Comparative Phytochemical Profiling of *Clerodendrum infortunatum* L. Using GC-MS Method Coupled with Multivariate Statistical Approaches

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Abstract

Clerodendrum infortunatum L. is extensively used in traditional and folklore medicine. It has also been recognized in Ayurveda and Unani medicinal systems. Different pharmacological properties of *C. infortunatum* have already been reported. However, the phytochemical constituents remained unknown. Thus, the present study was performed to investigate and compare the phytochemical composition of the major parts of *C. infortunatum* using gas chromatography-mass spectrometry.

GC-MS is one of the most reliable biophysical method for its specificity and repeatability, was utilized for the phytochemical profiling *C. infortunatum* leaf, stem, root, flower and seed. The phytochemical profiles were further compared using multivariate statistical techniques to evaluate the extent of analogy between the phytochemical profiles.

Several bioactive chemical species such as limonene, phytol, catechol, hexadecanoic acid, squalene, dodecanoic acid, vitamin E, hydroxymethylfurfural, stigmasterol, etc. were identified in different parts of *C. infortunatum*. Derivatives of pharmacological supplements such as cinnamic acid, guaiacol, eugenol, vanillic acid, vitamin D etc. were also detected. Moreover, a wide range of phenolics and phenolic acid derivatives were identified in *C. infortunatum*, which are known to be primarily responsible for bioactivities of herbal medicines. Principal component analysis coupled with hierarchical clustering revealed the extent of correlation and divergence among the major parts of *C. infortunatum* in terms of phytochemical fingerprints. Flower, leaf and root demonstrated equivalent phytochemical fingerprints.

The results provided insights into the presence of several pharmacologically active constituents. The pattern of phytochemical correlation between leaf, stem, root, flower and seed was established as well.

Introduction

Evidence based pharmacognostic studies on complementary and herbal medicine are booming. Synergistic activities of known phytochemicals or novel bioactive leads are constantly being identified by biophysical screening of medicinal plants. Traditionally known therapeutic uses of such plants are also being established through *in vivo* trails. However, in spite of recognized bioactivities, most medicinal plants still lack complete phytochemical standardization which would not only correlate with its potent bioactivities, but also serve as its individual phytochemical fingerprint. Besides, in most cases, phytochemical profiling is primarily focused on certain part of a plant and thus, the parallel phytochemical information of other parts of the same plant remain completely obscure. Thus, a comprehensive phytochemical profiling of the major parts of a plant becomes quintessential. This would not only provide a logical candidate for ancillary evaluation of bioactivities but also may be useful for *in silico* prediction of the bioactivities of ethnopharmacological plants

Clerodendrum infortunatum L. (syn. *Clerodendrum viscossum* Vent., *Clerodendrum calycinum* Turcz., *Ovieda infortunata* (L.) Baill.) is an ethnopharmacological plant, used for the treatment of diverse ailments such as wounds, inflammatory diseases, tumor, malaria, hyperglycaemia, fever, snake bite etc. Details of its therapeutic uses in Ayurveda, Unani, homeopathic and folklore medicine have previously been reported [1-5]. However, in spite of its tremendous medicinal uses, a systematic phytochemical profiling of the major parts of the plant is completely lacking. Therefore, the present study was initiated to investigate the phytochemical profiles of leaf, stem, root, flower and seed of *C. infortunatum* and to compare the individual phytochemical niche with other parts of the plant. To our knowledge, perhaps, this

is the first ever study of complete phytochemical metabolome of any medicinal plant.

Material and Methods

Chemicals

All chemicals and reagents were procured form HiMedia Pvt. Ltd. (Mumbai, India) unless otherwise indicated. HPLC grade solvents were obtained from Sigma Aldrich (USA). Milli-Q ultrapure water from the departmental facility was used in the experiments.

Plant material

Fresh and disease free plant materials were collected from the campus of University of North Bengal, India (26°42'N, 88°21'E). The plant was identified and authenticated by plant taxonomist Prof. Abhaya Prasad Das of Department of Botany, University of North Bengal. A voucher specimen was stored at the Botany Department Herbarium, University of North Bengal with an accession number of 09618.

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Preparation of extract

The extract was prepared according to a standard method [6]. Plant material was washed twice with double distilled water to remove dirt and foreign materials. The whole plant was divided into five major parts, i.e., leaf, stem, root, flower and seed. The parts were chopped into pieces (0.5-1.0 cm) and shade dried at laboratory temperature for 14 days. The dried parts were then grinded to powder using a blender-mixer (Hummer, Lords). The resultant fine powder (100 g) was mixed with 1000 ml 70% methanol (methanol : water 7:3, v/v) and kept in a shaking incubator (160 rpm) at 37°C. The mixtures were centrifuged for 15 min at 5000 rpm (Heraeus) after 12 h. The supernatant liquid was separately stored. The pellet was again mixed with 70% methanol and treated as previously described. After second phase of centrifugation, liquid supernatants of both phases were mixed together and concentrated in a rotary evaporator under reduced pressure (Rotavapor, Buchi). The resultant concentrated extract was lyophilized (SJIA-10N) and the anhydrous extract was stored at -20°C until further use.

GC-MS analysis

C. infortunatum leaf, stem, root, flower and seed extracts were passed through anhydrous Na_2SO_4 and activated charcoal (2:1; w/w) to remove any trace of moisture and colour. The samples were analysed using Thermo Scientific Trace 1300 gas chromatography instrument attached with Thermo Scientific ISQ QD single quadrupole mass spectrophotometer. The GC was equipped with TG-5MS column (30 m × 0.25 mm × 0.25 μm). The inlet temperature was maintained at 250°C. The initial temperature was set at 60°C (solvent delay 5 min) with a hold of 2 min, followed by a ramp of 5°C to 290°C with a hold of 6 min (54 min programme). Samples were (1 μl) injected in a splitless mode (split flow 50 ml/min) with splitless time of 0.80 min,

using a Thermo Scientific AI-1310 auto-sampler. The carrier gas was helium, with a constant flow of 1 ml/min. MS transfer line temperature was set at 290°C with an Ion source temperature of 230°C (electron ionization). The individual samples were analysed at electron energy 70 eV (vacuum pressure- 2.21e-0.5 Torr). The mass analyser range was set to 50-650 amu.

GC-MS data analysis

All samples were analysed thrice for confirmation. MS data analysis was performed by Automated Mass Spectral Deconvolution and Identification System (AMDIS) version 2.70. The major and essential compounds were identified by mass fragmentation patterns using the database of National Institute Standard and Technology (NIST) with a MS library version 2011.

Multivariate analysis

Principal Component Analysis (PCA) based on correlation matrix was performed in order to elucidate the correlated phytochemical profile in leaf, stem, root, flower and seed of *C. infortunatum*. The results were further analysed by multivariate statistical approach, employing a hierarchical cluster analysis (HCA) associated with proximity score matrix represented as proximity heat-map. PCA and HCA were performed using the IBM SPSS statistics version 20.0 software package for Windows.

Results

The results of the GC-MS analysis revealed the presence of several bioactive constituents in different parts of *C. infortunatum*. The GC spectra of *C. infortunatum* leaf, stem, root, flower and seed are presented Figure 1-5 and the identified compounds are enlisted in Table 1-5.

Figure 1: Complete GC chromatogram of *Clerodendrum infortunatum* leaf (corresponding to table 1).

Figure 2: Complete GC chromatogram of *Clerodendrum infortunatum* stem (corresponding to table 2).

Figure 3: Complete GC chromatogram of *Clerodendrum infortunatum* root (corresponding to table 3).
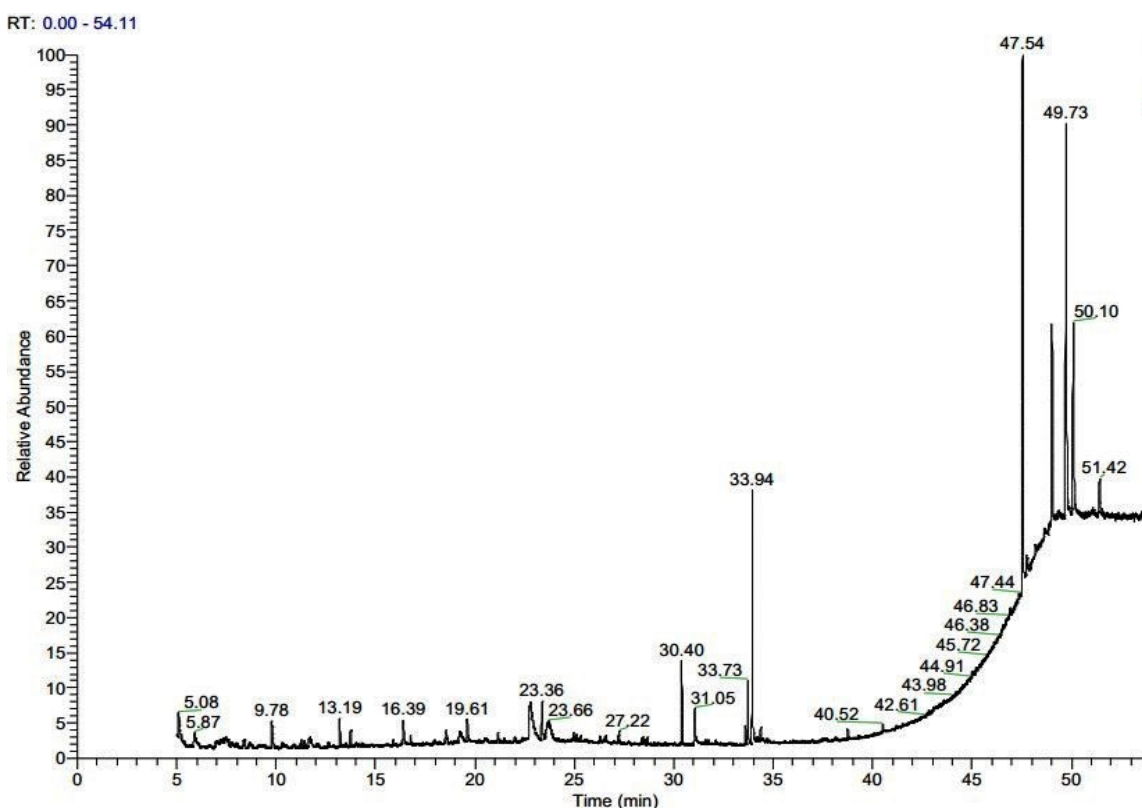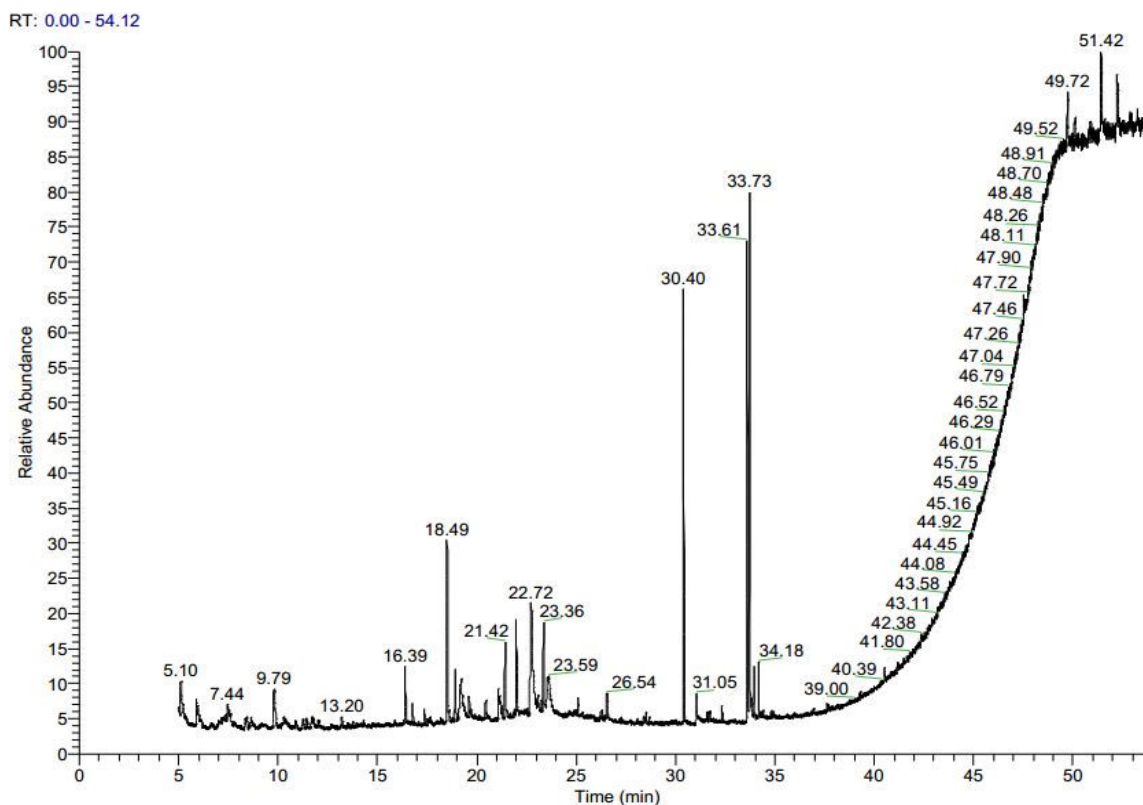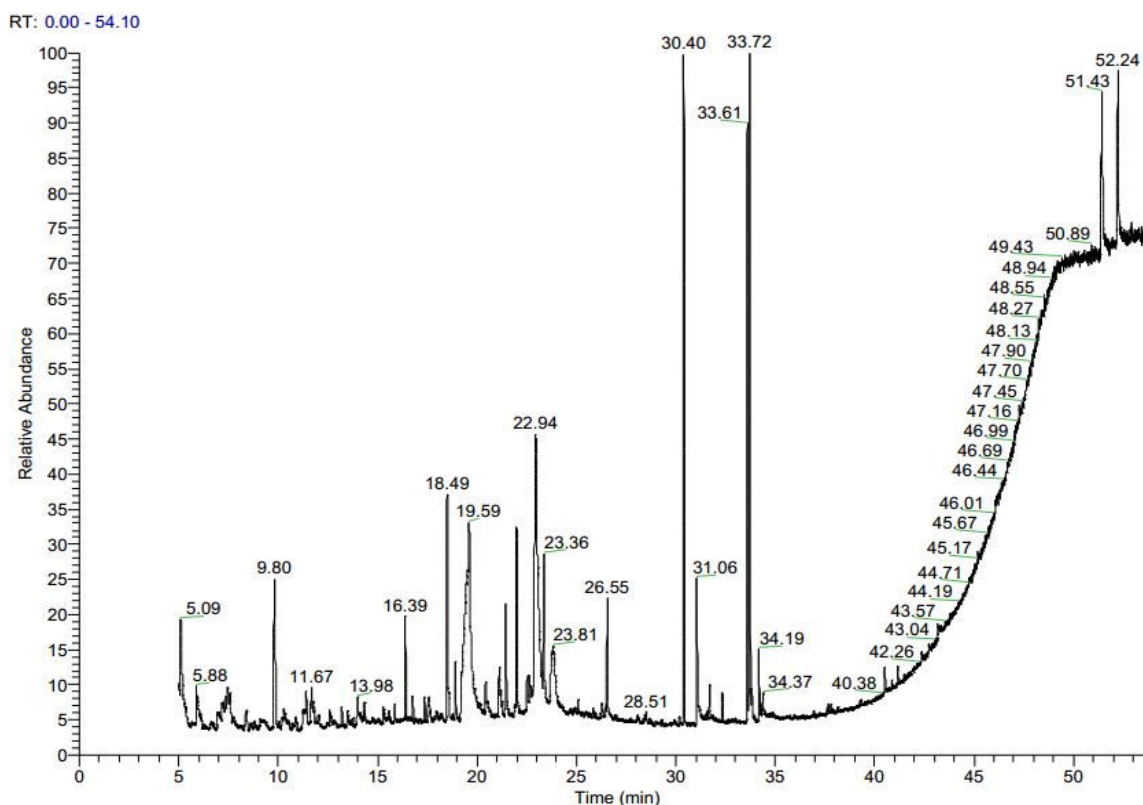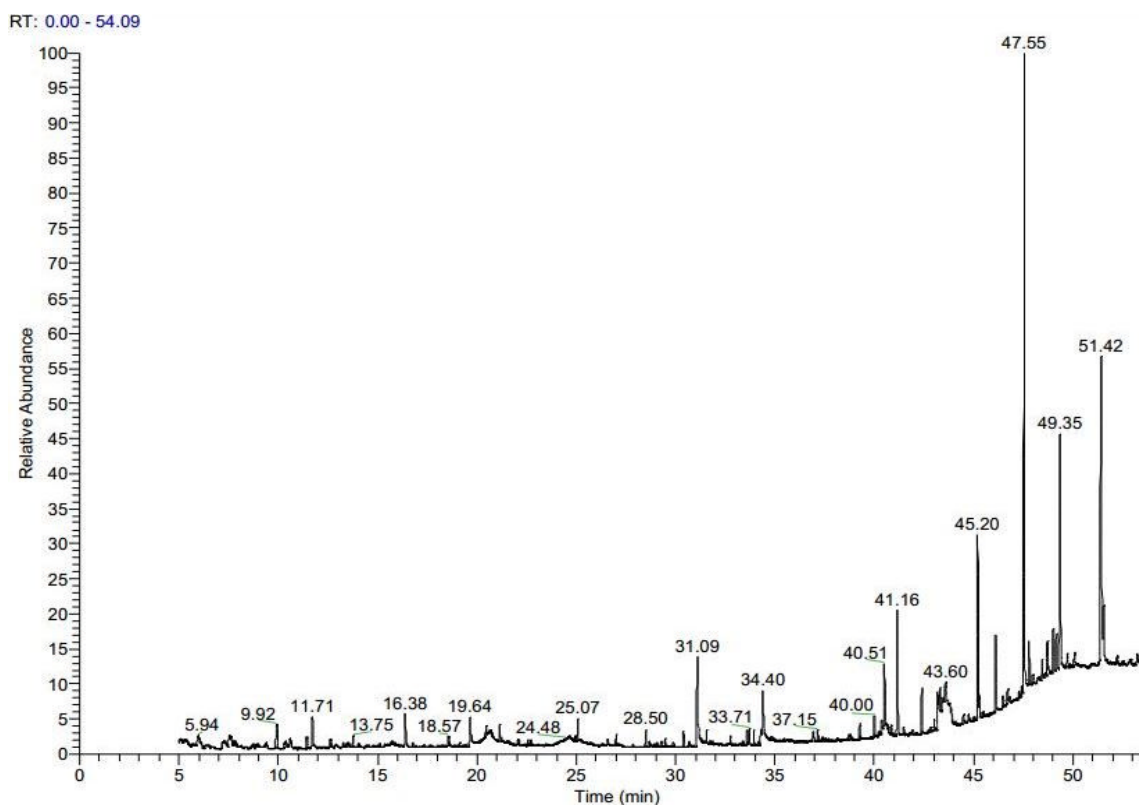
Figure 4: Complete GC chromatogram of *Clerodendrum infortunatum* flower (corresponding to table 4).
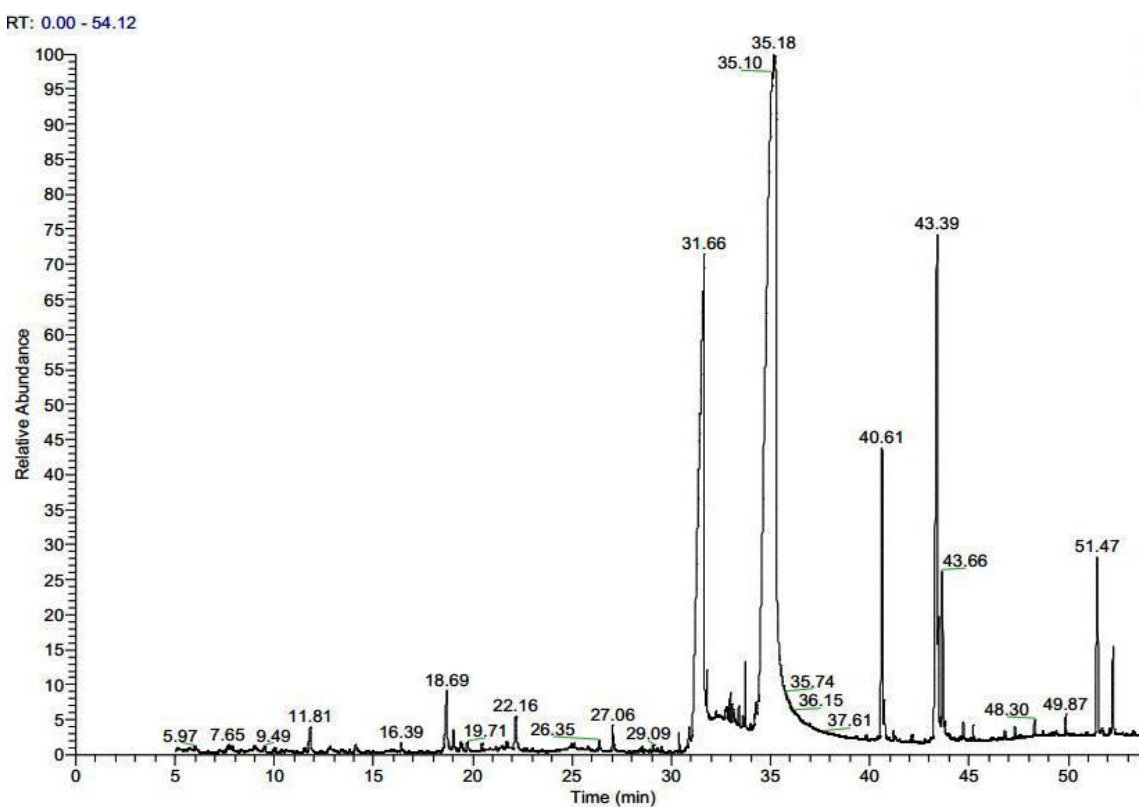

Figure 5: Complete GC chromatogram of *Clerodendrum infortunatum* seed (corresponding to table 5).

1.	Dihydroxyacetone	$C_3H_6O_3$	5.08
2.	6-Oxa-bicyclo[3.1.0]hexan-3-one	$C_5H_6O_2$	5.87
3.	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	6.63
4.	d-Gala-l-ido-octonic amide	$C_8H_{17}NO_8$	6.97
5.	4-Amino-1,5-pentandioic acid	$C_7H_{13}NO_4$	7.16
6.	9-Octadecenamide	$C_{18}H_{35}NO$	7.71
7.	3-[4-(2-Methoxy-ethoxymethoxy)-phenyl]-acrylic acid	$C_{13}H_{16}O_5$	8.38
8.	Methyl N-(N-benzyloxycarbonyl-beta-l-aspartyl)-beta-d-glucosaminide	$C_{19}H_{26}N_2O_{10}$	8.63
9.	Acetic acid, 6-morpholin-4-yl-9-oxobicyclo[3.3.1]non-3-yl ester	$C_{15}H_{23}NO_4$	9.78
10.	R-Limonene	$C_{10}H_{16}O_3$	10.03
11.	1,8-Di(4-nitrophenylmethyl)-3,6-diazahomoadamantan-9-one	$C_{23}H_{24}N_4O_5$	10.29
12.	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-	$C_{27}H_{44}O_3$	10.84
13.	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	11.41
14.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	11.67
15.	Hydrocinnamic acid, o-[(1,2,3,4-tetrahydro-2-naphthyl)methyl]-	$C_{20}H_{22}O_2$	11.76
16.	Octadecanoic acid, 4-hydroxy-, methyl ester	$C_{19}H_{38}O_3$	12.87
17.	Catechol	$C_6H_6O_2$	13.19
18.	6-Methylenebicyclo[3.2.0]hept-3-en-2-one	C_8H_8O	13.74
19.	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	13.99
20.	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	15.85
21.	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	16.39
22.	Furan-2-carbohydrazide, N2-(3-indolylmethylene)-	$C_{14}H_{11}N_3O_2$	18.56
23.	5,8,11-Eicosatriynoic acid, methyl ester	$C_{21}H_{30}O_2$	18.86
24.	L-Gala-l-ido-octose	$C_8H_{16}O_8$	19.21
25.	Adenosine, 4'-dehydroxymethyl-4'-[N-ethylaminoformyl]-N-[4-nitrobenzyl]	$C_{19}H_{21}N_7O_6$	19.61
26.	Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-	$C_{30}H_{48}O_2$	21.42
27.	Desulphosinigrin	$C_{10}H_{17}NO_6S$	22.76
28.	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	$C_{20}H_{13}N_5O_2$	25.09
29.	Pentaerythritol, bis-O-(9-borabicyclo[3.3.1]non-9-yl)-di-O-methyl-	$C_{23}H_{42}B_2O_4$	25.30
30.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	26.54
31.	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	27.22
32.	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	28.67
33.	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	$C_{28}H_{44}O_4$	29.54
34.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	30.40
35.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	31.05
36.	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	33.61
37.	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$	33.73
38.	Phytol	$C_{20}H_{40}O$	33.94
39.	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	34.37
40.	Betulin	$C_{30}H_{50}O_2$	47.54
41.	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	$C_{27}H_{40}O_4$	49.73

Table 1: Phytochemicals identified in *Clerodendrum infortunatum* leaf (corresponding to figure 1).

Sl. #	Compound name	Formula	RT
1.	Mannosamine	$C_6H_{13}NO_5$	5.10
2.	6-Oxa-bicyclo[3.1.0]hexan-3-one	$C_5H_6O_2$	5.89
3.	L-Gala-l-ido-octose	$C_8H_{16}O_8$	7.44
4.	Acetic acid, 6-morpholin-4-yl-9-oxobicyclo[3.3.1]non-3-yl ester	$C_{15}H_{23}NO_4$	9.79
5.	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	10.30
6.	10,13-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	10.87
7.	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	11.41
8.	Hydrocinnamic acid, o-[(1,2,3,4-tetrahydro-2-naphthyl)methyl]-	$C_{20}H_{22}O_2$	11.77
9.	3,6-Dimethoxy-2,5-dinitrobenzaldehyde oxime	$C_9H_9N_3O_7$	13.20
10.	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	14.29
11.	Methyl 8,10-octadecadiynoate	$C_{19}H_{30}O_2$	15.86
12.	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	16.39
13.	Octahydrochromen-2-one	$C_9H_{14}O_2$	18.49
14.	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	$C_{28}H_{44}O_4$	19.19

15.	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	21.42
16.	Hexadecane, 1,1-bis(dodecyloxy)-	$C_{40}H_{82}O_2$	21.99
17.	Desulphosinigrin	$C_{10}H_{17}NO_6S$	22.72
18.	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	$C_{27}H_{42}O_4$	25.09
19.	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	$C_{20}H_{13}N_5O_2$	26.26
20.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	26.54
21.	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	27.23
22.	Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-	$C_{30}H_{48}O_2$	28.41
23.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	68.40
24.	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	31.05
25.	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	$C_{24}H_{46}O_2$	32.34
26.	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	33.61
27.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	33.73
28.	Phytol	$C_{20}H_{40}O$	33.94
29.	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	34.18

Table 2: Phytochemicals identified in *Clerodendrum infortunatum* stem (corresponding to figure 2).

SI. #	Compound name	Formula	RT
1.	D-glucosamine	$C_6H_{13}NO_5$	5.09
2.	6-Oxa-bicyclo[3.1.0]hexan-3-one	$C_5H_6O_2$	5.88
3.	4-Amino-1,5-pentandioic acid	$C_7H_{13}NO_4$	7.18
4.	3-[4-(2-Methoxy-ethoxymethoxy)-phenyl]-acrylic acid	$C_{13}H_{16}O_5$	8.40
5.	4-Methylpiperidine-1-carboxylic acid, phenyl ester	$C_{13}H_{17}NO_2$	9.80
6.	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	$C_{11}H_{18}N_2O_2$	10.28
7.	10,13-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	10.86
8.	Desulphosinigrin	$C_{10}H_{17}NO_6S$	11.29
9.	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	11.40
10.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	11.67
11.	2-Cyclohexylpiperidine	$C_{11}H_{21}N$	12.59
12.	2-Oxaspiro[5.5]undecane-1,5-dione, 4,4-dimethyl-3-(4-nitrophenyl)-	$C_{18}H_{21}NO_5$	13.20
13.	5-Hydroxymethylfurfural	$C_6H_6O_3$	13.98
14.	Methyl 6-oxoheptanoate	$C_8H_{14}O_3$	15.29
15.	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	15.85
16.	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	16.39
17.	2,4-Dimethoxyphenol	$C_8H_{10}O_3$	17.37
18.	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	17.55
19.	Cyclobuta[1,2:3,4]dicyclooctene, hexadecahydro-	$C_{16}H_{28}$	18.49
20.	9-[2-Deoxy-β-d-ribohexopyranosyl]purin-6(1H)-one	$C_{11}H_{14}N_4O_5$	19.59
21.	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	21.44
22.	Hexadecane, 1,1-bis(dodecyloxy)-	$C_{40}H_{82}O_2$	21.99
23.	2-Oxabicyclo[3.3.0]oct-7-en-3-one, 7-(1-hydroxypentyl)-	$C_{12}H_{18}O_3$	22.52
24.	3-Oxabicyclo[4.1.0]heptane-7-carboxamide, 6-methyl-N-(1-naphthyl)-	$C_{18}H_{19}NO_2$	22.65
25.	α-D-Galactopyranoside, methyl	$C_7H_{14}O_6$	22.94
26.	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazine	$C_{20}H_{13}N_5O_2$	25.08
27.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	26.55
28.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	30.40
29.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	31.06
30.	Gibberellic acid	$C_{19}H_{22}O_6$	31.69
31.	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	32.34
32.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	33.61
33.	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	33.72
34.	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	33.82
35.	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	34.19
36.	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	34.26

Table 3: Phytochemicals identified in *Clerodendrum infortunatum* root (corresponding to figure 3).

Sl. #	Compound name	Formula	RT
1.	6-Oxa-bicyclo[3.1.0]hexan-3-one	$C_5H_6O_2$	5.94
2.	2-Propyl-tetrahydropyran-3-ol	$C_8H_{16}O_2$	7.19
3.	Oxirane, [(2-propenyloxy)methyl]-	$C_6H_{10}O_2$	7.52
4.	Dithiocarbamate, S-methyl-,N-(2-methyl-3-oxobutyl)-	$C_7H_{13}NOS_2$	9.35
5.	Thymine	$C_5H_6N_2O_2$	9.92
6.	1-Hexanethiol, 2-ethyl-	$C_8H_{18}S$	10.34
7.	n-Nonaldehyde	$C_9H_{18}O$	10.57
8.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	11.71
9.	5-Methoxypyrrolidin-2-one	$C_5H_9NO_2$	12.61
10.	Desulphosinigrin	$C_{10}H_{17}NO_6S$	13.36
11.	Benzofuran, 2,3-dihydro-	C_8H_8O	13.75
12.	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	16.38
13.	Carbamic acid, N-methyl-N-[6-iodo-9-oxabicyclo[3.3.1]nonan-2-yl]-, ethyl ester	$C_{12}H_{20}INO_3$	18.57
14.	2-Hydroxy-5-methylbenzaldehyde	$C_8H_8O_2$	19.64
15.	Dodecanoic acid, 3-hydroxy-	$C_{12}H_{24}O_3$	20.45
16.	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	22.05
17.	Undecanoic acid	$C_{11}H_{22}O_2$	22.55
18.	3-tert-Butyl-4-hydroxyanisole	$C_{11}H_{16}O_2$	22.69
19.	2-Methyl-9-β-d-ribofuranosylhypoxanthine	$C_{11}H_{14}N_4O_5$	24.62
20.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	26.57
21.	Tetradecanoic acid	$C_{14}H_{28}O_2$	26.99
22.	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	27.22
23.	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	28.67
24.	2-Bromotetradecanoic acid	$C_{14}H_{27}BrO_2$	29.06
25.	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	$C_9H_9F_3O_2$	29.29
26.	Hexadecane, 1,1-bis(dodecyloxy)-	$C_{40}H_{82}O_2$	29.47
27.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	30.39
28.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	31.09
29.	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	31.81
30.	2-Hexadecanol	$C_{16}H_{34}O$	33.37
31.	7,10-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	33.60
32.	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	33.71
33.	Phytol	$C_{20}H_{40}O$	33.93
34.	cis-Vaccenic acid	$C_{18}H_{34}O_2$	34.40
35.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	37.15
36.	Hexanoic acid, 2-ethyl-, hexadecyl ester	$C_{24}H_{48}O_2$	40.00
37.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	40.51
38.	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	43.19
39.	Lup-20(29)-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O_2$	43.60
40.	Squalene	$C_{30}H_{50}$	45.20
41.	Heptacosane	$C_{27}H_{56}$	46.11
42.	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	47.55
43.	Vitamin E	$C_{29}H_{50}O_2$	49.35
44.	Stigmasterol	$C_{29}H_{48}O$	51.42

Table 4: Phytochemicals identified in *Clerodendrum infortunatum* flower (corresponding to figure 4).

Sl. #	Compound name	Formula	RT
1.	Cyclopentanone, 2-methyl-	$C_6H_{10}O$	5.97
2.	2-Furanmethanol, 5-methyl-	$C_6H_8O_2$	6.64
3.	2-Propyl-tetrahydropyran-3-ol	$C_8H_{16}O_2$	7.22
4.	β-Allyloxypropionic acid	$C_6H_{10}O_3$	7.65
5.	2-Hexene, 1-(1-ethoxyethoxy)-, (E)-	$C_{10}H_{20}O_2$	7.84
6.	Desulphosinigrin	$C_{10}H_{17}NO_6S$	8.33
7.	β-Hydroxybutyric acid	$C_4H_8O_3$	8.73
8.	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	9.49
9.	13,16-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	10.86

10.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	11.81
11.	5-Methoxypyrrolidin-2-one	$C_5H_9NO_2$	12.80
12.	Cyclohexanone, 4-ethoxy-	$C_8H_{14}O_2$	12.93
13.	Catechol	$C_6H_6O_2$	13.35
14.	β-Hydroxydodecanoic acid	$C_{12}H_{24}O_3$	13.55
15.	5-Hydroxymethylfurfural	$C_6H_6O_3$	14.07
16.	D-Tyrosine, 3-hydroxy-	$C_9H_{11}NO_4$	14.98
17.	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	16.39
18.	2,4-Dimethoxyphenol	$C_8H_{10}O_3$	17.37
19.	2-Cyclohexen-1-one, 2-methyl-	$C_7H_{10}O$	18.69
20.	Octan-2-one, 3,6-dimethyl-	$C_{10}H_{20}O$	19.11
21.	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O_2$	19.37
22.	Octanoic acid, 7-oxo-	$C_8H_{14}O_3$	20.45
23.	2-Dodecenoic acid	$C_{12}H_{22}O_2$	21.69
24.	Cyclobutanecarboxylic acid, decyl ester	$C_{15}H_{28}O_2$	22.16
25.	Oleic Acid	22.58	22.58
26.	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	$C_{11}H_{14}O_3$	25.79
27.	Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester	$C_{10}H_{12}O_4$	26.35
28.	Tetradecanoic acid	$C_{14}H_{28}O_2$	27.06
29.	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	28.67
30.	Pentadecanoic acid	$C_{15}H_{30}O_2$	29.09
31.	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	29.47
32.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	30.40
33.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	31.66
34.	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	33.73
35.	cis-Vaccenic acid	$C_{18}H_{34}O_2$	35.58
36.	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	39.80
37.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	40.61
38.	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	43.39
39.	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	44.22
40.	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	44.71
41.	Squalene	$C_{30}H_{50}$	45.21
42.	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	46.80
43.	Pregna-3,5-dien-9-ol-20-one	$C_{21}H_{30}O_2$	47.31
44.	Stigmasterol	$C_{29}H_{48}O$	51.46

Table 5: Phytochemicals identified in *Clerodendrum infortunatum* seed (corresponding to figure 5).

Discussion

Phytochemical profiles of plants vary heavily depending on variation in season, soil constituents as well as cultivar. Complexity and variation in metabolites regarding polarity, molecular weight, abundance and different physiochemical properties makes it impossible to extract the whole metabolome using a single solvent extraction method. However, in the present study, phytochemical extraction was performed using a combination of water and methanol, because hydro-methanolic extraction method is one of the most reliable and highly preferred methods for extraction of phenolic metabolites which are known to be responsible for bioactivities of herbal medicines. Moreover, traditional plant based medicines are mostly prepared using polar solvents such as tinctures in Ayurveda.

C. infortunatum is an ethno pharmacological plant which is extensively utilized in traditional medicinal system to ameliorate a wide range of diseases. Moreover, several evidence based reports are available which have already demonstrated the therapeutic potentialities of *C. infortunatum* [4]. Previously, in a preliminary phytochemical analysis, Dey et al. [7] had quantified some major chemical species such as tannin, phenol, ascorbic acid, riboflavin, thiamine, alkaloid, flavonoid, sugar, lipid, protein etc. present in *C. infortunatum*. Moreover, Ghosh

and his group [8] studied the phytochemical profile of the methanolic extract of leaves using chromatographic approaches and identified few compounds.

Limonene, catechol, p-vinylguaiacol (2-methoxy-4-vinylphenol), 5,8,11-eicosatriynoic acid, stigmasterol, desulphosinigrin, guaiacol (2-methoxyphenol), tyrosol (4-hydroxy- benzeneethanol), vaccenic acid, hexadecanoic acid, phytol, betulin, hydroxymethylfurfural were the major bioactive constituents recognized in *C. infortunatum*. Metabolic intermediates and derivatives of several compounds such as vitamin D (9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-); eugenol (phenol, 2,6-dimethoxy-4-(2-propenyl)-); cinnamic acid (hydrocinnamic acid, o-[(1,2,3,4-tetrahydro-2-naphthyl)methyl]-); and vanillic acid (benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester) were identified as well. Previously, Dey et al, [7] also reported the presence of high amount of thiamine, riboflavin and ascorbic acid in the leaves of *C. infortunatum*. Moreover, Erukainure [9] also reported the presence of different vitamins including vitamin D in *Clerodendrum* species. Identification of hydrocinnamic acid has profound importance because cinnamic acid derivatives are integral part of our diet and has been attributed to the prevention of different diseases related to oxidative stress like atherosclerosis, inflammatory injury, cancer, and cardiovascular diseases [10]. The presence of 4H-pyran-4-one,2,3-

dihydro-3,5-dihydroxy-6-methyl- and n-hexadecanoic acid was supported by a previous study which reported both the compounds in *C. infortunatum* leaves [8]. The present study, revealing different phenolic species in *C. infortunatum*, also corroborated the previous findings that *C. infortunatum* leaf, stem and root contained high quantity of phenolic and flavonoid compounds [11]. These phytochemicals are considered to be the major determinant of bioactivities of herbal medicines.

Multivariate statistical analysis in the present study, revealed interconnected correlation patterns between the phytochemical profiles of *C. infortunatum*. Partial convergence of phytochemical profiles resulted due to synonymous primary and secondary metabolic pathways, giving rise to identification of common phytochemicals or their derivatives in multiple parts of the plant. For instance, several compounds were identified in multiple parts of *C. infortunatum* such as phytol, 6-oxa-bicyclo [3.1.0] hexan-3-one, 2-myristynoyl pantetheine, 2-methoxy-4-vinylphenol, desulphosinigrin, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, hexadecanoic acid, etc. representing common or overlapping metabolic pathways. Spatial arrangement of the phytochemical profiles of leaf, stem, root, flower and seed of *C. infortunatum* in the double-positive quadrant signifies presence of interrelated phytochemical species (Figure 6A). The loading plot of principal component 1 and 2 accounted for 97.60% and

1.78% variance, respectively. Moreover, clustering of phytochemical profiles of leaf, flower and root demonstrated similar phytochemical fingerprint with the phytochemical profile of flower highly overlapping with leaf and root. A previous report of antioxidant and free radical scavenging activities of leaf, stem and root of *C. infortunatum* [11] also demonstrated the comparable bioactivities of leaf and root extracts. Furthermore, the presence of fairly distinct phytochemical species in seed and stem of *C. infortunatum* are highlighted by their isolated spatial location in the component plot as well as through the correlation matrix (Table 6). It is interesting to note that the clustering analysis using hierarchical method (Figure 6B) also correlated with the PCA. Phytochemical profiles of the major parts of *C. infortunatum* were grouped into various statistically significant clusters according to their proximities. The proximity heat-map (Figure 6C) corroborates the correlation matrix demonstrating similar phytochemical profile in *C. infortunatum* leaf, flower and root.

Conclusion

Evaluation of a particular bioactivity of different parts of C. *infortunatum* has never been performed before. In fact, this remains a common scenario in case of most other ethnomedicinal plants. However, phytochemical analysis coupled multivariate statistical
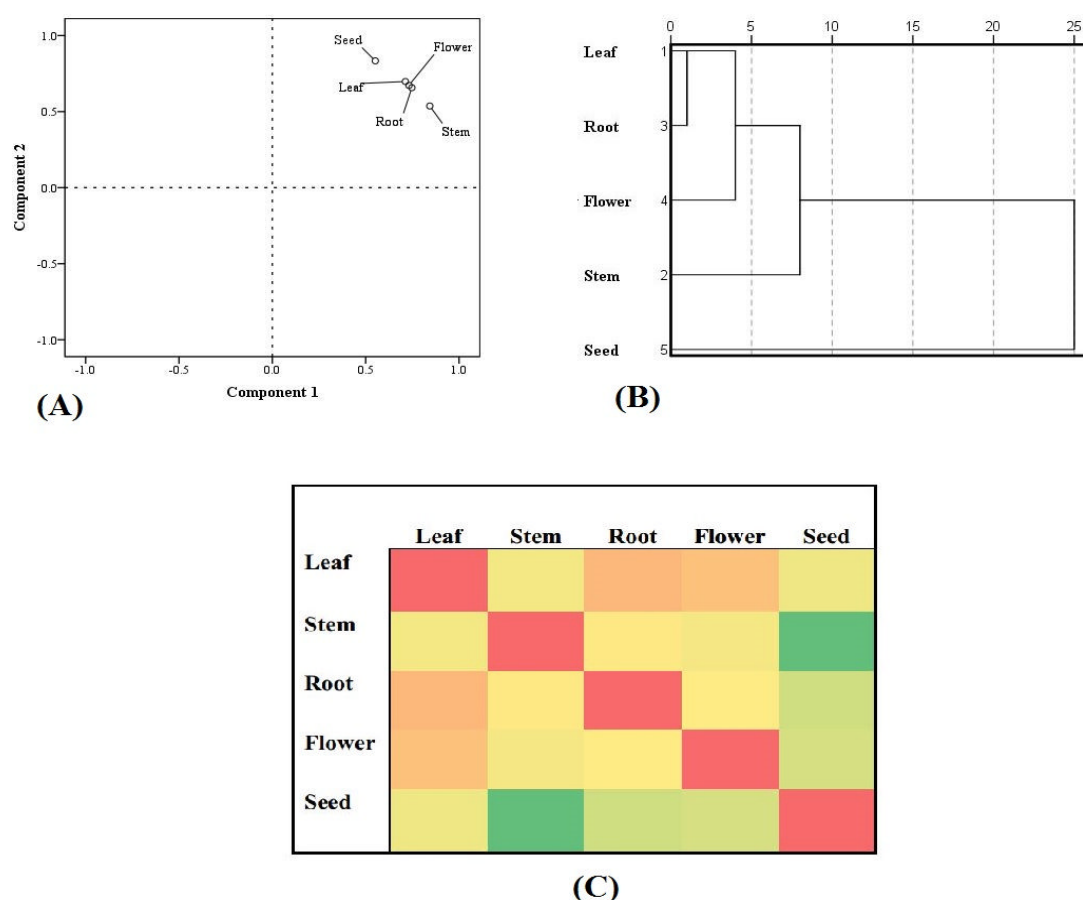

Figure 6: Multivariate statistical analysis of the phytochemical profiles of *C. infortunatum* leaf, stem, root, flower and seed. **(A)** Principal component loading plot corresponding to Table 1. Clustered spatial arrangement of leaf, flower and root in the double positive quadrant signifies comparable phytochemical profile whereas, unlike profile in case of seed and stem. **(B)** Dendrogram describing the hierarchical clustering of the phytochemical profiles. Phytochemical profiles of leaf, root and flower remains similar as shown by the clustered branching pattern. However, separation of the phytochemical profile of seed from the very beginning demonstrates highest variation. **(C)** Proximity heat map generated from proximity scores corresponding to Figure 6B. Shades of red to green colour demonstrates low to high proximity scores. Highest proximity phytochemical proximity resided between root and leaf whereas, lowest proximity between seed and stem.

	Leaf	Stem	Root	Flower	Seed
Leaf	1.000				
Stem	0.975	1.000			
Root	0.988	0.981	1.000		
Flower	0.987	0.976	0.981	1.000	
Seed	0.974	0.913	0.960	0.963	1.000

Table 6: Correlation matrix of phytochemical profiles of *C. infortunatum* leaf, stem, root, flower and seed corresponding to Figure 6. Where, $P < 0.001$ (1-tailed).

approach provides a simple yet comprehensive method for prediction of correlated bioactivities of different parts of a plant. The present study revealed occurrence of several bioactive phytochemicals in *C. infortunatum*. Moreover, comparable phytochemical profile of leaf, root and flower also predicts similar bioactivities of those parts as well as dissimilar bioactivities of seed and stem due to their distant spatial arrangement.

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