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Research Article

ANTIOXIDANT CAPACITY OF *N. INDICUM*: A CORRELATION STUDY USING PRINCIPAL COMPONENT ANALYSIS AND MULTIVARIATE STATISTICAL APPROACH

PRIYANKAR DEY, TAPAS KUMAR CHAUDHURI*

Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Siliguri–734013, West Bengal, India.
Email: dr_tkc_nbu@rediffmail.com, tapas.chaudhuri@gmail.com

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ABSTRACT

Objective: The aim of the present study was to evaluate the antioxidant capacity of the leaf, stem and root of *N. indicum* using principal component analysis and multivariate statistical approaches.

Methods: We have studied the antioxidant capacity by analysing various free radical scavenging activity of the leaf, stem and root of *N. indicum*. Quantification of total phenol and flavonoid species were also performed in order to get a definite picture of the antioxidant status of *N. indicum*. In the present study, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed to elucidate the inter-relationship of the outcome of various antioxidant assays. Correlation matrix was used to display the inter-relationship and a dendrogram coupled with corresponding icicle diagram expressed the similarity among different antioxidant assays.

Results: The loading plot of the PCA for *N. indicum* leaf, stem and root demonstrated 37.01% and 60.57% of the variance for PC1 and PC2, respectively. The results of hierarchical clustering also corroborated with the PCA loading plot and elucidated multiple clusters based on the proximity matrix.

Conclusion: The results obtained from the study demonstrated the underlying extensive interrelation between the different antioxidant and free radical scavenging capacities of *N. indicum*.

Keywords: *Nerium*, Antioxidant, Principal component analysis, Hierarchical cluster analysis, Phenol, Flavonoid, DPPH, Lipid peroxidation, Iron chelation, Oxidative stress

INTRODUCTION

The word antioxidant in general may be referred to a chemical species possessing reducing power. But in the biological domain, the word antioxidant is a general property to cumulatively perform a vast majority of functions encompassing from scavenging free radicals, inhibiting their activation, possessing reducing power or may be blocking peroxidation of lipid substances. It is paradox of metabolism that the key to survival i.e. oxygen, may act indirectly on our system through its reactive derivatives to destroy the biomolecules. The concept of ROS came to the lime light when Gershman in 1955 described the toxic property of reactive oxygen species (ROS) due to the partial reduced form of oxygen [1]. Two years later in 1956 after Harman's claim of free radicals being the cause of ageing, numerous other discoveries started to emerge correlating various diseases with ROS [2]. Since then, various free radicals with varying chemical class and physical nature have been found to be associated with various diseases such as neural disorders, cardiovascular diseases, cancer, diabetes and arthritis. In recent days, the main focus of nutraceutical companies is on natural products bearing potent antioxidant capacities and its pursuit have led the pharmaceutical companies to screen various herbal products which has been mentioned in the ancient texts having medicinal properties [3]. One such herb is *Nerium indicum* Mill (family Apocynaceae) which has been used in traditional medicine throughout the world for ages. Different groups of researchers have demonstrated its immunostimulatory and neuroprotective activity [4]; anti-anxiety properties [5]; the aqueous extract of the plant (Anvirzel™) has shown potent antitumor activity [6, 7]. An ethanol extract of this plant, SAOB-0401 (Xenavex™) is under Phase II clinical trial for anti-cancer activity [8]. Recently, a cardiac glycoside from the plant has been found to be a novel inhibitor of HIV infectivity [9]. The phytochemical analysis for all the major parts of the plant has already been performed [10]. We have previously demonstrated [11] the antioxidant and ROS scavenging capacity of the three major parts of *N. indicum* by studying nitric oxide, hydrogen peroxide, hydroxyl radical, peroxynitrite, superoxide, singlet oxygen, DPPH and hypochlorous acid scavenging activity. In addition, we have also demonstrated the lipid peroxidation inhibition, iron chelating capacity, trolox equivalent total antioxidant capacity (TEAC) and also quantified the total phenolic and flavonoid quantity of the 70% hydro-methanolic extract of the plant. Our present effort is to sketch the correlation between various antioxidant properties and the flavonoid and phenolic composition of the leaf, stem and root of *N. indicum* by using Principal Component Analysis (PCA) and Cluster Analysis.

PCA is a statistical method for data reduction dimensionally and thus, allow visualizing the underlying pattern in the variables of the experiments. To further ease the concept, PCA may be compared with the working of the brain. Our brain does not recognize a person by his/her facial variables (vis. nose, lips, eyes, ears), but recognizes the person by the cumulative effect of various variables, giving a simplified yet complete representation of the face. Similarly in PCA, the variables from the experimental data are arranged in the most simplified way to give the overall picture of the antioxidant profile of the plants. On the other hand, multivariate statistical data analysis approaches are powerful method for the relatively simple representation of the similarities between the experimental variables. The application of hierarchical cluster analysis (HCA) in this scenario has been performed to group different antioxidant properties according to the different variables, namely hydroxyl radical, nitric oxide, singlet oxygen, hypochlorous acid, superoxide, peroxynitrite, hydrogen peroxide scavenging activity, inhibition of lipid peroxidation, iron chelation, DPPH, TEAC and total phenolic and flavonoid content in the leaf, stem and root of *N. indicum*.

MATERIALS AND METHODS

Chemicals

The chemicals and reagents used in our previous experiments [11] were all of analytical grade. 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from Roche Diagnostics, Mannheim, Germany. 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (trolox) was obtained from Fluka, Buchs, Switzerland. Potassium persulfate ($K_2S_2O_8$), ethylenediamine tetraacetic acid (EDTA), ascorbic acid, 2-deoxy-2-ribose, trichloroacetic acid (TCA), mannitol, nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), sodium nitroprusside (SNP), sulfanilamide,

naphthylethylenediamine dihydrochloride (NED), L-histidine, lipoic acid, sodium pyruvate, quercetin and ferrozine were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Hydrogen peroxide, potassium hexacyanoferrate, Folin-Ciocalteu (FC) reagent, sodium carbonate (Na_2CO_3), butylated hydroxytoluene (BHT), sodium hypochloride (NaOCl), aluminium chloride ($AlCl_3$), ammonium iron (II) sulfate hexahydrate ($(NH_4)_2Fe(SO_4)_26H_2O$), potassium nitrite (KNO_2), N,N-dimethyl-4-nitrosoaniline and xylenol orange were obtained from Merck, Mumbai, India. 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Gallic acid and curcumin were obtained MP Biomedicals, France. Ferrous sulfate and catalase were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Evans Blue was purchased from BDH, England. Mangese dioxide was obtained from SD Fine Chemicals, Mumbai, India. Diethylene-triamine-pentaacetic acid (DTPA) was obtained from Spectrochem Pvt. Ltd., Mumbai, India. Thiobarbituric acid (TBA) was obtained from Loba Chemie, Mumbai, India. Sodium nitrite was obtained from Qualigens Fine Chemicals, Mumbai, India.

Plant Material

N. indicum plant was collected from the garden of University of North Bengal, India, during the month of June. The plant was identified by the Taxonomist Prof. A. P. Das of the Department of Botany, University of North Bengal and an accession number of 9618 was given. The voucher specimen was stored at the Herbarium of the Department of Botany, University of North Bengal.

Sample Preparation

The sample preparation for our previous antioxidant experiments [11] was performed in the following method. The whole plant was separated into three major parts: leaf, stem and root. The parts were washed properly with double distilled water. The parts were then shade dried at room temperature for 2 weeks and grinded to powder. The powder (100 g) was mixed with 70% methanol (1000 ml) and kept in a shaking incubator overnight (12h, 37^oC, 160 rpm). Then the mixture was centrifuged at 5000 rpm for 15 minutes. The pellet was mixed with 70% Methanol (1000 ml) and kept overnight at the shaking incubator and centrifuged. The supernatant liquid was collected from both the phases and filtered. The resultant filtrate was concentrated in a rotary evaporator under reduced pressure. The concentrated extract was lyophilized and stored at -20^oC until further use.

Antioxidant Assays

In our previous study [11], the leaf, stem and root extract of *N. indicum* was analysed for various ROS scavenging activity and the phenolic and flavonoid content quantification was performed. The details of the percentage of scavenging and the IC_{50} values were already shown in our previous study.

Statistical analysis

All the experiments were performed in six sets and the data were reported as the mean ± SD of six measurements. The IC_{50} values were calculated using KyPlot version 2.0 beta 15 (32 bit) for Windows by the formula $Y = 100*A1/(X + A1)$, where $A1 = IC_{50}$; Y = response (Y = 100% when X = 0); X = inhibitory concentration. The IC_{50} values were compared by paired t tests and $p< 0.05$ was considered significant. In the present study, in order to analyse the relationship between the antioxidant traits and the quantified phytochemicals, Principal Component Analysis (PCA) based on the correlation matrix was drawn. Two factors were extracted under varimax method. The data obtained from the study of the antioxidant profile were analysed by multivariate statistical approach, employing a hierarchical cluster analysis (HCA). The method employed was proximity matrix with between group linkages. The differences between the measured variables were calculated by square Euclidean distances. Transform values of variables (average zero and S.D. 1) called Z scores was carried out as a pre-treatment of the data. Horizontal dendrogram with all clusters icicle chart was carried out to elucidate the similarity or nearness of the various measured variables. PCA and HCA were performed using the IBM SPSS statistics version 20.0 software package for Windows.

RESULTS AND DISCUSSION

The tendency of the ROS to donate oxygen to various biomolecules leads to the destruction of cellular components. Antioxidants are chemical species which prevent the oxidation of the biomolecules and thus, prevent their degradation. Fruits and vegetables are potential source of antioxidant compounds. Our previous experiments with the three major parts of the *N. indicum* have already revealed the antioxidant and ROS scavenging capacity of the plant, which is evident from the IC_{50} values of different experiments. In this present study, PCA was used to identify the variation in the antioxidant capacity of the plant extracts and to demonstrate how the thirteen parameters namely DPPH, hydroxyl, singlet oxygen, super oxide, peroxynitrite, nitric oxide, hypochlorous acid, hydrogen peroxide, lipid peroxidation, iron chelation capacity, TEAC, phenol and flavonoid content contribute to the overall antioxidant capacity of *N. indicum*. The loading plot (Fig. 4) was used to draw an overview of the correlation among the various ROS scavenging potential of leaf, stem and root of *N. indicum* which describes how intricately the correlation between the various antioxidant capacities exist.

The loading of the first and second principal component (PC1 and PC2) accounted for 37.01% and 60.57% of the variance respectively when all the variables of leaf, stem and root are considered together (Fig. 4). When considered individually, PC1 for leaf, stem and root accounted for 54.65%, 34.29% and 43.26% of variance respectively and PC2 for leaf, stem and root accounted for 76.42%, 59.55% and 66.67% of variance respectively (Fig. 1, 2, and 3).

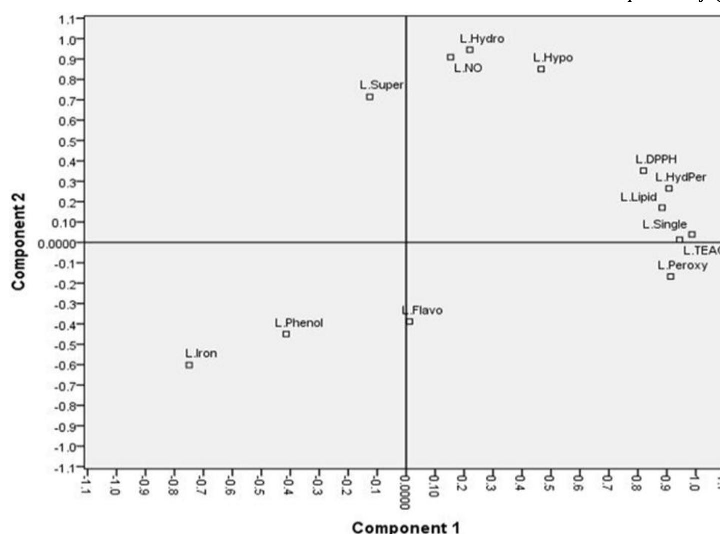

Fig. 1: Loading plot of different antioxidant capacities of *N. indicum* leaves

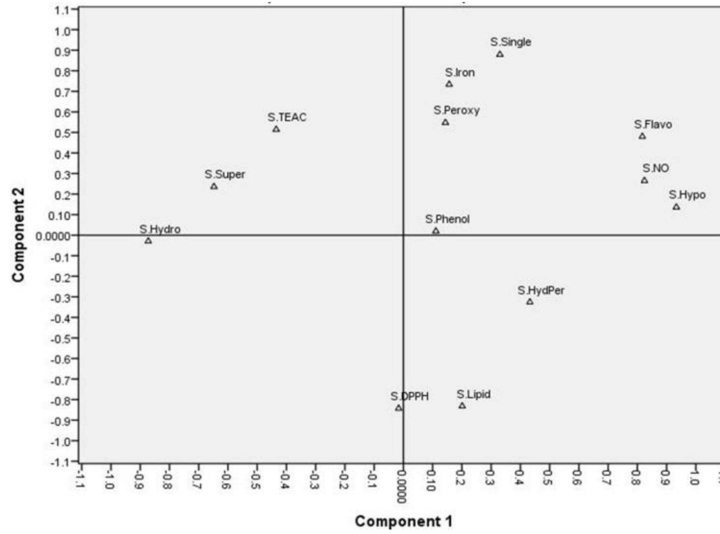

Fig. 2: Loading plot of different antioxidant capacities of *N. indicum* stem

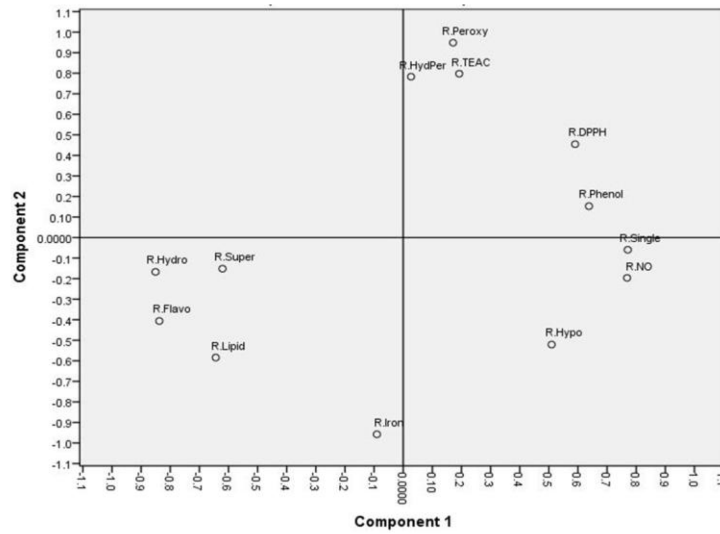

Fig. 3: Loading plot of different antioxidant capacities of *N. indicum* root

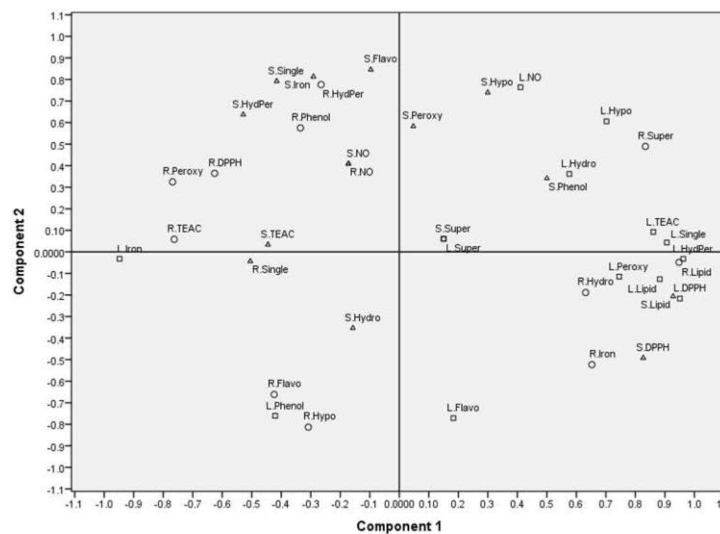

Fig. 4: Loading plot of different antioxidant capacities of *N. indicum* leaves, Stem and root all together

The loading plot of leaf, stem and root (Fig. 4) demonstrated that L.DPPH, L.HydPer, L.Lipid, L.Single, L.TEAC, S.DPPH, S.Lipid and R.Lipid were heavily loaded positively on the PC1 with squared cosine value of 0.974, 0.954, 0.891, 0.886, 0.833, 0.898, 0.949 and 0.948, respectively; whereas, L.NO, S.Hypo, S.Iron and S.Flavo were heavily loaded positively on the PC2 with squared cosine value of 0.822, 0.779, 0.753 and 0.817, respectively. A greater cluster of L.TEAC, L.Single, L.HydPer, L.Peroxy, R.Lipid, R.Hydro, L.Lipid,

S.Lipid, L.DPPH, R.Iron and S.DPPH may account for their comparable activity due to their positional grouping at the same region of the plot. Wong et al. have worked on aqueous extracts of edible tropical plants and have demonstrated that total phenolic content along with iron chelation and DPPH were heavily loaded on PC1 [12]. Working on antioxidant capacities of Mulberry fruits, Wang and Hu have demonstrated similar cluster [13] loaded heavily on PC1. It is interesting to note that in case of leaves; L.DPPH, L.HydPer, L.Lipid, L.Single, L.TEAC and L.Peroxy were loaded heavily on PC1 both in the Fig. 1 and Fig. 4. In the Fig. 1, L.DPPH, L.HydPer, L.Hypo, L.Lipid, L.Single and L.TEAC influenced PC1 with squared cosine value of 0.885, 0.917, 0.832, 0.849, 0.871 and 0.821, respectively. S.Hypo, S.NO, S.Single and S.Flavo were heavily loaded on PC1 with squared cosine value of 0.806, 0.804, 0.814 and 0.936, respectively. In the case of *N. indicum* root, only R.DPPH (0.744) and R.Peroxy (0.755) have found to be loaded highly on PC1 and R.Hypo was loaded onto PC2 having a squared cosine value of 0.728.

Table 1: Correlation matrix of the different antioxidant capacities of *N. indicum* leaf

	DPPH	HydPer	Hydro	Hypo	Iron	Lipid	Super	NO	Peroxy	Single	TEAC	Phenol	Flavo
DPPH	1.000												
HydPer	0.944**	1.000											
Hydro	0.602^{NS}	0.502^{NS}	1.000										
Hypo	0.586^{NS}	0.623^{NS}	0.873*	1.000									
Iron	-0.940**	-0.874*	-0.783^{NS}	-0.796*	1.000								
Lipid	0.788*	0.750*	0.346^{NS}	0.506^{NS}	-0.814*	1.000							
Super	0.164^{NS}	-0.013^{NS}	0.573^{NS}	0.470^{NS}	-0.384^{NS}	0.198^{NS}	1.000						
NO	0.274^{NS}	0.306^{NS}	0.823*	0.934**	-0.559^{NS}	0.258^{NS}	0.574^{NS}	1.000					
Peroxy	0.593^{NS}	0.698^{NS}	-0.038^{NS}	0.299^{NS}	-0.544^{NS}	0.846*	-0.034^{NS}	0.051^{NS}	1.000				
Single	0.775*	0.871*	0.246^{NS}	0.516^{NS}	-0.742*	0.896**	-0.140^{NS}	0.227^{NS}	0.895**	1.000			
TEAC	0.760*	0.923**	0.229^{NS}	0.498^{NS}	-0.671^{NS}	0.697^{NS}	-0.285^{NS}	0.195^{NS}	0.800*	0.930**	1.000		
Phenol	-0.148^{NS}	-0.328^{NS}	-0.345^{NS}	-0.741*	0.375^{NS}	-0.405^{NS}	-0.289^{NS}	-0.768^{NS}	-0.494^{NS}	-0.489^{NS}	-0.441^{NS}	1.000	
Flavo	-0.423^{NS}	-0.331^{NS}	-0.574^{NS}	-0.251^{NS}	0.358^{NS}	0.100^{NS}	0.143^{NS}	-0.142^{NS}	0.412^{NS}	0.023^{NS}	-0.123^{NS}	-0.388^{NS}	1.000

^{NS}Correlation is not-significant (1-tailed); *Correlation is significant at the 0.05 level (1-tailed) and **Correlation is significant at the 0.01 level (1-tailed)

Table 1, 2 and 3 represent the correlation matrix of the various antioxidant capacities of leaf, stem and root of *N. indicum*, respectively. Though interrelation between the variables were non-significant (p>0.05) for the most cases but it is very interesting to note that some of the variables share very close correlations among themselves. L.HydPer was very closely correlated with L.DPPH and L.TEAC with correlation coefficient of 0.944 and 0.923, respectively (Table 1). Similarly, L.NO with L.Hypo and L.Singlet with L.TEAC have close correlation coefficient of 0.93 and 0.930, respectively (Table 1). Close interrelations were also

Table 2: Correlation matrix of the different antioxidant capacities of *N. indicum* stem

	DPPH	HydPer	Hydro	Hypo	Iron	Lipid	Super	NO	Peroxy	Single	TEAC	Phenol	Flavo
DPPH	1.000												
HydPer	0.217^{NS}	1.000											
Hydro	-0.142^{NS}	-0.330^{NS}	1.000										
Hypo	-0.098^{NS}	0.296^{NS}	-0.725^{NS}	1.000									
Iron	-0.749*	0.065^{NS}	0.123^{NS}	0.394^{NS}	1.000								
Lipid	0.811*	0.235^{NS}	-0.061^{NS}	0.241^{NS}	-0.404^{NS}	1.000							
Super	0.128^{NS}	-0.142^{NS}	0.458^{NS}	-0.474^{NS}	0.165^{NS}	-0.121^{NS}	1.000						
NO	-0.175^{NS}	0.439^{NS}	-0.918*	0.657^{NS}	0.106^{NS}	-0.257^{NS}	-0.453^{NS}	1.000					
Peroxy	-0.114^{NS}	-0.598^{NS}	-0.350^{NS}	0.294^{NS}	0.161^{NS}	-0.211^{NS}	0.268^{NS}	0.213^{NS}	1.000				
Single	-0.653^{NS}	-0.408^{NS}	-0.382^{NS}	0.451^{NS}	0.566^{NS}	-0.602^{NS}	-0.005^{NS}	0.470^{NS}	0.791^{NS}	1.000			
TEAC	-0.285^{NS}	0.147^{NS}	0.182^{NS}	-0.454^{NS}	0.251^{NS}	-0.666^{NS}	0.687^{NS}	0.066^{NS}	0.062^{NS}	0.173^{NS}	1.000		
Phenol	0.328^{NS}	0.549^{NS}	-0.190^{NS}	0.205^{NS}	0.240^{NS}	0.210^{NS}	0.622^{NS}	0.169^{NS}	0.116^{NS}	-0.043^{NS}	0.466^{NS}	1.000	
Flavo	-0.500^{NS}	0.310^{NS}	-0.585^{NS}	0.892^{NS}	0.690^{NS}	-0.165^{NS}	-0.398^{NS}	0.697^{NS}	0.232^{NS}	0.625^{NS}	-0.145^{NS}	0.168^{NS}	1.000

^{NS}Correlation is not-significant (1-tailed) and *Correlation is significant at the 0.05 level (1-tailed)

Table 3: Correlation matrix of the different antioxidant capacities of *N. indicum* root.

	DPPH	HydPer	Hydro	Hypo	Iron	Lipid	Super	NO	Peroxy	Single	TEAC	Phenol	Flavo
DPPH	1.000												
HydPer	0.573^{NS}	1.000											
Hydro	-0.301 ^{NS}	-0.175 ^{NS}	1.000										
Hypo	0.104 ^{NS}	-0.643 ^{NS}	-0.212 ^{NS}	1.000									
Iron	-0.497 ^{NS}	-0.658 ^{NS}	0.173 ^{NS}	0.459 ^{NS}	1.000								
Lipid	-0.403 ^{NS}	-0.186 ^{NS}	0.711*	-0.193 ^{NS}	0.660 ^{NS}	1.000							
Super	-0.303 ^{NS}	0.302 ^{NS}	0.462 ^{NS}	-0.692 ^{NS}	0.271 ^{NS}	0.805 ^{NS}	1.000						
NO	0.543 ^{NS}	0.206 ^{NS}	-0.625 ^{NS}	0.149 ^{NS}	0.146 ^{NS}	-0.110 ^{NS}	0.008 ^{NS}	1.000					
Peroxy	0.402 ^{NS}	0.610 ^{NS}	-0.352 ^{NS}	-0.295 ^{NS}	-0.926**	-0.790 ^{NS}	-0.419 ^{NS}	-0.205 ^{NS}	1.000				
Single	0.832**	0.128 ^{NS}	-0.389 ^{NS}	0.571 ^{NS}	0.006 ^{NS}	-0.270 ^{NS}	-0.433 ^{NS}	0.693 ^{NS}	-0.015 ^{NS}	1.000			
TEAC	0.648 ^{NS}	0.586 ^{NS}	-0.152 ^{NS}	0.025 ^{NS}	-0.732 ^{NS}	-0.598 ^{NS}	-0.465 ^{NS}	-0.169 ^{NS}	0.822*	0.351 ^{NS}	1.000		
Phenol	0.195^{NS}	0.190 ^{NS}	-0.778 ^{NS}	-0.201 ^{NS}	-0.262 ^{NS}	-0.500 ^{NS}	-0.128 ^{NS}	0.661 ^{NS}	0.220 ^{NS}	0.135 ^{NS}	-0.186 ^{NS}	1.000	
Flavo	-0.543^{NS}	-0.417 ^{NS}	0.926 ^{NS}	0.000 ^{NS}	0.450 ^{NS}	0.757*	0.402 ^{NS}	-0.675 ^{NS}	-0.514 ^{NS}	-0.455 ^{NS}	-0.300 ^{NS}	-0.846*	1.000

^{NS}Correlation is not-significant (1-tailed); *Correlation is significant at the 0.05 level (1-tailed) and **Correlation is significant at the 0.01 level (1-tailed)

found in the case of *N. indicum* stem where, S.Lipid with S.DPPH (0.811), S.Single with S.Peroxy (0.791) and S.Flavo with S.Hypo (0.892) has been found to be closely correlated (Table 2). Proximal correlation between R.Single and R.DPPH (0.832), R.Super and R.Lipid (0.805), R.TEAC and R.Peroxy (0.822) for *N. indicum* root has been observed (Table 3). The correlation matrix of various antioxidant capacities of *N. indicum* leaf, stem and root have revealed a striking feature that S.Super with L.Super and S.No with R.NO share absolute correlation of 1.000. R.Lipid has close correlation with L.HydPer, L.Single and L.TEAC with correlation coefficient of 0.950, 0.959 and 0.950, respectively. Similar correlations of L.DPPH were found with S.DPPH and S.Lipid having correlation coefficient of 0.912 and 0.903. The correlation between L.Pheno and L.Hypo ($p<0.05$); R.Flavo with R.Lipid and R.Flavo ($p<0.05$) were found to be significant.

The cluster analysis (CA) was performed according to the hierarchical cluster analysis (HCA) method to distinguish similar groups among the various antioxidant capacities and also to confirm the CA of the PCA. Previously, Thiangthum et al [14] have utilized HCA to indicate antioxidant compounds from *Mallotus* and *Phyllanthus* species fingerprints and Rainha et al [15] have shown similar approach to study the antioxidant properties, total phenolic, total carotenoid and chlorophyll content of *Hypericum foliosum*. The HCA in our case rendered a dendrogram (Fig. 5) grouping the antioxidant capacities of leaf, stem, root of *N. indicum* into various statistically significant clusters. This grouping gave the evidence that various antioxidant capacities have different characteristics which can be correlated with the total phenolic and flavonoid content of plant extracts. The results of the HCA also corroborates with the results of PCA as S.NO with R.NO, S.Hydro with S.Phenol, S.Single with R.Lipid and S.Super with R.Super was found to be closely associated bearing 0.000 coefficient value, when average linkage was performed between groups. On the other hand, among all the variables, L.Iron and R.Flavo were merged at the highest distance of 12.394. The icicle diagram (Fig. 6) for the combined HCA of *N. indicum* leaf, stem and root indicated at which steps the various antioxidant capacities were merged to the cluster (Fig. 5) and gave the visual display of the agglomeration schedule table (Table 4).

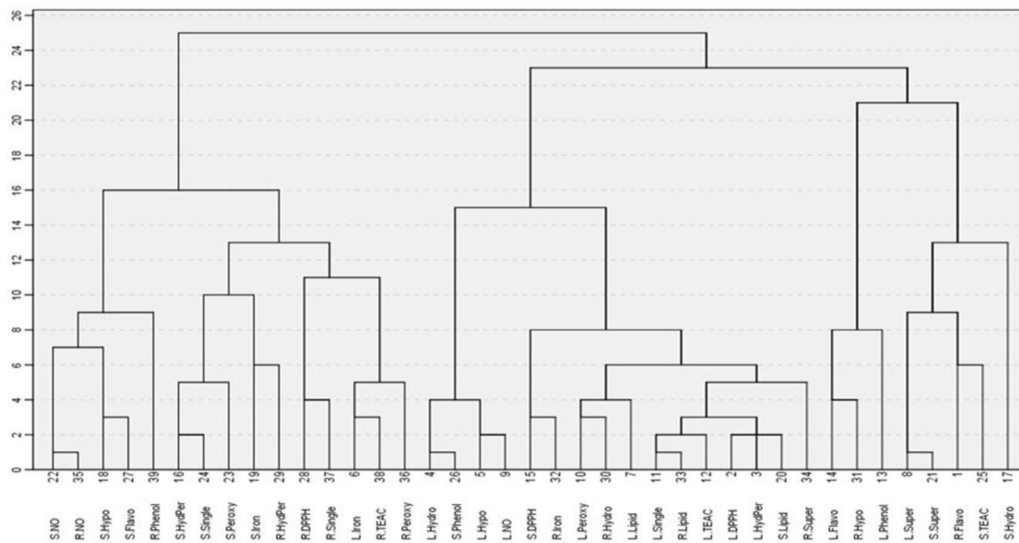

Fig. 5: The dendrogram describes the hierarchical clustering of the different antioxidant capacities of leaf, stem and root of *N. indicum*.

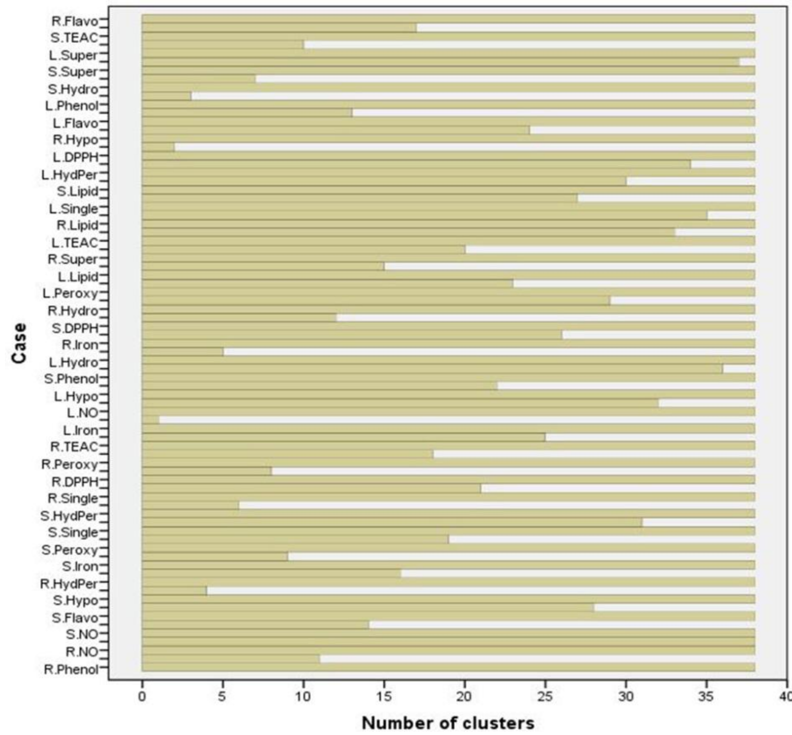

Fig. 6: Icicle diagram of the different antioxidant capacities of the leaf, stem and root of *N. Indicum*

Table 4: Agglomeration Schedule Table for the antioxidant capacities of *N. indicum* leaf, stem and root, which corresponds to the Fig. 2 and 3

Stage	Cluster Combined		Coefficient	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	22	35	.000	0	0	25
2	8	21	.000	0	0	29
3	4	26	.362	0	0	17
4	11	33	.407	0	0	6
5	2	3	.563	0	0	9
6	11	12	.602	4	0	12
7	5	9	.659	0	0	17
8	16	24	.874	0	0	20
9	2	20	.986	5	0	12
10	10	30	1.039	0	0	16
11	18	27	1.078	0	0	25
12	2	11	1.290	9	6	19
13	15	32	1.308	0	0	27
14	6	38	1.458	0	0	21
15	14	31	1.632	0	0	26
16	7	10	1.654	0	10	24
17	4	5	1.664	3	7	34
18	28	37	1.678	0	0	31
19	2	34	2.189	12	0	24
20	16	23	2.328	8	0	30
21	6	36	2.441	14	0	31
22	1	25	2.878	0	0	29
23	19	29	2.918	0	0	30
24	2	7	2.922	19	16	27
25	18	22	3.231	11	1	28
26	13	14	3.570	0	15	36
27	2	15	3.704	24	13	34
28	18	39	4.085	25	0	35
29	1	8	4.259	22	2	32
30	16	19	4.784	20	23	33
31	6	28	5.283	21	18	33
32	1	17	6.126	29	0	36
33	6	16	6.129	31	30	35
34	2	4	7.048	27	17	37
35	6	18	7.627	33	28	38
36	1	13	10.061	32	26	37
37	1	2	11.336	36	34	38
38	1	6	12.394	37	35	0

CONCLUSION

The antioxidant capacity of *N. indicum* leaf, stem and root have demonstrated considerable variations in their activity and the autonomous pattern recognition statistical approach enabled us to visualize the huge set of variables and their inter-relations in a much more simplified manner. The principal component analysis efficiently grouped different antioxidant capacities according to their phytochemical constituents. The cluster analysis efficiently separated different antioxidant capacities according to their similar activity. Being a natural source of antioxidant with high phenolic and flavonoid compounds, *N. indicum* has demonstrated satisfactory antioxidant profile. The consolidation of biochemical assaying techniques and the statistical approaches allowed us to visualize the complete correlation status of *N. indicum* antioxidant capacity. Information of this study may be advantageous in future to correlate the antioxidant profile of different plant species and to highlight the bioactivity of the plants in a more rational way.

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