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Pharmacognostical Studies on the Root of *Albizzia procera* (Roxb.) Benth.

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Abstract: *Albizzia procera* (Roxb.) Benth. belonging to the family Fabaceae is widely distributed from India and Myanmar through Southeast Asia to Papua New Guinea and northern Australia. This plant is commonly used for the treatment of various ailments in Siddha system of medicine. All parts of this plant are reported to show anti-cancer activity. They were reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc. The roots contain α-spinasterol and a saponin that has been reported to possess spermicidal activity at a dilution of 0.008%. Though the root of *A. procera* is used as medicine in the traditional systems, no scientific data is available to identify the genuine sample. The present investigation was therefore taken up to establish identity of the root of the plant morphologically, microscopically, physico-chemically, chromatographically and spectroscopically for the standardization of the drug. Diagnostic features by macroscopic and microscopic examinations not only confirm the true identity of the drug but also help in detecting any adulterant or substitute. Physico-chemical parameters like loss on drying at 105°C, total ash, acid insoluble ash, water soluble extractives and alcohol soluble extractives of the root powder were carried out. The HPTLC profile may serve as a characteristic fingerprint for qualitative evaluation. Spectroscopic technique like UV-Vis can be successfully used alone or with other methods for standardization purpose. The results achieved from all types of analysis performed will be useful for authentication, standardization and quality control assessment of the root of *Albizzia procera*.

***Index Terms*: *Albizzia procera*,macroscopic and microscopic examinations,physico-chemical parameters, HPTLC profile,UV-Vis Spectroscopy.**

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I. INTRODUCTION

Indian system of medicine is one of the oldest global traditional systems of medicine. India has the unique characteristic of having different well-acknowledged traditional systems of medicine such as Siddha and Ayurveda. Traditional systems of medicine always played an imperative role in global healthcare system. They are continuing to do so at present and shall play major role in future also. The major part of traditional therapy involves the use of plants and plant products as medicines and could be traced as far back as the beginning of human civilization. The deforestation and extinction of many species and incorrect identification of many plants has resulted in adulteration and substitution of raw drugs. It is therefore essential to establish internationally recognized guidelines for assessing their quality. In order to ensure the quality of raw drugs and medicinal plant products, it is mandatory to standardize the drugs by using modern control techniques and applying suitable standards. The present study is an attempt to standardize a well known plant used in Siddha system of medicine.

Albizzia procera (Roxb.) Benth. belongs to the family Fabaceae. It is commonly called white 'Srish' or tall Albizzia. The vernacular names[1, 2] of the plant are Hindi: Safed siris; Bengali: Kori; Marathi: Kinhai; Tamil: Konda vagai; Telugu: Tella chinduga; Malayalam: Chalavaka, Kottavaga, Jalavaka, Vellavaka and English: White siris. It is widely distributed from India and Myanmar through Southeast Asia to Papua New Guinea and northern Australia. The habitat ranges from monsoon forest, mixed deciduous forest, savannah woodlands, pyrogenic grassland, roadsides and dry gullies, to stunted, seasonal swamp forest. It is commonly found in open secondary forest and in areas with a pronounced dry season. It is a tree with an open canopy, height up to 30 m and trunk 35 - 60 cm in diameter; bole straight or crooked, up to 9 m. Bark - smooth, pale yellowish-brown or brown with horizontal ridges; Branches - terete, glabrous; Leaves - bipinnate, apex rounded or subtruncate, often emarginate, mucronate; both surfaces sparsely appressed puberulous, rarely glabrous on top side. Flowers - 15-30 per glomerule, sessile, uniform, bisexual; Fruits - rich red or reddish brown, flattened pods, chartaceous, glabrous, with distinct marks over the seeds; mature pods each containing 6-12 seeds, seeds are small, greenish-brown, elliptical to round, flat, with a hard, smooth seed coat[3]. Root - Lateral roots are wide spreading and the taproot is stout[4]. This species provides wood for a variety of purposes, nutritious fodder for live stock and shade for tea plantations. It is an important reforestation and agro-forestry species[5]. Biological compounds like α-spinasterol, hentriacontane and hexacosanol have been isolated from leaf. Heartwood and bark contain isoflavones, biochanin A, formononetin, genistein and daidzein. Bark also contains a new pterocarpan – demethylmedicarpin and β – sitosterol. Degraded gum contains galactose, mannose, glucuronic acid and 4-0- methyl –D- glucuronic acid. A saponin has been isolated from seeds, which on hydrolysis gives proceric acid. Seeds also contain procerogenin A, mechaerinic acid, proceranin A (hypotensive in animals) and oleanolic acid. Root contains α – spinasterol and oleanelic acid[6].

A.procera is commonly used for various diseases by traditional Indian Systems of Medicine like Siddha and Ayurveda and in Folk Medicine. All parts of the plant are reported to show anti-cancer activity[7]. The plant is also used for the treatment of pain, convulsions, delirium and septicemia[8]. The decoction of bark is given for rheumatism, haemorrhage and is considered useful in treating pregnancy problems, stomach-ache and sinus. They were reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc[9]. The roots contain α-spinasterol and a saponin that has been reported to possess spermicidal activity at a dilution of 0.008%. Traditionally, leaves of *A. procera* were extensively used for the treatment of variety of wounds[10]. In India, leaves are poulticed on to ulcers[11].Seeds are powdered and used in amoebiasis. It cures urinary tract infections including glycosuria, haemorrhoids, fistula and worm infestation. It also suppresses skin diseases. Fruits of *A. procera* act as astringent and diminish *Kapha and Sukra*[12]. In Siddha medicine and Folk medicine, parts of *A.lebbeck* and *A.procera* are used one for the other whether knowingly or unknowingly[13]. So the identification of the plant materials is essential and important. Though the root of *A. procera* is used as medicine in the traditional systems, no scientific data is available to identify the genuine sample. The present investigation was therefore taken up to establish identity of the root of the plant morphologically, microscopically, physico-chemically, chromatographically and spectroscopically for the standardization of the drug. Each and every drug has got its own physical and chemical characteristics which help for separating it from other closely related drugs or any adulterant drug by above mentioned evaluation techniques. High Performance Thin Layer Chromatography (HPTLC) is particularly valuable for the preliminary separation of plant constituents. The chromatographic profile may serve as a characteristic fingerprint for qualitative evaluation. Spectroscopic techniques like UV-Vis can be successfully used for standardization purpose.

Fig.1: ***Albizzia procera*** **(Roxb.) Benth.**

II. MATERIALS AND METHODS

Plant material

Fresh root of *A. procera* was collected from Thirunelveli. It was authenticated by the Pharmacognosy department, Siddha Regional Research Institute, Thiruvananthapuram. The fresh sample of the plant material was used for anatomical studies. The plant materials were cut, crushed, dried and kept in airtight containers and used for all other experimental purposes.

Botanical studies

(a) Macroscopy

Macroscopic identification of plant material is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface[14].

(b) Microscopy

Microscopic studies of root of *A. procera* were carried out by preparing thin sections of the plant material. The sections were further washed with water, stained with safranin and mounted in glycerine for observation[15].

(c) Powder microscopy

The powder microscopy of the powered root of drug was studied using standard procedure[16] by capturing the images of different fragments of tissues and the diagnostic characteristic features obtained were recorded.

Chemical studies

Physico- chemical analysis, chromatographic and spectroscopic studies were carried out using the shade dried and powdered plant material by adopting the standard procedures[14].

(a) Physico-chemical evaluation

Physico-chemical parameters like foreign matter, loss on drying at 105℃, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractives and volatile oil of the root powder were carried out.

(b) Chromatographic studies

High Performance Thin Layer Chromatographic (HPTLC) studies of the sample were carried out. HPTLC has become a routine analytical technique for herbal drug standardization due to its advantages of

low operating cost, speed, simplicity and minimum sample requirement, reproducibility, reliability and accuracy[17].

Preparation of extract: Powdered sample (4 g) was soaked in 40 ml chloroform for 16-18 h and then refluxed. The extract was filtered out and concentrated to 4 ml.

Selection of Solvent system: Chloroform extract was developed on chromatographic plates with many ratios of different solvents and the best mixture which gave the maximum separation was selected as the mobile phase for the study.

HPTLC analysis of plant extract: 10 and 20 µl of the chloroform extracts were loaded as 10 mm band length of two tracks in the Silica gel 60 F254 TLC plate using ATS4 instrument. The sample loaded plate was kept in TLC twin trough developing chamber after saturated with the solvent vapour of the selected mobile phase, Toluene: Ethyl acetate: Formic acid (5: 1.5: 0.2) and the plate was developed in the respective mobile phase up to 70 mm. The developed plate was air dried to evaporate the solvent from the plate. The plate was kept in photo-documentation chamber (Vizualiser) and captured the images at UV 254 nm and UV 366 nm. The plate was placed inside the Camag TLC Scanner 4 and scanning was done at 254 nm and 366 nm. The peak table, peak display and peak densitogram were noted. The developed plate was then dipped in the derivatisation reagent, vanillin- sulphuric acid and dried at 105°C on Camag hot plate. The derivatised plate was photo documented at white light using photo-documentation chamber. The plate was scanned at 575 nm and the results were documented[18].

(c). Ultra Violet-Visible (UV-Vis) Spectroscopy

UV-Vis Spectroscopic method is based on electronic absorption caused by the compounds present in the plant extract. The chloroform extract of the root of *A. procera* was subjected to UV-Vis Spectroscopic analysis. The extract was scanned at wave length ranging from 200 to 1100 nm using UV-VIS Spectrophotometer (Model: UV3120) and the characteristic peaks were detected and recorded.

III. RESULTS AND DISCUSSION

Botanical studies

(a) Macroscopy

Macroscopic study of the root indicated that it is taproot, pale white in colour when young and light brown colour when mature. Roots were branched.

(b) Microscopy

Anatomical features of *A. procera* root species were studied using fresh specimens. The cross sectional outline of root appears circular with well developed vascular system and fairly thick bark. Crystallized Parenchyma cells were found in the cortical zone and embedded with Sclerenchymatous cells. The root comprises of prominent superficial zone of periderm comprising 5 or 6 layers of wide, tabular, thin walled and suberized phloem cells. The cortical zone is encircling the xylem cylinder, very thick and solid occupying the major portion of the root. The vessels are diffusely distributed and are short radial multiples vessels. Intervessel pits and ring of porosity of cells were also seen in the cortical zone.

T.S.of root : P-Parenchymatous cell ; CrP- Crystal Parenchyma ;
Sc-Sclerenchyma cell. ; IV - Intervessel pits ; C- Cork; RP- Ring of porosity.

Fig.2: Anatomical features of root of *A. procera*

(c). Powder microscopy

The powder is pale grey-green in colour and bitter in taste. The following charactesr were seen in *A. procera*. Fibro vascular tissue, tannin cells, parenchyma tissue with fibres and fragments of a scalariformly thickened vessel, fibres with an associated reticulately thickened vessel, lower epidermis in surface view, fibres showing the thickened walls of parenchymatous cells, calcium oxalate cluster crystals, cork surface, phloem parenchyma with medullary ray, parenchymatous fibre cells and elements of the vascular tissue are present in root of *A.procera.*

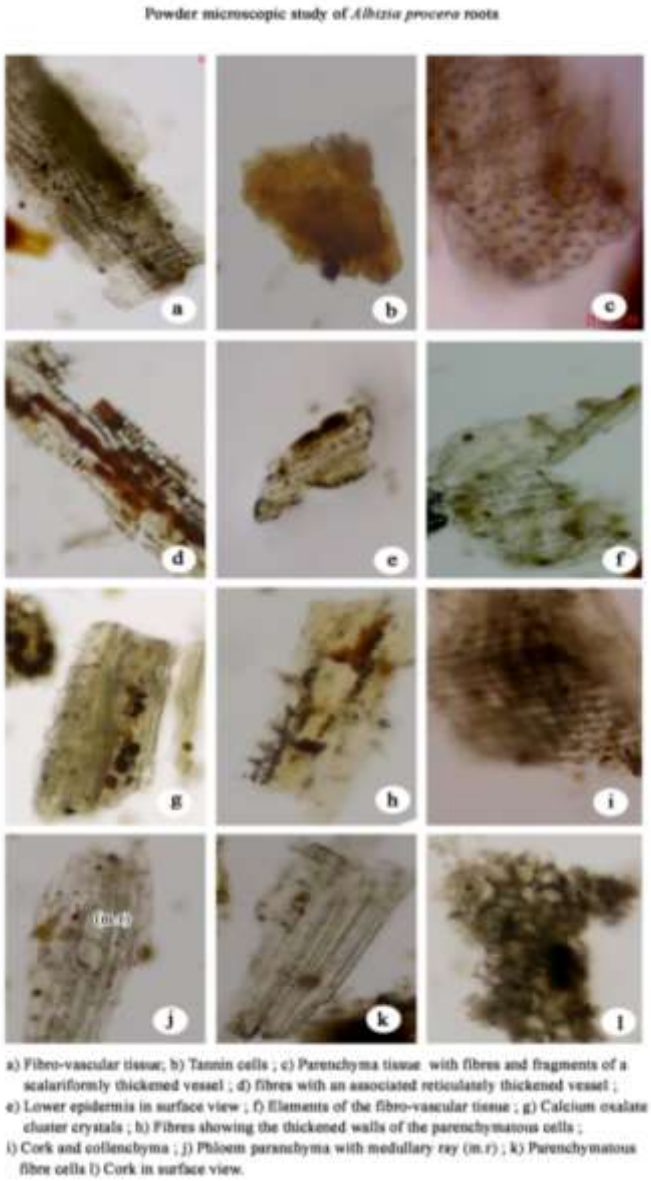


Fig.3: Powder microscopy of root of *A. procera*

Chemical studies

(a) Physico-chemical evaluation

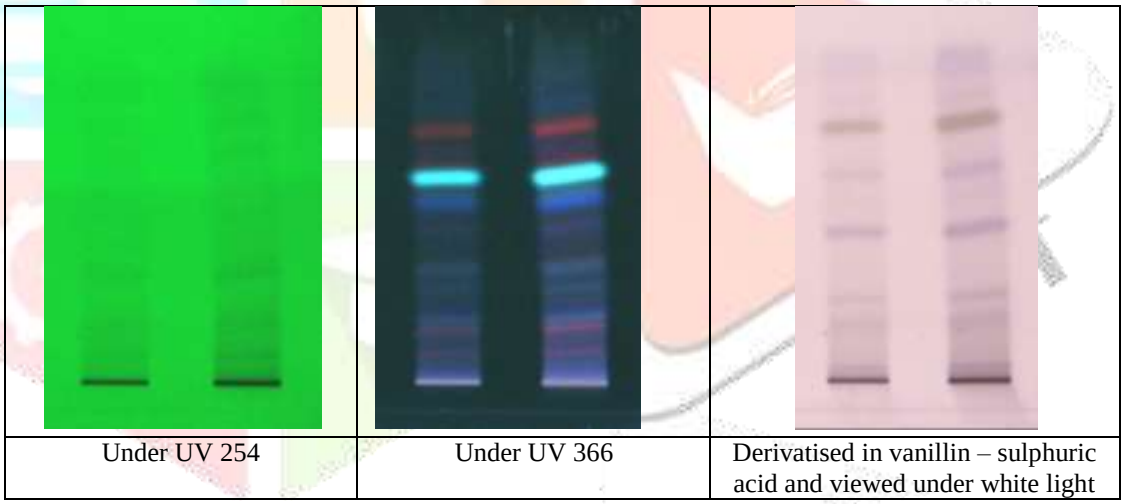
The loss on drying of the drug was found to be 8.11% which may attribute to the moisture content of the sample, since volatile oil is absent. The total ash was found to be 1.17 % which is due to the presence of inorganics in the drug. The acid insoluble ash showed that the drug contains only negligible amount of silicious matter. The extractive values, viz., water and alcohol were found to be 8.28 % and 10.20 % respectively. These values are the measures of high polar secondary metabolites such as glycosides, tannins, proteins, etc. in the drug. The physico-chemical values are shown in Table 1.

Table 1: Physico-chemical characters of root of *A. procera*

Sl. No.	Parameters	Results
1.	Foreign Matter %	<2
2.	Loss on Drying at 105^0C %	8.11
3.	Total Ash Content %	1.17
4.	Acid Insoluble Ash %	0.27
5.	Water Soluble Extractive %	8.28
6.	Alcohol Soluble Extractive %	10.20
7.	Volatile oil %	Nil

(b) Chromatographic studies

HPTLC photo documentation of the chloroform extract of the root of *A. procera* visible under UV 254 nm, 366 nm and visible light after derivatisation are given in Fig. 4. The solvent system Toluene: Ethyl acetate: Formic acid (5: 1.5: 0.2) opted for the study was ideal and gave well resolved sample peaks.



Under UV 254	Under UV 366	Derivatised in vanillin – sulphuric acid and viewed under white light

Fig.4: HPTLC photo documentation profile of 10 µl and 20 µl the chloroform extract of root of *A. procera*; Solvent system – Toluene: Ethyl acetate: Formic acid (5: 1.5: 0.2)

Table 2: R_f values and colour of major bands of chloroform extract of root of *A. procera*

Under UV 254 nm		Under 366 nm		After derivatisation under white light	
R_f values	**Colour**	**R_f values**	**Colour**	**R_f values**	**Colour**
0.07	Light Green	0.07	Pink	0.05	Purple
0.18	Light Green	0.18	Pink	0.17	Purple
0.33	Light Green	0.35	Light blue	0.26	Purple
0.52	Light Green	0.48	Blue	0.45	Purple
0.70	Light Green	0.63	Fluorescent blue	0.63	Purple
0.79	Light Green	0.77	Magenta	0.77	Light brown
0.91	Light Green	0.85	Blue	0.94	Purple
				0.97	Purple

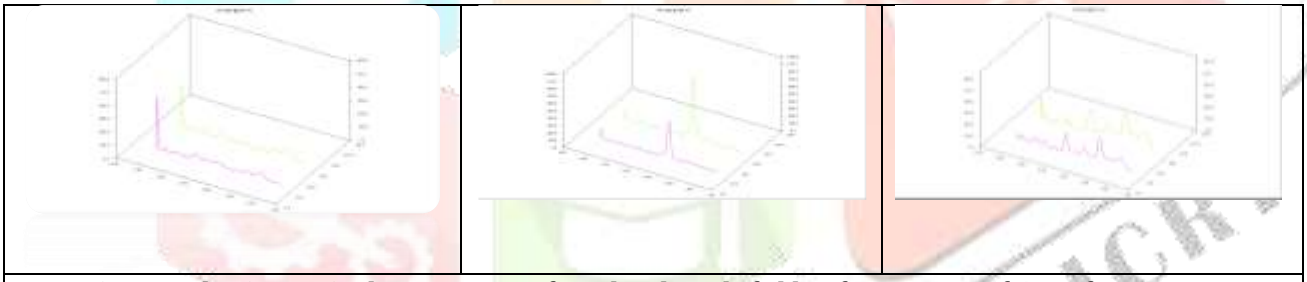
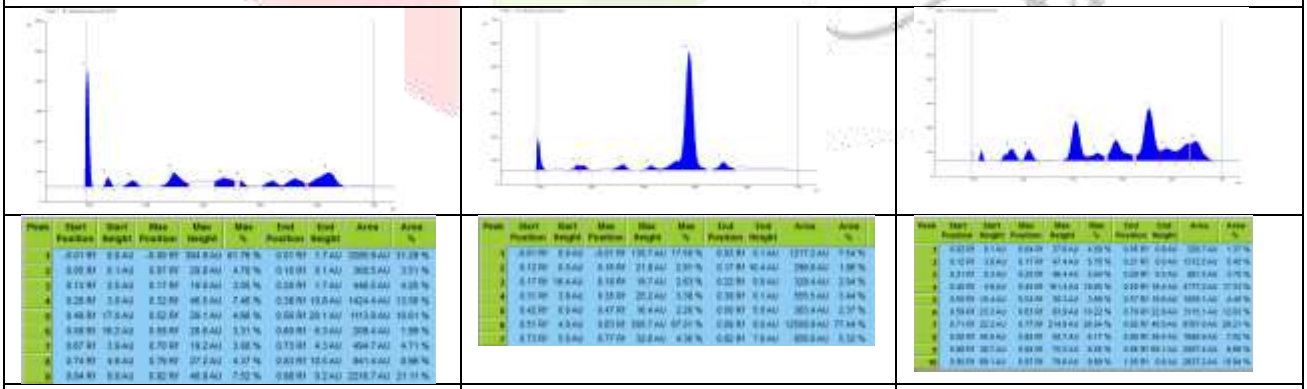


Fig.5: 3D densitometric chromatogram of 10 µl and 20 µl of chloroform extract of root of *A. procera*



At 254 nm | **At 366 nm** | **At 575 nm after derivatisation**

Fig.6: HPTLC finger print profile of 10 µl of chloroform extract of the root of *A. procera*

The chromatograms obtained with the chloroform extract of the root of *A. procera* revealed the presence of different phytoconstituents with respect to their zone position, colour and intensity of the bands. The observed R_f values of the bands and their colours are given in Table 2. The 3D densitometric chromatograms are shown in Fig 5. HPTLC fingerprinting pattern and table of R_f values and their relative peak areas of 10 µl chloroform extract of the

plant drug at 254 nm, 366 nm and 575 nm after derivatisation are shown in Fig 6. It is evident from the HPTLC finger printing pattern at 254 nm that there are 8 spots indicating the occurrence of at least 8 different major components in chloroform extract. It is also clear that out of 8 components, four components with R_f values 0.32, 0.52, 0.79 and 0.92 were found to be more predominant as the percentage area is more, with 13.56%, 10.61%, 8.96% and 21.11% respectively. Similarly the finger printing pattern at 366 nm showed 6 bands of 6 major chemical constituents. The band with R_f value 0.63 and peak area 77.44% represents the major constituent of the extract. The finger printing pattern at 575 nm after derivatisation showed the presence of six major peaks with R_f values 0.45, 0.63, 0.77, 0.85, 0.94 and 0.97 and percentage of peak area 17.33%, 12.93%, 28.21%, 7.02%, 8.66% and 10.94% respectively. The other peaks were found to be minor as the percentage area for the peaks were less. First peak of every fingerprint has not been taken into account since it is at the loading position.

(c). Ultra Violet-Visible (UV-Vis) Spectroscopy

The UV-VIS spectrum of chloroform extract of the root of *A. procera* is shown in Fig.7. The qualitative UV-VIS spectrum profile of the extract was selected at wavelength from 200 to 1100 nm. The profile showed peak at 260 nm with a shoulder peak at 293 nm. This spectrum can be considered as unique for the chloroform extract of the root of *A. procera*.

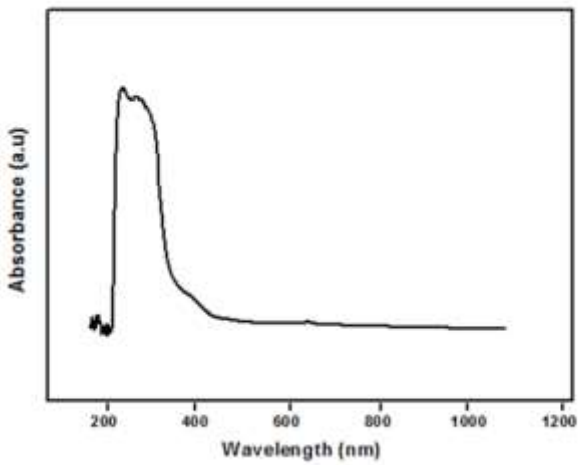


Fig 7:Ultra Violet-Visible Spectrum of chloroform extract of the root *of A. procera*

IV. CONCLUSION

The achieved results of macroscopic, microscopic and powder microscopic studies, physico-chemical analysis, HPTLC and spectroscopical studies may be useful as a tool for authentication, standardization and quality control assessment of the root of *Albizzia procera*. The HPTLC fingerprinting profile and UV-Vis spectrum are qualitative chemical evaluation parameters which indicate the spectrum of chemical constituents present in a plant drug and are unique for each drug. These detailed chemical profiles enable us to identify the plant material and distinguish from its substitute/adulterant. Hence, the result of the present study gives an insight into the evaluation of the root of *A. procera*.

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