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Phytochemical Analysis and Antioxidant potential of *Piper species* and its Molecular Characterization by RAPD Markers

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Abstract

BACKGROUD & OBJECTIVE: *Piper*, the pepper plants or pepper vines, are the genus of the *Piperaceae* family and are important economically and ecologically. It contains more than 1,000 species of shrubs, herbs and lianas, many of which are keystone species in their native habitat. The diversification of this taxon is of interest to understand the evolution of plants. In the scope of bioactive compounds, *Piper* species (*P. nigrum, P. retrofractum* and *P. longum)* were screened for phytochemicals. **METHODOLOGY:** Phytochemical analysis of these plants showed presence of phenolic compounds, anthraquinones, terpenoids, flavinoids and lignin. Also, the methanolic extracts of these plants were tested for their antioxidant potential by free radical scavenging activity by DPPH assay. All the tested plants showed antioxidant potential and the overall antioxidant activity of *P. nigrum* was found to be highest. Genomic DNA was extracted from the fresh leaves of selected cultivars and PCR was performed by using RAPD primers to check the genetic diversity among these cultivars. From the PCR generated fingerprint, dendrogram was plotted by cluster analysis of similarity matrix. **RESULTS:** Dendrogram constructed by cluster analysis of RAPD markers showed that *Piper nigrum* and *Piper retrofractum* are closely related. Since morphological differences among these species are indistinctive, RAPD characterization can be helpful in their Discrimination. **CONCLUSION:** This finding can be used as prerequisite for plant breeding activities as well as for conservation of genetic resources.

Keywords: Phytochemical, Antioxidant, RAPD, *Piper*

1. Introduction

Herbs and Spices have been used for thousands of years to enhance the flavor, color and aroma of food; additionally, they are known for their preservative and medicinal value[1,2,3]. In Recent time their use has been increased for the improvement of health and fitness[4]. Spices can be used as medicine because they are natural products easily absorbed by our bodies and generally do not have any adverse effects. Herbal remedies are an important source for the discovery of new antibiotics[5] and numerous studies have identified compounds within herbal plants that are effective antibiotics[6]. Phytochemicals are compounds present in plants that are used as food and medicine to protect against illness and to maintain human health[7]. Phytochemicals have antioxidant or hormone-like effect which helps in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues[8]. *Piper* species, widely distributed around the world are used for various medicinal purposes. Plants belonging to the genus *Piper* are reputed in the Indian Ayurvedic system of medicine for their medicinal properties[9]. Previous phytochemical studies of this genus have led to the isolation of a host of interesting chemical constituents which include lignins, amides, alkaloids and flavanoids[10].

Compounds isolated from *Piper* species have been reviewed but since then some new species have been investigated[11]. *Piper* species are good source for vitamin A and known to have catalase activity[12]. Black pepper (*Piper nigrum L.*, Piperaceae) is used to treat asthma, chronic indigestion, colon toxins, obesity, sinus, congestion and fever[13]. It has been shown that *Piper* has antimicrobial activity[14] and some have already produced compounds, effective against antibiotic resistant strains of bacteria[15]. Spices and herbs prolong the storage life of foods by preventing rancidity through their antioxidant activity or bactericidal activity[16]. The use of *Piper* genus in folk medicine is due to secondary metabolites with diverse biological activities. This study represents the phytochemical investigations and antioxidant potential of *Piper nigrum, Piper retrofractum* and *Piper longum.*

The study of genetic structure and diversity permits knowledge of the organization and distribution of the genetic variability among and within natural populations[17,18,19]. This understanding is indispensable in the choice of strategies for conservation and exploitation of natural populations in their natural habitats when the objective is the maintenance of diversity and guarantee of sustainability[19,20]. RAPD markers have been widely used in genetic diversity studies due to their great resolution power compared to morphological markers, the large number of DNA bands (markers) that can be obtained in relation to iso-enzymes and because of their simplicity and practicality compared to other DNA markers[21].

In recent years, Global interest in oriental medicine and production of medicinal plants has increased a lot. Since many species and varieties exist, development of molecular markers would be important for quality assessment in the medicinal industry[22]. The study of genetic relationships is a prerequisite for plant breeding activities as well as for conservation of genetic resources[23]. The main advantage of RAPD-PCR is the use of unique and short arbitrary primer for PCR amplification, without prior information about the sequence of DNA. Besides, it is more advantageous with respect to other similar molecular

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marker techniques such as the more recently introduced AFLP[23]. In fact, RAPD-PCR technology requires reduced time and cost, and no radioactive reagents for DNA fingerprinting analysis[24]. This study represents the phytochemical investigations, antioxidant potential and molecular characterization of *Piper nigrum, Piper retrofractum* and *Piper longum.*

2 Materials and Methods

2.1 Plant Material

The plant of *Piper nigrum, Piper retrofractum* and *Piper longum* were obtained from University of agriculture, Bangalore India. Fresh plant material (leaves) were washed with distill water, air dried under shade, powdered and stored in air tight bottles.

2.2 Phytochemical Analysis

Phytochemical screening was done according to the standard procedures described[25]. The powdered plant samples were subjected to preliminary screening for the presence of phenolic content, glycosides, anthraquinones, terpenoids, flavonoids, tannins, lignin and saponins.

2.3 Scavenging Activity of Extract

Scavenging activity on DPPH was assessed according to the method reported[25] with a slight modification. Briefly, 500µl of extracts (0.2 to 1mg/ml) were mixed with 3ml of 0.1mM DPPH. Then incubated at room temperature for 30 min and absorbance was measured at 517 nm in spectrophotometer. Percent inhibition was calculated from control using the following equation.

Scavenging activity (%) = [Absorbance of control – Absorbance of test sample] / Absorbance of control X 100

2.4 DNA Extraction and PCR Amplification

DNA was isolated from fresh leaf tissues as per the procedure described previously[26]. The polymerase chain reaction was carried out in final volume of 25 µl containing 100 ng DNA, 1 U of Taq DNA polymerase (Chromous Biotech, Bangalore), 2.5 mM MgCl (Chromous Biotech, Bangalore), 2.5 mM each dNTPs (Chromous Biotech, Bangalore) and 100 p mol of primers (GeNei, Bangalore). The DNA amplification was performed in the Corbett RG 6000 thermo cycler using the following conditions: complete denaturation (94°C for 5 min), 10 cycles of amplification (94°C for 45 sec, 35°C for 1 min and 72°C for 1.5 min) followed by 30 cycles of amplification (94°C for 45 sec, 38°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 1.5% (w/v) Agarose gel containing ethidium bromide (0.5 µg / ml). The gel was photographed with HP Alpha-imager.

Data Analysis

The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The genetic distance was calculated by the coefficient of similarity of Jacccard. The matrix of genetic distance was used for grouping the *Piper* cultivars based on the dendrogram constructed by UPGMA (unweighed pair group method with Arithmetic averages).

3 Results and Discussion

Phytochemical screening of the *Piper nigrum, Piper retrofractum* and *Piper longum* showed the presence of phenolic content, anthraquinones, terpenoids, flavinoids and lignin. Alternatively, glycosides, tannins and saponins are not detected in these species. The detailed results of all tests for each species are summarized in Table I. Since, the detected phytochemicals are known to have biological activity; these plants can be used for their extraction for medicinal use.

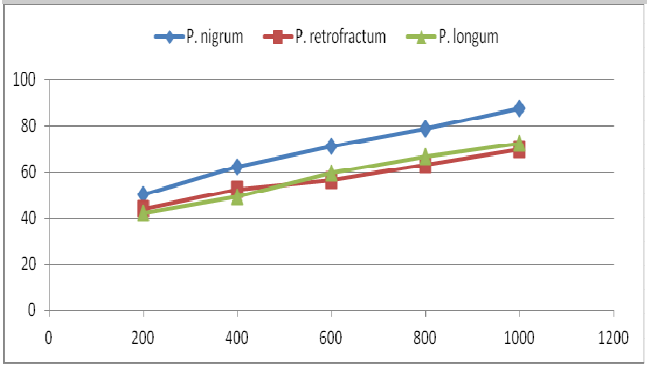
Antioxidants are used for preventing the deleterious consequences of oxidative stress; hence, there is increasing interest in the protective functions of natural antioxidants from plant source. In the present study the antioxidant activity of Piper species was determined by DPPH assay. In DPPH assay *P. nigrum* showed highest total antioxidant capacity followed by *P. longum* (Figure I).

Table I: Phytochemical Analysis of *Piper nigrum, Piper retrofractum* and *Piper longum*

Test	Plant Sample		
	P. nigrum	*P. retrofractum*	*P. longum*
Phenolic Compound	+	+	+
Glycosides	-	-	-
Anthraquinones	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Tannnin	-	-	-
Lignin	+	+	+
Saponin	-	-	-

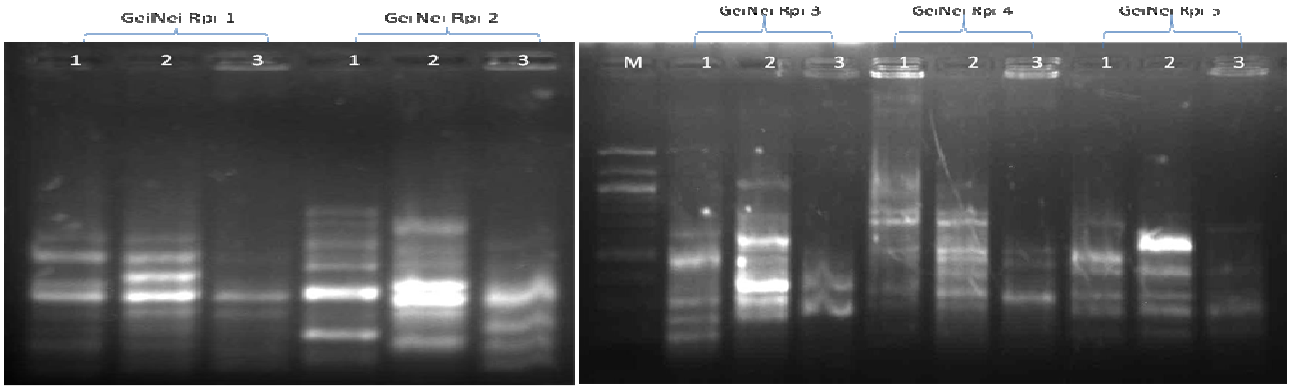
The RAPD patterns of genomic DNA of *Piper nigrum, Piper retrofractum* and *Piper longum* by GeNei primers 1 to 10 were analyzed for polymorphism, total five primers gave clear distinctive band pattern as shown (Figure II). Genetic similarity was calculated using Jaccard's similarity coefficient and dendrogram was generated to access the genetic relationship among three selected species (Figure III). Dendrogram constructed by cluster analysis of RAPD markers showed that *Piper nigrum* and *Piper retrofractum* are closely related.

Figure I: Antioxidant Activity by DPPH Assay



To conclude, the phytochemicals are either the product of plant metabolism or synthesized for defense purposes which are known to have bioactivity. The occurred bioactive phytochemical and the antioxidant potential of these *Piper* species can be used for medicinal purpose. Further chemical and pharmacological investigations are recommended to evaluate the potential of these *Piper* species for antioxidants. RAPD does not require prior genetic sequence information and can produce multi-locus profiles widely spanning the genome which can be used for the intra-species genetic diversity study among these species. This molecular analysis of different *Piper species* by RAPD will generate potential markers for phylogenetic analysis supported by RAPD derived Dendrogram. The knowledge of the genetic relationships is

Figure II: RAPD profiles of three selected cultivars obtained with GeNei (1-5) primers
(M- DNA Marker, 1- *Piper nigrum*, 2- *Piper retrofractum*, 3- *Piper longum*)



essential for developing breeding strategies, germplasm management, and utilization of genetic resources.

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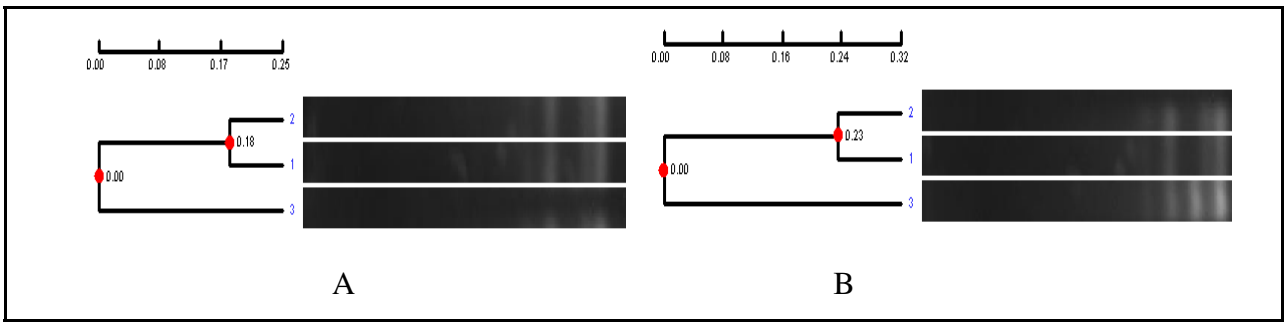

Figure. III (A,B): Dendrogram showing genetic relationship among three selected cultivars A. Dendrogram analysis for primer GeNei Rpi 1, B. Dendrogram analysis for primer GeNei Rpi 2