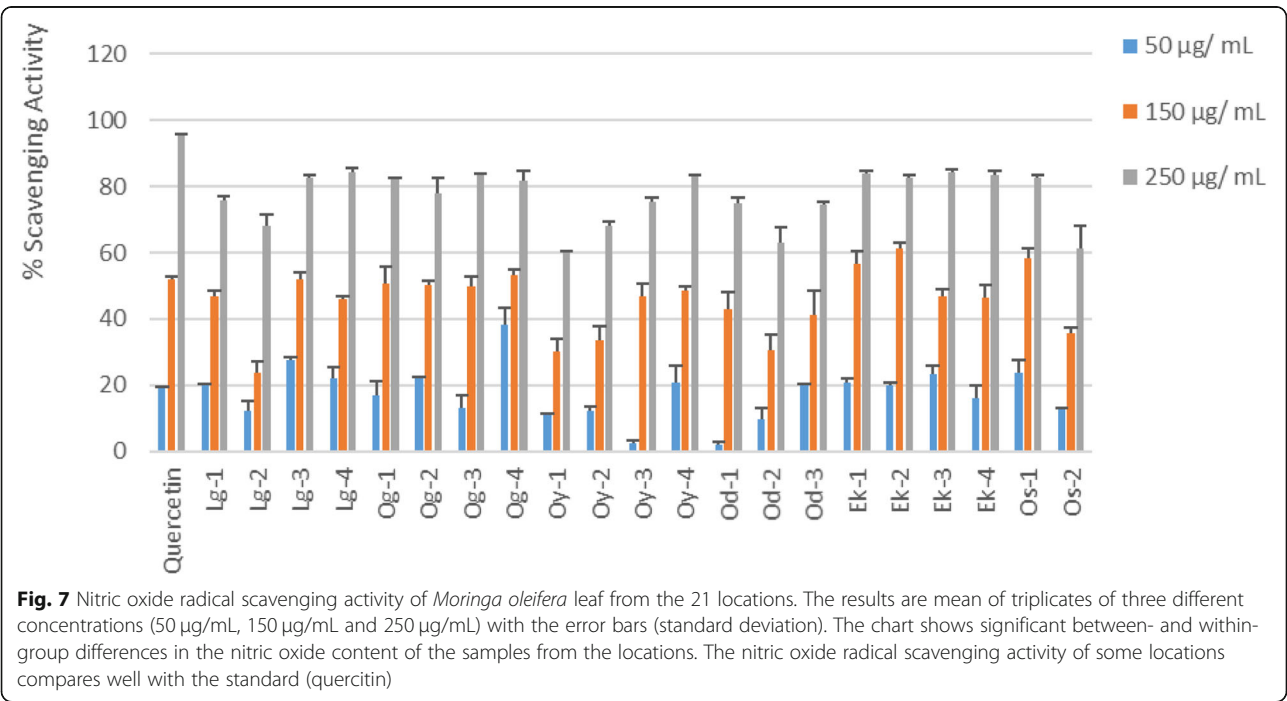

Fig. 4 Tannin content of *Moringa oleifera* leaf from the 21 locations. The results are mean of triplicates with the error bars (standard deviation). The chart shows significant between- and within-group differences in the tannin content of the samples from the locations

7.28%, 26.48% and 67.93% for 50, 250 and 500 μg/mL concentration of the samples respectively. While the sample with the highest activity is from the same state (Oy-4) with 20.48%, 51.03% and 68.14% nitric acid scavenging activity for 50, 250 and 500 μg/mL concentration of the samples respectively. The ANOVA also showed within- and between-group differences.
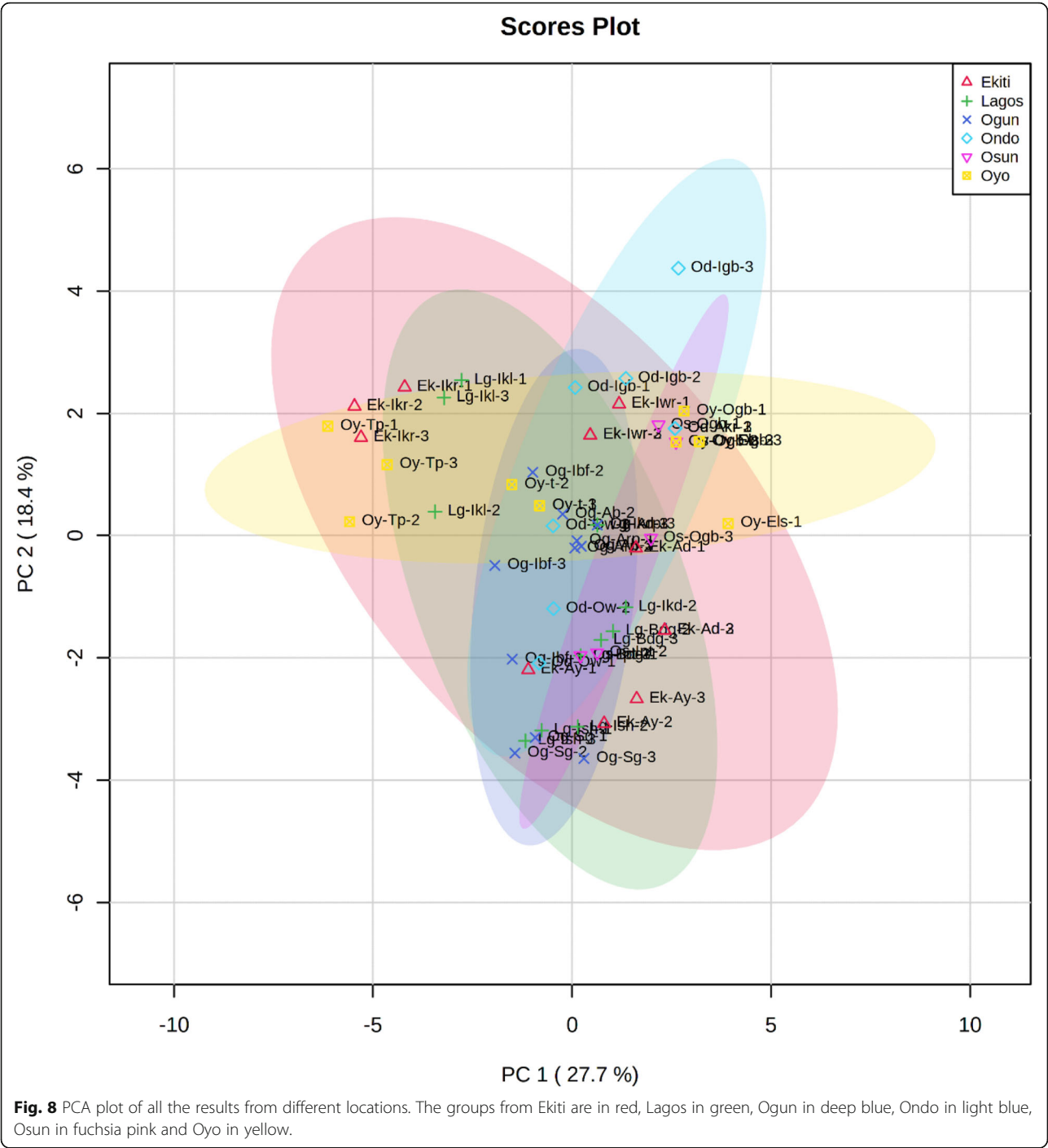
PCA plot of all the results from different locations is shown on Fig. 8. The PCA allows a more comprehensive comparison of all the parameters at a go. Also, PCA attempts to show differences within and between the different parameters analysed without considering the different groups each parameter falls. This is usually termed unbiased. The groups from Ekiti (red), Lagos (green) and Ogun (deep blue) had similarities in their grouping while groups from Ondo (light blue) and Osun (fuchsia pink) had similarities in their group and groups from Oyo cuts across them. PC1 was 27.7% and PC2 18.4% which explains almost 50% of the variation. The PLS-DA (Fig. 9) plot further pushes Ekiti, Lagos and Ogun together but this time around pushes the Oyo group towards the Ondo and Osun group unlike the PCA. Furthermore, the permutation test (Fig. 10) validates this prediction by PLS-DA. The observed statistics was $p = 0.008$. The heat map revealed at a glance the pattern of differences of the parameters checked between the different groups from different states (Fig. 11).
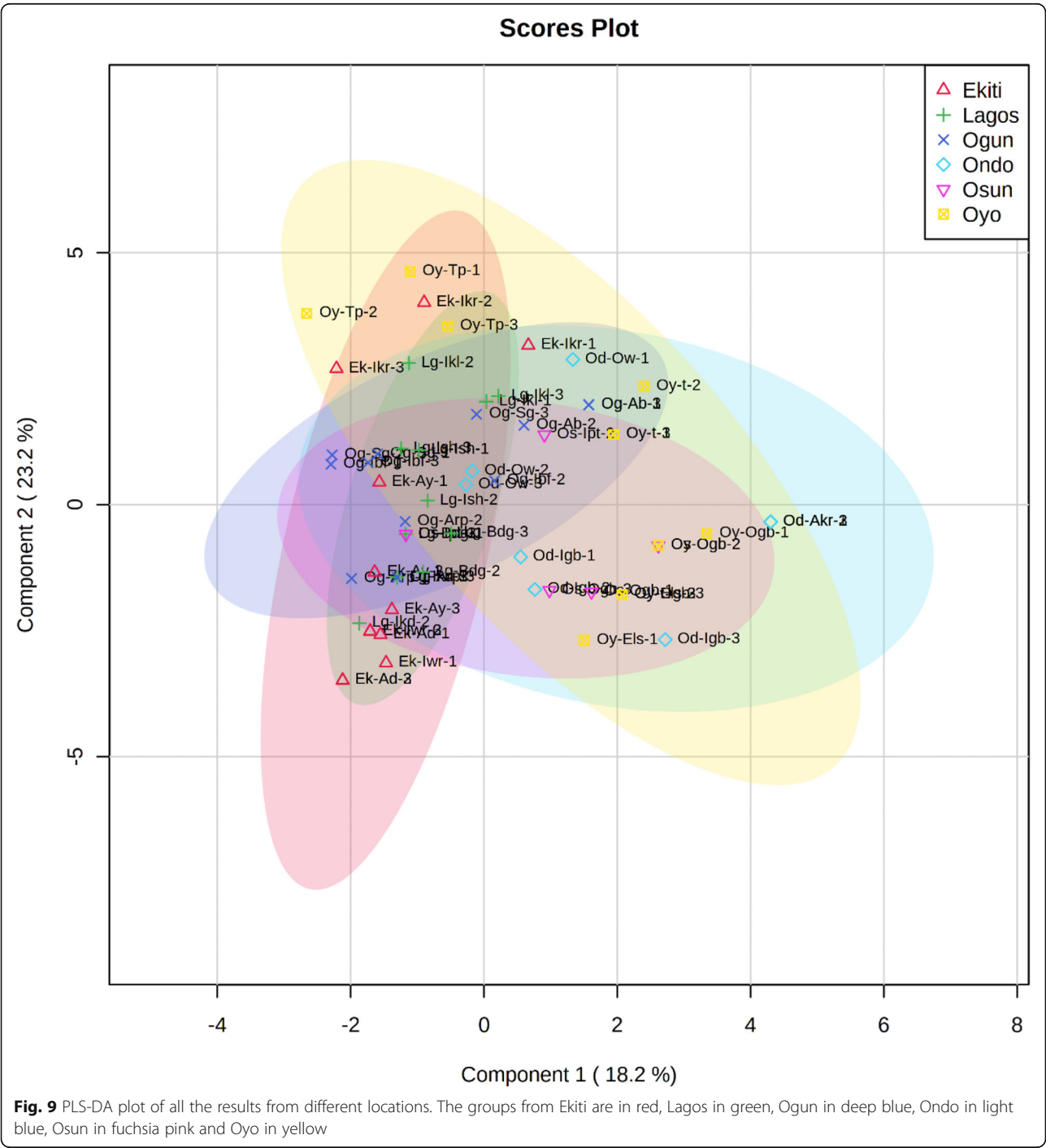
Probing further, the likely environmental influence on the parameters checked showed that annual precipitation, minimum temperature and maximum temperature based on average of 5 years record did not show notable influence on the parameters (results not shown). However, groups based on soil type according to global

Fig. 6 DPPH radical scavenging activity of *Moringa oleifera* leaf from the 21 locations. The results are mean of triplicates of three different concentrations (50 µg/mL, 250 µg/mL and 500 µg/mL) with the error bars (standard deviation). The chart shows significant between- and within-group differences in the DPPH content of the samples from the locations. Vitamin C, the standard used had higher scavenging activity than all the samples
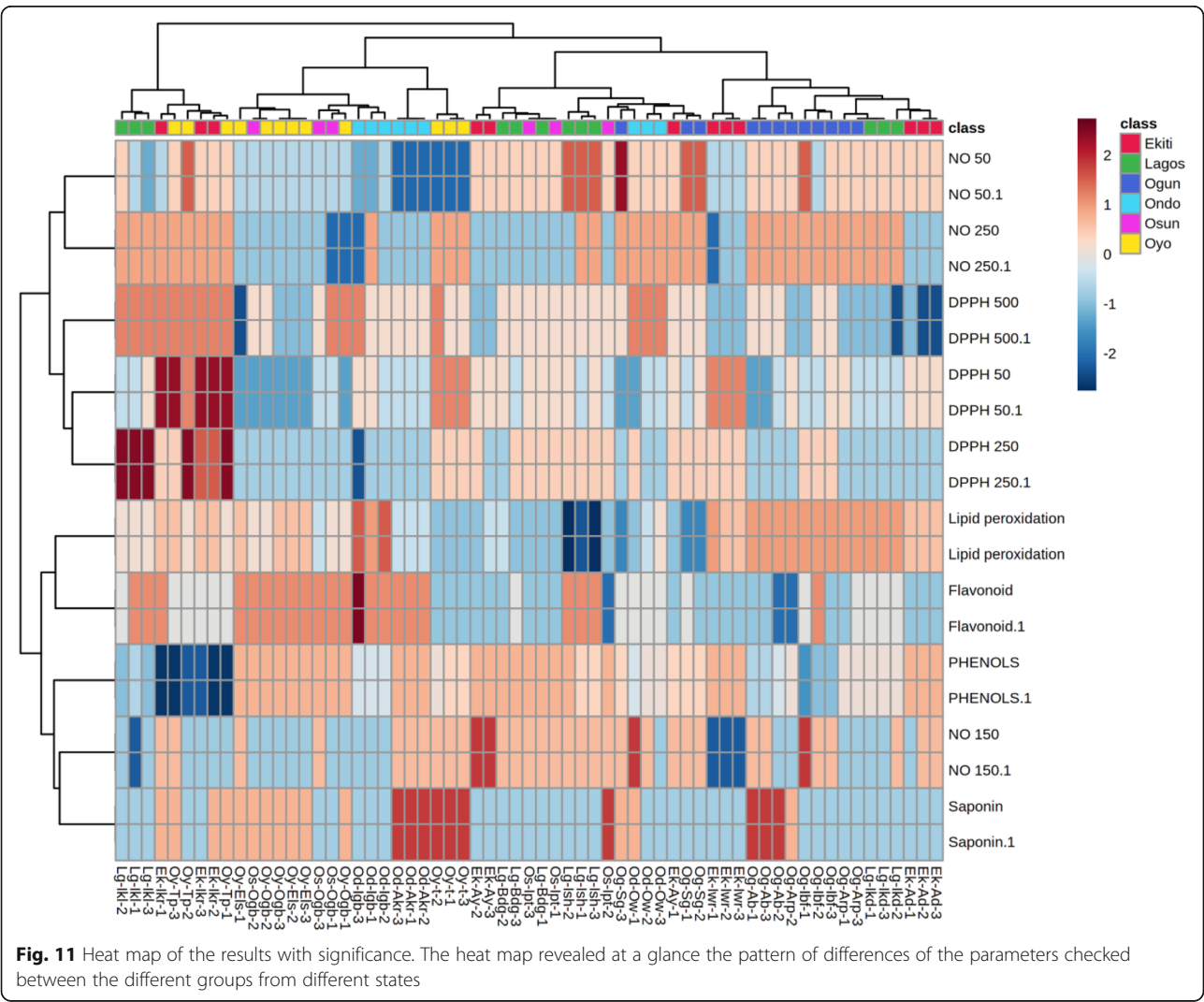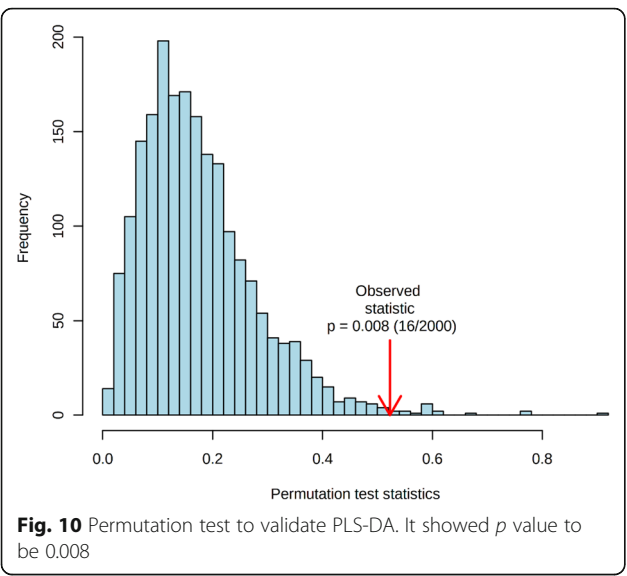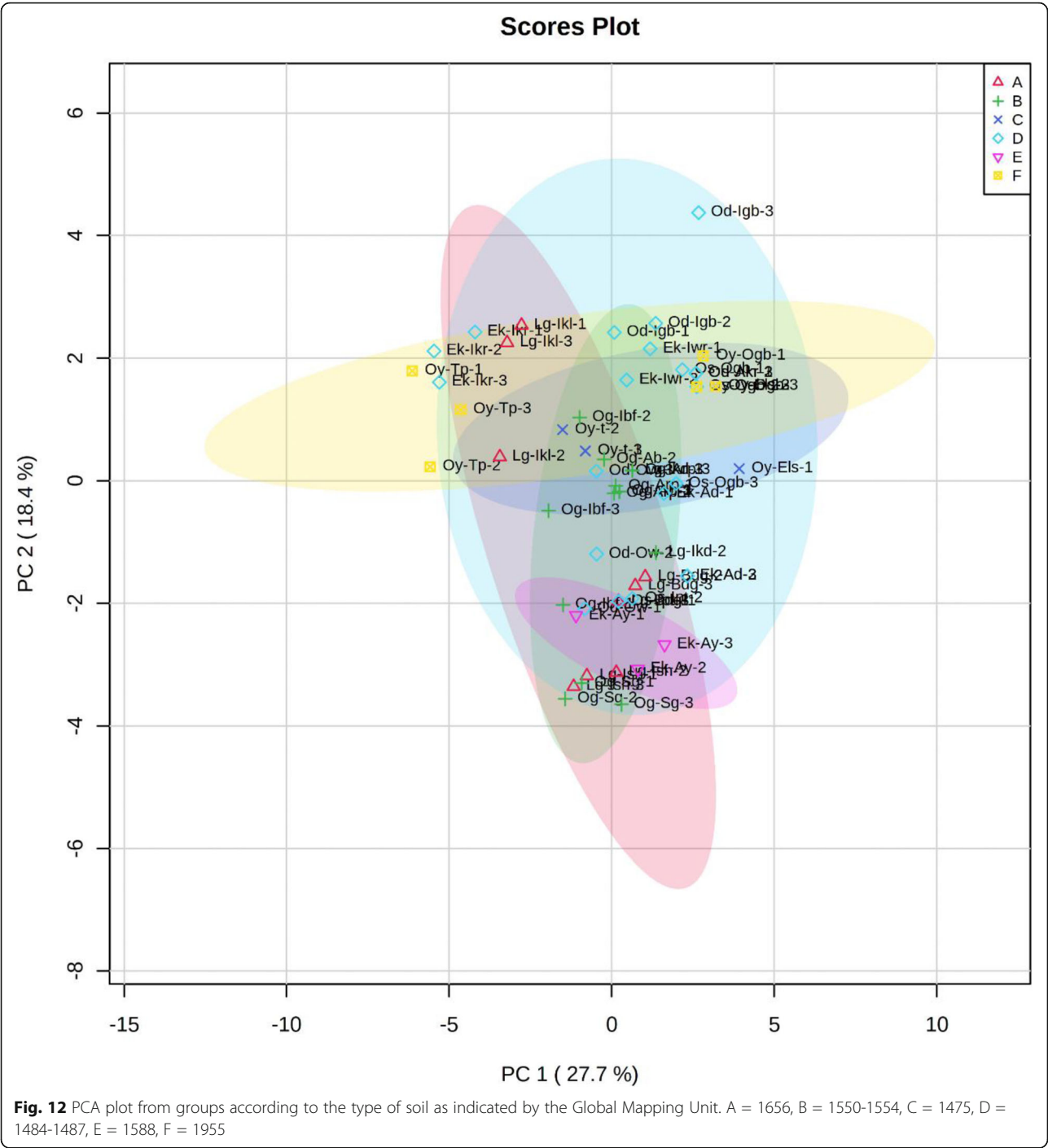
mapping unit (MU-GLOBAL) suggested influence of the soil on the observed differences. The results do not seem to really distinguish the groups with PCA (Fig. 12) with most of the groups being on the same cluster. However, the PLS-DA gave a better picture of the separation with two of the groups pushed further away from the others

(Fig. 13). The *p* value of the permutation validates the PLS-DA cluster at *p* = 0.0085 (Fig. 14). Also, the heat map (Fig. 15) showed at a glance the pattern of differences of the parameters checked between the different groups according to soil type using Global Mapping Unit classification.

Fig. 7 Nitric oxide radical scavenging activity of *Moringa oleifera* leaf from the 21 locations. The results are mean of triplicates of three different concentrations (50 µg/mL, 150 µg/mL and 250 µg/mL) with the error bars (standard deviation). The chart shows significant between- and within-group differences in the nitric oxide content of the samples from the locations. The nitric oxide radical scavenging activity of some locations compares well with the standard (quercitin)

Fig. 8 PCA plot of all the results from different locations. The groups from Ekiti are in red, Lagos in green, Ogun in deep blue, Ondo in light blue, Osun in fuchsia pink and Oyo in yellow.

Fig. 9 PLS-DA plot of all the results from different locations. The groups from Ekiti are in red, Lagos in green, Ogun in deep blue, Ondo in light blue, Osun in fuchsia pink and Oyo in yellow

Fig. 10 Permutation test to validate PLS-DA. It showed *p* value to be 0.008

Fig. 11 Heat map of the results with significance. The heat map revealed at a glance the pattern of differences of the parameters checked between the different groups from different states

Fig. 12 PCA plot from groups according to the type of soil as indicated by the Global Mapping Unit. A = 1656, B = 1550-1554, C = 1475, D = 1484-1487, E = 1588, F = 1955

Fig. 13 PLS-DA plot from groups according to the type of soil as indicated by the Global Mapping Unit. A = 1656, B = 1550-1554, C = 1475, D = 1484-1487, E = 1588, F = 1955

Fig. 14 Permutation test statistics of PLS-DA of groups according to soil type from the Global Mapping Unit (GMU). The permutation showed $p = 0.0085$

Fig. 15 Heat map of the parameters with significance. The heat map revealed at a glance the pattern of differences of the parameters checked when the different samples were grouped according to soil type using Global Mapping Unit classification

Discussion

Antioxidant activity observed in plant has been reported to be directly proportional to phenolic content [7, 8]. Three out of four samples from Ogun state had significantly lower flavonoids which also reflects in the total phenolic content. However, significantly low phenolic content are from different states. However, sizable number of samples has comparable phenolic and flavonoid content to each other. The antioxidant activities of phenols and flavonoids have been proved to be due to the high ability to donate hydrogen [32]. Among phytochemicals shown to be effective anti-carcinogenic, antimicrobial and antioxidant are saponin and tannin [33–35].

Lipid peroxidation is a major process that causes deterioration in food. In order to avoid synthetic antioxidants which are potentially injurious to health, natural sources are of interest. Studies have shown *Moringa oleifera* leaves as good preservative [36]. Deterioration of food has been linked to lipid peroxidation, and prevention of lipid peroxidative damage by an antioxidant is determined by the amount of hydroxyl group the molecule possess [37, 38]. The significance of between- and within-group lipid peroxidation values shows care had to be taken when the leaves are being used for preservative to ensure collection of leaves at the location that produces leaves with very good lipid peroxidation ability.

The ability of the samples to scavenge free radicals were demonstrated in both DPPH and nitric oxide scavenging activity. The result showed a concentration-dependent free radical scavenging ability of the samples (Figs. 6 and 7). Biochemical processes of aerobic life and its metabolism are known to generate free radicals [1]. Reactive oxygen species produced by group of enzymes known as nitric oxide synthases have been known to cause damage to biological macromolecules like proteins, DNA and membrane lipids when there is an imbalance in the generation and scavenging of the free radicals. This results in different disease conditions such as diabetes, arterosclerosis, inflammation and cancer [4, 39, 40]. Therefore, the ability of medicinal plants to scavenge nitric acid is seen as an advantage. The nitric acid scavenging activities of the sample compares well with the standard. These results suggest that the samples have good capacity to scavenge nitric oxide radicals.

The PCA plot of the parameters explained about 50% (PC1, 27.7% and PC2, 18.4%) of the variation while the PLS-DA explained over 40% (component 1, 18.2 % and component 2, 23.2%) of the variation. This further supports that samples sourced from locations in Ekiti, Lagos and Ogun had more similarities in the antioxidant content analysed. The prediction from the PLS-DA was further validated by the permutation which was set at 2000 and was significant at p = 0.008. Validation of PLS-DA

with permutation test has been seen as an important step in modelling [41]. These results suggest a possible common influence on the antioxidant parameters from samples collected from the locations that cluster around the same area in the plot. PCA is used to show separation when between-group variation is greater than within-group variation [41, 42]. Analysis of results is confirmed whenever unbiased methods like PCA are first used and then validated by PLS-DA [42]. Usually, PCA results are used for drawing up biological conclusions that are then confirmed or further tested using PLS or OPLS. This way, biologically relevant results are accomplished. The heat maps present clearly the notable differences in antioxidant activity of the plant from the different locations.

The literature have shown that geography, seasons, water stress, light factors, temperature, herbivory, soil factors and altitude influence the secondary metabolites found in *Camelia sinensis* (tea) [22]. Also, studies showed that climatic factors influenced wireworm risk in maize [43]. Therefore, the samples were placed into different groups other than states of the country the locations are situated. The groups based on the type of soil as classified by the GMU show input of the type of soil in the variation observed between and within the groups (Figs. 12, 13, 14, and 15). This could be seen in the clusters of group tending towards the clusters initially recorded in Fig. 13. This is unlike groups according to other parameters (annual precipitation, minimum temperature and maximum temperature based on average of 5 years record) that did not show distinct clusters according to the initial cluster (results not shown). This suggests that the soil type confer notable influence on the antioxidant component of the plant. And this is suggested to come from climatic influence on the soil as observed by Poggi et al. [43]. Also, environmental conditions connected with climate change have been shown to possibly cause as much as 50% increase or decrease in secondary metabolites of tea [22]. Furthermore, geographical differences have been shown to influence some mineral constituents of *Moringa oleifera* [44].

When the environmental conditions of the locations with highest and lowest parameters were compared (annual precipitation, minimum temperature and maximum temperature based on average of 5 years record and soil type), they were mostly different from each other with a few having the same environmental condition. However, the total flavonoids with a very wide difference between the highest and the lowest have notable differences in all these environmental parameter checked. At the cellular level of every plant, agro-environmental factors are said to affect homeostasis of organic acid [45] and thus other plant constituents. Presence of plants in different environment means exposure to diverse climatic and environmental

conditions [46]. For example, different seasons have been shown to have impact on the nutritional content of *Moringa oleifera*. Also, phenylpropanoid biosynthesis pathway in plants has been shown to be regulated in response to external factors at many levels [47, 48].

Conclusion
Different locations affected the different antioxidant parameters analysed. This is suggested to be from the influence of environmental parameters like annual precipitation, minimum temperature and maximum temperature on soil. Therefore, the type of soil seems to play a notable role in this. *Moringa oleifera* from different locations showed differences in its antioxidant activities and thus antioxidant content. Further work, analyzing the soil around the plant, might confirm the extent of the influence of soil.

Abbreviations
ANOVA: Analysis of variance; GIA: Geographic Information System; TBA: Thiobarbituric acid; TCA: Trichloroacetic acid; DPPH: 2,2-Diphenyl-2-picrylhydrazyl; PCA: Principal component analysis; PLSD-DA: Partial least squares-discriminant analysis; GAE: Gallic acid equivalent; GMU: Global Mapping Unit

Plant authentication
Leaves of *Moringa oleifera* were collected from four different locations each from six states in South Western Nigeria. The leaves were identified and deposited in the herbariums of the Department of Biology Federal University of Technology Akure, Nigeria, Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti Nigeria, Department of Pharmacognosy, Faculty of Pharmacy Obafemi Awolowo University Ile-Ife, Nigeria and Department of Botany Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria. All identified plants were assigned the voucher specimen numbers.

Authors' contributions
ABO conceived the study and participated in its design, execution and helped to draft the manuscript; CAO participated in the design, coordination and supervision; ORM participated in coordination and interpretation and data analysis; IN participated in the experimental, data analysis and the execution of the study. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission. All author(s) read and approved the final manuscript.

Availability of data and materials
The authors confirm that the data supporting the findings of this study are available within the article and its supporting attached files.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
No potential conflict of interest was reported by the authors.

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