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Research Article

VARIABILITY OF DNA, PROTEIN AND ALKALOID CONTENT IN *SOLANUM TORVUM* SW. ACCESSIONS A PHARMACOBOTANICAL HERB

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ABSTRACT
Solanum torvum Sw. is a traditional medicinal plant with wide morphometric variations. This investigation was carried out to assess the molecular diversity of six accessions based on random amplified polymorphic DNA markers, proteins by SDS-PAGE; IR finger prints ans the total alkaloid content. The methodologies adapted include random amplified polymorphic DNA, SDS-PAGE, IR finger printing and isolation and quantification of alkaloids. From 15 random primers used, 14 primers gave reproducible amplification banding patterns of 95 polymorphic bands out of 118 bands scored accounting for 74.5% % polymorphism across the genotypes. Three primers generated maximum polymorphic patterns (primer 8, 10 and 12), whereas primer 1 produced minimum polymorphism. The size of the amplified products varied from 450 to 3000 bp. Based on these markers, genetic similarity coefficients were calculated and a dendrogram was constructed. UPGMA dendrogram cluster analysis indicated two distinct clusters, one comprising accessions of Chembooru, Korani, Kazhakuttom and Kaimanam. Accessions from Kanthallur and Rajamalai formed second cluster indicating its genetic relatedness. Torvoside content also varied among the accessions. SDS denatured protein gels could resolve 48 bands most of them are shared and some are unique and can be correlated with the DNA polymorphism. Fourier transform infrared spectra showed unique IR finger prints between the accessions. Genetic diversity among the accessions, a finding which strongly substantiate the morphological variability noticed among the *S. torvum* accessions across the study sites. Designing of a primer that can apparently generate accession-specific profiles is significant for further taxonomic and phylogenetic studies.

Key Words: *Alkaloids, Dendrogram, Genetic Polymorphism, Infra Red Finger Print, Protein Banding Pattern, RAPD, Solanum Torvum*

INTRODUCTION
Solanum torvum Sw. (Solanaceae), commonly known as Turkey berry, a medicinal plant in tropical and subtropical countries. The fruits and leaves are widely used in Cameroonian folk medicine (Kamble *et al.,* 2009). The fruits are utilized as vegetable and regarded as an essential ingredient in the South Indian population's diet. It is a pantropical weed that invades disturbed areas and forms large thorny impenetrable thickets with wide morphological variations. An enormous amount of naturally occurring morphological variation observed among the plants affecting quality of the drug within wild and domesticated habitats. This diversity is presumably involved in plant adaptation to different natural environments or in human preferences. In addition, such intraspecific variation provides the basis for the evolution of plant development at larger evolutionary scales. Natural phenotypic differences are now amenable to genetic dissection up to the identification of causal DNA polymorphisms (Guillermo *et al.,* 2011). Information on genetic diversity and relationships among populations is important for breeding programs as it helps to select the right genetic material to be used. Genetic diversity in species can be determined by using the agro-morphological as well as biochemical and molecular markers. PCR-based techniques such as amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), inter simple sequence repeats (ISSR) and random amplified polymorphic DNA (RAPD) also have been

widely used in genetic diversity studies (Gracelin *et al.*, 2011). The plants are used in liver diseases, tuberculosis and as antianaemic. The biological potentiality is due to the alkaloids like solasodine, torvogenin, torvoside and steroids such as sisalogenene and sisalogenin (Nurit-Silva *et al.*, 2011). In spite of the economic and medicinal value of *Solanum torvum*, no serious attention has been paid to evaluate the taxonomical diversity at the molecular level. This is a prerequisite to the exploitation of the vast genetic variability available for the improvement of the quality and quantity of their drug contents. The present study was aimed at analyzing molecular variability between six accessions species of *Solanum torvum* from different localities of Kerala.

MATERIALS AND METHODS

Plant Materials

Fresh *Solanum torvum* Sw. were collected from Kazhakuttom, Korani, Chembooru, Kaimanam, Rajamalai and Kanthallur, Kerala, India, and were identified by comparison with the voucher specimen from Kerala Forest Research institute (KFRI, Kerala). Voucher specimens were deposited at the herbarium of the Department of Botany, University College, Thiruvananthapuram and Kerala.

IR Spectroscopy

The leaves of each accession (approximately 3-4 cm) taken from plants were pooled as one sample. The samples were immediately dried in an oven for 2d 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 4500cm^{-1}. At least three spectra were obtained for each sample (Batten, 2009). 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.

Protein Band Patterns

Total soluble proteins were extracted from the accessions and protein banding pattern analysis was conducted using SDS-PAGE (John De Britto *et al.*, 2012).

Random Amplification of Polymorphic DNA (RAPD) Analysis

DNA extraction was carried out using the CTAB method with minor modifications in *Solanum torvum* leaf accessions (Alam *et al.*, 2012). Fresh leaf samples were thoroughly washed with double distilled water and stored at -20°C. 1g of tissue was taken, finely chopped and ground into a fine powder in a pre-chilled mortar using liquid Nitrogen. The ground sample was added to 1 ml extraction buffer (2% (w/v) CTAB; 100 mM Tris-HCl buffer (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, (1% 8w/v) PVP-40] and incubated at 65 °C for 90 min. The homogenate was mixed with 500 μ L 24:1 chloroform: isoamyl alcohol (v/v) and mixed well by gentle inversion. Following centrifugation at 10,000 rpm for 10 min, the upper aqueous layer was transferred to a fresh tube containing 600 μ L of isopropanol. The mixture was then allowed to settle at room temperature for 40 min. After centrifugation at 5,000 rpm for 3 min, the pellets were washed twice with 76% ethanol. The pellets were allowed to settle overnight at room temperature and resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA pH 8.0). The selected 15 arbitrary primers were purchased from Operon Technologies (Alameda, California). Amplification reactions were performed using the protocol reported by Xiao Xia *et al.*, (2010). Primers with reproducible results were selected for calculations (Table 1). At least three or four samples representing each taxon were used for amplification. Amplification was repeated twice for each sample. For the DNA amplification, a Biometra thermocycler (T Personel) was programmed for 45 cycles at 96 °C for 30 s, 35

°C for 30 s and 72 °C for 30 s, for denaturing, annealing and primer extension, respectively (Xiao Xia *et al.*, 2010)15 μ L of reaction products were separated alongside molecular weight markers (100 bp DNA ladder) by electrophoresis, on 1.5% agarose gels containing ethidium bromide. The gels were photographed under UV light, and the amplification patterns were examined. RAPD images were scored used for the analysis of the amplification products. Only the reproducible bands in multiple runs were considered in this study.

***Alkaloid Isolation*:** 10 g coarsely powdered fruit samples was Soxhlet-extracted with 50 mL 2% methanolic acetic acid for 3 h, and the extract was diluted to 100 mL with 2% methanolic acetic acid (Sutkovic *et al.*, 2011).

Procedure for Assay of Alkaloids

5 mL of the extract solution was taken and the pH was maintained at 2–2.5 with dilute HCl. 2 mL amount of DR (Prepared by mixing (1) solution of 0.8 g bismuth nitrate pentahydrate in 40 mL distilled water and 10 mL glacial acetic acid, and (2) solution of 8.0 g potassium iodide in 20 mL distilled water) was added to it, and the precipitate formed was centrifuged. The centrifugate was checked for complete precipitation by adding DR. After centrifugation, the centrifugate was decanted completely and meticulously. The precipitate was further washed with alcohol. The filtrate was discarded and the residue was then treated with 2 mL disodium sulfide solution. The brownish black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulfide. The residue was dissolved in 2 mL concentrated nitric acid, with warming if necessary. This solution was diluted to 10 mL in a standard flask with distilled water; 1 mL was then pipetted out, and 5 mL thiourea solution was added to it. The absorbance was measured at 435 nm against the blank containing nitric acid and thiourea. The amount of bismuth present in the solution was calculated by multiplying the absorbance values with the factor, taking suitable dilution factor into consideration. The factor is obtained from the standard curve, which is a constant for different concentrations.

Factor = concentration/absorbance

A comparison of total alkaloids in all the plant materials taken was made by using the method given in the CCRUM publication, Physicochemical Standards of Unani Formulations.

Solosodine Estimation by HPLC

The model used for HPLC analysis was Shimadzu, Japan. All samples were filtered through 0.2 μ m membranes. Main column used was analytical-shim-pack CLCOCTA DECYL SILANE (ODS-C18) (46mm10x25cm) and guard column was shim-pack G-ODS (4mm 10x1cm), flow rate: 1mL per minute, run time: 15 minutes, detector wavelength: 254nm, stationary phase: Silica gel (reversed phase) and mobile phase: Methanol (100% HPLC grade). Column head pressure was 125Kg+1cm 220 microlituce samples was injected to the column using Hamilton Microlituce Syringe, made in Japan. UV spectra were recorded with a diode array spectrophotometer coupled online. The experimental results were calculated as follows (mg/100g D.wt) =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard weight}}{\text{Dilution Sample}} \times \frac{\text{Dilution}}{\text{weight purity}} \times \text{Standard} \times 100$$

***Extraction and Isolation of Torvoside*:** The fresh fruits (500 g) were extracted with refluxing methanol, which was evaporated under reduced pressure to give a methanol extractive. The extractive was passed through Diaion HP-20 and successively eluted with water, 50% methanol, methanol and acetone. The methanol elute was chromatographed on silica gel with $CHCl_3$: methanol: water-8: 2: 0.1, 7: 3: 0.2, 7: 3: 0.5, methanol, gradient, to afford six fractions. Fraction 2 was further separated with silica gel ($CHCl_3$: MeOH: water system) and HPLC (50% methanol) to provide torvosides.

HPLC was conducted on an ODS column (Waters Cosmosil 5C18-MSII, f 20_250 mm, 5m m) using a Hitachi L- 6000 pump equiped with a differential refractometer (JASCO 830-RI). The FAB-MS were measured with a JEOL JMS-DX303HF spectrometers (Xe atom beam, accel. voltage 2—3 kV, matrix glycerol), 200—300 mA, ioni. Sugars were analyzed by using HPLC with 80% acetonitrile (flow rate: 0.8

mL /min, column oven 30 °C) equipped with optical rotatory instrument (column oven: JASCO CO-2060, pump: Pu-2080, detection of specific rotation: OR-2090 for and column: YMC-Pac R&D Polyamine II).

Statistical Analysis

The data was statistically evaluated by one way ANOVA and *t*-test. The results are average of 6 replications and are represented as mean ± SD.

RESULTS

RAPD Analysis

The 15 selected RAPD primers allowed us to obtain 118 DNA fragments, an average 7.86 per primer (Table 1). OPA 01 primer gave the fewest bands (3 fragments) and OPA 12 the most (16 fragments) (Figure 1). Optimization by the magnesium ion concentration produced a sufficient number of bands for each primer used.

98 DNA fragments were polymorphic for all investigated accessions (Table 2). Maximum polymorphic bands were displayed by the primers OPA 8, 10, 12 and minimum by OPA 1 (3 bands). OPA 6 did not produce any polymorphic bands (Table 2). The primers OPA 5, 7, 8, 9 and 14 produced unique bands for the accession collected from Kanthallur (850 bp; 450 bp; 900 bp, 800 bp and 900 bp respectively). Thus, categorization of individuals of Kanthallur accession was done by selected OPA markers. The number of polymorphic bands was 74.5 % on average. The number of shared bands between accessions from Kazhakuttom and Korani are 23; Kazhakuttom and Chembooru are 15; Kazhakuttom and Kaimanam- 9; Kazhakuttom and Rajamalai – 10 and Kazhakuttom and Kanthallur are 10. Similarly, Korani and Chembooru are 15; Korani and Kaimanam- 9; Korani and Rajamalai – 10 and Korani and Kanthallur are 12. Chembooru and Kaimanam- 9; Chembooru and Rajamalai – 10 and Chembooru and Kanthallur are 8. Kaimanam and Rajamalai – 9 and Kaimanam and Kanthallur are 8. Meanwhile, Rajamalai and Kanthallur are 12 (Table 3). The total bands among the accessions also showed variations i.e., Kazhakuttom -24; Korani - 25; Chembooru - 18; Kaimanam- 13; Rajamalai – 17 and Kanthallur- 21 (Table 10). The percentage of genetic distance among the accessions also showed diversity from 75% (Chembooru and Kanthallur) to 11.5% (Kazhakuttom and Korani) (Table 3).

The size of the amplified products varied from 450 to 3000 bp. Based on these markers, genetic similarity coefficients were calculated and a dendrogram was constructed. UPGMA dendrogram cluster analysis indicated two distinct clusters. The first larger cluster bifurcates into two. One lead ends in accessions Kaimanam and the other lead again branches comprising Chembooru accession. The second branch further divides into two includes accessions of Kazhakkuttom and Korani. The second major cluster includes highland accessions from Rajamalai and Kanthallur (Figure 2).

***Protein Profiling*:** The *S.torvum* accessions exhibited substantial changes in protein levels. SDS denatured protein gels could resolve a total of 48 bands. Highest number of protein bands was observed in the Kaimanam accessions. These SDS protein bands belong to different molecular weights ranging from 3.5 kDa to 84.5 kDa. Using Total Lab 100 protein analyzer software the molecular weights and the Rf values of each band was calculated. The least molecular weight protein with 3.5 kDa was with highest Rf value (0.91) and highest molecular weight protein with 84.5 kDa was with lowest Rf value (0.19). 39.8 and 32 kDa at Korani and 12.1 kDa at Chembooru are unique may be used to identify the respective plants. Similarly, 84.5, 48, 37 and 28.2 kDa are shared bands between Kazhakuttom and Kaimanam accessions. 3.5 kDa shared by all the accessions (Table 4).

Based on SDS-PAGE, the Kazhakuttom accession expresses 9 bands with; MW ranged from 3.5 to 84.5 kDa; Korani with 9 bands with MW ranged from 3.5 to 64 kDa; Chembooru showed 6 bands with MW ranged from 3.5 to 28.2 kDa; Kaimanam accession displayed 11 bands with MW ranged from 3.5 to 84.5; Rajamalai with 6 bands with MW ranged from 3.5 to 28.2 kDa and Kanthallur 7 bands with MW ranged from 3.5 to 28.2 kDa (Figure 3).

***FTIR Spectral Analysis*:** FTIR detected all compounds, including polymers and low-molecular weight compounds in whole samples, subsequently providing biochemical profiles of extremely high-density

data sets. Representative baseline-corrected and normalized FTIR spectra for *Solanum torvum* accessions are shown in Figure 4 a, b, c, d, e and f. Absorption bands in the range of 4000–1500 cm^{-1} are due to functional groups (e.g., –OH, C=O, N–H, CH3, etc.), while the region 1500–675 cm^{-1} is referred to as the finger print region, which is highly specific for each taxon. Similarly, in mid-IR region (2000-1000 cm^{-1}) appeared large numbers of sharp peaks, indicating that the leaves have a rich chemical composition, such as carbohydrates, proteins and lipids. However, this region yielded broad and overlapped bands.

Knowitall software was used to find the function groups for preliminarily analyzing IR spectra collected. The bands around 3370 cm^{-1} represent O-H and N-H stretching vibrations that are mainly generated by proteins and carbohydrates. The bands between 3000 and 2800 cm^{-1} represent C-H stretching vibrations that are mainly generated by lipids. The proteins absorption bands mainly located between 1800 and 1500 cm^{-1} contained amide-I and amide-II bands (Xu *et al.,* 2012), (Natalie *et al.,* 2011) but overlapped with other absorption bands within this region. Amide III, the function group of nucleic acid and carbohydrates contributed to these absorption bands in the leaves. Amide-I and amide-II bands are particularly useful for determining the protein IR absorption changes. Amide-I region (1700-1600 cm^{-1}) mainly represent C=O stretching vibrations of polypeptide, which can detect changes of the overall protein conformation and content (Salman *et al.,* 2012), (Pavlík *et al.,* 2010). Among the *S.torvum* accessions Chembooru, Kanthallur and Rajamalai displayed a band at 1508. Meanwhile, accession from Kazhakuttom, Korani and Kaimanam showed bands viz. 1516 – 1583.56. The protein banding pattern show diversity between the accessions and this may be used to demark the plants at this level. Further analysis by de-convolution and curve fitting process in amide-I region between 1700 and 1600 cm^{-1} can give additional information about the protein structure: the band around 1685 cm^{-1} assigned to the turn structure, the band around 1656 cm^{-1} assigned to the α-helix structure, and the band around 1621 cm^{-1} assigned to the β-sheet structure.

The bands around 2850 cm^{-1} and 2921 cm^{-1} represents C-H asymmetric or symmetric stretching vibration, which belongs to the -CH_2 group of lipids. The results show the total band areas (3000-2800 cm^{-1}) were similar. This implies that lipid profiles in the accessions are similar. The IR spectra between 1200 and 1000 cm^{-1} mainly occur from carbohydrates. The band size at 1022 was shared between Kazhakuttom and Kaimanam.

The band around 1700 - 1726 cm^{-1} represents -COOR stretching vibration (Figure 4), which belongs to characteristic group of cell wall pectin. Chembooru, Kanthallur and Rajamalai possess the characteristic band width at this region while, Kazhakuttom and Korani accessions showed only a higher band width of 1829-1859.

The peak 3200- 3300 may represent NH group of *Solanum* alkaloids. Similarly the peak at 1635 forms C=N group contain alkaloids. Chembooru and Rajamalai showed peak at 3342 but the peak in this range was absent in others. Both the accessions at Kazhakuttom, Korani and Kaimanam possess peaks between 3421 -3442.

The bands at 408.91 – 486.06 are unique for high land accessions such as Kanthallur and Rajamalai. 447.49 in Chembooru accession, 470.63 for Kazhakuttom and 420.48 for Korani accession. Kaimanam accession has no bands at these regions. 707.88-721.38 was used to identify this accession (Table 5). The broad peaks in the accession Kazhakuttom viz., 3300-3500 indicative of p henols, 1629 –ketones, 1408, 1151 that of flavonoids. Korani accession has 3441- alcohols, 1859 – aliphatic ketones/aldehydes, 1637 – aromatic compounds, 1159-1459 flavonoids. Chembooru has 3342- alcohol or phenols, 1730 – aromatic ketones, 1161- ethers. Rajamaali accession has 3379 – alkaloids, 1423, 1159- flavonoids/ secondary amines, 1726 – aliphatic ketones. Similarly, in Kanthallur accession has 3346- alkaloids/ secondary amines, 1726- aliphatic ketones, 1429-1159 flavonoids.

*Alkaloid, solasodine and torvoside content***:** Alkaloid content in plants expressed on a fresh weight basis varied among the accessions (ANOVA, $P < 0.05$) and was lower in Kaimanam accession than others (Table 6). Plants in Rajamalai and Kanthallur accessions showed higher alkaloid content. Similarly, the

HPLC data of solasodine and torvoside are significantly higher in highland accessions i.e., Rajamalai and Kanthallur species.

DISCUSSION

In this work, RAPD analyses provided insight into the genetic diversity, genetic structure and distribution of the six native accessions of *S.torvum* in Kerala. It produced enough polymorphic markers for estimation of population genetic parameters, meeting the critical number of dominant markers suggested for reliable estimation of population genetic parameters (Mohapatra *et al.,* 2009). Suwanchaikasem *et al.,* (2012) reported that random amplified polymorphic DNA (RAPD) in *Thunbergia laurifolia* facilitates the rapid detection of the medicinal materials. Out of 80 screened primers, nine gave clear and reproducible RAPD patterns. Among 164 amplified bands, 70 bands were polymorphic in six species. The dendrogram constructed using unweighted pair group method with arithmetic averages grouped the genotypes into three main clusters. Gwata and Wofford (2012) stated that the RAPD fragment reported in their study co-segregated with promiscuous nodulation. This indicated the possibility of using RAPD markers, in conjunction with SCAR markers, in laboratory-based selection approaches for this trait in soybean. Similarly, in *Pogostemon cablin* 45 decamer random primers used for PCR reactions, 10 primers showed reproducible results. Out of 98 amplification products recorded, 16.7 per cent were monomorphic and 83.3 per cent were polymorphic. Dendrogram produced two major clusters. Overall, RAPD analysis revealed the existence of considerable genetic variations in the cultivars. This information regarding genetic variability at the molecular level could be used to identify and develop genetically unique germplasm that complements existing cultivars (Kumara Swamy and Anuradha, 2011). Similarly, RAPD analysis for genetic diversity of medicinal plant *Coptis omeiensis* by Zhang *et al.,* (2010) showed 132 amplified bands, out of that 98 showed polymorphism, the percentage of polymorphic bands reached to 74.24% less than the present result. RAPD analysis of seven medical Asteraceae plants revealed 337 clear polymorphic bands. Clustering analysis showed that the phylogenetic tree established by RAPD molecular marker was identified with traditional classification (Xia *et al.,* 2010). RAPD analysis of *Angelicae sinensis* seeds from six different regions in Gansu provided with high polymorphism, good repeatability and stability. The results provided the experimental basis for molecular identification (HeFeng *et al.,* 2010). All these data supports the present genetic variation observed in *S.torvum* accessions.

The different values for *S.torvum* may be attributable in part to the use of different RAPD primers, ecology or to a difference in their reproductive biology, as the species was insect pollinated. In general, geographically widespread species tend to possess higher genetic polymorphism within populations than species with a restricted distribution (HeFeng *et al.,* 2010). The genetic structure of plant populations reflects the interactions of various evolutionary processes including the long-term evolutionary history, shifts in distribution, habitat fragmentation and population isolation, mutation, genetic drift, mating systems, gene flow and selection (Xia *et al.,* 2010).

From the present DNA polymorphism by RAPD technique provide a minimal profile for each of the accessions, thus allowing them to be differentiated from each other. The technical simplicity of the RAPD technique has facilitated its use in the analysis of genetic relationship in several genera. The major concern regarding RAPD-generated phylogenies include homology of bands showing the same rate of migration, causes of variation in fragment mobility and origin of sequence in the genome. In spite of this limitation, RAPD markers has the greatest advantage of its capability to scan across all regions of the genome hence highly suited for phylogeny studies at species level.

In general, the cluster analysis grouped the accessions according to many morphological traits and showed a clear separation between the accessions. The RAPD marker revealed considerable genetic diversity among the accessions, a finding which strongly agrees with the great morphological variability observed among the *S.torvum* in this study. Similar results were observed for the oriental *Cucurbita moschata* using RAPD marker (Mohammed *et al.,* 2012). RAPD marker systems proved to be useful for

analyzing the genetic diversity of some genera of the family Cucurbitaceae. The knowledge of the diversity of this germplasm will facilitate its use in breeding programmes and the improvement of management of large collections of the species of this potential medicinal plant.

Protein profiles of a few medicinal plants in Apocynaceae by using SDS-Polyacrylamide gel showed bands varied from 7 to 13**.** More number of peptide bands was produced in *Rauwolfia tetraphylla* and less number of bands was observed in *Nerium indicum*. Molecular weight of the five plants varied from 1.26 kDa to 89.12 kDa. Highest molecular weight protein was observed in *N.indicum* (89.12 kDa). *R.tetraphylla* has the low molecular weight (1.26 kDa) protein (John De Britto *et al.,* 2012). The variations in protein synthesis in plants suggest that proteins could be used as biomarkers. Similarly, the synthesis of additional protein repressed or induced in response to stress indicates that the proteins have an important role in the maintenance of vital cellular function, they could be abnormal proteins synthesized in response to stress. Specific binding proteins are considered to play an important role in plants correlated with total RNA, enzyme activity and proteins levels of several key photosynthesis proteins including RUBISCO. Harish and Murugan, (2011) reported protein analysis of *Clerodendron inerme* across three mangrove habitats revealed remarkable variations in the protein band pattern. There was variation both in the number and mass of the polypeptides. The major components of all the three accessions were in the range of 11-117 KD. It was evident that gel electrophoresis of proteins could be a useful tool in species identification. Similarly, Mahmoodzadeh (2009) reported protein Profiles in response to salt Stress in seeds of *Brassica napus*. Except for some minor differences in the intensity of the bands, major differences were not recorded in the protein profiles. On the other hand, no protein polymorphism was observed in *Manihot esculenta* varieties collected from Adira1 and Cabak makao in Ngawi, East Java (Tribadi *et al.,* 2010).

FTIR spectroscopy allows detecting the whole range of infrared spectrum simultaneously providing speed and accuracy in measurements of biological specimens (Smith-Moritz *et al.,* 2011). Recently, Anil kumar *et al.,* (2012) identified protein absorption bands located between 1800-1500 and the bands between 1500-1000 cm^{-1} (finger print region) showed variation between the two species *Solanum nigrum* and *S. giganteum*. Infrared spectra of leaves are of taxonomic value in genus *Solanum*, and this technique can be widely used for identification and classification of other taxa when standard spectra are available. Ran Xu *et al.,* (2012) applied Fourier transform infrared (FTIR) spectroscopy, to differentiate the four medicinal plants effectively and inferred that their habitats could be judged preliminarily, and also the genetic relationships of the plants. The application of FTIR spectroscopy in crude medicine authentication and quality evaluation deserved to be further emphasized. Wang *et al.,* (2012) reported that two important plant pathogenic bacteria *Acidovorax oryzae* and *Acidovorax citrulli* are closely related and often not easy to be differentiated from each other, which often resulted in a false identification between them based on traditional methods such as carbon source utilization profile, fatty acid methyl esters and ELISA detection tests. MALDI-TOF MS and Fourier transform infrared (FTIR) spectra have recently been successfully applied in bacterial identification and classification, which provide an alternate method for differentiating the two species. Svecnjak *et al.,* (2011) used IR technique in 144 samples of nine different unifloral honey types (black locust, sweet chestnut, lime, sage, heath, rosemary, lavender, mandarin and strawberry tree) collected from different Croatian regions directly from the beekeepers. Results of this study showed that IR spectroscopy provides reliable results, but also represents rapid and cheap analytical tool in comparison to commonly used standard analytical methods. This research has also provided the first insight in infrared spectra of Croatian honeys. Baseri and Baker (2011) used FTIR spectroscopy to identify the cellular components of the medicinal plants of *Mimosa pudica* and *Caesalpinia pulcherrima.* FTIR spectroscopy is proved to be a sophisticated instrument to analyze the components of the plant cells. The cellular constituents in the leaves and stem of these plants were monitored for the qualities of medicinal applications. Various functional groups present in the medicinal plants were identified. The results indicate that plants contain carotenoids, polysaccharides, carbohydrates and glycogen.

Table 1: Arbitrary primers of P1-P15 and their sequences used for Random Amplified Polymorphic DNA (RAPD) analysis of *Solanum torvum* accessions

Primer	Sequence
P1	AACCGACGGG
P2	GGGGGTCGTT
P3	TGCCCTGCCT
P4	CCAGACCCTG
P5	AAGCTCCCCG
P6	TACCACCCCG
P7	GGCGGACTGT
P8	GTCACTCCCC
P9	ACCGCGAAGG
P10	GGACCCAACC
P11	GTCGCCGTCA
P12	TCTGGTGAGG
P13	TGAGCGGACA
P14	ACCTGAACGG
P15	TTGGCACGGG

Table 2: Polymorphic bands in the six *S.torvum* accessions using 15 RAPD primers

Primers	Number of bands
Primer 1	3
Primer 2	8
Primer 3	9
Primer 4	5
Primer 5	5
Primer 6	0
Primer 7	9
Primer 8	11
Primer 9	6
Primer 10	10
Primer 11	6
Primer 12	10
Primer 13	4
Primer 14	5
Primer 15	7
Total polymorphic band	98

The results of this work recommend FTIR spectroscopy as a potential analytical rapid, economic and nondestructive tool to particular, several specific characteristic peaks were determined for each of the species. Compared to the traditional time consuming method, FTIR spectroscopy is easy to implement and is an emergent physico-chemical technique in modern research. Therefore, result from this study may give a new strategy for the rapid identification and differentiation of plant accessions.

Table 3: Shared bands between different accessions of *Solanum torvum* Sw. N1- Kazhakkuttom, N2- Korani, N3-Chembooru, N4- Kaimanam, N5- Rajamalai, N6- Kanthallur

Accessions	Shared bands	Percentage of genetic distance
N1-2	23	11.5
N1-3	15	44.4
N1-4	9	67.8
N1-5	10	67.7
N1-6	10	70.6
N2-3	15	46.4
N2-4	9	68.9
N2-5	10	68.8
N2-6	12	63.6
N3-4	9	59.1
N3-5	10	60
N3-6	7	77.4
N4-5	9	57.1
N4-6	8	68
N5-6	12	52

Table 4: Protein banding pattern among *Solanum torvum* accessions

Kazhakkuttom	Korani	Chembooru	Kaimanam	Rajamalai	Kanthallur
84.5	64	28.2	84.5	28.2	28.2
64	39.8	25	64	20.1	25
48	32	18.7	48	18.7	20.1
37	20.1	12.1	37	17.2	18.7
28.2	18.7	6.5	28.2	8	10.6
20.1	17.2	3.5	25	3.5	8
14.3	14.3		20.1		3.5
6.5	8		14.3		
3.5	3.5		10.6		
			8		
			3.5		
9	9	6	11	6	7

**kDa-kilodalton*

Alkaloids are one of the most important groups of secondary metabolites due to the great number of isolated products and their pharmacological activity. They have a restricted distribution and are readily affected by the plant growth location and atmospheric conditions. It is important to know if these factors can affect either the presence or absence of certain compounds, mainly if they have pharmacological properties.

Alkaloid content in plants depends on numerous factors such as species variety, age (developmental stage), environment and geographical location. Alkaloid content in plants has been found to impact the central nervous system of living organisms, with low levels acting as stimulators and higher levels as suppressors. Therefore, the aim of plant selection could be development of competitive accession with low alkaloid content. Needs further more comprehensive selective and genetic studies related to alkaloid content among the accession from different altitude ranges from Kerala.

Table 5: Unique IR finger printing peaks used to identify the *S.torvum* accessions

Kazhakuttaom	Korani	Chembooru	Kaimanam	Rajamalai	Kanthallur
470.63	420.48	447.49	707.88-721.38	486.06	408.91
518.85	1516.05	605.65	945.12	538.14	422.41
1336.67	3738.06-3905.85	900.76	3039.81	580.57	607.58
1408.04		2077.33-2123.63		611.43	1056.99
1477.47				669.31	1639.49
1563.13				1510.26	
1829.85				2335.8-2360.87	
3450.3					

Table 6: Total alkaloids, solasodine and torvoside content among the different *S. torvum* accessions

Accessions	Total alkaloids (mg/g)	Solasodine (mg/g)	Torvoside (μ g/g)
Kazhakuttaom	0.89	0.077	266
Korani	0.98	0.09	300
Chembooru	1.1	0.10	349
Kaimanam	0.65	0.056	170
Kanthallur	1.8	0.28	878
Rajamalai	1.68	0.19	743

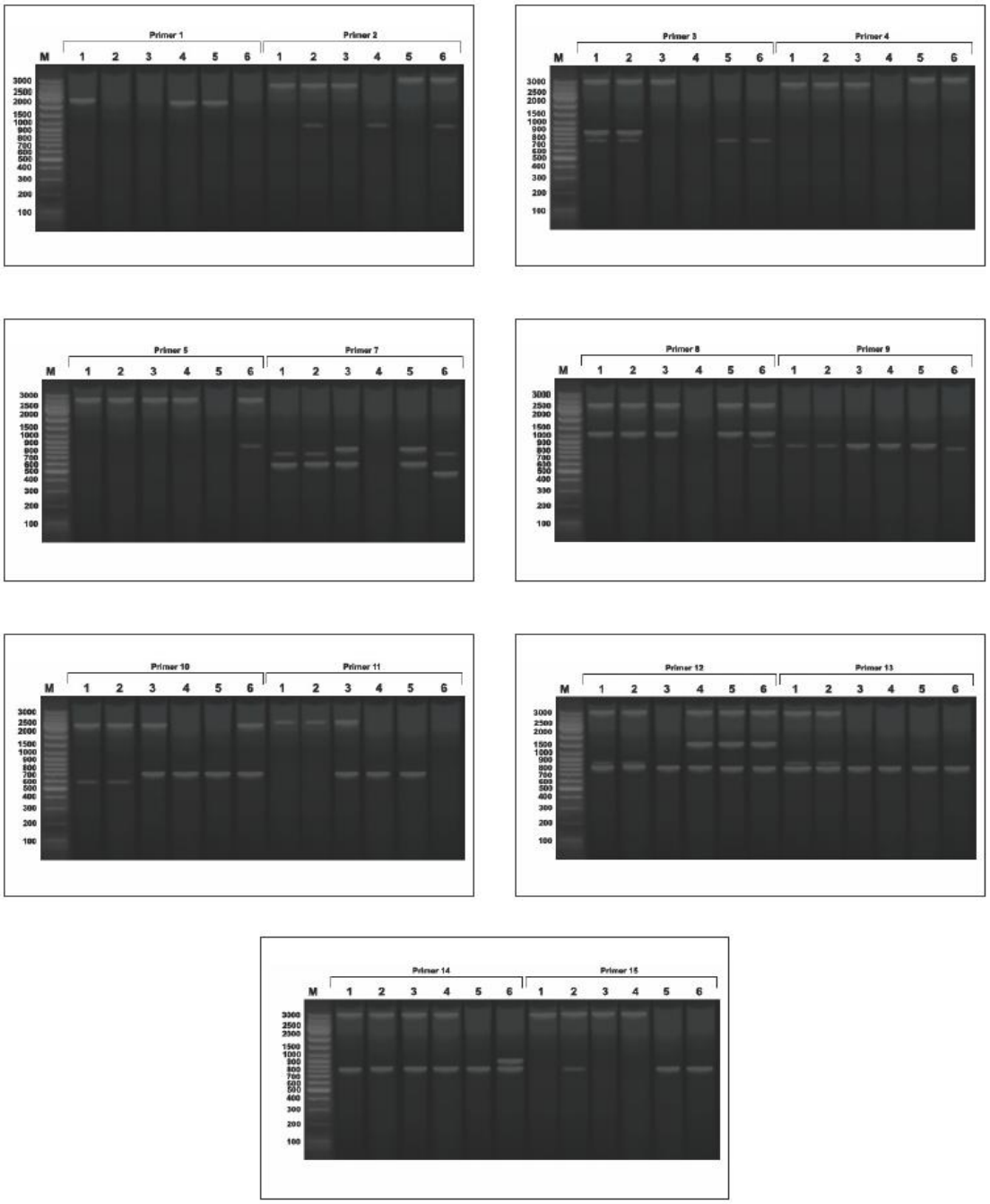

Figure1: RAPD profile in *S. torvum* accessions using 15 primers. 1. Kazhakkuttom 2. Korani 3. Chembooru 4. Kaimanam 5. Rajamalai 6. Kanthallur

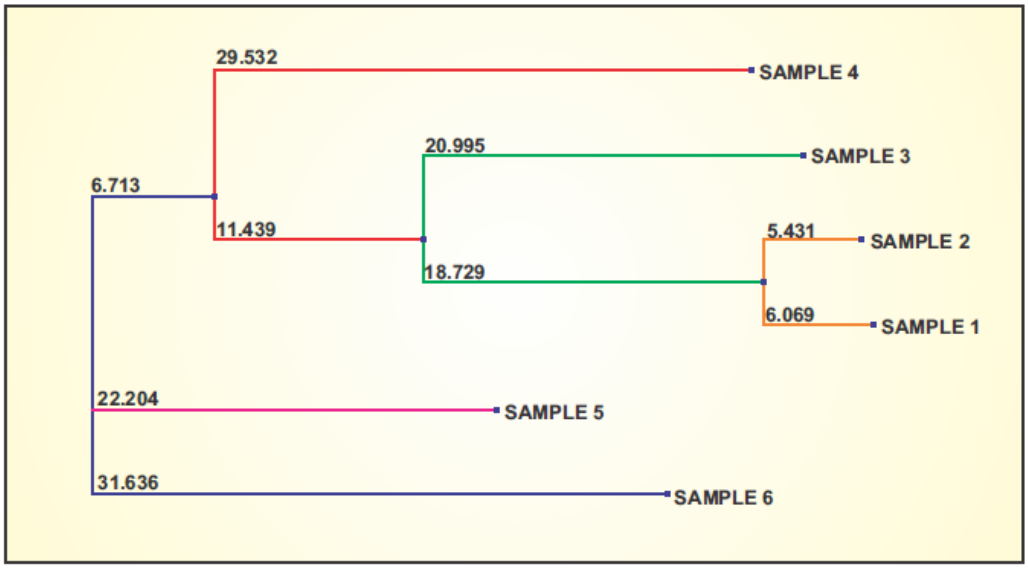

Figure 2: Dendrogram of *S. torvum* accessions. 1. Kazhakkuttom 2. Korani 3. Chembooru 4. Kaimanam 5. Rajamalai 6. Kanthallur

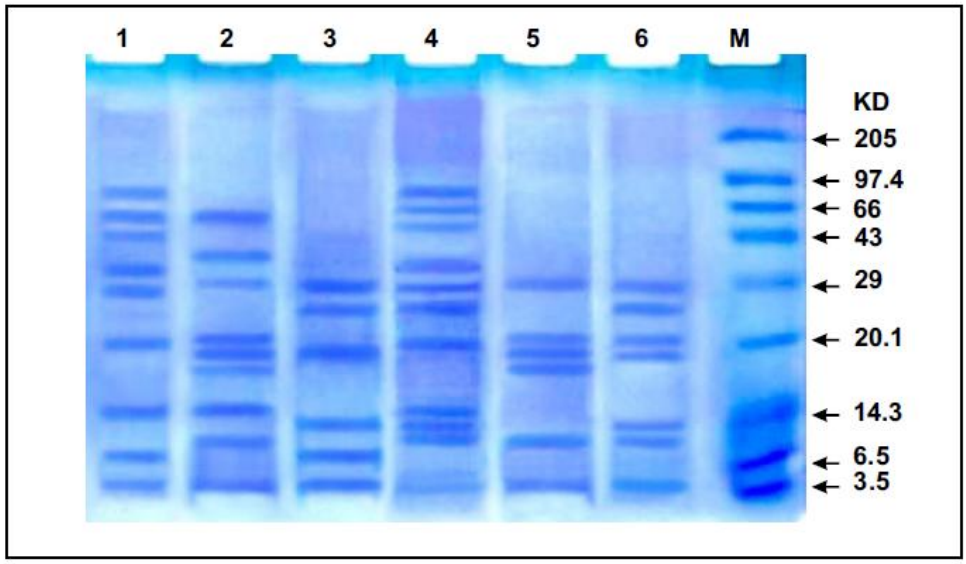

Figure 3: Protein banding pattern in *S. torvum* accessions. 1. Kazhakkuttom 2. Korani 3. Chembooru 4. Kaimanam 5. Rajamalai 6. Kanthallur

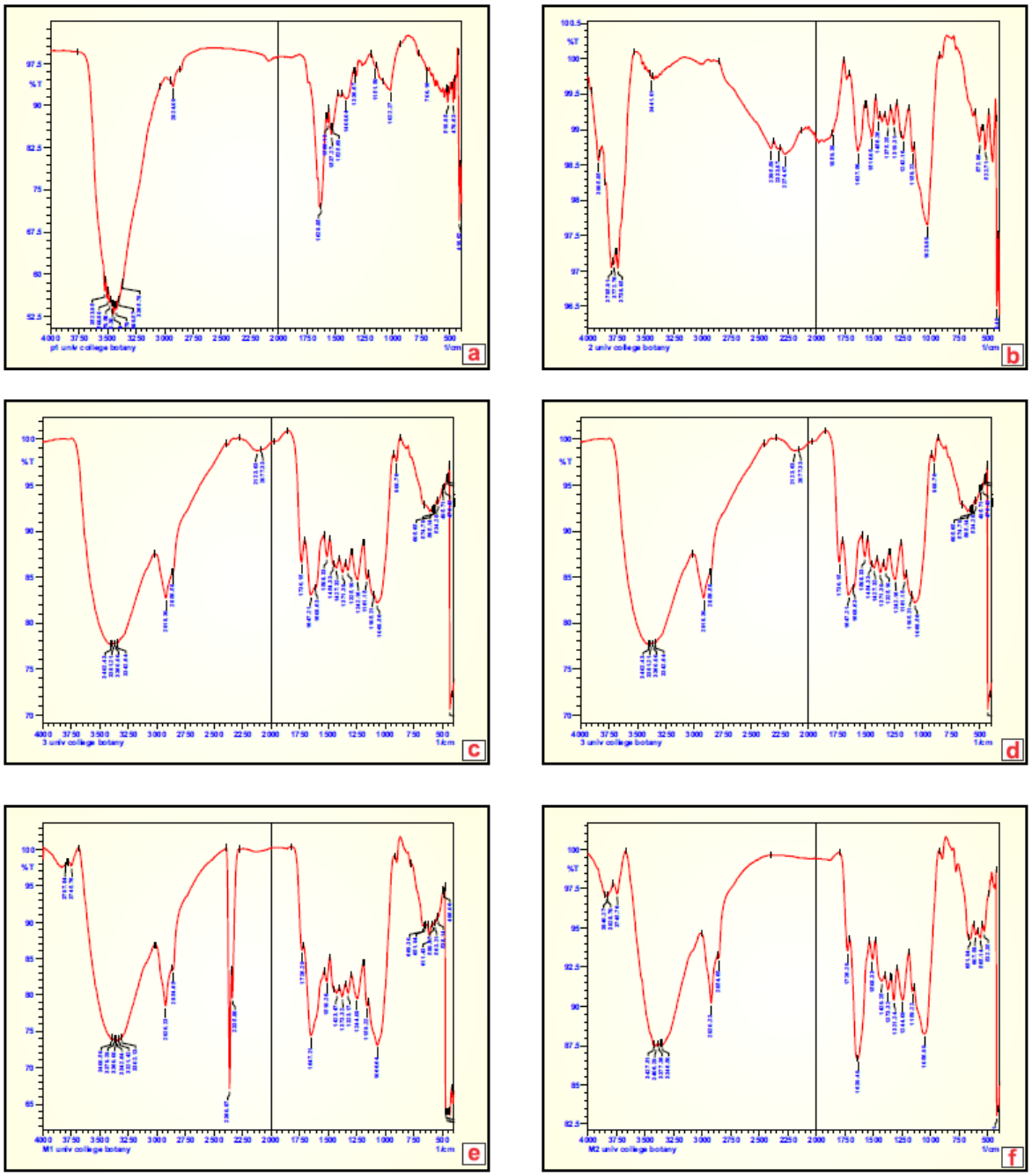

Figure 4: Infrared finger printing pattern in *S. torvum* accessions. a. Kazhakkuttom b. Korani c. Chembooru d. Kaimanam e. Rajamalai f. Kanthallur

Conclusion

In conclusion, taxonomic classification of plant species basically depends on the morphological and anatomic characters, these features are not static and sometimes difficult to observe, so it is necessary to be supported by molecular techniques. This study represents molecular markers as a tool to study systematics in medicinal plants. In the future studies, the analysis of additional accessions, more primers and also the use of different types of molecular markers such as AFLP, SSR, ITS will improve the accuracy of resolution of categorization of the accessions of *S.torvum*.

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