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ANATOMICAL AND ANTI-MICROBIAL SCREENING OF CASSIA SENNA AND CASSIA OBOVATA

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Introduction

Medicinal plants have been used in Indigenous medicine for thousands of years. In recent years there has been a tremendous range of interest in the medicinal plants especially those used in indigenous system of medicine. Drugs obtained from plants are believed to be much safer and exhibit a remarkable efficacy in the treatment of various ailments. Siddha system is one of the indigenous medical system. In the siddha system chronic diseases can be treated successfully rather than the acute conditions. Skin diseases are the common problem in the community. The medicinal plants which possess blood pacificator, antiseptic, disinfectant, and anti purities pharmacological actions are used in treatment of skin diseases. Two different plants belonging to family Leguminosae are used as a main ingredient in Siddha herbal drugs used for skin diseases. Normally two different species are identified by the name of "Nilavarai" according to siddha practice in Sri Lanka. In Sri Lanka *Cassia obovata*(KaadduNilavarai) is commonly identified as Nilavarai. Another species *Cassia senna*(Naaddu Nilavarai)genuine species, imported from India is commonly identified as Nilavarai. As both plants are used to cure skin diseases in the name of Nilavarai, a comparative anatomical and antimicrobial study was carried out to see whether there is any anatomical difference and antibacterial property in these two plants.

Aim

- To find out the anatomical and antimicrobial screening of Cassiasenna and Cassia obovata.

Justification

- This research will help promote cultivation and utilization of effective species in Sri Lanka.

Literature Review

Cassia senna, a branched, erect shrub, up to 1.8m in height, introduced into India, naturalized in some parts; also cultivated. Leaves pinnate,pubescent, leaflets pale green to bluish green, 3-9pairs, lanceolate or elliptic, varying on the same plant, 1.5-5.0cm x 0.4-2.0cm.

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Flowers brilliant yellow, in erect terminal racemes; pods light green when young to dark brown or black when mature, flat, thin, oblong, pubescent, 3.5-7.0cm x 0.2-2.5cm; Seeds dark brown, obovate-oblong, The crop can be grown both as a dry and wet crop. In Tamil Nadu, the season for the unirrigated crop starts from July and for the irrigated one from December after the north-east monsoon.The unirrigated crop grows either as the main rainy season crop from the month of july or in the later monsoon from September onwards. The spring- sown(March- April) crop is reported to yield better-quality seeds. Usually seeds at dough stage are collected for sowing. The seeds are small and 14,000 to 35,000 seeds weight to a Kg. The rate of seed employed for sowing under the rain-fed conditions is 10-27 Kg/ha and 7-15Kg/ha under irrigated conditions. The seeds are reported to have environment controlled dormancy and also inhibitions of germination; hence they are soaked in water for 10-12hrs. The swollen seeds are separated for sowing to ensure highest germination. The young leaves and 3-5days old pods contain high percentage of sennasoides (6.93 and 11.9% respectively). The physiological stage of the plant also affects the quality of the harvested leaves. If the harvesting is started after 50 days and subsequently continued at 70 and 90 day intervals, the yield is poor but the quality is reported to be high with 3.3-3.4% sennasoides.The leaves are picked once in 15-20days. Eight to ten pickings can be obtained under very favourable conditions.When the crop is 50-70days old, the first harvest is generally carried out, the second between 90 and 110days, and the third after 130days. In the third picking, the pods are also harvested before attaining maturity, and are still green. A second crop of pods can also be obtained at the end of the season when the plants are uprooted in August or during April – May. The method of harvest for both the dry and irrigated crop is almost similar in Tamil Nadu. The dry crop is harvested thrice during Oct-Dec. When planted after rains, i.e during Dec-Jan, the first picking is in March and others before May; more pickings can be taken later in Oct- Dec. The young and immature leaves and pods contain more sennasoids than mature ones. The flowers show a higher concentration than the leaves. The concentration of sennasoides is reported to be more in the shells of the pod. The concentration of sennasoides also varies in the leaves and pods according to the stage of maturity and collection. The midrib, stem, peduncles, flowers and roots which are rejected, possess considerable quantities of anthroquinones.In tissue culture experiments it was found that the maximum synthesis of sennasoides occurs in callus at the time of rapid growth of cells. The leaves in the form of Confection of senna are used externally used for certain skin diseases, and the powdered leaves in vinegar are applied to wounds and burns, and to remove pimples. and bronchitis, and probably leprosy. Cassia obovata, a semi shrubby perennial, sometimes an annual with erect, smooth, pale green, zigzag stems and spreading,terete or angular branches. Leaves alternate, abruptly pinnate. Petiole without a gland, linear lanceolate, leaflets opposite in 3-6pairs nearly sessile. Flowers irregular, bisexual, bright yellow, large stalked, arranged in few flowered, orange colored, petals 5, ovary superior. Fruit pod 3.7 cm long 1.5cm broad, shortly stalked, flat,oblongreniform. Seeds about 10, Obovate, wedge shaped in outline, scantily albuminous. The leaflets and pods contain oxymethylanthraquinone (Chrysophanic acid). An infusion of the leaves and pods is used as a purgative.An infusion of the root in milk as a cure for influenza.Cassia with high levels of anthraquinones and chrysophanic acid are very effective inhibitors of skin fungus, mite infestations, bacterial and microbial diseases.Also used to treat eczema, itching and skin infections in human.

Cassia obovata

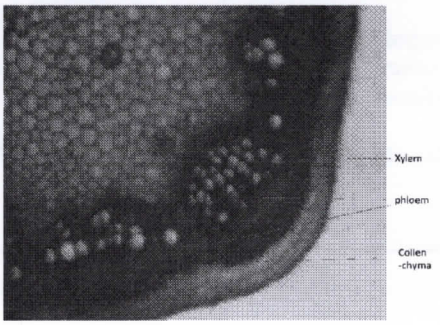
Cassia senna

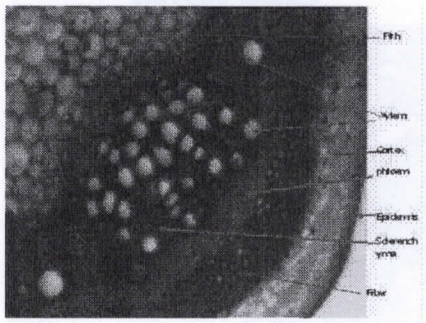
Materials and methods

Anatomical study

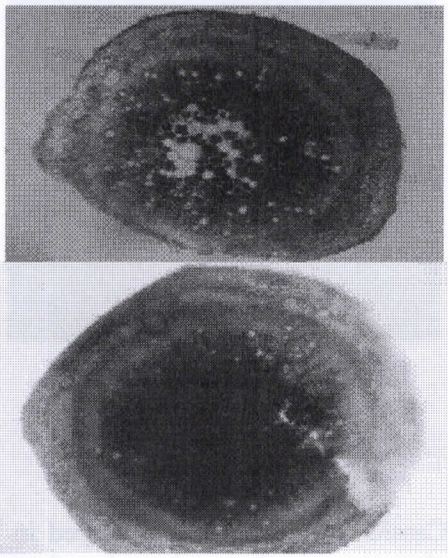
Thin section of the stem of Cassia spwere taken and placed in water in a watch glass, Thin transparent sections were selected and observed under the medium power of the light microscope.One good section was selected and treated with 25% alcohol. Then it was treated with 50% alcohol. The section was stained with saffrannin. Excess stain was removed by washing with 50% alcohol. It was mounted in 50% glycerine and observed under the microscope. Another section was stained with KI/I_2 and observed under the microscope.

Transverse Section of Stem of Cassia Obovata





Transverse section of stem of Cassia senna



Cassia senna	Cassia obovata
Similar characters	
• Dicot • Vascular bundles are arranged in a regular manner in the cortex. • Difinite pith is found. • Collenchyma is found below the epidermis.	• Dicot • Vascular bundles are arranged in a regular manner in the cortex. • Difinite pith is found. • Collenchyma is found below the epidermis.

Different characters	
• Starch grains in the centre of the stem. • Starch grains are found in the pithparenchyma. • High density of starch grains.	• Starch grains are not found. • Starch grains are not found in the pith parenchyma.

The starch is amply available in the center part of the stem of Cassia senna. This is a specific anatomical character of Cassia senna.

Antimicrobial study

Cassia obovata (KaadduNilavarai-KN) and Cassia senna (NaadduNilavarai -NN) leaves were shade dried, ground and made into fine powder. 20 g of each powder was soaked in 60 ml sterile distilled water and ethanol separately through double layered muslin cloth and further filtered through Whatman No 1 filter paper into Mac Cartney bottles. This procedure was repeated twice and all the water extracts and ethanol extracts were collected separately and dried in an oven at 45 C until the solvent was removed. For the antibacterial screening Nutrient agar plates were freshly prepared and spreed plates of diff. bacteria Pseudomonas aerogenosa, E.coli,Bacillus sp., Klebsiella sp., and Proteus vulgaris were prepared by spreading 0.1 ml bac. Suspension ($1x10^6$ /ml). 6mm diameter wells were cut on the bacteria culture plates. The dried water and ethanol cold extracts were dissolved in water and ethanol respectively. Then wells were filled with 100µl of (150 mg) cold extracts separately save volume of Streptomycin (50µg/100µg) was used as positive control distilled water and ethanol were used as negative control: Plates were incubated at 37 °C for 24 hours and the zones of inhibition in different bacterial plates were measured including the well diameter of 6mm and the results were subjected to ANOVA followed by LSD test.

Treatment	Pseudomonas sp.	E.coli	Bacillus sp.	Klebsiella sp.	Proteus sp.
Streptomycin	28.66 ± 0.57^{a}	30.33 ± 0.57^{a}	34.00 ± 0.00^{a}	34.66 ± 0.57^{a}	35.00 ± 0.00^{a}
KN water	12.00 ± 1.00^{c}	13.00 ± 0.00^{d}	24.66 ± 0.57^{c}	14.66 ± 0.57^{c}	9.66 ± 0.57^{d}
KN ethanol	13.33 ± 0.5^{b}	14.00 ± 1.00^{c}	23.00 ± 1.73^{d}	19.66 ± 0.57^{b}	16.00 ± 1.00^{c}
NN water	6.00 ± 0.00^{d}	16.66 ± 0.57^{b}	27.66 ± 0.57^{b}	6.00 ± 0.00^{dc}	17.66 ± 0.57^{b}
NN ethanol	13.33 ± 0.57^{b}	13.33 ± 1.52^{cd}	15.66 ± 0.57^{e}	9.00 ± 0.00^{d}	15.66 ± 0.57^{c}

Values are mean ± SD

Values with deferent superscript on the same column are significantly ($p<0.05$) different.

All the bacteria were highly inhibited by standard antibiotic Streptomycin which is very much higher than the inhibition by other extracts tested and differently inhibited by different extracts.