WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 5, 990-1028. Research Article ISSN 2277– 7105

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION, STUDY AND CHARACTERISATION OF THE PLANT *SAPINDUS MUKOROSSI; A.K.A.* THE SOAPBERRIES

***Dr. Saurabh Dilip Bhandare[1], Miss. Niphade Srushti Sunil[1], Miss. Pagar Pranali Anil[1]**

Nashik Gramin Shikshan Prasarak Mandal's, College of Pharmacy, Bramha Valley Educational Campus, Affiliated to SPPU; formerly known as: university of Pune. Ganeshkhind, Pune, Maharashtra-411007. India.

Article Received on
24 Jan. 2023,

Revised on 13 Feb. 2023,
Accepted on 05 March 2023

DOI: 10.20959/wjpr20235-27401

***Corresponding Author**
Dr. Saurabh Dilip Bhandare
Nashik Gramin Shikshan Prasarak Mandal's, College of Pharmacy, Bramha Valley Educational Campus, Affiliated to SPPU; formerly known as: university of Pune. Ganeshkhind, Pune, Maharashtra-411007. India.

ABSTRACT

The world is home to the highly valued medicinal plant. *Sapindus mukorossi*, which is found in tropical and subtropical areas are quite important. Research and investigation of the plant, examination and documentation of *Sapindus mukorossi's* phytochemistry and pharmacognosy was the current study's objective that was to compile information on the pharmacological effects and phytochemical makeup of this tree as well as the advantages of soapberries. The identification of phyto-constituents through phytochemical analysis is important and helpful for advancing the field of pharmacognosy. Triterpenoidal saponins of the oleanane type are the primary phytoconstituents that have been extracted and identified from different portions of this plant. All of the triterpenoidal saponin kinds found in *Sapindus mukorossi* have undergone a brief structural and chemical naming study. Numerous studies have been done to demonstrate the plant's potential as a spermicidal, contraceptive, hepatoprotective, emetic, anti-inflammatory, and anti-protozoal agent; however, these studies have had little real-world impact. Terpenoids, particularly triterpenoid saponins and sesquiterpenoid glycosides, phenylpropanoids, steroids, and saccharides are the main chemical components of the soapberry pericarp. These components were determined through a variety of laboratory chemical reactions and established tests to determine the presence or identification of any unidentified chemicals in the plant. For this plant, a phenological study was conducted using a variety of plant studies that involved observation. These studies assist in providing a

significant amount of the information needed by plant-based companies to gather and store raw resources. One important component examined by this study is the season and its impact on the plant materials or metabolites such as: gum, fruits, resins, husks, flowers etc.

KEYWORDS: *Sapindus mukorossi*, soapberry, phyto-chemical analysis, pharmacognostic study of *sapindus mukorossi*, Indian soap-cherry.

I. INTRODUCTION

The soapberry (*Sapindus mukorossi Gaertn*., *Sapindaceae*, Sapindus) is a commercially applicable tree with numerous uses in landscape, biomedicine, toiletries etc.[11][12] **Synonym: or local names includes:** Sabangoty/Shabungoty. Also called as Laundry-nut or[33] **Soapberries**—the fruit that soap-nuts are. Ayurveda uses soapnuts frequently because of their purifying and softening qualities. These fruits were used as a naturally occurring shampoo in ancient India to wash hair and get rid of dandruff. Additionally, it was used to wash wool clothing and expensive silks. The species is known by some botanists as *Sapindus* detergens because of this.[5]

More than 1000 species in 125 genera make up the *Sapindaceae* (or soapberry) family, which is widely distributed in the tropics and warm subtropics. Most of these species are trees and shrubs, but a small number are also herbs. Although there are a few species in South America, Africa, Australia, and Europe, the bulk of species are endemic to Asia.

The lathering substances called saponins are found in many plants, but are exceptionally concentrated in the fruits. In addition to being a potent natural alternative to conventional shampoos in terms of overall hair health, soapberries are a simple, vitamin-rich, 100% natural product that can help with a number of hair and scalp-related problems.

Tropical tree *Sapindus mukorossi Gaertn*., often known as the wash-nut, belongs to the *Sapindaceae* family. The plant got its name since it is used as a natural detergent for cleaning and washing purposes. Triterpenoid saponins, which are present in numerous plant sections, including the fruits, galls, and roots, are the most vital components of the plant. Additional useful, biologically active chemicals that were extracted from the tree are also present. A valuable plant product that can be employed in the food industry, pharmaceutical, cosmetic, and chemical sectors is separated saponins and raw or refined extract. The most significant

biological and surfactant characteristics of isolated saponins and extracts produced from various plant sections are significant and notable.

The only soapberries on the market that are "Certified Organic" are those from "That Red House,"[9] and, soapberries are a natural substitute for conventional fabric softener and laundry detergent. Although it has a name "soapnuts," they are actually not nuts at all but are termed so is one exceptional fact about soapberries. They are a fruit, after all.

The fruit's shell is gathered, dried, and packaged for use. The shell contains a lot of "saponin," the soap found in nature. This saponin lowers the water's surface tension, allowing dirt to be removed and soft, clean materials to be left behind. *Sapindus Mukorossi* trees, which are found all over the world but are most common in the Himalayas, produce soapberries, which are gathered there by small, local populations.

The fruit has significant medicinal value as well. The Ayurvedic schools of medicine regard it as beneficial for treating a range of conditions, including: the common cold, acne, epilepsy, constipation, nausea, etc. However, it is unclear how important this medication is for internal use, and there are no clear-cut research on its toxicity and safety studies which are evident and clear, thus can be studied for safety evaluation of this fruit/ *Sapindus mukorossi*.[5]

The tree *Sapindus mukorossi*, also referred to as Indian Soapberry, Washnut, or Ritha, Rishthak; belongs to the Sapindaceae genus. It is also called or named as Phenila (frothy fruits) in Sanskrit.[2]

A member of the *Sapindaceae* family called *Sapindus mukorossi Gaertn* is also known as soapnut, soapberry, washnut, reetha, aritha.

The fruit is reputed for the pericarp saponins (10.1%), which make up, up to 56.5% of the drupe and are known to suppress the formation of tumour cells.

II. Geography

It is a deciduous tree that can be found up to 1,200 metres above sea level in the lower foothills and midhills of the Maharashtra in the Deccan region and also in the Nashik regions. It grows automatic and is wildly fruit bearing trees that are found in the forest places in hilly and mountainous area of Nashik and its judicial operating division. It is also native to western and hilly areas of Maharashtra, and Goa; also in the Nashik region and Nashik-

division; (Rural and North Maharashtra.) It is tolerant to reasonably poor soil, and one tree can produce 30 to 35 kilograms (66 to 77 lb) of fruit per year. It is also found in some of the hilly ranges and regions of Nashik that is near the Trimbakeshwar in Torangan area. (Nashik division is one of the six divisions of India's Maharashtra state and is also known as: North Maharashtra. The historic Khandesh region covers the northern part of the division, in the valley of the Tapti river. Nashik division is bound by Konkan division and the state of Gujarat to the west, Madhya Pradesh state to the north, Amravati division and Marathwada (Aurangabad division) to the[27] east, and Pune division to the south. The city of Nashik is the largest city of this division.)[10]

Instead of being actual nuts, soap-nuts, also known as: soapberries are the fruit's outer shell from the Sapindus tree, which belongs to the Sapindaceae family.[2] [3]

It is a deciduous tree that is also commonly cultivated at altitudes ranging from 200 m to 1500 m in the higher regions of the Indo-Gangetic plains, Shivaliks, and sub-Himalayan tracts. The *Sapindus mukorossi* is a sizable, deciduous tree with a straight trunk that can grow to a height of 12 metres, occasionally reaching 20 metres in height and a girth of 1.8 metres, a globose crown, and quite fine leathery leaf. Dark to pale yellow, somewhat smooth bark with numerous vertical lenticels and small cracks that exfoliate as irregular wood scales.

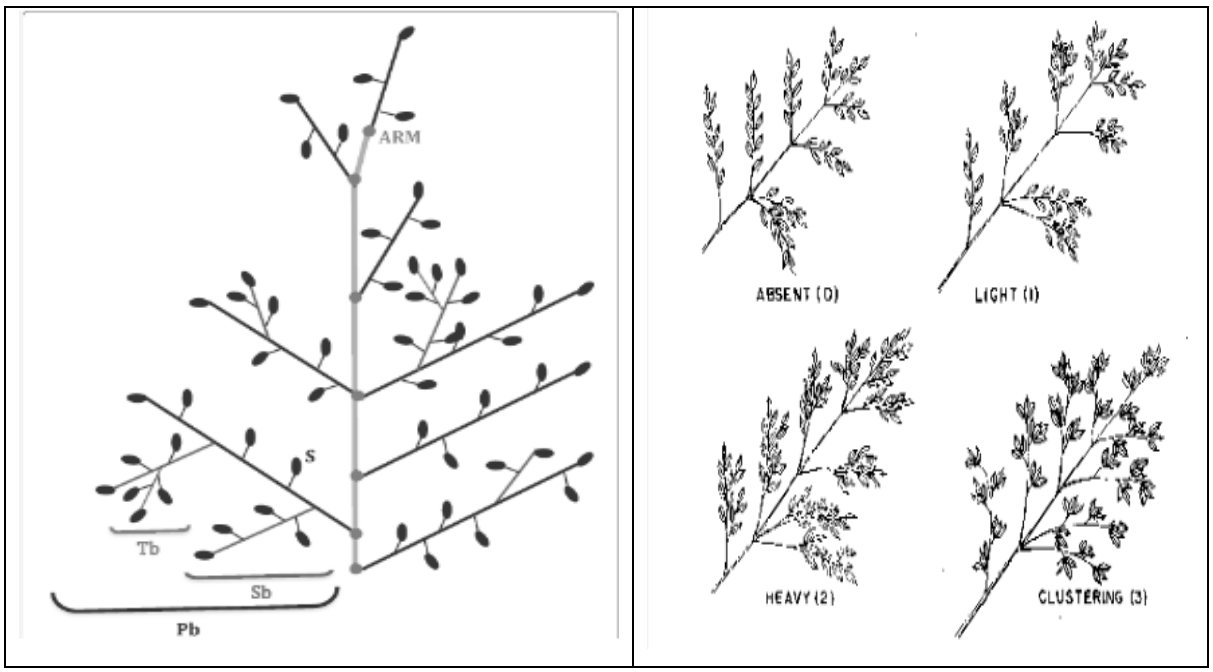


Figure 1: Structure of panicles.

Figure 2: Dorsal surface of the leaf of *Sapindus mukorossi.*

Figure 3: Inflorescence[6] of *Sapindus mukoross.*[34]

III. Macroscopic description

The blaze is firm, not fibrous, pale yellow to olive green, hairy brown or looks very pubescent, brittle, and granular bunch or the cluster hanging together, measuring 0.8 to 1.3 cm very attractive and though delectable to eyesight. Petioles are 2-5 cm long, or up to 7 cm compound stalk with leaves and the leaves are alternating, paripinnate, and 7–8 cm long. The leaflets are 5 pairs, opposite or alternate, and 5–10 by 2.5–5 cm in size. Four opposite and

one alternate on same petioles. They are lanceolate, acuminate, found whole, also glabrous, and frequently somewhat falcate or oblique. Compound terminal panicles that are at least 20 cm long, extending up to 25 cm and have pubescent branches make up the inflorescence. The majourity of the 5 mm-wide, terminal, polygamous, greenish-white, subsessile flowers are bisexual. Five sepals, each with a woolly scale above the claw on either side. The fruits are globose, meaty, full of juice that comes out upon its squeezing. It is a 1-seeded drupes that measure between 1.8 and 2.5 cm in diameter. Dry fruit contains dry sap 0.8–1.3 cm in diameter, globose, smooth, turns black or brown to ochre, and have loosely arranged seeds that sometimes makes sounds upon shaking.[7][8]

IV. The fruit of the tree, which has several medicinal and cleaning uses, is the source of most of the tree's value

It is a common ingredient in most Ayurvedic and herbal shampoos as it is known to make hair shinier and softer with regular use. It is often used in herbal preparations with other ingredients like neem, shikakai and amla.[5]

IV.a. Detergent/Insecticide: Because soap-nuts contain the chemical saponin, which has natural cleaning powers, they can be used to wash clothes, upholstery, skin, and hair. These saponins can also be used as pesticides to get rid of lice on the scalp, among other things like using of *Sapindus mukorossi* with other herbs to combine and fix an anti-hair lice shampoo. The Ritha fruit or the *Sapindus mukorossi* can be used in a method to increase oil recovery, according to research. A useful organic surfactant is soap-nut. Additionally, studies have shown that soap-nut has the potential to be used as a natural surfactant to remove arsenic from iron-rich soils.

V. Scientific classification

Scientific classification:[1] [4]
Kingdom: Plantae,
Clade: Tracheophytes, **Clade:** Angiosperms, **Clade:** Eudicots, **Clade:** Rosids,
Order: Sapindales,
Family: Sapindaceae,
Sub-family: Sapindoideae,
Genus: Sapindus,
Species: S. mukorossi,
Binomial name: *Sapindus mukorossi* Gaertn.

After the trees have grown to maturity, which takes around 9 to 10 years, they naturally give their berries for the next 90 years. The inside seed of the soap-nut fruit is removed and the outer shell is dried naturally in the sun without the use of any chemicals.

A compound called saponin, found in the soap nut shell, acts as a surfactant, or a chemical that attracts dirt off your clothes, shroud; (A burial shroud.) and, prevents it from re-entering them.

When the dirt is rinsed away into your sewage system or grey water system, it leaves no evidence of chemical poisons behind. The dirt will stay raised and suspended in the water.

Because they are hypoallergenic, odourless, leave no trace, and do not harm fabrics or shroud, soap-nuts are frequently used as a cleaning agent or detergent. Thus, soap-nuts are an essential component of one's home due to their sustainability and adaptability.

VI. Soap-nut detergent is a natural laundry detergent[2] [3]

Delicate clothing washing they function as a handmade natural detergent and are mild in nature. As a result, they can be applied to delicate fabrics like wool, cashmere, and silk. The colours stay vivid because to its mildness.

VII. Clothing durability

Due to their mild nature, they also serve as a fabric softener, preserving the fabric structure of clothing for longer periods of time.[2][3]

Because; soap-nuts don't contain any chemicals, they can be used right away to wash cloth diapers and napkins. Unlike chemical detergents, they do not clog the fabric, causing the diaper to lose its absorbency, making them excellent for washing cloth diapers.

Additionally, soap nuts don't cause diaper irritation and clean and eliminate detergent residue from diapers. Conserving water and energy they rinse more easily, requiring less water, which conserves water. One might use a quicker rinse cycle when using the washing machine to conserve energy. Low foam content makes them ideal for high-efficiency machines.[2][3]

VIII. Soap-nut detergent is a natural laundry detergent

They are soft by nature and work as a handmade natural detergent when washing delicate clothing. As a result, they can be applied to delicate fabrics like wool, cashmere, and silk, cotton, and other natural fibres, also synthetic. The colours stay vivid because to its mildness.

VIII.a. Clothing durability: Due to their mild nature, they also serve as a fabric softener, preserving the fabric structure of clothing for longer periods of time.

VIII.b. Cleaning cloth napkins and diapers: Since soap-nuts don't contain any chemicals, you can wash cloth napkins and diapers with them right away. Unlike chemical detergents, they do not clog the fabric, causing the diaper to lose its absorbency, making them excellent for washing cloth diapers. Additionally, soap nuts don't cause diaper irritation and clean and eliminate detergent residue from diapers.

VIII.c. Saving water and energy: Because they rinse more easily, less water is needed. One might use a quicker rinse cycle when using the washing machine to conserve energy. Low foam content makes them ideal for high-efficiency machines.

VIII.d. Dish washing: Washing pans, glasses, silverware, and dishes with Soap-nut washing liquid.

VIII.e. Detoxifying and cleaning food: fruits and vegetables can be steeped in the soap-nut cleaning solutions for ten to fifteen minutes before being rinsed. This gets rid of residue and dangerous substances.

VIII.f. Cleanser for the skin: the natural product soap-nuts are safe for the skin and free of toxins. As a result, it is especially suitable for use during bathing for those with extremely sensitive skin. It is a terrific option for those who prefer odourless cleaners because it has no smell. Soap nuts are used in Ayurvedic medicine to wash the skin, get rid of tan, and treat eczema and psoriasis. This can be done with mild change in the soapnut preparations.

VIII.f. Shampoo: Soapberry is a traditional medicinal plant. A substitute of soap-nut washing liquid or soap-nut liquid for any typical shampoo.

IX. Several advantages include

Makes hair strong, wholesome, supple, and lustrous: In addition to acting as a natural detergent, soap nuts are rich in vitamins A, D, E, and K, which maintain the hair's health and impart shine and lustre.

Soap-nuts have been used in Ayurvedic medicine for centuries to treat sensitive skin, eczema, hair and scalp problems, as a hair conditioner and to alleviate parasites such as: lice. Soap-nuts have a mild anti-bacterial and anti-microbial function and because of this they have been used in Ayurveda to treat infections, and to help keep the scalp free from bacteria which may cause dandruff or inhibit the growth of new hair.[5]

IX.a. A natural conditioner: Soap-nuts give the hair natural conditioning and moisturisation.

Stops dandruff: To treat a variety of scalp issues, including dandruff, eczema, and psoriasis, apply powdered soap-nuts to the scalp. In Ayurveda, this treatment is applied.

IX.b. Eliminates head lice: Soap-nuts are helpful at removing lice from the scalp because they have insecticidal characteristics. They have historically served this function.

IX.c. Prevents hair loss: Soap-nuts are utilised in Ayurveda to stop hair loss shaving lotion. Grinding the shells off a few soap-nuts and ground the flesh in a mixer to make shaving cream. Blending the flesh into a paste and then add 3 teaspoons of the soap-nut liquid and 1 tablespoon of olive oil. After being prepared, the blended paste is subsequently utilised as shaving cream. With *Rose oil, Jasmine, Leechi, Jasminum samabac. Lemon. Also with alovera extracts and rose petal extracts to make the shaving cream soft and mild and tender to skin.*

IX.d. Pest-and-insect repellent: certain type of insects can be repelled by soap-nuts. In the garden, the crushed shells can be utilised to deter pests and insects. An alternative that is both efficient and natural is the soap-nut liquid. To get rid of the bothersome pests, it can be sprayed on the plants close to the affected areas.

IX.f. Pet shampoo: Pets can be washed with Soap-nuts liquid, and spraying their coats with the product can help them stay pest- and insect-free.

IX.g. General cleaner: The house can be made clean and pathogen-free by using the Soap-nut liquid as a floor cleaning. The liquid can be used to clean porcelain, tiles, sinks, toilets, bathtubs, window panes, bathroom fixtures, and windows in a home.

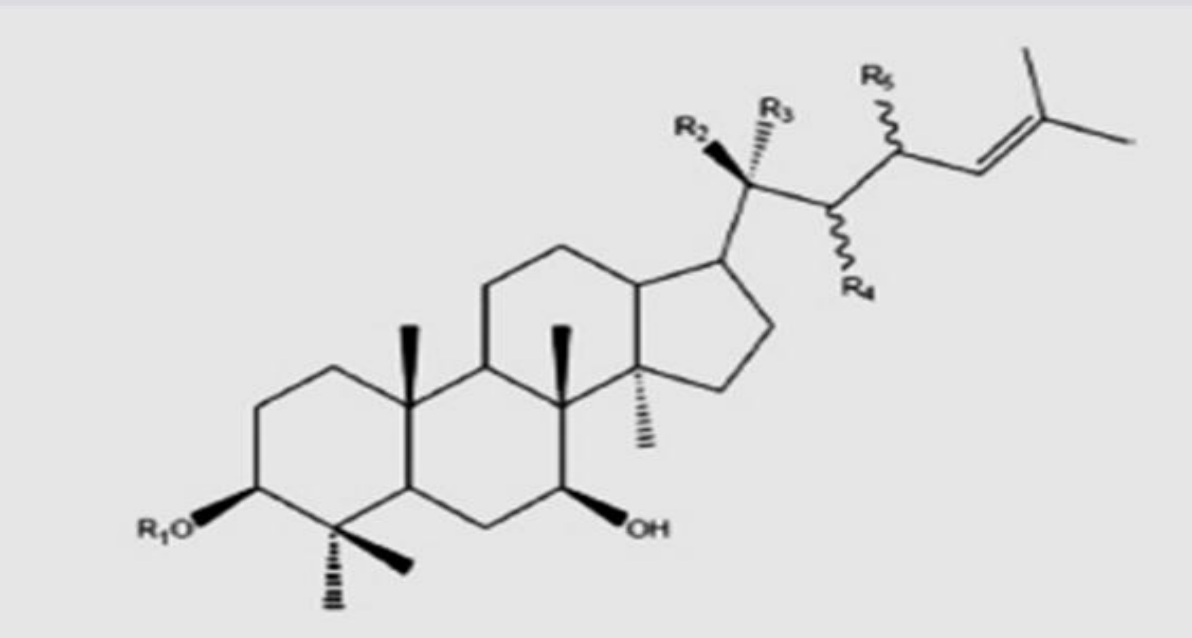
IX.h. The carwash: Large quantities of the soap-nut liquid can be used to wash the automobile, the wheels, the dashboard, the steering wheel, and even the windows. The wash water can then be recycled and utilised to water trees and plants.

IX.i. Cleaning jewellery: Soap-nuts are frequently used in Indian homes to clean jewellery. The Soap-nut liquid is applied to the jewellery, which is then polished with a cloth after soaking. In India, people have used soap-nuts for cleaning and laundering for hundreds of years. They undoubtedly increase the selection of waste-free cleaning supplies.

When compared to other types of detergents, they are both affordable and environmentally friendly. One can always choose a powerful, deliberate substitute for harsh synthetic chemicals. 'Why not try soap nuts at your house if you have all of these options?'

Soap-nuts have been used in Ayurvedic medicine for centuries to treat sensitive skin, eczema, hair and scalp problems, as a hair conditioner and to alleviate parasites such as lice.[5]

Chemical constituents of *Sapindus mukorossi:* From the fruits of the Sapindus mukorossi plant, a member of the Sapindaceae family, six new fatty esters of tetracyclic triterpenoid, as well as a recognised acyclic sesquiterpenoidal glycoside, have been identified.



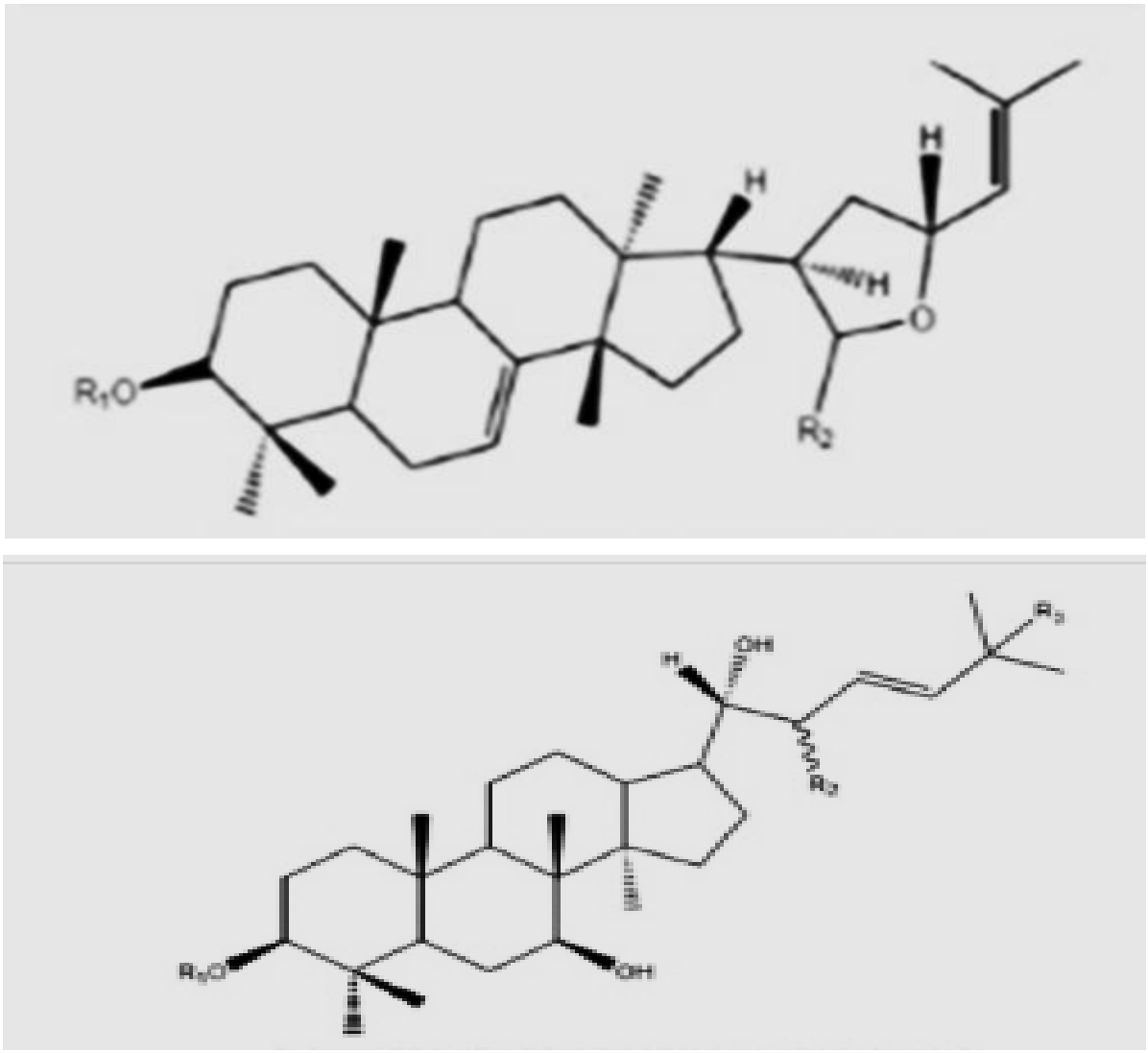


Figure 4: Sapimusaponins.[12][13][15]

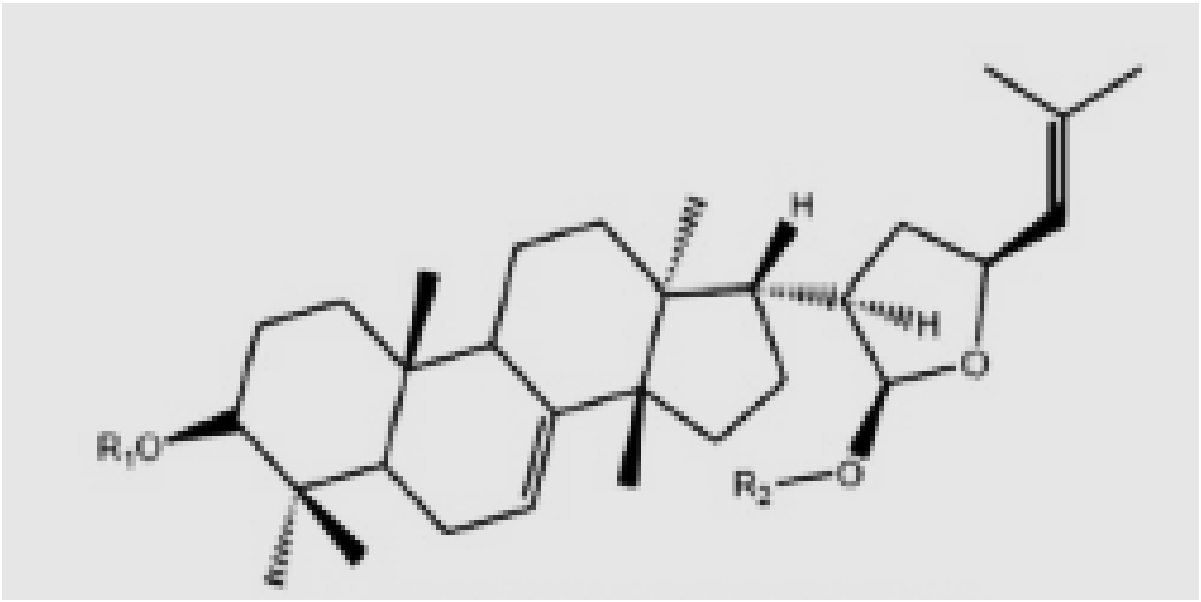


Figure 5: Sapimukosides.[14]

Their structures were elucidated with the help of extensive spectroscopic techniques and by chemical means.

X. Toxicity of the *Sapindus mukorossi*

Excess consumption can cause Jamaican vomiting sickness. (Also cause by the ***Ackee*** apple or **Ayee** from *Sapindaceae* family).

Figure 6: Ackee apple.[17] [18] [19]

XI. Toxicity

Hypoglycin toxins, also referred to as "soapberry toxins," such as hypoglycin A and hypoglycin B are present in the unripened aril and the inedible fruit parts. While hypoglycin B is exclusively present in the seeds, hypoglycin A is present in both the seeds and the arils. Ripe arils contain just trace amounts of the toxin. Depending on the time of year and exposure to the sun, the quantities in the unripe fruit may be up to 10–100 times higher.

A naturally occurring amino acid derivative called hypoglycin A is present in the unripe fruit of the ackee tree (Blighia sapida) and the box elder tree's seeds (Acer negundo). It is poisonous if consumed and the cause of the Jamaican vomiting disease. According to a 2017 Lancet study, eating unripe lychees that contain hypoglycin A or methylenecyclopropylglycine (MCPG) can cause hypoglycemia, which can lead to death from acute toxic encephalopathy.

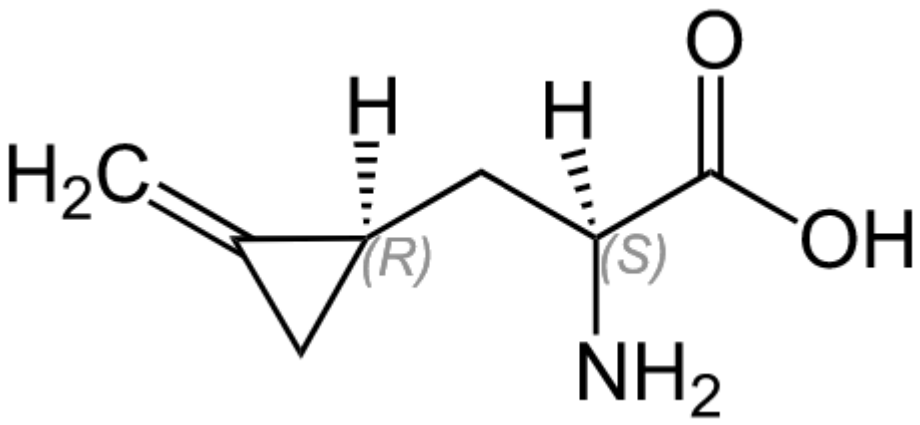


Figure 7: Structure of hypoglycin.[20]

Since hypoglycin A is a protoxin, when consumed, it degrades into hazardous byproducts rather than being poisonous as a whole. Leucine, isoleucine, and valine are ordinarily converted into acyl-CoA derivatives by the branched-chain alpha-keto acid dehydrogenase complex, but Hypoglycin A is converted into the extremely poisonous MCPA-CoA. The MCPA-alpha CoA's carbon interacts with the FAD cofactor, which is required for the beta oxidation of fatty acids, to form an irreversible compound that renders the enzyme inactive. In addition, MCPA-CoA inhibits several gluconeogenesis-related enzymes.

The majority of the symptoms of Jamaican vomiting sickness are assumed to be caused by a decrease in gluconeogenesis and a decrease in fatty acid oxidation. Cells start utilising

glycogen for energy when the metabolism of fatty acids is blocked. Severe hypoglycemia results from the body's inability to create additional glycogen once it is used up. Acidosis and an overabundance of medium chain fatty acids in the urine are indicators of these metabolic consequences. Key therapies, including as intravenous fluids, glucose, and hemodialysis in the event of renal failure, are focused at avoiding or reversing the metabolic alterations.

A naturally occurring chemical substance called hypoglycin B is found in the species *Blighia sapida*. It is contained notably in the seeds of the plant's fruit. One of the causes of Jamaican vomiting illness is the toxic substance hypoglycin B,[23] which is also harmful if consumed. It is a glutamic acid and hypoglycin -A dipeptide.

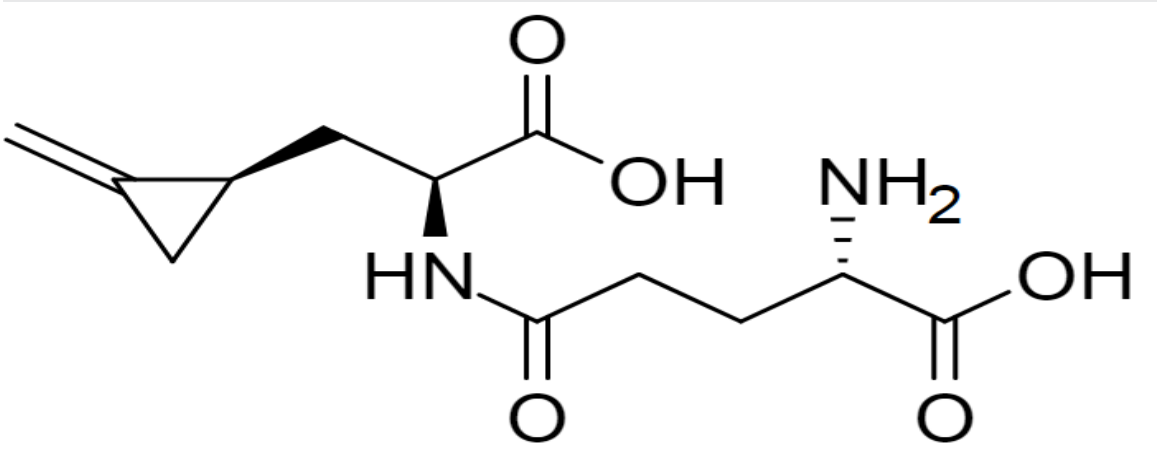


Figure 8: hypoglycin-B.[22]

XII. Laboratory synthesis of this toxins hypoglycin includes a step wise procedures

XIII. Synthesis

In **1958, John Carbon, William Martin, and Leo Swett** were the first to synthesise hypoglycin A, in racemic form, starting from 2-bromopropene and ethyl diazoacetate to form the cyclopropane ring.

The first asymmetric total synthesis of the individual diastereoisomers of hypoglycin A was completed in **1992 by Jack Baldwin, Robert Adlington, David Bebbington, and Andrew Russell**. They did this by using the Sharpless epoxidation to enable an asymmetric methylene cyclopropane synthesis. The predominant diastereoisomer of natural hypogycin A is (2S, 4R) according to 1H NMR and circular dichroism investigations, whereas the minor diastereoisomer is (2S, 4S).

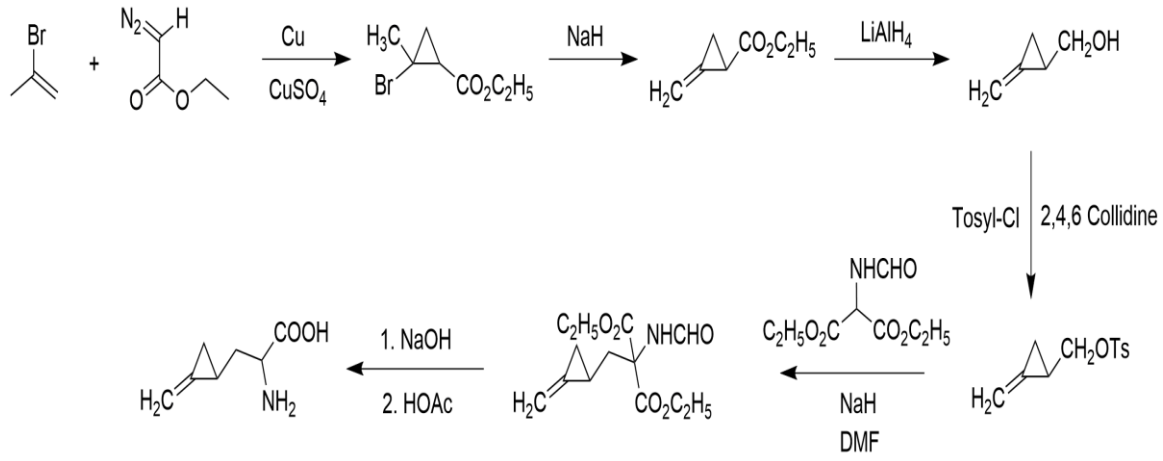


Figure 9: synthesis; John Carbon's synthesis of Hypoglycin A.[24]

XIV. Reportable uses

The fruit's "soap" characteristics can be used as a washing agent or fish poison, among other applications, in rural areas of the Caribbean Islands and West Africa. The resilient heartwood is utilised for building, pilings, oars, paddles, and casks, while the fragrant blooms can be used as decorations or cologne. Ripe arils, leaves, or bark were used in traditional African medicine to heal mild illnesses.[25][26]

XV. Research methodology

The research methodology includes pharmacognostic study of the plant *Sapindus mukorossi* collected from the areas of: Torangan, Triambak, Nashik, and Maharashtra. 422212. The fresh areal parts were collected using a scissors and blade cutting techniques. The collected plant materials like leaf were immediately stacked inside the books. The fruits were collected and picked-up from underneath the tree that has been fallen in tremendous over the dried foliage of various trees in the jungle that have been a part of abscission. Few fruits were collected and the newer leaf was isolated from the steams of the tree and were subjected to be placed inside the stacking paper and some in the surgical compass-box and bought intact into the laboratory of the institute for further examination.

Identification of the plant is very important to maintain the same plant inside the institute botanical garden, or have the knowledge of these plant in the form of its morphological studies and also macroscopic and microscopic studies. The macroscopic studies are documented as above in the brief pharmacognostic description or the study of the plant through macroscopic observational studies. **(Refere/check Macroscopic description).**

XVI. Scientific/pharmacopoeia descriptions and definitions

XVII. Description of the terms as per pharmacopoeia

XVII.a. Water: The term water is used without qualification in the formulae for formulated preparations means either potable water freshly drawn direct from the public supply and suitable for drinking or freshly boiled and cooled purified water.[35]

XVII.b. Excipients: Where an excipient for which there is a pharmacopoeial monograph is used in preparing an official preparation it shall comply with that monograph.

Antimicrobial preserbatives: When the term, 'suitable antimicrobial preservatives' is used it is implied that the preparation concerned will be effectively preserved according to the appropriate criteria and interpreted as described in the test for efficacy of antimicrobial preservation. (Appendix XVI C). Of British Pharmacopoeia, 2005.[35]

XVII.c. Crude drugs: The macroscopical characteristics of a crude drug includes those features that can be seen by the unaided eye or by the use of a hand-held lens.

Vegetable drugs are required to be free from insects and other animal matter, and from animal excreta. Not more than traces of foreign organic matter may be present in powdered vegetable drugs. Microbial contamination should be minimal.[35]

XVII.d. Odour: Term such as odourless, practically odourless, a faint characteristic odour or variations thereof, apply to examination after exposure to the air for 15 min. either of a freshly open package of the article or good.[36]

XVII.e. Paper chromatography: In paper chromatography the adsorbent is a sheet of paper of suitable texture and thickness chromatographic separation may be proceeded through the action of single liquid phase in a process analogous to adsorption chromatography in columns. Since the natural water content of paper, or selective imbibition of hydrophilic component of liquid phase by the paper fibres, may be regarded as a stationary phase, a partitioning mechanism may contribute significantly to the separation. Alternatively, a two-phase system may be used. The paper is impregnated with one of the phases, which then remains stationary (usually the more polar phase in case of unmodified paper). The chromatography is developed by slow passage of the other, mobile phase over the sheet. Development may be ascending in which case the solvent is carried up the paper by capillary forces or descending, in which case the solvent flow is also associated by gravitational force.

(The machine direction is usually designated by the manufacturing on packages of chromatography.).

XVII.f. Types: Ascending chromatography: In ascending chromatography, the lower edge of the sheet (or strip) is dipped into the mobile phase to permit the mobile phase to permit the mobile phase to rise of the chromatographic sheet by capillary action.

XVIII. Macroscopic study

The macroscopic study was done visually through observation. The observation was made using a magnifying glass or examining direct under a zooming camera that covers picturisation of such plant and plant surfaces studies.

XIX. Microscopic study

Microscopic study includes study of plant under the microscopic observation to examine the microscopic characteristic of the plant materials.

XX. Chemical tests

Confirmatory test for saponins was performed and the revealed results were documented.

XXI. pH of the soapberry

The pH study of the soapberry was performed with accurately with 1% of the solutin and, the results were noted.

XXII. Test for Alkaloids

XXII.a. Test for alkaloids: XXII.a.1.Wagner test: specific amount about 2-3 mL of filterate + few drops of Wagners reagent was utilised to define the test results. **(For fruits or the soapnut.)**

XXIII. Test for alkaloids was performed for leaf extract of *Sapindus*

XXIII.a. Wagner test was performed for the leaf extract of *Sapindus* and the test results were recorded and discovered results were documented.

XXIV. Test for protenis: for the fruits of *sapindus* were carried out using the ninhydrin method for the common pharmacognosy tests.

XXV. Test for the carbohydrates: The test for the carbohydrates have been carried out using the reducing test to confirm is any of the results produced are functional with the

carbohydrates. The obtained results were justified and documented also recorded for the purpose of the visual inspection also in the form of the photorecord.

XXVI. Phenological stage studies

The various stages of development (germination, budbloom, flowering, etc.) that make up a plant's life cycle are referred to as phenological stages. The stages of a plant's lifespan can be ordered in many back-to-back arrangements, annular arrangements to trace the years of the years of the plant age in living.

XXVII. TLC studies of the plant materials[30]

Separation by chromatographic method is very important process in modern and advanced method of analysis. The success of the method is significantly dependent on how pure is the sample that is given for analysis. Therefore, a small fraction of impurity can affect the resolution to distorted in results of analysis which shows shabby spotted development of the TLC plates or the TLC stripes. The process including other method than non-Chromatographic method are: solvent extraction, Zone refining, froath floation, ring oven, dialysis, precipitation and reversed osmosis. Chromatography has stationary and mobile phase independently.[37] TLC studies of the plant materials are based on the carried-out experiment in the field of analytical chemistry and its application in determination of phyto-constituents experimented in the laboratory. Such studies are done to meta-analyse different phyto-constituents present in the plant materials and to separate them for individual studies. Some of the phytochemicals present in them can be separated if they are justified and done with the help of analytical experiments with the help of a chemical analysts. Such studies were performed to justify the presence of any significant separations in plant components and pigments. For this purposes, a suitable medium was utilised to separate the plant component on the chromatographic papers. A++ grade chromatographic papers, Whatman 3001-604 Cellulose Chromatography paper were utilised to perform this tests.[28][29][30]

A chromatography technique called thin layer chromatography (TLC) is used to separate mixtures. A thin layer of adsorbent material, typically silica gel, aluminium oxide, or cellulose (blotter paper), is coated on a sheet of glass, plastic, or aluminium foil, and this layer of adsorbent is known as the stationary phase.

After the sample has been applied to the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Separation is accomplished because various analytes ascend the TLC plate at various speeds.[30]

Thin layer chromatography can be used to: monitor a reaction's development; identify the components that are present in a given substance; and assess a substance's purity.

Measure R_f values, compare samples in mixtures with known substances, and look for coloured and colourless compounds using the techniques of thin layer chromatography (T.L.C.). TLC can be used to separate mixtures of up to a few hundred milligrammes on a modest semi-preparative scale. Instead of "spotting" the mixture as spots on the TLC plate, the mixture is placed to the plate as a thin, even layer that extends to and just above the solvent level. When combined with a solvent, the chemicals separate in bands that are horizontal.

The starting point and solvent front—the level the solvent reached when the plate was taken from the developing tank—are marked, and all spots observed on the plate are circled in lead pencil, after which the retention factors (Rf) for thin layer chromatography are calculated. Then, a Retention Factor is computed to reflect each spot's location on the plate numerically (R_f).[30][31]

Apart from the TLC method a large number of species can be developed or explored using simple column chromatographic techniques. This method of separation with course of reagents which cleans or removes the species from column are used. These are known as 'eluant.'

V_t is the total removal of volume of an eluant run in a column. The volume when 50% of the component has been removed it is called as 'V_{max}.' Also, termed as peak elution volume when elution starts it is V_0.

Cm is the concentration of solute in the mobile phase and Cs is the concentration of solute in the stationary phase.

V_m is the volume of mobile phase.

Vs is the volume of stationary phase.

$R/1\text{-}R = C_m V_m/C_s V_s$ if

R= equilibrium from of the solute in mobile phase.

(1-R) is solute fraction in the stationary phase partition coefficient ad K_d; when related.

$R = [V_m/V_m+K_d\ V_s]$ similar of solvent extraction method.

$D = (V_w/V_0-E)/(100+E)$ or $E=100D/(D+V_w/V_0)$.

Instead of volume time can also be used such as: tm and ts with same notations. The column capacity is usually expressed in terms of K as:

So, $K = (C_s\ V_s/C_m\ V_m)$ if $\beta = V_m/V_s$ phase.

Ratio and if $K_d=C_s$ Km then (K) is $K=K_d/\beta=K_d\ \beta^{-1} = [V_R - V_m/V_m]$.

The majourity of chromatographic work represents the Van Deemeter equation, which is mathematically state that A+B/u +Cu =H.

If H =height of theoretical plate in chromatography A, B, C are constant,

U=rate of flow of liquid.

So, if a column consists of (N) number of plates and if (H) is height of one plate, and (L) is the length of the column.

Then, N=L/H or H=L/N. i.e., height equivalent of theorised plate. (HETP).

Relate (N) which is peak evaluation volume (V_{max}).

Therefore: In elution curve, V_{max}=peak elution volume,

V_t = total volume of an eluant,

V_0 = commencement volume for elution,

W = Represent base of the plot while, We=width of elution curve. If N= number of theoretical plates then N is related to W and V_{max} as N=16 (V max 2/w) or N=8 (V_{max} 2/We).

This equation represents $V_{max} = V_R$=retention volume.

This help calculate the max number of plates in a column.

Increased number of plates is much efficient in the separation.

Similarly, in the thin layer and paper chromatography (U.S.P, B.P.), R_f is the retardation factor, therefore the retardation is the measurement of the solute front with that of measurement of solvent front in a mathematical ratio of each plate individually as an average of 3 developed chromatographic plates.

The resolution factor (R) is generally involved to give efficiency i.e., $R=Z/4\ \sigma$.

Z = Separation of zone centres.

σ = Standard deviation of the zone.

So, R = (V_{R2} - V_{R1})/0.5 (W_2+W_1), two individual components separate elution peak volumes are represented with V_{R1}, V_{R2}.

The rate of flow Fc of a mobile phase in the column, represented as: V_R=(T_R×F_c) and R=[t_m/t_m+t_s].

XXVIII. Stereochemistry /3D chemistry: Chemistry's subdiscipline of stereochemistry examines and manipulates the relative spatial arrangement of the atoms that make up molecules. Stereochemistry is the study of the interactions between stereoisomers, which by definition have the same molecular formula and order of linked atoms (constitution) but have different structural formulas (the three-dimensional orientations of their atoms in space). Due to the fact that the prefix "stereo-" signifies three-dimensionality, it is also known as 3D chemistry. So, it is also generally referred as 3D chemistry that is because of the word "stereo-" that denotes three-dimensionality.

XXIX. RESULT

XXX. Macroscopic study

The macroscopy of the plant was done using a magnifying glass and revealed that its leaves have small hairy covering that is like velvet fur coating. This hairy covering are coloured in metallic hue of golden or copper tint in colour. Covers throughout the leaves. The leaves are large and as described above in the pharmacognistic description of the plant. The fruits are round and contain sap inside the fruit when ripe and wet or unripe but well formed. The fruits are dried and the sap too that is present in it. They can be powdered and stored by removing seeds inside of it. The leaves are enough well-formed and gives good bio-fuel during winters to keep oneself warm and also used inside the bonfire chimney of the house during the time of winters. After complete drying the fruits shed the hairy part that is present on the unripe fruits and the ripe and dried fruit sheds off the plants. The fruit sheds and fall on the ground of fall in the collecting net or cloth present under the tree, in care cultivation of the plant is done for the medicinal and its scientific values.

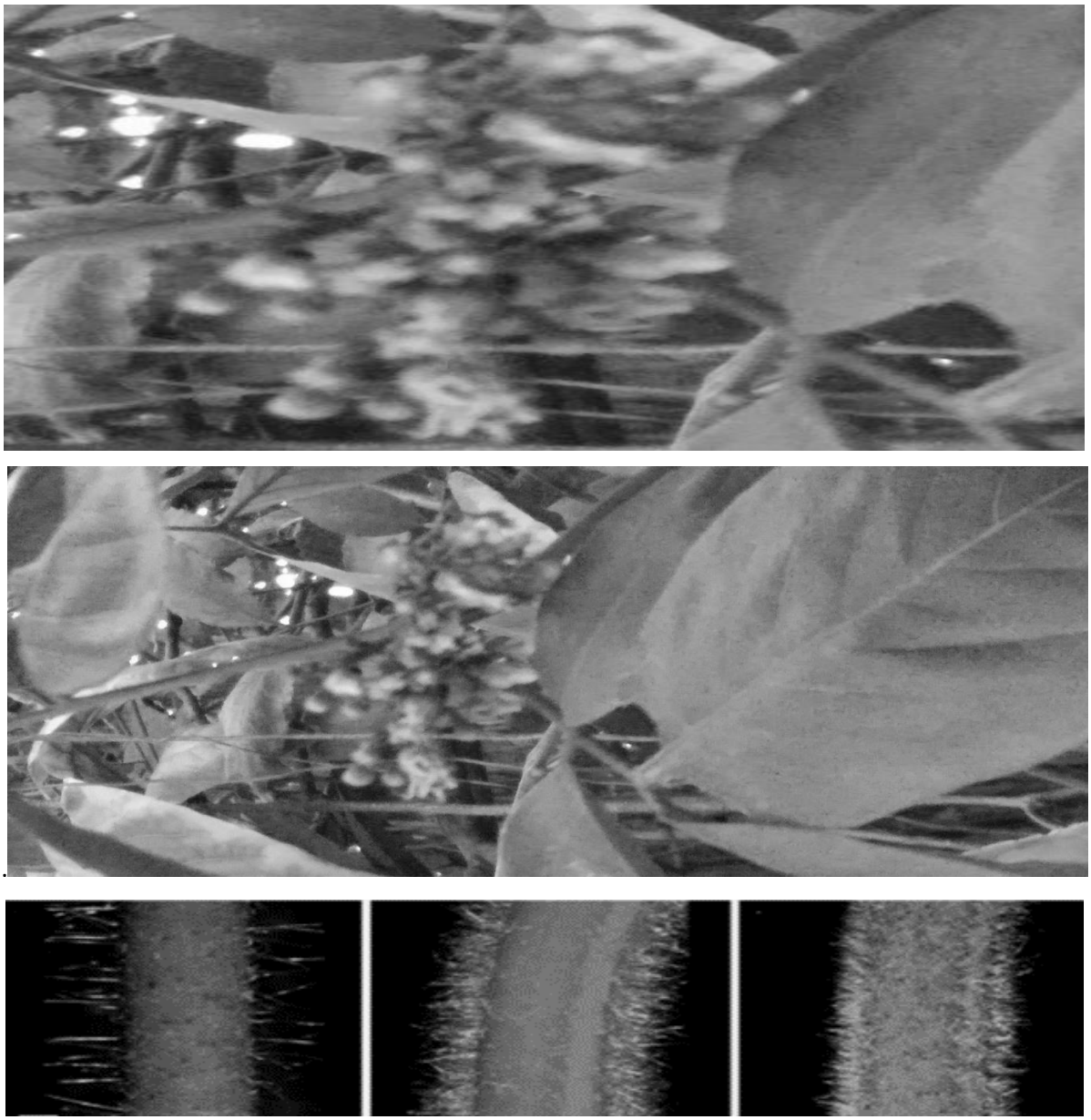


Figure 10: Macroscopy. (Macroscopic investigation on *Sapindus mukorossi*).

XXXI. Microscopic study

Microscopic study revealed data on its microscopy and potential identification of microscopic characters that are observed through microscopy of the plant material leaf.

The investigation is documented below in the form of microscopic images. The staining of these transverse section of the plant was done with the help of the scalpel or the bisturi. These small sections cut from the plant body was subjected to the chemical dying using synthetic and laboratory prepared staining mediums to give accurate data on the microscopy of the plant. The genuine idea is to understand the plant botanically and in prospective of pharmacognosy standards before utilising any plant materials in the study of pharmaceutical

preparations for the quality control purposes in the pharmaceuticals or the medicinal business.

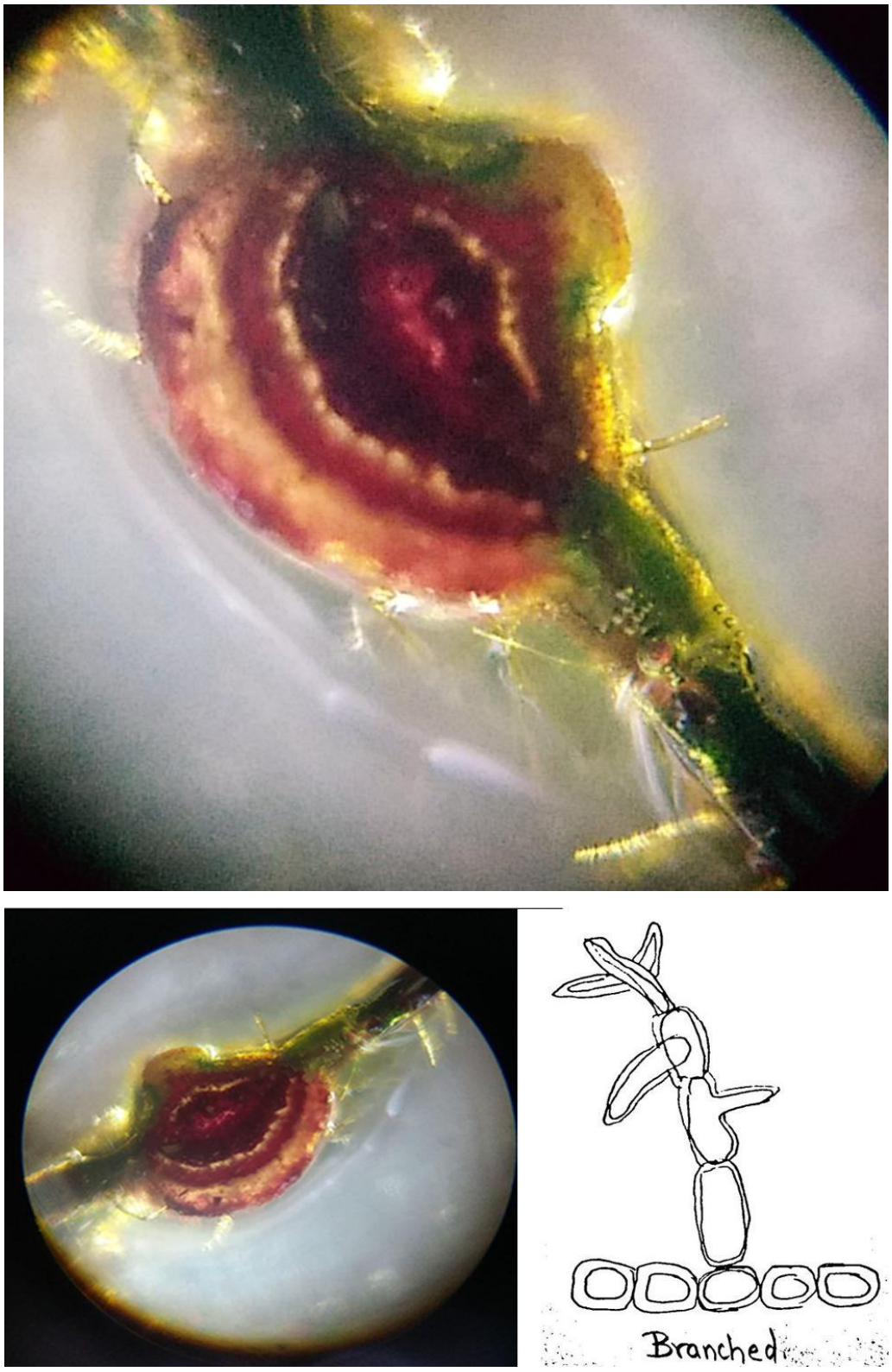


Figure 11: Microscopy of the leaf. (Microscopic investigation on the leaf of *Sapindus mukorossi*).

XXXII. Chemical tests

Confirmatory test for saponins was performed and the revealed results were positive and thus saponins were present in its fruit or the soapberry itself.

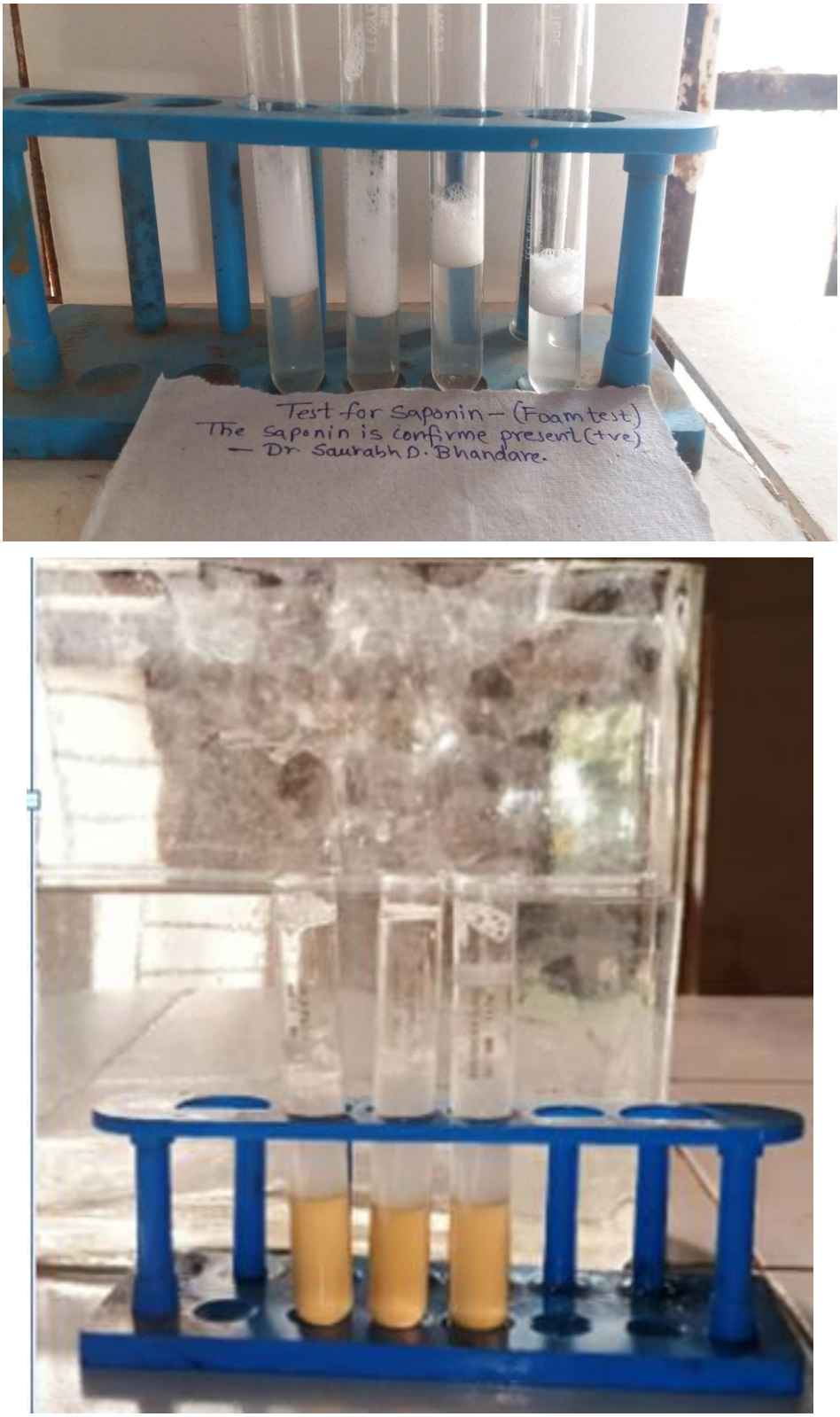


Figure 12: Saponin test for the Soapberry.

XXXIII. pH of the soapberry

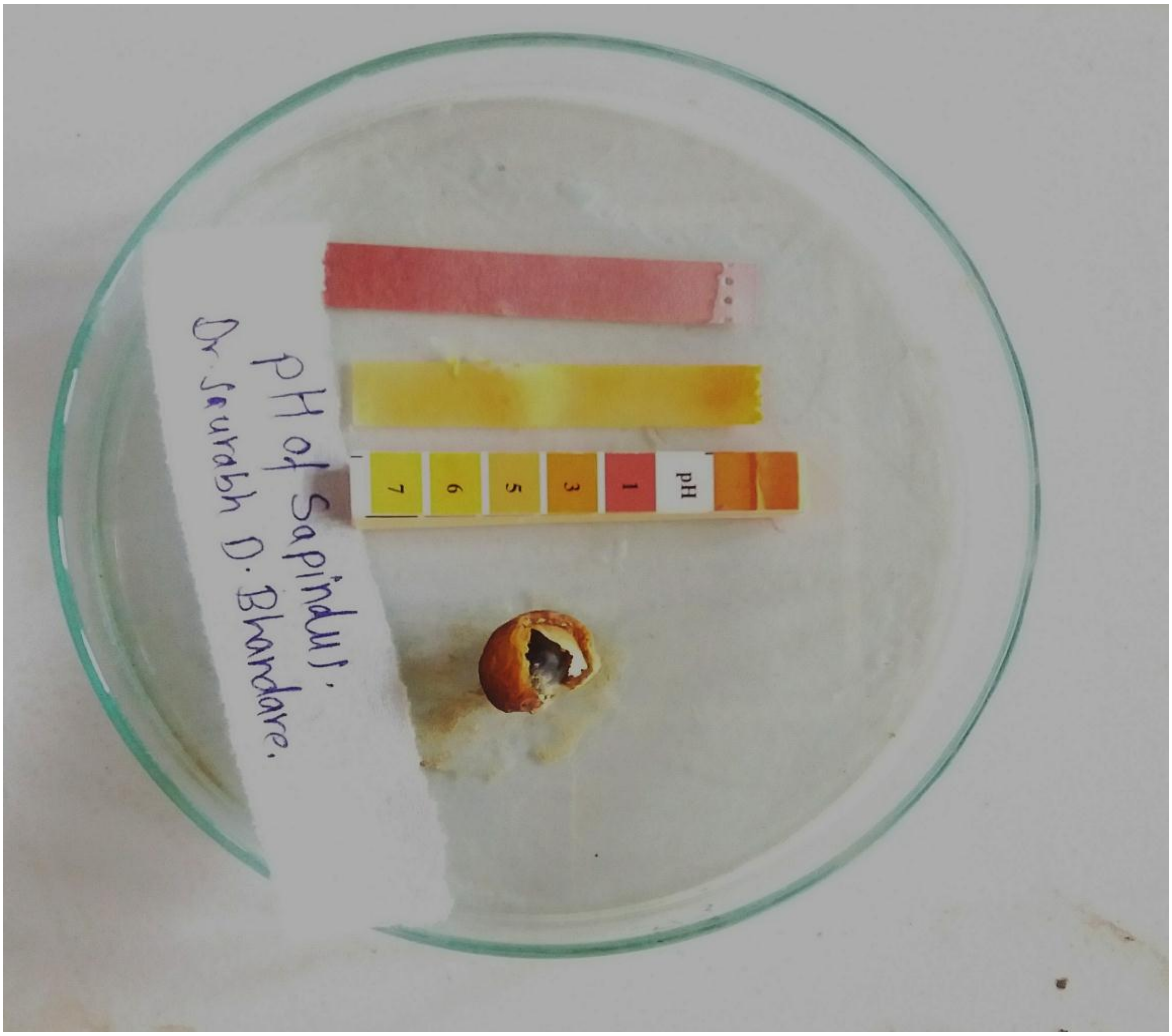


Figure 13: The recorded pH was found to be 6 pH.

XXXIV. Test for Alkaloids

XXXV. a. Test for alkaloids was performed for fruits: Dragondroff test

Figure 14: Dragondroff test resulted negative, presents absence of alkaloids.

XXXV.b. Test for alkaloids was performed for fruits: Wagner test: specific amount about 2-3 mL of filterate + few drops of Wagners reagent was utilised to define the test results. **(The recorded results show the test to be nrgative -$_{ve}$).**

Figure 15: Test for alkaloids performed for fruits/soapberry.

XXXV. c. Test for alkaloids was performed for leaf extract of *Sapindus*

The discovered test results were observed and recoreded in the document. It show the presence of the of the alkoloids in the plant of *Sapindus*. The test results showed positive results. The colouration have been produced and the observation have been pictured with the help of camera as below:

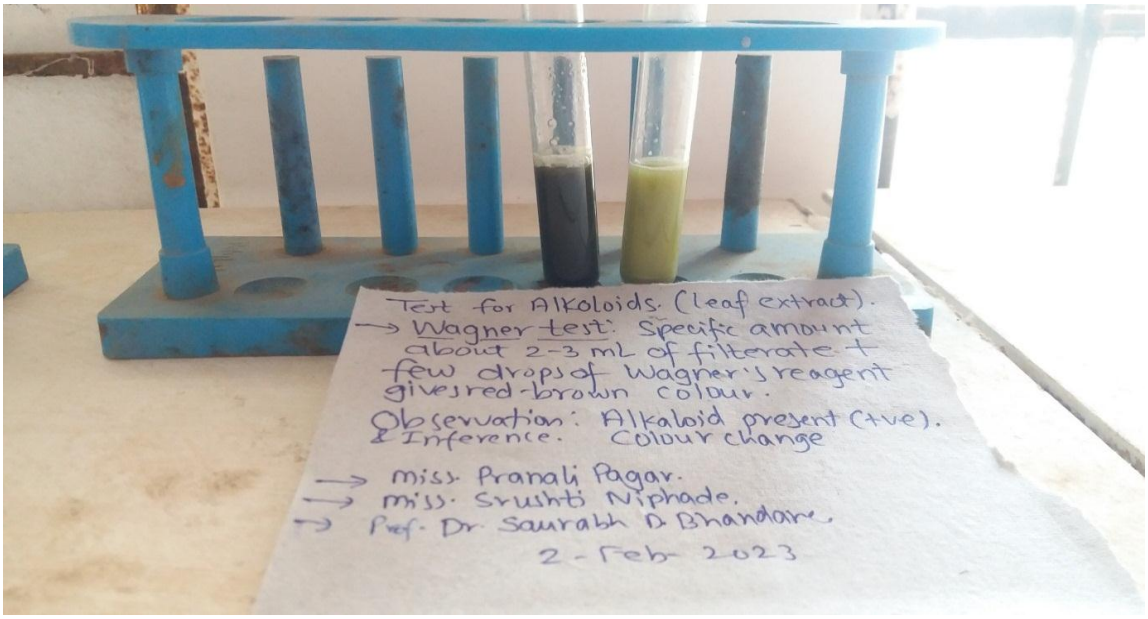
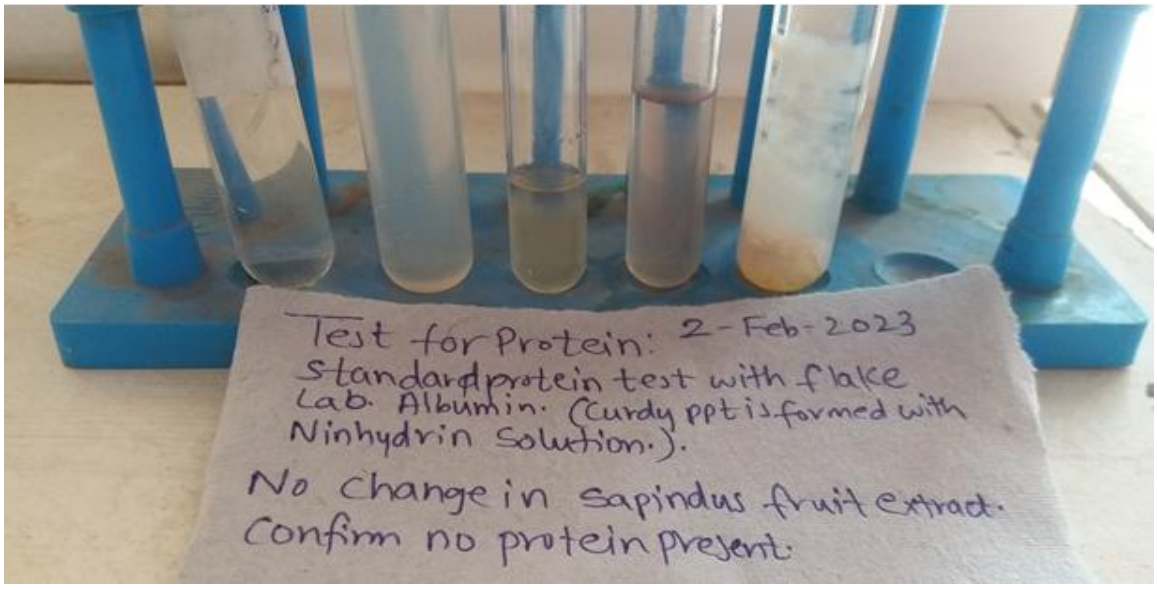


Figure 16: Test for alkaloids (Leaf extract).

XXXVI. Test for protein: Test for protein was carried out using the typical methods and the discovered results were observed and documented.



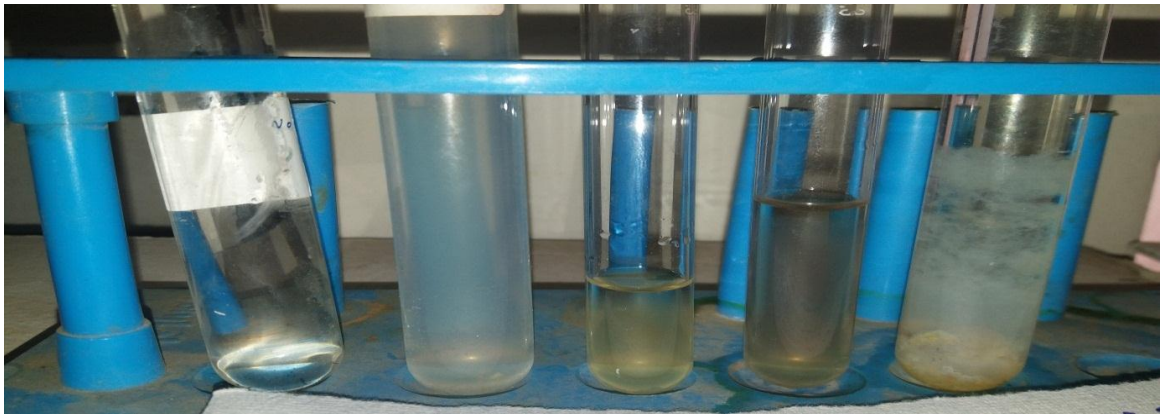
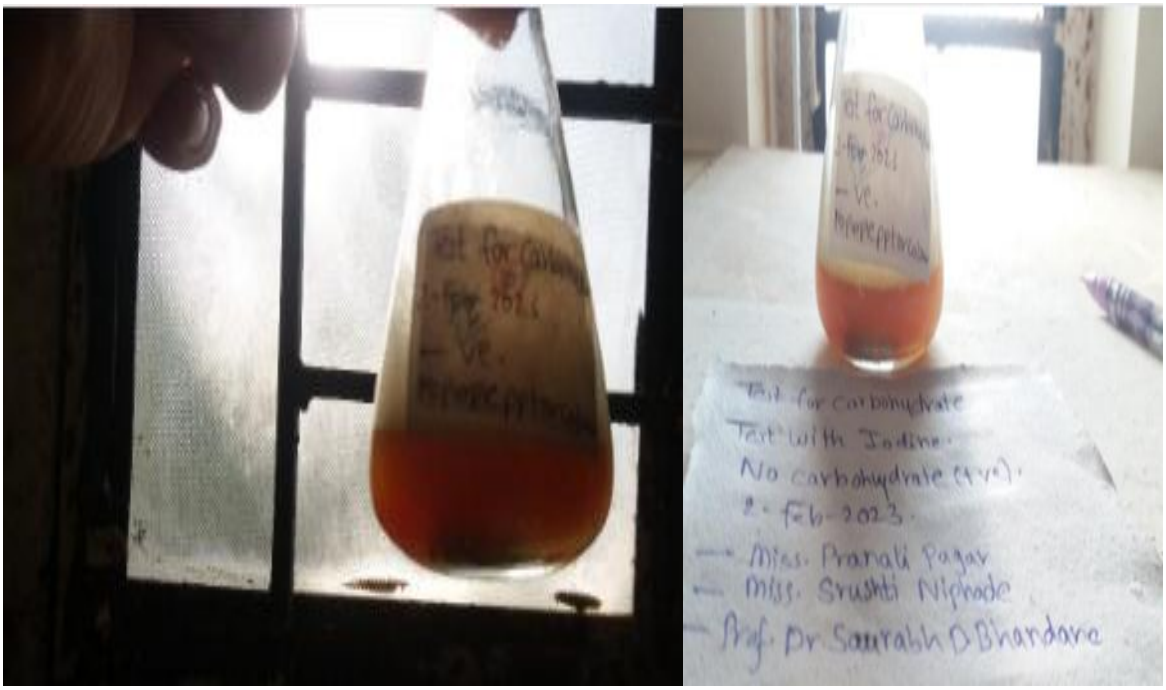


Figure 17: Test for protein.

XXXVII. Test for the carbohydrates: The discovered results were documented and the photograph of the produced results have been recorded in this file to sufficiently record the results for future purpose. The test for the carbohydrate has shown to be negative $-_{ve}$.



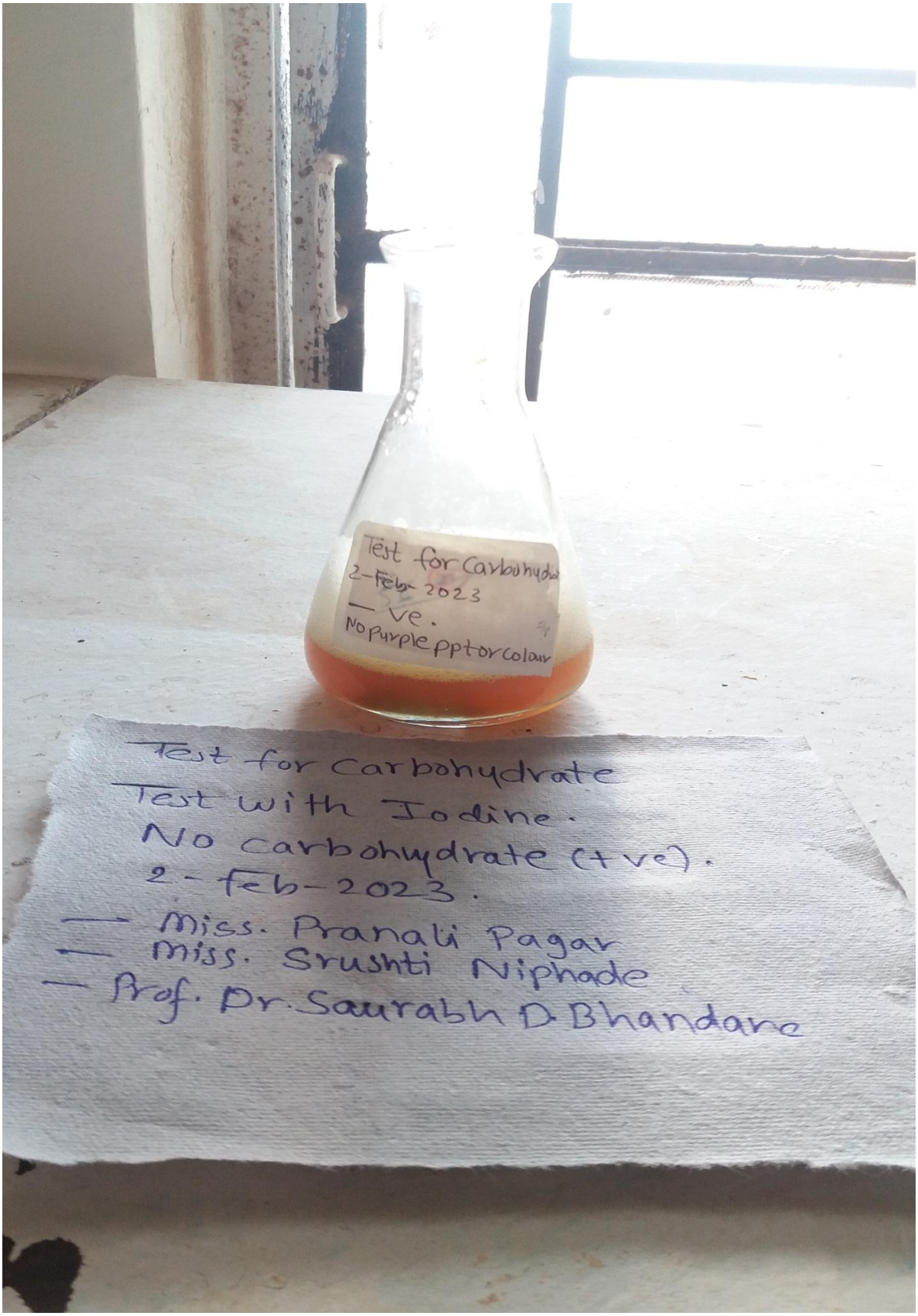


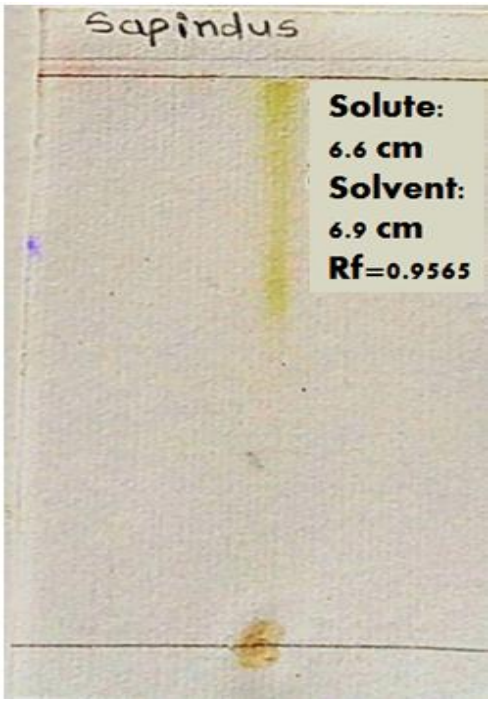
Figure 18: Test for carbohydrates.

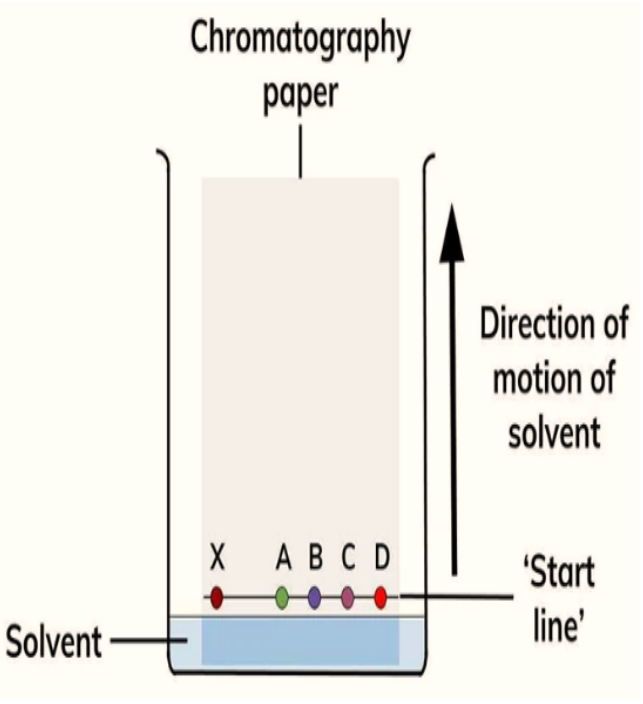
XXXVIII. Phenological stage studies

During the yearly observatory studies, in the month of February, new buds sprouts, in the month of March, new shoots and leaves sprouts, in the month of April to May, new inflorescence develop, during the month of June, July and, August; the inflorescence stages to development and develops to reproduce sexually by exchanging the sexual gametes within the reproduction cycle either by any of reproductive mediators. Further, from half of the August to September green tender soap berries develop; which ripens in the month of the hues or turns to ripe from its raw stage in the month of the October. In the month of the November, the soapberries are completely ripened and are ready to harvest stage. These Soapberries fall down till the stages of its complete maturity on the tree up till the month of December and also there is notable abscission observed.

XXXIX. Thin layer chromatography (TLC)

The TLC studies were performed and the study gave the idea and data was discovered on chromatographic methods on the ***sapindus.*** Solvents selection method for the TLC of ***Sapindus*** of the solvents have been giving the accurate results for the performed TLC and were done in triplicate to get the accurate results that were obtained through this research carried out that signifies the importance of the TLC for the plant-based materials in the phyoto-pharmaceutical developments. The Rf value was recorded to be: **0.9565.**





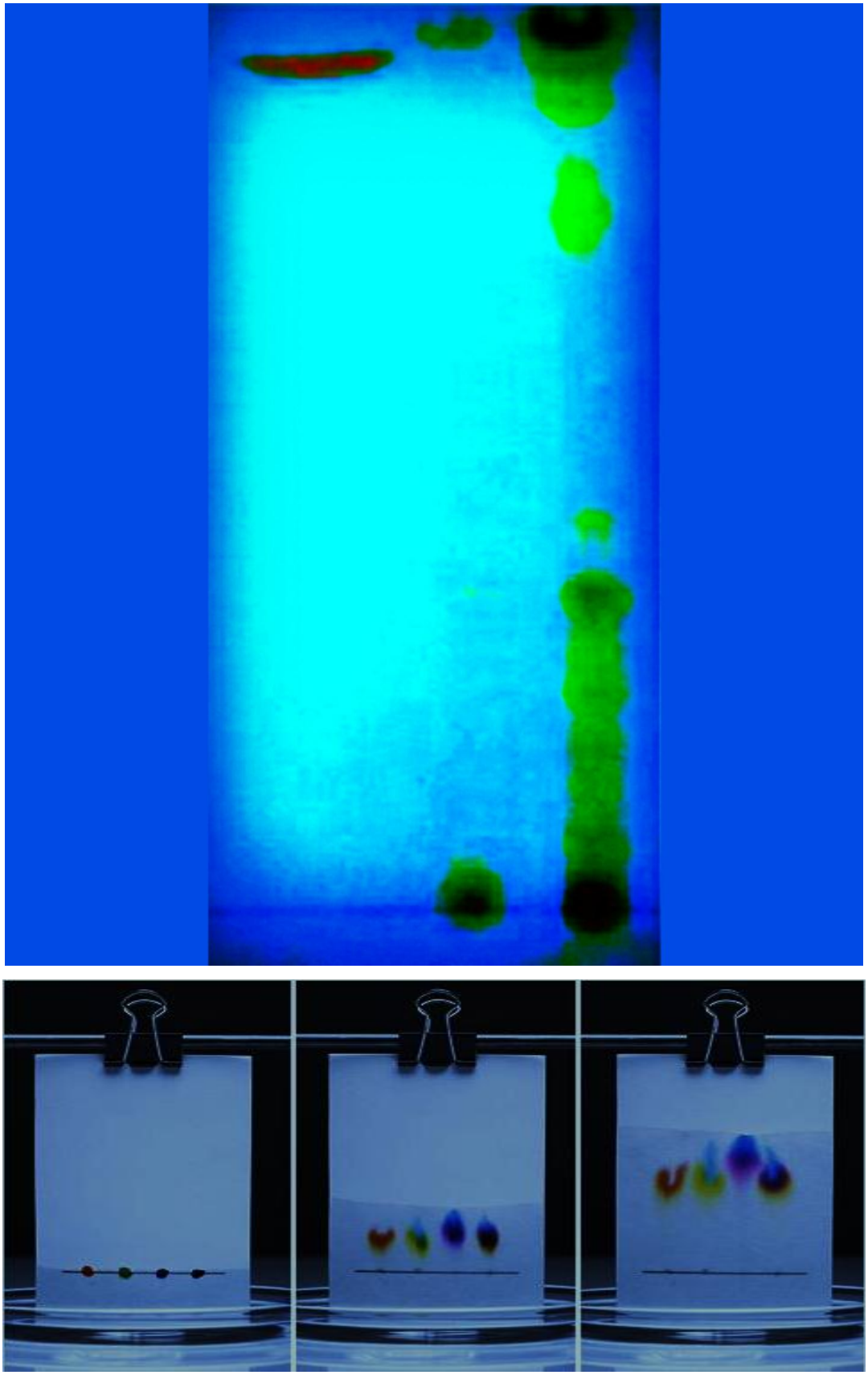
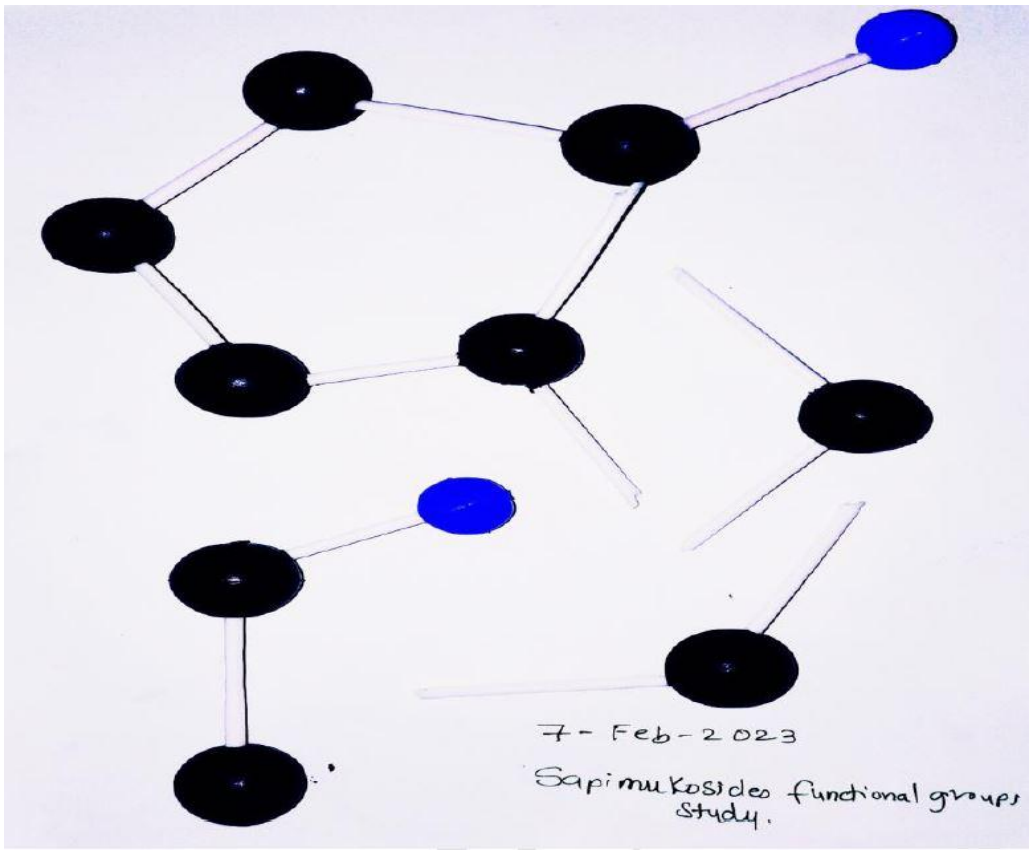
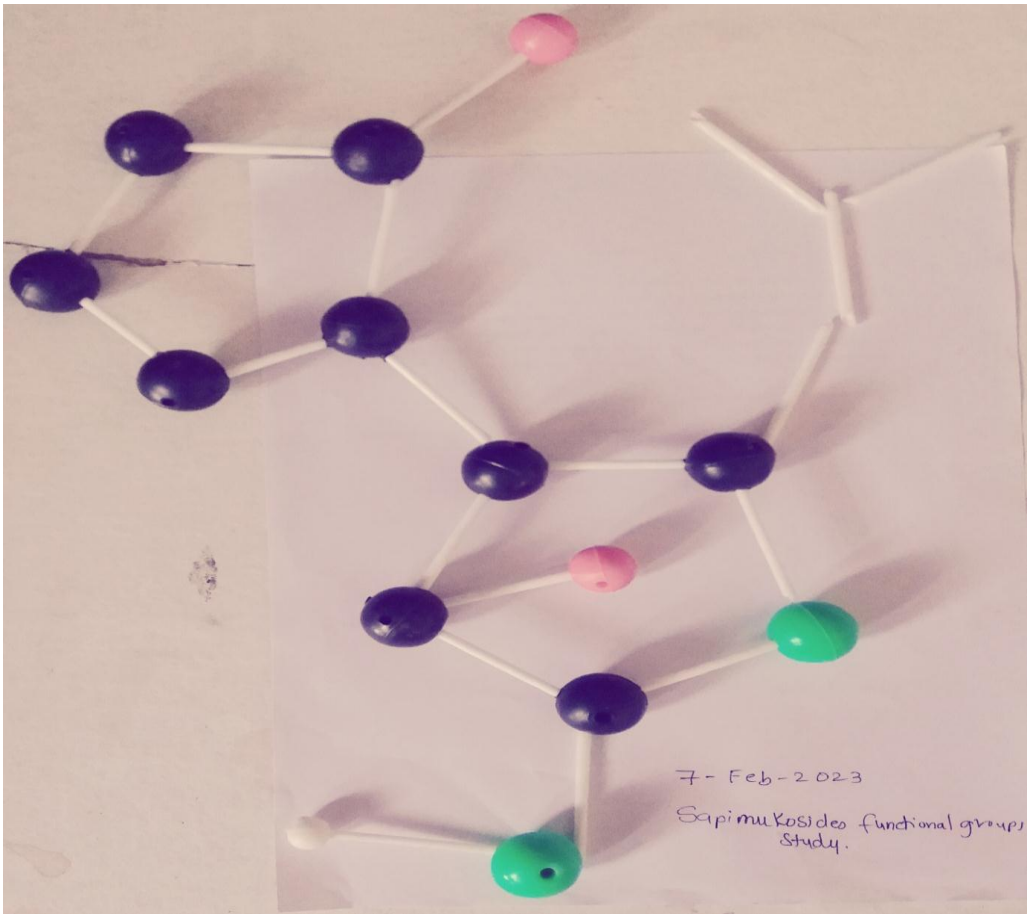


Figure 19: Thin layer chromatography (TLC) of *Sapindus,* in ratio of suitable solvents.[32]

XL. Stereochemistry /3D chemistry: (Stereochemistry /3D chemistry-study)





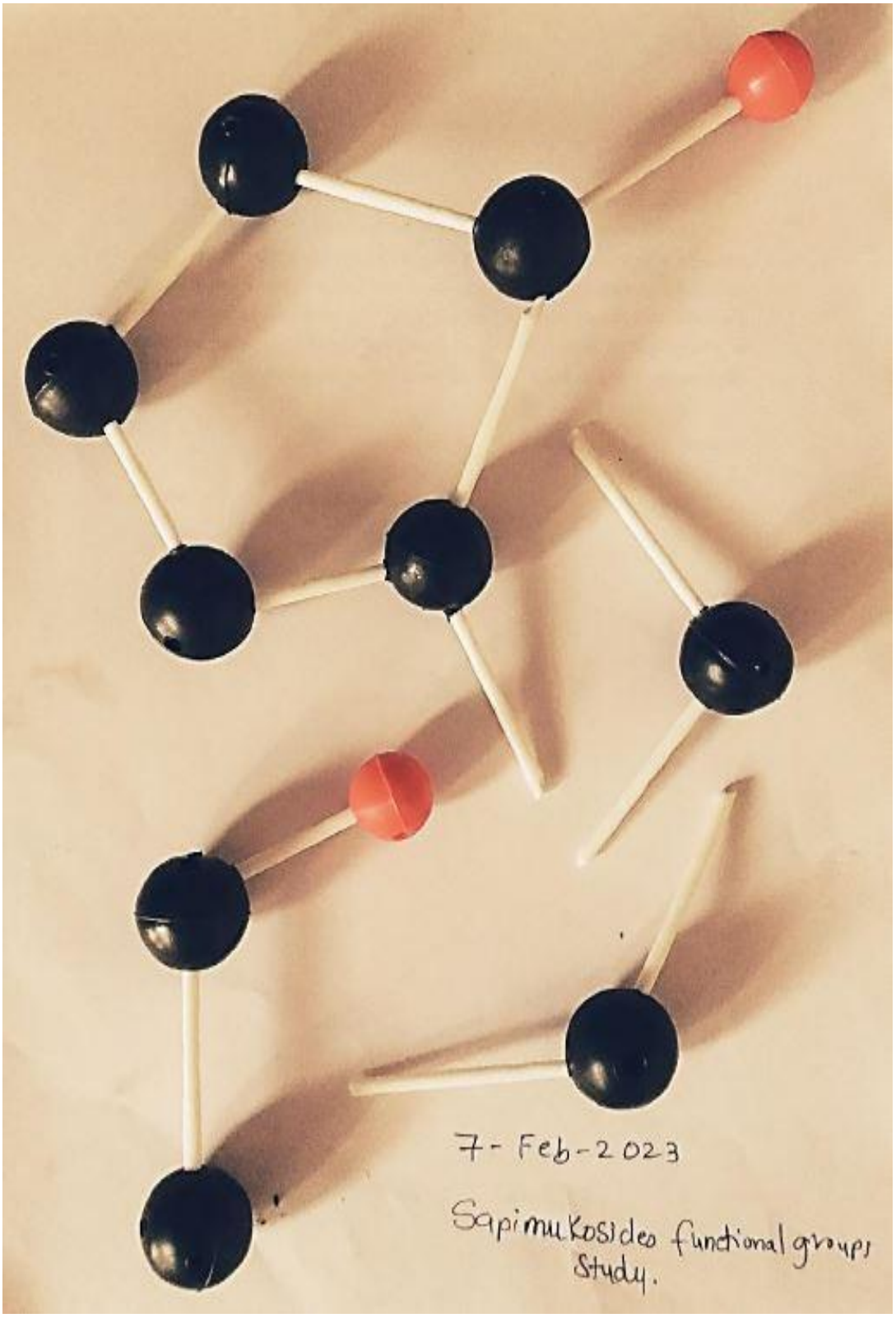


Figure 20: Stereochemistry /3D chemistry: (Stereochemistry /3D chemistry-study).

XLI. CONCLUSION

The main chemical constituents of the soapberry pericarp are terpenoids (especially triterpenoid saponins and sesquiterpenoid glycosides), phenylpropanoids, steroids, and saccharides, observed through various laboratory chemical reaction and standard tests to process the presence or identification of any unknown chemicals in the plant. The Phenological study was done for this plant through various plant studies carried through observation. These studies help in large required information to be discovered and required for to process the plant-based industries to collect and store the raw materials with these studies. The season and the climatic influence are also one of the majour factors that are done through these studies. The study of the timing of life cycle events, such as the production of flowers and fruits, can be summed up as phenology. These occurrences are frequently brought on by environmental cues, making them ideal for examining the potential effects of temporal and geographical variation on the life cycle of plants. The various phytochemical studies based on the chemical test gave a summed up on a discovered data on the plant chemistry in details. These studies revealed the presence of saponins and various other test that give a perfect information on its phytochemical presence in it.

These studies signify the value of the plant materials in the goal to development valuable methodologies and its significance and value in the pharmaceuticals to develop valuable medicinal products that are sufficient to meet the primary objectives and requirements of the pharmaceutical industries. The studies are important to forecast scientific information that are majour requirements to signify the justified value of such plant-based product in the daily life usage.

Herbal caustics may be definitely different than that of the chemical caustics that are required to be studied before usage and therefore is important. Through these studies it had reveal that the majourity of the *sapindus* component have been saponin. An estimate of about 99.99 percent and remaining to be the water component that is naturally present in it. Along with Sapimukosides, Sapimusaponins presence. The acidity or basicity of the sap of *sapindus* fruits or soapberry have been slightly acidic that turns the blue litmus paper to light faint red on its immersion in the *sapindus* liquid 1% which though that, disappears within seconds. This reveals that *sapindus*, or soapberry are mildly acidic in nature. The pH study of the sap of *Sapindus* reveal it to be of pH 6.

It confirms a lot of physical and chemical data that have been structured in this research study from the carried-out research. The microscopy revealed its huge data on its microscopic appearance and also help to understand it in a distinct manner, distinguishing individual cells and its structure been studied. Apart from the regular cells identified in the mid-rib of the leaf and the leaf itself, it was found that it has excess of the trichomes on the surface of the leaf, and the microscopic view of its surface study revealed that it has a branched trichomes type of the trichomes.

XLII. ACKNOWLEDGEMENTS

1. Foremost, I would like to express my sincere, gratitude to my project advisor and supervisor honourable prof. Dr. Saurabh D. Bhandare, sir from the bottom of my heart. His on-going and continuous assistance with my research studies is appreciated also for his patience's in redirecting immense of knowledge and support in the form of guidance his advice was helpful to me throughout the entire research and writing for this dissertation or thesis. Thank you for the mentorship and mentoring me. Mentoring is reciprocal and collaborative of guide and the student's efforts towards the achievements of goals, working, decision making and submitting quality work. (Feb. 2023). - Miss. Pagar A. Pranali.
2. I express my immensurable appreciation and thanks for valuable guidance and support, encouragement during the toughest decision in choosing a project topic and working and achieving the goals to our honourable prof. Dr. Saurabh D. Bhandare, sir. It helps me in writing the manuscripts also driven by the mind-set of study goals. That could be prompt to achieve. Studying medicines and developing them is one such of my goal that was better achieved through my handwork and sincere advice from you as a mentor.

Thank you for the betterment, support in this research work. -Miss. Srushti S. Niphade. (Feb. 2023).

XLIII. Conflict of interest

None. The authors produce no data on any conflict of interest amongst them and in publication of this research carried out to be publish in the suitable journal of scientific interest.

XLIV. Appendix

XLV. Herbarium of the Indian soapberry. *(Sapindus Mukorossi.).*

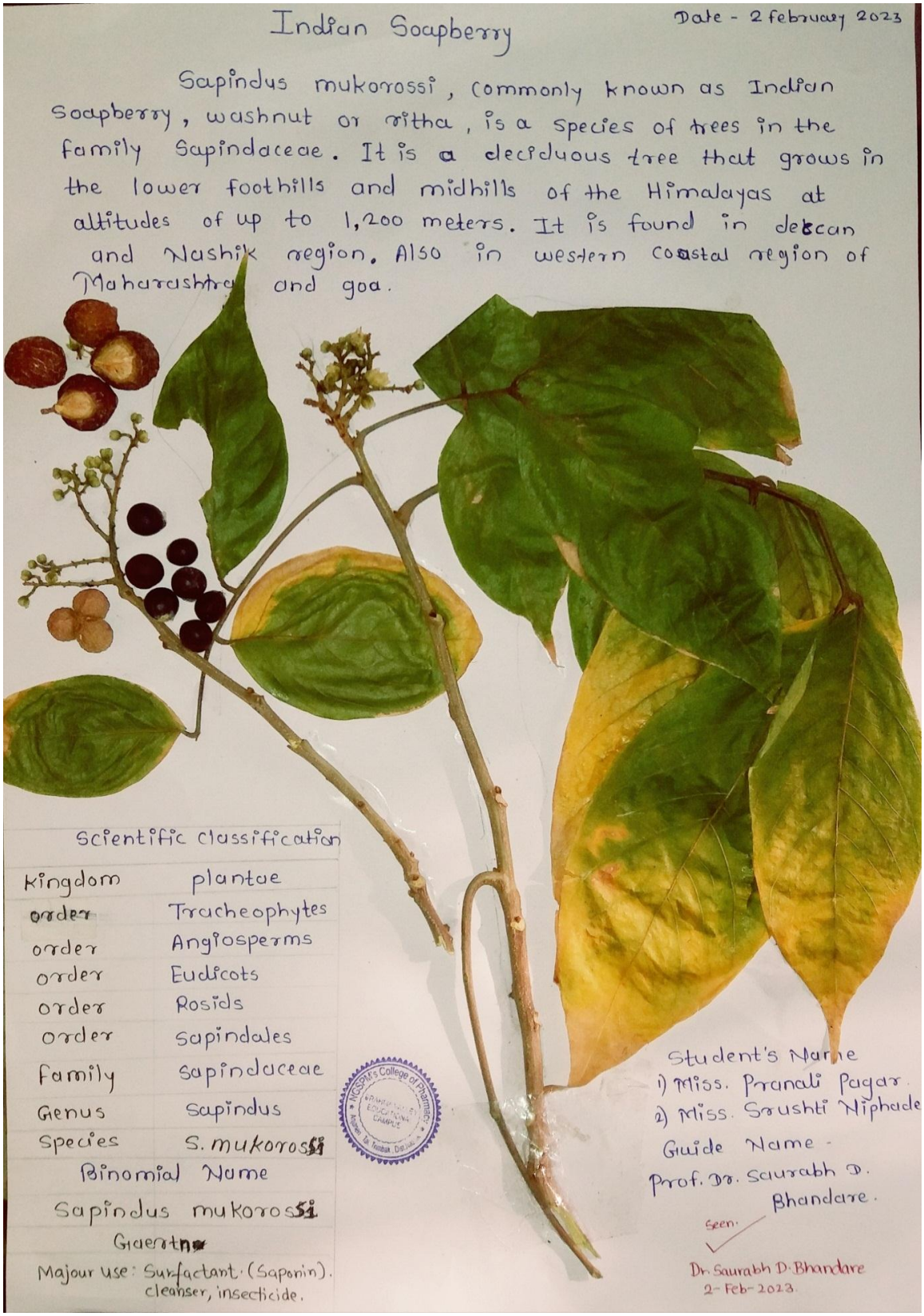


Figure 21: Herbarium of the *(Sapindus Mukorossi.)* the whole part of the plant. Chemically treated.

XLVI. REFERENCE

1. https://en.wikipedia.org/wiki/Template:Taxonomy/Sapindus [22-January-2023]
2. https://reethainfo.blogspot.com/2023/01/soapberries.html [22-January-2023]
3. https://indiansoapberries.blogspot.com/2022/10/apply-these-8-secret-techniques-to.html [22-January-2023]
4. http://www.mobot.org/MOBOT/research/APweb/genera/sapindaceaegen.html [22-January-2023]
5. https://www.soapnuts.co.uk/blogs/news/18979835-the-many-uses-of-soapnuts-in-ayurveda [25-January-2023]
6. https://tree-nation.com/projects/trees-for-tribals/species [26-January-2023]
7. https://reethainfo.blogspot.com/2023/01/soapberry.html [26-January-2023]
8. https://cutt.ly/89v4fHS [26-January-2023]
9. https://www.thatredhouse.com.au/pages/what-are-soapberries [27-January-2023]
10. https://divcomnashik.maharashtra.gov.in/htmldocs/At_Glance-English.pdf [29-January-2023]
11. https://reethainfo.blogspot.com/2023/01/soapberries_26.html [29-January-2023]
12. https://tinyurl.com/595zcc57 [29-January-2023]
13. https://tinyurl.com/2p9b3buf [29-January-2023]
14. https://tinyurl.com/4tksdzuy [29-January-2023]
15. https://tinyurl.com/2p9b3buf [29-January-2023]
16. https://tinyurl.com/3hzw6dxb [29-January-2023]
17. https://tinyurl.com/2p8anvfk [29-January-2023]
18. https://tinyurl.com/pje8enfn [29-January-2023]
19. https://tinyurl.com/4775aszh [29-January-2023]
20. https://rb.gy/lq0nyy [29-January-2023]
21. https://rb.gy/tihvj2 [29-January-2023]
22. https://rb.gy/yoepvn [29-January-2023]
23. https://rb.gy/sulsit [29-January-2023]
24. https://rb.gy/oagpl5 [29-January-2023]
25. https://rb.gy/aeoq4f [29-January-2023]
26. https://rb.gy/ukcztr [29-January-2023]
27. https://en.bharatpedia.org/wiki/Nashik_division [29-January-2023]
28. https://www.mn-net.com/filter-paper-sheets-mn-214-chromatography-90-100-mm/30-min-smooth-817001a?c=3633 [29-January-2023]

29. https://www.amazon.in/100-Chromatography-Paper-Strips-Laboratories/dp/B081RJGSHH [29-January-2023]
30. https://rb.gy/vmwuc7 [29-January-2023]
31. https://chromatography-techniques.blogspot.com/2023/01/chromatography.html [29-January-2023]
32. https://rb.gy/qleemx [10-Februry-2023]
33. https://indiansoapberries.blogspot.com/2023/02/sapindus-berry.html [12-Februry-2023]
34. https://rb.gy/phmusc [12-Februry-2023].
35. British pharmacopoeia-2005, Volume-I, medicinal and pharmacopoeial substances 5th edition of the European pharmacopoeia, London, the stationary office. 2005, 0113226829. Page number. 10, 16.
36. United States Pharmacopoeia, USP-NF 2008, Volume-I. The official compendia of standard, USP-NF, P.P. 8, 233, 234. Twin brook, Rock Ville 20852, 1-889788-54-6.
37. https://chromatographicseparationtechniques.blogspot.com/2023/02/chromatographic-sepatartion-techniques.html [19-Feb-2022.]