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Research Paper

RP-HPLC AND FT-IR FINGER PRINTING OF *Pilea microphylla* (L.) Liebm. IN CONNECTION WITH DESICCATION

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Abstract

Pilea microphylla (L.) Liebm an annual herb, belongs to urticaceae commonly known as Gun powder or artillery plant is acclimatized to grow in extreme and diverse habitats. A major factor that limits the productive potential of higher plants is the availability of water. The most severe form of water deficit is desiccation i.e., most of the protoplasmic water is lost and only a very small amount of tightly bound water remains in the cell. So, an attempt was made to study the desiccation tolerance mechanism of *P. microphylla* in terms of total phenol content, phenolic acids using RP-HPLC and FT-IR spectroscopic analysis. The plant materials were subjected to desiccation treatment using polyethylene glycol (PEG) from 1D to 7D followed by rehydration for 30 min. All the values were compared with respective controls. The total phenol content (TPC) increased gradually with duration i.e. TPC was increased approximately by 3 fold on the 5D of desiccation [11.28 mg g^{-1}] when compared to the control [3.9 mg g^{-1}], whereas on rehydration the phenol level is regained to the control value. The RP-HPLC analysis of phenolic acids in the control plant showed the presence of chlorogenic acid [211.553 µg g^{-1}], sinapic acid [0.569 µg g^{-1}], coumaric acid [0.744 µg g^{-1}], hydroxyl benzoic acid [7.784 µg g^{-1}], gallic acid [81.331 µg g^{-1}], vanillic acid [211.553 µg g^{-1}], cinnamic acid [211.553 µg g^{-1}], catechol [241.775 µg g^{-1}] and ferulic acid [1.686 µg g^{-1}]. Whereas, the 3D desiccated plant showed the presence of only few phenolic acids such as hydroxyl benzoic acid [70.271µg g^{-1}], gallic acid [124.451 µg g^{-1}], ferulic acid [456.321 µg g^{-1}] and phloroglucinol [601.515 µg g^{-1}]. Similarly the 3D rehydrated plants showed phenolic acids such as HBA [86.794 µg g^{-1}], gallic acid [145.453 µg g^{-1}], ferulic acid [533.328 µg g^{-1}] and phloroglucinol [703.023 µg g^{-1}]. The level of these phenolic acids was higher in the desiccated and rehydrated plants when compared to the control. The FT-IR spectra of the control plant showed 23 bands, range between 3514.30 – 420.48 cm^{-1}. Similarly the 1D plant showed the presence of additional bands like 1732.0cm^{-1}[carbonyls] and 1643.53 cm^{-1} [α, β-unsaturated aldehydes and ketones]. The 3D and 5D desiccated plants showed a more or less same trend meanwhile the 7D plant showed the presence of some additional groups indicating the breakdown of certain compounds due to stress. Further studies are warranted at molecular level to analyze the mechanism of desiccation tolerance in *P. microphylla*.

Key words: FT-IR, RP-HPLC, functional groups, desiccation and rehydration.

INTRODUCTION

The process of extreme drying or the state of dryness is known as desiccation that leads to the generation of free radicals in the cells. Most of the higher plants are unable to survive desiccation to an air-dried state whereas, seeds and pollen grains can withstand for certain periods of time. Pollen grains loose tolerance quickly, whereas seeds can stay for longer in the desiccated state. Similarly, group of vascular angiosperm plants termed "resurrection plants" have evolved desiccation tolerance, and also they can revive from an air-dried state. Desiccation tolerance and prolonged longevity in the desiccated state depend on the ability to scavenge free radicals, using antioxidants such as glutathione, ascorbate, α-tocopherols and free radical-processing enzymes. Lower plants such as lichens and mosses often contain pool of polyphenolics with antioxidant properties. *Pilea microphylla* (L.) Liebm is an annual plant belonging to Urticaceae . It has light green, almost succulent, stems and tiny 1/8" leaves which contribute to its nickname, "Artillery Fern", though it is not related to ferns. It is grown as a ground cover and also in many moist areas of cement walls.

The plant grows in extreme and diverse habitats and run wild in waste places. Abiotic stresses such as desiccation leads to the generation of free radicals in the cells that ultimately cause damage to the membranes, proteins and nucleic acids which causes the death of the plant. Polyphenols are powerful detoxifiers of ROS and are reported in resurrection plants. In *Selaginella lepidophylla*, phenolics (e.g., caffeate), flavonols (e.g., apigenin and naringenin), and phenylpropanoids (e.g., coniferyl alcohol) accumulated in desiccated tissues. In *H. rhodopensis*, phenols reached around 15–20% of the total dry weight of the desiccated plant. In contrast, the phenolic acids in *Ramonda serbica* decreased under desiccation and increased upon rehydration suggesting the diverse mechanisms in the plants. The present study focuses the tolerance mechanism of *Pilea microphylla* (L.) Liebm induced by desiccation.

MATERIALS AND METHODS

Pilea microphylla (L.) Liebm. was chosen for the present study.

Desiccation treatment

All the experimental *P. microphylla* samples were collected from the natural habitat. Before desiccation, the samples were fully hydrated and equilibrated in a controlled environment chamber for 48 h at 20°C and a radiant flux intensity 75 μM m^{-2} s^{-1}. The samples were desiccated in a desiccator over PEG in a controlled environment chamber using the same light and temperature regimes as described above. The selected plants were subjected to four different desiccation regimes 1 D, 3 D, 5 D and 7 D. After the desiccation exposure a set of desiccated samples were subjected to rehydration for 30 min. The samples were divided into two groups: desiccated and desiccated subsequently rehydrated. Control plants were maintained in an optimal water conditions in each case during the whole experimental period.

Quantification of total phenol

Total phenol content in the samples was estimated by the method of Mayr, *et al.,* [1995]. The reaction between phenols and an oxidizing agent phosphomolybdate in Folin-Ciocalteau reagent resulted in the formation of a blue complex. A standard graph of phenols was constructed with pyrocatechol by taking absorbance against different concentration. The total phenols g^{-1} tissue was calculated from the standard graph.

Reverse phase high performance liquid chromatography (RP-HPLC) of phenols

Quantitative fractionation of various phenolic acids in the samples was studied by RP-HPLC analysis. Phenolic acids extracted from fresh tissues in aqueous methanol were used for the study.

Preparation of the sample

1 g fresh finely chopped tissue was reflexed in boiling 80% methanol for 10 min. The tissue was homogenized, filtered through cheese cloth and centrifuged at 15000 rpm for 10 min. The resultant supernatant was made up to 5 ml with 80% methanol and used for RP-HPLC analysis.

Procedure of RP-HPLC

A modified method of Beta, *et al.,* [1999] was followed for HPLC analysis. An HPLC system (Waters Associates) equipped with a 7725 Rheodyne injector and Waters 510 HPLC pump, 486 tunable absorbance detector and Millennium 2010 software data module were used for the study. An HPLC column of 4.6 × 250 mm id reverse phase (RP) C8 was used for the fractionation of phenolic acids. Potassium hydrogen phosphate and acetonitrile in a ratio of 75:25 was used as the mobile phase for the isocratic elution. An elution period of 20 min with a flow rate of 0.8 ml min^{-1} was given. 10 µl of the sample was injected and the absorbance at 254 nm was recorded. Standard phenolic acids such as gallic, vanillic, p-hydroxybenzoic, ferulic, chlorogenic sinapic, para coumarate and cinnamic acids were injected in to the column separately. Comparing with the retention time of the standard identified phenolic acids in the sample. Height of the peaks was taken for quantification. Concentration of the standard and height of the standard peak were taken as the standard parameters.

FT-IR spectroscopy

The plant sample of each experimental condition (approximately 3-4 cm) was taken. The samples were immediately dried in an oven for 2 days at 60°C. Tablets for FTIR spectroscopy were prepared in an agate mortars, by mixing leaves powder (2 mg) with KBr (1:100 p/p). The absorbance spectra were measured between 300 and 4500 cm^{-1}. At least three spectra were obtained for each sample [Anilkumar, *et al.,* 2012].

A FTIR spectrometer (FTIR Shimadzu Prestige 21) was used to collect spectra. Spectra were obtained in 32 scans co-added, 4000 resolution, and 2.0 gains. The parameters for the Fourier self-deconvolution were a smoothing factor of 15.0 and a width factor of 30.0 cm^{-1}. De-convolved and second-derivative spectra were calculated for Fourier self-deconvolution and the bands were selected and normalized to unity with Omnic 7 software. Curve-fitting of the original spectra was performed with Origin 7 software. The band position of functional groups was monitored with Knowitall 7.8 software. The spectral region between 3000 and 2800 $cm^{-1}$was selected to analyze lipids. The spectral region between 1800 and 1500 $cm^{-1}$was selected to analyze proteins. The spectral region between 1200 and 1000 $cm^{-1}$was selected to analyze carbohydrates. Triplicate experiments (N = 3) were conducted and spectra from the first two times of experiments were used for establishment of chemometric models and the spectra from the third time of experiment were used for model validation.

Statistical analysis: Three independent replicate trials were conducted and significant differences ($P < 0.05$ or $P < 0.01$) between control plant and different desiccated samples, band area of spectra, and regression coefficient of loading plot were determined by one-way analysis of variance (ANOVA) followed by *t*-test using Matlab.

RESULTS AND DISCUSSION

Total phenols and fractionated phenolic acid by RP-HPLC

The total phenolic content in the tissues varied during the experimental periods. After desiccation (5 D), the total phenols increased approximately by 3 fold while on rehydration, it was maintained back to the level of control. These values are statistically significant at 1% ($P < 0.01$) (Table 1). The waxing and waning pattern of phenols was further investigated by fractionating the phenols by reverse phase high performance liquid chromatography (RP-HPLC). The figures 1a, b & c represent the HPLC chromatogram of phenolic extracts of tissues of *P. microphylla* of control, 3 D desiccation and rehydration. Phenolic acids such as cinnamic acid, caffeic acid, ferulic acid, sinapic acid, coumaric acid, hydroxy benzoic acid, chlorogenic acid, gallic acid and vanillic acid were used as standards for detecting the compounds. It is evident from the figures that phenolic extract of *P. microphylla* samples, contain the peaks of some of the standards, indicating the functional compartmentation of phenolic acids during plant growth (Table 2). The role of phenolic acids in lignin synthesis is well established by several authors [Murugan, *et al.,* 2004]. Lignin serves an important function in plant defense, mechanical support and water transport.

Drought stress is one of the remarkable abiotic stresses that affect growth and development of plants [Xu, *et al.,* 2010]. Drought stress initiates when the absorbable water in the soil is reduced to critical levels and unsaturated atmospheric conditions add to continuous

loss of water. Drought stress tolerance is common in plants but its tolerance varies from species to species. The drought stress occurs due to the water deficit, usually accompanied by acute temperatures and solar radiation [Xu, *et al.,* 2010]. Water deficit and salt stress are universal issues to ensure survival of agricultural crops and sustainable food production [Gosal, *et al.,* 2010]. Drought leads to oxidative stress and was reported to show increase in the amounts of polyphenols in *Willow* leaves [Larson, 1998]. Further, it also influenced changes in the ratio of chlorophyll a and b and carotenoids [Anjum, *et al.,* 2003]. A reduction in chlorophyll content was reported in cotton under desiccation stresses [Massacci, *et al.,* 2008] and *Catharanthus roseus [*Soliz-Guerrero, *et al.,* 2002]. Similarly, desiccation decreased the saponin content in *Chenopodium quinoa* from 0.46% dry weight (dw) in plants growing under low water deficit conditions to 0.38% in high water deficit plants *[*Soliz-Guerrero, *et al.,* 2002]. Anthocyanins are also reported to accumulate under drought and cold stress. Plant tissues containing anthocyanins are usually rather resistant to drought [Chalker-Scott, 1999]. For example, purple cultivar of chilli resists water stress better than green cultivar [Bahler, *et al.,* 1991]. Polyphenols have protective functions during drought stress. They are implicated to provide protection to plants growing in soils that are high in toxic heavy metals such as aluminum.

Polyphenolic compounds such as phenolic acids, flavonoids, anthocyanins have been already found to play an important role in scavenging free radicals produced during salt/abiotic stress in plants [Hichem, *et al.,* 2009]. The antioxidant activity of phenolics is mainly due to their redox properties, allowing them to function as reducing agents, hydrogen donors and singlet oxygen quenchers. They are implicated in response to different abiotic/biotic stresses. Polyphenol oxidase is one of the polyphenolic compounds redox catalyzing enzyme and the induction of its activity under salinity indirectly could indicate the involvement of antioxidative polyphenols in salt-challenged plants.

Gall, *et al.,* [2015] pointed out that polyphenols function in two ways such as an increased level in xyloglucan endotransglucosylase / hydrolase (XTH) and expansin proteins, associated with an increase in the degree of rhamnogalacturonan I branching that maintains cell wall plasticity and (ii) an enhanced cell wall thickening by reinforcement of the secondary wall materials such as hemicellulose and lignin deposition i.e., polyphenols could be targeted to enhance biomass production under stress situations.

Further, Gholizadeh and Kohnehrouz [2010] analyzed differential antioxidative potentialities in the leaves of two maize inbreeds under salinity stress and the subsequent recovery period. Total antioxidation test revealed that in both inbreeds, this potentiality was increased during stress period, but was reduced back to the normal level during recovery period. The enzymatic antioxidative activities showed differential patterns in catalase, peroxidase and polyphenol oxidase in both maize inbreeds. Comparative analysis of the activity of phenylalanine ammonia lyase (PAL), a key enzyme at the gateway of propanoid biosynthetic pathway, suggested that propanoid compounds might be antioxidants of pivotal importance to the salt-challenged maize antioxidation system. As for drought-stressed plants, PAL-dependent antioxidative strategy is proposed as a promising target for maize salt resistance engineering.

Senaratna, *et al.,* (2007) empolyed salicylic acid (SA) for its potential to induce tolerance to salinity (NaCl) stress in plants. Pre-treatment with 0.5 mM SA as a soil drench, remarkably increased the ability of garden bean, tomato, desert pea**,** corn, tuart and jarrah**,** wheat to tolerate saline conditions. Results suggest that SA induced tolerance to salinity stress in control species as well as in cultivars within a species. Furthermore, the degree of SA-induced tolerance appeared to be positively related to the inherent salt tolerance capacity of the taxon. This implies that intrinsic salt tolerance characteristics of particular genotypes may be a determinant in the successful utilization of SA.

Kang, *et al.,* [2006] reported the role of phloroglucinol, the phenolic acid to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydrogen peroxide (H_2O_2), hydroxy radical, intracellular reactive oxygen species (ROS), and thus prevented lipid peroxidation. As a result, phloroglucinol reduced H_2O_2 induced apoptotic cells formation in V79-4 cells. In addition, phloroglucinol inhibited cell damage induced by serum starvation and radiation through

scavenging ROS. Phloroglucinol increased the catalase (CAT), activity and its expression of protein. In addition, CAT inhibitor inhibited the protective effect of phloroglucinol from H_2O_2 induced cell damage. Furthermore, phloroglucinol increased phosphorylation of extracellular signal regulated kinase (ERK). Thus, the results suggest that phloroglucinol protects V79-4 cells against oxidative burst by enhancing the cellular CAT activity and modulating ERK signal pathway.

FT-IR spectroscopy

The IR spectrum of plant samples are shown in figures 2a, b, c, d and e. IR spectral absorption bands for the control ranges from 3514.30 to 420.48/cm. Alcohols and phenols are represented by 3514.30 cm^{-1} and 3458.37 cm^{-1} O-H (H-bonded); Primary, secondary amines, amides 3381.21 cm^{-1} and 3342.64 cm^{-1} N-H; Alkynes by 3323.35 cm^{-1}, 3302.13 cm^{-1}, 3284.77 cm^{-1}; Alkanes 2916.37 cm^{-1} C-H; Aldehydes, saturated aliphatic 1735.93 cm^{-1} C=O; Alkenes 1641.42 cm^{-1} C=C; Aromatics 1556.55 cm^{-1}, 1419.61 cm^{-1}, 873.75 cm^{-1}, 707.88 cm^{-1} C=C, C-C & C-H bending; Nitrocompounds 1546.91 cm^{-1} N-O; Carboxylic acid 1263.37 cm^{-1} C-O; Esters, ethers 1263.37 cm^{-1} C-O; Aliphatic amine 1060.85 cm^{-1} C-N; Alkyl halides 821.68 cm^{-1}, 667.37 cm^{-1}, 563.21 cm^{-1} C-Cl stretch & C-Br. The control plant showed 23 bands (Table 3).

Similarly, the 1 D desiccated plant also showed the presence of the above functional groups, in addition to some new groups like Carbonyls 1732.08 cm^{-1} C=O; α, β-unsaturated aldehydes and ketones 1643.53 cm^{-1} C=O. But some of them like saturated aliphatic, alkenes, aldehydes, nitrocompunds, esters, ethers and aliphatic amines were disappeared.

The 3 D desiccated plant showed a more or less similar functional groups with the disappearance of carbonyls, α, β-unsaturated aldehydes and ketones that noticed in the 1 D desiccated plants. Further, the 5 D desiccated plants also showed the same trend. Meanwhile, the 7 D desiccated plants showed the presence of some additional groups like Nitrocompounds 1533.41 cm^{-1} N-O; Alcohols & Phenols 3522.02 cm^{-1} O-H; indicating the formation of new compounds in the *P. microphylla* plant body due to desiccation stress (Table 3). Ahmed, *et al.,* [2013] reported that salt stress in *Jojoba* plant resulted in the emergence of some new peaks in FT-IR analysis; is may be due to the breakdown of certain compounds due to oxidative stress created by extreme salt exposure.

Yang and Yen [2015] attempted FT-IR spectrometry to detect the corresponding changes in chemical profile associated with the rapid changes in sodium and water content in 200 mm NaCl-stressed halophyte ice plants (*Mesembryanthemum crystallinum*). The changes in glycophyte *Arabidopsis* stressed with 50 mm NaCl were also analysed for comparison. Absorption bands corresponding to carbohydrates, cell wall pectin, and proteins were identified, with distinct IR spectra representing each developmental stage. Within 48 h of mild salt stress, the absorption band intensities in the fingerprint region increased continuously in both plants, suggesting that the carbon assimilation was not affected at the initial stage of stress. The intensities of ester and amide I absorption bands decreased marginally in *Arabidopsis* but increased in ice plant, suggesting that the cell expansion and protein synthesis ceased in *Arabidopsis* but continued in ice plant. In both plants, the shift in amide I absorption band was noticed regularly after salt stress, indicating a drastic conformational change of cellular proteins. Analyses of the ratio between major and minor amide I absorption band revealed that ice plant was able to balance complex form of proteins under stress. Furthermore, the changes in protein conformation showed a positive correlation to the leaf sodium contents in ice plant, but not in Arabidopsis.

FT-IR spectral signature of crystalline mannitol, a fungal polyol display its complex protective roles in *C. protuberata* hyphae. Similarly, protein content in cells remains fairly constant throughout the length of hypha, whereas the mannitol is found at discrete, irregular locations. Since the concentration of mannitol in cells varies with respect to position and is not present in all hyphae, this discovery may be related to habitat adaptation to stress, fungal structure and growth stages [Susan, *et al.,* 2010].

Similarly, the IR spectra demonstrated that salt stress can alter the protein structure, and protein became less ordered, but following prolonged stress, this response did not persist, and protein refolded slowly. In addition, FT-IR provides direct evidence for lipid peroxidation damage of salt stress on plants.

In the case of carbohydrates, the total band areas decreased 19%, and the bands at 1199 $cm^{-1}$and 1060 cm^{-1}lowered gradually, indicated the reduction in their synthesis and the structure kept changing, showing salt stress changed the structure of carbohydrate in the plant. After salt stress, the total band areas at1743 cm^{-1}decreased by 12% compared with the *Jojoba* by curve fitting analysis, and the band intensity was lowered, indicating the pectin synthesis decreased. After stress, this band just had a little change compared with the *Jojoba,* indicating the pectin synthesis returned to the *Jojoba* level [Ahmed, *et al.,* 2013]. The results are supportive to the present analysis in *P. microphylla*.

Wolkers and Hoekstra, [2003] analyzed FTIR studies on the overall protein secondary structure indicate that during the acquisition of desiccation tolerance, plant embryos exhibit proportional increases in α-helical structures and that β-sheet structures dominate upon drying of desiccation sensitive-embryos. During ageing of pollen and seeds, the overall protein secondary structure remains stable, whereas drastic changes in the thermotropic response of membranes occur, which coincide with a complete loss of viability. Properties of the cytoplasmic glassy matrix in desiccation-tolerant plant organs can be studied by monitoring the position of the OH-stretching vibration band of endogenous carbohydrates and proteins as a function of temperature. By applying these FTIR techniques to maturation-defective mutant seeds of *Arabidopsis thaliana* was able to establish a correlation between macromolecular stability and desiccation tolerance. Taken together, *in situ* FTIR studies can give unique information on conformation and stability of endogenous biomolecules in desiccation-tolerant tissues.

FTIR-ATR spectroscopy is suitable as a high-throughput method for lignin and energy estimations in large data sets. Zhou, *et al.,* [2011] studied the intra-specific variations in lignin and energy contents were unrelated to each other and that the lignin content, therefore, was no predictor of the energy content. Principle component analyses revealed that factor loadings for the energy content were mainly associated with carbohydrate ring vibrations, whereas those for lignin were mainly related to aromatic compounds.

Table 1: The total phenol content (mg g^{-1}) of the desiccated (1, 3, 5 and 7 D) and rehydrated plant body.

	Control	1 D	1 R	3 D	3 R	5 D	5 R	7 D	7 R
	3.9	5.69	3.96	10.22	3.59	11.28	4.2	5.78	4.2
SE	0.67	0.71	0.29	0.45	0.38	0.29	0.66	0.72	0.38
F ratio	1.35**	1.79**	2.56**	2.72**	2.79**	1.38**	1.29**	2.89**	2.59**
CD (0.05)	2.02	1.99	1.66	1.85	1.38	1.09	1.21	1.44	1.69

Table 2: RP-HPLC profile of phenolic acids (µg g^{-1} tissue) in control, and 3 D desiccated and rehydrated *P. microphylla* and their biological activities.

SL.NO	Phenolic acid	Control	Desiccated	Rehydrated	Biological activity
1.	Chlorogenic acid	211.553	-	-	Is an important intermediate in lignin biosynthesis.
2.	Sinapic acid	0.569	-	-	most common hydroxycinnamic acid and potent antioxidant
3.	Coumaric acid	0.744	-	-	Major component of lignin
4.	Hydroxyl benzoic acid (HBA)	7.787	70.271	86.794	It is an inducer of systemic acquired resistance in plants against diseases, soil borne pathogens (*Phytophthora* in *Banksia attenuate*) & also induces tolerance to a variety of abiotic stresses.
5.	Gallic acid	81.331	124.451	145.453	Acts against stress & pathogens
6.	Vanillic acid	211.553	-	-	It is an intermediate in the production of vanillin from ferulic acid & have medicinal properties(*Angelica sinensis)*
7.	Cinnamic acid	211.553	-	-	cinnamic acid is the first metabolite of the phenylpropanoid pathway and is consequently a precursor for lignin and flavonoid biosynthesis
8.	Catechol	241.775	-	-	Has a role in plant defence mechanism
9.	Ferulic acid	1.686	456.321	533.328	It is the component of lignocelluloses, where it confers rigidity to the cell wall by making the crosslink between polysaccharides and lignin.
10.	Phloroglucinol	-	601.515	703.023	Scavenge ROS and prevents lipid peroxidation (*Ecklonia cava)*

Table 3: FT-IR profile of functional groups (cm^{-1}) in control and (1, 3, 5 and 7 D) desiccated *P.microphylla*

COMPOUNDS	CONTROL	1 D	3 D	5 D	7 D
Alcohols / phenols	3514.30, 3458.37	3338.78	1060.85	-	3522.02
Primary, secondary amines, amides	3381.21, 3342.37	3400.50, 3271.27	3294.42	3327.21, 3311.78	3500.80, 3479.58, 3460.30, 3441.01, 3419.79, 3383.14, 3360.00, 3304.06, 873.75
Alkynes	3323.35, 3302.13,3284.77	3321.42, 3304.06,3282.84	3294.42	698.23	-
Alkanes	2916.37	2949.16, 2848.86	2920.23	2920.23	2916.37
Aldehydes, saturated aliphatic	1735.93	-	-	-	-
Alkenes	1641.42	-	-	1647.21	1664.57, 1643.35
Aromatics and aromatic amines	1556.55, 1419.61, 873.75, 707.88	1556.55, 1423.47, 678.94	1544.98, 1425.40, 873.75	1554.63, 1431.18, 873.75,1259.52	1448.54, 1419.61
Nitrocompounds	1546.91	-	-	-	1533.41
Carboxylic acids	1263.37	3246.20	2920.23	-	3288.63, 2916.37, 1058.92
Esters, ethers	1263.37	-	1060.85	-	1058.92
Aliphatic amines	1060.85	-	1060.85	-	-
Alkyl halides	821.68, 667.37, 563.21	823.60,796.60, 578.64,613.36, 678.94	786.96,665.44, 613.36, 543.93	545.85	792.74, 709.80
Carbonyls	-	1732.08	-	-	-
α, β unsaturated Aldehydes, ketones	-	1643.35	-	-	-
Fingerprint regions	439.77, 420.48	418.55	420.48	472.56	439.77

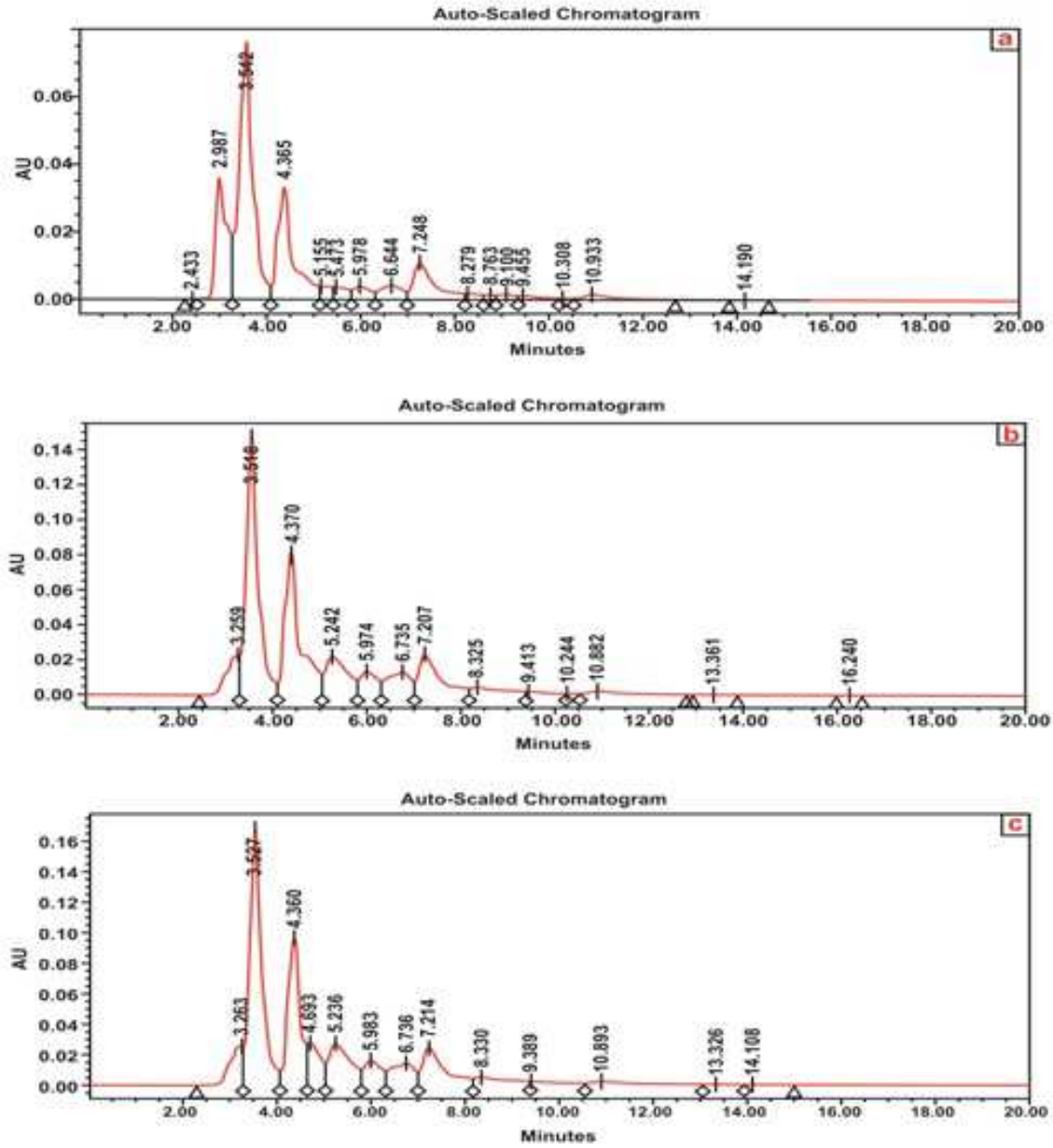

Figure 1: RP-HPLC profile of phenolic acids in *P. microphylla* a- control, b-3 D desiccated, c-3 D rehydrated

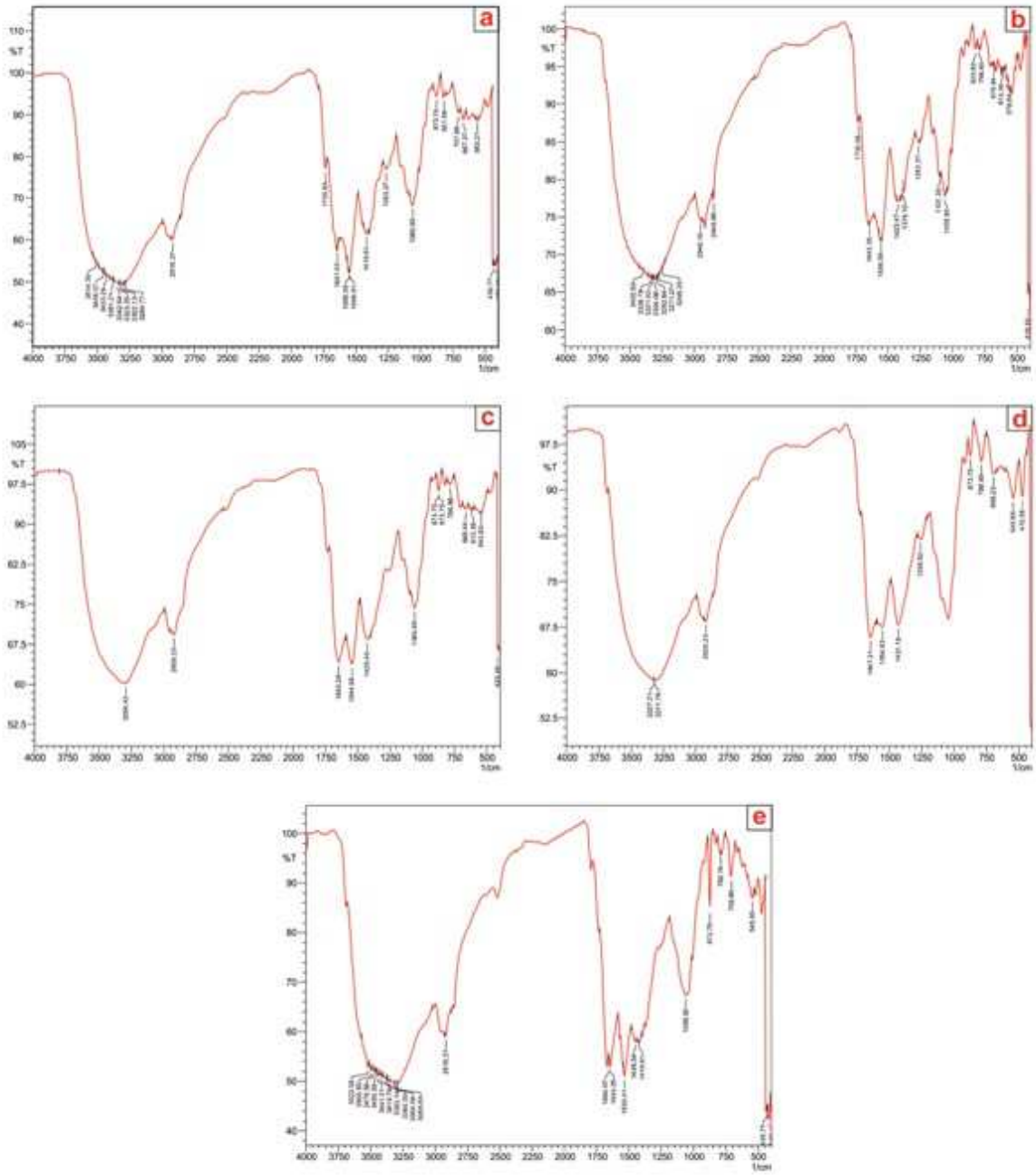

Figure 2: IR finger printing of control and desiccation treated *Pilea microphylla* a-Control, b-1 D, c- 3 D, d- 5 D and e-7 D (Days)

CONCLUSION

In summary, *P. microphylla* of urticaceae showed desiccation tolerance for 5D effectively. The total phenol content, phenolic acids and FT-IR functional group analysis support the tolerance mechanism in the plant. Further studies are designed at genetic level to trace the resistance mechanism of the plant against drought.

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