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*Research Article*

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# Genetic diversity studies in twelve accessions of *Hemidesmus indicus* (L.) R. BR. by RAPD analysis

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**ABSTRACT**

*Hemidesmus indicus is an important medicinal plant which is widely used in traditional and modern medicinal systems. In order to identify the genetic similarity and diversity among the collected accessions of H. indicus, Randomly Amplified Polymorphic DNA(RAPD) analysis was carried out. A total of 12 accessions growing in the Botanical garden of Department of Botany, Osmania University have been taken up for the study and the RAPD markers have been identified for them.The dendrogram was generated by UPGMA whichshowsa wide range of variability among the accessions.The maximum genetic similarity among the collected species was 64% which was calculated by Jaccard's coefficient. These results could be very useful to study and manage the genetic resources of this important medicinal plant.*

***Keywords****: Hemidesmusindicus, accessions, RAPD, dendrogram, genetic similarity.*

## INTRODUCTION

*Hemidesmus indicus* R. Br. belongs to family *Asclepiadaceae*. It is known as Indian Sarasaparilla or Anantmul, and is a well-known plant in Ayurvedic system of medicine. The plant is a perennial climber and distributed throughout India in plains and low hills[1] and growing widely in upper Gangetic plains and Eastwards of Bengal and from Central to South India[2]. *H. indicus* is an important plant particularly due to its use to make beverages[3] and also in traditional medicine[4].

Genetic variation has implications for the conservation the species level. These molecular markers can characterize plants with greater precision than the biochemical parameters[5]. Among these, Random Amplified Polymorphic DNA (RAPD) markers are efficient to assess genetic variation and have been used extensively to evaluate natural genetic diversity in plant populations[6,7,8].

Due to its procedural simplicity, the use of RAPD as molecular marker for taxonomic and systematic analyses of plants[9], as well as in plant breeding and the study of genetic relationships, has considerably increased[10]. It is informative and fast for the assessment of population structure, genetic diversity and phylogenetic analysis. The RAPD molecular markers can characterize plants with greater precision than biochemical parameters[5]. The RAPD markers are efficient to assess genetic variation and have been used extensively to evaluate natural genetic diversity in plant populations[7]. The present study on *H. indicus* has been taken up to study the genetic diversity among the collected germplasm.

## MATERIAL AND METHODS

### Molecular analysis of the germplasm for study of genetic diversity by RAPD

The twelve accessions of *H. indicus* collected from different places were raised in the Botanical garden, Department of Botany, Osmania University (Table-1). After acclimatization and growth for one year, these were used in the genetic diversity studies through Randomly Amplified Polymorphic DNA (RAPD) analysis.

The RAPD analysis was taken up from the DNA isolated from young leaves of the accessions. The technique comprised the isolation of DNA, qualitative and quantitative analysis of DNA by electrophoresis, followed by the polymerase chain reaction (PCR) by employing random primers.

The DNA isolation was carried out according to the protocol of Doyle and Doyle[11]. The leaves were ground in the extraction buffer and after homogenization treated with RNase and precipitated. It was washed with 70% ethanol and dried. The DNA was dissolved in 100ml of Tris EDTA. The purity was checked by electrophoresis and by spectrophotometric analysis. The concentration of DNA was adjusted to 50 ng/µl. Twentyrandom primerswere used for PCR. The primers were OPJ - 1, OPJ – 2, OPJ – 3, OPJ – 4, OPJ – 5, OPB – 6, OPB – 7, OPB – 8, OPB – 9, OPB – 10, OPB – 11, OPC – 1, OPC – 5, OPC – 6, OPC – 7, OPC – 8, OPC – 9, OPD – 1, OPD – 2 and OPD – 3.

**The PCR conditions followed were:** Initial denaturation at 94$^{o}$C for 5minutes, denaturation at94$^{o}$C for 1 minute, annealing at 35$^{o}$C for 1 minute, extension at 72$^{o}$C for 2 minutes followed by the final extension at 72$^{o}$C 10 minutesfor 40 cycles.

### RAPD data analysis

The amplified fragments were scored as 1 for present and 0 for absent. The binomial data generated was used to estimate level of polymorphisms by dividing the polymer bands by the total number of scored bands. The RAPD Data of 12 accessions of *H. indicus* was analyzed using CERVUS (3.03 version). Dendrogram was constructed by UPGMA (Unweighted Pair Group Method with Arithmetic mean) method to measure the resulting phenotypic groups. Pairwise comparision between accessions based on the proportion of shared bands produced by the primers used, were calculated using Jaccard's similarity matrix.

## RESULTS AND DISCUSSION

In the present study of 12 accessions of *H. indicus* were characterizedby RAPD analysis to find the genetic relationship among them. The DNA from 12 samples was checked for quality by electrophoresis (Fig-1). Total of twenty random primers were used for DNA amplification. Among these, four primers (OPB- 06, OPB- 9, OPC -01, OPC – 07 and OPJ – 15) showed good amplification. Nine primers (OPB - 07, OPB – 08, OPB – 11, OPC – 05, OPC – 06, OPC –09, OPD – 03, OPJ – 15) showed medium amplification.

In the present study, RAPD primers generated good number of polymorphic bands and the polymorphism was clearly distributed.Good quality genetic profiling was obtained (Table-2).

### Polymorphism

In the present study of 12 accessions of *H. indicus*, a total number of 88 bands appeared in which 8 bands were polymorphic bands and 5 monomorphic bands (Table-2). The total number of bands in each accession ranged from 4-8. The maximum number of 8 bands were obtained with four primers (OPB – 06, OPB – 07, OPC – 06, OPC – 07). Hence, these were presented (Fig 2-5). Among the 8 bands produced by OPB - 06 primer, 2 were polymorphic and 1 was monomorphic band, in case of OPB-07 1 polymorphic and 1 monomorphic band, in case of OPC-06 no polymorphic or monomorphic bands were seen and with OPC-07 primer only 2 polymorphic bands and no monomorphic bands were observed. (Fig. 2,3,4,5 and Table - 2). The highest percentage (25%) of polymorphism was recorded with three primers (OPB-06, OPB-9,OPC-07). 20% of polymorphism appeared with OPC-01 followed by 16.6% of polymorphism with OPJ-15. The lowest percentage (12.5%) of polymorphism was shown in OPB-07 (Table-2).

**Polymorphic information content (PIC)**

The polymorphism level of markers is compared by polymorphic information content. The PIC is recorded as the mean which was calculated from frequency of polymorphic bands among all the genotypes. The PIC values of 12 accessions of *H. indicus* ranged between 0.0046 and 0.128. The highest PIC value was obtained in OPB-11 as 0.128 followed by 0.112 in OPC-01 and 0.101 in OPB-08. The lowest PIC value (0.046) was recorded in OPJ-01 (Table-2).

**Cluster analysis**

The genetic similarity matrix were prepared on the basis of all amplified products of 12 accessions of *H. indicus* with 20 primers, with the help of dendrogram which was generated by UPGMA cluster analysis of Jaccard's similarity coefficient. It showed two clusters with a wide range of variability among 12 accessions. Cluster A consists of 10 accessions (HCU-1,HCU-2,MK-1,MK-2,WGL-1,WGL-2,APMPB-2,PP-1,PP-2,OU-1) and cluster B consists of 2 accessions (OU- 2 and APMHPB- 1) (Fig- 6).

**Genetic similarity**

The genetic similarity among the twelve accessions of *H. indicus* has shown the range between 20%-64%. Maximum similarity (64%) was observed among the accessions HCU1 and HCU2 which was calculated by Jaccard's coefficient (Table-3). The genetic distance between the 12 accessions ranged from 0.53 to 0.819. The genetic distance was calculated by Jaccard's distance coefficient (Table-4). Among the 12 accessions the highest genetic distance was identified between APMHPB (0.81) and PP-2 (0.53). Further, the accessions could be identified clearly with the help of the RAPD markers.

A single report on RAPD analysis of *H. indicus* is available along with its substituted plants which revealed unique fingerprint (band) pattern in both[12].However, the results agreed with our earlier studies in Jatropha wherein, several RAPD primers were employed[13] and in *Aristolochia indica*, where five RAPD primers were used to analyze the genetic variability in different populations[14].

**Table. 1: Particulars of twelve accessions of *Hemidesmus indicus* collected from different places**

| S. No. | Accession number / code | Collected place & District |
|---|---|---|
| 1 | PP-1 | Parvathapuram, Ranga Reddy |
| 2 | PP-2 | Parvathapuram, Ranga Reddy |
| 3 | OU-1 | OsmaniaUniversity Campus |
| 4 | OU-2 | OsmaniaUniversity Campus |
| 5 | HCU-1 | University of Hyderabad Campus |
| 6 | HCU-2 | University of Hyderabad Campus |
| 7 | WGL-1 | Mahboobabad, Warangal |
| 8 | WGL-2 | Mahboobabad, Warangal |
| 9 | MKR-1 | Mothkur, Nalgonda |
| 10 | MKR-2 | Mothkur, Nalgonda |
| 11 | APMPB-1 | Andhra Pradesh Medicinal Plants Board, Hyderabad |
| 12 | APMPB-2 | Andhra Pradesh Medicinal Plants Board, Hyderabad |

**Table. 2: List of amplified primers used for RAPD analysis, unamplified primers, total no. of bands, polymorphic and monomorphic bands and polymorphism information content of 12 accessions of *Hemidesmus indicus***

| primer | Sequence | GC (%) content | Maximum no. of bands | Total no. of bands | Polymorphic bands | Monomorphic bands | Percentage polumorphism | PIC | Range of molecular size (bp) |
|---|---|---|---|---|---|---|---|---|---|
| OPB -06 | TGCTCTGCCC | 70 | 8 | 78 | 2 | 1 | 25 | 0.081 | 200-2000 |
| OPB-07 | GGTGACGCAG | 70 | 8 | 40 | 1 | 1 | 12.5 | 0.056 | 200-2000 |
| OPB-08 | GTCCACACGG | 70 | 5 | 21 | 0 | 1 | 0 | 0.101 | 400-1500 |
| OPB-09 | TGGGGGACTC | 70 | 4 | 19 | 1 | 1 | 25 | 0.092 | 250-2000 |
| OPB-11 | GTAGACCCGT | 60 | 7 | 64 | 0 | 1 | 0 | 0.128 | 200-2500 |
| OPC-01 | TTCGAGCCAG | 60 | 5 | 27 | 1 | 0 | 20 | 0.112 | 250-1500 |
| OPC-05 | GATGACCGCC | 70 | 7 | 24 | 0 | 0 | 0 | 0.076 | 400-1500 |
| OPC-06 | GAACGGACTC | 60 | 8 | 45 | 0 | 0 | 0 | 0.068 | 500-1300 |
| OPC-07 | GTCCCGACGA | 70 | 8 | 49 | 2 | 0 | 25 | 0.094 | 500-1500 |
| OPC-09 | CTCACCGTCC | 70 | 5 | 53 | 0 | 0 | 0 | 0.057 | 475-1500 |
| OPD-03 | GTCGCCGTCA | 70 | 4 | 20 | 0 | 0 | 0 | 0.069 | 750-1500 |
| OPJ-01 | CCCGGCATAA | 60 | 6 | 40 | 0 | 0 | 0 | 0.046 | 200-2000 |
| OPJ-05 | CTCCATGGGG | 70 | 7 | 35 | 0 | 0 | 0 | 0.059 | 300-2000 |
| OPJ-15 | TGTAGCAGGG | 60 | 6 | 38 | 1 | 0 | 16.6 | 0.089 | 200-1500 |
| TOTAL | | | 88 | 553 | 8 | 5 | | | |

**Table. 3:Similarity matrix calculated by Jaccard's coefficient of 12 *Hemidesmusindicus* accessions**

| | HCU1 | HCU2 | MK1 | MK2 | OU1 | OU2 | WGL1 | WGL2 | APMHPB1 | APMHPB2 | PP1 | PP2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HCU1** | **1** | **0.634** | **0.513** | **0.471** | **0.291** | **0.329** | **0.383** | **0.378** | **0.181** | **0.244** | **0.383** | **0.31** |
| **HCU2** | | **1** | **0.529** | **0.462** | **0.392** | **0.271** | **0.405** | **0.364** | **0.242** | **0.222** | **0.333** | **0.309** |
| **MK1** | | | **1** | **0.469** | **0.36** | **0.294** | **0.431** | **0.405** | **0.188** | **0.289** | **0.411** | **0.329** |
| **MK2** | | | | **1** | **0.286** | **0.31** | **0.4** | **0.314** | **0.255** | **0.229** | **0.338** | **0.348** |
| **OU1** | | | | | **1** | **0.273** | **0.303** | **0.22** | **0.22** | **0.343** | **0.222** | **0.263** |
| **OU2** | | | | | | **1** | **0.269** | **0.344** | **0.289** | **0.25** | **0.269** | **0.225** |
| **WGL1** | | | | | | | **1** | **0.443** | **0.327** | **0.267** | **0.37** | **0.378** |
| **WGL2** | | | | | | | | **1** | **0.233** | **0.333** | **0.443** | **0.373** |
| **APMHPB1** | | | | | | | | | **1** | **0.259** | **0.327** | **0.23** |
| **APMHPB2** | | | | | | | | | | **1** | **0.439** | **0.47** |
| **PP1** | | | | | | | | | | | **1** | **0.545** |
| **PP2** | | | | | | | | | | | | **1** |

**Table. 4:Distance matrix calculated by Jaccard's coefficient of 12 *Hemidesmusindicus* accessions**

| | HCU1 | HCU2 | MK1 | MK2 | OU1 | OU2 | WGL1 | WGL2 | APMHPB1 | APMHPB2 | PP1 | PP2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **HCU1** | **0** | **0.366** | **0.487** | **0.529** | **0.709** | **0.671** | **0.617** | **0.622** | **0.819** | **0.756** | **0.617** | **0.69** |
| **HCU2** | | **0** | **0.471** | **0.538** | **0.608** | **0.729** | **0.595** | **0.636** | **0.758** | **0.778** | **0.667** | **0.691** |
| **MK1** | | | **0** | **0.531** | **0.64** | **0.706** | **0.569** | **0.595** | **0.812** | **0.711** | **0.589** | **0.671** |
| **MK2** | | | | **0** | **0.714** | **0.69** | **0.6** | **0.686** | **0.745** | **0.771** | **0.662** | **0.652** |
| **OU1** | | | | | **0** | **0.727** | **0.697** | **0.78** | **0.78** | **0.657** | **0.778** | **0.738** |
| **OU2** | | | | | | **0** | **0.731** | **0.656** | **0.711** | **0.75** | **0.731** | **0.775** |
| **WGL1** | | | | | | | **0** | **0.557** | **0.673** | **0.733** | **0.63** | **0.622** |
| **WGL2** | | | | | | | | **0** | **0.767** | **0.667** | **0.557** | **0.627** |
| **APMHPB1** | | | | | | | | | **0** | **0.741** | **0.673** | **0.77** |
| **APMHPB2** | | | | | | | | | | **0** | **0.561** | **0.53** |
| **PP1** | | | | | | | | | | | **0** | **0.455** |
| **PP2** | | | | | | | | | | | | **0** |

**Fig. 1: Extracted DNA from 12 accessions of *Hemidesmus indicus***

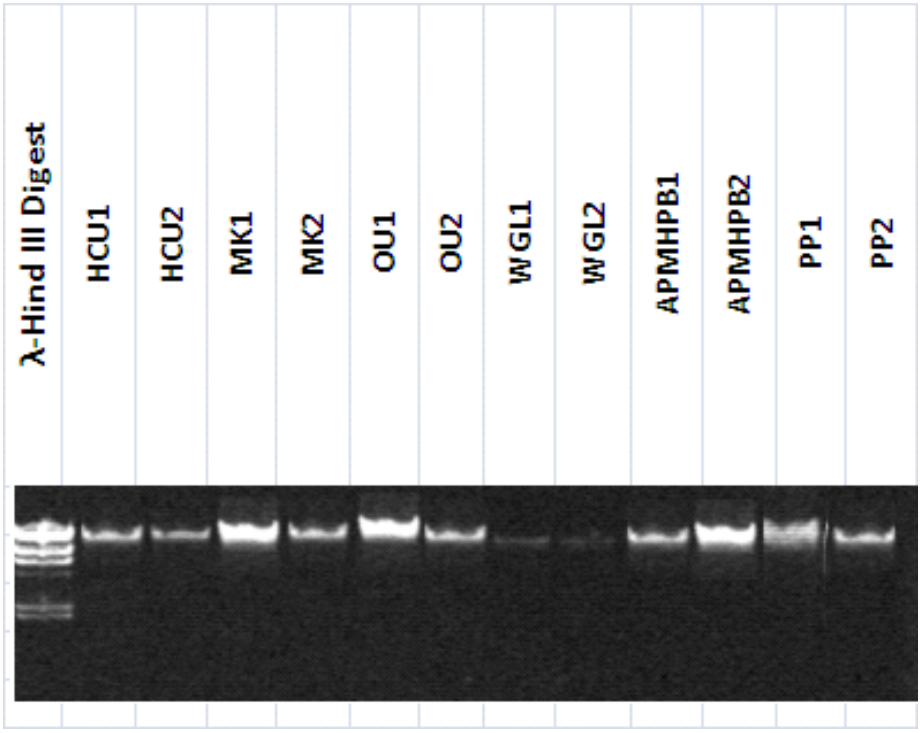



**Fig. 2: RAPD profile generated by twelve accessions of *Hemidesmusindicus*using OPB-6 primer**

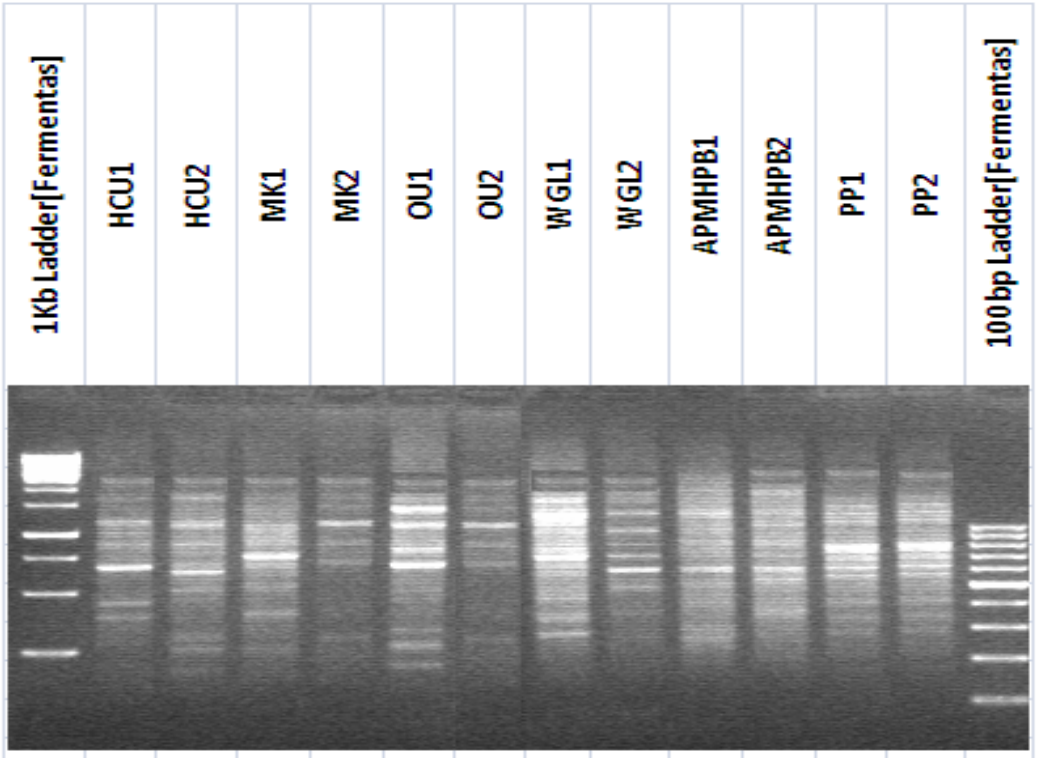


**Fig.3: RAPD profile generated by twelve accessions of *Hemidesmus indicus* using OPB-7primer**

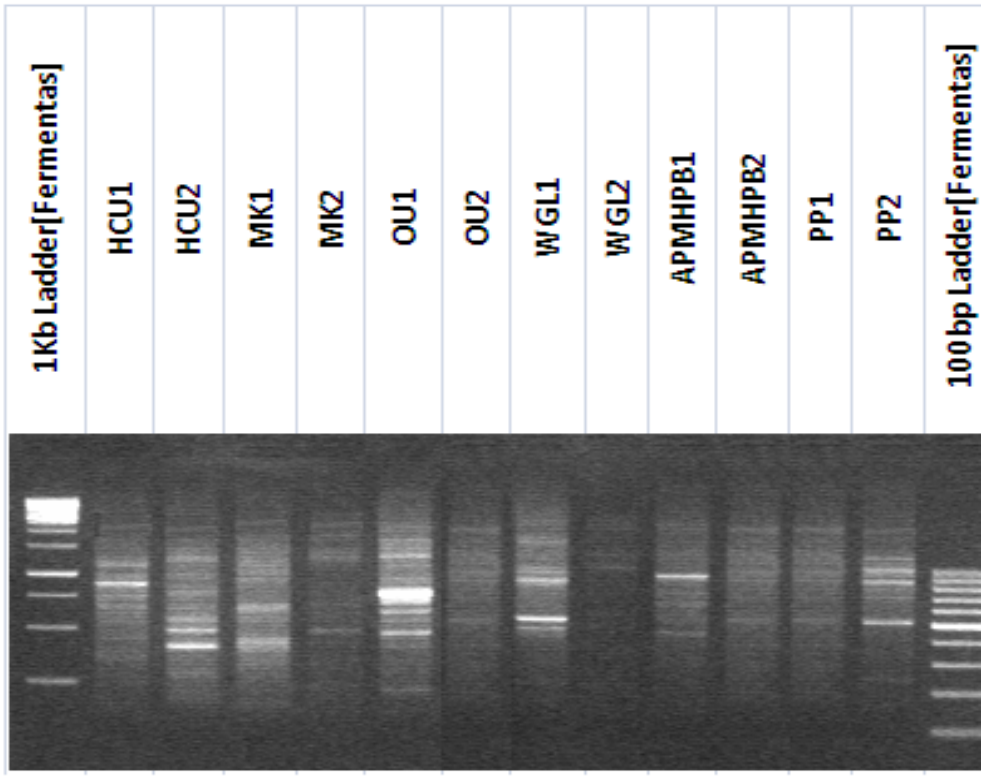



**Fig. 4: RAPD profile generated by twelve accessions of *Hemidesmus indicus* using OPC-6 primer**

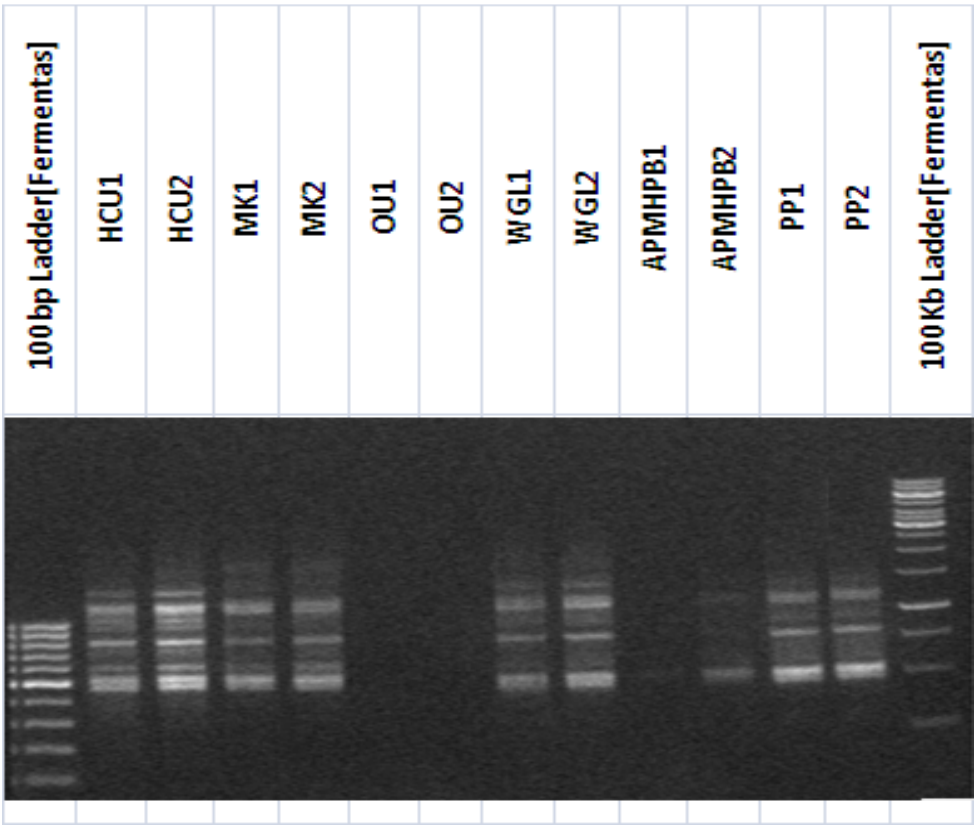


**Fig. 5: RAPD profile generated by twelve accessions of *Hemidesmus indicus* using OPC-7primer**

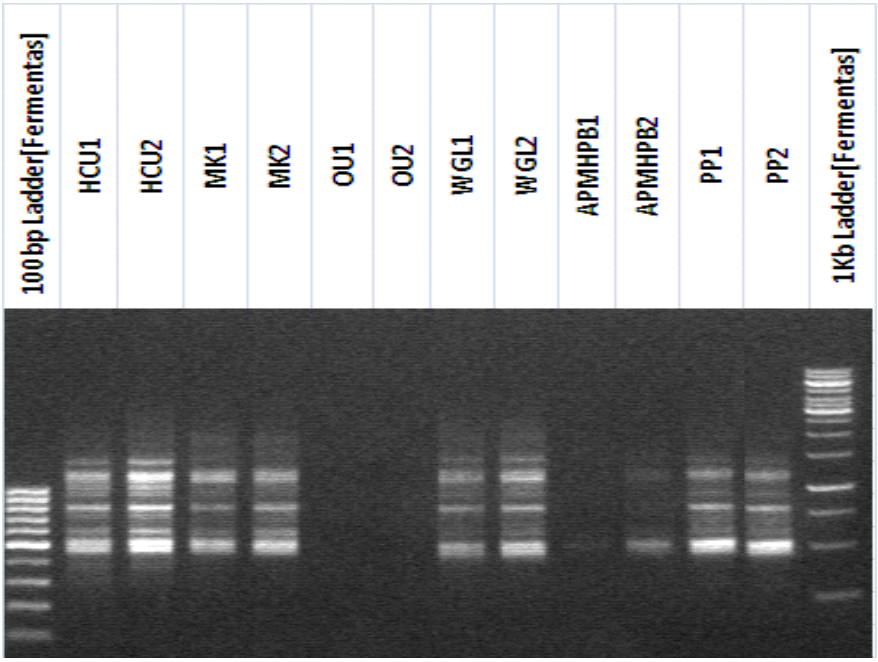
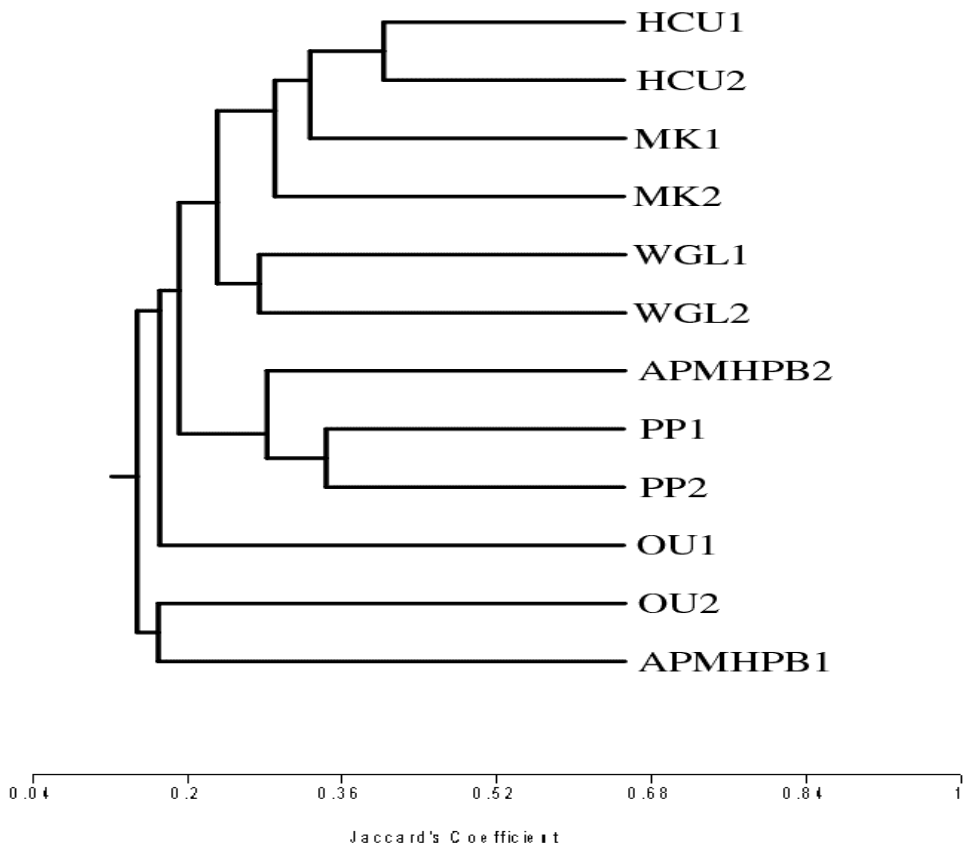



**Fig.6: Dendrogram of 12accessions of *Hemidesmus indicus* based on genetic distance generated by twenty random primers**



## CONCLUSION

As there was very little work done in molecular studies of *H. indicu*s, the present study of genetic relationships obtained among the twelve accessions of *H. indicus* may provide an important source of genetic information for improvement and conservation of this important medicinal plant.

## REFERENCES

1. Warrier, P.K. Nambiar, V.P.K. and Ramankutty, C. Indian medicinal plants - Compendium of 500 species. Orient Longman, Delhi, **3**: 141(1994)
2. Rekha, S. and Parvathi, A. Evaluation of phytochemical constituents of the roots of Licorice, Indian Ginseng, Indian Madder and Indian Sarasaparilla. *Int Jour Pharm Bio Sci*, **3**: issue 2b-357-362(2012)
3. Gurudutt, K.N. Rao, M.J.L. and Nagarajan, S. Chemical composition of the volatiles of *Hemidesmus indicus* R. Br. Plantation products, species and flavor technology Department, CFTRI, Mysore India. **16**: 212-214 (2001)
4. George, S. Tushar, K.V. and Balachandran, I. Morphological and phytochemical characterization of different accessions of *Hemidesmus indicus, J. Trop. Med. Plants*, **8(1)**: 97-101(2007).
5. Polanco, C. and Ruiz, M.L. Economic importance of some medicinal plants. *Plant Sci.*, **162**: 817-824 (2002)
6. Williams, J.G.K. Kubelik, A.R. Livak, K.L. Rafalski, J.A. and Tingey, S.V. DNA polymorphism amplified by arbitrary primers are used in genetic markers, *Nucleic Acid Research*, **18**: 6531-6533 (1990)
7. Nybom, H. and Bartish, I.V.*Hemidesmus indicus*. *Plant Ecol. Evol. Syst*, **3/2**: 93-114(2000)
8. Li, J.M. and Jin, Z.X. Extensive use of medicinal plants. *Biochem. Syst. Ecol*., **34**: 725-735 (2006)
9. Bartish, I.V. Garkava, L.P. Rumpunen, K. And Nybon, H. (2000) Phylogenetic relationship and differentiation among and within population of *Chaenomeles*Lindl (Rosaceae) estimated with RAPDs and isozyme. *Theor. Appl. Ge*net. **101**: 554-563 (2000)
10. Ranade, S.A. Farooqui, N. Bhattacharya, E. and Verma, A. Gene tagging with random amplified polymorphic DNA (RAPD) marker for molecular breeding in plants. Crit. Rev. *Plant Sci*, **20**: 251-275 (2001)
11. Doyle, J.J. and Doyle, J.L. A rapid DNA isolation procedure from small quantity of fresh leaf material. *Phytochemistry Bull*. **119**: 11-15 (1990)
12. Matharu, G.K. Thappa, D. Kamble, V. Krishnan, A. and Sane, R.T. Use of genetic and protein markers for characterization of medicinal ayurvedic plants. *Int J Pharm Sci Res*. **1(12)**: 95-110 (2010)
13. Leela, T. Naresh, B. Reddy, M.S. Madhusudhan, N.Ch. and Prathibha Devi Morphological, physico-chemical and micropropagation studies in *Jatrophacurcas* L. and RAPD analysis of the regenerants. *Applied Energy*, **88**: 2071–2079 (2011)
14. Sundar, S.K. and Akashari, C., Genetic variability studies of anti-poisonous plan *Aristolochia indica* L. *J. Biosci Res*., **2(4)**: 284-288 (2011)