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Pharmacognostic Evaluation and Phytochemical Investigation of Stem Bark of Nothopegia Racemosa (Dalz)

Poornima.C.H[*1], Rajesh Shastri[2], Prasanna Habbu[2], G.S. Hiremath[3], Y. Srinivas[1]

[1]Department of Pharmacognosy, BVVS Hanagal Shri Kumareshwar College of Pharmacy, Bagalkot, 587101 Karnataka, India

[2]Postgraduate Department of Pharmacognosy and Phytochemistry, SET's College of Pharmacy, S.R. Nagar, Dharwad-580 002, Karnataka, India

[3]Department of Pharmacology, BVVS Hanagal Shri Kumareshwar College of Pharmacy, Bagalkot, 587101 Karnataka, India

***Correspondence Author:**

Poornima Hosamath,

Research Scholar, Department of Pharmacognosy and phytochemistry, BVVS Hanagal Shri Kumareshwar College of Pharmacy, Bagalkot -587101, Karnataka, India

Email ID: pornimaraj.06@rediffmail.com

ORC ID: 0000-0001-7189-1216

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ABSTRACT

Nothopegia racemosa **(Dalz)**. (Anacardieae) a Small trees, up to 7 m tall, generally found in Karnataka and other tropical regions. Traditionally the plant fruits are used to prepare pickles. Scientifically the plant possesses hepatoprotective, antioxidant, and lipid lowering activity. In the present study systematic Pharmacognostic work this includes Organoleptic, Macroscopy, Microscopy and Phytochemical screening was carried out.

Methods: The stem bark of plant *Nothopegia racemosa.* Dalz. Were collected from Dandeli, Tinaighat , Malsang forest area Karnataka and authenticated by Taxonomist. Washed stem bark were Shade dried and powdered to courser size and store in air tight container. Organoleptic, macroscopic and microscopic characters were studied. Extractive values, moisture content and ash values were carried out, with powdered crude drug were subjected to successive soxhlet extraction with Pet ether, Ethyl acetate, Ethanol and Aqueous extraction. Phytochemical screening of all these extracts were carried out.

Results: Microscopically the TS of leaves and stem bark show the presence of stomata, xylem vessels, ca-oxalate crystals, cork, cortex, phloem, pith etc. These diagnostic features of the plant will help in the identification and authentification process of the drug. Preliminary Phytochemical Investigation of the successive extract of the stem bark revealed the presence of tannins, Polyphinolic compounds, steroids, flavonoids, glycosides.

Conclusion: The present study enabled us to set up the parameters for standardization of the drug. The results of the investigations justify the folklore use of *Nothopegia racemosa* **(Dalz)**.in the treatment of diseases of head, kideny and lever. The plant is worth for further chemical and pharmacological investigations.

Keywords: *Nothopegia racemosa, Pharmacognostic, Extractions, Phytochemical screening*

1. INTRODUCTION

The majority of Western Ghats plants include phytochemicals that are not well known and for which there is insufficient scientific data. However, due to a lack of understanding regarding their attractiveness and nutraceutical composition, these are not commercialized. The Pharmacognostic and phytochemical investigation of stem bark of plant *Nothopegia racemosa*. Dalz., is a member of the Anacardiaceae family.[1] Herbal medicine serves as the foundation for primary health care for approximately 75–80% of the global population, particularly in developing countries. This is attributed to its safety, efficacy, cultural acceptability, and reduced side effects. Ancient literature references herbal remedies for age-related ailments such as memory loss, osteoporosis, diabetic wounds, and immune and liver disorders, for which modern medicine offers limited options or only enhances the quality of life for patients. The production of these drug raw materials utilizes

eco-friendly processes, contributing to economic prosperity in the sector.[2] The significance of traditional medicinal systems surpasses that of archaeological or anthropological perspectives. According to the World Health Organization (WHO), the bioactive substances derived from these plants may be less harmful to humans, and the plant medications have the advantage of being used for a long time[3]. Plants have a variety of secondary metabolites, such as tannins, glycosides, alkaloids, and others. Because of this chemical diversity, plant metabolites have evolved to have contents that are either superior to or on par with those of synthetic drugs made in labs.[4,5] Interest in the study of ethnomedicine has surged, and its acceptance has grown in various regions globally during the past few decades. Secondary metabolites such as glycosides, alkaloids, tannins, and resins derived from medicinal plants are crucial in the creation of effective therapeutic medicines due to their low toxicity and cost feasibility[5-8].

Based on the literature survey and as ethnic medicines *Nothopegia racemosa* stem bark is used externally as an antimicrobial, anti-diabetic, anticancer, anti-inflammatory, and antioxidant.[9-12]

Figure:1 Nothopegia racemosa stem bark

There is no much research carried on ***Nothopegia racemosa*** stem bark. Many evidences are of therapeutic activities for the other species of the genus ***Nothopegia*** very limited literature is available on phytochemical profile of bark. Hence the present investigations aim to explore the Pharmacognostic Evaluation and Phytochemical Investigation of stem bark of *Nothopegia racemosa* (Dalz).

2. MATERIALS AND METHODS

The stem bark of plant *Nothopegia racemosa* (Dalz). Were collected from dandeli, Tinaighat , Malsang forest area Karnataka, and authenticated by taxonomist. The stem bark are collected and thoroughly washed with running water to remove the adherent impurities. Shade dried and powdered to courser size and store in air tight container. Organoleptic, macroscopic and microscopic characters were studied.[13] Extactive values, moisture content, ash values and foaming index were carried with powdered crude drug of *Nothopegia racemosa* (Dalz),[14] plant were subjected to successive soxhlet extraction with Pet ether, Ethyl acetate, Ethanol and Aqueous extraxts.[15] Qualitative chemical tests of all these extracts were carried out using different solvents.[16-19]

Morphological Features: [16,17]

Morphology is the study of the form of an object whilst morphography is the description of that form where the material is known to occur in a particular form. Morphological features and organoleptic features viz. color, odor, taste, shape and size were observed and evaluated botanically.

3. STUDY OF MICROSCOPICAL CHARACTERS: [16-19]

a. Transverse section of stem bark and Leaf of Nothopegia racemosa (Dalz).

Transverse section of stem bark and leaf was taken and observed under low & high power Microscope.

b. Powder microscopy

The dried stem bark of plant *Nothopegia racemosa* (Dalz). were coarsely powdered and boiled with chloral hydrate for 5-10 minutes and then stained with phloroglucinol and HCl in 1:1 ratio, observed under high power (40 x), for different diagnostic characters such as Cork & Cortex region (a) Starch grain, Ca-Oxalate crystal Prismatic (b) Medullary rays, (c) Compound Starch grains etc. .

4. PROXIMATE VALUES:

The following proximate values were determined for the powder drug of stem bark of plant *Nothopegia racemosa* (Dalz)

a. Extractive values: [19, 20]

The determination of Extractive values helps to determine the amount of soluble constituents in a given amount of medicinal plant material, when extracted with solvents.

The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of drug and solvent used. The use of single solvent can also be used by means of providing preliminary information of quality of a particular drug sample. There are two methods of determination of extractive value.

I. Cold maceration method

II. Hot extraction method

Alcohol soluble extractive value

Cold maceration method:

4g of shade-dried stem bark of plant *Nothopegia racemosa* (Dalz) powder was macerated with 100ml of 95% ethanol in a closed flask, shaking frequently during the first 6hrs and allowed to stand for 18hrs. Thereafter it was filtered rapidly taking precaution against loss of ethanol. Evaporated 25ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105^0C and weighed. Percentage ethanol soluble extractive was calculated.

II. Hot extraction method: 4g of shade-dried stem bark of plant *Nothopegia racemosa* (Dalz) powder was macerated with 100ml of 95% ethanol in a closed flask, shaking and allowed to stand for 1hr. attach a reflux condenser to the flask, boil it 1hr and cool. Thereafter it was filtered rapidly taking precaution against loss of ethanol. Evaporated 25ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105^0C and weighed. Percentage ethanol soluble extractive was calculated.

The above procedure was followed for Water soluble extractive value and for Ether soluble extractive value.

b. Moisture content:

Accurately weighed quantities of the shade-dried coarsely powdered stem bark of plant *Nothopegia racemosa* (Dalz). powder was taken in a tared glass bottle and the initial weight was taken. The crude drug was heated at 105^0C in an oven and weighed. This procedure was repeated till a constant weight was obtained. The moisture content of the sample was calculated as percentage with reference to the shade-dried material.

c. Ash values: [18-20]

- **Total ash**

2g of accurately weighed quantity of the shade-dried coarsely powdered stem bark of plant *Nothopegia racemosa* (Dalz). Was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled and weighed. The percentage of total ash was calculated with reference to shade-dried whole plant powder.

- **Acid-insoluble ash**

Total ash obtained was boiled for five minutes with 25 ml of dilute Hydrochloric acid. The insoluble matter was collected on an ash-less filter paper, washed with hot water and ignited, cooled and weighed. The percentage of acid insoluble ash was calculated with reference to shade-dried whole plant powder.

- **Water-soluble ash**

Total ash obtained was boiled for five minutes with 25ml of distilled water, cooled and collected the insoluble matter on an ash-less filter paper, washed with hot water and ignited for 15 minutes at temperature not exceeding 450^0C. Subtracted the weight of the insoluble ash. The percentage of water-soluble ash was calculated with reference to shade dried whole plant powder.

- **Sulphated ash**

Silica crucible is heated to redness for 10 minutes; cooled and weighed. 1 gram of air-dried leaf powder is placed in silica crucible, moistened with sulphuric acid, ignited gently, again moistened with sulphuric acid and ignited at about 800^0C. Cooled and weighed, once again ignited for 15 minutes and weighed. The percentage of sulphated ash was calculated with reference to air-dried whole plant powder.

d. Foaming index:[20-22]

The foam index is determined by measuring the height of the foam produced by the equivalent of

1 g of herbal drug or herbal drug preparation under the stated test conditions. Weigh accurately about 1 g of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml of boiling water maintain at moderate boiling at

80- 90 ^{0}C for about 30 min. Then make it cold, filter into a volumetric flask and add sufficient water through the filter to make the volume up to 100 ml (V1). Cleaned stopper test tubes 10 numbers are taken and marked with 1 to 10. Take the successive portions of 1, 2 ml up to 10 ml drug in separate tubes and adjust remaining volume with the liquid up to 10 ml in each test tube. After closing the tubes with stoppers, Shake them for 15 seconds and allowed to stand for 15 min. then measure the height. If the height of the foam in each tube is less than 1cm, the foaming index is less than 100 (not significant). Here, if the foam is more than 1cm height after the dilution of plant material in the sixth tube, then corresponding number of the test tube is the index sought. If the height of the foam in every tube is more than 1 cm, the foaming index is more than 1000. In this case, 10 ml of the first decoction of the plant material needs to be measured and transferred to a volumetric flask of 100 ml capacity (V2) and volume is to be maintained up to 100 ml and follow the same procedure. Foaming index is calculated by using the following formula,

Foaming index = 1000/a in case of V1

Foaming index = 1000 × 10/a in case of V2

Where, a = Volume (ml) of decoction used for preparing the dilution in the tube where exactly 1cm or more foam is observed

PHYTOCHEMICAL INVESTIGATIONS

Extraction:[17-20]

Successive Extraction:

70-80 g per batch of shade-dried stem bark of plant *Nothopegia racemosa* (Dalz)**.**Powder was successively extracted with petroleum ether (60-80^{o}C), ethyl acetate, ethanol and Aqueous in increasing order of polarity. The extracts were concentrated under reduced pressure using Rotary flash evaporator and the residues were dried in desecrator over Calcium carbonate. After drying, the respective extracts were weighed and percentage yield and their characteristic were determined.

Qualitative chemical identification:[20-22]

All the extracts of Stem bark of plant *Nothopegia racemosa* (Dalz) was subjected to qualitative chemical tests to detect the presence of various phytoconstituents.

Tests for Carbohydrates:

Molisch's test: Treat the extract solution with few drops of alcoholic □-napthol. Add 0.2 ml of concentrated H2SO4 slowly through the sides of the test tube, **purple to violet** colored ring appears at the junction.

Benedict's test: Treat the extract solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms if reducing sugars are present.

Barfoed's test: General test for monosaccharides: Heat the test tube containing 1ml reagent and 1 ml of extract solution in a beaker of boiling water; if red cuprous oxide is formed within two minutes, a

monosaccharide is present. Disaccharides on prolonged heating (about 10min) may also cause reduction, owing to partial hydrolysis to monosaccharides.

Selwinoff's test: Hydrochloric acid reacts with ketose sugar to form derivative of furfuraldehyde, which gives red colored compound when linked with resorcinol. Add extract solution to about 5 ml of reagent and boil. Fructose gives **red** color within half minute. The test is sensitive to 5.5 mmol/lt. if glucose is absent. If glucose is present it is less sensitive and on addition of large amount of glucose it gives similar color.

Fehling's test: Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed along with few drops of extract solution, boiled, a **brick red** precipitate of cuprous oxide forms, if reducing sugars are present.

Tests for Proteins & Aminoacids

Millon's Test: Extract solution + 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) **white precipitate** appears, which turns **red** upon gentle heating.

Ninhydrin Test: Amino acids and proteins when boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate), produces violet color.

Tests for Sterols and Triterpenoids

Libermann-Burchard test: Extract is dissolved in pet ether and treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added from the side of the test tube, and a **brown ring** at the junction of two layers and the upper layer turns **green** indicates the presence of sterols and formation of **deep red color** indicates the presence of

triterpenoids.

Salkowski's test: Treat extract in chloroform with few drops of concentrated Sulfuric acid, shake well and allow to stand for some time, red color appears in the lower layer indicates the presence of sterols and formation of yellow colored lower layer indicating the presence of triterpenoids.

Tests for Glycosides

Test I: Extract 200 mg of the drug by warming in a test tube with 5 ml of dilute (10%) sulphuric acid on a water bath at 100ºC for two minutes, centrifuge or filter, pipette out supernatant or filtrate. Neutralize the acid extract with 5% solution of Sodium hydroxide (noting the volume of NaOH added). Add 0.1 ml of Fehling's solution A and B until alkaline (test with pH paper) and heat on a water bath for 2 minutes. Note the quantity of red precipitate formed and compare with that formed in Test II.

Test II: Extract 200 mg of the drug using 5 ml of water instead of sulphuric acid, and boil on water bath. After boiling add equal volume of water to the volume of NaoH used in the above test. Add

1. 1 ml of Fehling's A and B until alkaline (red litmus changes to blue) and heat on water bath for two
2. minutes. Note the quantity of the red precipitate formed. Compare the precipitates of Test II with
3. Test I. If the precipitate in Test-I is greater than in Test-II, then Glycoside may be present. Since Test
4. II represent the amount of free reducing sugar already present in the crude drug, whereas Test-I
5. represents the Glycoside after acid hydrolysis.

Tests for Alkaloids

Mayer's test: (Potassium mercuric iodide solution): To the extract/sample solution, add few drops of Mayer's reagent, creamy white precipitate is produced

Dragendroff's test: (Potassium bismuth iodide solution) : To the extract/sample solution, add few drops of Dragendroff's reagent, reddish brown precipitate is produced.

Wagner's test: (Solution of Iodine in Potassium Iodide): To the extract/sample solution, add few drops

of Wagner's reagent, reddish brown precipitate is produced.

Hager's Test: (Saturated solution of Picric acid): To the extract/sample solution, add few drops of Hager'sreagent, yellow precipitate is produced.

Tests for Phenolic Compounds

Ferric chloride test: Extract solution gives **blue-green** color with few drops of Fecl3.

Shinoda Test (Magnesium Hydrochloride reduction test): To the extract solution, add few fragments of magnesium ribbon and concentrated Hydrochloric acid drop wise, yellowish; yellow- orange occasionally orange color appears after few minutes.

Zinc-Hydrochloride reduction test: To the extract solution, add a mixture of Zinc dust and concentrated Hydrochloric acid. It gives yellowish, yellow- orange occasionally orange color appears after few minutes.

Tests for Flavonoids

Shinoda Test (Magnesium Hydrochloride reduction test): To the extract solution add few fragments of magnesium ribbon and concentrated Hydrochloric acid drop wise, pink scarlet, **crimson red** or occasionally green to blue color appears after few minutes.

Zinc-Hydrochloride reduction test: To the extract solution, add a mixture of Zinc dust and conc Hydrochloric acid. It gives red color after few minutes.

Alkaline reagent test: To the extract solution, add few drops of Sodium hydroxide solution, formation of an intense yellow color that turns to colorless on addition of few drops of dilute acetic acid indicates the presence of flavonoids.

Tests for Tannins

Gelatin test: Extract solution with 1% gelatin solution containing 10% sodium chloride gives **white** precipitate.

Ferric chloride test: Extract solution gives blue-green color precipitate with FeCl3.

Vanillin Hydrochloride test: Extract solution when treated with few drops of Vanillin Hydrochloride reagent gives **purple red color.**

Test for Steroidal Glycosides

Kedde's test: Extract the bark powder with chloroform, evaporate to dryness, and add one drop of 90% of alcohol and 2 drops of 2% 3,5-dinitro benzoic acid(3,5, dinitrobenzene carboxylic acid - Kedde's reagent) in 90% alcohol. Make alkaline with 20% sodium hydroxide solution. A purple color is produced. The color reaction with dinitrobenzoic acid depends upon the presence of □,□ unsaturated – lactones in the aglycone.

5. RESULTS AND DISCUSSION

Table No: 01 Morphological Evaluation of Stem bark of plant *Nothopegia racemosa* (Dalz

Sl.No.	Features	Observation
a	**Stem bark**	
1	**Color**	Blaze –brown
2	**Odor**	Characteristic
3	**Taste**	Bitter
4	**Size**	2.5-11.0 cm long
5	**Shape**	Obiate or oblong
6	**Texture**	Externally dark brown, finely fissured internally it has smooth surface.

Study of Microscopical Characters

Transverse section of stem bark of plant Nothopegia racemosa (Dalz

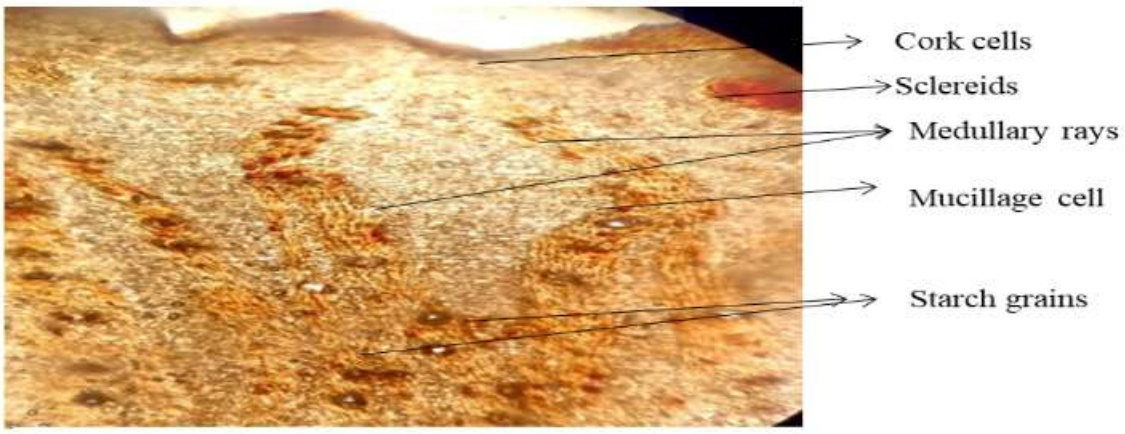
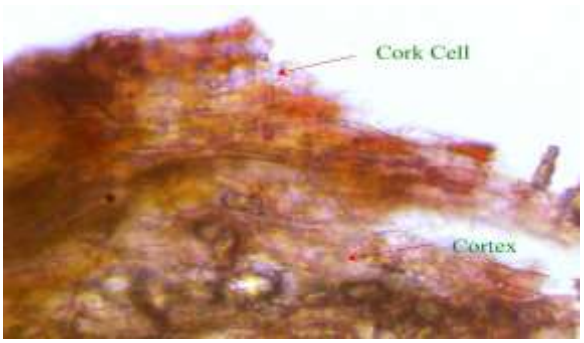
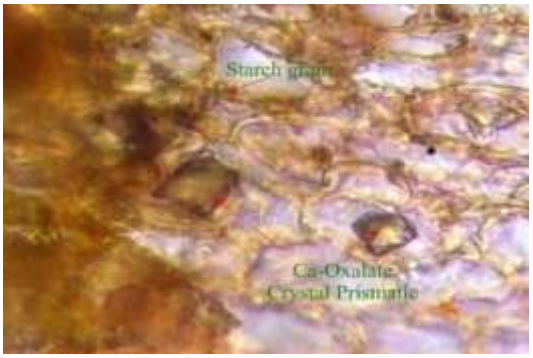


Figure: 02 Transverse section of stem bark of plant *Nothopegia racemosa* (Dalz).

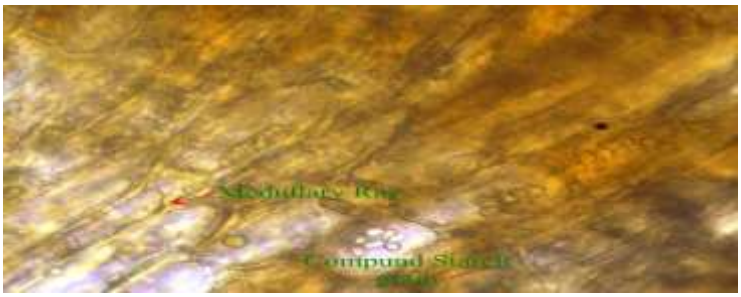
ii. Transverse section of stem bark of plant *Nothopegia racemosa* (Dalz). With different elongated parts



Cork & Cortex region- Starch grain



Ca-Oxalate crystal Prismatic



Medullary rays, Compound Starch

Figure: 03 Transverse section of stem bark of plant *Nothopegia racemosa* (Dalz). With different elongated parts

The Transverse section of stem bark of plant *Nothopegia racemosa* (Dalz). Shows the Cork cells: 4 to 5 layers tangential elongated cork cells, Cortex region: contains patches added of scalireds. Cortex contains medullary rays, mucilage matter, brown content material and compound starch grains.

iii. Transverse section of leaf of plant *Nothopegia racemosa* (Dalz

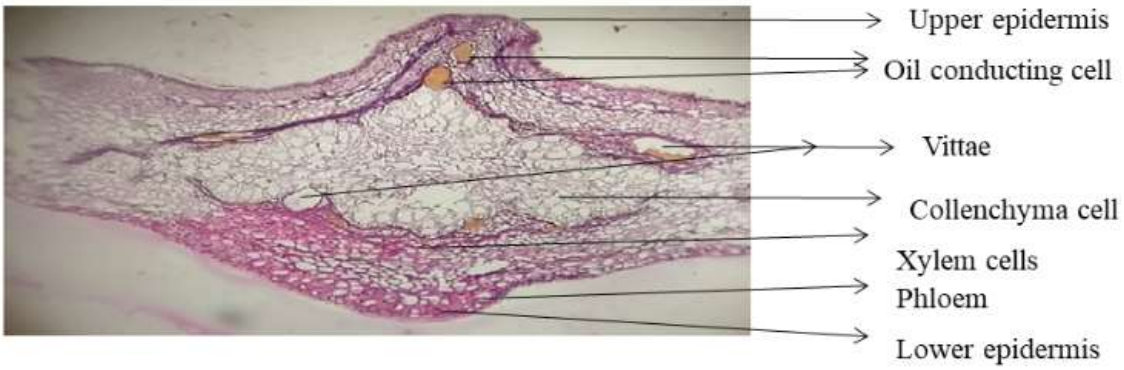


Figure: 04 Transverse section of leaf of plant *Nothopegia racemosa* (Dalz)

iv. Enlargement of transverse section of leaf of plant *Nothopegia racemosa* (Dalz)

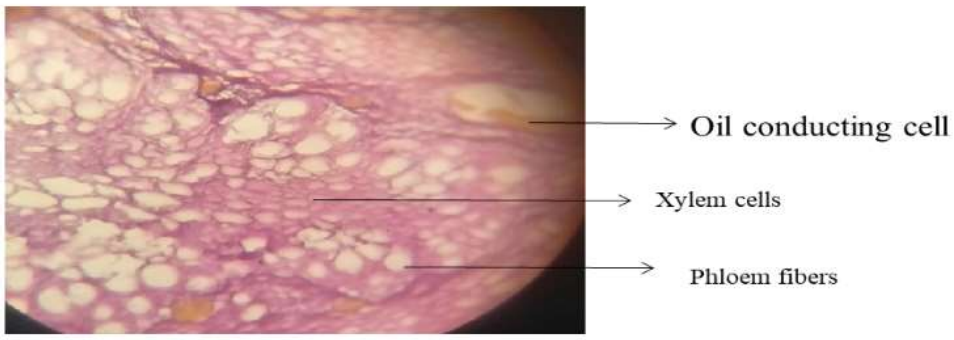


Figure: 05 Enlargement of transverse section of leaf of plant *Nothopegia racemosa* (Dalz)

The transverse section of leaf of plant *Nothopegia racemosa* (Dalz). Shows the presence of Upper epidermis: Oval shaped epidermal cells, oval shaped collenchyma cells.Lower epidermis: show Xylem and Phloem cells , Oil conducting cells, oil droplets and vittae are observed in collenchyma cells, Tringle shaped midrip is observed , Tringle structured collenchyma cells are arranged.

b. Study of Powder microscopy

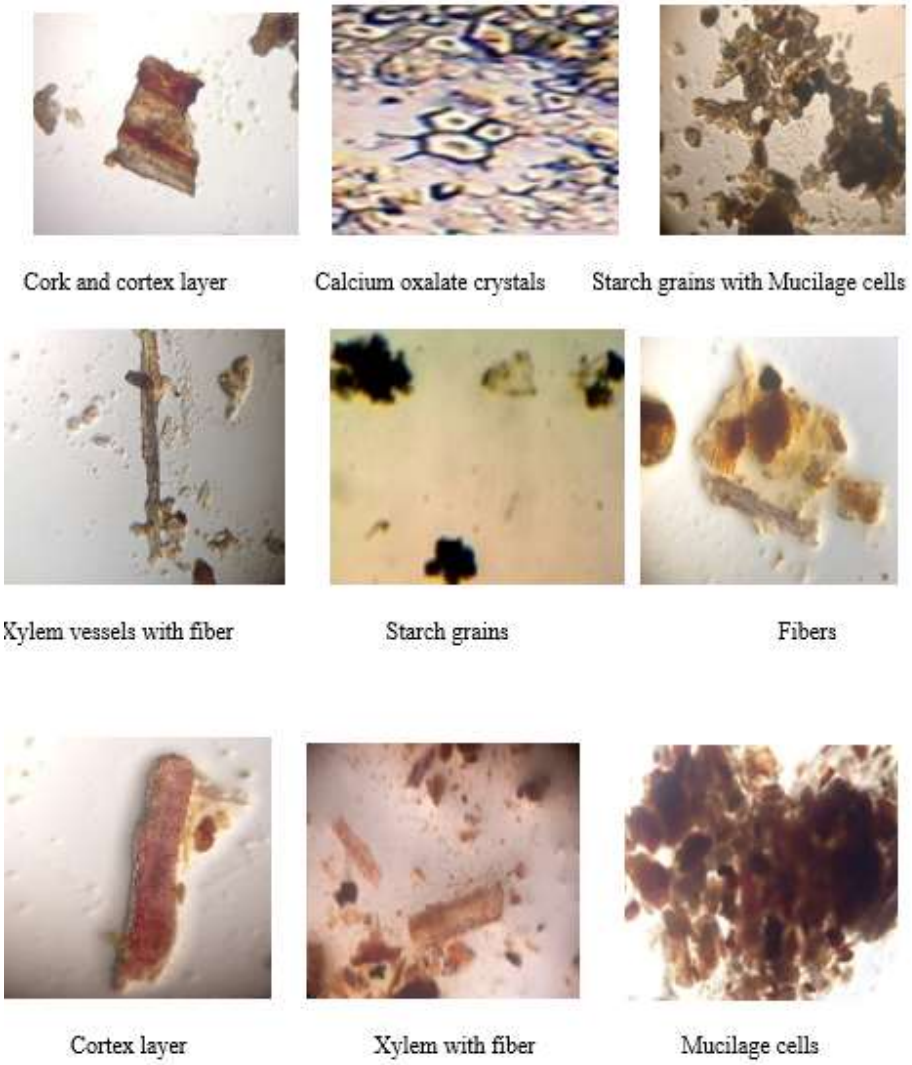


Figure: 06 Powder microscopy of stem bark of plant *Nothopegia racemosa* (Dalz).

6. PROXIMATE VALUES

Table No: 2 a. Extractive values, b. Moisture content and c. Ash values of Stem bark of plant *Nothopegia racemosa* (Dalz)

Sl.No.	Parameter	Determined Value % w/w
a	**Extractive values for** **I: Cold maceration**	
1	Alcohol soluble extractive value	5.00
2	Water soluble extractive value	8.00
3	Ether soluble extractive value	1.00
	II: Hot extraction method	
1	Alcohol soluble extractive value	12.00
2	Water soluble extractive value	16.00
3	Ether soluble extractive value	5.00
b	**Moisture content by** Loss on drying	6.5
c	**Ash Values**	
1	Total ash	6.50
2	Acid insoluble ash	1.20
3	Water soluble ash	2.32
4	Sulphated ash	6.00
d	**Foaming Index**	
1	Foaming index	is more than 100

b. PHYTOCHEMICAL INVESTIGATIONS

Table No: 3 Percentage yield and physical characteristics of various extracts of Stem bark of plant *Nothopegia racemosa* (Dalz).

Extract	% Dry wt in gms.	Colour	Odour	Consistency
Successive extraction				
Petroleum Ether (60-80^{0}C)	2.08	Brownish black	Characteristic	Sticky wax
Ethyl acetate	3.91.	Reddish Brown	Characteristic	solid
Ethanol	8.24	Reddish Brown	Characteristic	solid

Aqueous	5.10	Dark brown	Characteristic	semisolid

Table No: 4 Qualitative chemical analysis of various extracts of Stem bark of plant *Nothopegia racemosa*(Dalz)

Nature	**Successive Extraction**		
	EtOAc	**EtoH**	**Aq**
Alkaloids	--	--	--
Steroids	--	--	--
Carbohydrates	--	+	++
Phenolic compounds	++	++	+++
Tannins	++	+++	+++
Glycoside	--	+	+
Flavonoids	++	++	+++
Triterpinoids	+	+	--

EtOAc= Ethyl acetate EtOH =Ethanol, Aq=Aqueous

Present; -- = Absent

7. CONCLUSION

The present study is focused on the stem bark of plant *Nothopegia racemosa* Dalz. to determine its Pharmacognostic and Phytochemical studies. Morphological, Microscopical, powder microscopy and Proximate values like: Extractive value, Ash value, moisture content and foaming index were determined and successively extracted with petroleum ether (60-80^{0}C), ethyl acetate, ethanol and Aqueous in increasing order of polarity to determine their physical characteristics and their percentage yield. Based on the above results, the preliminary Phytochemical analysis of Stem bark of plant *Nothopegia racemosa* (Dalz), revealed the presence of phenolics, tannins and flavonoid compounds.

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