Research Article

Phytochemical evaluation and assessment of genetic diversity amongst *Artemisia* species collected from foothills of Himalayas

Aqsa Mir Alam¹, Khan Irfan Khan^{2,3*}, Amna Mustafa¹ and Zohaib Asad⁴

1. Department of Botany, University of Poonch. Rawalakot 12350, Azad Jammu and Kashmir-Pakistan

2. Department of Soil and Environmental Sciences, University of Poonch. Rawalakot 12350, Azad Jammu and Kashmir-Pakistan

3. Key lab of Environmental Engineering, Shaanxi Province, Xi'an University of Architecture and Technology, No. 13 Yanta Road, Xi'an 710055, PR-China

4. Department of Plant Pathology, PMAS- Arid Agriculture University Rawalpindi-Pakistan

*Corresponding author's email: irfankhan10711@gmail.com

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Abstract

Artemisia is a significant genus in family Asteraceae and has large number of taxa having economic and ecological importance. The aim of the study was to analyze highly medicinal products as well, antioxidant activity and genetic variability in three species of *Artemisia* from various sites of district Poonch. For the evaluation of phytochemicals and antioxidants, different volumes of extracts and solvents were used from leaves. The species were analyzed for genetic diversity within and between various *Artemisia* species. For molecular investigation, random amplification of polymorphic DNA (RAPD) fragment was used to determine genetic variations between and within species. From of ten primers only six primers gave amplified products. By using all six primers, total 331 bands were amplified from which 30 were monomorphic, 301 were polymorphic and percentage of polymorphism was 91% in *Artemisia japonica*, 321 bands were amplified in *Artemisia roxburghiana* from which 21 were monomorphic, 300 were polymorphic and percentage of polymorphism was 93% as well as 36 bands were amplified in *Artemisia absinthium* from which 14 were monomorphic, 22 were polymorphic and percentage of polymorphism was 61%. Based on obtained results, it was observed that a significant genetic diversity occurs among the species while little variation was present within the species. *Artemisia* species showed significant variation phytochemical such and phenols, flavonoid and antioxidant. Chemical derivatives of artemisinin and sesquiterpenoid showed high percentage in these species and also has medicinal properties such as anti-malarial and anti-cancerous agent.

Keywords: Antioxidants; *Artemisia*; Genetic Diversity; Phytochemical Activities; RAPD

Introduction

The *Artemisia*, in the family Asteraceae is one of the bigger genera and in the tribe Anthemideae the biggest genus, contains 200-500 taxa present at or sub areas [1] and

they are scattered in five sub genera [2]. *Artemisia* species are generally herbs, and bushes, with solid fragrance. These species are mostly found in roadsides, fields, waterless areas and on waste lands. The

species of *Artemisia* are located mostly in grassland atmosphere where direct precipitation is present [3]. Most species of *Artemisia* are perennial; biannual and annual species are only ten [4].

The entire plant is a fragrant tonic and formally appreciated a high value in debility of the digestive organs. It is additionally viewed as anthelmintic. Prior to the revelation of cinchona, it is to a great extent utilized irregular. It is recommended in type of a poultice or fomentation as a hostile to septic. By refining, it yields dull green or yellow oil, having a solid odor of the plant and harsh taste [5]. Basic oils obtained from the plant have a wide range of bioactivity, inferable from the nearness of a few dynamic ingredients or secondary metabolites, which work through different methods of activity [6]. Chemicals derived from plants are profitable hotspots for an assortment of pharmaceuticals, flavors, colors, oils, and gums. Larger part of the individuals from this family are essential and they have been utilized as a part of drug (*Artemisia annua*), nourishment (*Artemisia Japonica* and *Artemisia absinthium*), oils (*Artemisia roxberghiana* and *Artemisia absinthium*) and as ornamentals.

Antioxidant is mostly utilized for two distinct gatherings of substances; chemical and mechanical, which are added to items and avoid oxidation and common chemicals, found in Vegetables (some *Artemisia* species) fruits and other tissues of body, which are helpful for fitness [7]. In the genetic composition of species, genetic diversity is the quantitative aggregate of genetic characterization. Some time ago, morphological investigation and some of the time chemical and cytogenetic examination were utilized as a part of request to scrutinize the plant diversity [8]. It has additionally been very much recorded that geological condition influences the dynamic constituents of the therapeutic plants.

Extensive contrasts in substance of artemisinin and precursor of artemisinin were watched [9]. RAPD markers are used to observe chemotype variations of *Artemisia annua*.

Significance of RAPD markers in various breeding projects, current investigation was started for determination of genetic diversity between various *Artemisia* genotypes for future reproducing. SSRs have demonstrated more important in deciding genetic diversity and parentage since they are universal all through plant genomes, are exceedingly polymorphic, and are co-predominant. Random amplified polymorphic DNA has various focal points over other. RAPD are fast, can simply assessed and require low measure of DNA template. They are circulated irregularly all through the genome and have high genomic quality. RAPDs are leading markers and show restrictions for mapping, but RAPD is not as much reproducible that they are touchy to the response conditions thus high standardized strategies are required [10].

Here in this research study we calculate the phytochemical activity, antioxidants of three species of *Artemisia* (*Artemisia japonica*, *Artemisia roxberghiana*, *Artemisia absinthium*). Additionally, assess hereditary variability in three types of *Artemisia* and their phylogenetic relationship was also studied. Larger part of the individuals from this family are essential and they have been utilized as a part of drug (*Artemisia annua*), nourishment (*Artemisia Japonica* and *Artemisia absinthium*), oils (*Artemisia roxberghiana* and *Artemisia absinthium*) and as ornamentals. In disturb living spaces *Artemisia* plants also act as soil stabilizers. Few species are harmful or causing allergy while several are weeds which antagonistically influence crops. Sesquiterpene lactone (artemisinin) containing unordinary peroxide, disconnected from *Artemisia annua* L.

(Asteraceae).

Basic oils obtained from the species of *Artemisia* have an expansive range of bioactivity. The method of extraction utilized, from the sweet-smelling plants, basic oils contain an assortment of unpredictable particles, for example, terpenes, phenolic components with strong aroma and aliphatic segments [6]. In a further examination, from *Artemisia absinthium* a senescence-advancing substance was discovered. In Korea, wormwood (*A. absinthium*) is generally utilized as a flavor and pigment representative for cooking Korean rice cake. Two unpleasant substances (anabsinthine and absinthin), tannic thujone, and resinous substances like succinic and malic acid are also present in species of *Artemisia*. The plant extracts have appeared to repress solid antimicrobial movement [11].

Materials and Methods

Sample collection and identification

Plant materials (*Artemisia* species) were collected from different locations of district Poonch Azad Kashmir which is located at an average elevation of 1750-2500 meter from sea level. Collected species of *Artemisia* (*Artemisia roxberghiana*, *Artemisia japonica* and *Artemisia Absinthium*) were identified and confirmed in the herbarium lab of botany department, University of Poonch Rawalakot, AJK, in herbarium lab of NARC (National Agriculture Research Center) and Agriculture University of Faisalabad.

Preparation of plant extract for phytochemical analysis

For preparation of plant extracts fresh leaves of mentioned species of *Artemisia* were collected and washed thoroughly with distilled water to remove the dust particles and other contaminants. The leaves were air dried under shade for 2-3 weeks followed by grinded into powdered form. Grinded plant powder was soaked in the methanol and

chloroform at 1:1. Resultant extracts were filtered through filter paper following by drying in the water bath at temperature 40 °C until it became semi-liquid. These dried plant extracts were preserved for further phytochemical analysis.

Estimation of phenolic content

Estimation of phenolic content was performed by using Folin-Ciocalteu reagent technique [12]. Aliquots of different extracts (10, 100 and 1000 ppm) were mixed quickly in Folin-Ciocalteu reagent (0.75 mL, 10-fold diluted with d H₂O). The resultant blended product was left at room temperature for 5 min followed by addition of sodium carbonate (0.75 mL of 6 %). It was trailed by a 90 min of response time and thereafter absorbance values were recorded at 415 nm. The standard adjustment (0 µg/mL-25 µg/mL) curve was plotted by using Gallic acid as standard and aggregate phenolic content were expressed as mg Gallic acid equivalent (GAE/g) per gram weight of extract.

Evaluation of total flavonoids content

Aluminum chlorides colorimetric method [12] was adapted to measure total flavonoids content. Quickly, an aliquot of 0.5 mL of different extracts (10, 100, and 1000 ppm) was added to 1.5 mL of methanol, potassium acetate (1 M, 0.1 mL), aluminum chloride (0.1 mL, 10 %), and d H₂O (2.8 mL) followed by blending and incubation at room temperature for 30 min. After incubation periods absorbance value was recorded at 415nm by spectrophotometer. The adjustment curve (0 µg/mL-8 µg/mL) was plotted by utilizing quercetin as a reference. The aggregate flavonoid was measured as mg quercetin equivalent (QE/g) dry weight.

Evaluation of antioxidant assay

To evaluate the antioxidant plant extracts were set up in dimethyl sulfoxide (DMSO) with ascorbic acid as a positive control. Two diverse strategies were adopted to get better

result such as DPPH, total antioxidant assay and Reduce Power TRAP (total peroxyl radical trapping antioxidant parameter) Assay. Antioxidant activities of extracts were measured with DPPH assay.

i. Diphenyl-1-Picryl-Hydrazyl Radical (DPPH) Assay

In this measurement, the purple chromogen radical is reduced by antioxidant substances to the relating light-yellow hydrazine. The falling capacity of antioxidants towards DPPH can be assessed by observing the absorbance reduced at 515–528 nm until the

point that the absorbance stays stable in natural media. Officially detailed strategy was taken after for DPPH examine with a few adjustments [13]. Quickly, DPPH (8.98 mg/100 mL C₂H₅OH) solution was blended with 50 µL of each extract in Eppendorf tubes, vortex gently and kept in dark for one hour at 37 °C. Variation in absorbance was recorded at 517 nm with spectrophotometer. IC₅₀ value was computed by table curve technique and inhibition percentage was estimated by following equation.

$$\text{Percentage inhibition} = \left\{ \frac{\text{Absorption of control} - \text{Absorption of samples}}{\text{Absorption of control}} \right\} \times 100$$

ii. Total antioxidant capacity

Total antioxidant capacity was measured as the proportion of aggregate antioxidant capacity per total dissolved phenolics in micrograms and Trolox counterparts per milligram chlorogenic acid. The total antioxidant capacity gives data of the efficiency of phenolics to kill free radicals. A higher total antioxidant capacity implies phenolic mixes have a higher ability to settle free radicals.

iii. Reduce Power TRAP (total peroxyl radical trapping antioxidant parameter) Assay

Reduce Power TRAP (total peroxyl radical trapping antioxidant parameter) Assay method was used for the determination of reducing power TRAP (total peroxyl radical trapping antioxidant parameter) assay [14]. Extracts of *Artemisia* plants were prepared by treating with methanol. Different concentrations of *Artemisia* plant extracts (2500 µl) were mixed with 2500 µl of 2000 µmol sodium phosphate buffer pH 6.6 and 2500 µl of 1% potassium ferric cyanide. The mixture was incubated at 35°C for 15 min following by addition of trichloroacetic acid at 2500 µl of 10%. The mixture was centrifuged at 1000 rpm for 7 min. After centrifugation 500 µl of supernatant was

mixed with 500 µl distilled H₂O and 1000 µl of 0.1% of ferric chloride, followed by absorbance measurement at 515 nm. Higher absorbance indicates higher reducing power and vice versa. As a standard solution α-tocopherol was used.

Molecular characterization

DNA extraction

Genomic DNA was extracted from fresh leaves samples of three *Artemisia* species by CTAB (Cetyltrimethylammonium bromide) method [15] with few modifications. For extraction of DNA grinded 50-60 mg of plant leaves in 400 CTAB buffer by using mortar and pestle. Sap was transfer in 1.5 µL Eppendorf tube. Thereafter added 500 µL CTAB buffer again in Eppendorf tube and mixed gently. After mixing incubation was done at 55°C for one hour. Vortex the sample tubes after 30 minutes for few seconds. Following the incubation and vertexing placed the Eppendorf tubes in fume hood then added 500 µL Chloroform and mixed gently by inverted the tubes 4-5 times followed by Centrifugation at 10,000 rpm for 7 minutes. Transfer the supernatant into new Eppendorf tube gently with Pipette. Measured the volume of aqueous layer. After measuring added 28 µL cold 7.5 molar ammonium acetate and 204 µL of ice chilled

isopropanol and mixing was done gently by inverting Eppendorf tubes 25-30 times. Afterward Incubation was done for 30-40 minutes on ice followed by centrifugation at 13,000rpm for 3 minutes. Discard supernatant and added 70% EtOH (700 μ L) and mix gently. Then again Centrifugation was done at 13000rpm for 1 minute and discard supernatant. Again added 700 μ L 95% EtOH to wash the pellet and inverted the tubes for 5-10 times followed the Centrifugation for 1 minute at 13,000 rpm and discarded supernatant. In the final step invert tubes on dry tissue paper for air dry. In hydrated pellet added 50 μ L TE buffer and re-suspended over night at room temperature. Next day the DNA was used for further processes.

DNA quality confirmation and quantification

Quality of isolated DNA was checked by gel electrophoresis and bands were visualized under gel documentation system. Quantification of DNA was measured by taking absorbance at 260nm in UV through spectrophotometer. Conc. (μ g/ml) = $A_{260} \times 50 \times \text{Dilution factor}$ (one absorbance unit = 50 μ g/ml). To evaluate DNA purity, measure absorbance from 230nm to 320nm to detect other possible contaminants. The most common purity calculation is the ratio of the absorbance at 260nm divided by reading at 280nm. Good-quality DNA have an A_{260}/A_{280} ratio of 1.7-2.0.

PCR (Polymerase Chain Reaction) amplification

PCR reactions were done by utilizing 10 RAPD primers of OPC series (Table 1). Total volume of 25 μ L was used for PCR amplification. The amplification reaction mixture of 25 μ L for each sample contained 1 μ L of primer (forward and reverse) 10.5 μ L of nuclease free water, 1 μ L of DNA template and 12.5 μ L of Master Mix (MBI Fermentas). Initial denaturation for one min at 94° C followed by 44 cycles of denaturation at 94°

C for 30s, annealing at 38-40° C for 1min and Extension at 72° C for 2 min. Final extension was carried out at 72° C for 7 min. For annealing temperature different concentrations were tried for amplification utilizing gradient PCR and lastly a standard technique was applied. PCR products were analyzed by on a 1.5% agarose gel and viewed with a UV transilluminator after staining with ethidium bromide.

RAPD data scoring

RAPD data was scored as absence or presence of bands for every sample individually and polymorphic and monomorphic bands were checked. All visible bands between ~100-1000 bp were counted for each primer and absence or presence of bands was calculated as (0) if absent and (1) when present for every sample. The coefficient of similarity among *Artemisia* species were determined by [16] method.

Results

Evaluation of phenolic and flavonoid content

Diverse range of phenolic and flavonoid contents were present in the leaves of three species of *Artemisia* as shown in (Table 2).

Determination of antioxidant assay

In DPPH assay prepared plant extracts were treated with DPPH. Specific antioxidant capacity method was used to determine total antioxidant ratio. In Reduce Power TRAP assay plant extracts were treated with sodium phosphate buffer, potassium ferric cyanide, trichloroacetic acid and distilled water. Estimated results are shown in (Table 3).

Molecular characterization on the basis of ten RAPD primers

Molecular data indicated that high genetic variability was observed among *Artemisia* species. Ten RAPD Primers were used to evaluate the monomorphic and polymorphic properties of three species of *Artemisia*.

Evaluation of All Amplified Products in *Artemisia* species by using RAPD primer OPC-7

A total 331 bands were produced in *Artemisia* species from which 30 were monomorphic and 301 were polymorphic with a polymorphism of 91% except two samples collected from different areas of AJK such as Rawalakot, Banjoa and Toli peer etc. Average of amplified fragments per primer was 33.1 while an average of 9.1 was observed in polymorphic fragments per primer. It was also observed that the ratio of polymorphic bands per whole number of amplified bands varied from 88 (OPC-7) to 28 (OPC-2) with an average of 30.1 shown in (Table 4). The PCR product gel documentation picture showing all bands by using primer OPC-7 are shown in (Fig. 1, 2, 3).

Evaluation of All Amplified Products in *Artemisia* species by using RAPD primer OPC-4

The sums of 321 bands were produced in *Artemisia* species from which 21 were monomorphic and 300 were polymorphic with a polymorphism of 93% except five samples collected from different areas of AJK such as Rawalakot, Banjoa and Toli peer etc. Average of amplified fragments per primer was 32.1 while an average of 9.3 was observed in polymorphic fragments per primer. It was also observed that the ratio of polymorphic bands per whole number of amplified bands varied from 77 (OPC-4) to 18 (OPC-1) with an average of 30 shown in (Table 5). The PCR product gel documentation picture showing all bands by using primer OPC-7 are shown in (Fig. 4, 5, 6).

Table 1. RAPD primers, their names and sequence used in this study

S. No.	Primer Code	Sequence	Bp
I	OPC-1	TTCGAGCCAG	10
II	OPC-2	GTGAGGCGTC	10
III	OPC-3	GGGGGTCTTT	10
IV	OPC-4	CCGCATCTAC	10
V	OPC-5	GATGACCGCC	10
VI	OPC-6	GAACGGACTC	10
VII	OPC-7	GTCCCACGA	10
VIII	OPC-8	TGGACCGGTG	10
IX	OPC-9	CTCACCTCG	10
X	OPC-10	TGTCTGGGTG	10

Table 2. Quantitative analysis of phytochemicals

Specie name	Plant part used	Phenolic contents	Flavonoid contents
<i>Artemisia japonica</i>	Leaves	1.4236±1.504	1.6649±0.6639
<i>Artemisia roxburghiana</i>	Leaves	1.4793±0.234	0.1575±0.1157
<i>Artemisia absinthium</i>	Leaves	1.7245±0.478	0.0300±0.0293

Table 3. Quantitative analysis of Antioxidants of *Artemisia* species

Species names	plant part used	Antioxidants		
		DPPH	Specific antioxidant assay	TRAP
<i>Artemisia japonica</i>	Leaves	0.60075±0.1785	1.0648±1.369	0.5618±0.5585
<i>Artemisia roxberghiana</i>	Leaves	0.6118±0.1148	1.764±0.1955	0.3126±0.0664
<i>Artemisia absinthium</i>	Leaves	0.4268±0.0338	2.0615±0.477	0.2121±0.213

Table 4. List of primers, size of amplified products, monomorphic and polymorphic bands in *Artemisia japonica* species

Sr. No	Primers	Bands size (bp)	Polymorphic bands	Monomorphic bands	Total bands	Percentage polymorphism
1	OPC_4	360-1600	77	0	77	100
2	OPC_2	330-2300	28	1	29	97
3	OPC_7	300-1600	88	0	88	100(0)
4	OPC_8	200-1200	61	1	62	98
5	OPC_9	700-1500	47	1	48	98
6	OPC_1	300-1300	0	8(0)	8	0
7	OPC_5	1100-1700	0	5(0)	5	0
8	OPC_6	600-1200	0	6(0)	6	0
9	OPC_10	350-1500	0	5(0)	5	0
10	OPC_3	400-1200	0	3(0)	3	0
Total			301	30	331	91
Mean			30.1	3	33.1	9.1

Figure. 1. RAPD profile of twelve site of *Artemisia japonica* species through OPC-7

Figure 2. RAPD profile of twelve site of *Artemisia roxburghiana* species through OPC-7

Figure 3. RAPD profile of twelve site of *Artemisia absinthium* species through OPC-7

Table 5. List of primers, size of amplified products, monomorphic and polymorphic bands in *Artemisia* species

Sr. No	Primers	Band size (bp)	Polymorphic bands	Monomorphic bands	Total bands	Percentage Polymorphism
1	OPC_4	360-1600	77	0	77	100
2	OPC_2	330-2300	70	1	71	98
3	OPC_7	300-1600	59	0	59	100
4	OPC_8	200-1200	39	1	40	98
5	OPC_9	700-1500	37	0	37	100(0)
6	OPC_1	300-1300	18	3	21	86
7	OPC_5	1100-1700	0	4(0)	4	0
8	OPC_6	600-1200	0	5(0)	5	0
9	OPC_10	350-1500	0	3(0)	3	0
10	OPC_3	400-1200	0	4(0)	4	0
Total			300	21	321	93
Mean			30	2.1	32.1	9.3

Figure 4. RAPD profile of twelve site of *Artemisia japonica* species through OPC-4

Figure 5. RAPD profile of twelve site of *Artemisia roxburghiana* species through OPC-4

Figure 6. RAPD profile of twelve site of *Artemisia absinthium* species through OPC-4

Evaluation of all amplified products in *Artemisia* species by using RAPD primer OPC-2

The total of 36 bands were produced in *Artemisia* species from which 14 were monomorphic and 22 were polymorphic with a of 61% except five samples collected from different areas of AJK such as Rawalakot, Banjoa and Toli peer etc. Average of amplified fragments per primer was 3.6 while an average of 6.1 was observed in polymorphic bands per primer. It was also observed that the ratio of polymorphic bands per whole number of amplified bands varied from 7 (OPC-4 and OPC-7) to 2 (OPC-2 and OPC-1) with an average of 2.2 shown in (Table 6). The PCR product gel documentation picture showing all bands by using primer OPC-7 are shown in (Fig. 7, 8, 9).

Coefficient of similarity

Development of similarity coefficient depends upon the amplified bands of different *Artemisia* genotypes using six RAPD primer because these shows much more accuracy/ purity of DNA out of ten primers. According to Nei's Lei's method similarity coefficient matrix was calculated to estimate genetic similarity and diversity among different *Artemisia* genotypes. The genetic similarities values among different *Artemisia* species varied from 0.30 to 0.85. Maximum genetic similarity was observed between *Artemisia absinthium*, *Artemisia roxberghiana* and *Artemisia japonica* as shown in (Table 7).

Polymorphic information content

The PIC (polymorphic information content) was performed through software Power Marker V.3.25. PIC values were calculated as: $PIC = 1 - \sum P_{ij}^2$ whereas P_{ij} is frequency of i th allele in j th population for each locus [17]. Genetic diversity, PIC, major allele

frequency and number of alleles amplified for each RAPD primer were determined as given in the (Table 8). Minimum number of amplified alleles was 4 while maximum number of amplified alleles was 5. Total number of amplified alleles was 36.7 with mean value of 3.7 alleles per primer. Each of the primers OPC-4, OPC-2, OPC-7, OPC-8 and OPC-9 amplified maximum number 5 of alleles. However, each of the OPC-1 and OPC-2 has amplified 5 alleles. Major allele frequency varied from 0.3 (OPC-1, OPC-5 and OPC-6) to 0.5 (OPC-2) with an average of 0.3. Maximum gene diversity value (0.71) was found for primers OPC-8, OPC4- and OPC-7 and minimum gene diversity calculated was 0.65 for OPC-2. However, average gene diversity was found to be 0.71. PIC value for different primers varied from 0.040 (OPC-7) to 0.73 (OPC-5) with a mean of 0.61.

Principle component and variance analysis

PCA analysis shown that all *Artemisia* species can be sub divided into four aggregates. The first group comprise of plants of *Artemisia japonica*, group second comprise of plants of *Artemisia roxberghiana* while third one consists *Artemisia absinthium* plants. The fourth group consists of plants of *A. japonica*, *A. roxberghiana* and *A. absinthium*. PAC analysis showed that there is genetic dissimilarity among plants of different *Artemisia* species and a close relationship in the plants of same *Artemisia* species. Variance analysis for *Artemisia* plants analyzed in terms of RAPD amplified fragments. Low values of variance were obtained in same *Artemisia* species but, high significant values of variance were obtained for different *Artemisia* species.

Table 6. List of primers, size of amplified products, monomorphic and polymorphic bands in *Artemisia absinthium* species

Sr. No	Primers	Band size (bp)	Polymorphic bands	Monomorphic bands	Total bands	Percentage Polymorphism
1	OPC_4	360-1600	7	1	8	88
2	OPC_2	330-2300	2	2	4	50
3	OPC_7	300-1600	4	2	6	67
4	OPC_8	200-1200	7	0	7	100(0)
5	OPC_9	700-1500	0	3	3	0
6	OPC_1	300-1300	2	2	4	50
7	OPC_5	1100-1700	0	2(0)	2	0
8	OPC_6	600-1200	0	0(0)	0	0
9	OPC_10	350-1500	0	1(0)	1	0
10	OPC_3	400-1200	0	1(0)	1	0
Total			22	14	36	61
Mean			2.2	1.4	3.6	6.1

Figure 7. RAPD profile of twelve site of *Artemisia japonica* species through OPC-2

Figure 8. RAPD profile of twelve site of *Artemisia roxburghiana* species through OPC-2

Figure 9. RAPD profile of twelve site of *Artemisia absinthium* species through OPC-2

Table 7. The similarity coefficient of different *Artemisia* species

G	1	2	3	4	5	6	7	8	9	10
1	1	1								
2	0.76	2	1							
3	0.30	3	0.51	1						
4	0.62	4	0.6	0.69	1					
5	0.69	5	0.58	0.44	0.67	1				
6	0.67	6	0.64	0.51	0.64	0.67	1			
7	0.51	7	0.53	0.53	0.67	0.64	0.67	1		
8	0.67	8	0.64	0.6	0.69	0.8	0.69	0.71	1	
9	0.76	9	0.73	0.47	0.73	0.8	0.64	0.62	0.78	1
10	0.51	10	0.53	0.62	0.85	0.6	0.67	0.73	0.71	0.71

Table 8. Primers, major allele frequency, allele number, gene diversity and polymorphic information contents

Primer	Major allele frequency	Allele number	Gene diversity	PIC
OPC-4	0.4	5	0.65	0.63
OPC-2	0.5	5	0.55	0.60
OPC-7	0.5	4	0.78	0.040
OPC-8	0.4	4	0.66	0.73
OPC-9	0.5	4	0.73	0.69
OPC-1	0.4	4	0.65	0.73
OPC-5	0.4	5	0.74	0.73
OPC-6	0.3	5	0.65	0.72
OPC-10	0.4	4	0.73	0.71
OPC-3	0.3	4	0.66	0.69
Mean	0.3	4	0.71	0.61

Discussion

In phytochemical analysis, flavonoids and phenols were studied as well as antioxidant

activity of various *Artemisia* species by different methods. The phytochemical analysis in flavonoids were present

1.6649±0.6639 in *Artemisia japonica*, 0.1575±0.1157 in *Artemisia roxberghiana*, 0.0300±0.0293 in *Artemisia absinthium* while in phenols varying from 1.4236±1.504 in *Artemisia japonica*, 1.4793±0.234 in *Artemisia roxberghiana* and 1.7245±0.478 in *Artemisia absinthium*. Quantitative analysis of phytochemical showed that large quantity of flavonoids and phenolic compounds were present in *Artemisia japonica* and *Artemisia roxberghiana* as compared to *Artemisia absinthium*.

Phenotypic and genetic diversity is essential for preservation, evaluation and improvement of hereditary properties. It is additionally useful to decide hereditary composition of genotypes and to examine diversity. For choice reason, high genetic diversity, recognizable proof of different ancestral combinations and production of segregation offspring is significant. Examination of *Artemisia* genomic diversity is critical to distinguish parental arrangements as well as produce offspring having large genomic diversity [18].

The molecular marker RAPD is utilized effectively to determine genetic diversity inside germplasm of different yields by DNA profiling [19]. RAPD markers are dominant, utilized for traits identification [20] and chromosomal mapping. RAPD have been utilized for discovering differences in different yields [21, 22]. In RAPD analysis from out of ten only six OPC series primers gave countable and reproducible amplified bands. The sums of 331 bands were produced in *Artemisia japonica* from which 30 were monomorphic and 301 were polymorphic with a polymorphism of 91%. Average of amplified fragments per primer was 33.1 while an average of 9.1 was observed in polymorphic fragments per primer. The sums of 321 bands were produced in *Artemisia roxberghiana* from which 21 were monomorphic and 300 were polymorphic

with a polymorphism of 93%. Average of amplified fragments per primer was 32.1 while an average of 9.3 was observed in polymorphic fragments per primer. The sums of 36 bands were produced in *Artemisia absinthium* from which 14 were monomorphic and 22 were polymorphic with a polymorphism of 61%. Average of amplified fragments per primer was 3.6 while an average of 6.1 was observed in polymorphic bands per primer. It was also observed that the ratio of number of polymorphic bands per total number of amplified bands ranged from 77 (OPC-4) to 21 (OPC-1).

In the literature reported a low degree of polymorphism of 88 % [23] and is a high degree of polymorphism according to [24] 93.5 %. [25, 26] revealed polymorphism of low level showing 46.67 % and 46.97 % correspondingly. Our study showing polymorphism percentage 91, 93 and 61 in different plants of *Artemisia japonica*, *Artemisia roxberghiana* and *Artemisia absinthium* respectively. Furthermore, the reproducibility of RAPD profile may be affected by various factors, including quality and quantity template, primer sequence polymerase concentration, and type of thermocycler utilized [19]. It shows variations in results; however, the RAPD reproducibility can be improved by a standardized RAPD protocol.

In our study, an average of amplified fragments per primer was 33.1 whereas per primer polymorphic bands were shown an average of 30.1. In previous study, [27] reported an average number of 21.35 polymorphic fragments per primer. It was also observed that the ratio of polymorphic bands per whole number of amplified bands varied from 88 (OPC-7) to 28 (OPC-2) with an average of 30.1. It was also observed that the ratio of polymorphic bands per whole number of amplified bands varied from 77 (OPC-4) to 18 (OPC-1) with an average of

30. It was also observed that the ratio of polymorphic bands per whole number of amplified bands varied from 7 (OPC-4 and OPC-7) to 2 (OPC-2 and OPC-1) with an average of 2.2. These results revealed that RAPD markers are useful to collect information for identification and selection of *Artemisia japonica*.

In our study development of similarity coefficient depends upon the amplified bands of different *Artemisia* genotypes using six RAPD primers. According to Nei's Lei's method [16] similarity coefficient matrix was calculated to estimate genetic similarity and diversity among different *Artemisia* genotypes. The genetic similarities values among different *Artemisia* species varied from 0.30 to 0.85. Maximum genetic similarity was observed between *Artemisia absinthium* and maximum genetic dissimilarity in *Artemisia roxberghiana* and *Artemisia japonica*.

PCA analysis shown that all *Artemisia* species can be sub divided into four aggregates. The group first comprise of plants of *Artemisia japonica*, group second comprise of plants of *Artemisia roxberghiana* while third one consists *Artemisia absinthium* plants. The fourth group consists of plants of *A. japonica*, *A. roxberghiana* and *A. absinthium*. PAC analysis showed that genetic dissimilarity among plants of different *Artemisia* species and a close relationship in the plants of same *Artemisia* species.

Variance analysis for *Artemisia* plants analyzed in terms of RAPD amplified fragments. There are low values of variance were obtained in same *Artemisia* species but, high significant values of variance were obtained for different *Artemisia* species. According to our study, the qualitative and quantitative analysis showed that significant genetic dissimilarity is present among different *Artemisia* species and a maximum similarity occur within the species.

Conclusion

Our study focused on phytochemicals, antioxidants and genetic variation present in *Artemisia* species. The phytochemical analysis showed that flavonoids and phenols were present in large amount in leaves of *Artemisia* species as well as *Artemisia* having a maximum antioxidant capacity. In the results of genetic diversity, RAPD markers are useful in germplasm characterization to develop and improve variety of *Artemisia* species. According to the present investigation RAPD analysis has been effectively utilized for assessment of genetic diversity between *Artemisia* species. RAPD based analysis grouped *Artemisia* species into four main groups based on similarity and dissimilarity among and within species. The genotypes of different *Artemisia* species were observed to be more different and the genotypes of plants of one *Artemisia* species are more similar. Our study shows that there is great genetic diversity among *Artemisia* species as compared to within species. Among the species genetic relatedness is useful in collection of genetically divergent plants in germplasm enhancement.

Authors' contributions

Conceived and designed the experiments: AM Alam, Performed the experiments: AM Alam & A Mustafa, Analyzed the data: Z Asad, Contributed materials/ analysis/ tools: KI Khan, Wrote the paper: KI Khan.

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