Assessment of phytochemical, antioxidant and genetic diversities among selected medicinal plant species of Mimosoideae (Mimosaceae)

Manas Ranjan Saha, Pallab Kar & Arnab Sen*
Molecular Genetics Laboratory, Department of Botany, University of North Bengal, Siliguri- 734 013, India
E-mail: senarnab_nbu@hotmail.com

Received 25 January 2017, revised 14 August 2017

Mimosoids form a major group in legumes. Of which, most of the members are traditionally reported remedies to diseases in rural India. However, proper justification and validation of their traditional practice are lacking. Hence, we intended to explore a comprehensive account of phytochemical profiling, antioxidant activity and genetic variation among selected ethnomedicinal species of Mimosoideae (Mimosaceae). The phytochemical study confirmed the presence of alkaloid, tannin, phenolic and flavonoid in all selected members. Antioxidant profiling through DPPH (2,2-diphenyl-1-picrylhydrazyl), OH• (Hydroxyl radical), NO (nitric oxide), and TAA (total antioxidant activity) assays was found to be significant (p < 0.001). In fact, all the extracts exhibited higher scavenging potentiality than the respective standards underlining their ethnomedicinal significance. An attempt was also made to analyze the genetic diversity of nine selected Mimosoids employing RAPD and DNA barcode analysis. A total of 330 amplified distinct bands, ranging from 190-1763 bp with 100 % of polymorphism were yielded through RAPD analysis. The dendrogram constructed clearly revealed the genetic relatedness among the species of Mimosoids validating conventional classification. We further correlated the dendrogram with phytochemical profiling. What's more, two phylogenetic dendrograms of matK and TrnL-F loci of selected taxa clearly showed inter-generic and intra-generic diversity among the nine studied species as a first-hand information.

Keywords: Mimosaceae, Phytochemical, Antioxidant, RAPD, DNA barcode.

IPC Int. Cl*: A61K 36/00, C09K 15/00, C07, C08, C12

Since time immemorial, men used to depend on plant resources for their basic needs. In essence, practices of medicinal plants are deeply rooted in the society of indigenous community, and the traditional knowledge passes from generation to generation verbally. A plethora of evidence reflected the therapeutic significance of plants in India as well as in other countries. In fact, medicinal plants are considered to be the main sources of several phytochemical compounds (PCs) like alkaloids, tannins, phenols, steroids and flavanoids curing diversified chronic diseases. The most obvious role of PCs is protection from free radicals or reactive oxygen species (ROS) that is produced continuously in human body. In fact, medicinal plants are the best known scavenger of ROS without any adverse effects. In this context, Mimosoids play a major role within legumes having multidisciplinary medicinal value. According to Cronquist, they have usually been recognized either as the family Mimosaceae or as the subfamily Mimosoideae of the order Fabales. Mimosoideae consist of about 80 genera and 3370 species of trees, shrubs, and lianas found mainly in tropical, subtropical, and warm temperate regions of the world. The members of Mimosoideae are characterized by their valvate aestivation of petals, bipinnate leaves, regular flowers grouped into spicate or capitate inflorescence. Besides, they have been acknowledged as vegetables (e.g. Pisum sativum, Glycine max, etc.), oil (e.g. Arachis hypogea, Glycine soya, etc.), fruits (Tamarindus indica, Phaseolus coccineus, etc.) and timber (e.g. Dalbargia sisso, Acacia auriculiformis, etc.). Recently, in our preceding studies we have already documented the local traditional practices of Mimosa pudica, Acacia nilotica, A. catechu and A. concinna curing leucorrhea, bone crack, ankle sprain, breast cancer or tumor, eczema, leucoderma and other skin disorders by the indigenous people of Malda and Uttar Dinapur districts. Besides, Mimosa, Acacia, Albizia, and Samanea under Mimosoideae were accounted to be effective as anti-diuretic, anti-dysenteric, anti-
More surprisingly, hardly any step was undertaken so far to represent a comparative as well as a comprehensive account of phytochemicals and antioxidant activities among these ethnomedicinal members of Mimosaceae. Also, information is hardly available regarding the genetic variation of the above mentioned populations within the order Fabales. Since the morphological variation between species is difficult to distinguish; an appropriate knowledge of molecular documentation would be a rational way to understand the genetic relationship among different families. Hence, a first initiative step was carried out to explore the genetic variations of the above mentioned populations through DNA fingerprinting techniques. Of the various DNA fingerprinting techniques developed for plant research, random amplified polymorphic DNA (RAPD) analysis has become increasingly popular which are being used to evaluate the genetic relationship among species, cultivars, and varieties. Subsequently, a new modified molecular technique, i.e. DNA barcoding was developed recently to explore the evolution, identification and genetic relatedness of unknown plants and animal species resolving various anomalies in the taxonomic levels by using a short stretch of DNA sequence. Considering all of these facts, the present study was designed to investigate a comprehensive phytochemical and antioxidant profiling among nine medicinal plant species of Mimosoideae. Besides, we intended to explore whether this antioxidant activity of selected species corroborates with the genetic diversity or not.

Materials and methods
Plant materials
Germplasm was collected from various places of northern parts of Bengal province in India. Herbarium specimens were prepared and deposited at North Bengal University Herbarium (acronym: NBU). *Mimosa pudica* (M1), *M. invisa* (M2) and *Acacia nilotica* (M3) were collected from Malda district (Voucher No. NBU/MLD/308, NBU/MLD/311 & RUC/MLD/433, respectively). *A. nilotica var. indica* (M4), *A. catechu* (M5) and *A. concinna* (M6) were collected from Uttar Dinajpur district (Voucher No. NBU/UD/454, NBU/UD/1039 and NBU/UD/756, respectively). Similarly, *Albizia lebbeck* (M7), *A. chinensis* (M8) and *Samanea saman* (M9) were obtained from Darjeeling district (Voucher No. NBU/DJ/902, NBU/DJ/1002 and NBU/DJ/1022). The habit, habitat, locality, collection time and morphology of each plant specimen were also recorded. Fresh leaf materials were used for DNA isolation.

**Preparation of plant extracts**
The leaves of each plant were washed thoroughly in double distilled water followed by shade-dry separately for three weeks and then grounded into powder by electric grinder (Lords Hummer 1100). Extensive extraction was further performed in Soxhlet apparatus for 9-11 h using ethanol as a solvent (dried leaf material:ethanol, 1:10 v/v). Each extract was then concentrated under reduced vacuum pressure at 40 °C ± 2 °C in a rotary vacuum evaporator (Buchi Rotavapor R-3, Switzerland). The concentrated extracts were further lyophilized separately using Eyela Freeze Dryer (FDU-506, USA) and weighed subsequently. Finally, the lyophilized extracts were stored in a sterile container and placed at -20 °C until further use. The extracts were freshly dissolved in double distilled water prior to experiments.

**Chemicals**
Chemicals used in the present study were of analytical grade and/or molecular grade and procured either from HiMedia (India), or E-Merck (India), or Promega unless mentioned otherwise.

**Phytochemical diversity screening**
A preliminary phytochemical screening was carried out as per the standard methods described by Harborne and Evans.

**Diversity of antioxidant activity among nine Mimosoids**
Antioxidant potentioplicity of different plant extracts were performed to measure the scavenging capacity of each extract comparing with respective potent known standards (positive control), corresponding to the assays. The concentrations of each extract were selected based on the physiological standards to reflect their dose-dependent inhibitory effects.

Initially, free radical scavenging activity of each extract was performed through DPPH (2,2-diphenyl-1-picrylhydrazyl) assay employing different concentrations of extracts (0–100 μg/mL, dissolved in distilled water) and freshly prepared DPPH solution (1 mM; diluted in 95 % methanol). The Optical density (OD) value was measured after 30 min of reaction at 517 nm using UV-Vis Spectrophotometer.
Ascorbic acid was taken as positive control and the percentage inhibition was calculated as per Saha et al.\textsuperscript{17} The hydroxyl radical (\(\text{OH}^\bullet\)) scavenging assay was carried out on the basis of Fenton reaction with a slight modifications\textsuperscript{17}. In this assay, a reaction mixture was prepared containing 2-deoxy-2-ribose (2.8 mM), ferric chloride (FeCl\(_3\); 100 \(\mu\)M), hydrogen peroxide (H\(_2\)O\(_2\); 1.0 mM), monopotassium phosphate-potassium hydroxide buffer (KH\(_2\)PO\(_4\)-KOH; 20 mM; pH 7.4), ethylenediaminetetraacetic acid (EDTA; 100 \(\mu\)M), ascorbic acid (100 \(\mu\)M) and different concentrations of plant extracts (0–100 \(\mu\)g/mL, dissolved in distilled water) up to a final volume of 1 mL and left for 1 h of incubation at 37 °C. Afterward, 0.5 mL of incubated mixture was transferred into another test tube and mixed with 1 mL of each tricarboxylic acid (TCA; 2.8 %) and aqueous thiobarbituric acid (TBA; 1 %). Finally, the mixture was incubated for 15 min at 90 °C and cooled down to room temperature and the absorbance was measured at 532 nm. Mannitol was used as standard. Subsequently, nitric oxide (NO) quenching activity was performed following the Griess-Ilosvoy reaction with a few changes\textsuperscript{17}. In essence, a reaction mixture was prepared containing phosphate buffered saline (\(\text{pH} 7.4\)), sodium nitroprusside (SNP; 10 mM) and various concentrations of extract (0–100 \(\mu\)g/mL) and left for 150 min at 25 °C. After incubation, 1 mL of each forward and reverse primer (0.25 \(\mu\)M), 8 µL of Pyrogen free (PF) water and 2 \(\mu\)L of template DNA (25 ng/\(\mu\)L). The PCR reactions were performed on Applied Biosystems Thermocycler-2720. The RAPD-PCR amplification cycle consisted of the following specifications: 4 min at 94 °C, 30 sec at 48 °C, 1 min at 72 °C, 34 cycles of 1 min at 94 °C, 30 sec at 48 °C, 1 min at 72 °C, and a final extension time of 10 min at 72 °C. The matK-PCR amplification cycle followed in the present study are: 4 min at 94 °C, 1 min at 37 °C, 2 min at 72 °C, 44 cycles of 1 min at 94° C for, 1 min at 37 °C, 2 min at 72 °C and a final extension time of 10 min at 72 °C. The matK-PCR amplification cycle followed in the present study are: 4 min at 94 °C, 30 sec at 48 °C, 1 min at 72 °C, 34 cycles of 1 min at 94 °C, 30 sec at 48 °C, 1 min at 72 °C, and a final extension time of 7 min at 72 °C whereas 5 min at 95 °C, 45 sec at 54 °C, 2 min at 72 °C, 34 cycles of 45 sec at 95 °C, 45 sec at 54 °C, 2 min at 72 °C, and a final extension time of 7 min at 72 °C was considered for trnL-F region. The amplified products of RAPD, matK and TrnL-F were resolved on 1.8 % (w/v) agarose gel containing Ethidium bromide solution (0.5 µg/mL) run in 0.5 X TBE (Tris-borate-EDTA) buffer. The fragment size was estimated using 100 bp DNA ladder and \(\lambda\) DNA/Eco RI/Hind III double digest as molecular weight marker.

### Statistical analysis

All the data in the present study were prepared as the mean ± SD of six measurements. The statistical analysis was executed by one-way analysis of variance (ANOVA) with Dunnett’s test using KyPlot version 5.0 beta 15 (32 bit) for windows where \(p < 0.05\) was considered as significant. The graphs were also generated using KyPlot (version 5.0 for windows).

### Molecular diversity study

#### DNA extraction, amplification, and sequencing

Total genomic DNA from each plant was isolated using the standardized protocol developed by Doyle & Doyle\textsuperscript{19} with a slight modification of Goyal & Sen\textsuperscript{20}. Initially, a total of 45 RAPD markers and two phylogenetic markers such as matK and trnL-F region were amplified using standard PCR protocols. Primers (matK and TrnL-F) used for amplification and sequencing were as follows, matK F: CGATCTATTCATTCAATATTTTC; matK R: TCATGCAACGAAAAGTCCAAGT and TabC (forward): CGAAATCGGTAGACGCTACG; TabF (reverse): ATTTGAACTGTTGACACGAG. Briefly, 25 \(\mu\)L of PCR reaction mixture were prepared containing 12.5 \(\mu\)L of PCR Master Mix (2X), 1.25 \(\mu\)L of each forward and reverse primer (0.25 \(\mu\)M), 8 \(\mu\)L of Pyrogen free (PF) water and 2 \(\mu\)L of template DNA (25 ng/\(\mu\)L). The PCR reactions were performed on Applied Biosystems Thermocycler-2720. The RAPD-PCR amplification cycle consisted of the following specifications: 4 min at 94 °C, 1 min at 37 °C, 2 min at 72 °C, 44 cycles of 1 min at 94° C for, 1 min at 37 °C, 2 min at 72 °C and a final extension time of 10 min at 72 °C. The matK-PCR amplification cycle followed in the present study are: 4 min at 94 °C, 30 sec at 48 °C, 1 min at 72 °C, 34 cycles of 1 min at 94 °C, 30 sec at 48 °C, 1 min at 72 °C, and a final extension time of 7 min at 72 °C whereas 5 min at 95 °C, 45 sec at 54 °C, 2 min at 72 °C, 34 cycles of 45 sec at 95 °C, 45 sec at 54 °C, 2 min at 72 °C, and a final extension time of 7 min at 72 °C was considered for trnL-F region. The amplified products of RAPD, matK and TrnL-F were resolved on 1.8 % (w/v) agarose gel containing Ethidium bromide solution (0.5 \(\mu\)g/mL) run in 0.5 X TBE (Tris-borate-EDTA) buffer. The fragment size was estimated using 100 bp DNA ladder and \(\lambda\) DNA/Eco RI/Hind III double digest as molecular weight marker.

#### Sequencing and BLAST analysis

The PCR products were sequenced from Chromous Biotech Pvt. Ltd. Company (Bangalore, India) in both directions using matK R, matK F and Tab C, Tab F primers. The sequences were first aligned using CLUSTAL W method of Bio-Edit software. Subsequently, the aligned sequences were further deposited in NCBI GenBank as query sequences in nucleotide BLAST tool to obtain similarity level with respective species already present (sequences) in
GenBank. Finally, the authenticated sequences (inhouse) were submitted to the GenBank.

Data collection

Genome sequences of matK (17) and TrnL-F (15) region of Fabaceae were downloaded from NCBI (National Centre for Biotechnology Information) including one outgroup taxa from Cannabaceae.

Data analysis

In the present study, each polymorphic band was considered as a binary character and was scored as 1 (presence) or 0 (absence) for each sample and accumulated in a data matrix. Further, a similarity matrix was calculated on the basis of band-sharing from the binary data using Dice coefficient while a dendrogram of similarities was generated using the group average agglomerative clustering tool. The analysis was done using the software package NTSYSpc (version 2.0).

Phylogenetic analysis

The matK and TrnL-F region sequences of selected Mimosoids generated in the present study are as follows: matK region (accession no.)- LM643807, LM643808, LM643809; TrnL-F region (accession no.)- LM643811, LM643810, LM643812. The other reference sequences of matK and TrnL-F regions of different families or subfamilies (Mimosoideae, Caesalpinioideae, Papilionoideae and Canabaceae) were retrieved from GenBank and used to construct a phylogenetic tree by means of MEGA 4.0 software version with neighbour joining (NJ) and UPGMA methods after the proper alignment of DNA sequences using CLUSTAL W2 software. Parsimony analysis, various clades, transition/transversion (ns/nv) ratio and variability in different regions were also determined by MEGA 4.0.

Results and discussion

Comparative phytochemical screening and antioxidant potentiality of nine plant species of Mimosoids

Since PCs or secondary metabolites contribute a significant role towards the biological activities of medicinal plants, we performed a preliminary phytochemical screening of nine Mimosoids to justify their ethnobotanical claim significantly. Result exhibited the presence of tannins, flavonoids, alkaloids, saponins, phenolics and terpenoids in all cases (Table 1) attributing their antioxidant, anticancer, anti-mutagenic, antihyperglycemic, anti-inflammatory, immunomodulatory and tumor promotion inhibitory activity. This finding may lead in locating the source of pharmacologically active compound facilitating new drug discovery and thereby validates ethnomedicinal aspect of selected Mimosoids.

We further explored the antioxidant potential and therapeutic values of those plants using four different antioxidant assays namely DPPH, OH, NO and TAA. In the present investigation, free radical scavenging activity (DPPH) of A. catechu extract was found to exhibit higher (86.30±0.18 % at 100 µg/mL) inhibitory activity than the others followed by A. nilotica with 83.79±0.92 % of inhibition at 100 µg/mL (Fig. 1a). Moreover, we found that all the extracts revealed greater scavenging activities (p < 0.001) than the standard, ascorbic acid (27.93±1.10 % at 100 µg/mL) with lower IC50 (half maximal inhibitory concentration, it indicates how much of a particular substance is needed to inhibit the effectiveness of a given biological process by half) values (Table 2). In fact, all the nine Mimosoids exhibited higher DPPH quenching activity than many other studied plants, so far signifying their effective role as potent antioxidants and free radical scavengers. The underlying mechanism of this enhancing activity was probably due to the presence of electron or hydrogen donating capacity of extracts. Besides, hydroxyl radical (OH●), the most hazardous free radical causing severe damage to biomolecules found in living cells, was efficiently scavenged by all of the extracts (Fig. 1b) in a dose-dependent manner.

Table 1 — Comparative phytochemical screening of nine Mimosoids

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Plant Sample(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) = Presence, (-) = Absence
extract exhibited the highest OH\textsuperscript{*} scavenging capacity (56.70 ± 2.15 % at 100 µg/ml) among the nine studied species. Hence, the result justifies the OH\textsuperscript{*} quenching potential of all extracts considerably. Subsequently, Fig. 1c represented enhanced NO scavenging activity of all nine different Mimosoids. Of which, M5 extract revealed the highest inhibitory activity (63.04 ± 0.36 % at 100 µg/mL) with lower IC\textsubscript{50} value of 45.57 ± 1.3 µg/mL. indicating beneficial function of extracts as potent antioxidant which might be due to presence of phenolic compounds, that we have obtained in phytochemical investigation (Table 1). Eventually, total antioxidant activity (TAA) of nine extracts were further determined. Results demonstrated significant TAA quenching ability (p < 0.001) in all the studied extracts (Fig. 1d) highlighting their potent role against free radicals and thus the ethnomedicinal value of these species are established.

**Study of genetic diversity**

We further intended that whether the antioxidant activity of selected Mimosoids corroborates with genetic diversity or not. The genetic diversity study of selected Mimosoids was performed by means of RAPD and DNA barcoding analysis. Initially, 45 different decamer primers have been used to study the genetic diversity (RAPD analysis) of nine plant species under Mimosoideae\textsuperscript{24}. Out of the 45 primers screened, 23 (OPA 01-OPA 04, OPA 07, OPA 10-OPA 13, OPA 16-OPA 20, OPB 01, OPB 11-OPB 13, OPF 09, OPG 19, OPN 05, OPN 13 and OPN 19) resulted distinct and scorable bands ranging from 190 bp to 1763 bp. A total of 330 bands were generated of which all are polymorphic bands, no monomorphic bands were observed. Interestingly, the percentage of polymorphism was found to be 100 % and the number of polymorphic bands generated by each decamer primers ranged in between 2 (OPB 13)

<table>
<thead>
<tr>
<th>Extract</th>
<th>DPPH (µg/mL)</th>
<th>OH (µg/mL)</th>
<th>NO (µg/mL)</th>
<th>TAA (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>29.82±0.7</td>
<td>108.02±2.05</td>
<td>58.71±0.6</td>
<td>9.65±0.5</td>
</tr>
<tr>
<td>M2</td>
<td>37.67±1.2</td>
<td>114.43±4.2</td>
<td>52.57±1.9</td>
<td>9.32±0.1</td>
</tr>
<tr>
<td>M3</td>
<td>22.33±0.6</td>
<td>77.24±2.51</td>
<td>56.40±2.1</td>
<td>5.42±0.1</td>
</tr>
<tr>
<td>M4</td>
<td>37.87±1.7</td>
<td>91.35±3.1</td>
<td>63.67±0.7</td>
<td>5.90±0.0</td>
</tr>
<tr>
<td>M5</td>
<td>18.26±0.4</td>
<td>101.41±2.11</td>
<td>45.57±1.3</td>
<td>8.92±0.0</td>
</tr>
<tr>
<td>M6</td>
<td>34.04±0.7</td>
<td>104.41±3.53</td>
<td>52.14±0.3</td>
<td>5.38±0.2</td>
</tr>
<tr>
<td>M7</td>
<td>39.22±2.2</td>
<td>113.89±0.63</td>
<td>72.82±1.6</td>
<td>8.53±0.2</td>
</tr>
<tr>
<td>M8</td>
<td>43.50±1.8</td>
<td>120.87±1.46</td>
<td>77.29±1.5</td>
<td>9.73±0.1</td>
</tr>
<tr>
<td>M8</td>
<td>45.26±1</td>
<td>138.93±4.94</td>
<td>94.73±1.2</td>
<td>16.91±1.1</td>
</tr>
<tr>
<td>Stnd</td>
<td>266.88±29</td>
<td>512.77±48.1</td>
<td>95.26±4.8</td>
<td>81.42±4.7</td>
</tr>
</tbody>
</table>

Stnd=Standard, AA= Ascorbic acid, M= Mannitol, C=Curcumin.

![Fig. 1 — Free radical scavenging activity of nine different Mimosoids. (a). DPPH scavenging activity and standard ascorbic acid. (b). Hydroxyl radical quenching activity and standard mannitol. (c). Nitric oxide radical scavenging aptitude of nine Mimosoids and standard Curcumin. (d). Total antioxidant activity of different Mimosoids and standard ascorbic acid [Each value represents mean ±SD (n=6)]. Where, γ = p < 0.001, β = p < 0.01 and α = p < 0.05 Vs standard (µg/mL).](image-url)
and 20 (OPA 01). The RAPD profile of the nine accessions of Mimosoideae generated using primers OPA 01 and OPB 13 are depicted in Figs 2a & b. A similarity matrix was further drawn using Dice coefficient of similarity\(^21\) ranging from 0.528 to 0.867. The lowest similarity was observed between *Mimosa pudica* and *Albizia lebbeck*, while the highest value was recorded between *Acacia catechu* (*Senegalia catechu*) and *Acacia nilotica*. A dendrogram was constructed on the basis of the data obtained from RAPD analysis using NTSYSpc (Fig. 3). The dendrogram prepared from RAPD analysis revealed that the members of *Mimosa, Acacia, Albizia* and *Samanea* formed a group in which members of *Acacia* were found to form a loose sub-group. *Acacia nilotica* and *A. catechu* shared a node at 86.7 % whereas *A. concinna* exhibited a cluster with *A. nilotica* and *A. catechu* sharing a node at 84.9 % and 85.8 %, respectively. In addition, *Albizia lebbeck* appeared as a distinct outgroup in the dendrogram. Similar results were also documented by Sulain *et al.*\(^25\) using RAPD analysis where *M. pudica* was found to be closely related to *M. pigra* and *M. invisa*. Nanda *et al.*\(^26\) also reported the genetic relationships of six *Acacia* species using RAPD in which *A. farnesiana* and *A. catechu* were the closest members sharing with 30 % similarity whereas *A. auriculiformis* and *A. concinna* shared about 28 % and 18 % similarity, respectively, with the cluster formed by *A. farnesiana* and *A. catechu*. Hence, the RAPD markers exhibited the potentiality to conserve the identified clones as well as to characterize the genetic relatedness among the species of Mimosoideae. Interestingly, this genetic relatedness was also supported by the antioxidant profiling, examined in the present study. For instance, M1 (*M. pudica*) and M2 (*M. invisa*) exhibited similar kind of antioxidant activities (Table 2) out of four different antioxidant assays, thereby clubbed together as found in Fig. 3. Subsequently, all four *Acacia* species were
found to reveal quite comparable antioxidant activities corroborating with RAPD dendrogram. Antioxidant activities (Table 2) of two Albizia species and one Samanea species also evidenced as per the molecular screening observed in Fig. 3. Thus, we may infer that the antioxidant activities of selected Mimosoids probably played a distinct role in discriminating different taxa as found in molecular profiling.

Apart from RAPD analysis, DNA barcoding is another kind of taxonomic method that has become a rational approach for identifying million species of animals and plants, based on the analysis of short, standardized and universal DNA regions. Molecular documentation of different taxa and their validated systematic position in the respective family of plant kingdom had always been a challenging task. Chloroplast gene like matK and IGS region like TrnL-F could be pivotal to resolve this problem.

In the present study, we employed selected species (Figs 4a & b) to explore inter-generic and intra-generic differences between Mimosoideae, Caesalpinioideae, Papilionoideae and Canabaceae using matK and TrnL-F locus. The phylogenetic analysis (Figs 4a & b) of the matK and TrnL-F region revealed a close relationship among the selected taxa. Interestingly, Fig. 4a discloses that M. pudica and M. invisa share 99% similarity while Senegalia catechu syn. Acacia catechu and Acacia concinna share 96% similarity reflecting their close genetic relatedness as found in traditional classification.

Result also exhibited that a total of 2 major clades were formed; one of which consisted of Mimosoideae and Caesalpinioideae while another single one is Papilionoideae. Therefore, it can be attributed to the fact that all the selected taxa under the subfamily Mimosoideae and Caesalpinioideae were clubbed together and shared more similarities with each other.

![Fig. 4](image-url) — Most parsimonious tree (neighbour joining method) showing the relationship of matK region of 16 different taxa (a) and TrnL-F region of 14 different taxa (b) belonging from subfamilies, Mimosoideae, Papilionoideae and Caesalpinioidae within the family Fabaceae and one outgroup taxa from Cannabaceae. The tree indicates the phylogenetic evolution of different subfamilies Mimosoideae; Papilionoideae; Caesalpinioideae [Numbers at nodes indicate the bootstrap values; sequence numbers were provided within parentheses; *Present Study (inhouse sequence number submitted to GeneBank) and the rest of sequences were downloaded from NCBI].
(94 %) than the subfamily Papilionoideae (Fig. 4a). We also observed a similar trend in Fig. 4b that the seven species from Mimosoideae and one species from Caesalpinioideae grouped together forming the first clade whereas the remaining six genera of Papilionoideae clubbed together to make the second clade demonstrating their different place within the family. The present genetical approach through matK and TrnL-F clearly reflected that the members of Mimosoideae and Caesalpinioideae are much closer than the members from Papilionoideae validating the traditional classification. Hence, from the above illustration, we may conclude that DNA barcode serves a reliable genetical approach to place the morphologically similar or dissimilar or disputed taxa into its appropriate systematic position.

Conclusion
Since most of the members of the Mimosoideae have great medicinal importance in Indian traditional therapeutic system, no such remarkable comprehensive accounts of their different properties have been carried out so far. Hence, we designed the present study with an aim to explore the phytochemical, antioxidant and genetic diversities among nine ethnomedicinal plant species of Mimosoideae. The antioxidative properties of the plants have gained great interest due to the presence of certain phytochemical compounds. Among the nine extracts examined, Acacia catechu, A. nilotica, Mimosa pudica and M. invisa exhibited higher free radical scavenging activity than the rest, and can be used as a good source of natural antioxidants for health benefits. Further isolation of unknown bioactive compounds would be an appreciable one for pharmaceuticals. Additionally, DNA profiling in selected Mimosoideae clearly showed that it was possible to analyze the RAPD patterns for correlating their similarity and distance between species by which one could predict the origin of the species to a great extent. Further, the application of DNA barcode like matK and TrnL-F appeared more practical for defining the uniqueness and validation of the systematic position of species and taxa.

Acknowledgement
The work was supported by Department of Biotechnology, Govt. of West Bengal, India through grant no. 206/Bt(Estd.)/RD-22/2014 (AS). MRS acknowledges the receipt of BSR, UGC fellowship, India. PK is thankful to Department of Biotechnology, Govt. of India for fellowship.

Conflict of interest
The authors declare that they have no conflict of interest.

References
8. Saha MR, Kar P, Sen A & De Sarker D, Ethnobotany of Chanchal Block of Malda District of West Bengal (India); plants used in local healthcare, Plieone, 8(2) (2014b) 381-390.
Possible Implications in Alleviating Selected Cognitive Disorders, PloS one, 11(3) (2016) e0150574.

18 Prieto P, Pineda M & Aguilar M, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, Anal Biochem, 269(2) (1999) 337-341.


26 Nanda RM, Nayak S & Rout GR, Studies on genetic relatedness of Acacia tree species using RAPD markers, Biologia (Bratisl), 59(1) (2004) 115-120.