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Research Article

SIMILARITIES BETWEEN SPECIES OF OCIMUM AND MENTHA ON THE BASIS OF PHYTOCHEMICAL AND DNA QUANTIFICATION STUDY

Singh Charan[1]*, Sharma Nevadita[1] and Singh Sukhjinder[2]
[1]Assistant Professor in Biotechnology Department of Seth. G.L. Bihani S.D (P.G) College, Sri Ganganagar, Rajasthan, India
[2]Student in Biotechnology Department of Seth. G.L. Bihani S.D (P.G) College, Sri Ganganagar, Rajasthan, India
*Corresponding Author Email: charan.singh201080@gmail.com

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ABSTRACT

Ocimum species (*Ocimum tenuiflorum, Ocimum gratissimum* & *Croton bonplandianus*) and *Mentha spicata* are having various medicinal properties. The substances derived from the plants remain on the basis for a large proportion of the commercial medications used today for the treatment of diseases. Therefore, one of the main objectives of the study was to analyze the phytochemical content, genetic similarity and distance between these three species of ocimum and one of mentha. All the prepared plant extracts were subjected to preliminary phytochemical screening for the presence of glycosides, saponins, alkaloids, flavonoids, tannins, proteins, free amino acids, carbohydrate and vitamin C. Another objective of the study is to provide a simple method of isolation of DNA, using the CTAB extraction protocol by phenol chloroform method. DNA quantity obtained was comparable to the known standard DNA concentration curve at 600nm. Good quality of DNA was isolated and suitable for various molecular biology applications.

Keywords: CTAB, DNA extraction, Ocimum species, Mentha, Rama tulsi, Shyama tulsi, Ban tulsi

INTRODUCTION

Medicinal plants are continued to be a major source of medicine, as they have throughout human history [1]. *Ocimum sanctum* commonly known as Tulsi in Hindi and Holy Basil in English and *Mentha spicata* commonly known as Mint, these are popular herb was used for this study. *Ocimum sanctum* is 30-75 cm high erect herb which found throughout the semitropical and tropical parts of India. Leaves are 2.5 – 5 cm long and 1.6 – 3.2 cm broad, elliptical, oblong obtuse. Inflorescence is verticillate and flowers are in racemes 15-20 cm long in close whorls. Odour and taste are aromatic and sharp [2]. *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. These species are mainly found in temperate regions of Europe, Asia (Middle East, Himalayana, China, etc.), Australia, South Africa and North America [3].

The genus *Ocimum sanctum* and *Mentha spicata* belongs to the same family Lamiaceae (Labiatae). These herbs has been reported in Indian Traditional Systems of Medicine and its modern applications are receiving wide spread attention day by day [4]. It has been observed that tulsi has antioxidant, antibiotic, antiatherogenic, immunomodulatory, anti-inflammatory, analgesic, antiulcer, chemopreventive and antipyretic properties [5], whereas mint observed as an antispasmodic, aromatic, antibacterial, use in digestive problems and antiseptic in the treatment of cancers, colds, cramps, indigestion, nausea, sore throat and toothaches [6]. The objective of study to find the genetic and phytochemical similarities between *Ocimum sanctum* species (*Ocimum tenuiflorum*, *Ocimum gratissimum* & *Croton bonplandianus*) and *Mentha spicata* which genus belongs to the same family. The comparison of DNA concentration of both species is known by standard curve.

MATERIALS AND METHODS

Sample Collection

Plant sample such as Rama tulsi (*Ocimum gratissimum*), Shyama tulsi (*Ocimum tenuiflorum*), Ban tulsi (*Croton bonplandianus*) and *Mentha spicata* were collected from northern Rajasthan. The identification was done by Prof. Suman Sharma, The Floras and Literature of Botany Department, Dungar College, Bikaner, Rajasthan (India).

Solvent Extraction

The dried plant material was powdered using motor and pestle and subjected to soxhlet extraction with 50% ethanol for 2 hours. The mixture was evaporated to dryness in a rotary flash evaporator and stored in refrigerator. The condensed extracts were used for preliminary screening of phytochemicals.

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 Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C. Following standard procedures were used [7-9].

Test for Glycosides

Keller Killiani Test – Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Test for Saponins

Foam Test – Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Test for Alkaloids

Hager's Test – Test solution was treated with few drops of Hager's reagent (saturated picric acid solution).Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Test for Flavonoids

Ferric chloride test – Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Test for Tannins

Gelatin Test – Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

Test for Proteins

Biuret Test – Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

Test for Free Amino Acids

Ninhydrin Test – Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

Test for Carbohydrate

Benedict's test – Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for Vitamin C

DNPH Test – Test solution was treated with Dinitrophenyl hydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

DNA Isolation and Quantification

DNA was isolated from cetyl trimethyl ammonium bromide (CTAB) protocol [10, 11] with some modifications. Grind 350 mg of freshly cut leaves along with 4 ml of preheated CTAB buffer in mortar and pestle. The finely crushed leaf tissues were then transferred to 15 ml centrifuge tube, which were then incubated for 60 min at 65ºC. After incubation, centrifugation at 2,300 rpm for 5 min was performed; the supernatant was transferred to new centrifuge tubes to which an equal quantity of chloroform/saturated phenol (50:50) was added. These tubes were inverted for 5 to 6 times then again centrifugation on above mention conditions was performed and supernatant was collected. This step was performed 2 to 3 times then collects to supernatant and adds 25µl of RNase A. Mix the sample gently by inversion and incubates for 30 min at room temperature. After it add an equal volume of isopropanol was added for DNA precipitation, again centrifugation at 2,300 rpm for 5 min was done, supernatant was discarded and to DNA pellet 70% cold ethanol was added for washing the pellets. The pellets were air dried at room temperature and then resuspended in 30 to 40 µl of 0.1 × TE (Tris EDTA) buffer. For short-term storage (24-48 hours) of the DNA, 2-8ºC is recommended. For long term storage, -20^{0}C or lower temperature (-80ºC) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA.

Gel Electrophoresis

The quality of DNA was checked by agarose gel electrophoresis. DNA presence in the samples and its quality was assessed by running DNA samples mixed with 2 µl of 6X gel loading buffer to 10 µl of DNA sample on 0.8 % agarose gel and 0.5µl Ethidium bromide prepared in 1x TAE (Tris-Acetate-EDTA). Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized. Gel were photographed and documented under UV light using a BIORAD gel documentation system.

DNA Quantification

DNA concentration was estimated by the standard curve formation of known DNA concentration by measuring the absorbance at 600 nm by visible spectrophotometer (double- beam vis- spectro 1203).

RESULT AND DISCUSSION

Phytochemical analysis of *Ocimum sanctum* species and *Mentha spicata*

Phytochemical screening provides basic information about the medicinal importance of the plant extracts. Secondary metabolites including flavonoids, alkaloids, saponins and tannins possess antioxidant, anticancer and anti-inflammatory activity [12]. Tannin possessed spasmolytic activity in smooth muscles cells, free radical scavenger and antioxidant. Flavonoids have antioxidant and antimicrobial properties while saponins are glycosides possessed antimicrobial and inhibit Na+ efflux, by blockage of the entrance of the Na+ out of the cell, reducing congestive heart failure [13,12] alkaloids have possessed analgesic, antisplasmodic and bactericidal activities, antioxidant and are useful in renal disorder [14,15]. The current investigation showed the presence of certain constituents in ethanolic extract of *M. spicata* and *O. sanctum* (*O. gratissimum*, *O. tenuiflorum* and *Croton bonplandianus*). The *Mentha spicata* revealed the presence of flavonoids, tannins, alkaloids and, while saponins and vitamin C found absent while extract of *O. gratissimum* and *tenuiflorum* consisted of glycosides, saponins, flavonoids, tannins, protiens, free amino acid, carbohydrates and vitamin C whereas alkaloids were found absent. In *Croton bonplandianus* extract alkolids and flavonoids were found absent whereas glycosides, saponins, tannins, proteins, free amino acid, carbohydrates and vitamin C were found present (Table 1). Similar phytochemicals were reported during characterization of medicinal plants [16]. Similar results were reported by various studies that ethanolic fraction possesses highest amount of phenolic and poly phenolic compounds [17,18]

DNA extraction and DNA quantification

DNA was isolated from three species of O. *sanctum* and M. *spicata* species by using the protocol of Richards (1997), with some modifications[11] and then it was run on 0.8% agarose in 1× TAE buffer to check the quality and quantity of isolated DNA. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized. Gel were photographed and documented under UV light using a BIORAD gel documentation system (Figure 1). It was found that the bands of ban tulsi (*Croton bonplandianus*) and shyama tulsi (*Ocimum tenuiflorum*) appear at 200bp which is 40ng/5µl and by relative front versus intensity graph it was observed that these lane band (Figure 2) have relative front 0.896, 0.890; volume (intensity) 8, 774 and

66,447; lane percent 0.1, 0.7 and band percent 0.9, 14.2 respectively whereas in rama tulsi (*Ocimum gratissimum*) bands are found between 500bp and 600bp, which is consistent with 550bp so, DNA mass is 30ng/5 µl, relative front was observed 0.658, volume (intensity) 10,379, lane percent 0.4 and band percent 7.1. Mint (*Mentha spicata*) bands are found between 300bp and 400bp, which is consistent with 350bp so, DNA mass is 30ng/5 µl, relative front was observed 0.825, volume (intensity) 127,865, lane percent 1.1 and band percent 14.4. Therefore, there are similarity in band percent of shyama tulsi (*Ocimum tenuiflorum*) and Mint (*Mentha spicata*). DNA concentration was estimated by the standard curve method by measuring the optical density at 600 nm in visible spectrophotometer concentration was found (µg//200µl) 480µg, 410 µg, 389 µg, 379 µg for ban tulsi, rama tulsi, shyama tulsi and mint respectively (Graph 1).

Table 1: Preliminary Phytochemical analysis table

Phytochemicals	Samples			
	Rama tulsi (*Ocimum gratissimum*)	Shyama tulsi (*Ocimum tenuiflorum*	Ban tulsi (*Croton bonplandianus*)	Mint (*Mentha spicata*)
Glycosides	+	+	+	+
Saponins	+	+	+	-
Alkaloids	-	-	-	+
Flavonoids	+	+	-	+
Tannins	+	+	+	+
Proteins	+	+	+	+
Free Amino Acids	+	+	+	+
Carbohydrate	+	+	+	+
Vitamin C	+	+	+	-

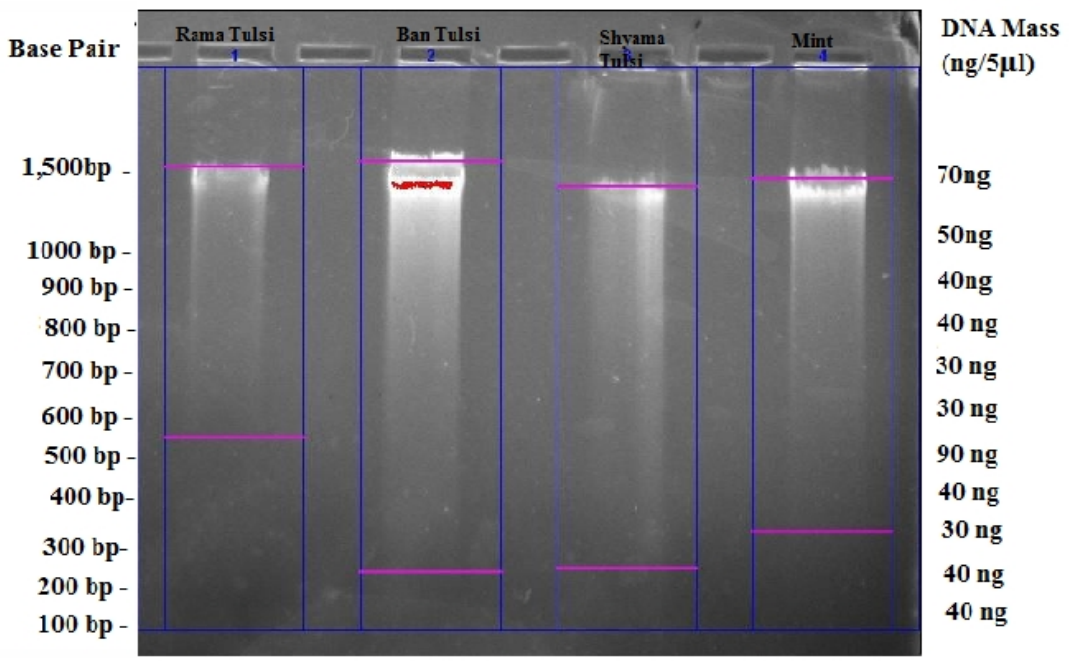
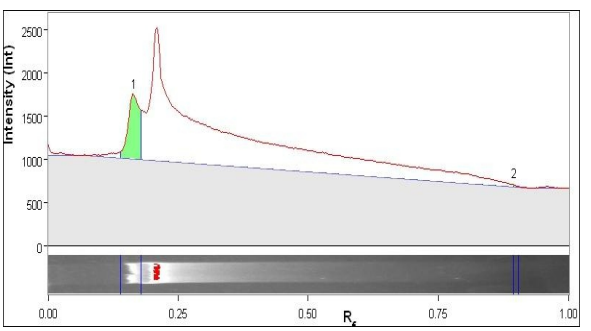
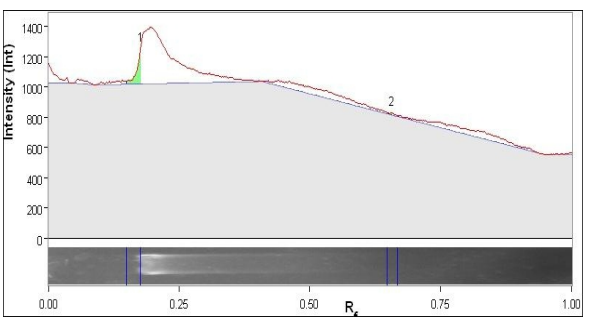


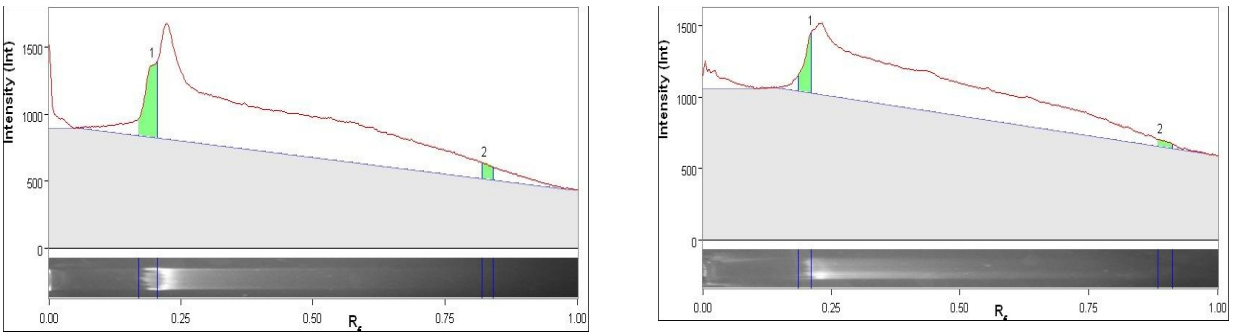
Figure 1: Gel Electrophoresis of Rama Tulsi, Ban Tulsi, Shyama Tulsi and Mint



Lane 1

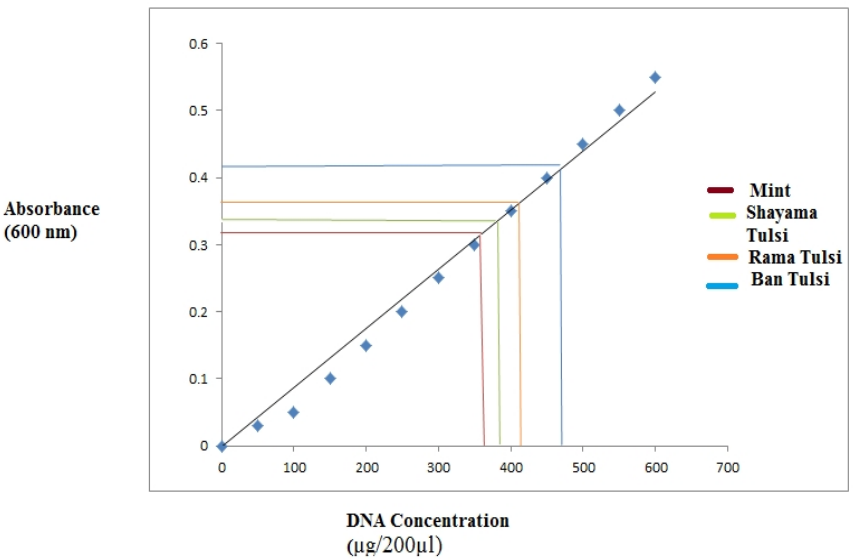


Lane 2



Lane 3 **Lane 4**

Figure 2: Lane 1 (rama tulsi), Lane 2 (ban tulsi), Lane 3 (shyama tulsi) and Lane 4 (Mint) Rf (relative front), Int (intensity)



Graph 1: Absorbance (600 nm) versus DNA concentration (µg/ 200µl)

CONCLUSION

Based on our results, we may conclude that at phytochemical and DNA quantification level ban tulsi (*Croton bonplandianus*) and shyama tulsi (*Ocimum tenuiflorum*) band found at same base pair, phytochemical test are same whereas (*Mentha spicata*) and rama tulsi (*Ocimum gratissimum*) show somewhat same results. Therefore we can say that at this level ban tulsi and shyama tulsi show so many similarities, further we are work in a genetic level for more confirmative study on similarities by RAPD method.

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