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Comprehensive investigation of free radical quenching potential, total phenol, flavonoid and saponin content, and chemical profiles of twelve *Chlorophytum* Ker Gawl. species

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India represents eighteen species of genus *Chlorophytum* Ker Gawl., which are cumulatively known and used as *Safed Musali*. Out of these, *Chlorophytum borivilianum* Santapau & R.R.Fern is extensively utilized by virtue of a wide array of bioactivities. In the present study, comparative analysis of twelve species of the genus *Chlorophytum* including *C. borivilianum* was done to find its alternative with respect to phytochemical content and antioxidant potential. Among all species analyzed, *C. amaniense* Engl. showed highest phenol (654.5 mg/g), flavonoid (191.1 mg/g) and saponin (7.42 %) contents. Highest DPPH free radical quenching activity was observed in *C. amaniense* Engl. (86.97 %) which was nearly 30 % more than that of *C. borivilianum*. HPTLC chemical fingerprint of all the C*hlorophytum* species was established showing a visible difference in the band pattern and intensities. HPTLC fingerprint of *C. amaniense* showed presence of maximum number of bands with high intensity. GC-MS analysis of all the extracts of twelve species revealed presence of 35 different molecules and/or fragments of molecules. Present study may be the first systematic and comparative evaluation of the genus *Chlorophytum*, indicates *C. amaniense* Engl. as an alternative to *C. borivilianum.*

Keywords: Antioxidant activity, *Chlorophytum* Ker Gawl., GC-MS, HPTLC, Saponins.

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Introduction

Family Asparagaceae covers one of the major genera, *Chlorophytum* Ker Gawl., a member of subfamily Agavoideae[1]. Genus *Chlorophytum* comprises of more than 215 species, 6 subspecies and 8 varieties which are distributed around the world[2]. This genus is represented by 18 species in India; of which 16 species are found in Western Ghats alone[3]. Most of the members of genus *Chlorophytum* are known under general term *Safed Musali* and *Chlorophytum borivilianum* Santapau & R.R.Fern is the most popularly marketed medicinal herb under this common nomenclature since roots of this plant are regarded as a '*Rasayana*' class of drug in *Ayurveda*[4]. More than 100 health related issues like rheumatoid arthritis, hemorrhagic shock, CVS disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases, gastrointestinal ulcerogenesis, etc. are managed or treated by using *Rasayana* drugs[5-8]. A systematic review of sixteen *Chlorophytum* species with special reference to their physiology, occurrence, chemical constitution, ethnobotanical usage and different biological activities in Ayurvedic perspectives has been reported earlier[9].

A number of researchers have investigated various species of *Chlorophytum* with respect to their biological activities; however, species like *C. borivilianum* and *C. tuberosum* (Roxb.) Baker have been studied extensively while others were largely overlooked. In India, out of available eighteen *Chlorophytum* species, only *C. borivilianum* is commercially cultivated, which maybe because of sufficient knowledge generated in respect to its phytochemical content and medicinal activities. A wide array of actions of *C. borivilianum*, viz. aphrodisiac, immunomodulatory, anthelmintic, antioxidant, antiulcer, anti-stress, anti-tumor and antimutagenic activities are reported[10-16].

The major consumers of root powder of *Safed Musali* (*Chlorophytum* species) are from USA, UK,

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Australia, Japan, China and European Union countries. China and Japan are the top consumers having highest per capita consumption of herbal medicines. In the last decade, annual demand for *C. borivilianum* in India alone was 35000 tones as against supply of 5000 tones[17]. There is huge gap in supply because only *C. borivilianum* is in demand; which may be due to presence of detailed scientific studies on it. This situation arose because the need for finding an alternative to *C. borivilianum* and scientific comparison of rest of the species with respect to their phytochemical and pharmacological activities is largely overlooked. Reports, though scanty, suggest that all the species found in India demonstrate parallel bioactivities but due to lack of reliable scientific data, the demand is creating pressure on *C. borivilianum* only[17]. Hence, a systematic comparison of twelve *Chlorophytum* species with special reference to their antioxidant activity, total phenols, saponins and flavonoids content, HPTLC fingerprinting and GC-MS analysis was undertaken with the aim to find a suitable alternative to *C. borivilianum.*

Materials and Methods

Analytical grade organic solvents, chemicals, reference compounds, 2,2-diphenyl-1-picrylhydrazyl (DPPH) etc. were used and procured from Sigma-Aldrich (India). HPTLC silica gel 60 (without fluorescence) plates were purchased from Merck (Mumbai, India).

Plant material

Twelve *Chlorophytum* species (Plate 1) were collected during rainy seasons of 2009 to 2012 from different locations of Western Ghats and after

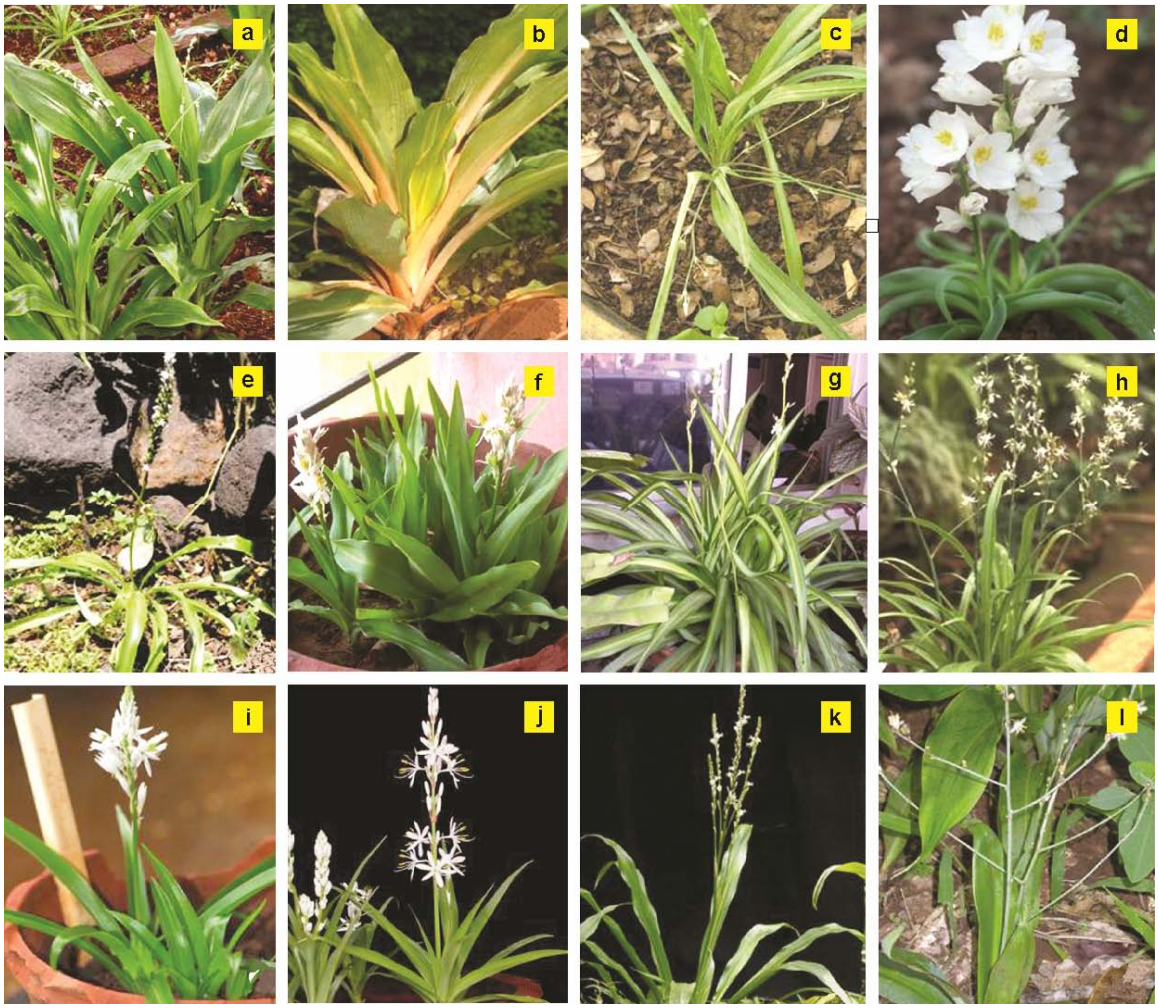

Plate 1—*Chlorophytum* Ker Gawl. species growing in natural habitat. a) *C. nimmonii* Dalzell, b) *C. amaniense* Engl., c) *C. laxum* R. Br., d) *C. tuberosum* (Roxb.) Baker, e) *C. glaucoides* Blatt., f) *C. belgaumense*, g) *C. comosum* (Thunb) Jacq., h) *C. bharuchae* Ansari, i) *C. gothanense* Malpure & S.R.Yadav, j) *C. borivilianum* Santapau and R.R.Fern, k) *C. glaucum* Dalzell and l) *C. kolhapurense* Sardesai)

phenotypic characterization, identification was done with the help of expert taxonomist Prof. S R Yadav, Department of Botany, Shivaji University, Kolhapur. Specimens were maintained in the botanical garden of the Department of Botany, Shivaji University and voucher specimens were deposited in the herbarium at Shivaji University. Details of specimen collected, location of collection with GPS coordinates along with voucher specimen number are given in Table 1.

Preparation of plant extracts

Tubers were harvested from intact plant body in the month of November 2012, washed with tap water and dried in hot air oven at 40 °C for 2-3 days. For analysis of each plant, 5-7 similar size rhizomes were pooled together to avoid inter species difference in the chemical fingerprint. Dried tubers were homogenized in an electric blender to pass through 1 mm sieve and stored in airtight containers for further use. Powdered material (5 g) of each plant was defatted three times with 50 mL n-hexane and dried. Defatted powdered tubers were extracted with 50 mL of 60 % methanol in water. Extracts were filtered, concentrated and dried under vacuum. Dried extracts were dissolved in 30 mL water and extracted three times with equal volume of n-butanol. After partitioning, n-butanol extract was concentrated to dryness and the resultant residues were dissolved in 2 mL methanol and again precipitated using 40 mL diethyl ether. Precipitate so formed was collected by centrifugation (2000 rpm at 5 °C for 20 min) and re-dissolved in methanol to achieve stock concentration at 10 mg/mL for further analysis[18].

Total phenol content

The amount of total phenolics present in extracts of all *Chlorophytum* species was determined (as gallic acid equivalent μg/mg of extract) using the Folin-Ciocalteau reagent as described earlier[19]. A gallic acid standard curve (R^2 = 0.9) was used to measure the phenolic content.

Total flavonoid content

The amount of total flavonoids (as Quercitin equivalent μg/mg of extract) present in extracts of

Table 1—List of *Chlorophytum* Ker Gawl. species, voucher information and GPS coordinates of collection location

S. No.	*Chlorophytum* species	Voucher number (Collection year)	Location (GPS)
1.	*C. nimmonii* Dalzell	SUK 104 (2011)	Kondushi (16°12'36.3"N 74°00'01.3"E)
2.	*C. amaniense* Engl.	SUK 749 (2009)	Ornamental plant
3.	*C. laxum* R. Br.	SUK 105 (2012)	Shivaji University, Kolhapur (16°40'24.9"N 74°15'15.1"E)
4.	*C. tuberosum* (Roxb.) Baker	SUK 101 (2011)	Ratnagiri (17°00'19.9"N 73°19'39.5"E)
5.	*C. glaucoides* Blatt.	SUK 111 (2012)	Tillari (17°56'23.7"N 73°37'57.9"E)
6.	*C. belgaumense* Chandore	Chandore 1113 (2010)	Khanapur belgaum (15°40’49.0”N 74°30’09.5”E)
7.	*C. comosum* (Thunb) Jacq.	SUK 765 (2012)	Ornamental plant
8.	*C. bharuchae* Ansari	ANC 700 (2009)	Adi Chikkodi (16°29'54.8"N 74°21'06.1"E)
9.	*C. gothanense* Malpure & S.R.Yadav	SUK 103 (2011)	Kondushi, Gargoti (16°12'41.1"N 73°59'24.1"E)
10.	*C. borivilianum* Santapau & R.R.Fern	SUK 100 (2011)	Kasedi Poladpur (17°54'07.8"N 73°26'14.2"E)
11.	*C. glaucum* Dalzell	SUK 110 (2012)	Tillari (15°46'42.6"N 74°10'18.4"E)
12.	*C. kolhapurense* Sardesai	SUK 106 (2012)	Sutagatti ghat (16°02'31.9"N 74°29'21.5"E)

collected *Chlorophytum* species was determined using aluminium chloride reagent[20]. A Quercetin standard curve (R^2 = 0.9) was used to measure the total flavonoid content

Total saponin content

Total Saponin content (as Quillaja saponin equivalent µg/mg of extract) in final saponin rich extracts was determined by the colorimetric method using vanillin sulphuric acid. In this, extract (10 µL) diluted with distilled water (90 µL) was mixed with vanillin (8 % w/v in absolute ethanol) and sulphuric acid (72 %). This mixture was incubated for 10 min at 60 °C and cooled in ice bath. Following vigorous shaking, absorbance was measured at 538 nm. Quillaja saponin was used as a reference standard and standard curve (R^2 = 0.9) was used to measure the total saponin content[21].

DPPH free radical scavenging assay

Comparative antioxidant capacity of all the species of *Chlorophytum* collected was confirmed by the DPPH scavenging assay with slight modification[22]. Different concentrations (10 to 100 µg/mL) of the extracts and ascorbic acid (standard) were thoroughly mixed with 5 mL of methanolic DPPH solution (33 mg/L) in test-tubes and the resulting solution was kept standing for 10 min at 37 °C before the optical density (OD) was measured at 517 nm. The measurement was repeated with three sets and an average of the readings was considered. The percentage radical scavenging activity was calculated using the following formula:

$$\% \text{ scavenging [DPPH]} = [(A_0 - A_1)/A_0] \times 100$$

where; A_0 was the absorbance of the control and A_1 is the absorbance in the presence of the samples.

HPTLC fingerprint analysis

Sample solutions (1 mg/mL) along with standard saponin (1 mg/mL) obtained from Sigma-Aldrich, USA were applied (band length 10 mm, 150 nL/s delivery speed, distance from the edge 10 mm) with the help of a Camag Linomat 5 applicator (CAMAG, Muttenz, Switzerland) on HPTLC silica gel 60 pre-coated plates 20 cm x 10 cm (Merck). Nitrogen was used as carrier gas for the sample application. A mobile phase comprising of chloroform: acetic acid : methanol: water (6.4:3.2:1.2:0.8) was used. Solvent system was added to twin trough chamber for saturation for about 20 min and then the plate was allowed to develop in an upward direction with migration distance of 90 mm. After development, the plates were derivatized by vanillin sulphuric acid reagent, anisaldehyde reagent and phosphomolybdic acid reagent separately. It was air-dried at room temperature and then heated at 100 °C for 15 min[23,24]. Data was processed by win CATS 1.4.4.6337 (CAMAG) software.

GC-MS analysis

Gas chromatography coupled with mass spectrometry was used for identifying compounds present in the saponin-rich extracts of all *Chlorophytum* species. The analysis was executed with the help of Agilent Technologies 7890 instrument comprising of head space injector and combipal auto sampler coupled to MS and was operating in 70 eV Electron Impact mode. HP5 column (30.0 m x 0.25 mm x 0.25 µm) was used for separation of compounds. Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. The split ratio of 20:80 was used for injection of 1 µL of sample solution (1 mg/mL) at 280 °C. The temperature program of oven commenced at 80 °C, was held for 1 min and further increased to 280 °C (80 to 200 °C at 3 °C/min and 200 to 280 °C at 7 °C/min), injector temperature was kept at 280 °C[25]. The qualitative analysis was done in full scan acquisition mode. Interpretation of mass spectrum from GC-MS was done using NIST/EPA/NIH Mass spectral database (NIST 11) with NIST MS search program v.2.0 g. The mass spectra of unknown compounds were compared with the spectrum of known molecules stored in NIST library.

Results and Discussion

The tubers of the twelve *Chlorophytum* species were processed and used for extraction and comparative radical scavenging, HPTLC finger print development and GC-MS profiling.

Phytochemical analysis (Total phenol, flavonoids and saponin contents)

Plant-derived compounds belonging to phenol group contribute largely in demonstrating free radical scavenging[26]. Among all the species analyzed, *C. amaniense* gave highest total phenol contents (654.5 mg/g) followed by *C. borivilianum* (429.5 mg/g) whereas *C. glaucoides* Blatt. had least total phenol content (57.5 mg/g) of the total extract. The total phenol content of remaining species ranged in between 72.5 to 159.5 mg/g of total extract.

Table 2—Total phenol content (TPC), Total flavonoid content (TFC), Total saponin content and % DPPH scavenging activity of twelve different *Chlorophytum* species

S. No	Name of *Chlorophytum* species	TPC (mg GAE/g extract)	TFC (mg quercetin/ g extract)	Total saponin content (%)	% DPPH scavenging (at 100 µg/mL)
1.	*C. nimmoni* Dalzell	83.5	48.0	2.22	32.18
2.	*C. aminense* Engl.	654.5	191.1	7.42	86.97
3.	*C. laxum* R. Br.	113.0	50.3	6.53	33.43
4.	*C. tuberosum* (Roxb.) Baker	93.5	104.3	4.76	28.33
5.	*C. glaucoides* Blatt.	57.5	74.6	4.61	29.75
6.	*C. belgaumense* Chandore	139.5	74.6	3.78	33.96
7.	*C. comosum* (Thunb) Jacq.	118.5	57.4	3.20	32.60
8.	*C. bharuchae* Ansari	159.5	154.3	2.01	34.53
9.	*C. gothanense* Malpure & S. R. Yadav	154.5	110.1	3.05	33.00
10.	*C. borivilanum* Santapau and R. R. Fern	429.5	93.4	4.85	62.86
11.	*C. glaucum* Dalzell	72.5	56.5	2.33	31.88
12.	*C. kolhapurense* Sardesai	78.5	48.0	1.89	31.24

The phenol content of individual sample is shown in Table 2.

Total flavonoid contents of the twelve species reported as quercetin equivalent were found between 48 to 191.1 mg/g (Table 2). *C. amaniense* had the highest flavonoid content (191.1 mg/g), where as *C. nimmonii* Dalzell had lowest flavonoid content (48.0 mg/g). Flavonoid content of most utilized and studied *C. borivilianum* was found to be 93.4 mg/g, which was far lower than *C. amaniense* even *C. bharuchae* Ansari was superior to it with respect to flavonoids content (154.3 mg/g).

A wide range of biological activities are attributed to saponin class of plant-derived secondary metabolites and comprehensive studies have been conducted by researchers to validate bioactivities of saponins[27]. The presence of saponins, viz. stigmasterol and hecogenin is considered to be the molecules responsible for imparting aphrodisiac properties to members of the genus *Chlorophytum*, especially *C. borivilianum*[28,29]. These qualities of *Musali* may be responsible for gaining it a very respectable place as *Vajikaran Rasayana* in the Indian System of medicine i.e. *Ayurveda*[30]. Hence, the saponin content was evaluated in the twelve species which ranged from 1.89 to 7.42 % (w/w). The most investigated *C. borivilianum* ranked third with 4.85 % saponin content. *C. amaniense* had highest total saponin content (7.42 %) followed by *C. laxum* R. Br. (6.53 %). *C. glaucoides* and *C. tuberosum* (Roxb.) Baker showed comparable saponin content (4.61 and 4.76 %, respectively) to that of *C. borivilianum*. Other species, viz. *C. gothanense* Malpure & S.R.Yadav, *C. comosum* (Thunb) Jacq. and *C. belgaumense* showed 3.05, 3.20 and 3.78 % total saponins, respectively. Whereas, *C. kolhapurense* Sardesai, *C. bharuchae*, *C. nimmonii* and *C. glaucum* Dalzell showed less than 3 % saponin content i.e. 1.89, 2.01, 2.22 and 2.33 %, respectively (Table 2). The saponin content in various species of genus *Chlorophytum* with special reference to their types, content and biological activities have been reviewed with major focus on *C. borivilianum* by a number of researchers and are systematically compiled and documented[31]. Variation in the saponin content (2-17 %) in *C. borivilianum* has been reported[32], which may be due to change in the genotypes. Literature search revealed maximum number of reports on saponin content focused on *C. borivilianum* indicating that less attention has been paid towards the remaining species.

DPPH free radical scavenging activity

DPPH free radical scavenging potential was evaluated at various concentrations (10-100 µg/mL) of methanolic extracts. For the ease of comparison, antioxidant activity and radical scavenging activities at 100 µg/mL only are given here. A significant

variation in all the species was observed ranging from 28.33 to 86.97 % (Table 2). Highest DPPH free radical quenching activity was observed in *C. amaniense* (86.97 %). *C. borivilianum* showed 62.86 % antioxidant activity, which was around 30 % lower than that of *C. amaniense*. The remaining species showed much lower antioxidant activities ranging from 29.75 to 34.53 % at 100 µg/mL concentration. Saponins, phenolics, flavonoids, alkaloids, pigment molecules, etc. are the major groups of compounds which demonstrate antioxidant properties[33]. A highly significant correlation between total phenols and radical scavenging potential has been reported in various studies in a number of plant species[26,25]. Similarly a correlation between saponin content and antioxidant activity has been reported earlier[27]. The present observations are in partial agreement with these reports regarding the total phenol and saponin content and its positive correlation with antioxidant activity. The present study indicates that total phenol plays a vital role in imparting radical scavenging activity and *C. amaniense* emerged as a superior alternative to *C. borivilianum* with respect to total phenols, flavonoids, saponins and antioxidant activity.

HPTLC profiling

In the present study, a chemical fingerprint of twelve different *Chlorophytum* species with respect to saponins was established. A solvent system comprising of chloroform, glacial acetic acid, methanol, water (6.4: 3.2: 1.2: 0.8) showed optimum resolution and better separation as compared to all other solvent systems tried. There was a need of derivatization with the help of chemical reagent for visualization of saponins since they do not show fluorescence quenching in UV range (254 and 366 nm). Saponins were derivatized using three different reagents, viz. anisaldehyde, phosphomolybdic acid and vanillin-sulphuric acid[23]. As far as the stability of colour developed on chromatogram was concerned, vanillin-sulphuric acid reagent gave better colour stability of bands than that of anisaldehyde reagent. Use of phosphomolybdic acid could not display colour variation in chromatogram as in vanillin-sulphuric acid and anisaldehyde reagent. Display of various characteristic colour bands of varying intensities were observed in a qualitatively developed HPTLC plate (Plate 2). Distinct bands of blue, violet, grey, reddish brown and brown colours representing

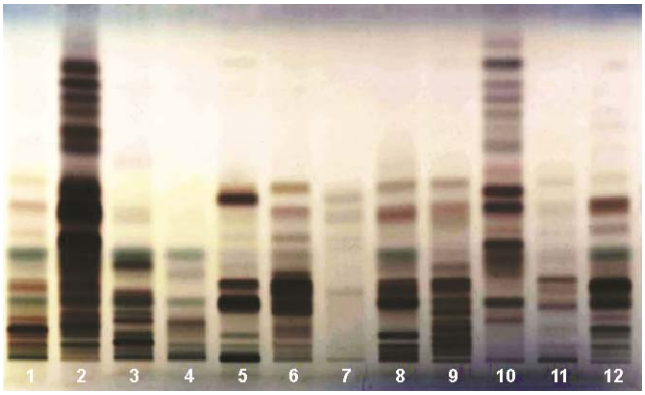

Plate 2—HPTLC analysis for separation of saponins, HPTLC plate after derivatization by vanillin sulphuric acid reagent (white light), profiles of 12 Chlorophytum Ker Gawl. species (where 1- *C. nimmonii* Dalzell, 2- *C. amaniense* Engl., 3- *C. laxum* R. Br., 4- *C. tuberosum* (Roxb.) Baker, 5- *C. glaucoides* Blatt., 6- *C. belgaumense*, 7- *C. comosum* (Thunb) Jacq., 8- *C. bharuchae* Ansari, 9- *C. gothanense* Malpure & S.R.Yadav, 10- *C. borivilianum* Santapau & R.R.Fern, 11- *C. glaucum* Dalzell and 12- *C. kolhapurense* Sardesai).

different saponins could be observed and are in agreement with earlier reports[34,35]. Each track corresponding to individual *Chlorophytum* species was scanned at 540 nm as a function of R_f value; each peak at different R_f value represented individual compound. Number of bands in different species of *Chlorophytum* ranged in between 7 to 12. The chromatogram revealed that most of the molecules are common in all the species though there are variations in the area under peak of the respective band. *C. amainense* showed maximum 12 distinct peaks where as other species showed merging of major bands of lower R_f values suggesting presence of more than one molecule having similarity in structures. The peak pattern of lower R_f value molecules in *C. borivilianum*, *C. belgaumense*, *C. laxum*, *C. gothanense*, *C. nimmonii*, *C. glaucum*, *C. kholapurense*, *C. glaucoides* and *C. tuberosum* was almost similar with slight difference in the concentrations of corresponding molecules as observed by variation in the area under peak. A track wise comparative HPTLC profile scanned at 540 nm after application of equal concentration of extracts of all the *Chlorophytum* species studied is presented in Plate 3.

GC-MS analysis

Extracts of the twelve *Chlorophytum* species under study were subjected to GC for separation and further to MS analysis of separated molecules.

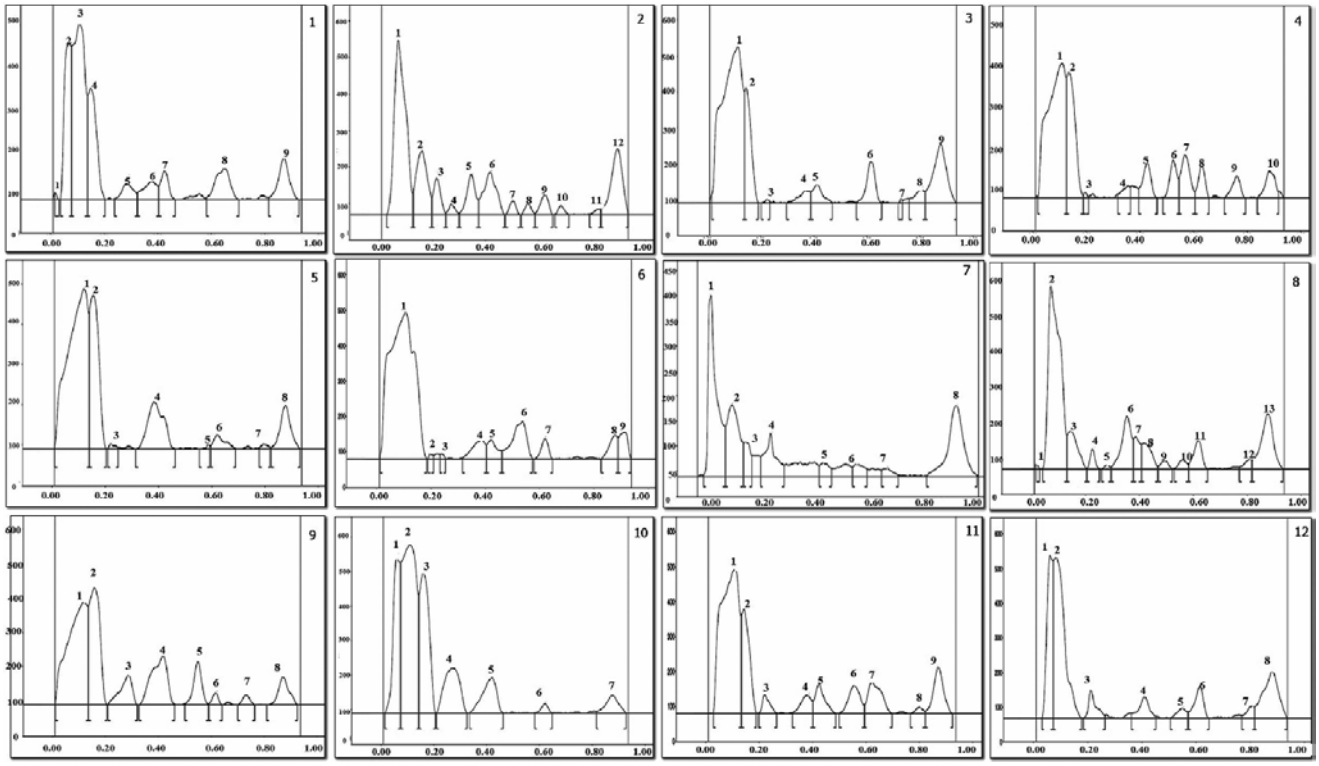

Plate 3—A track wise comparative HPTLC profile scanned at 540 nm after application of equal concentration of extracts of all the species of *Chlorophytum* Ker Gawl. (where 1- *C. nimmonii* Dalzell, 2- *C. amaniense* Engl., 3- *C. laxum* R. Br., 4- *C. tuberosum* (Roxb.) Baker, 5- *C. glaucoides* Blatt., 6- *C. belgaumense*, 7- *C. comosum* (Thunb) Jacq., 8- *C. bharuchae* Ansari, 9- *C. gothanense* Malpure & S.R.Yadav, 10- *C. borivilianum* Santapau & R.R.Fern, 11- *C. glaucum* Dalzell and 12- *C. kolhapurense* Sardesai).

Interpretation of mass spectrum from GC-MS was done using NIST/EPA/NIH mass spectral database (NIST 11) with NIST MS search program v.2.0 g. The mass spectra of unknown compounds were compared with the spectrum of known molecules stored in NIST library. An array of molecules was observed to be present in the extracts subjected to GC-MS analysis. Cumulatively, peaks with retention times (Rt) ranging from 3.1 to 32.2 min were detected. NIST 11 database could detect nearly 35 molecules, out of which nearly 40 % compounds appeared to be fragments of some other molecules which were not available in the database. A comprehensive list of all compounds and their presence or absence in the respective *Chlorophytum* species is presented in Table 3.

World Health Organization estimates that nearly 80 % world population still relies on plant based medicines to cater its primary health care needs[36]. In developed countries, phyto-pharmaceuticals are regarded as complementary or alternative medicine and their popularity is observed to be increasing. United State Food Drug Administration regulates such botanicals as food rather than drugs[37]. In the last twenty years, pharmaceutical sector all over the world has invested a lot of money to conduct research on pharmacological, clinical and phytochemical analysis in anticipation of developing more potent plant drugs[38]. As far as genus *Chlorophytum* is concerned, its members harbor a wide array of secondary metabolites, saponins being the dominant with varying concentrations. *C. borivilianum* is considered to be economically important probably due to higher content of saponins and is utilized to prepare a variety of drug formulations like tonic, massage oil, capsule, powder, etc. by different phytopharmaceutical companies[39]. More than 95 % of the available quantum of *C. borivilianum* is collected from the forests[17]. Continuous increase in the magnitude of medicinal plant market indicates a very high economic importance of *Safed Musali* at international level[30].

Table 3—Compound and/or fragments of molecules detected in extracts of twelve *Chlorophytum* Ker Gawl. species by GC-MS

S. No.	Compound name	R_t (min)	Plant species											
			1	2	3	4	5	6	7	8	9	10	11	12
1.	Formic acid, ethenyl ester	3.1	+	+	-	-	-	+	+	-	+	-	-	+
2.	1H-Tetrazole, 1-methyl-	3.4	-	-	-	-	-	+	-	-	-	-	-	-
3.	Glycerin	3.5	-	-	-	-	+	-	-	-	-	-	-	-
4.	2-Cyclopenten-1-one, 2-hydroxy-	3.5	-	-	-	-	-	+	-	-	-	-	-	-
5.	Benzeneacetaldehyde	4.6	-	-	-	-	-	-	+	-	-	-	-	-
6.	1-amino-2,6-dimethylpiperidine	4.7	-	-	-	-	-	+	-	-	-	-	-	-
7.	Guanethidine	4.9	-	+	-	-	-	-	-	-	-	-	-	-
8.	Cyclopropylcarbinol	5.5	-	-	-	-	-	-	-	-	+	-	-	-
9.	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	6.4	-	-	-	-	-	-	+	-	-	-	-	-
10.	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	7.4	-	-	-	+	-	+	+	-	-	-	-	-
11.	Hydroquinone	7.9	+	+	-	+	-	-	-	+	-	-	-	-
12.	DL-Proline, 5-oxo-, methyl ester	9.6	+	+	-	-	+	-	-	+	-	+	-	-
13.	4-Hydroxy-2-methylacetophenone	10	-	-	-	-	-	-	-	-	-	-	+	-
14.	Benzene, 1-chloro-4-methoxy-	10.1	-	-	-	+	-	-	-	-	-	-	-	-
15.	L-phenylalanine, methyl ester	10.4	-	-	-	-	-	-	-	-	-	+	-	-
16.	1,3-Propanediol,2-(hydroxymethyl)-2-nitro	10.9	-	-	-	-	-	-	-	-	-	+	-	-
17.	Ethanone,1-(4-hydroxy-3-methoxyphenyl)-	11.5	-	-	+	-	-	-	-	-	-	-	-	-
18.	Methyl-α-d-ribofuranoside	11.6	-	+	-	+	-	-	-	-	-	-	-	-
19.	Phenol, 2,4-bis(1,1-dimethylethyl)-	11.8	+	-	-	-	+	-	-	-	-	+	-	-
20.	Adipic acid, diphenyl ester	11.9	-	+	-	-	-	-	-	-	-	-	-	-
21.	Benzoic acid, 4-ethoxy-, ethyl ester	12	-	-	-	-	-	-	-	+	-	-	-	-
22.	2-Butenedioic acid (Z)-, dibutyl ester	12.2	-	-	-	-	-	-	-	+	+	-	-	-
23.	Lactose	12.4	-	-	-	-	-	-	-	-	-	-	-	+
24.	3',5'-Dimethoxyacetophenone	12.6	-	-	+	-	-	-	-	-	-	-	-	-
25.	Benzeneacetic acid, 4-hydroxy-3-methoxy-	13.9	-	-	+	-	-	-	-	-	-	-	-	-
26.	Tertadonium bromide	14.3	-	-	-	+	-	-	-	-	-	-	-	-
27.	1-Tetradecanamine, N,N-dimethyl-	14.6	-	-	-	-	-	+	-	-	-	-	-	-
28.	Phenol,4-(3-hydroxy-1-propenyl)-2-methoxy-	15.1	-	-	-	+	-	-	-	-	-	-	-	-
29.	1-Nonanamine, N,N-dimethyl-	17.7	-	-	-	-	-	-	+	-	-	+	-	+

(*contd.*)

Table 3—Compound and/or fragments of molecules detected in extracts of twelve *Chlorophytum* Ker Gawl. species by GC-MS (*contd.*)

S. No.	Compound name	R_t (min)	Plant species											
			1	2	3	4	5	6	7	8	9	10	11	12
30.	1-Naphthalenecaboxaldehyde, 2-methoxy-	19.7	-	-	-	-	-	-	-	-	-	-	+	-
31.	9,12-Octadecadienoic acid, methyl ester, (E, E)-	20.9	-	-	-	-	-	-	-	-	-	+	-	-
32.	Dibutyl phthalate	22.3	-	-	-	-	-	-	-	+	+	-	-	-
33.	Hexadecanoic acid, 2,3-dihydroxypropyl ester	26.7	-	-	+	-	-	-	-	-	-	-	-	-
34.	1,2-benzenedicarboxylic acid, diisooctyl ester	27.3	-	+	-	-	-	-	-	-	-	-	-	-
35.	Squalene	32.2	+	-	-	-	-	-	-	-	-	+	-	-

*Where 1: *Chlorophytum nimmonii* Dalzell; 2: *C. amaniense* Engl.; 3: *C. laxum* R. Br.; 4: *C. tuberosum* (Roxb.) Baker; 5: *C. glaucoides* Blatt.; 6: *C. belgaumense* Chandore ; 7: *C. comosum* (Thunb) Jacq.; 8: *C. bharuchae* Ansari; 9: *C. gothanense* Malpure & S. R. Yadav; 10: *C. borivilianum* Santapau & R.R.Fern; 11: *C. glaucum* Dalzell and 12: *C. kolhapurense* Sardesai

Conclusion

Twelve *Chlorophytum* species were collected from different locations of Western Ghats and analyzed comparatively for total saponin, flavonoid and phenol contents. *In vitro* comparative antioxidant potential of all the species was also determined along with chemical fingerprinting with HPTLC and GC-MS. Of the twelve species analyzed, *C. amaniense* Engl. showed highest phenol, flavonoid and saponin contents. It also demonstrated 30 % higher DPPH free radical scavenging activity than that of *C. borivilianum*. HPTLC chemical fingerprint of all the studied *Chlorophytum* species showed a significant variation in saponin and flavonoid content. HPTLC fingerprint of *C. amaniense* showed presence of maximum number of bands with high intensity. In present study, better performance of *C. amaniense* was found over *C. borivilianum* in terms of total saponin, flavonoid, phenol content and *in vitro* antioxidant activity. Considering the vulnerability of *C. borivilianum*, *C. amaniense* can serve as a potential alternative for this valuable plant.

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