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MICROSCOPICAL STUDY AND QUANTITATIVE ESTIMATION OF STEROIDS IN SOYMIDA FEBRIFUGA LEAVES.

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ABSTRACT

The selected plant was reported to have wide ethnomedicinal use. The literatures revealed that there is lack of scientific reports on its leaf. So it is important to provide scientific means in a systematic manner. The present study mainly focuses on the ethnomedicinal importance of *Soymida febrifuga*. The ethnomedicinal documentation confirms about the potent activity of the leaf part of *Soymida febrifuga*. The present study provides evidence that For steroids, pet. Ether extract contains 22.46% and chloroform 23.97% steroids results obtained by Liebermann-burchard color reaction. The detailed microscopy reveals anomocytic stomata, spherical peltate scales, cuticle striations, stomatal index and palisade ratio etc. as identifiable characters.

KEYWORDS: *Soymida febrifuga,* steroids, microscopy.

INTRODUCTION

Soymida febrifuga A. Juss is a tall tree. Leaves 23-45cm. long, crowded towards the ends of the branches; leaflets 3-6 pairs,opposite,5-11.3 by 2.5-6.3 cm., elliptic or oblong, obtuse, glabrous penninerved, the nerves numerous and conspicuous beneath, base rounded, inequilateral, the lower side generally extending further down the petiolule than the upper; petiolules 3-6mm. long. Flowers in large terminal or axillary divaricately branched panicles often equaling the leaves, the branches of the panicle alternate; pedicels very short; bracts minute, triangular, acute. Sepals 5, rotund, the margins membranous, slightly lacerate. Petals 5, obovate, 6mm. long, clawed, often notched at the apex. Staminal tube about half as long as the petals, slightly urceolate; anthers attached by the middle of the back. Ovary glabrous;

stigma, discoid, 1.5mm. diam., 5-lobed, the lobes radiating to the centre. Capsules 2.5-6.3 cm. long, obovoid, 5-celled, 5-valved.Seeds winged. Distributed in Dry forests of the W.peninsula, extending northwards to merwara, the mirzapur hills and chota Nagpur, Ceylon. Chemical constituients –Bark contains bitter substances. Lupeol, sitosterol, methyl angolensate, deoxyandirobin from wood bark.Leaves contains Quercetin-3-O-L-rhamnoside, 3-O-rutinoside from leaves. Three tetratriterpenoids from fruits of soymida febrifuga epoxyfebrinin B, 14, 15 – dihydroepoxyfebrinin B,febrinolide. Properties and uses the bark is acrid; refrigerant, anthelmintic, aphrodisiac, laxative; good for sore throat; removes "vata " cures "tridosha" fevers, cough, asthma; removes blood impurities; good for ulcers, leprosy, dysentery (Ayurveda). The bark is the bowels and useful in fevers in fevers (Yunani). The bark is astringent, tonic and antiperiodic. In intermittent fevers and general debility, in the advanced stages of dysentery, in diarrhea, and in other cases requiring the use of astringents, it has been used with success. The decoction forms a good substitute for oak-bark and is well adapted for gargles, vaginal infections and enemas. The bark of this tree is said to be a bitter tonic and a good antimalarial like cinchona. A decoction of the bark 1 in 20 was given in one ounce doses three times a day in cases of malarial fever and found to be beneficial. The action was not only very slow but very inferior to that of the alkaloids of cinchona (Koman).

Plant material and extraction a) Plant Material: Fresh leaves of *soymida febrifuga* collected in the month of August to September from Amravati District, Maharashtra. A voucher specimen was botanically authentified by Mrs.P.Y. Bhogaonkar head Botany Department, Vidarbha Institute of Science and Humanities College Amravati & deposited in the herbarium. The fresh leaves were dried in a hot air oven for 24 hr. at 55^{o}C under shed & powder in a mixture grinder. The powder sieved (40 mesh) leaves packed in a paper bags & stored in air tight container until use.

b) Extraction was carried out by solvent extraction-50 gm of dry powder was extracted with 200 ml of solvent by Soxhletion for 20 cycles for Pet. ether, chloroform, methanol and water. And also the total aqueous extract was obtained.

Determination of total steroids
(Liebermann-Burchard color reaction)

Reagents

1. Ethanol:Ether Mixture: Mix 3 volumes of ethanol and 1volume of ether (Bloor's reagent).

2. Acetic anhydride-Sulphuric acid reagent:Mix 20ml acetic anhydride with 1ml conc. Sulphuric acid (AR).Prepare fresh just before use.
3. Chloroform
4. Cholesterol std.(1mg/ml):Dissolve 100mg of cholesterol in chloroform and make up to 100 ml with chloroform in a standard flask. Mix, keep well stoppered.
5. Cholesterol (working std)(0.2mg/5ml):Dilute 4 ml stock std to 100 ml in a standard flask, with chloroform.

Method

In a glass stoppered conical centrifuge tube about 5ml ethanol:ether mixture was taken. 1 ml sample was addaed stoppered and mixed vigorously for 1 minute. The tube were left in a slanting position for 30 minutes for the ppt. to settle centrifuge. The supernatant was decanted into a 25ml dry beaker completely. Chloroform was evaporated to dryness at room temperature. 5ml chloroform was added and the residue was dissolved.

Transfered the contents to a dry tube marked T.

In a dry test tube marked S, 5ml working STD was added.

In a third dry test tube marked B, 5ml chloroform was taken.

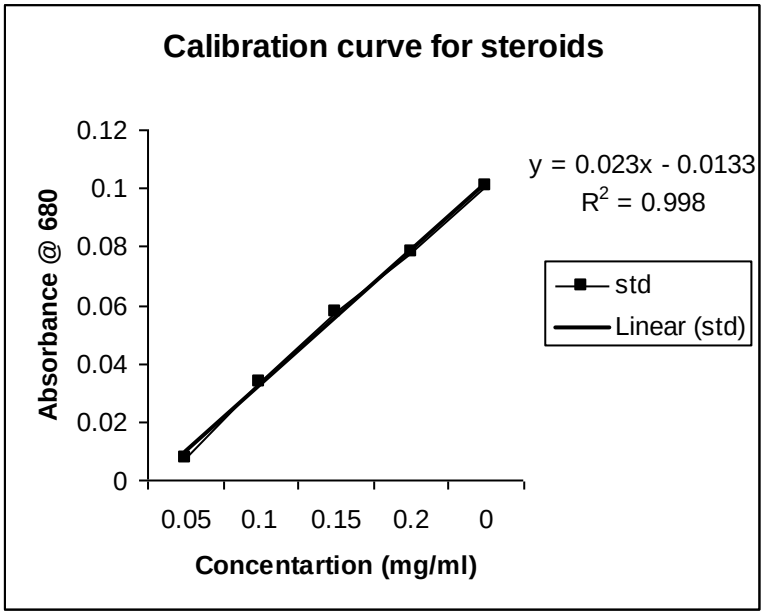
To each 2ml mixture of acetic anhydride sulphuric acid (freshly prepared) was added and mixed. The tubes were kept in dark for 15mins for color development.

The emerald green color formed was measured at 680nm with the reagent blank at 100% T.

RESULT AND DISCUSSION

Table no 1: Standard curve of Cholesterol at 680 nm.

Sr. No.	Conc.(mg / ml)	Absorbance
1	0.40	0.00781
2	0.08	0.03381
3	0.12	0.05786
4	0.16	0.07837
5	0.20	0.10059



Graph No.1: Calibration curve of cholesterol @ 680nm

Table no: 2. Results of Total steroid content (%) in each extract.

Extract	Abs. at 765 nm	% Content	Mean (%) ± SD
Pet ether	0.0903	22.44	22.46±0.023
	0.0905	22.48	
	0.0905	22.48	
Chlorofom	0.1000	24.00	23.97±0.020
	0.0998	23.96	
	0.0998	23.96	

Microscopic study

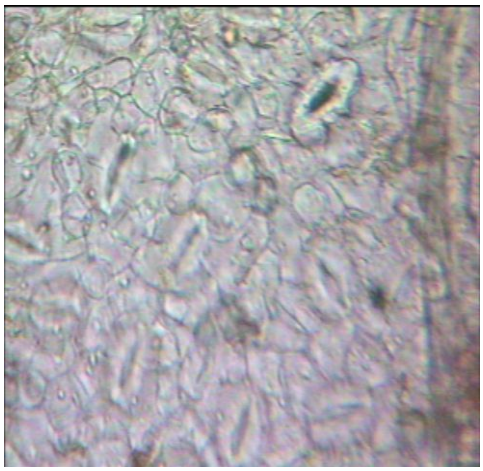
Procedure for surface study

A leaf whose stomata has to be observed peel off a thin section from the leaf. Clean the thin section by warming it slightly with chloral hydrate to remove the epidermal part. Wash the section place it on glass slide & place a drop of glycerine on it. Put a coverslip & observe the stomata and other characteristics.

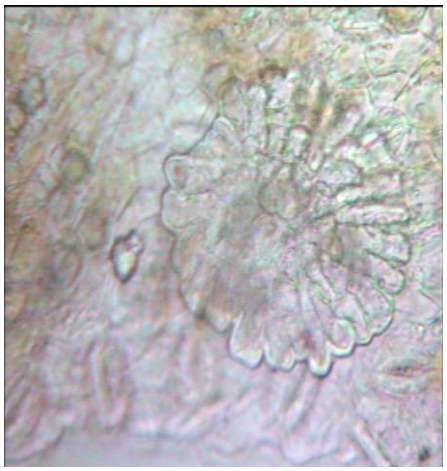
Procedure for Transverse section

Take a leaf give a rectangular cut part through the midrib. Take thin transverse section of the leave, put the section in water than in bleeching solution for 2 mins. Transfer to staining solution(1:1 phloroglucinol:HCl) for 2 mins. Mount the section on slide with help pf glycerin, put coverslip and odserve under microscope.

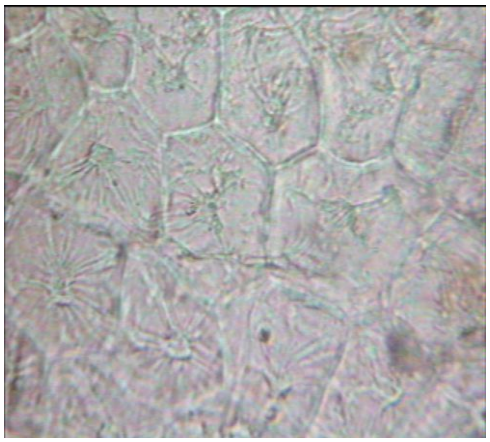
Microscopic study



Anomocytic stomata

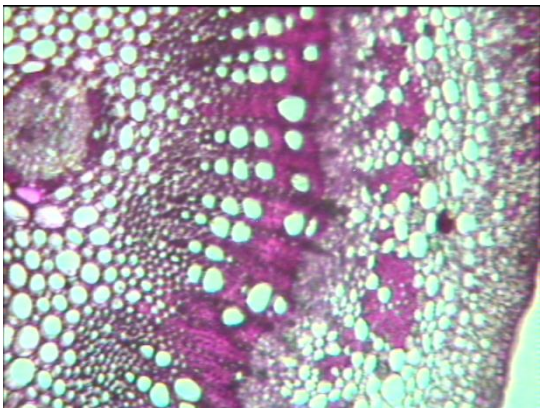


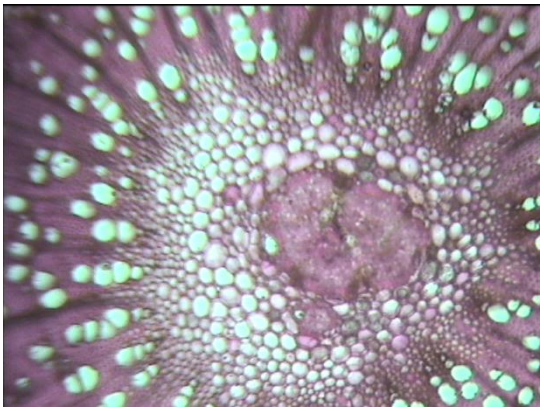
Spherical peltate scales



Cuticle striations

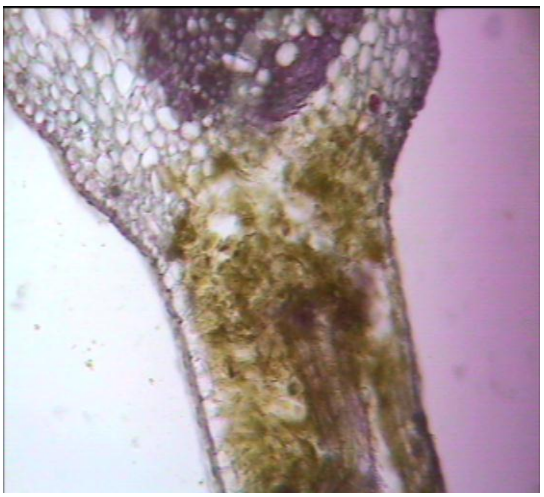
- Cuticle with prominent striations
- Cuticle striations forming more uniform pattern with centre elevation and striations radiating from it
- Vein cells with oil globules Scales and spheraphides absent

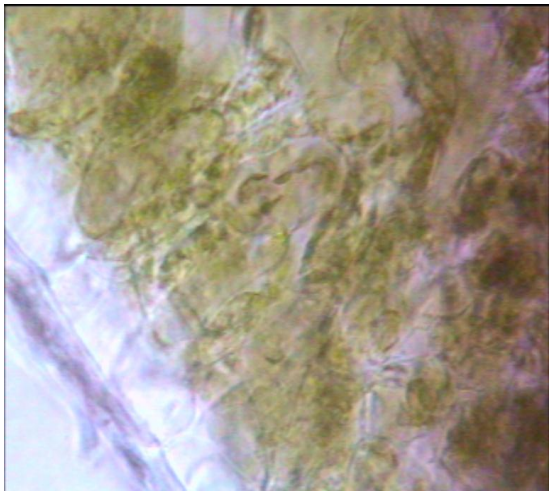




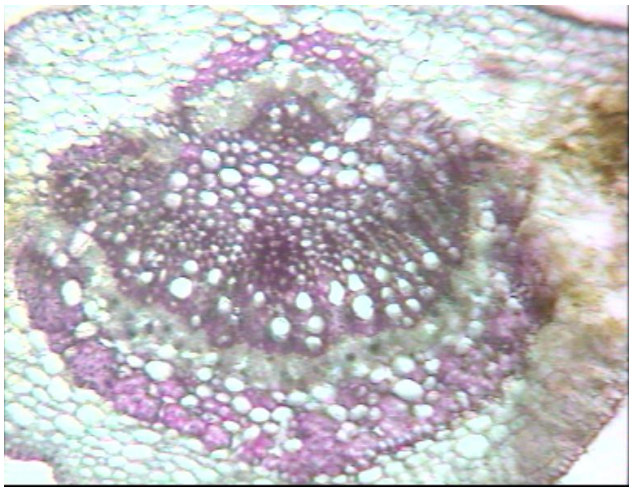
- Petiole

– Roughly trigonal in transverse section
– Epidermis single layer followed by narrow zone collenchymatus hypodermis
– Cuticle thick
– Brown tissue collenchyma
– vasculature in form of roughly trigonus ring
– Secondary growth takes place to some extend producing a continuous cylinder of xylem and phloem outside vascular cylinder .several patches of fibers simulating the pericyclic zone present
– Pith- Parenchymatus, medullary vascular bundles present in pith
– Medullary vasculature some what irregular in orientation
– Bundles concentric emphphollic or with irregular orientation





- Lamina
- Dorsiventral, hypostomatus .
- Palisade-1,2 layered followed by spongy parenchyma
- Intracellular spaces in spongy tissue large



Midrib

- Epidermis with thick cuticle follwed by board collenchymatus zone
- Brown parenmchyma few layered
- Vasculature in form of a shallow arc of xylem and phloem encircled by fibrous pericyclic zone
- Ambiting the protoxylum pole of main xylem arc inversely oriented vascular bundle present

CONCLUSION

1. Microscopy of leaves of *Soymida febrifuga* shows microscopic characters from anomocytic stomata, spherical peltate scales, cuticle striations, stomatal index and palisade ratio etc. as an identifiable characters.
2. For steroids, pet. Ether extract contains 22.46% and chloroform 23.97% steroids results obtained by Liebermann-burchard color reaction.

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