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# DNA Fingerprint in identification of*Securinega Leucopyrus* (willd.)Through rapidmethod Including Root Analysis.

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## ABSTRACT

*Securinega leucopyrus* (Willd.) Muell has long been used by the tribes of Sri Lanka traditionally known as 'Katupila'and also in India as 'Humri'. Common in scrub jungls, limited to India, Sri Lanka and Burma. DNA fingerprinting for cultivar or varietal identification has become an important tool for genetic identification in plant breeding and germplasm management.Several different PCR based techniques have been developed during the last decade, each with specific advantages and disadvantages. The Random Amplified Polymorphic DNA (RAPD) marker technique is quick, easy and requires no prior sequence information.Pharmacognostical evaluation of leaf and stem of *Securinega leucopyrus* (Willd.) already established but no research work have been established on its roots and DNA fingerprints; for the first time leaf samples were subjected to fingerprints and studied the pharmacognostical aspects of roots. Pharmacognostical study showed that presence of oil globules, rosette crystals and uni to multiserriate medullary rays. DNA finger prints showed that OPC 6, OPD 8 and OPB 10 showed that merging 2 bright bands and one light band.

**Keywords:** Finger prints, Katupila, RAPD, Root, *Securinega leucopyrus.*

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## INTRODUCTION

"Katupila" as commonly known in Sri Lanka *Securinega leucopyrus* is regarded as an important herb used by the native folklore practitioners and also a favorite with the sick monkeys. When the monkeys get wounded they stand by this tree chewing leaves and plucking fruit on and off until cured. DNA fingerprinting provides an objective evaluation of genetic identity of plants based on species, cultivars, or geographic origin. It can ensure genetic uniformity of raw herbal materials. For medical herbs, synthesis and accumulation of chemical constituents rely on both genetic makeup and environmental conditions[1]. Thus molecular evaluation characterization is an important tool in ensuring that the plant material used is of desired attribute.

The DNA fingerprints exclusively using for species genus and traits level. To find out similarity and dissimilarity the DNA fingerprint may play a significant role. Pharmacognostical evaluation of leaf and stem already established[2], but no research work have been established on its roots and DNA fingerprints. For the first time leaves samples were subjected to fingerprints and studied the pharmacognostical aspects of roots. Data collected, calculated and scientifically studied. The obtained data explained in the form tables and with fingerprints.

## MATERIALS AND METHODS:

**Collection and preservation of the sample:**

After identification with the help of Forest flora of Gujarat state (R I Patel, 1984) the whole plant of *Securinega leucopyrus,*was collected from Colombo, Sri Lanka (Attidiya wetland forest area) during October 2012 and After authentication by expert taxonomist sample specimen was cross verified with Institute Pharmacognosy Museum (SPECIMEN NO- PHM 3612/11/09/1961) for future references. The roots were separated from the collected plant washed, shade dried, powdered, sieved through 80 mesh and preserved in an air-tight container. Fresh sample of root were preserved for Microscopical evaluation, in a solution prepared from 70% ethyl alcohol: glacial acetic acid: formalin (AAF) in the ratio of 90:5:5 [3] (Johnson Alexander Donald, 1940).

**Pharmacognostic studies:**

Free hand thin transverse sections of Root were taken for detailed microscopical observation. Sections were observed as such for the presence of any crystals, then were stained with Phloroglucinol and Hydrochloric acid (HCl) to notice the lignified elements and other parts (Khandelwal K.R., 2008). Canon digitalcamera attached to Zeiss microscope was used to take photographs of the sections of root. Powder characters were studied according to the guidelines[4]. The histo-chemical tests were carried out as per the standard guidelines[5] .

**Plant DNA isolation and RAPD**

**Place of experiment**-The study was carried out at Aristogene Biosciences Pvt, Ltd. Bangalore, Karnataka State, India.

**DNA Isolation from Plant: SOURCE:** leaves

**PROTOCOL**

500mg of Leaves were cut into small pieces and homogenized using tissue homogenizer and dry ice.15ml of lysis buffer was added. The tubes were incubated at 65˚C for 1 hour in a dry bath with intermittent mixing. Centrifuge at 10000 rpm for 10 minutes to separate out the unlysed cells. Supernatant was transferred to a fresh 30 ml centrifuge tube carefully. Equal volumes of Chloroform was added and mixed well. Centrifuge this at 10000 rpm for 15 minutes. The aqueous layer was pipette out into the fresh 30 ml centrifuge ube without taking the interface.

Equal volumes of Isopropanol and 1/10$^{th}$ volumes of 3M Sodium acetate was added and mixed well. Left at room temperature to stand for 5-10 minutes. Centrifuged at 10000 rpm for 10-15 minutes. The supernatant was discarded. The pellet was washed with 500 µl of 70% ethanol. The pellet air dried and suspended in 500 µl of 1X Tris- EDTA buffer. To remove inhibitors for PCR further the DNA sample was column purified following the below protocol[6].

**Protocol:**

**Column purification**

The column was placed in collection tube, 400µl of equilibration buffer was added to the column and centrifuge at 10000rpm for 1min. Collected buffer was discarded. 400µl of equilibration buffer was added to the DNA samples , mixed and loaded into the column (This step was repeated till the DNA sample was completed).Flow through was collected. 500µl of wash buffer 1 was added, centrifuge at 10000rpm for 1min and buffer was collected. 500µl of wash buffer 2was added, centrifuge at 10000rpm for 1min and buffer was collected.

The column was centrifuge with empty collection tube to completely remove the wash buffer for 2 min. 50µl of elution buffer was added to the column placed in new collection tube. Incubated at room temperature for 2 min and centrifuge at 10000rpm for 1min and eluted sample was saved(elution 1). Previous step was repeated.(DNA may elute in this fraction also) (elution2). Quantization of eluted DNA samples was done by loading into the Agarose gel[7].

## RESULTS AND DISCUSSION:

**T.S of Root**

Transverse section of root showed an outer cork, middle cortex, centrally located vascular bundles

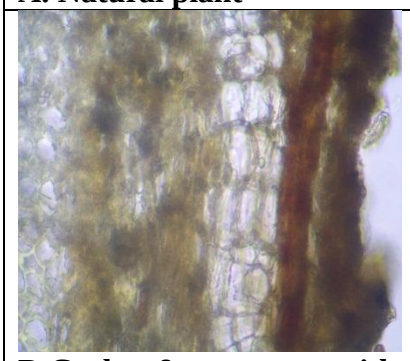
devote of pith. (Plate :1-C)

Detailed section showed cork the outer most layer consisting of 8-10 layers of tangentially elongated barrel shaped cells with brownish colouring matter. Beneath the cork a reduced cortex zone of 6-7 layers of parenchyma cells consisting of oil globules, rosette crystals of calcium oxalate and yellowish brown contents (tannin).Cortexmade up ofcortical parenchyma filled with simple and compound starch grains.Some of the isolated 6-8 celled pericyclic fibres was also observed. Endodermis is single layered followed by phloem region. Beneath the phloem, xylem is arranged radially consisting xylem parenchyma and fibers.Beneath the phloem, xylem is arranged radially consisting xylem parenchyma and fibers.Xylemreaches upto the central most part of the root without any pith. Medullary rays uniserriate to multiserriate somewhat elongated. The medullary rays heavily deposited with oil globules and tannin materials, ray cells starting from the center reaching upto cortical zone. (Plate: 1 D-G).

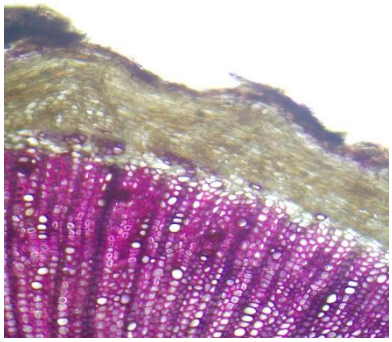
**A. Natural plant**

**B. Roots morphology**

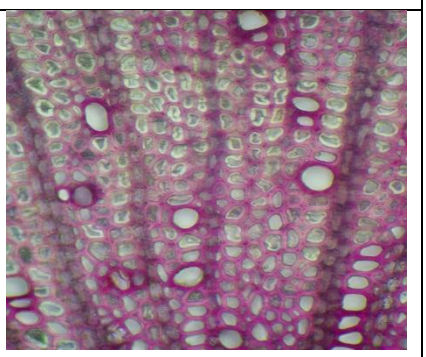
**C. T.S. of Root**



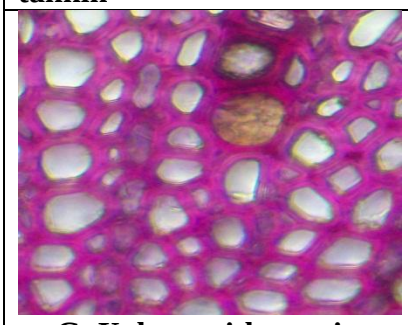
**D.Cork & cortex with tannin**



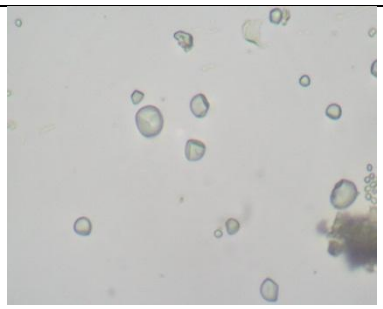
**E. Cortex,phloem,Xylem**



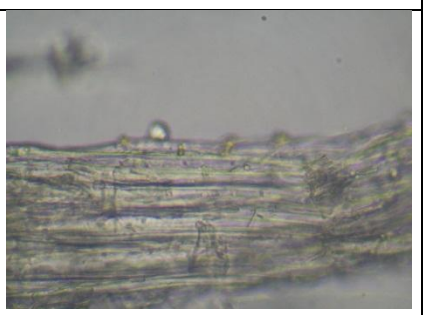
**F. Xylem, medullary rays**



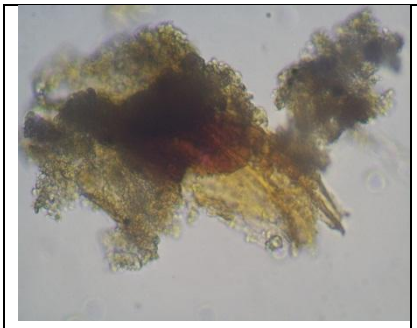
**G. Xylem, with tannin**



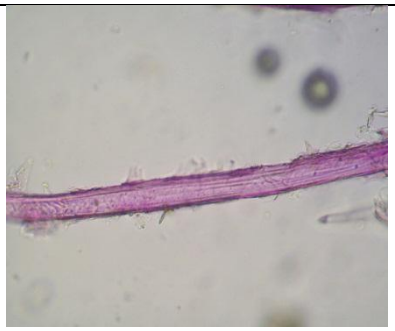
**H. Simple starch grains**



**I. Simple fibres**



J. Tannin contants



K. Lignified fibre

L. Border pitted vessel

**Plate 1. Microphotographs of root**

**Powder microscopy**

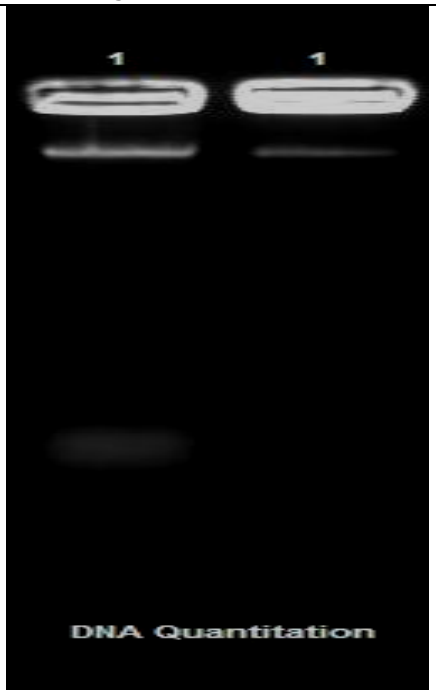
**Organoleptic characters**

The powder color was light brown taste was astringent bitter and fine powder in touchThe diagnostic characters of the root are fragment of cork with tannin content simple starch grains, simple fibres, tannin contents, lignified fibres and pitted vessels. (Plate: 1 H-L)

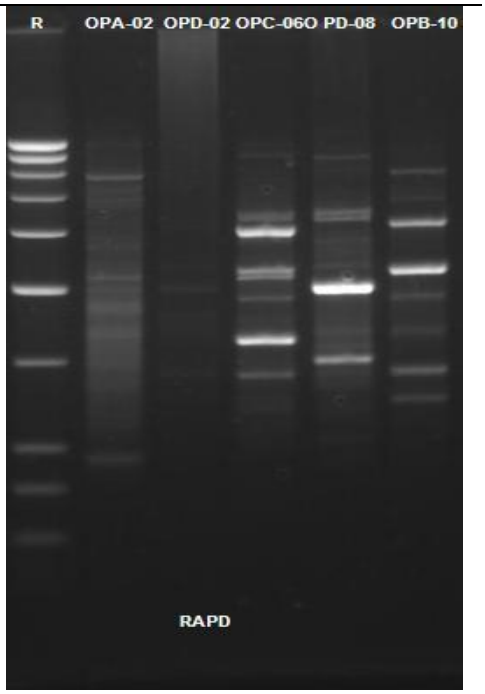
**Histo-chemical evaluation:** Thick sections were treated with various reagents to locate the chemical which were present in the plant Results were tabulated in the table 1.

**Table 1: Histo-chemical test of root of *Securinega leucopyrus***

| Sr. no | Reagent | Observation | Characteristics | Results |
|---|---|---|---|---|
| 1. | Phloroglucinol+Conc. HCl | Red | Lignified cells | ++ |
| 2. | Iodine | Blue | Starch grains | ++ |
| 3. | Phloroglucinol+Conc. HCl | Dissolved | Calcium oxalate crystals | ++ |
| 4. | $Fecl_3$ solution | Dark blue to black | Tannin cells | ++ |




**DNA Quantification**




**Comparative DNA with polymers**

**Plate 2. DNA Fingre prints**

**DNA Finger Prints**

2µl loaded from pooling the elutions. DNA was seen Conc50ng/µl .1µl of sample was used for PCR

**Amplification:**

A cocktail was made with PCR master mix and respective Random primer.

**Table 2: Random Primers Results**

| Sl.no | Primers | sequence |
|---|---|---|
| 1. | OPA-02 | TGCCGAGCTG |
| 2. | OPD-02 | GGACCCAACC |
| 3. | OPC-06 | GAACGGACTC |
| 4. | OPD-08 | GTGTGCCCCA |
| 5. | OPB-10 | CTGCTGGGAC |

**Table 3:PCR Condition**

| | Mastermix | |
|---|---|---|
| Components | 1X | 5X |
| D.D.H20 | 18ul | 90ul |
| 2X PCR Master MIX | 20 µl | 100 µl |
| *Random Primer | 1µl | 5µl |
| Template | 1ul | 5ul |
| Total Volume | 40 µl | 200 µl |

39µl of this was aliquoted into 5 different labeled PCR vials and to this 1 µl of template DNA was added and PCR was set.

**Table 4: PCR Cycle conditions:**

| Temperature | Time | No. of cycles |
|---|---|---|
| `94˚C | 5 minute | 1 |
| 94˚C | 30 second | |
| 45˚C | 1minute | 40 |
| 72˚C | 1.30minute | |
| 72˚C | 7 minutes | 1 |

**Note:**

**R**-mid range ruler ranging from 0.1, 0.2, 0.3, 0.61, 1.5, 2.02.5, 3.0,3.5kb

Medicinal plant species were authentified by RAPD analysis the advantages of RAPD technique include their simplicity, rapidity and low amount of genomic DNA required. RAPD marker provide to easily reproducible under a wide variation of amplification conditions as it was clearly visible up to an nailing temperature of $38^0$ C and result were not affect with changes in the origin of the primer. Plant DNA extraction was done using CTAB method. The DNA was further purified using spin columns. RAPD results showed that OPA2, OPD2 does not exhibit any bands OPC6exihibit 4 bright bands and no of light bands OPD8 showed 2 bright bands where as OPB 10 exhibit 2bright bands and many light bands. OPC6 OPD8 and OPB 10 exhibit nearby 2

bright bands of particular family and species characters (Plate 2 A & B). Analysis of DNA fingerprints of selected samples can contribute significantly which further need to be elaborated by bar-coding.

## CONCLUSION:

Scientific study of the Pharmacognosy, phytochemistry and the advanced DNA finger printing gives the specific differentiate characters it is scientifically necessary in this type of works. This work is an superficial work to implicate for the further research works.

## REFERENCES:


1. Shinde, V.M., Dhalwal, K., Mahadik, K.R., Joshi, K.S., Patwardhan, B.K. RAPD Analysis for Determination of components in Herbal Medicine. eCAM 2007;4 (S 1):P.21-23.
2. Ajmeer etal, Micromorphological and micrometric evaluation of *Securinega lecopyrous* Leaf and stem- Unexplored drug, WWW. IJSIT, 2013, 2(2), P. 140-149.
3. Johnson Alexander Donald. Plant Micro technique. Macgrow Hill Book Company, New York, London 1940: 105.
4. Trease and Evans, Pharmacognosy, 15th Ed., W.B. Sunders Company Ltd. 1996 P.569,570.
5. Krishnamurty KV. Methods in the plant histo-chemistry, Vishwanadhan Pvt Limited, Madras1988:1– 77.
6. Baum B.R, Mechanda S, Livesey JF, Binns SE.Arnason JT. Predicting quantitative phytochemical markers in single Echinacea plants or clones from their DNA fingerprints. Phytochemistry 2001; 56: (6): 543-549.
7. Chavan P, Joshi K, Patwardhan B. DNA Micorrays in Herbal Drug Research, Evid Based Complement Alternate Med 2006; 3: 447-457.