



# Quantification of a bioactive homo isoflavanoid bonducillin in *Caesalpinia bonducella* (L) Roxb. - an ethno medicinal plant.

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## Abstract:

*Caesalpinia bonducella* (L.) Roxb. is a perennial climber belonging to the family Caesalpniaceae. The present study deals with the quantification of a unique homoisoflavanoid in the various plant organs and developmental stages of *Caesalpinia bonducella* (L.) Roxb. The methanolic extracts of various plant organs of *Caesalpinia bonducella* (L.) Roxb. viz root, stem, leaf, flower, pod, seed coat and seed kernel were subjected to HPLC analysis for the quantification of bonducillin. The bonducillin was present in flower, seed coat and seed kernel. The seed coat and seed kernel showed  $0.001 \pm 0.31 \mu\text{g/g DW}$  and  $0.004 \pm 0.27 \mu\text{g/g DW}$ . Maximum amount of bonducillin  $1.834 \pm 0.04 \mu\text{g/g DW}$  was reported in the flowers of *Caesalpinia bonducella* (L) Roxb. The bonducillin was absent in other plant organs. The methanolic extracts of various developmental stages of *Caesalpinia bonducella* (L.) Roxb. viz 30 days old seedling plants, 60 days old seedling plants, 90 days old seedling plants, 120 days of the seedling plants, shoot before flowering, shoot at flowering and shoot after flowering were subjected to HPLC for the quantification of bonducillin. The amount of bonducillin in 30 days old seedling plants was  $0.003 \pm 0.06 \mu\text{g/g DW}$  While it was  $0.002 \pm 0.74 \mu\text{g/g DW}$  in 60 days old seedling plants. The seedling plants of 90 days old produced  $0.001 \pm 0.32 \mu\text{g/g DW}$  while it was  $0.002 \pm 0.93 \mu\text{g/g DW}$  in 120 days old seedling plants. The extract of shoot before flowering did not showed the presence of bonducillin. The shoot extract of plant at flowering and after flowering showed  $0.013 \pm 0.40 \mu\text{g/g DW}$  and  $0.013 \pm 0.40 \mu\text{g/g DW}$  bonducillin respectively. This study reveals the suitable plant organs and developmental stages of the plant to be used for the extraction of bonducillin which imparts many pharmacological activities of the plant.

**Key words:** *Caesalpinia bonducella* (L.) Roxb., bonducillin, plant organs, developmental stages, HPLC.

## Introduction:

The plants are natural factories, which manufacture various chemicals known as metabolites through biochemical reactions. These metabolites are broadly classified into primary metabolites and secondary metabolites. The secondary metabolites are accessory compounds synthesized in response to specific chemical stimulus, environmental conditions, infections or in particular developmental stages of the plant. These products are an expression of individuality of a species (Hussain *et al.*, 2012). Among the major secondary metabolites flavonoids occupies a significant position as it has innumerable pharmacological activities and it takes away a lion share of human diet.

The bonducillin present in *Caesalpinia bonducella* (L.) Roxb. is a significant bioactive secondary metabolite which has been used for years to treat various diseases. The plant is over exploited for the preparation of medicines and became critically endangered. The strategy of applying the principles of tissue culture and phytochemistry for the production of *in vitro* plant material for the extraction of bonducillin can be an important measure for the conservation of *Caesalpinia bonducella* (L.) Roxb.

*Caesalpinia bonducella* (L.) Roxb. is an ethno medicinal plant which also contains an important homoisoflavanoid viz. 7- hydroxyl -(E) -3-phenylmethylene- chroman-4-one popularly known as bonducillin. It is the bitter constituent of seed kernel. Bonducillin is not only isolated in *Caesalpinia bonducella* (L.) Roxb. but in *Caesalpinia pulcherrima* (L.) Sw. and *Caesalpinia digyna* Rottl. Bonducillin is usually isolated using reverse phase counter current chromatography. Plenty of literature are available on the pharmacological properties of *Caesalpinia bonducella* (L.) Roxb. The structure, extraction and isolation of bonducillin were studied extensively (Das *et al.*, 2009; Tumminkatti, 1930). On contrary the reports on quantification of bonducillin in *Caesalpinia bonducella* (L.) Roxb. was scanty. We found scarce research work on specific developmental stages and plant organs which can be used for the maximum extraction of bonducillin.

Plant scientists did not leave an ovoid space in *in vitro* studies of *Caesalpinia bonducella* (L.) Roxb. All plant parts like roots, node, internode, leaves, pulvinus, epicotyl region etc. are explored for the callus production and plant regeneration of *Caesalpinia bonducella* (L.) Roxb. All these facts led us to study the *in vitro* production and the elicitation of bonducillin with biotic and abiotic elicitors. The objectives of the present phytochemical study includes a) to find out the proper developmental stage and plant organ that can be used for the production of bonducillin. b) to provide an alternative tool to extract the compound through *in vitro* cultures without disturbing the natural populations of *Caesalpinia bonducella* (L.) Roxb.

## Materials and methods:

### A) *In vivo* plant material:-

*Caesalpinia bonducella* (L) Roxb. was collected from Manjri and authenticated from BSI, Regional office, Western circle, Pune-01 (BSI /WRC/Cert./2015). The shoot at flowering, before flowering, after flowering and the seeds to raise the seedling plants for analysis were also obtained from the above mentioned source.

The seeds of *Caesalpinia bonducella* (L) Roxb. were washed thoroughly with running tap water followed by labolene for 5 minutes. Further washing was done with running tap water for 30 minutes with frequent stirring to remove the traces of detergent trapped on the seed coat. The seedling plants of 30, 60, 90 and 120 days old were obtained from the acid scarified seeds. The shade dried seedling plants of 30, 60, 90, 120 days, shoot before flowering, at flowering and after flowering were pulverized and used to carry out phytochemical experiments.

### B) Extraction and quantification of bonducellin:

#### i) Preparation of standard solution

The bonducellin standard was procured from Bio Bio Pha. A stock solution was prepared by dissolving 1 mg of standard bonducellin in 1 ml of HPLC grade ethanol. The solution was then stored in 4° C in refrigerator. The standard solution was centrifuged twice before HPLC. The standard solution was run trice to check the repeatability and precision of results. The standard curve was prepared by taking 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm of standard solution of bonducellin.

#### ii) Preparation of sample solution

Samples were prepared by using 1g of *in vivo* 30, 60, 90, 120 days old seedling plants; shoot at flowering, before flowering after flowering and *in vitro* plants by soaking separately in ethanol for 24 hours. The ethanolic extract was filtered and centrifuged at 5000 rpm for 10 minutes. The supernatant was transferred in to ependoffs tube and the residue was re extracted thrice with ethanol. There after the residue was filtered and supernatant was evaporated to dryness in a rotatory evaporator at 40 ° C. The ethanolic extract thus obtained was used for HPLC analysis with solvent system methanol and mille Q water. The quantification of bonducellin was done by calculating the area of standard and the area of each sample.

### C) HPLC Analysis

The facilities for HPLC was provided by NCL Venture Center, 100, NCL Innovation Park, Dr. Homi bhabha Road, Pune 411 008, India. HPLC analysis of the sample was carried out with the ethanolic extract of samples. HPLC was performed on the instrument Shimadzu LC - 2010 HT and the software used was LC solution. The mobile phases for bonducellin elution was mille Q water and Methanol, at a flow rate of 0.50 ml/ min with other samples and the UV detection was done in 369 nm at a temperature 30°C. The standard was prepared by dissolving

1 mg bonducillin in 1 ml ethanol. The authenticity of standard was confirmed by noting the retention time provided along with the samples. The validation of quantitative method was done for each sample in three replicates. The standard was run along with samples each time. The accuracy was confirmed by running the standard after every five samples. The profiles of the standard and extracts were compared on the basis of retention time. The ethanolic extracts of 30, 60, 90, 120 days old seedling plants, shoot of *Caesalpinia bonducella* (L) Roxb. at flowering, before flowering and after flowering along with *in vitro* samples were used for HPLC analysis. The area of the peaks were recorded and compared with that of standard bonducillin. The amount of bonducillin was thus calculated for different samples used for the study.

#### D) Statistical Analysis:

Each experiment was repeated thrice. All the investigated parameters were analyzed using ANOVA. The level of significance of the experiments was determined at  $P - \text{value} < 0.05$ . Variability in data has been expressed in terms of mean  $\pm$  standard error.

#### Results and conclusion:

The samples of *Caesalpinia bonducella* (L) Roxb. viz. seed coat, seed kernel, shoot at flowering, shoot after flowering, 30 days old seedling plants, 60 days old seedling plants, 90 days old seedling plants and 120 days of the seedling plants were subjected to HPLC for quantification of bonducillin. Among the different mobile phases used for HPLC, Methanol: Mille Q water (1: 1) was found to be more suitable for the quantification of bonducillin. The standard sample of bonducillin 1 mg/ ml in methanol was analyzed for five times and prepared a standard curve.

For the calculation of the amount of bonducillin in the samples following values were considered -

1. Retention time of pure bonducillin.
2. Retention time of compounds in samples which showed the same retention time.
3. Peak area of bonducillin.
4. Dry weight of the plant material used to prepare the extract.

1 ml of extract was dissolved in methanol and used for HPLC analysis. The amount of bonducillin in 30 days old seedling plants was  $0.003 \pm 0.06 \mu\text{g/g DW}$  While it was  $0.002 \pm 0.74 \mu\text{g/g DW}$  in 60 days old seedling plants. The seedling plants of 90 days old produced  $0.001 \pm 0.32 \mu\text{g/g DW}$  while it was  $0.002 \pm 0.93 \mu\text{g/g DW}$  in 120 days old seedling plants. The extract of shoot before flowering did not showed the presence of bonducillin. The shoot extract of plant at flowering and after flowering showed  $0.013 \pm 0.40 \mu\text{g/g DW}$  and  $0.013 \pm 0.40 \mu\text{g/g DW}$  bonducillin respectively(Table – 1). Among the plant organs used for the quantification of bonducillin only seed coat and seed kernel showed  $0.001 \pm 0.31 \mu\text{g/g DW}$  and  $0.004 \pm 0.27 \mu\text{g/g DW}$ . Bonducillin was absent in other plant organs.

HPLC analysis showed maximum amount of bonducillin  $1.834 \pm 0.04 \mu\text{g/g DW}$  in the flowers of *Caesalpinia bonducella* (L) Roxb. followed by callus obtained from 1 mg /l 2, 4-D which produced  $0.083 \pm 0.44 \mu\text{g/g DW}$  bonducillin. The use of flower for the extraction of bonducillin was not considered as it might lead to the overexploitation of flowers for the extraction of bonducillin. The callus obtained from leaf explant on MS medium supplemented with 1 mg /l 2, 4-D was selected for the elicitation studies of bonducillin with a perspective to find out the potential to enhance the amount of bonducillin. This would help to conserve the natural population of *Caesalpinia bonducella* (L) Roxb. Thus the important findings of our HPLC analysis are as follows:-

1. The biosynthesis of bonducillin might be responsible for the flowering as it was absent in shoot before flowering and in different plant organs of mature plant like root, stem, leaf and pod.
2. Our study revealed the significance of different developmental stages of *Caesalpinia bonducella* (L) Roxb. in the synthesis of bonducillin.

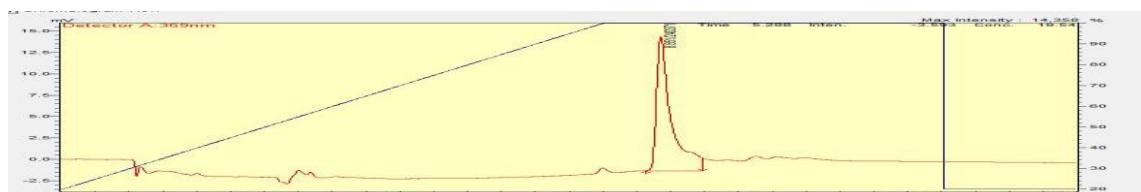
Table – 1: The production of bonducillin in different developmental stages of *Caesalpinia bonducella* (L) Roxb.

Sr. No.	Developmental stages	Amount of bonducillin ( $\mu\text{g/g DW}$ )
1.	30 days old seedling plant	$0.003 \pm 0.06$
2.	60 days old seedling plant	$0.002 \pm 0.74$
3.	90 days old seedling plant	$0.001 \pm 0.32$
4.	120 days old seedling plant	$0.002 \pm 0.93$
5.	Shoot before flowering	–
6.	Shoot at flowering	$0.013 \pm 0.40$
7.	Shoot after flowering	$0.013 \pm 0.40$

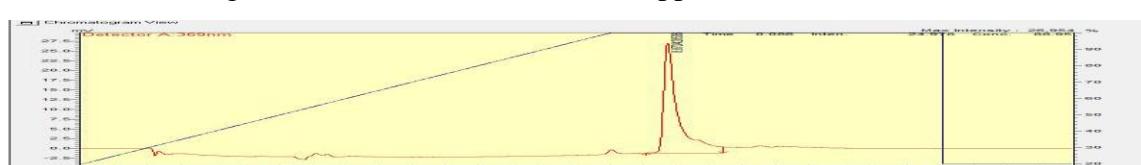
The values represented as the mean  $\pm$  SE calculated on three independent experiments. The p - value was  $< 0.05$  in all experiments.

#### I. HPLC chromatograms of Standard bonducillin:

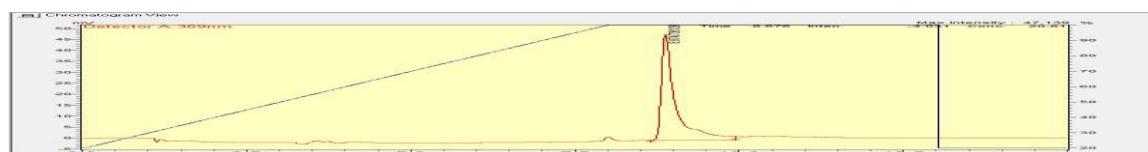
HPLC Chromatogram of Standard bonducillin (5ppm).



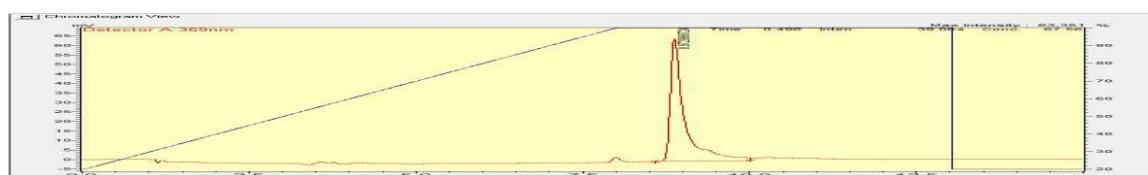
HPLC Chromatogram of Standard bonducillin (10ppm).



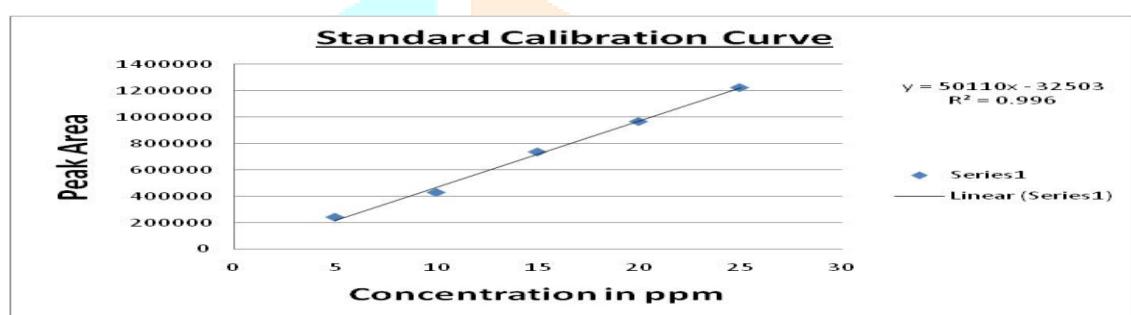
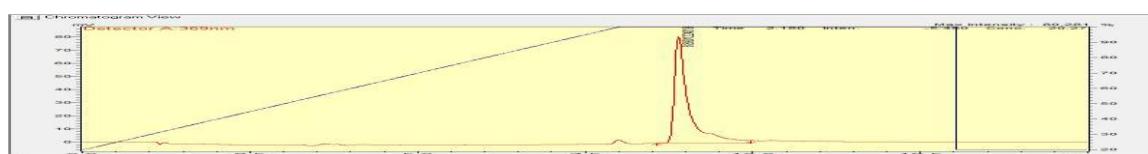
HPLC Chromatogram of Standard bonducellin (15ppm).



HPLC Chromatogram of Standard bonducellin (20ppm).

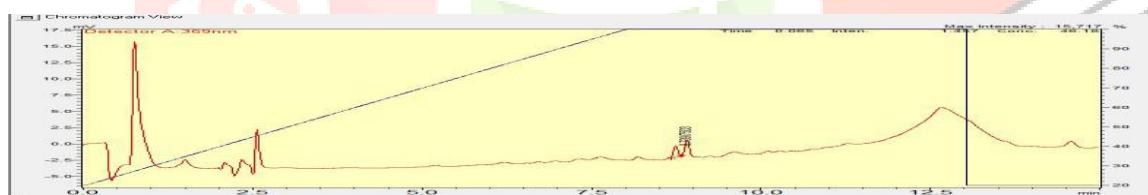


HPLC Chromatogram of Standard bonducellin (25ppm).

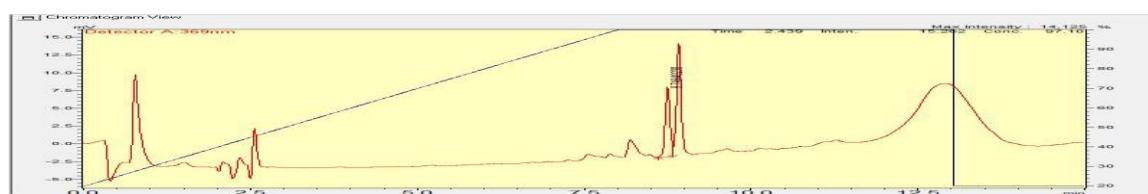


II. HPLC chromatogram of different plant organs of *Caesalpinia bonducella*(L) Roxb.:

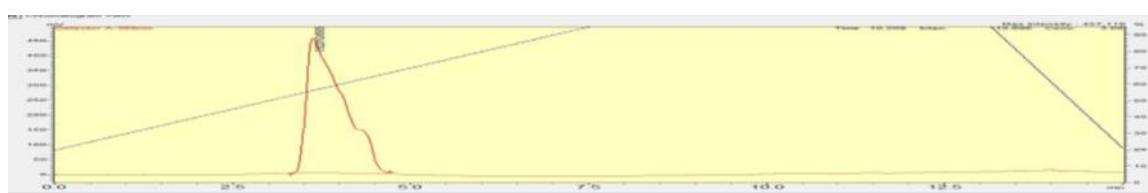
HPLC Chromatogram of Seed coat .



HPLC Chromatogram of Seed kernel.

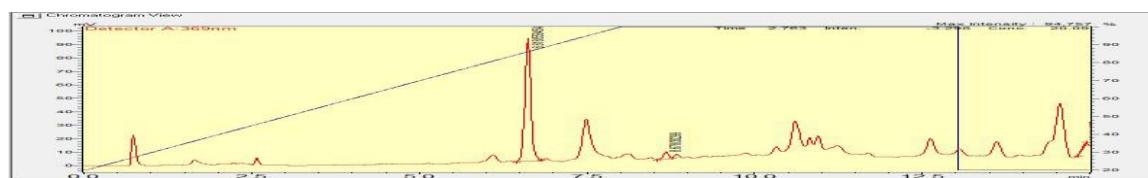


HPLC Chromatogram of flower.

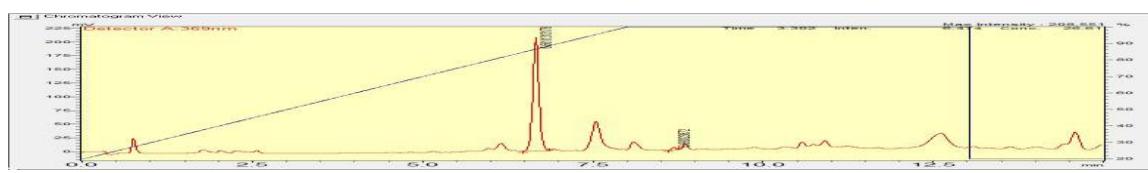


### III. HPLC chromatogram of different developmental stages of *Caesalpinia bonducella*(L) Roxb.:

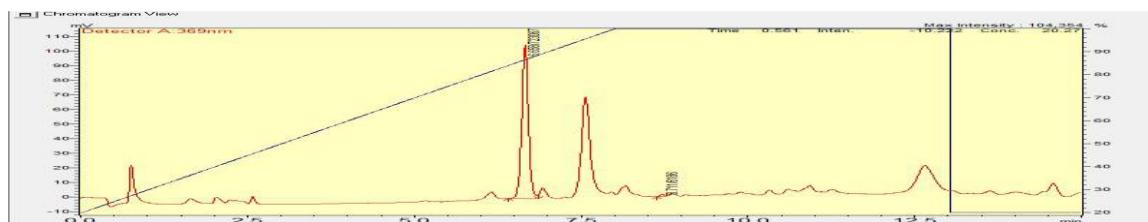
HPLC Chromatogram of 30 days old Seedling plant.



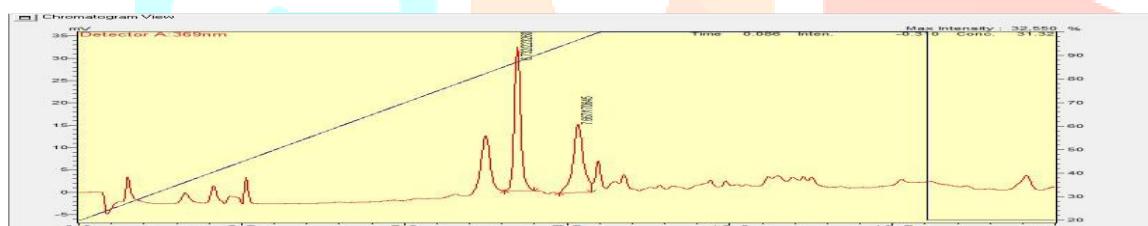
HPLC Chromatogram of 60 days old Seedling plant.



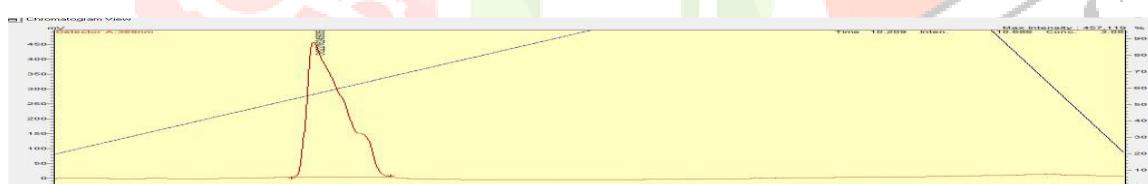
HPLC Chromatogram of 90 days old Seedling plant.



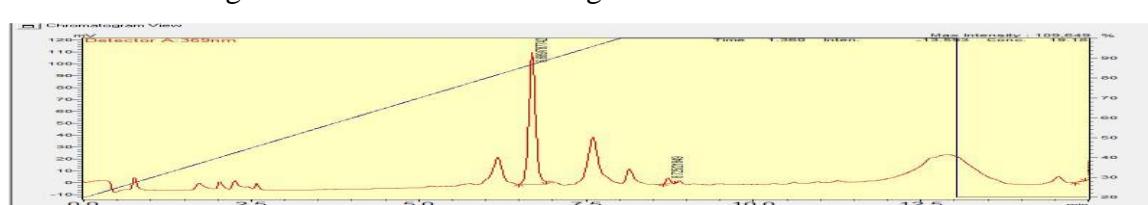
HPLC Chromatogram of 120 days old Seedling plant.



HPLC Chromatogram of shoot at flowering.



HPLC Chromatogram of shoot after flowering.



In conclusion, the extraction method of bonducillin was designed with reference to the earlier studies in the extraction of flavonoids and quantification of bonducillin was done with HPLC. The present study revealed that the plant organ, seed kernel and the shoot at flowering can be selected for the extraction and quantification of bonducillin from *Caesalpinia bonducella* (L.) Roxb. From the literature review of *Caesalpinia bonducella* (L.) Roxb., the plant species under study it appears that the flavonoid - bonducillin have not been quantified in different

plant organs and developmental stages before and are reported for the first time. Thus it can be useful in the industrial production of bonducillin without disturbing the natural population of *Caesalpinia bonducella* (L.) Roxb., as it an endangered medicinal plant.

## References:

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