



Pharmacognostical Exploration And Pharmacological Potential Of *Solanum Indicum* Berries Belongs To The Family Solanaceae

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Abstract

Solanum indicum, also recognized as Vanbhanta or Brihati, holds a significant place in traditional medicinal practices, notably Ayurveda. This review concentrates on a thorough examination of *Solanum indicum* fruits, encompassing their Pharmacognostical evaluation and potential pharmacological applications. The assessment encompasses various facets such as the plant's morphological traits, microscopic attributes, chemical constituents, and physical constants, offering valuable insights into the plant's quality and purity. *Solanum indicum* fruits exhibit distinct Pharmacognostical characteristics facilitating their identification and quality assessment. Noteworthy features include the presence of small, spherical berries in shades of yellow to orange, typically measuring 1-2 cm in diameter. The fruit's exterior is smooth, housing numerous small seeds within the inner pulp. Microscopic scrutiny reveals abundant parenchyma cells, vascular bundles, and distinct glandular trichomes. The pharmacological potential spans diverse therapeutic areas, including anticancer, antioxidant, and antipyretic properties, showcasing their versatility as a natural remedy. Research indicates that *Solanum indicum* fruit extracts may possess antipyretic activity, making them particularly intriguing in regions where they are indigenous. These fruits hold promise in alleviating fever symptoms and contributing to the overall well-being of individuals with febrile conditions. Additionally, preliminary studies suggest that certain compounds within *Solanum indicum* fruits may exhibit anti-cancer properties by impeding cancer cell growth and inducing apoptosis. Abundant in antioxidants, especially flavonoids and polyphenols, *Solanum indicum* fruits play a role in scavenging free radicals and shielding cells from oxidative damage.

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Keywords: - *Solanum indicum*, Phytoconstituents, Antipyretic, Hydroalcoholic, Microscopic, Pharmacognostical

Introductions

In traditional medicinal systems such as Ayurveda, Siddha, Unani, and Homeopathy, *Solanum indicum* plays a pivotal role as a medicinal plant. In Ayurveda, it is utilized to balance Vatta, Pitta, and Kapha, with the fruits of *Solanum indicum* serving as a potent remedy for various diseases. The peak time for the growth of *Solanum indicum* fruits is June, during which the maximum concentration of bioactive phytoconstituents is present. These fruits are known to have therapeutic potential for conditions like difficult breathing, ulcers, abdominal pain, and cough, attributed to the bioactive phytoconstituents present in the natural compounds [1,2]. *Solanum indicum*'s plant profile includes features such as a height ranging from approximately 0.3 to 1.2 meters, with a highly branched structure and formidable prickles. The fruits turn yellowish when ripe and are of a globous size. Local or vernacular names for *Solanum indicum* include Bruhati, Barhanta, Vanabharata, Poison berries, among others. [3] This plant species is widely distributed across India and is found in various tropical and subtropical regions. The rich traditional medicinal use of *Solanum indicum* has been passed down through generations, addressing a wide range of health conditions. Traditional practitioners employ its fruits, leaves, and roots to treat ailments such as anorexia, cough, sore throat, abdominal pain, and more. *Solanum indicum* is also used for managing pain and fever, reducing inflammation, treating insomnia, addressing urinary complications, and managing cardiac weakness, showcasing its remarkable and diverse therapeutic potential in traditional medicine. The plant contains various phytoconstituents like Solanine, solasornine, solamargine, solasidine, waxy material, fatty acids, etc., contributing to its therapeutic potential in human health [4, 5, 6].

Plant profile

Solanum indicum is a robust and densely branched shrub, reaching heights of 0.3 to 1.5m. The branches are notably stiff, adorned with short, hooked prickles that are sharp, slightly curved, and feature a broad compressed base. Its fruits transition from green with a white lining in their youth to a ripe, yellow hue. The stems and branches are coated with brown, hairy stellate structures. [7, 8, 9]

Botanical source: *Solanum indicum* Linn.

Family: Solanaceae.

Taxonomic classification: Taxonomic classification shown [10]

Kingdom:	Plantae
Division:	Magnoliophyta
Phylum:	Tracheophyta
Class:	Magnoliopsida
Order:	Solanales
Family:	Solanaceae
Genus:	<i>Solanum</i>
Species:	<i>Indicum</i>

Geographical distribution and habitat: *Solanum indicum* mainly grow in torrid or sub torrid area of India, Nepal, and Pakistan up to mount of 1500m. This herbs usually grow waste place, on roadside [11] this adaptable plant exhibits a broad distribution across tropical regions, encompassing India, Sri Lanka, Malaysia, China, and the Philippines. In India, it flourishes in diverse tropical habitats, including wastelands and roadsides, spanning from sea level up to an elevation of approximately 1500-2000 feet. [12-19]



Fig. No. 1 Berries of *Solanum indicum*

1. Powder microscopy:

An essential technique for quality control is powder microscopy, which studies particular microscopic characteristics using staining agents such as hydrochloric acid (1:1), iodine, chloral hydrate, and phloroglucinol. It is the most effective method for assessing crude medication adulteration. Fresh *Solanum indicum* fruits were gathered, let to dry in the shade, and then roughly ground using a pulverizer.[20] The freshly powdered fruits of *Solanum indicum* were steeped in 20% nitric acid for the entire night before being cleaned the next day with distilled water. The powder is transferred on a slide, and then the staining reagents hydrochloric acid, safranin, iodine, and phloroglucinol are added. [21]

1.1. Reaction of chemicals with powdered crude drugs & fluorescence analysis:

Fluorescence, also known as luminescence, is the process by which light is absorbed and then released. There are a few materials with longer wavelengths that have lower energy than absorption radiation. Therefore, light emitted is visible while radiation absorbed is invisible to the eye. When this light comes into touch with uv radiation, it gives off the characteristic color of a fluorescence material. An essential factor in determining the medicinal plants' quality is fluorescence analysis. After being powdered and treated with various solvents, the fruits' fluorescence under uv light was detected [22,23]. The dried fruits that had been roughly ground were first examined in daylight and under two distinct uv light wavelengths (254 and 365 nm). One gram of powder was placed in a test tube and exposed to two to five milliliters of various bases, acids, and solvents are given in table no.1 and 2.[24]

2. Standardization parameters

Standardization refers to the establishment of predefined criteria for assessing the quality, safety, and effectiveness of crude drugs. This term encompasses various measures and parameters applied throughout the processing or manufacturing stages. Key factors in this evaluation include assessing attributes such as loss on drying, ash value, foreign matter, foaming index, swelling index, and others. These diagnostic features play a crucial role in verifying the distinctive characteristics of crude drugs [25] are given in table no.3

2.1. Foreign organic matter:

The meticulous preparation of herbal drugs requires careful attention to ensure that only the validated portion of the plant is utilized, free from any infestations or fungal growth. Special care is taken to exclude visible impurities such as stones, sand, harmful substances, or chemical residues, ensuring the absolute purity and safety of the herbal drugs. In addition to addressing visible contaminants, herbal medicine must also be safeguarded against the presence of animal elements, including insects and concealed microbial agents.[26] Some of these agents have the potential to produce toxins that could jeopardize the quality and safety of these valuable remedies. To commence the examination process, a 100-gram sample of powdered *Solanum indicum* fruits is evenly spread in a thin layer on a suitable platform. The inspection is carried out under daylight conditions, utilizing either the naked eye or a 6X magnifying glass, to meticulously identify and separate any foreign matter from the herbal materials. If needed, an appropriate sieve can be used to effectively isolate foreign matter from the herbal sample for the isolation of dust, classified as a mineral admixture, a 250 µm sieve can be employed to sift the sample, ensuring the refinement of the herbal preparation. Once the foreign matter has been sorted and separated from the powdered *Solanum indicum* fruits, it becomes essential to accurately weigh the collected foreign matter. This weight allows for the calculation of the percentage of foreign matter present relative to the weight of the original drug sample of powdered *Solanum indicum* fruit. [27,28]

2.2. Ash value studies

The examination of ash values in herbal drugs primarily focuses on quantifying the inorganic residue, including phosphates, carbonates, and silicates that may be present. These values play a crucial role as indicators, providing insights into both the estimated quality and purity of the herbal medicine under scrutiny. By removing all traces of organic matter during the ashing process, any potential interference in subsequent analytical evaluations is effectively mitigated. This ensures the generation of accurate and reliable results in the analysis of herbal medicines. Ash is mainly two types physiological ash and non-physiologically Ash. Physiological ash plant produced itself through biochemical reaction but non-physiological ash does produce itself through biochemical reaction [29, 30]

a. Total ash content

To accurately determine the ash content, take a precise weight of approximately 2-3 grams of powdered *Solanum indicum* fruits and incinerate it in a silica crucible. Conduct the incineration process carefully, ensuring the temperature does not exceed 600 degrees Celsius until all carbon content is eliminated.[31] Once the incineration is complete, allow the dish to cool inside a desiccator for 30 minutes to remove any residual moisture. Without delay, weigh the dish containing the ash sample to obtain the ash content of the *Solanum indicum* powdered materials. If achieving carbon-free ash content through the initial incineration proves challenging, the following steps can be taken: subject the charred mass to hot water treatment, then gather the remaining material using an ashless filter paper. Incinerate both the residue and the filter paper. Next, combine the filtrate with the ash sample and subsequently evaporate it to dryness. Finally, ignite the dried mixture at a temperature not exceeding 600 degrees Celsius. The total ash value is then calculated as a standard for the dried sample, providing crucial information about its ash content.[32]

b. Water-soluble ash studies

Continuing the ash analysis, the ash from the preceding step should undergo a 5-minute boiling process with 25ml of water. This step is designed to extract water-soluble components. Following this, the insoluble residue should be collected, either in a silica crucible or on ashless filter paper, ensuring thorough retrieval to achieve complete purification, meticulously wash the collected insoluble residue with hot water. Subsequently, ignite both the insoluble residue and the original sample, placing them either in the silica crucible or on the ashless filter paper, and maintain a temperature below 450 degrees Celsius. This crucial additional step enhances the comprehensive assessment of the inorganic residues within the powdered *Solanum indicum* sample. [33]

c. Acid-insoluble ash analysis

After subjecting the entire ash to a 5-minute treatment with 25 ml of dilute hydrochloric acid, meticulously gather the non-soluble components by employing either a silica crucible or an ashless filter paper. Following a thorough rinse of the accumulated residue with hot water, proceed to incinerate it and meticulously document its mass.[34] To determine the percentage of acid-insoluble ash, divide the weight of the acid-insoluble residue by the weight of the air-dried drug and then multiply by 100. This calculation provides valuable insights into the amount of inorganic residue that persists after treatment with HCl, facilitating the evaluation of the quality and purity of the *Solanum indicum* fruits. This analytical process holds crucial significance in ensuring the safety and effectiveness of the herbal material across various applications.[35]

2.3. Extractable matter studies

These extracts play a crucial role in the field of pharmacognosy, providing researchers and scientists with essential tools for identifying and quantifying the chemical compounds present in different crude drugs. The use of a diverse range of solvents for extraction enables a comprehensive analysis of various constituents. This includes water-soluble compounds like glycosides, tannins, and mucilage; alcohol-soluble compounds such as tannins, glycosides, and resin; as well as ether-soluble compounds found in drugs containing volatile constituents and fats [36]. Through this analytical approach, a deeper understanding of the chemical composition of crude drugs is achieved, paving the way for their potential application in pharmaceutical and medicinal preparations. Extractive value plays a crucial role in identifying exhausted or adulterated drugs, making it a fundamental tool for quality control and purity assessment of crude drugs. By evaluating the extractive value of the drug, one can ascertain its overall quality and purity, ensuring that it meets the required standards for safe and effective use in various medicinal applications. The information obtained from these solvent extracts not only provides insights into the nature of chemical constituents within the crude drugs but also proves valuable for estimating the quantity of these constituents extracted using the chosen solvent. Furthermore, this extraction method becomes particularly advantageous when dealing with materials for which no appropriate chemical or biological assay has been developed, as it offers a reliable means to assess and evaluate the composition and potential properties of the crude drug. [37]

a. Cold maceration method for extractive value determination

To determine the content of extractable matter in the air-dried *Solanum indicum* fruits powder, precisely weigh about 5 grams of coarsely powdered *Solanum indicum* fruits and place it in a flask with a glass stopper. Conduct maceration by adding 100 ml of specified solvents such as Ethanol, Methanol, Water, Petroleum ether, Ethyl acetate, Chloroform, Benzene, n-hexane, etc., and shake the mixture regularly for 6 hours. After maceration, allow the flask to stand undisturbed for 18 hours.[38] To isolate the extractable constituents, rapidly filter the mixture, ensuring no solvent loss. Carefully transfer 25 ml of the filtrate to a tared flat-bottom dish and gently

evaporate it to dryness on a water bath. Subsequently, place the dish with the dried residue in an oven set at 105 degrees Celsius for 6 hours. Once completed, allow it to cool in a desiccator for 30 minutes before promptly weighing it. By adhering to this meticulous process, the content of extractive matter can be accurately calculated in milligrams per gram of air-dried materials. This provides valuable information about the concentration of desirable constituents in the sample of *Solanum indicum* fruits. This in-depth analysis offers crucial insights into the potency and composition of the herbal extract, further aiding in its potential applications across various medicinal formulations. [39]

b. Moisture content analysis

Moisture content analysis in herbal or crude drugs is crucial not only for preventing decomposition, ensuring chemical stability, and controlling microbial growth but also for directly impacting the physical attributes of the products. Maintaining correct moisture levels is vital to prevent issues like mold growth, caking, or degradation of active compounds. Rigorous monitoring and maintenance of optimal moisture content are paramount to guarantee the efficacy, safety, and shelf life of herbal medications, thereby bolstering the confidence of consumers in their medicinal benefits.[40] To conduct moisture content analysis, a small amount (2 grams) of powdered *Solanum indicum* fruits is placed in a crucible with a lid. The sample is then subjected to a hot air oven at a temperature of 105 degrees Celsius, where it is left for 30 minutes to remove any moisture content. Once the drying process is complete, the crucible with the dried sample is taken out of the oven and cooled in a desiccator for 15-20 minutes [41]. Subsequently, the cooled sample is weighed inside the crucible with the lid, providing the precise weight of the dry powder, free from any moisture. This procedure ensures accurate measurement and facilitates further analysis of the sample constituents without interference from water content [42]

2.4. Swelling index analysis

The swelling index serves as a valuable parameter for assessing the water-absorbing capacity of plant materials and detecting the presence of mucilaginous content. This analysis is crucial for researchers and scientists to understand how the material interacts with water, providing insights into its potential applications.[43] To determine the swelling index, 1 gram of *Solanum indicum* fruits is weighed and immersed in water. Two readings are then recorded at different time intervals: the initial reading measures the size of the plant material before water absorption, and the final reading is taken after 3 hours. By comparing the initial and final readings, one can quantitatively evaluate the extent of swelling that occurred during water absorption. A higher swelling index indicates a greater water uptake capacity, potentially indicating the presence of mucilaginous compounds within the plant materials. Mucilage, a gelatinous substance, contributes to increased swelling properties. [44]

2.5. Foaming index analysis[45,46,47]

Certainly, here's a summary of the procedure for evaluating the foaming ability of a *Solanum indicum* decoction

1. Weigh 1 gram of *Solanum indicum* powder and place it in a 500ml conical flask.
2. Add 100ml of boiling water and gently boil the mixture for 25-30 minutes
3. After cooling, filter the mixture into a 100ml volumetric flask and adjust the volume as needed with water
4. Divide the solution into ten test tubes, incrementally filling them with 1ml, 2ml, and so on, up to 10 ml, each topped up to 10ml with water
5. Shake each tube vigorously for 15 seconds and allow them to stand for 15 minutes
6. Measure the foam height in each tube. A foam height less than 1cm in every tube indicates a foaming index below 100
7. If a tube has a foam height of 1cm, note the volume 'a' (in ml) of the decoction for precise results
8. If the foam height exceeds 1cm in all tubes, the foaming index is 1000 or more
9. Calculate the foaming index using the formula: $\text{Foaming Index} = 1000/a$, where 'a' is the volume (in ml) of the decoction used in the tube with a 1 cm foam height

This analysis provides insights into the foaming characteristics of the *Solanum indicum* decoction, aiding in the understanding of its medicinal properties and potential applications

3. Test for heavy metal

Sl. No.	Heavy metal contents	Limits
1.	Lead	NMT10 ppm
2.	Arsenic	NMT 3 ppm
3.	Cadmium	NMT 0.3 ppm
4.	Mercury	NMT 1 ppm

To ensure accurate testing, transfer approximately 3gm of the test substance into a clean, dry 300 ml kjeldahl flask. Securely clamp the flask at 45 degree angle and moisten the substances by adding concentration nitric acid. Gently warm flask to initiate the reaction, then carefully add the nitric acid mixture, heating after each addition. Continue this process until total of 18ml of acid has been added. Heat and boil the solution until it darkens. After cooling down, add 2ml of nitric acid and apply heat until the solution darkens again. Keep adding nitric acid until no further darkening occurs and white fumes are produced [48]. To cool add 2-5ml of water, then continue heating until the volume is reduced by few ml. Next, cool the solution by adding 5ml of water and observed the colour. If the colour appears dark yellow, add 30% hydrogen peroxide solution. Apply heat to evaporate the density, and if the colour remains yellow add 2-5ml of water with hydrogen peroxide solution. Dilute the solution with a few ml of water and rinse it into a 50 ml comparison colour tube. Prepare a blank solution but withdrawn the sample by follow this method. Prepare minimum 3 standard solution of elements, each with different concentration, covering a range of 25% to 200% of the range present in the sample solution. Add the corresponding reagents to both the blank and test solution. Measure the absorbance of each solution at different concentration and the blank reference separately. Finally record the reading and construct the calibration curve are given in table no.4 [49]

Instrument name: Inductive coupled plasma-atomic emission spectroscopy.

Detector system: uv visible detector with temperature -40 degree c

Nebulizer: Cross flow gem tip.

Standard injector: 1.0-2.0mm inner diameter

4. Extraction process of *Solanum indicum* fruits

According to the explanation by Gaurav Saxena et al., a 1000 g powdered, air-dried sample was blended with 95% ethanol in a 2:1 ratio and left to macerate at room temperature for one week. Following this, the mixture underwent filtration, and the remaining residue was mixed with fresh 95% ethanol for an additional week of maceration. The ethanolic extracts were gathered, filtered, and concentrated under reduced pressure. The resulting extracts were dried and preserved in a sealed container in a refrigerator. Subsequently, the dried extract underwent successive maceration using different solvents. [50] According to Manoj M. Gadewar et al., about 500 grams of *Solanum indicum* powder underwent extraction with methanol utilizing the Soxhlet extraction method over a 24-hour period. Following the extraction, the resulting extract was concentrated using a rotary vacuum evaporator. Subsequently, the concentrated extract underwent lyophilization to yield a powder, which was then employed for subsequent experimental procedures. [51] As per the description by Rizwan Ul hasan et al., a 25-gram sample of dried *Solanum indicum* fruits in powdered form was subjected to extraction with 250 ml of ethanol for 8 hours using a Soxhlet apparatus. Subsequently, the mixture underwent reflux at 60 degrees Celsius, followed by filtration and evaporation to dryness. This process led to the formation of crude extracts are given in table no.5. [52]

5. Preliminary phytochemical screening [53,54,55,56,57]

The chemical tests or preliminary phytochemical screening for various phytoconstituents in the dried powder and extracts of *Solanum indicum* fruits were conducted as outlined below, and the outcomes were documented in table no.6.

5.1. Carbohydrates Test:

a. Molisch's Test: Combine the extracts with 5 ml of distilled water and filter the solution. Treat the filtrate with 2 ml of Molisch's solution, followed by the addition of concentrated sulfuric acid to form a layer without shaking. The presence of a purple coloration indicates the presence of carbohydrates.

b. Benedict's Test: Apply Benedict's solution to the filtrate and gently heat. The formation of an orange color precipitate indicates the presence of carbohydrates.

c. Fehling's Test: In a test tube, add 2 ml of the filtrate to a mixture of equal volumes of Fehling's solutions I and II. Heat or boil the solution for 2-3 minutes. The appearance of a brick-red color indicates the presence of reducing sugars or carbohydrates.

5.2. Alkaloids Test:

a. Dragendorff's Test: Dissolve extracts in concentrated hydrochloric acid and use the filtrate for further testing. Treat the filtrate with Dragendorff's reagent; a reddish-brown color indicates the presence of alkaloids.

b. Mayer's Test: Take an acidic solution of the filtrate and add Mayer's reagent. The presence of a yellowish precipitate indicates the presence of alkaloids.

c. Wagner's Test: Add Wagner's reagent to the filtrate; a reddish color indicates the presence of alkaloids.

d. Hager's Test: Treat the filtrates with Hager's reagent; confirmation of alkaloids is indicated by the formation of a yellow-colored precipitate.

5.3. Detection of Glycosides:

a. Modified Bontrager's Test: Hydrolyze the extracts with dilute sulfuric acid, treat the resulting filtrate with ferric chloride solution, and submerge it in boiling water. A rose-pink to red color indicates the presence of glycosides.

b. Test for Cardiac Glycosides: Add three drops of strong lead acetate solution to the aqueous solution of extracts. Follow further steps to observe bluish-green and reddish-brown colors, indicating the presence of cardiac glycosides.

c. Legal Test: Treat the extracts with sodium nitroprusside in pyridine and sodium hydroxide. A pink to red color indicates the presence of cardiac glycosides.

5.4. Test for Phytosterols:

a. Liberman Burchard's Test: Dissolve 0.5-1g of extracts in anhydrous chloroform, filter, and mix the solution with acetic anhydride and concentrated sulfuric acid. The formation of a brown ring junction indicates the presence of phytosterols.

b. Salkowski's Test: Shake the extracts with chloroform, filter, and add sulfuric acid to the filtrate. A reddish-brown color precipitate indicates the presence of a steroid ring.

5.5. Flavonoids Test:

a. Shinoda's Test: Treat 0.5-1g of extracts with magnesium and concentrated hydrochloric acid. A pink to crimson red color indicates the presence of flavonoids.

b. Alkaline Reagent Test: Treat the extracts with sodium hydroxide; the formation of a yellow color indicates the presence of flavonoids.

5.6. Saponins Test:

a. Froth Test: Dissolve the extracts in 2-5ml of distilled water, transfer to a graduated cylinder, and shake vigorously. The formation of a 1cm foam indicates the presence of saponins.

b. Foam Test: Combine 1g of extracts with 2ml of water; the presence of foam for 10 minutes indicates the presence of saponin.

5.7. Phenolic Compounds Test:

a. Gelatin Test: Treat the extracts with a solution of 1% gelatin and 10% sodium chloride. The formation of a precipitate indicates the presence of phenolic compounds.

b. Ferric Chloride Test: Dissolve the extracts in water, warm the solution, and add 1-2ml of a 5% ferric chloride solution. The formation of a green or blue color indicates the presence of phenolic compounds.

c. Iodine Test: Treat individual fruit extracts with iodine solution; a red color indicates the presence of tannin.

d. Nitric Acid Test: Dissolve 0.5-1g of extracts in nitric acid; a reddish to yellowish color indicates the presence of phenolic compounds.

5.8. Proteins and Free Amino Acids Test:

a. Millon Test: Mix 2ml of the test solution extracts with Millon's reagent. The formation of a brick-red color indicates the presence of protein.

b. Biuret Test: Dissolve fruit extracts with 1 ml of 10% sodium hydroxide solution and heat. Add copper sulfate solution, and the formation of a purple-violet color indicates the presence of amino acids.

c. Ninhydrin Test: Add 0.25% w/v ninhydrin solution to the extracts, boil, and the formation of a blue color indicates the presence of both protein and amino acids.

5.9. Diterpenes Test: Copper Acetate Test: Treat aqueous extracts with 2-3 drops of copper acetate solution. The formation of a green color indicates the presence of diterpenes.

5.10. Volatile Oils Test: Tincture of Alkane Test: Add tincture of alkane to a thin section of crude drugs. The development of a brick-red color indicates the presence of volatile oil

6. Chromatographic evaluation [58]

From the Greek words "chroma," meaning color, and "graph," meaning write, chromatography is a laboratory technique utilized for the separation of mixtures. The terms "mobile phase" and "stationary phase" are employed to describe the mixture of compounds when passing a dissolved mixture through the stationary phase. This process separates molecules based on the differential partitioning of the mobile and stationary phases. The alteration in separation is achieved by varying the compounds' partitioning coefficients, leading to distinct retentions on the stationary phase. Analytical chromatography, focused on smaller amounts of materials, measures the relative proportions of analytes in a mixture. Chromatography, being a physical method, involves the distribution of components between two phases: one mobile and the other stationary. The stationary phase remains fixed, while the mobile phase moves in a targeted direction.

6.1. Thin layer chromatography:

Principle:

Separation of compound or mixture of compound based on adsorption. To separate the non-volatile mixture thin layer chromatography is more useful method. A thin glass plate which is coated with aluminum or silica gel used as stationary phase. According to the properties of the components in the mixture chose of mobile phase as a solvent. After the sample applied on the plate due to capillary action drawn up through mixture of solvent. Principle is based on solid stationary phase applied on the plate either glass or plastic and mobile phase is moving over the stationary phase. Sample is applied on the bottom of the TLC plate just above 2cm of bottom. Tlc plate may be performed on the analytical scale for monitoring the progress of a reaction or preparative for small scale purify the compounds. According to affinity components are moved towards the stationary phase, components has longer affinity towards the adsorbent travel slower and components has less affinity towards the stationary phase travel faster given table no.7 [59,60]

Plate preparation:

TLC (Thin-Layer Chromatography) plates are typically manufactured with standard particle size ranges to enhance reproducibility. These plates are created by blending silica gel and water to form a thick slurry. The resulting mixture of the stationary phase is evenly spread on a glass plate. Subsequently, the plates are transferred to a heating chamber, often a hot air oven, and exposed to temperatures ranging from 105 to 110 °C for 30 minutes [61, 62]

Selection of mobile phase:

Stationary phase - Silica gel g

Mobile phase – Acetic acid: ethanol (1:3) & Chloroform: Methanol: Water 7:4:1

Detecting agent – 10% Sulphuric acid in methanol

The Rf values were calculated using the formula =
$$\frac{\text{Distance progress by solute}}{\text{Distance progress by solvent}}$$

7. Pharmacological effects of *Solanum indicum* and discussion[63,64]

1. The leaves serve as an anti-emetic, with the juice demonstrating anti-emetic effects when combined with ginger. Additionally, when the leaves are mixed with sugar and applied to the skin, they exhibit anti-itching effects

- The juice of the fruit is advantageous for treating baldness, while the dried powder serves as an anthelmintic drug for children. Moreover, it has the ability to stimulate appetite and is employed in the treatment of bitter stomach conditions
- Roots are used in the treatment of dysuria and urinary retention or incontinence. These are also useful in nasal ulcer treatment
- Whole plant is used as anti-pyretic, asthma, dry cough

7.1. Anti-pyretic effects of *Solanum indicum*

It was determined that *Solanum indicum* possesses robust anti-pyretic activity, as evidenced in Albino rats using Brewer's yeast-induced pyrexia method. The methanolic extract of *Solanum indicum* was administered at doses of 250mg/kg and a higher range of 500mg/kg relative to the animals' body weight. After a 4-hour period following drug administration, body temperature was measured.[65] The methanolic extract demonstrated a noteworthy anti-pyretic effect with a p-value less than or equal to 0.05. Post drug administration, rectal temperature measurements revealed a reduction in temperature to (38±0.18) for the 250mg/kg methanolic extract and (36.76±0.12) for the 500mg/kg methanolic extract. Based on these findings, it can be concluded that *Solanum indicum* exhibits potent inherent anti-pyretic activity are given in table no.8 [66, 67]

7.2. Anti-inflammatory evaluation of *Solanum indicum* fruits.

The investigation into the anti-inflammatory properties of hydroalcoholic extracts from *Solanum indicum* fruits employed the carrageenan-induced paw edema method. Animals were distributed across four groups, each comprising six subjects, in this experimental setup. Following acclimatization to the laboratory environment, the test rats underwent oral administration of various treatments, including two doses of the test extract (250 and 500 mg/kg), Diclofenac Sodium (1 mg/kg), and normal saline (1 ml p.o) as a control. [68, 69] All treatments were orally administered to both the test and control groups. One hour after drug administration, a 0.1 ml injection of 1% Carrageenan suspension in normal saline was administered to the right hind paw of all animals. Paw volumes were measured at 0, 2-, 4-, 6-, and 24-hours post-edema induction using a mercury Plethysmometer, and the results are summarized in Table 9. [70, 71]

The percentage of inhibition was determined by the formula: Percentage of Inhibition

$$(V_c - V_t) / V_c \times 100$$

Here, V_c represents the average increase in paw volume for control rats, while V_t represents the average increase in paw volume for treated rats.

Results and discussion:

1. Powder analysis and microscopy evaluation

Powder characteristic: Microscopic analysis of *Solanum indicum* fruit. Oil globules (OG) in parenchyma cells. Calcium oxalate crystals (CO). -starch granules (SG). Spongy parenchyma cells. -TS of epidermis: - epidermis with scleroids, coats of epidermis, epidermal layer, hypodermis of collenchyma cells. - TS of epidermis palisade parenchyma, hypodermis of collenchyma cells

1.1. Fluorescence analysis of powder and extracts:

Fluorescence characteristic of powdered fruits with different chemical

S.no	Test	Day light	Short uv 254nm	Long range 366nm
1	Powder	Dark brown	Brown	Dark brown
2	Powder + water	Light brown	Green	Brown
3	Powder + 1M hydrochloric acid	Gray	Black	Light Black
4	Powder + Glacial acetic Acid	Light Brown	Green	Dark brown
5	Powder + 1M sulphuric Acid	Greenish brown	Light green	Light Black
6	Powder + Concentrated Nitric Acid	Fluorescent green	Yellowish green	Light brown
7	Powder +5% NaOH	Brown	Brown	Black
8	Powder +10%FeCl3	Greyish green	Greenish black	Black
9	Powder+dil.NH3+Conc.HNO3	Yellowish	Light Yellowish green	Light brown
10	Powder + dil. NH3	Dark Brown	Brown	Black
11	Powder + +Acetone	Colourless	Light pink	Colourless

The characteristic fluorescent properties or colours emitted by the fruit's powders of *Solanum indicum* before and after treating with various reagents were observed and recorded. The fluorescence analysis of fruits powder and various extracts were carried out and Given in **table 1 and 2**.

1.2. Fluorescent analysis of the extracts:

The characteristic of various extracts in natural light and under uv light at 254nm and 366 nm and presented in **table.2**

Table 2: fluorescence analysis of extracts of *Solanum indicum*

Sl no	Extracts	Consistency	Day light colour	Short uv 254 nm	Long uv 366nm
1.	Methanol	Semisolid	Brown	Dark Brown	Green
2.	Petroleum ether	Semisolid	Light yellow	Golden yellow	Whitish
3.	Benzene	Semisolid	Wine colour	Golden yellow	Orange yellow
4.	Water	Semisolid	Dark Brown	Black	Dark green
5.	Chloroform	Semisolid	Greenish brown	Coffee colour	Yellowish green

2. Standardization parameters evaluation: Various standardization or physiochemical parameter studies evaluated as per above mention different procedure. Evaluation of physiochemical parameter shown in **table no3**.

Table.3: standardization parameters of *Solanum indicum* .

Sl no	Parameters	Percentage (%w/w)
I	Foreign organic matter	0.2
II	Loss on drying	8.14%
III	Ash value	
	Total ash	8.62
	Acid insoluble ash	3.58
	Water soluble ash	5.17
	Sulphated ash	4.63
IV	Extractive values	
	Petroleum ether	4.77
	Chloroform	5.84
	Ethanol	13.58
	Methanol	14.71
	Water	17.57
	Acetone	4.54
	Benzene	3.89
	Hexane	2.54
V	Foaming index	Less than 100
VI	Swelling index	4.00

3. Evaluation result of Heavy metal. Powdered fruits of *Solanum indicum* individual heavy metals were done by inductive coupled plasma-atomic emission spectroscopy method. Heavy metals like lead, arsenic, cadmium, mercury were detect and quantified. **The result are shown table no 4**

Table 4. Analysis of heavy metals

Sl no	Element	Results (ppm)	Specification (not more than)
1	Lead	Not found	10 ppm
2	Arsenic	0.0021	3 ppm
3	Cadmium	0.0017	0.3 ppm
4	Mercury	Not detect	1 ppm

4. Extraction:

The percentage of yield of successive extraction fruits of *Solanum indicum* fruits tabulated in table no 5.

Table 5. Colour of successive extracts of fruits of *Solanum indicum* .

Sl no	Extract	Method of Extraction	Nature of extract	Colour
01	Hydroalcoholic	Continuous Extraction Using Soxhlet Apparatus	Semi solid	Brownish green

5. Preliminary phytochemical screening

Qualitative phytochemical analysis for the fruits powder and various extracts were carried out and the results are tabulated in table 6

Table .06. Preliminary phytochemical screening of powdered fruits and extracts of *Solanum indicum* Linn.

S.no	Test	Result	
		Powdered drug	Hydroalcoholic extracts
01	Test for carbohydrates		
	A. Molisch's test	+	+
	B. Benedict's test	-	-
	C. Fehling's test	+	+
02	Test for alkaloids		
	A. Dragendroff's reagent	+	+
	B. Mayer's reagent	+	+
	C. Wagner's reagent	+	+
03	Test for glycosides		
	A. Modified bontrager's test	+	+
	B. Cardiac glycosides	+	+
	C. Legal test	-	-
04	Test for phytosterols		
	A. Liebermann- burchard's test	-	-
05	Test for flavonoids		
	A. Shinoda test	+	+
06	Test for saponins		
	A, froth test	+	+
07	Test for phenolic compounds		
	A.gelatin test	-	-
	B. 5% ferric chloride solution	-	-
	C. Dilute iodine solution	+	+
08	Test for proteins and amino acid		
	A. Millon's test	+	+
	B. Biuret test	-	-
09	Test for diterpenes		
	Coppper acetate test	-	-
10	Test for volatile oils	+	-

(+) indicates positive reaction

(-) indicate negative reaction

6. Thin layer chromatography of extracts:

The number of spots, rf value of the same and the colour of the spots under uv light 366nm and visible light is presented in **table 7** and the photograph of the plate is presented in **figs. 2 & 3**.

Table. 7. thin layer chromatography studies.

Sl no	Extracts	Solvent system	No of spots	Rf value
1	Hydroalcoholic	Acetic acid: ethanol (1:3)	01	0.46
		Chloroform: Methanol: Water 7:4:1	02	0.88,0.91



Fig. 2- Hydroalcoholic extracts solvent system acid : ethanol (1:3)



Fig. 3- Hydroalcoholic extracts Acetic Chloroform :Methanol: Water 7:4:1

7. Antipyretic effect of *Solanum indicum*

Table 8. Antipyretic effect of *Solanum indicum* Linn in rats

Sl no	Treatment	Initial temperature	Rectal Temp. after Yeast induction				
			19h	20h	21h	22h	23h
1	Control	37.41±0.14	39.59 ± 0.19	39.51± 0.63	39.28± 0.15	39.31± 0.04	39.27± 0.07
2	Hydroalcoholic extracts(250mg/kg)	37.34±0.32	39.25± 0.11	38.41± 0.17 *	38.21± 0.17*	38.07± 0.42*	37.64± 0.03*
3	Hydroalcoholic extracts(500mg/kg)	37.39±0.62	39.15± 0.22*	36.79± 0.11*	36.53± 0.19*	37.77± 0.17*	37.35± 0.04*
4	Paracetamol (150 mg/ kg)	37.29±0.42	39.23± 0.16*	35.87± 0.13*	36.51± 0.24*	37.69± 0.20*	37.60± 0.09*

7.2 Antipyretic effect of *Solanum indicum*

Table 9. Anti-inflammatory activity of *Solanum indicum* Linn. Fruits. Hydroalcoholic extract.

Treatment	Duration post-administration of the edema-inducing agent (volume displaced in ml)				
	0 h	2 h	4 h	6 h	8 h
Control	0.43± 0.004	0.83± 0.01	1.6± 0.07	1.76± 0.07	1.89± 0.02
Hydroalcoholic extracts(250mg/kg)	0.42± 0.004	0.79± 0.001	1.5± 0.001	1.05± 0.005	0.74± 0.005*
Hydroalcoholic extracts(500mg/kg)	0.43± 0.004	0.75± 0.001	1.3± 0.001	0.89± 0.004*	0.61± 0.008*
Diclofenac Sodium (1 mg/ kg)	0.42± .004	0.65± 0.001	0.96± 0.001	0.76± 0.006*	0.45± 0.004*

The data presented depicts the mean ±SEM of six rats for each value, with statistical significance denoted by *p value ≤ 0.05.

SUMMARY AND CONCLUSION

The current investigation, titled "Exploration of the Pharmacognostical and Pharmacological Evaluation of the Fruits of *Solanum indicum* Linn (Family-Solanaceae)," is centered around a plant commonly found across India. This plant has been traditionally utilized in the treatment of diverse ailments. However, despite its widespread use, there is a notable gap in research specifically concerning the fruits of *Solanum indicum*. The study aims to bridge this gap by conducting a comprehensive exploration of the pharmacognostical and pharmacological aspects of the plant's fruits

Pharmacognostical studies:

The Pharmacognostical study of *Solanum indicum* plant was conducted and reported for the first time, as indicated by the review literature. This study encompassed various aspects, including macroscopy, microscopy, determination of physicochemical constants, and heavy metal analysis of the plant's fruits. Macroscopically, the fruits were identified as globose or round berries. In their young stage, the fruits appeared green with a white lining, transitioning to a yellow color as they ripened. Additional characteristics noted during macroscopic studies included the brownish-yellow color, unpleasant odor, approximate diameter of 0.8-1.0 cm, globous shape, and bitter taste. This comprehensive pharmacognostical study provides valuable insights into the macroscopic and microscopic characteristics of *Solanum indicum* fruits, contributing to a better understanding of the plant's pharmacological properties. The study conducted various physicochemical tests, including loss on drying, Foaming index, Ash value, swelling index, and extractive studies, to aid in confirming the identity and purity of the *Solanum indicum* plant. Any significant deviation in the percentage of these parameters could potentially indicate adulteration or substitution in the drug. Additionally, quantitative estimation of heavy metals was performed, and the results fell within acceptable limits, indicating the absence of excessive heavy metal contamination. Furthermore, fluorescence analysis was carried out to detect fluorescent chromophores in the powdered drug and its extracts. Interestingly, no fluorescence was observed in either the powder or the extracts. This finding provides valuable information about the absence of specific fluorescent compounds in

Solanum indicum In conclusion, this work represents the first report on the Pharmacognostical standardization of the fruits of *Solanum indicum*. The comprehensive analysis of physicochemical constants, heavy metals, and fluorescence not only contributes to understanding the plant's characteristics but also establishes a baseline for future quality control and standardization efforts.

Phytochemical studies:

Phytochemical evaluation involves the chemical analysis of extracts used for pharmacological screening. In this study, successive solvent extraction was performed using solvents such as aqueous and ethanol (hydroalcoholic extraction). The qualitative preliminary phytochemical analysis played a crucial role in identifying the phytoconstituents present in the various extracts. Thin-layer chromatography (TLC) was conducted for the extracts to further analyze and identify individual or mixed constituents within the extract. Different solvent systems were employed to separate the components of the extract, and the R_f (retention factor) value was measured. The R_f value is a ratio representing the distance traveled by a compound relative to the solvent front in the chromatographic system.

This chromatographic technique aids in the separation and identification of different compounds present in the extracts, providing valuable information about the chemical composition of the plant material. The combination of successive solvent extraction, qualitative phytochemical analysis, and TLC allows for a comprehensive understanding of the phytoconstituents present in the studied plant extracts.

Pharmacological Studies:

Effects of *Solanum indicum* fruits extract shows Anti-Pyretic activity:

The hydroalcoholic extract of *Solanum indicum* fruits demonstrated significant antipyretic activity in rats, particularly when assessed using the brewer's yeast-induced pyrexia method. The antipyretic effect observed in the hydroalcoholic extract surpassed both the control group and the standard group. The results indicated a marked reduction in rectal temperature in the treated group following yeast induction and treatment. Specifically, the hydroalcoholic extract, administered at a dose of 250mg/kg, resulted in a reduction in rectal temperature to 38.41±0.17 degrees Celsius. Furthermore, at a higher dosage of 500mg/kg, the extract exhibited a further decrease in rectal temperature to 36.79±0.11 degrees Celsius. In comparison, the standard drug paracetamol, used for reference, showed a rectal temperature of 35.87±0.13 degrees Celsius at the 20-hour mark. These findings suggest that the hydroalcoholic extract of *Solanum indicum* fruits has promising antipyretic effects in rats, with a dose-dependent response similar to or even surpassing the standard drug paracetamol in this experimental setup.

Effects of *Solanum indicum* fruits extract shows Anti-inflammatory activity:

The *Solanum indicum* fruit demonstrated significant anti-inflammatory effects in Wistar rats, comparable to the reference drug Diclofenac sodium (1mg/kg), six hours post-treatment. The inhibition of paw edema was quantified, revealing a 40.34% inhibition for Hydroalcoholic 250 mg/kg, 49.43% for HA 500 mg/kg, and 56.77% for Diclofenac sodium. *Solanum indicum*, commonly known as Bruhati, holds popularity in indigenous systems of folk medicine. The fruit extract of *Solanum indicum* was found to contain various bioactive compounds such as flavonoids, phenolic compounds, tannins, glycosides, carbohydrates, alkaloids, and phytosterols. These findings suggest that the extract from *Solanum indicum* fruits has the potential to serve as a lead medicinal plant for the synthesis of various semi-synthetic drugs. These drugs could be utilized in the treatment of various life-threatening diseases, including but not limited to anti-inflammatory, antipyretic, and hepatotoxicity-related conditions. The multifaceted pharmacological properties make *Solanum indicum* a promising candidate for further exploration and development in the field of medicinal research and drug discovery.

CONFLICTS OF INTEREST:

The authors declare no conflicts of interest.

References

1. Jayanthi A, Maurya A, Verma SC, Srivastava A, Shankar MB, Sharma RK(2016). A brief review on pharmacognosy, phytochemistry and therapeutic potential of *Solanum indicum* L. used in Indian Systems of Medicine. Asian Journal of Research in Chemistry. ;9(3):127-32.

2. Iyer KN, Kolammal M(1960). Pharmacognosy of Ayurvedic Drugs. Department of Pharmacognosy. Govt. Ayurveda College, Trivandrum. ;I(4):99
3. Deb PK, Ghosh R, Chakraverty R, Debnath R, Das L, Bhakta T(2014). Phytochemical and pharmacological evaluation of fruits of *Solanum indicum* Linn. Int. J. Pharm. Sci. Rev. Res. Mar;25(2):28-32.
4. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY(2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary and alternative medicines.;8(1).
5. Asolkar LV, Chopra RN(1992). Second supplement to glossary of Indian medicinal plants with active principles. Publications & Information Directorate;
6. Kirtikar KR, Basu BD(1975). Indian Medicinal Plants, 2nd edn., Vol. I. Bishen Singh Mahendra Pal Singh: Dehra Dun,India.151.
7. <https://vikaspedia.in/agriculture/crop-production/package-of-practices/medicinal-and-aromatic-plants/Solanum-indicum#:~:text=leaves%20are%20ovate%20in%20outline,about%201.3%e2%80%932.5%20cm%20long>
8. <https://www.iafaforallergy.com/herbs-a-to-z/bruhati-Solanum-indicum/#:~:text=it%20is%20commonly%20known%20as,the%20small%20variety%20of%20brinjal>.
9. https://en.wikipedia.org/wiki/Solanum_indicum.
10. Sharma V, Hem K, Seth A, Maurya SK. Current Research Journal of Pharmaceutical and Allied Sciences.
11. <https://www.planetayurveda.com/library/brihati-Solanum-indicum/>
12. Shah Biren, Sethak(2014). Text book of Pharmacognosy and phytochemistry, 2nd edition, CBS Publishers & Distributors Pvt. Ltd., New Delhi, Chennai, Pune, 115.
13. Singh GK, Bhandari Anil. The text book of Pharmacognosy, cbs publisher and distributors, 1st edition, New Delhi, Bengaluru, Chennai, Kochi, Kolkata, 32-33.
14. Murthy GS, Inamdar JA, Rao VS(1980). Organographic study of vessels in some Solanaceae. Phytomorphology.30(2/3):149-58.
15. Anjana V, Kumari NO, Mathew PM(2005). Trichomes in some south Indian species of *Solanum* L. Indian Journal Botanical Research. 1(1):65-72.
16. Anjana VN, Kumari NO, Mathew PM(2008). Intra-specific variations of trichomes in the genus *Solanum* L. Biodiversity conservation. Jun 1:109-4.
17. Jayanthi A, Maurya A, Verma SC, Srivastava A, Shankar MB, Sharma RK(2016). A brief review on pharmacognosy, phytochemistry and therapeutic potential of *Solanum indium* L. used in Indian Systems of Medicine. Asian Journal of Research in Chemistry.9(3):127-32.
18. Kirtikar KR, Basu BD (1999). Indian medicinal plants. vol 3. International Book Distributors.
19. Sivarajan VV, Balachandran I (1994). Ayurvedic drugs and their plant sources. Oxford and IBH publishing.
20. Ashish m, vinit s, kritika h, kumar ms. Plants used for treatment of diarrhea: an ayurvedic prospective.
21. Kumar S, Malhotra R, Kumar D(2010). *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacognosy reviews. Jan;4(7):58.
22. Maurya SK, Seth AN(2014). Potential medicinal plants and traditional ayurvedic approach towards urticaria, An allergic skin disorder. Int J Pharm Pharm Sci. 6(5):172-7.
23. Kumar S, Malhotra R, Kumar D(2010). *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacognosy reviews. Jan;4(7):58.
24. Deb PK, Ghosh R, Chakraverty R, Debnath R, Das L, Bhakta T(2014). Phytochemical and pharmacological evaluation of fruits of *Solanum indicum* Linn. Int. J. Pharm. Sci. Rev. Res. Mar;25(2):28-32.
25. Patwekar SL, Suryawanshi AB, Gaikwad MS, Pedewad SR, Potulwar AP(2016). Standardization of herbal drugs: An overview. The Pharma Innovation. Apr 1;5(4, Part B):100.
26. Gautam A, Kashyap SJ, Sharma PK, Garg VK, Visht S, Kumar N. Identification, evaluation and standardization of herbal drugs: A review. Der Pharmacia Lettre. 2010;2(6):302-15.
27. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants.
28. Bharatkumar G(2019). *A Study on Ameliorative Effects of Karisalai Karpam (KK) on Hepato-Toxicity in Animal Models* (Doctoral dissertation, The Tamil Nadu Dr. MGR Medical University, Chennai).Aziz NA, Wal P, Wal A, Saxena MS. Evaluation of a polyherbal powder for treatment of Diabetes Mellitus. Indian Journal of Pharmaceutical Sciences. Nov 1;81(6):1070-7.
29. Unit P, World Health Organization(1992). Quality control methods for medicinal plant materials. World Health Organization;
30. Trease and evans(2009). Text book of Pharmacognosy. 16th edition. 124-125
31. British pharmacopoeia. 2012; vol-5.

32. Bhar K, Mondal S(2022). Proponents Implemented for Quality Standardization and Toxicity Evaluation of Classical Ayurvedic Formulation: Dhatupaustik Churna. *Current Overview on Pharmaceutical Science* Vol. 1. Dec 24:163-209.
33. <https://www.yourarticlelibrary.com/medicine/ayurvedic/determination- of-ash-values/49966>.
34. <http://www.jiwaji.edu/pdf/ecourse/pharmaceutical/Evaluation%20of%20crude%20drugs.pdf>.
35. Chaudhari RK, Girase NO(2015). Determination of soluble extractives and physicochemical studies of bark of *Sesbania sesban* (L) Merr. *Journal of chemical and pharmaceutical research*. 7(8):657-60.
36. Bora KS(2016). Pharmacognostic evaluation and physico-chemical analysis of *Ulmus wallichiana* Planch. *Journal of Applied Pharmaceutical Research*. Nov 24;4(3):01-7.
37. Shah Biren, Sethak AK(2014). *Text book of Pharmacognosy and phytochemistry*, 2nd Edition, CBS Publishers & Distributors Pvt. Ltd., New Delhi, Chennai, Pune, 118
38. Krishna TA, Krishna TA, Kumuthakallavalli R, Raj VS, Juliet S, Rani TS, Darsana U, Nair SN(2014) Ravindran R. Physico-chemical evaluation and biochemical quantification of crude drug powder (stem) of *Chassalia curviflora* (Wall. ex Kurz.) Thwaites; A folk medicinal plant. *Journal of Pharmacognosy and Phytochemistry*. 3(4):121-4.
39. Dwiti M(2016). Pharmacognostic evaluation, phytochemical investigation and antioxidant activity on leaves of *Marsilea minuta* Linn.(Marsileaceae). *Journal of Pharmacognosy and Phytochemistry*. 5(2):134-40.
40. <https://www.chips.ac.in/pages/downloads/PPTs/Pcognosy/standardisatio n%20of%20herbal%20drugs.pdf>.
41. Gavali J(2020). WHO (world health organization) guidelines for standardization of herbal drugs. *Education*. Jul.
42. Rao MM. *Comparative study of efficacy of jatyadi ghrita pichu and yasthimadhu ghrita pichu in the management of parikartika (fissure-inano)* (Doctoral dissertation, Tilak Maharashtra Vidyapeeth).
43. Saxena G, Mittal A, Siddiqui AW(2019). Acute Toxicity Studies and In-vitro Antioxidant Potential of *Solanum indicum*. *Journal of Drug Delivery and Therapeutics*. Jun 22;9(3-s):736-9.
44. Gadewar MM, GK P, Mishra PC, Ashraf GM, Almashjary MN, Harakeh S, Upadhye V, Dey A, Singh P, Jha NK, Jha SK(2023). Evaluation of Antidiabetic, Antioxidant and Anti-Hyperlipidemic Effects of *Solanum indicum* Fruit Extract in Streptozotocin-Induced Diabetic Rats. *Current Issues in Molecular Biology*. Jan 19;45(2):903-17.
45. Hasan RU, Prabhat P, Shafaat K, Khan R(2013). Phytochemical investigation and evaluation of antioxidant activity of fruit of *Solanum indicum* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*. 5(3):237-42.
46. Bhattacharya AS, Banaushadhi C. 2nd volume, 3rd reprint.
47. Jaiswal V, Singh SK, Singh AK, Maurya SK(2014). A critical review on antiasthmatic Ayurvedic medicinal plants and compound Formulations. *International Journal of Pharmacy Archive*. 3(5):400-9.
48. Gu G, Du Y, Linhardt RJ(2004). Facile synthesis of saponins containing 2, 3-branched oligosaccharides by using partially protected glycosyl donors. *The Journal of organic chemistry*. Aug 6;69(16):5497-500.
49. Gopalakrishna SM, Thimappa GS, Thylur RP, Shivanna Y, Sreenivasan A(2014). In-vitro anti-cancer screening of *Solanum indicum*, *Rhus succedanea*, *Rheum emodi* and *Gardenia gummifera* medicinal plants in cancer cells. *Research Review Journal of Pharmaceutical Sciences*. 3(4):22-30.
50. Ashish m, vinit s, kritika h, kumar ms. Plants used for treatment of diarrhea: an ayurvedic prospective.
51. Kumar S, Malhotra R, Kumar D(2010). *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharmacognosy reviews*. Jan;4(7):58.
52. Melakyrkhu niangmih, samiran chutia, banyashree das, bapi roy sarkar, biplab kumar dey, atanu Bhattacharjee(2018), preliminary phytochemical analysis of selected medicinal plants of north eastern region of India, *international journal of pharmaceutical research and bio-science* · january , volume 7(4): 91-93
53. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A(2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). *In Veterinary research forum: an international quarterly journal* (Vol. 5, No. 2, p. 95). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
54. Kumar A, Jha KK, Kumar D, Agrawal A, Gupta A(2012). Preliminary phytochemical analysis of leaf and bark (mixture) extract of *Ficus infectoria* plant. *The pharma innovation*. Jul 1;1(5, Part A):71.
55. <https://www.Pharmacy180.com/article/chemical-tests-for-volatile-oils-218/>
56. Bajpai VK, Majumder R, Park JG(2016). Isolation and purification of plant secondary metabolites using column-chromatographic technique. *||| Bangladesh Journal of Pharmacology|||*. Oct 1;11(4):844-8.
57. <http://reports.ias.ac.in/report/18980/analytical-techniques-involved-in-extraction-and-purification-of-phytochemicals-from-a-medicinal-plant.:3.2.2>

58. https://en.wikipedia.org/wiki/thin-layer_chromatography
59. Kumar S, Jyotirmayee K, Sarangi M(2013). Thin layer chromatography: a tool of biotechnology for isolation of bioactive compounds from medicinal plants. International Journal of Pharmaceutical Sciences Review and Research. Jan;18(1):126-32.
60. Sankar ravi S. A text book of pharmaceutical analysis ,5th edition, rx publication, tirunelveli -India : 14-2.
61. Maurya SK, Seth AN(2014). Potential medicinal plants and traditional ayurvedic approach towards urticaria, An allergic skin disorder. Int J Pharm Pharm Sci.6(5):172-7.
62. Kumar S, Malhotra R, Kumar D(2014). Euphorbia hirta: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacognosy reviews. Jan;4(7):58.
63. Deb PK, Ghosh R, Chakraverty R, Debnath R, Das L, Bhakta T(2014). Phytochemical and pharmacological evaluation of fruits of *Solanum indicum* Linn. Int. J. Pharm. Sci. Rev. Res. Mar;25(2):28-32.
64. Deb PK, Das N, Bhakta RG(2013). Evaluation of in-vitro antioxidant and anthelmintic activity of *Solanum indicum* Linn. berries. Indo American Journal of Pharmaceutical Research. 2013a.3(2):4123-30.
65. Hasan RU, Prabhat P, Shafaat K, Khan R(2013). Phytochemical investigation and evaluation of antioxidant activity of fruit of *Solanum indicum* Linn. International Journal of Pharmacy and Pharmaceutical Sciences. 5(3):237-42.
66. N'Dri D, Calani L, Mazzeo T, Scazzina F, Rinaldi M, Rio DD, Pellegrini N, Brighenti F(2010). Effects of different maturity stages on antioxidant content of Ivorian Gnagnan (*Solanum indicum* L.) berries. Molecules. Oct 15;15(10):7125-38.
67. Bhuvaneshwari B, Suguna MM (2014). Hepatoprotective and antioxidant activities of *Solanum indicum* Linn. Berries. International journal of clinical toxicology.2:71-6.
68. Narayanaswamy N, Balakrishnan KP(2011). Evaluation of some medicinal plants for their antioxidant properties. Int J Pharm Tech Res. Jan;3(1):381-5.
69. Chiang HC, Tseng TH, Wang CJ, Chen CF, Kan WS(1991). Experimental antitumor agents from *Solanum indicum* L. Anticancer research. Sep 1;11(5):1911-7.
70. Gopalakrishna SM, Thimappa GS, Thylur RP, Shivanna Y, Sreenivasan A(2014). In-vitro anti-cancer screening of *Solanum indicum* Rhus succedanea, Rheum emodi and Gardenia gummifera medicinal plants in cancer cells. Research Review Journal of Pharmaceutical Sciences.3(4):22-30.
71. Rahman MS, Begum B, Chowdhury R, Rahman KM, Rashid MA(2008). Preliminary cytotoxicity screening of some medicinal plants of Bangladesh. Dhaka Univ. J. Pharm. Sci. Oct 7;7(1):47-52.