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PHYTOCHEMICAL ANALYSIS AND DNA FINGERPRINTING OF *Mentha species* USING RAPD MOLECULAR MARKERS

Prasad M. P

Sangenomics Research Labs, Domlur Layout, Bangalore-560071, India
Corresponding Author:prasad_m_p@hotmail.com

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ABSTRACT:

Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of diseases. There are different species of *mentha* having various medicinal properties. The species used are Peppermint (*Mentha piperita*), Bergamot mint (*Mentha citrate*), Spanish mint (*Mentha requienii*), Spearmint (*Mentha spicata*), and Japanese mint (*Mentha arvensis*). One of the main objectives of the study was to analyze the phytochemical content and genetic similarity and distance between these species of *mentha*. All the prepared plant extracts were subjected to preliminary phytochemical screening for the presence of phenolic content, glycosides, anthraquinones, terpenoids, flavinoids, tannins, lignin and saponins. The DNA was isolated from the *mentha* species by phenol chloroform method. The genetic variability among 5 *mentha* species was determined by RAPD technique using of random primers OPW 1-3. Maximum numbers of bands were produced by the primer OPW3 (43) and minimum by primer OPW1 (32). Genetic fingerprinting and phylogenetic diversity between different *mentha* species was determined by phylogenetic tree. The distinctive RAPD profiles demonstrate that RAPD can be used in mint to distinguish between species and between genotypes within a species of *mentha*.

KEYWORDS: *Mentha*, Phytochemical analysis, DNA fingerprinting, RAPD-PCR, Phylogenetic tree

INTRODUCTION:

Herbal medicine is the oldest form of healthcare known to mankind which had been used by all cultures throughout history. The genus *mentha* has attracted serious attention from plant breeders for genetic improvement of quality and yield traits. *Mentha* is usually taken after a meal for its ability to reduce indigestion and colonic spasms by reducing gastrocholic reflux [1]. A large volume of literature is available on the medicinal properties of essential oils present in *Mentha*. However, no much study has been directed toward the pharmacological properties of the *mentha* leaves which are locally available. The distinctive smell and flavour, a characteristic feature of *mentha* is due to the naturally occurring cyclic terpene alcohol called menthol. Menthol is prescribed as a medication for gastrointestinal disorders, common cold and musculoskeletal pain [9]. Species belonging to genus *mentha* can be found in diverse

environmental conditions and grow best in wet and moist environments where they usually grow 10 to 120 cm tall and are invasive in nature [2]. The genus *mentha* belongs to the family Lamiaceae (Labiatae) consisting of about 25 to 30 species mainly found in temperate regions of Eurasia, Australia , South Africa and North America. The presence of tannins and flavanoids in the methanolic *mentha* leaf extract has been reported between the phytochemicals and the free radical scavenging activity and antibacterial activity [5].

The antibacterial and antifungal activity of methanolic leaf extract of *mentha* is attributable to the presence of tannins and flavanoids [4,8]. The genus *Mentha* produces secondary metabolites such as alkaloids, flavanoids, phenols, gummypolysaccharides. Terpens and quinines are used in food and pharmaceutical, cosmetics and pesticide industries [6]. Tannins and flavanoids have therapeutic uses due to their anti-inflammatory, antifungal, antioxidant and healing properties [10]. PCR technique is one of the best available DNA-based tools for scoring variations between cultivars within species [7]. Phylogenetic relationships among *mentha* taxa have been reported by using random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) analyses [3]. The objective of the study was to assess the phytochemical contents and Phylogenetic relationships of the locally grown *mentha* plant leaves.

MATERIALS AND METHODS:

Sample Collection

Plant samples such as Peppermint (*Mentha piperita*), Bergamot mint (*Mentha citrate*), Spanish mint (*Mentha requienii*), Spearmint (*Mentha spicata*), and Japanese mint (*Mentha arvensis*) were collected from University of Agriculture, Bangalore, India and used in the present study.

Solvent Extraction

Plant leaves were dried under shade and the dried leaves were crushed using mortar and pestle. Soxlet extraction of the powdered plant material (10 g) was done with 100 ml of Methanol for 2h. The extract thus obtained was concentrated by solvent evaporation and used for phytochemical analysis.

Preliminary Phytochemical Analysis

Phytochemical screening for qualitative analysis of plant extracts was done according to the standard procedure. All the prepared plant extracts were subjected to preliminary phytochemical screening for the presence of phenolic content, glycosides, anthraquinones, terpenoids, flavinoids, tannins, lignin and saponins

DNA Isolation and PCR Amplification

DNA was isolated from fresh leaves by phenol: chloroform extraction method. The polymerase chain reaction was carried out in a final volume of 25µl of the reaction mixture containing 100 ng DNA, 3 U of Taq DNA polymerase, 2.5mM MgCl, 2.5mM each dNTPs and 100 pmol of primers. The DNA amplification was performed using the Corbett RG 6000 thermo cycler instrument under the following conditions: complete denaturation (94°C for 5 min), 10 cycles of amplification (94°C for 45 sec, 32°C for 1 min and 72°C for 1.5 min) followed by 30 cycles of amplification (94°C for 45 sec, 34°C for 1 min and 72°C for 1 min) and the final elongation step (72°C for 5 min). All PCR products were separated on 2% (w/v) agarose gel containing ethidium bromide. The gel was photographed with HP Alpha-imager. The genetic distance was calculated by the coefficient of frequency similarity matrix.

RESULTS AND DISCUSSION:

The phytochemical analysis of *mentha* leaves revealed positive for tannins, cardiac glycosides, anthraquinones, terpenoids, flavonoids, protein

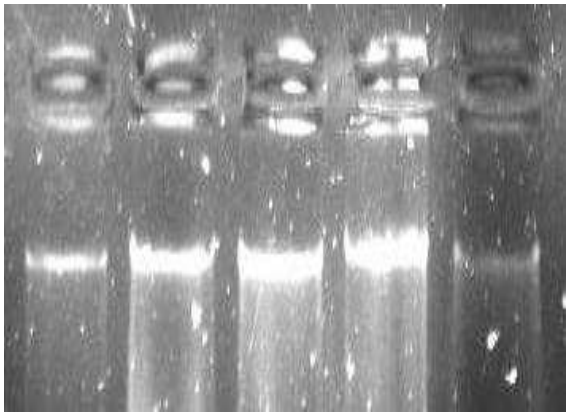
and lignin; while negative for reducing sugar, saponin and phenol (Table 1).

Table1. Phytochemical analysis

Species\ Test	Spear mint	Bergamot mint	Spanish mint	Pepper mint	Japanese mint
Reducing sugar	-	-	-	-	-
Tannin	+	+	+	-	-
Cardiac glycosides	-	+	+	+	+
Anthraquinones	-	-	-	+	+
Terpenoids	-	+	+	+	+
Flavonoid	-	+	+	+	+
Protein	-	-	-	+	+
Saponin	-	-	-	-	-
Lignin	-	+	+	+	+
Phenol	-	-	-	-	-

One of the main objectives of the study was to analyze the genetic similarity and distance between these species of *mentha.* Molecular analysis is considered one of the best methods of studying molecular taxonomy to identify and differentiate between species. The DNA was isolated from the *mentha* species by phenol chloroform method (figure 1).

Figure 1. Genomic DNA



The genetic variability among 5 *mentha* species was determined by RAPD technique using of random primers OPW 1-3. All the three primers produced distinguishable band patter with some exception (figure 2-4).

Figure 2. DNA Fingerprint for OPW1

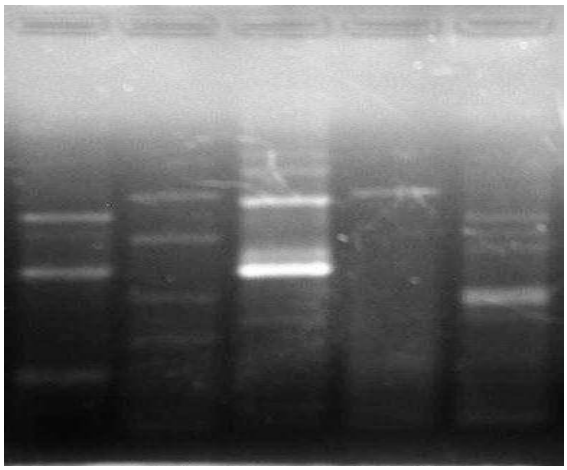


Figure 3. DNA Fingerprint for OPW2

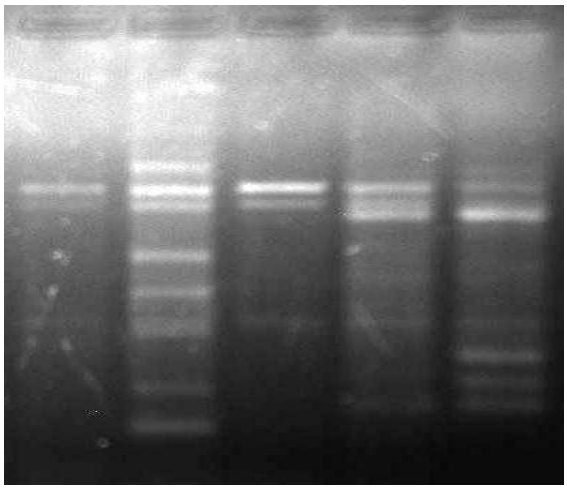
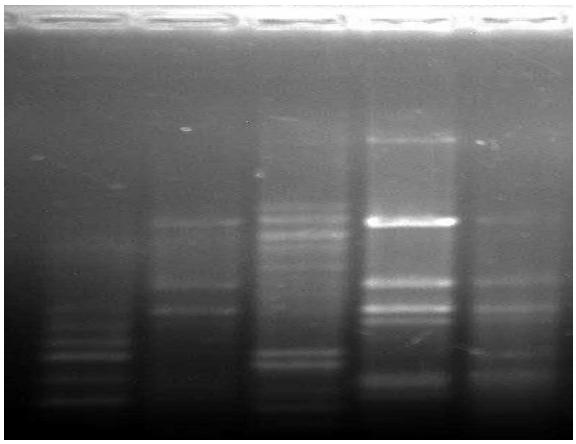


Figure 4. DNA Fingerprint for OPW3



The set of three primers on all genotypes generated a total of 109 bands. Maximum numbers of bands were produced by the primer OPW3 (43) and minimum by primer OPW1 (32). The failure primers to amplify distinct scorable bands of DNA may be due to their need to special requirements for amplifications in terms of PCR-reagents or temperature profile, since all of the reaction parameters were identical for all primers. Genetic fingerprinting and phylogenetic diversity between different *mentha* species were determined by UPGMA to produce a phylogenetic tree (figure 5-7) and converting RAPD data into a frequency similarity matrix (Table 2-4).

Figure 5. Dendrogram for OPW1

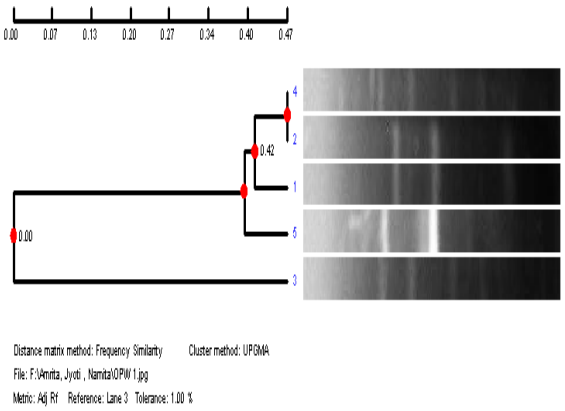


Figure 6. Dendrogram for OPW2

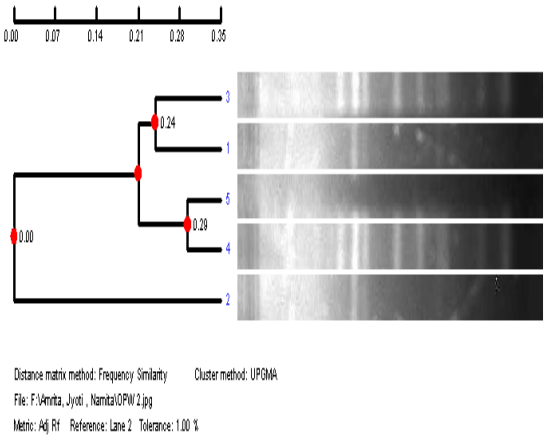


Figure 7. Dendrogram for OPW3

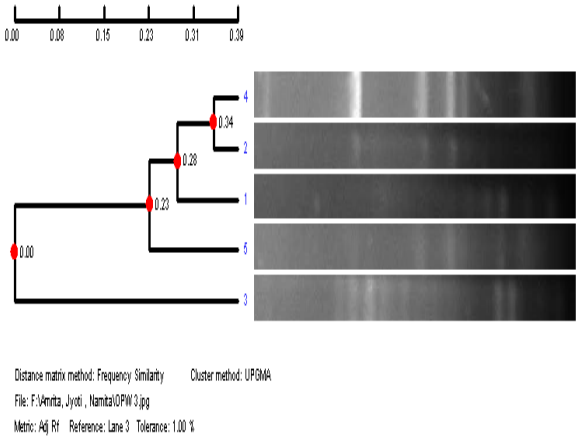


Table 2. Similarity matrix for OPW 1

	1	2	3	4	5
1	100	88.89	11.11	88.89	77.78
2	88.89	100	0	100	88.89
3	11.11	0	100	0	11.11
4	88.89	100	0	100	88.89
5	77.78	88.89	11.11	88.89	100

Table 3. Similarity matrix for OPW 2

	1	2	3	4	5
1	100	33.33	77.78	77.78	66.67
2	33.33	100	33.33	33.33	22.22
3	77.78	33.33	100	77.78	66.67
4	77.78	33.33	77.78	100	88.89
5	66.67	22.22	66.67	88.89	100

Table 4. Similarity matrix for OPW 3

	1	2	3	4	5
1	100	83.33	16.67	75	66.67
2	83.33	100	16.67	91.67	66.67
3	16.67	16.67	100	25	33.33
4	75	91.67	25	100	75
5	66.67	66.67	33.33	75	100

CONCLUSION:

The distinctive RAPD profiles demonstrate that RAPD can be used in *mentha* to distinguish between species and between genotypes within a species. The RAPD technique can also be used to estimate degrees of relatedness within the genus of *mentha*.

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