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RESEARCH ARTICLE

Classification of different edible plants based on total phenolic and *in vitro* antioxidant activities using principal component and hierarchical cluster analysis

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Abstract

An acetone-aqueous extracts of 16 edible plants was prepared; their Total Phenolic Content (TPC), 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging (ABTS), Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) were measured. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to clarify the correlations between antioxidant assays and plant extracts. The first (PC1) and the second (PC2) principal components accounted for 91.60 % of the overall variability, whereas HCA classified samples into three main groups based on measured parameters. The 16 plants were classified into high, medium and low antioxidant activity groups based on according to TPC and the antioxidant activities results. Those in the high-antioxidant activity group were *Punica granatum*, *Rheum officinale*, *Phyllanthus niruri* and *Melaleuca leucadendra*. The plants in the medium-antioxidant activity group were *Thymus vulgaris*, *Datura metel*, *Menthae arvensis*, *Parameria laevigata*, *Glycyrrhiza glabra*, *Curcuma xanthorrhiza* and *Zingiber officinale*. The plants in the low-antioxidant activity group were *Plantago major*, *Guazuma ulmifolia*, *Centella asiatica*, *Sericocalyx crispus* and *Piper nigrum*. Positive relationships existed between TPC and the antioxidant activity of the extracts, indicating that the principal contributors to the antioxidant characteristics of the studied plants are phenolic compounds. Some of these plants may serve as valuable sources of natural antioxidants.

Keywords: antioxidant activity; hierarchical cluster analysis; Principal Component Analysis; Total Phenolic Content

Introduction

Reactive Oxygen Species (ROS) are detrimental by-products generated during cellular respiration. Excess ROS in cells can damage proteins, lipids and DNA, cumulatively resulting in oxidative stress (1). Oxidative stress, characterised by an imbalance favouring oxidants over antioxidants, has been proposed as a contributing factor to ageing and numerous diseases in humans (2, 3). Antioxidant activity reflects the power of a compound to stop oxidative damage. Dietary antioxidants can enhance cellular defences and protect biological components from oxidative damage (4). Natural antioxidants have received much attention recently because of their scavenging properties (5) and because they are natural products, they are favoured by consumers (6). Like synthetic antioxidants, natural antioxidant containing higher phenolic compounds can scavenge free radicals effectively, chelate transition metals and absorb ultraviolet light, so stopping progressive oxidative damage and preventing undesirable odours and flavours (7). Recently, there have been considerable efforts to identify new natural sources of active antioxidant compounds (8). Phenolic-rich plant extracts are utilised in the food industry to enhance food quality by inhibiting the oxidative deterioration of lipids. There are two types of *in vitro* antioxidant activity assays: those based on Hydrogen Atom Transfer (HAT) and those based on Electron Transfer (ET). In HAT assays, the thermally generated peroxyl radicals will be a target to compete between the antioxidant and substrate and ORAC assay is an example. ET assays measure the capacity of a compound to reduce an oxidant and resulted in a color change. The antioxidant concentration in sample is correlated with extent of the change in color. Examples of ET-based assays include DPPH, ABTS and FRAP (9). Due to different mechanisms of antioxidant action; antioxidant activity for a sample should be evaluated using several methods (10). PCA is a statistical technique which basically reduces the dimensionality of a large datasets in an interpretable way. The aim of this study was to analyse extracts prepared from 16 edible plants commonly used in Indonesia and worldwide for their total phenolic content and antioxidant activity. PCA was used to report the details variations in the antioxidant activity among the studied plants and to classify them.

Material and Methods

Chemicals

Sodium carbonate was from RDH (Germany), glacial acetic acid from Mallinckrodt Baker (USA), Trolox (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid), fluorescein disodium salt (FL), sodium acetate trihydrate, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), gallic acid, 2,2'-azo-bis(2-amidinopropane) dihydrochloride, (AAPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and potassium persulfate were purchased from Sigma (USA), Folin-Ciocalteu reagent, ferric chloride ($FeCl_3.6H_2O$) and HCl were obtained from Merck, (Germany). All chemicals and reagents used in this study were of analytical grade.

Preparation of plant extracts

Plant samples were collected from Jember District, East Java; all plants were properly authenticated by the Indonesian Institute of Sciences at Purwodadi Botanical Garden, East Java, Indonesia. Prior to extraction, the materials were cleaned, diced, dried for three days and grinded into a fine powder. Table 1 presents the common names, scientific names, family classifications and the part used for extracts preparation of all plants. Following the methods described previously (11), 0.1 g of dried plant powder were extracted with 10 mL of 70 % aqueous acetone using a magnetic stirrer at 1000 rpm (IKA, Germany). Extract was centrifuged for 10 min at 4750 g using thermo-line, a mini centrifuge (China) and the collected supernatants were used.

Determination of Total Phenolic Content (TPC)

TPC was reported following the method of Slinkard and Singleton (12). Folin-Ciocalteu reagent (500 µL) was added to each plant extract, gallic acid standard, or blank (100-µL), followed by the addition of 1 mL sodium carbonate (7.5 %, w/v). Results were compared to gallic acid at absorbance of 765 nm after 120 min of incubation using microplate reader spectrophotometer (Biotek, USA).

Determination of Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay method (13) was followed to determine reducing/antioxidant power. FRAP reagent was prepared as using 300 mM acetate buffer, 10mM TPTZ (2,4,6- tri(2-pyridyl)-s-triazine) in 40 mM HCl and 20mM $FeCl_3$ $6H_2O$ in the ratio of 10:1:1. For assays, 1900 µL FRAP reagent was mixed with 100 µL of each plant extract, Trolox standard, or blank and incubated for 30 min before reading absorbance at 595 nm using microplate reader spectrophotometer (Biotek, USA).

Determination of DPPH radical scavenging activity assay

The DPPH assay method (14) was followed to determine radical scavenging activity. A 3 mL of DPPH solution (40 mg/L) was mixed with 100 µL of each plant extract, Trolox standard or blank and incubated for 30 min before reading absorbance at 517 nm using microplate reader spectrophotometer (Biotek, USA).

Determination of ABTS radical scavenging activity

The ABTS method (15) was followed to determine radical scavenging activity. ABTS radical cations were generated by oxidizing 2.45 mM potassium persulfate and 7 mM ABTS in the dark. Using distilled water; ABTS solution was adjusted to an absorbance of 1 ± 0.02 at 734 nm. A 100 µL of each plant extract, Trolox standard, or blank was added to 1 mL of ABTS solution and incubated for 5 min before reading absorbance using microplate reader spectrophotometer (Biotek, USA).

Determination of Oxygen Radical Absorbance Capacity (ORAC)

A 150 µL fluorescein (10 nM) was mixed with 25 µL Trolox (6.5 µM to 50 µM), buffer (blank), or sample in 96-well black plate (Thermo Scientific, USA). An automatic injector was used to add 25 µL AAPH (153 mM) to the solution. The measurement was conducted at 485 nm excitation wavelength and 520 nm emission wavelengths. ORAC values were calculated by subtracting the area under the curve (AUC) of the blank from the AUC of each sample and by comparison with the Trolox standard curve. The absorbance of solutions was measured using FLUO star Omega microplate fluorescence reader (BMG Labtech, Germany).

Table 1. Common name, scientific name, family name of the plants collected from Jember, East Java, Indonesia

No.	Common name	Scientific name	Family name	Part used
1	Stonebreaker	*Phyllanthus niruri*	Euphorbiaceae	Aerial part
2	Thyme	*Thymus vulgaris*	Lamiaceae	Aerial part
3	Pomegranate	*Punica granatum*	Punicaceae	Fruit rind
4	Centella	*Centella asiatica*	Umbelliferae	Aerial part
5	Bastard cedar	*Guazuma ulmifolia*	Sterculiaceae	Leaves
6	Yellow strobilanthus	*Sericocalyx crispus*	Acanthaceae	Leaves
7	Broadleaf plantain	*Plantago major*	Plantaginaceae	Leaves
8	Black pepper	*Piper* nigrum	Piperaceae	Fruit
9	Cajeput tree	*Melaleuca leucadendra*	Myrtaceae	Leaves
10	Devil's trumpet	*Datura metel*	Solanaceae	Leaves
11	Wild mint	*Mentha arvensis*	Lamiaceae	Aerial part
12	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
13	Tight wood	*Parameria laevigata*	Apocynaceae	Wood
14	Liquorice	*Glycyrrhiza glabra*	Leguminosae	Root
15	Rhubarb	*Rheum officinale*	Polygonaceae	Root
16	Java Ginger	*Curcuma xanthorrhiza*	Zingiberaceae	Rhizome

Statistical analysis

Results are expressed as means with Standard Deviation (SD) of three measurements. The significance of differences of $p < 0.05$ among mean values was evaluated by Analysis of Variance (ANOVA) using MINITAB® (16.00) software. Correlation analyses were performed using Pearson's correlation coefficient (r). PCA and HCA were applied to the data for classification.

Results and Discussion

As can be seen in Table 2 the TPC varied clearly among plant extracts tested. The range of the TPC value is from 126 to 6833 mg GAE /100 g (DW), a dissimilarity of almost 54-fold between the TPC values of the samples. *R. officinale*, *P. granatum*, *M. leucadendra* and *P. niruri* showed the highest TPC values (>5000 mg GAE/100 g DW) and *P. nigrum* showed the lowest value. FRAP values of the studied samples ranged from 246 to 20969 mg TE /100 g DW, a dissimilarity of nearly 85-fold between the FRAP values. *P. granatum*, *R. officinale*, *M. leucadendra* and *P. niruri*, also showed the highest antioxidant activities in the FRAP assay. *P. nigrum* and *P. major* showed the lowest FRAP values.

Table 2 showed the DPPH radical scavenging activities of the samples. The DPPH value ranged from 4377 to 41156 mg TE/100 g DW, with a difference of about 9-fold between the DPPH scavenging activities. *P. granatum* showed the highest antioxidant activity, followed by *R. officinale*, *P. niruri* and *M. leucadendra*, while the lowest antioxidant activity was shown by *P. nigrum*. ABTS radical scavenging activities are shown in Table 3. The activities determined in this assay ranged from 24 to 1741 mg TE/100 g DW, representing the largest difference between the lowest and highest activities (approximately 73-fold) among the four assays. *P. niruri* showed the highest antioxidant activity, followed by *M. arvensis*, *P. laevigata*, *M. leucadendra*, *P. granatum* and *R. officinale*. Consistent with the results of the TPC, FRAP and DPPH assays, the *Piper nigrum* extract showed the lowest ABTS value (24 mg TE/100 g DW). The ORAC values are shown in Table 3, values ranged from 9997 to 69035 mg TE /100 g DW, showing about 7-fold variation between the lowest and highest ORAC values. The extract from *R. officinale* showed the highest ORAC value, followed by those from *Z. officinale*, *P. granatum* and *P. niruri*. *C. asiatica* and *G. ulmifolia* extracts showed the lowest ORAC values.

Several studies have evaluated the antioxidant activity of *R. officinale* (16-18) and *P. niruri* (19-21). *P. granatum* is one of the oldest known edible fruits and its antioxidant activity has been determined in other studies (22, 23). *M. leucadendra* is considered one of the plants with the highest antioxidant activities (24). Other studies have analyzed the antioxidant activities of *T. vulgaris* (25), *D. metel* (26), *M. arvensis* (27), *C. xanthorrhiza* (28-30), *G. glabra* (31, 33), *P. laevigata* (34) and *Z. officinale* (35, 36). Other studies have investigated the antioxidant activities of *C. asiatica* (37,38); *P. major* (39, 40), *G. ulmifolia* (41) and *S. crispus* (42). High antioxidant and radical scavenging activities were reported for *P. nigrum* in other studies (43, 44), but in this study where the *P. nigrum* extract showed low antioxidant activity.

The correlation coefficient between total phenolic content and antioxidant activities

Table 3 displays the correlation coefficient (R) values between TPC and antioxidant activities. TPC was strongly correlated with FRAP and with DPPH. TPC values for all plant extracts showed a medium correlation with ABTS and a good correlation with ORAC. These results show the importance of phenolic compounds in the radical scavenging activity as well

Table 2. Total Phenolic Content (TPC), Ferric Reducing Antioxidant Power (FRAP), DPPH radical scavenging activity (DPPH), ABTS radical scavenging activity (ABTS) and Oxygen Radical Absorbance Capacity (ORAC) in different plant extracts

Plant name	TPC[a]	FRAP[b]	DPPH[b]	ABTS[b]	ORAC[b]
P. niruri	5270.85 ± 275.92 ^{D}	9617.19 ± 1296.49 ^{D}	29501.2 ± 0 ^{D}	1740.77 ± 1.99 ^{A}	39550 ± 1552 ^{D}
T. vulgaris	2195.74 ± 93.07 ^{H}	3204.12 ± 123.95 ^{G}	14923 ± 0 ^{G}	1707.57 ± 11.28 ^{AB}	19248 ± 862 ^{GH}
P. granatum	6477.69 ± 27.18 ^{B}	20968.8 ± 1060.88 ^{A}	41156.3 ± 106.18 ^{A}	1732.47 ± 1.99 ^{A}	47439 ± 3537 ^{C}
C. asiatica	826.39 ± 14.67 ^{L}	1101.27 ± 60.78 ^{HI}	8529.11 ± 270.4 ^{J}	1148.96 ± 75.58 ^{D}	9997 ± 943 ^{L}
G. ulmifolia	926.91 ± 23.79 ^{KL}	1339.65 ± 72.49 ^{HI}	7624.05 ± 43.85 ^{K}	1170.75 ± 50.9 ^{D}	11287 ± 759 ^{L}
S. crispus	794.77 ± 47.79 ^{L}	1253.39 ± 29.98 ^{H}	6782.28 ± 95.01 ^{L}	988.69 ± 57.53 ^{E}	18284 ± 1100 ^{HI}
P. major	973.39 ± 7.4 ^{KL}	831.97 ± 73.36 ^{H}	7212.66 ± 197.32 ^{K}	1004.77 ± 32.32 ^{E}	12195.5 ± 1200.5 ^{KL}
P. nigrum	126.01 ± 6.42 ^{M}	245.5 ± 14.22 ^{I}	4377.22 ± 7.31 ^{M}	24.48 ± 3.59 ^{F}	11590 ± 276 ^{KL}
M. leucadendra	5893.11 ± 95.2 ^{C}	13803.8 ± 411.1 ^{C}	29018.4 ± 291.99 ^{C}	1735.06 ± 4.15 ^{A}	31172 ± 1188.19 ^{E}
D. metel	1467.54 ± 104.88 ^{J}	1520.74 ± 164.41 ^{H}	9117.72 ± 14.62 ^{HI}	1448.24 ± 16.28 ^{C}	13515 ± 9 ^{JK}
M. arvensis	3425.18 ± 202.54 ^{F}	5069.72 ± 526.84 ^{F}	13342.5 ± 325.17 ^{F}	1738.69 ± 1.99 ^{A}	32280 ± 416 ^{E}
Z. officinale	1100.36 ± 40.84 ^{K}	2839.39 ± 88.99 ^{G}	8836.78 ± 165.91 ^{I}	1679.56 ± 22.18 ^{B}	50496 ± 2672 ^{B}
P. laevigata	4460.21 ± 151.55 ^{E}	6312.66 ± 68.17 ^{E}	18549.4 ± 537.53 ^{D}	1738.17 ± 5.49 ^{A}	21535 ± 979 ^{G}
G. glabra	3158.75 ± 152.42 ^{G}	1671.8 ± 63.38 ^{H}	10647.1 ± 212.36 ^{G}	1727.28 ± 3.54 ^{AB}	26045 ± 549 ^{F}
R. officinale	6833.73 ± 175.52 ^{A}	18686.9 ± 992.71 ^{B}	32593.1 ± 491.08 ^{B}	1731.95 ± 3.59 ^{A}	69034.67 ± 1415.66 ^{A}
C. xanthorrhiza	1817.5 ± 12.32 ^{I}	1540.85 ± 78.31 ^{H}	9560.92 ± 205.72 ^{H}	1656.22 ± 55.85 ^{B}	15745 ± 1295 ^{IJ}
Minimum	126.010	245.500	4377.220	24.480	9997.000
Maximum	6833.730	20968.800	41156.300	1740.770	69034.670
Mean	2859.258	5625.484	15735.734	1435.852	26838.323
Std. deviation	2259.711	6644.706	11152.196	471.768	17140.787

There are no significant differences among values marked with the same superscript capital letters in individual columns

[a] mg gallic acid equivalents (GAE) per 100 g DW of sample

[b] mg Trolox equivalents (TE) per 100 g DW of sample

Table 3. Correlations (R^2) between Total Phenolic Content (TPC) and antioxidant values obtained using Ferric Reducing Antioxidant Power (FRAP), DPPH radical scavenging activity (DPPH), ABTS radical scavenging activity (ABTS) and Oxygen Radical Absorbance Capacity (ORAC)

	TPC	FRAP	DPPH	ABTS	ORAC
TPC	-	0.924	0.948	0.655	0.739
FRAP	-	-	0.971	0.483	0.785
DPPH	-	-	-	0.557	0.724
ABTS	-	-	-	-	0.543

as the reducing ability of the extracts. There were strong correlations among the values obtained using the various assay methods. In particular, the values obtained using the DPPH and FRAP were very strongly correlated. ORAC showed a good correlation with FRAP and DPPH. The weakest correlations were between the ABTS and those obtained using FRAP, DPPH and ORAC. These differences might result from the different antioxidant mechanisms detected in each assay and this should be studied further.

Positive relationships between TPC, free radical scavenging activity and ferric reducing power have been reported in other studies (45, 46). However, some researchers found no or only weak correlations between phenolic contents and antioxidant activities (47) and between different antioxidant assay methods (48). The link between antioxidant activities and TPC could be affected by the various testing methods and by other substances with antioxidant properties, such as ascorbic acid, sugars and carotenoids, present in the extracts. (49).

Principal Component Analysis

PCA was applied to the standardized results of the 16 plants and 79.359 % of the variability in the dataset was explained by the first principal component (PC1). This significant proportion indicates that the PC1 has successfully captured most of the patterns in the data. PC2 (second principal component) adds another 12.237 % to the total explained variability, bringing the cumulative total to 91.60 %. Adding the third principal component (PC3) into the mix raises the cumulative percentage to 98.62 %. The last two components, PC4 and PC5, contribute very little to the explained variability (only about 1.383 % collectively). Fig. 1 shows a sample score plot for PC1 vs PC2. *P. granatum* and *R. officinale* are located on the righthalf of the plot and *P. nigrum* is located away from other samples. This indicates that certain antioxidant

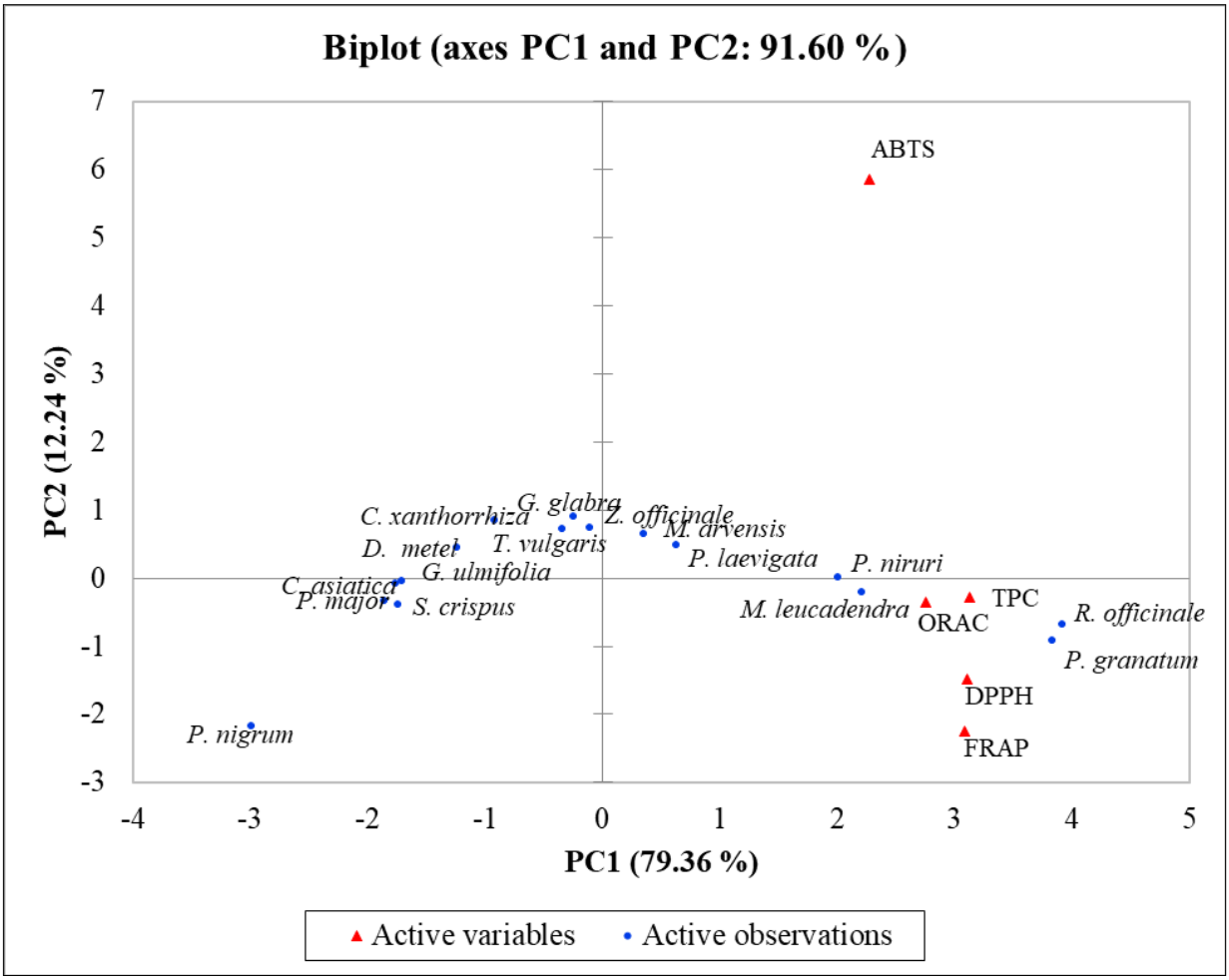

Fig. 1. Principal Component Analysis (PCA) scores plot for different plants and loading plots for different variables on PC1 and PC2. The locations of the plants in the plot are based on their antioxidant activity.

TPC: Total Phenolic Content; FRAP: Ferric Reducing/Antioxidant Power; DPPH: DPPH radical scavenging activity; ABTS: ABTS radical scavenging activity; ORAC: Oxygen Radical Absorbance Capacity; PC: Principal component analysis factors

characteristics are considerably different from those of the other samples.

The plot clusters TPC, FRAP, DPPH and ORAC on the right side, while ABTS occupies the upper right-hand side. Fig. 1 illustrates how the antioxidant activity of the plants determines their placement. The positioning of *P. granatum* in the lower right quadrant of Fig. 1 may be attributed to their elevated antioxidant activities, which are situated in this area of the principal component space. In contrast, *P. nigrum*, characterised by low antioxidant levels, is positioned in the lower left quadrant of Fig. 1 diametrically opposite to *P. granatum*.

The relations between TPC, FRAP, DPPH and ORAC and PCs are shown as an evidenced by their Pearson correlation coefficients (Table 4). PC1 positively correlates with TPC, FRAP, DPPH, ORAC and moderately with ABTS, suggesting it essentially represents the variance in these variables.

The second component (PC2) has a strong positive relationship with ABTS and a moderate negative relationship with FRAP and DPPH, meaning it reflects different information, especially related to ABTS. PC3 has a notable positive correlation with ORAC, capturing some unique variance not explained by PC1 or PC2.

The fourth and fifth components (PC4 and PC5) show low correlation with all variables, indicating their small contribution to the total variance. This result implies that most of the original variables are significantly associated with the first three principal components, corroborating their contribution to over 98 % of the total variability.

The squared cosines of the variables in PCA, presented in Table 5, show how well each principal component represents each variable. TPC, FRAP and DPPH have high squared cosines with PC1 (0.933, 0.906 and 0.917 respectively), signifying that this component predominantly represents them. PC1 and PC2 approximately equally represent ABTS, with squared cosines of 0.492 and 0.503, respectively, indicating that these components split its variance. ORAC's variance is mainly accounted for by PC1 and PC3, with squared cosines of 0.720 and 0.278 respectively. Low squared cosines show that PC4 and PC5 contribute little to the total variance and have weak connections with the original variables.

The given factor scores (Table 6) are the coordinates of the original observations (different plants) in the space defined by the principal components (PC1-PC5), indicating how each plant corresponds to the principal components. Plants like *R. officinale, P. granatum*, *P. niruri*and *M. leucadendra*, show high positive scores on PC1, implying high values for the variables most strongly associated with this component, while *Piper nigrum* shows a notably negative score, indicating low values for the same variables. Similarly, *C. xanthorrhiza*, *G. glabra*, *Z. officinale* and *T. vulgaris* present positive scores on PC2, suggesting high values for variables associated with PC2. Despite PC3-PC5 accounting for a smaller proportion of the total variance, they also provide valuable insights. Factor scores are instrumental for comparing observations and identifying patterns, with similar scores implying similarities in original variable values.

Table 2 also summarizes the statistics representing five variables, all with 16 observations and no missing data. The variables TPC, FRAP, DPPH and ORAC all have high standard deviations, suggesting substantial variability in these measures among the samples. TPC and FRAP have lower average values compared to DPPH and ORAC, which have the highest mean values, indicating they generally have higher measures in the dataset. In contrast, ABTS has the lowest mean value and least variability, suggesting it has more consistent, lower measurements across the samples.

Table 4. Correlations between TPC, FRAP, DPPH, ABTS radical scavenging activity (ABTS), ORAC and the PCA factors

	PC1	PC2	PC3	PC4	PC5
TPC	0.966	-0.033	-0.175	-0.186	0.007
FRAP	0.952	-0.271	-0.065	0.087	0.093
DPPH	0.957	-0.179	-0.189	0.083	-0.092
ABTS	0.702	0.709	-0.050	0.045	0.012
ORAC	0.848	-0.042	0.527	-0.017	-0.018

Table 5. Squared cosines of the variables (TPC, FRAP, DPPH, ABTS and ORAC)

	PC1	PC2	PC3	PC4	PC5
TPC	0.933	0.001	0.031	0.035	0.000
FRAP	0.906	0.074	0.004	0.008	0.009
DPPH	0.917	0.032	0.036	0.007	0.008
ABTS	0.492	0.503	0.002	0.002	0.000
ORAC	0.720	0.002	0.278	0.000	0.000

TPC: Total Phenolic Content

FRAP: Ferric Reducing/Antioxidant Power

DPPH: DPPH radical scavenging activity

ABTS: ABTS radical scavenging activity

ORAC: Oxygen Radical Absorbance Capacity

PC: Principal Component Analysis Factors

Table 6. Factor scores for the sixteen plant extracts indicating how each plant extract corresponds to the principal components (PC1 to PC5)

	PC1	PC2	PC3	PC4	PC5
Phyllanthus niruri	2.005	0.010	-0.176	-0.123	-0.437
Thymus vulgaris	-0.348	0.725	-0.302	0.228	-0.113
Punica granatum	3.830	-0.915	-0.453	0.460	0.015
Centella asiatica	-1.761	-0.079	-0.285	0.199	0.004
Guazuma ulmifolia	-1.711	-0.035	-0.211	0.148	0.084
Sericocalyx crispus	-1.745	-0.394	0.242	0.054	0.033
Plantago major	-1.862	-0.332	-0.117	0.011	0.017
Piper nigrum	-2.991	-2.168	0.240	-0.223	-0.068
Melaleuca leucadendra	2.213	-0.202	-0.765	-0.087	0.133
Datura metel	-1.240	0.456	-0.263	0.117	0.056
Menthae arvensis	0.351	0.653	0.240	-0.221	0.120
Zingiber officinale	-0.109	0.739	1.712	0.257	-0.050
Parameria laevigata	0.628	0.489	-0.651	-0.309	0.034
Glycyrrhiza glabra	-0.249	0.897	0.081	-0.392	-0.035
Rheum officinale	3.913	-0.688	0.949	-0.198	0.146
Curcuma xanthorrhiza	-0.924	0.845	-0.242	0.081	0.060

Dendrogram of HCA of plants extract

Dendrogram of HCA of plants extract is shown in Fig. 2; three well-defined clusters are grouped in based on their similarity. *M. leucadendra*, *P. niruri*, *P. granatum* and *R. officinale* comprise a group of samples (A) associated with high antioxidant activity. A second cluster (B) consists of *T. vulgaris*, *D. metel*, *M. arvensis*, *Z. officinale*, *P. laevigata*, *G. glabra*and *C. xanthorrhiza* because of their medium antioxidant activity. A third cluster (C) includes *C. asiatica*, *G. ulmifolia*, *S. crispus*, *P. major* and *Piper nigrum*.

HCA results agree with PCA results indicating that the samples are distinct from others. It was observed that the plants with high antioxidant activity had TPC > 5000 mg GAE/100 g DW, FRAP > 9000, DPPH > 25000, ABTS > 400 and ORAC > 40000 (mg TE/ 100g DW). Medium antioxidant activity group had TPC ranging from 5000 to 1000 mg GAE/100 g DW, FRAP from 9000 to 1000, DPPH from 9000 to 25000, ABTS from 400 to 1000 and ORAC from 4000 to 15000 (mg TE/ 100g DW). The plants with low antioxidant activity had TPC < 1000

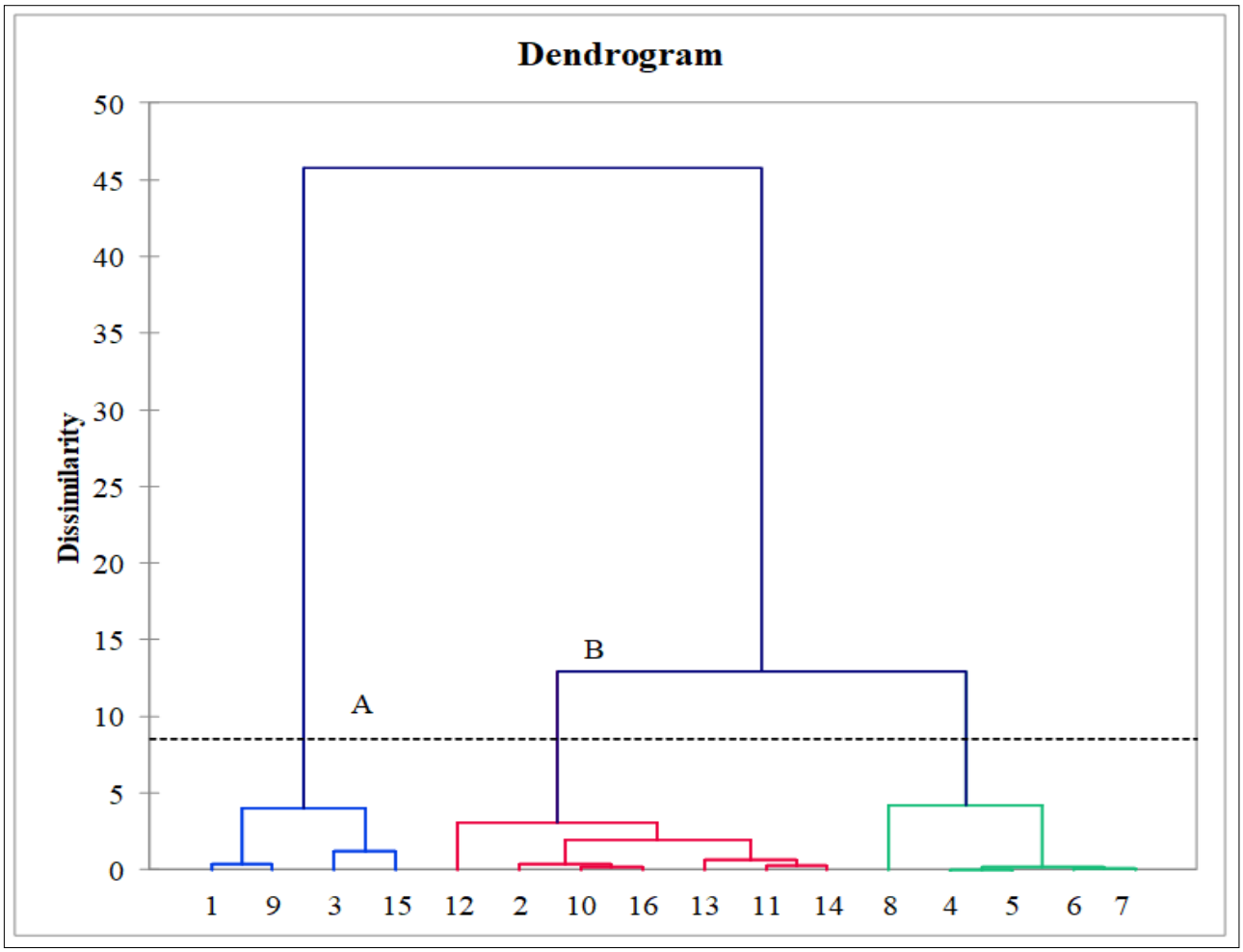

Fig. 2. Dendrogram of Hierarchical Cluster Analysis (HCA) of plants extract (The sample code as in Table 1). Three well-defined clusters are grouped in based on their similarity; (A): a group of samples associated with high antioxidant activity, (B): a second cluster for medium antioxidant activity group, (C): a cluster includes low antioxidant activity group.

mg GAE/100 g DW, FRAP < 1000, DPPH < 9000, ABTS < 100 and ORAC < 15000 (mg TE/ 100g DW).

Conclusion

The total phenolic contents and antioxidant activities of 16 plant extracts were differed significantly. The studied plants showing strong antioxidant activities are widely distributed, making them a promising source of natural antioxidants for food industries. The extracts from *P. granatum*, *R. officinale*, *P. niruri* and *M. leucadendra* showed the strongest antioxidant activities among the tested plants. The antioxidant activities of these plants are linked to their phenolic compounds, as higher levels of phenolic content were associated with stronger antioxidant activities. PCA and HCA facilitated the visualisation of the dataset and the underlying observed grouping relationships. Easy interpretation of differences and similarities in antioxidant activity of plants could be achieved by using a combination of sample characterization and multivariate data analysis. PCA and HCA provide powerful insights into the antioxidant profiles among different plants. Further investigations are required to identify the active compounds in these plant extracts and to demonstrate their biological activities.

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Authors' contributions

BK contributed the concepts. AA and KHM provided the overall design. BK, KHM and MAH verified the methodology part of the study. KHM and MAH checked the data curation. The original draft of the work was prepared and wrote by KHM and AA. BK, KHM and MAH edited the review part of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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