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ORIGINAL ARTICLE

Identification and Characterization of *Memecylon* Species Using Isozyme Profiling

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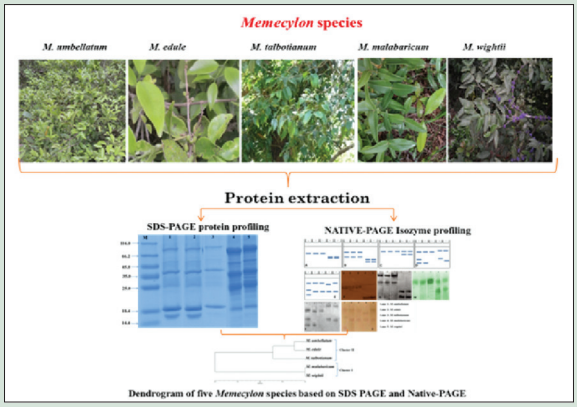
ABSTRACT

Background: The protein/isozyme fingerprint is useful in differentiating the species and acts as a biochemical marker for identification and systematic studies of medicinal plant species. **Objective:** In the present study, protein and isozyme profiles for peroxidase, esterase, acid phosphatase, polyphenol oxidase, alcohol dehydrogenase, and alkaline phosphatase of five species of *Memecylon* (*Melastomataceae*), *Memecylon umbellatum*, *Memecylon edule, Memecylon talbotianum, Memecylon malabaricum*, and *Memecylon wightii* were investigated. **Materials and Methods:** Fresh leaves were used to prepare crude enzyme extract for analyzing the five enzymes isozyme variations. Separation of isozymes was carried out using polyacrylamide gel electrophoresis (PAGE) and the banding patterns of protein were scored. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to regenerate similarity coefficients. The similarity coefficients were then used to construct dendrograms, using the unweighted pair group method with arithmetic averages. **Results:** A total of 50 bands with various Rf values and molecular weight were obtained through PAGE analysis. Among the five *Memecylon* species, more number of bands was produced in *M. wightii* and less number of bands was observed in *M. edule*. The results of similarity indices grouped *M. malabaricum* and *M. wightii* in one cluster with 98% similarity and *M. umbellatum*, *M. edule*, and *M. talbotianum* are grouped in another cluster with 79% similarity showing close genetic similarities which is in accordance with the morphological identification of *Memecylon* species. **Conclusion:** The protein/isozyme fingerprint is useful in differentiating the species and acts as a biochemical marker for identification of *Memecylon* species.

Key words: Acid phosphatase, esterase, native polyacrylamide gel electrophoresis, peroxidase, polyphenol oxidase, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SUMMARY

- Biochemical characterization of *Memecylon* species was evaluated by SDS-PAGE of extracted protein and isozyme profiling on native PAGE.
- After electrophoresis, each gel was stained with specific stains. Genetic distance relationships were evaluated based on the banding patterns of protein on isozymes.
- Unique banding pattern of esterase, peroxidase, acid phosphatase, alcohol dehydrogenase and polyphenol oxidase are observed in all the five species of *Memecylon*, which represent the fingerprint of *Memecylon* species.
- SDS-PAGE and isozyme profiling of five *Memecylon* species revealed that *M. malabaricum* and *M. wightii* grouped in one cluster and *M. umbellatum*, *M. edule* and *M. talbotianum* grouped in another cluster showing close genetic similarities which is in accordance with the morphological identification of *Memecylon* species.
- This is the first report on the comparison of protein and isozyme profile of five different *Memecylon* species.



Abbreviations Used: SDS-PAGE: Sodium docecyl sulfate polyacrylamide gel electrophoresis; NTSYS PC2: Numerical taxonomy system, version 2.2 for Windows XP, Vista, Win7, Win 8 and Win10 including 64 bit

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INTRODUCTION

The genus *Memecylon* L. (*Melastomataceae*) consists of 300–400 species, distributed in the tropical areas of Asia, Africa, America, and in India, mainly distributed in the Western Ghats. Genus *Memecylon* has great importance in traditional medicine. Several *Memecylon* species were used in the treatment of herpes, leucorrhea, gonorrhea, conjunctivitis, snake bite, and skin diseases.[1] Several pharmacological and phytoconstituents are reported such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and antipyretic properties.[1-5] Identification of *Memecylon* species is complex due to close morphological features which has been described in our previous paper.[1] The diversity revealed by biochemical profiles such as protein and more importantly isozyme profile of selected enzymes are highly valuable in the accurate identification of the plant, predominantly medicinal plants.[6,7] Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins is one of the molecular tools to study the molecular systematic and identification of genotypes in medicinal plants. Isozymes are

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used as classical biochemical markers, which can be separated using nondenaturing native polyacrylamide gel electrophoresis (native PAGE) and also used to analyze the genetic diversity before the introduction of DNA markers for solving various problems of plant taxonomy to distinguish or to authenticate species.[8] Previous studies on isozymes signify that about 57 enzymes are attributed in plant system for biodiversity analysis.[9] Since the biochemical markers have not been developed to know the generic and taxonomic status of *Memecylon* species, the present study was taken up.

MATERIALS AND METHODS

Plant materials

Five *Memecylon* species, namely, *Memecylon umbellatum* Burm, *Memecylon edule* Roxb, *Memecylon talbotianum* Brandis, *Memecylon malabaricum* Clarke, and *Memecylon wightii* Thwaites, have been collected from different parts of Karnataka. Fresh leaf samples were collected from these plants, frozen in liquid nitrogen, and stored at −80°C until used for total protein isolation.

Isolation of total protein and sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The total protein was isolated based on the method described by Wang *et al*.[10] The protein was quantified by the Bradford method[11] with bovine serum albumin as standard. Protein extracts isolated from *Memecylon* species were analyzed by SDS-PAGE.[8]

Native polyacrylamide gel electrophoresis

Fresh leaves were used to prepare crude enzyme extract for analyzing the isozyme variations among *Memecylon* species using five enzymes according to the method described by Smila *et al*.[12] For this study, 500 mg of leaf tissue was taken and homogenized using 1.5 mL of cold homogenizing buffer, 0.1 M sodium phosphate buffer (pH 7.0) for peroxidase and esterase, 50 mM citrate buffer (pH 5.3), for acid phosphatase, 50 mM citrate buffer (pH 9.0) for alcohol dehydrogenase, and 0.01 M potassium phosphate buffer (pH 7.0) containing 1% Tween 80 for polyphenol oxidase in a prechilled pestle and mortar. The homogenate was centrifuged for peroxidase and esterase at 10,000 rpm for 20 min, for acid phosphatase and alcohol dehydrogenase at 10,000 rpm for 20 min, and for polyphenol oxidase at 18,000 rpm for 25 min. Supernatant was stored at 4°C.

Separation of isozymes was carried out using native PAGE.[13] Isoenzyme analysis for peroxidase, esterase, acid phosphatase, alcohol dehydrogenase, and polyphenol oxidase was carried out as described by the previous studies.[14-18] The gels are incubated at 37°C until the bands developed sufficiently to permit scoring; later, the bands were fixed by 7% acetic acid. The Rf of each bands was calculated,[19] and the similarity index or pairing affinity was analyzed by the method described by Sneath and Sokal.[20]

Genetic distance relationships

The banding patterns of protein on SDS-PAGE and isozymes on native PAGE were scored, and data were fed to the PC as 1 and 0 for the presence and absence of bands, respectively. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to regenerate similarity coefficients, according to Jaccard.[21] The similarity coefficients were then used to construct dendrograms, using the unweighted pair group method with arithmetic averages (UPGMA) employing the Sequential, Agglomerative, Hierarchical, and Nested clustering from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.1 Program Rohlf.[22]

RESULTS

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis

In the present study, protein profiles of five *Memecylon* species were studied using SDS-PAGE. The total number of bands in all five *Memecylon* species varied from 7 to 14 [Figure 1]. More number of bands was produced in *M. wightii* (14); *M. malabaricum* (13) followed by *M. talbotianum* and *M. umbellatum* (8) and less number of bands was observed in *M. edule* (7). The molecular weights of the bands were calculated using standard curve. The molecular weight of the five plants varied from 14.4 to 85.08 kDa. The highest molecular weight protein was observed in *M. talbotianum* (~85.08 kDa) [Table S1].

The similarity index [Table S2] represents the similarity between the species. The dendrogram [Figure 2] was constructed based on UPGMA. The dendrogram shows a distinct separation of the collected species into two major groups. In cluster I, the *M. malabaricum* and *M. wightii* clustered in the same cluster with 91% similarity showing a close genetic relationship with each other, cluster II is subdivided into two subclusters, in which *M. umbellatum* and *M. edule* are grouped together with 60% similarity with *M. talbotianium* as out-group.

Isozyme analysis

Peroxidase

A total of 7 bands were present in the *Memecylon* species. The bands of Rf = 0.102 and 0.125 were commonly shared by *M. umbellatum*, *M. edule*, and *M. talbotianum*, and Rf = 0.397 band was present in *M. malabaricum* and *M. wightii* as marker bands [Table S3 and Figure 2].

Esterase

A total of 10 isozyme bands were observed. The Rf = 0.190, 0.220, and 0.710 bands was commonly shared by *M. umbellatum*, *M. edule*, and *M. talbotianum*, and Rf = 0.424 band was present in *M. malabaricum* and *M. wightii* [Table S3 and Figure 2].

Alcohol dehydrogenase

In the alcohol dehydrogenase enzyme system, a total of 7 bands at two were observed in the enzyme system of *Memecylon* species. The

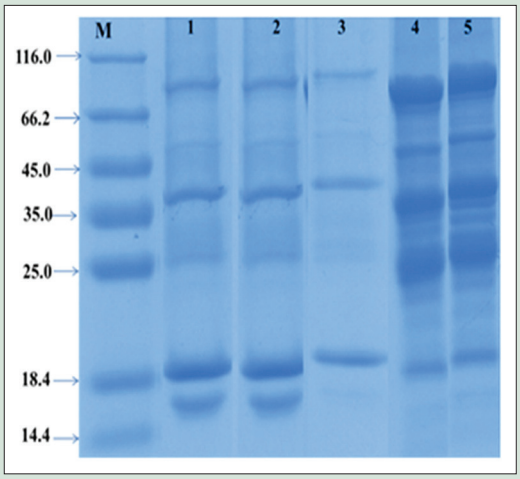

Figure 1: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein banding patterns of *Memecylon* species M: Marker; (1) *Memecylon umbellatum*; (2) *Memecylon edule*; (3) *Memecylon talbotianum*; (4) *Memecylon malabaricum*; (5) *Memecylon wightii*

Rf = 0.196 bands were common for all five *Memecylon* species and Rf = 0.223 band was present only in *M. malabaricum* and *M. wightii* [Table S3 and Figure 2].

Acid phosphatase

In the acid phosphatase enzyme system, a total of 10 bands were observed for *Memecylon* species. The Rf = 0.213 band was present in *M. malabaricum* and *M. wightii*, Rf = 0.138 band was present in *M. umbellatum*, *M. edule*, and *M. talbotianum*, Rf = 0.816 band was present in *M. wightii*, Rf = 0.912 band was present in *M. umbellatum* and *M. malabaricum*, and Rf = 0.232 band was present in *M. umbellatum* and *M. edule* [Table S3 and Figure 2].

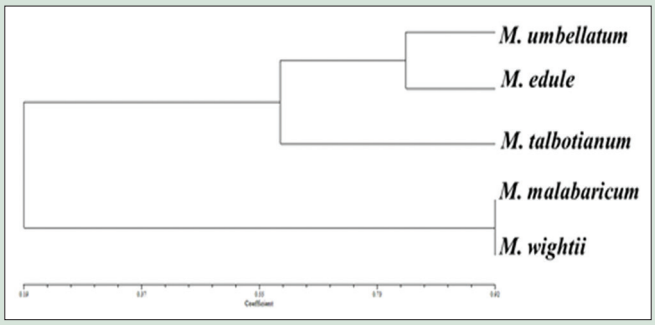

Figure 2: Dendrogram of five *Memecylon* species based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein banding pattern

Polyphenol oxidase

A total of 11 bands were found in polyphenol oxidase enzyme system of *Memecylon* species. The Rf = 0.439 and 0.826 bands were common in *M. malabaricum*, *M. wightii, M. edule*, and *M. talbotianum*, and Rf = 0.231 and 0.534 bands were observed only in *M. umbellatum* [Table S3 and Figure 2].

The isozyme analysis revealed that the genetic similarity indices ranged from 70% to 80% [Table S4]. The closest relationship was observed between *M. malabaricum* and *M. wightii* with 80% similarity and also between *M. umbellatum*, *M. edule*, and *M. talbotianum* with 78% similarity. As shown in Figure 3, the dendrogram based on the similarity matrices of isozyme banding patterns classified the *Memecylon* species into two main clusters. *M. malabaricum* and *M. wightii* grouped in cluster I, whereas *M. umbellatum*, *M. edule*, and *M. talbotianium* grouped in cluster II showing close genetic relationships among these *Memecylon* species.

Combined analysis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isozyme systems

The combined data on the banding patterns of protein on SDS-PAGE and isozyme profiles of *Memecylon* species were analyzed using the NTSYS-PC2 software [Figure 4]. The similarity indices were 79% between *M. umbellatum*, *M. edule*, and *M. talbotianum*, followed by 98% similarity between *M. malabaricum* and *M. wightii*. The dendrogram resulted from the combination of the two techniques [Figure 5] revealed two different clusters: in cluster I, *M. malabaricum* and *M. wightii* grouped together,

Table S1: Protein banding patterns of the five *Memecylon* species as revealed by SDS- PAGE

Band No.	Molecular weight (KD_a)	*Memecylon* species				
		M. umbellatum	*M. edule*	*M. talbotianum*	*M. malabaricum*	*M. wightii*
1	85.08	0	0	1	0	0
2	78.37	1	1	0	1	1
3	58.2	0	0	0	1	1
4	56.2	0	0	0	1	1
5	52.0	0	0	0	1	1
6	45.0	1	0	1	0	0
7	42.0	0	1	0	0	0
8	38.0	0	0	0	1	1
9	35.0	1	1	1	0	0
10	34.0	0	0	0	1	1
11	32.0	0	0	0	0	1
12	30.2	1	1	1	1	1
13	25.0	1	1	1	1	1
14	20.2	1	1	1	0	0
15	20.1	0	0	0	1	1
16	20.0	0	0	0	1	1
17	19.0	0	0	0	1	1
18	18.4	1	1	1	1	1
19	17.0	1	1	0	0	0
20	14.4	0	0	0	1	1
Total		8	8	7	13	14

Table S2: Similarity matrix among the five *Memecylon* species based on SDS-PAGE protein data

	M. umbellatum	*M. edule*	*M. talbotianum*	*M. malabaricum*	*M. wightii*
M. umbellatum	1.0000000				
M. edule	0.6666667	1.0000000			
M. talbotianum	0.7777778	0.5000000	1.0000000		
M. malabaricum	0.1875000	0.2000000	0.1875000	1.0000000	
M. wightii	0.1764706	0.1875000	0.1764706	0.9166667	1.0000000

and in cluster II, *M. umbellatum*, *M. edule*, and *M. talbotianum* grouped together showing close genetic relationships among different *Memecylon* species [Table S5].

DISCUSSION

The traditional taxonomic system of plants depends on morphological characters; however, the morphological characters between different species are sometimes difficult to distinguish; hence, the study of biochemical (total protein and isozymes) variations gains importance in the study of identification of inter- and intra-specific genetic variation among plant species.[23]

In the present study, the protein and isozyme profiles of five species of *Memecylon* such as *M. umbellatum*, *M. malabaricum*, *M. wightii*, and *M. edule* of family *Melastomataceae* were investigated. SDS-PAGE and isozyme analysis revealed that *M. malabaricum* and *M. wightii* are grouped in one cluster, and *M. umbellatum*, *M. edule*, and *M. talbotianum* are grouped in another cluster, showing close genetic similarities which is in accordance with the morphological identification of *Memecylon* species.[1] Similarity index was 98% between *M. malabaricum* and *M. wightii* indicating that these two species are sister species and 79% similarity observed in *M. umbellatum*, *M. edule*, and *M. talbotianum* showing close genetic relationships. Similar studies are carried out

Table S3: Distribution of Peroxidase, Esterase, Alcohol dehydrogenase, Acid phosphatase and Polyphenol oxidase Isozymes of five *Memecylon* species according to their relative mobility

Band No	RF	*Memecylon* species				
		M. umbellatum	*M. edule*	*M. talbotianum*	*M. malabaricum*	*M. wightii*
Peroxidase						
1	0.102	1	1	1	0	0
2	0.125	0	0	0	0	0
3	0.397	0	0	0	1	1
4	0.397	0	0	0	1	1
Total		1	1	1	2	2
Esterase						
1	0.190	1	1	0	0	0
2	0.220	1	1	1	0	0
3	0.424	0	0	0	1	1
4	0.710	1	1	1	0	0
Total		3	3	2	1	1
Alcohol dehydrogenase						
1	0.196	1	1	1	1	1
2	0.223	0	0	0	1	1
Total		1	1	1	2	2
Acid phosphatase						
1	0.138	1	1	1	0	0
2	0.213	0	0	0	1	1
3	0.232	1	1	0	0	0
4	0.816	0	0	0	0	1
5	0.912	1	0	0	1	0
Total		3	2	1	2	2
Polyphenol oxidase						
1	0.231	1	0	0	0	0
2	0.439	1	1	1	0	1
3	0.459	0	0	0	1	0
4	0.534	1	0	0	0	0
Total		3	2	2	2	2

Table S4: Combined similarity matrix of isozyme systems of five *Memecylon* species

	M. umbellatum	*M. edule*	*M. talbotianum*	*M. malabaricum*	*M. wightii*
M. umbellatum	1.0000000				
M. edule	0.7692308	1.0000000			
M. talbotianum	0.9090909	0.6923077	1.0000000		
M. malabaricum	0.2222222	0.2777778	0.2352941	1.0000000	
M. wightii	0.2222222	0.2777778	0.2352941	1.0000000	1.0000000

Table S5: Similarity matrix based on combined analysis of SDS-PAGE protein and isozyme systems of five *Memecylon* species

	M. umbellatum	*M. edule*	*M. talbotianum*	*M. malabaricum*	*M. wightii*
M. umbellatum	1.0000000				
M. edule	0.7272727	1.0000000			
M. talbotianum	0.8500000	0.2000000	1.0000000		
M. malabaricum	0.2058824	0.2424242	0.2121212	1.0000000	
M. wightii	0.2000000	0.2352941	0.2058824	0.9565217	1.0000000

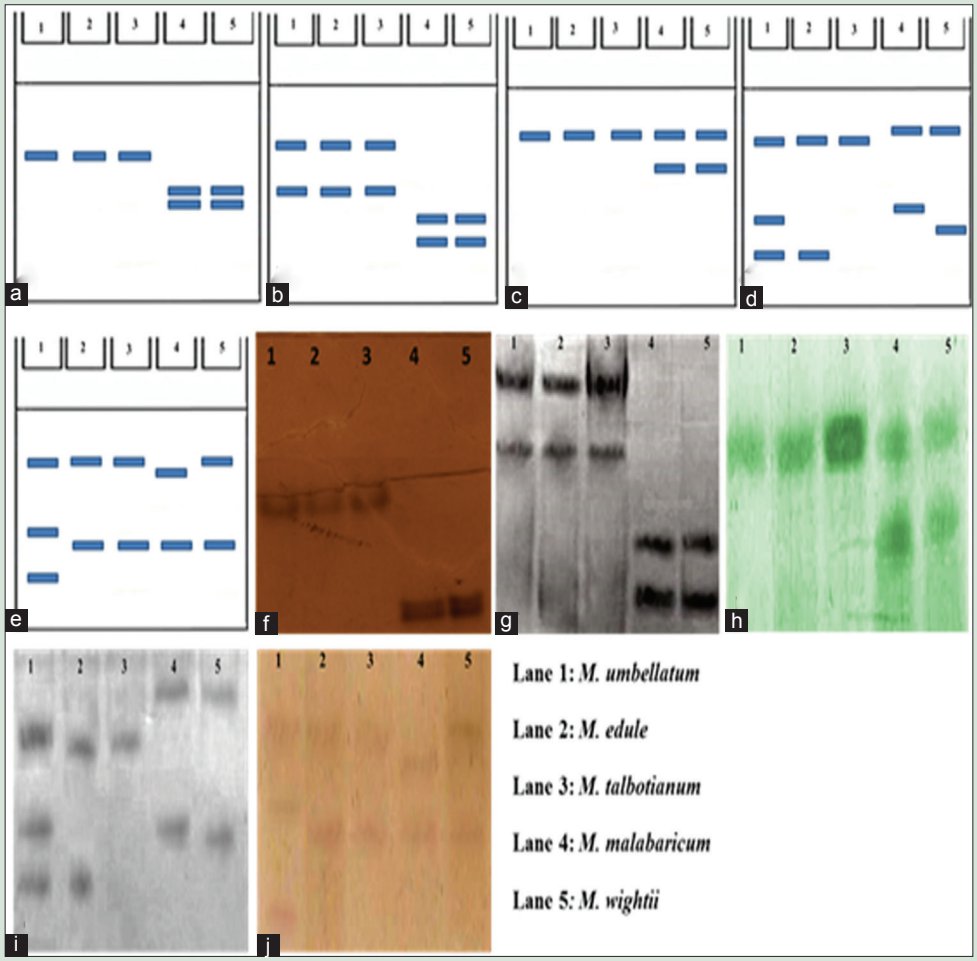

Figure 3: Isozyme profiles of *Memecylon* species (a) zymogram of peroxidase; (b) zymogram of esterase; (c) zymogram of alcohol dehydrogenase; (d) zymogram of acid phosphatase; (e) zymogram of polyphenol oxidase; (f) peroxidase banding pattern of *Memecylon* species; (g) esterase banding pattern of *Memecylon* species; (h) alcohol dehydrogenase banding pattern of *Memecylon* species; (i) acid phosphatase banding pattern of *Memecylon* species; (j) polyphenol oxidase banding pattern of *Memecylon* species

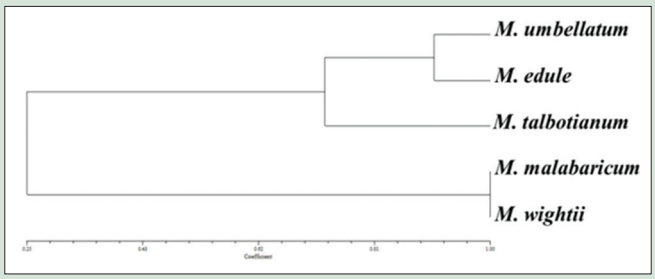

Figure 4: Isozyme profile-based dendrogram of five *Memecylon* species

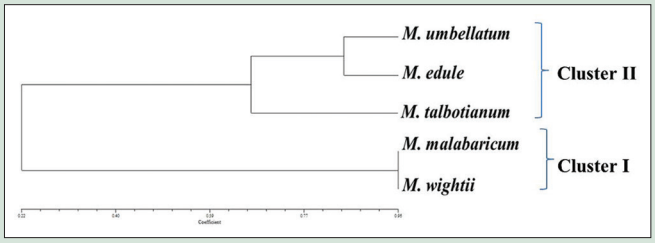

Figure 5: Dendrogram of five *Memecylon* species based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and native polyacrylamide gel electrophoresis data

to study the genetic variability among different plant species which include *Brassica rapa*, *Cucurbitaceae*, *Calotropis procera*, and *Gymnema sylvestre*,[24-27] also in *Solanum* species.[28] In addition to leaf proteins, seed proteins were also analyzed by several workers to know the taxonomic and generic status in several plant species including *Datura*, *Hyoscyamus*, *Withania, Atropa*, and *Ebenus*.[29,30] The dendrogram based on UPGMA revealed the generic status and interrelationships of these species.

In the present study, isozymes of peroxidase, esterase, acid phosphatase, alcohol dehydrogenase, and polyphenol oxidase have been used as biochemical markers to identify the systematic position of five different *Memecylon* species. Since 1930, electrophoresis and zymogram technique together are being used as a tool to study genetic variation and population genetics. In PAGE analysis, each zone is engaged by a specific gene locus coding for that isozyme. In certain enzyme systems, more than one distinct band could be resolved which represent allelic isozymes, coded by different alleles of the same gene at one locus.[31] In the present observations, similar kind of banding profiles is detected in all the enzyme systems, specifying the existence of multiple alleles.

The occurrence of common banding profiles suggests that protein shares similar functional properties. Similar observations were made in *Brassica* species, *Plumbago* species, *Ocimum sanctum*, *Thelypteris ciliata*, *Nephrolepis*, *Aegle marmelos, Naringi crenulata*, and *Plantago ovata*[32-35] which supports the present observations. The results from the present study suggest that stable expression of several proteins and isozyme systems such as peroxidase, esterase, acid phosphatase, alkaline dehydrogenase, and polyphenol oxidase have been utilized to assess the genetic similarity and differences at the various taxonomic levels.

CONCLUSION

The results from the present study suggest that unique banding profiles of isozymes are observed in all the five species of *Memecylon*, which represent the fingerprint of *Memecylon* species. Such fingerprinting is useful in differentiating the species and acts as a biochemical marker for these species in plant identification and systematic studies. These conclusions can be further used for accurate identification of the most important proteins present in different species with two-dimensional gel electrophoresis and several molecular marker systems.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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