Experiment No.1

Aim 1: PURIFICATION AND EVALUATION OF ALKALOIDS FROM SOLANUM XANTHOCARPUM IS NECESSARY FOR FOLLOWING STUDY.

3.1 CHEMICALS:

Sodium carbonate, Copper Sulphate, Potassium tartarate, Sodium Molybdate, Potassium iodide, Ferric Chloride, Sodium Sulphate, Sodium hydroxide, concentrated hydrochloric acid; Potassium Permagnate was used from Paul laboratory. Chloroform, Acetone, Methanol, Butanol, n-propanol, Ninhydrin etc from BDH, E.D.T.A., S.D.S. from Qualigen fine chemicals. Ammonium hydroxide, ethyl acetate, Petroleum ether, Diethyl ether, Silica gel, Ammonium Sulphate, Cellulose Power, cerrick sulphate and Mercuric Chloride are used from Loba chemical company Mumbai..AR grade chemicals like ethyl alcohol ,Picric acid H$_2$SO$_4$ β napthol ,HCl were obtained from research laboratory of college.

Pure grade of all the above mention regents were used .Distilled water was used in whole experimental work.

Buffer solution preparation: It is prepared by sorense’s method.

39 ml sodium dihydrogen ortho phosphate and 60 ml sodium phosphate anhydride dissolve in 100 ml distilled water.

3.2 Reagents Preparation:

The following reagents are used to precipitate alkaloids from the source.

1) Hager’s reagent:

1 g of picric acid and 100 ml distilled water mixed together for preparing hagers reagent.

2) Mayer’s reagent: Two solutions were prepared

A.Solution: 1.358g of HgCl$_2$ dissolved in 60ml of Distilled water.
**B solution:** For this solution preparation 5 gms fo KI was dissolved in 40 ml of Distilled water.

After preparation of both solutions mixed together up to 100 ml volume.

3) **Potassium Iodo palatinate reagent:**

One gram platinate chloride completely dissolved in ten ml distilled water. 250 ml of water KI (4%) is added in this prepared solution and volume made up to 500 ml with distilled water.

The Reagent five and six were used for the detection of spot of TLC.

5) **Ninhydrin reagent:** It was prepared by using 0.8 g of ninhydrin and 0.12g of hydrindatin. These chemicals dissolved in 10 ml Acetic Buffer and 30 ml of Methyl cellulose.

6) **Dragndoff’s reagent:**

Two solution A and B were prepared for this reagent.

**Solution A:** 20 gms of Tartaric acid and 1.7 gms of bismuth nitrate were dissolved in 60 ml of distilled water. It was considered as solution A.

**Solution B:** In 40 ml of distilled water 16 gms of potassium Iodide was dissolved. The solution was prepared and considered as solution B.

Dragndoff’s reagent was prepared by mixing solution A and solution B in beaker.

3.3 **Source:**

The *Solanum xanthocarpum* was collected from the campus of Saraswati Engineering College, kharghar navi Mumbai. It is hilly area. Various types of waste land plants are found in this area. Solanum xanthocarpum plants are spread over the waste land of college campus.
**Storage of source:**

Solanum xanthocarpum plant was washed with water to remove soil foreign material and other contamination. It was dried for 8 to 10 days in sunlight. The purpose of drying is to remove moisture from plant material and make it resistant against growth of micro organism. Different dried parts of the plant were separated and cut into small pieces. It was grinded by blender into fine powder and sieved through botanical sieve size mesh number 85. The green coloured leaves powder and yellowish coloured fruit powder was stored properly in air tight glass bottles. These bottles were kept in a dark place to get protection from sunlight. It was observed that there was no seasonal change in colour of leaves and fruit powder.

Figure 3.1 A. Image of Morphology of *Solanum Xanthocarpum* plant
B. Dried plant of Solanum xanthocarpum

Figure 3.2 Dried powders of leaves of Solanum xanthocarpum plant.
In the history of medicine, it was noted that many medicinal plants are acts as tonic, restorative and also as poisons. The reasons for the use of herbs in the treatment of sickness and afflicted has survived for more than thousands of years due to medicines. Medicines are organic in nature and its sources are on earth. Medicinal plants and herbs contains many organic compounds. These organic compounds are provides particular physiological action on body of human being. These bioactive compounds include various compounds like proteins carbohydrates, tannins, terpenoids, flavonoids, alkaloids and steroids.

Secondary metabolites are shows taxonomically and chemically adverse effects because of its content. These compounds are widely used various types of therapy, agriculture, scientific research, veterinary and various research area.

Modern medicines are also prepared from the medicinal plant extract. A department of medicine consultancy always maintains syrup of Ipecace to induce vomiting in patients who have swallowed certain type of poisons. Castor oil is used as purgative. Many plants are known as indigenous medicines are used for indigestion and stimulation of digestion. as antispasmodic, purgative, carminatives, antihelmintics, liver disorders and emetics. The evaluation of drugs and function of quality control are assess the value of raw material and assure the final product is of desired standard. Evaluation of drugs involves authentication, proper identification of plant materials and determination of purity and quality.

Now a day the modern techniques of instrumentation provide the possibility of development of suitable criteria of quality control. For an analyst, evolution of plant material and their derived products are very important. In earlier time the authentication was sufficient with comparing with botanical description and monograph of plants.

Later it was realized that for determination of adulterants the above data must be supplemented with both confirmatory chemical tests and microscopically analysis for the
Many pharmacopoeias have introduced a requirement that certain drugs must confirm to a chromatographic fingerprint in order to be acceptable.

In order to make traditional remedies more acceptable in modern therapeutics and to promote wider application of their knowledge and practice for human use, quality control of traditional medicines are essential. Plants contain chemical compounds that show greater variation in solubility and stability. They are broadly categorized into tannin, lipids, phenols, proteins, volatile oil, carbohydrates, and glycosides.

### 3.4 Methods: Extraction of Carbohydrates and Proteins from *Solanum xanthocarpum*

1) **Protein:**

Proteins are nitrogenous organic compounds produced by and associated with living matter. They occur in plants and animals. Proteins are derived from the amino acids which are their building units. In metabolism, proteins play an important role.

Protein detection was carried out by the Lowry method (O.H. Lowry et al; 1951).

2) **Carbohydrates:**

Carbohydrates are compounds containing elements like hydrogen, carbon, and oxygen. They are aldehydic or ketonic alcohols in which hydrogen and oxygen are present in the same ratio as in water. They are used widely in pharmaceutical for several applications.

Carbohydrates detection was carried out by the method of Phenol Sulphuric acid (Dubois M; 1956).

3) **Alkaloids:**

Crude alkaloids were precipitated by Manske R.H.F. Method (K. Scheiber et al; 1956). In the present study, modification of procedure was carried out.
3.5 Preparation of extract of leaves of *Solanum xanthocarpum:*

The extract of solanum xanthocarpum leaves was prepared by using 10 gms of leaves. These leaves were extracted with sodium phosphate solution at pH 7. The pH of solution was maintain by using Buffer solution at room temperature.

The extract of the leaves used to detect the percentage of proteins, carbohydrates and amine. UV spectrum and TLC of the sample was carried out.

3.5.1 Protein detection: Proteins are very essential for animals as well as human being. Proteins are presents in animals, plants and in human. The presence of protein can be measured by using many methods. Some methods are very economical but some are expensive.

**Present Study:** In the present study 0.1 ml sample of *Solanum xanthocarpum* leaves was used for detection of proteins. It was measured by Lowry method (O.H. Lowry; 1951). It was done by using standard casein at 0.16 O.D. It is corresponds to 0.1 miligrams of proteins.

3.5.2 Amine Detection:

By transamination of aldehydes and decarboxylation process of amino acids, simple amines are forms in plants. These processes are take place in plants and gives protection against pathogens. It shows physiological effects in animals. In plants these compounds may be involved as protection against pathogens. This often occurs because of their structural similarity to neuro transmitters. In plants amines are present in the form of amides. In family Asclepiadaceae and Araceae amines attracts insects and flies in pollination. For the solanaceae family the amine detection was done. For the detection of amine, 0.1 g *Solanum Xanthocarpum* Leaves powder and 1ml 1:1 HCl solution, shaked well and filtered. In that filtrate 20% NaOH solution was added drop wise. The compound was dissolved and solid reappeared.
3.5.3 Detection of Carbohydrate:

Manjunath and Shadaksharaswamy (Yu.B.W et al;2002) used dried fruits of solanum xanthocarpum for extraction of glycoalkaloids. On its hydrolysis they obtained the a glycone solanidine-s and sugars. Therefore carbohydrates studies carried out from Solanum xanthocarpum leaves.

3.5.4 UV Spectrum of Extract of Solanum Xanthocarpum Leaves.

20 µl of sample was used and for uv spectrum determination uv 2100 spectrophotometer was used.

3.5.5: TLC of Extract of Solanum xanthocarpum leaves.

1) Stationary Phase: Silica gel was applied on the glass plate and fixed in oven.

2) Mobile Phase: Various mobile phases were tried and finally 60 % Butanol: Acetone: Water (1:3:1) was selected for the separation of sample.

3) Test Solution:

10µl extract of Solanum xanthocarpum leaves (wet) was applied on the TLC plate by capillary.

4) Detection and Evaluation: Electric oven dried plate has been evaluated in UV 254nm. Brown spots were generated with spraying ninhydrine reagent.

Present study:

In the present study, dry powder of Solanum Xanthocarpum leaves have been used which is shown in figure 3.3. Further, classical methods of isolation have been employed in view of the fact that once a method of obtaining uniform constituents has been ultimately worked out; they can be conveniently obtained in larger quantities, with optimum yields. The following methods were used for the isolation of alkaloids from Solanum xanthocarpum leaves

  I) Solvent extraction
  II) Soxhlet method
  III) New method /Modern method
I) Solvent extraction method:
The isolation for alkaloid is reported in literature through thin layer and column chromatography. It has been obtained in sizable quantity, through solvation in methanol, ethanol and fractional precipitation with water. This procedure has been made by several groups of workers. For working out experimental procedures for the isolation of alkaloids as a result of these studies.

Procedure:
10 g powdered plant material was wetted with 15 ml of NH₄OH (25%, m/m) at room temperature. Solvent extraction was performed with 300 ml of ethyl acetate for 72h. The extract was filtered and the solvent was evaporated in rotary evaporator under reduced pressure at 40°C. The residue dissolved in water and acidified with H₂SO₄ to pH 3-4. This was again extracted with petroleum ether and diethyl ether to remove lipophilic, acidic and neutral material. After basifying the aqueous solution to pH 9-10 with NH₄OH (25%m/m), it was extracted with chloroform, the extract washed with distilled water to neutral pH, dried with Na₂SO₄ and concentrated to dryness under reduced pressure to obtained crude alkaloid. TLC, UV, IR and physical constant of crude alkaloids was carried out. The detailed flow chart diagram of extraction of alkaloids from *Solanum Xanthocarpum* by solvent method is shown in flow chart no.3.1
Flow chart diagram 3.1. Extraction of alkaloids by solvent extraction method.
II) Soxhlet method:

In the soxhlet method 10 g of powdered dry plant material was kept in thimble and extracted with 300 ml of ethyl acetate solution in a Soxhlet apparatus for 18 hrs. The final extract was filtered concentrated under vaccum. The residue was dissolved in distilled water and acidified with H$_2$SO$_4$, pH 3-4. It was extracted with petroleum ether and diethyl ether to remove lipophilic, acidic and neutral layers. After basifying the aqueous solution to pH 9-10 with 25% NH$_4$OH, it was extracted with chloroform. The extract washed with distilled water to neutral pH. It was dried with Na$_2$SO$_4$ and concentrated to dryness under reduced pressure. The crude alkaloid was obtained which is shown in figure no 3.4. The detailed flow chart diagram of extraction of alkaloids from *Solanum xanthocarpum* by Soxhlet method is shown in flow chart no.3.2

The layers obtained from the extraction process were used for the thin layer chromatography. For the thin layer chromatography the solvent system of water, acetone and butanol was used.

1) TLC of each layer was carried out with water : acetone : butanol (1:3:1) system results are produced in Table 3.1.

2) The UV spectrum and IR spectra of crude alkaloid were carried out.

3) Melting point of crude extract was carried out in paraffin oil and also confirmed by electronic thermometer.
10 g dried powder of source +300 ethyl acetate

18 hours

Filter (suction pump)

Residue

Liquid discarded

(Dissolve in distilled water Check pH 3-4)

Extraction with petroleum ether

or Diethyl ether

1) lipophilic layer

2) Acidic layer

3) Aqueous layer (separated with funnel)

Aqueous layer (pH 9-10) separated with funnel

Extracted with chloroform

Wash with distilled water (neutral pH)

Dry with Na₂SO₄
Concentrate under reduced pressure

Crude alkaloid

TLC UV IR Physical constant

Flow chart 3.2. Soxhlet extraction method of alkaloids

III) New method/Modern method for extraction:
Natural product are extracted by conventional methods such as soxhlet and room temperature solvent extraction (Yu, B et al; 2002) or by ultrasound (Sargenti et al; 2000), microwaves (Kaufman B. et al; 2002), supercritical solvents or other methods (Teixeira et al; 2005), but in the present study, natural product extracted by using surfactant EDTA.

A sample of 10 g of powdered dry plant material was suspended in 400 ml of 0.3 % (m/v) EDTA surfactant solution in glass beaker. It was sonicated for 120 min in an ultrasonic bath at a constant temperature. The extract was separated by simple filtration. The residual material washed with 20 ml of pure water and acidified with sulphuric acid solution to pH 3-4. The alkaloids were precipitated with 15 ml of Mayer reagent. The precipitate was dissolved in an alkaline solution of sodium carbonate (5%; m/m) and extracted with CHCl₃. In that extract two layers were formed, one was organic and other was aqueous layer. These two layers were separated by separation funnel. Then the organic layer was washed with water to neutral pH, dried with Na₂SO₄ and concentrated to dryness under reduced pressure to obtain alkaloids. The process was repeated thrice. The crude alkaloid was obtained which shown in figure 3.6. The characterization of crude alkaloids was done by UV, NMR, IR and TLC. The flow chart of extraction of alkaloids from kantkari shown in flowchart number 3.3.

UV/VIS Spectra: The UV of crude alkaloid was recorded on UV-2100 spectrophotometer in 10 mm quartz cuvettes (SHEMADZU CORP.)
IR and NMR of sample was carried out in IIT Pawai, Mumbai which is shown in result and discussion chapter.

### 3.6 Extraction of alkaloid from *Solanum Xanthocarpum* leaves:

Because of the interest in natural products, different types of the extraction methods of alkaloids were studied. Alkaloids are extracted by conventional methods like solvent and soxhlet extraction process (Yu.B.W et al.;2002), or by ultrasound (Sargeni S.R et al.;2000) by using microwave (Keren Z. et al.;2005). Extraction also done with supercritical solvents (Dean J.R et al.;2000) or other methods (Lee H.K.et al 2002). Djilani Abdelouaheb in 2006 has developed extraction method for natural products. Some plants were selected like Datura, hyoscyamus and ruta. They have shown good yield of alkaloids using SDS. In the present study, the extraction methods were used with combination of ultrasound and different types of surfactants. Some properties like emulsification solibilisation, wetting and dispersion (Rosen, M.J.et al;1978) reduces extraction time and required solvents from the leaves of solanum xanthocarpum.

**THE METHODS ARE AS FOLLOWS.**

**i) Effect of various concentration of SDS on alkaloids:**

Various concentrations of SDS ranging from 0.1 to 0.3% (m/v) with 5g dry powder of *Solanum xanthocarpum* leaves followed Mayer reagent was used for the extraction. The same experiment was carried out without addition of SDS as a control. The TLC analysis was done silica coated plates.

**ii) Effect of sonication with varying time on extraction of alkaloids:**

A concentration of 0.1%(m/v) of SDS kept constant for extraction of alkaloids from *Solanum Xanthocarpum* leaves with varying time 0 to 150 min with the sonication. The same experiment was carried out without sonication as a control.

Effect of sonication on extraction of alkaloids is shown in figure 2.2. Total alkaloids are increase up to 120 min and total alkaloids are fall down after 150 min and onward hrs. According to the figure 2.2 indicates that sonication (ultrasound) affect the alkaloids percentage.
iii) Effect of varying time on extraction of alkaloids (without sonication): The same above experiment was carried out with 0.1% (m/v) of SDS at varying time without sonication.

3.7 Modified Method for extraction of alkaloids: Various concentrations of surfactant were used to determine the plant alkaloid. Surfactant with 5g dry powder of plant followed by sonication was carried out for 120 min at room temperature. By the filtration method, extract was separated. The obtained residue. The extract was separated by simple filtration method. The residual material was washed with distilled water and acidified with sulphuric acid to maintain the pH in between 3-4. Mayer’s reagent was used for the precipitation of alkaloids. The obtained precipitate of alkaloids was dissolved in Na$_2$CO$_3$ solution. The extraction was done with CHCl$_3$.

The organic layer from solution was washed with distilled water to pH neutral and Na$_2$SO$_4$ was used for drying the product of crude alkaloid. The crude alkaloid was stored properly in glass bottle. The same experiment was carried out without addition of Surfactant, for various time interval, wave factor and different concentration of source. The percentage of alkaloids was checked and it was compared with respective control.

3.7.1 TLC Identification:
1) Stationary Phase:
Silica gel slurry was applied on the glass plate and dried at room temperature.

2) Mobile Phase:
Various mobile phases were tried and finally butanol was selected for the separation of sample.

3) Test Solution:
Extraction sample Solanum xanthocarpum leaves containing 0.1 % (m/v) 0.2 % (m/v) and 0.3% (m/v) concentration of SDS was applied on the TLC plate by capillary.

4) Detection and Evaluation:
After the plate has been dried in hot air drier it was evaluated in UV 254nm. The brown spots were generated with spraying iodine reagent.

3.8 UV Structure of sonicated sample: The crude alkaloids obtained after sonication. UV spectra of each sample were carried out using 10mm quartz cuvette on SHEMAZDU spectrophotometer.

B) Extraction of alkaloid with EDTA
For the extraction of alkaloids ethylene diamine tetra acetic acid was used. EDTA surfactant acts as a chelating agent. It binds to metals via four carboxylate and two amine groups. It prevents joining of cadherins between cells and cell clumping. In the field of agriculture and pharmaceutical it is very useful.

**i) Effect of various concentration of EDTA:**

Various concentrations of EDTA ranging from 0.1 to 0.3% (m/v) were prepared for the experimental work. 5g dry powder of *Solanum xanthocarpum* leaves followed Mayer's reagent was used for the extraction of alkaloid.

The same experiment was carried out without addition of EDTA as a control. The TLC analysis and UV of sample was carried out.

**ii) Effect of sonication with varying time on extraction of alkaloids:**

0.3% (m/v) of EDTA kept constant for extraction of *Solanum xanthocarpum* leaves alkaloids. The above reaction mixture was kept for sonication from zero min to 120 min. The same experiment was carried out with 0.3% (m/v) of EDTA at varying time without sonication as a control.

**iii) Effect of varying time on extraction of alkaloids (without sonication):** The extraction was carried by EDTA for comparison of the amount of alkaloid precipitated from *Solanum Xanthocarpum leaves*.

**TLC Identification:**

1) Stationary Phase: Silica gel was applied on the glass plate.

2) Mobile Phase: Various mobile phases were tried and finally butanol was selected for the separation of sample.

3) Test Solution:

The test solution used for the determination of thin layer chromatography. The extraction samples of various concentrations was used.
Extraction sample of *Solanum xanthocarum leaves* containing different concentration of EDTA 0.1 % (m/v) 2.2 % (m/v) and 0.3% (m/v) was applied on the TLC plate by capillary.

10 g dry powder of source + 400 ml EDTA solution

Sonication in ultrasonic bath for 2 ½ hours

FILTRATION

Washing with distilled water (pH 3-4)

RESIDUE

FILTRATE

Addition Mayers reagent

FORMATION OF PRECIPITATED

Dissolve in 5% Na₂CO₃

CHCl₃ extract

Separation of layers
Detection and Evaluation: After the thin layer chromatography plate has been dried in hot air current. The plates was evaluated in UV 254nm and it was observed that brown spots were generated with spraying iodine reagent.

3.9 Comparative study of different surfactant:  
The effect of EDTA in comparison with SDS in relation to time on *Solanum xanthocarpum* leaves alkaloids study was carried out.

ISOLATION OF ALKALOIDS  
Many compounds with alkaloids type structure have been isolated from lower animal, marine and microbial sources. Alkaloids also isolated from mammals. Muscopyridine alkaloid isolated from scent gland of musk deer. The individual alkaloids are usually separated from the very complex mixture by chromatographic methods. Alkaloids from the seed, root, and bark of plant are isolated by extraction with dilute acids such as HCl, H₂SO₄, acetic acid or alcohol. They are liberated by treatment with calcium hydroxide before extraction. The extraction of alkaloids can be done by using acidic solution and water. Some alkaloids isolated directly by using alcohol extract with chromatographic method. For extraction of tropane alkaloids this method is applicable. First time erythiina alkaloid was extracted from Erythrina Americana seeds. Extraction of *Tricocereus cacti* was carried with MeOH. This was carried out by using hot Solvent-extraction apparatus and Soxhlet method. The extraction of the aerial parts of *Solanum Jabrense* was done.
by using ethyl alcohol, hexane, methanol and chloroform. Solasodine alkaloid was isolated by column chromatography with methanol fraction on silica gel. (Olatundeet et al; 2000). In isolation and purification of alkaloids from Evodia rutaecarpa high speed counter current chromatography method was used. Two solvent systems were used in this method. The composition was made with proportion 5:5:7:5 of n hexane : ethyl acetate : methanol : water. (Renmin Liu et al; 2005). The leaves of *Solanum pseudo capsicum* extracted with methanol. It was used for anti tumor activity. It was tested against mice (Weissenberge M et al; 2001). Wissenberg M. used direct hydrolysis method for isolation of sapogegenins solasodine and steroidal alkaloids. (Weissenberge M et al; 2001) Karawya M. S. et al, isolated solasodine from *Solanum laciniatum* leaves by extraction and hydrolysis of glycoalkaloids (Karawya M. S. et al; 1975). Roosen Runge developed modified analytical method. By this method quantitative determination of chaconine and solanine alkaloids from tumor, leaves and sprouts of potato. Glycoalkaloids determined by gas chromatography after silylization (Roosen Runge C et al; 1977) Nikolic N.C et al isolated solanidine from the *Solanum tuberosum* vine by using solid liquid solid systems (Nikolic N.C et al; 2003) In 1953 Sato and Lathem isolated diosgenin along with the alkaloids solasodine, solanidine and solacarpidine as the hydrolysed product of the crude base from the dry fruits of the plants (Y. Sato et al; 1953). In 1973, Kusano et al isolated different types of constituents like solamargine, cycloartenol, sitosterol, β solanomargine from of *Solanum Xanthocarpum* fruits. (Kusano et al; 1973) In the same year two new sterols were isolated through thin layer chromatography of crude preparation of *Solanum xanthocarpum*, namely norcarpesterol and 4a methyl-24 E methylcholest -7ene -3 β, 22E-diol. Tupkari isolated coumarins, scopoletin, esculetin and their glycoalkaloids, scopolin and esculin from the dry leaves, roots and fruits of the plants. (Tupkari et al; 1972) Dubey and Gupta reported the isolation of quercetin, apigenin and sitosterol from the flowers of *Solanum xanthocarpum*. (Dubey et al; 1978). Hellenas K.E isolated glycoalkaloids from potato. They also isolated aglycones from blood serum by high performance liquid chromatography. In view of the importance of steroids and alkaloids of *Solanum Xanthocarpum*. These compounds used in synthesis of sex hormones and cortico steroids. Various groups of workers carried out biosynthetic studies for
increasing the production of secondary constituents of the plant (Hellenas K.E et al; 1992). For the isolation of alkaloids from dried plant powder solution like hexane and ethers were used to remove waxes, oil, and fats from the alkaloids. After the discardation of extract, remaining material used for methanol or ethanol extraction. After evaporation of extract the crude alkaloid was obtained. To isolate the alkaloids from plants, dried powder of plant material is extracted with pet ether or hexane coleman etc. This removes the fats, oils; terpene waxes etc. The extract is discarded and the material subjected to an alcohol extraction, methanol or ethanol. The extract was evaporated to leave the crude alkaloid mixture.

Purification of Alkaloid:

4.1 Introduction:
In 1968, Seth and Chaterjee isolated three glycoalkaloids from the berries of Solanum surattense by thin layer and column chromatography. They were identified solasodine, solamargine, and solasurine. Later Mukharjee isolated 0.9 percentage of solasodine from the same plant. Heble et al carried out studies in the tissue cultures of Solanum Xanthocarpum and reported the isolation of solasodine along with diosgenin, ß-sitosterol, and lupeol (M.R Hemble et al; 1968). Later Khanna reported the hydrolysed base solasodine occurs in the tissue culture along with diosgenin (P. Khanna et al; 1976). In 1979 Chowdhury isolated solasodine in differentiated tissue of Solanum Xanthocarpum (A.R. Choudhary et al; 1980) There are many methods for purification of alkaloids. There is no satisfactory method available which permits quantitative determination of the alkaloid with accuracy. In solanum species steroid are determined by calorimetrically as well as spectrophotometrically. The method of assay of alkaloid in the majority of pharmacology are based upon lime treatment other methods use for polarographic, Colorimetric (Rasmussen et al; 1946) and ion-exchange (Jindva; 1940) procedure have also been described. The methods advocating adsorption of alkaloids onto alumina (Eder et al; 1940) and Florisil (Florence C et al; 1953) columns have been published. The glycoside is extracted by the usual methods and the partially pure product hydrolyzed to aglycone. Aglycone is complexed with methyl orange and colored complex extracted into chloroform and determined calorimetrically. Solasodine, steroidal
alkaloids, sapogenesis isolated by direct hydrolysis (Weissenberg M et al; 2001). Solasodine is isolated from Solanum laciniatum leaves by rapid one step extraction followed by hydrolysis of the glycoalkaloids. The resulting steroidal aglycone directly estimated by the formation of a colored complex with bromo cresol green (Hellensas K.E.et al; 1992). Glycoalkaloids from potato aglycone in blood serum were purified by High – performance liquid – chromatography. Solanidine hydrolytic extraction and separation was carried out from the Solanum tuberosum using solid – liquid system (Nikolic N.C.et al; 1849). A new analytical method for quantitative determination of Solanine and Chaconine in potato tubers, sprouts and leaves has been developed. The extraction with pyridine causes a careful isolation of glycoalkaloids.

There are various techniques for the purification of alkaloids but most convenient is adsorption chromatography.

4.2 Adsorption Chromatography:
This technique of chromatography is based on the difference in the adsorbing power of the components on an adsorbent. It is a solid-liquid partitioning technique. The stationary phase is a solid, which separates the components of a liquid passing through it by selective adsorption on its surface. This type of interaction, causing adsorption, are the same as those that cause attractions between any molecules, electrostatic attraction, complexation, hydrogen bonding, Vander Walls forces etc. It is also known as the solid liquid chromatography with solid as the stationary and the liquid as the mobile phase. This type of chromatographic technique was introduced by Twesett in 1903. Adsorbent like silica and alumina are used as the solid phase and any suitable liquid as the mobile phase. The nature of the processes involved is believed to be adsorption of the solute on the solid phase and its solubility in the liquid phase.

Elution: In the elution process, molecules are separated and the individual components are recovered. There are two ways of elution:

i) Physically: The solvent is drained of, the bands are physically extruded and are cut by a spatula and the adsorbed component is recovered by means of a suitable solvent.

ii) By flow of solvent: In this method, the flow of the solvent is continued till the bands are washed out one by one and the components are recovered by distillation of the solvent. The elution normally begins with a non-polar solvent like hexane or petroleum
ether and the polarity of the elution solvent can be increased gradually by adding successively greater percentage of either ether or benzene or some other solvent polarity. The transition from one solvent to another solvent should not be too rapid in solvent changes. Elution is successful when the components of the mixture are colored.

**Adsorbent:**

A few of the solid adsorbents commonly used include alumina, silica gel, florisil, charcoal, magnesia, calcium carbonate, starch and sugar. Alumina (Al₂O₃) is a highly active, strongly adsorbing polar compound and is available in three forms neutral, basic and acidic. Basic and acidic alumina offer good separating power for acids and bases respectively. For compounds that are sensitive to chemical reactions under acidic or basic conditions, neutral alumina should be used. Being highly polar, alumina adsorbs polar compounds quite tenaciously, so that they may be difficult to elute from the column. The activity of alumina may be reduced by addition of small amounts of water. Silica gel and florisil are also polar but less than alumina. Fraction of alkaloids from the stems of *Illigera luzonesis* was carried out with silica gel column chromatography with CHCl₃ MeOH to yield dicentrinone and liriodenine (Kangasundaram Y.et al.;1997). The alkaloids form the aerial part of *Efedra transitoria* was purified with the silica gel column in CHCl₃. While it was reported from the stems of *Fuligo Septica* with the column chromatography over open silica gel by eluting MeOH (Atta ur .Rehman et al.;2000). The alkaloids from the root bark of *Tripterygium hypoglaucum* were purified with silica gel using CHCl₃ (V.K.Ahluwalia et al.;2006). The alkaloids from the aerial parts of *Solanum alopecuroides* were purified with silica gel column using CHCl₃. Silica gel was used for purification of alkaloids from various plants.

**Column chromatographic studies:**

Packed column is a stationary phase thorough which mobile phase is allowed to flow under the gravity and under the pressure. The suitable solvents are selected in column on the basis of characterization of the mixture. Commonly the non polar solvent is used for passing the mixture in to the column. The initial solvents are used for the chromatogram development. With increasing the polarity of eluting solvent applied for
solvent selection. These solvents have low boiling point which allow the recovery of eluted material. The solvents like trichloroethylene, hexane, diethyl ether, cyclo hexane, toluene, dichloromethane, carbon tetrachloride chloroform, ethyl acetate, acetone, ethanol, and methanol are used in column chromatography. In the present study silica gel used as a adsorbent material.

1) Extraction of alkaloids: The Solanum xanthocarpum crude alkaloids product from soxhlet was extracted by the method of manske and Ashford (Manke et al ;1954).

2) Column packing with silica:
The column was packed with slurry of silica gel. Silica gel slurry was prepared by using silica powder with 5:2:1 ratio of chloroform, ethyl acetate and butanol. By mixing this quantity a homogenous mixture was formed. The column is packed with silica gel slurry which was free from air bubbles.

3) Adsorption column:
The obtained crude alkaloids from soxhlet extraction method were used for loading on column of silica gel (4.5X 2 cm). The column was filled with the solvents chloroform: Ethyl acetate: Butanol in a ratio (5:2:1) and allowed to become soaked. As soon as the first drop of solvent started to drop from the bottom end of the tube, it was connected with the receiving flask, adjusted the rate of flow to approximate 1 drop per 2 minutes. Total thirteen fractions were collected with volume 50ml of each. Recovered fraction containing alkaloids from column It was followed by uv, IR melting point and TLC.

Experiment No.2

Aim: PHYTOCHEMICAL SCREENING OF AERIAL PARTS OF SOLANUM XANTHOCARPUM

4.3 Volumeric analysis of alkaloids: From the obtained fraction, 10 ml solution mixed and acidified with HCl (concentrated) solution. NaOH solution with normality 0.1 filled in the buret and titrated against acidified fraction solution. Methyl orange was used as a indicator in titration process. It was observed that the colour was changes
from red to yellow. It was considered as end point of titration. The above procedure was carried out for each fraction.

### 4.4 Structure determination of alkaloids by MP, TLC, IR and UV.

TLC of each fraction from adsorption column chromatography and monitored with iodine vapours, and ninhydrine reagent was carried out. The fraction three, five, seven, and nine were matches with the standard alkaloids solamargine, solasodine, solanine, and solasodine respectively. The Rf values of fraction three, five, seven, and nine are shown in table no.4.1

(ii) **Physical Constant:**
Physical constant of each active fraction obtained from silica gel column was carried out and shown in table no.4.1

(iii) **U.V:** For UV determination of obtained alkaloids, Shimadzu's spectrophotometer (UV-2100) was used

Result: UV of fractions three, five, seven, and nine are shown in table no.4.1.

(iv) **IR:** IR of fraction three, five, seven, and nine was carried out in IIT Pawai, Mumbai

### 1.5 Structure elucidation of alkaloids in fraction:

**Fraction Three:**

TLC of fraction three is shown in figure 4.1

IR of fraction three is shown in figure no.4.2

Mass Spectrum of fraction three is shown in figure no.4.5; is carried from IIT Pawai, Mumbai.

**Fraction Five:**

TLC of fraction Five: fraction five was used for the TLC determination. This fraction was used on TLC plate and the rf value was found 0.78. Glycoside solasodine was separated by silica gel TLC plate. The obtained Rf value was matches with the standard alkaloids of solasodine.
IR (KBr) of fraction five: characteristics peaks at wave number in cm\(^{-1}\) were taken. IR of fractions five shows the peak at 666.9 cm\(^{-1}\) region spectra. It indicates that -N-H wagging out of plane. 1522.4 cm\(^{-1}\) and 1254.7 cm\(^{-1}\) shows simple open secondary amides absorbs near 1580 cm\(^{-1}\) shows competition between the ring and C-O for non bonded electron pairs of nitrogen. 1610 cm\(^{-1}\), 1630 cm\(^{-1}\) and 1660 cm\(^{-1}\) peaks confirms -C-H and N-H stretching with benzene ring. 2380 cm\(^{-1}\) region shows that strong absorption band results from superimposed –OH and NH\(_3^+\) stretching band which characterized multiple fine structure. 3360 cm\(^{-1}\) (NH stretching) 3700 cm\(^{-1}\) to 3584 cm\(^{-1}\)(superimposed OH and NH\(_3^+\) stretching bands) IR of fraction five is shown in figure 4.7 From the above study, fraction five contains Solasodine detailed of UV and Physical Constant shown in table 4.1. The solid form of Solasodine and structure of Solasodine are shown in figure no 4.8 and 4.9 respectively.

The molecular formula of solasodine is \(\text{C}_{27}\text{H}_{29}\text{NO}_2\)

From the IR data the values are found 1580 and 1610 cm\(^{-1}\)

3360(N-H) stretching found. 1630, 1660 also observed. and IR \(\lambda_{\text{max}}\) (KBr) cm\(^{-1}\)

The UV \(\lambda_{\text{max}}\) (Methyl alcohol) in nm are measured like 283, shoulders at 208 and 240. The mass m/e was found : 409.2981.

The melting point of solasodine was measured and it was in between the range of 200-202 °C. On hydrolysis of solasodine the needle like crystal were formed.
Figure 3.3 Structure of Solasodine

**Fraction Seven:**
The thin layer chromatography was done for the fraction seven. The rf value was noted 0.40. The Dragendorff reagent was used for the visualization of spot. It was observed the the spot was not exactly matches with standard Solanine alkaloid.

**IR of fraction seven (Solanine):** The IR values like 700 cm$^{-1}$, 3500-3200 cm$^{-1}$ shows the starching of -NH (-NH stretching)

**UV of fraction seven:** UV spectra of fraction seven was noted and found $\lambda_{max}$ 235nm and $\lambda_{min}$ 205nm.

Solanine are crystalline form and the structure of Solanine is shown in figure no.4.10 and 4.14 respectively.
It is observed that structure of solasodine contains β hydroxyl group at c3 position in hexacyclic ring. The double bond is present in 5 and 6 carcon atoms. In ether linkage secondary nitrogen and oxygen are present which form spiroaminoketel system.

Molecular formula: $\text{C}_{45}\text{H}_{73}\text{NO}_{15}$

Figure no.3.4 Structure of Solanine

**Fraction Nine:**

**TLC:** TLC of fraction nine shows brown spot in iodine fumes. The Rf value 0.98 which matches with standard Solanidine.

**UV of fraction nine:** UV spectra of fraction nine was $\lambda_{\text{max}}$ 225nm and $\lambda_{\text{min}}$ 218.3nm. The needle shaped crystalline form of Solanidine is shown in figure no.4.11 and structure of Solanidine is shown in figure no.4.12

**IR:** c=O from COOH group at $1760 \text{ cm}^{-1}$ C=O from COOR $1245 \text{ cm}^{-1}$, (C-O) 860 cm$^{-1}$, 825 cm$^{-1}$ (lactum)
Experiment No.3

**Aim:** ISOLATION OF DYE FROM AERIAL PARTS/EXTRACTION OF COLOURANT

Green extraction methods were used for isolation of dyes/colourants from leaves. The fresh leaves of *Solanum xanthocarpum* were selected for the experimental part.

**Source:** For the extraction of colourants, fresh leaves of *Solanum xanthocarpum* plant were collected.
Figure 3.6  Fresh green leaves of solanum xanthocarpum

Methods:

Green Extraction methods of dyes:

For the extraction of colourants the fresh leaves were collected and washed with water to remove the foreign particles and dust. Colourants / Dyes were extracted by using green leaves of solanum xanthocarpum. Three different methods were used for extraction of colourants. Very economical methods were used for the extraction of colourants.

1) FIRST METHOD:

The first method is used for the extraction of colourant in which only water was used for the extraction of colourants. In this method, dye was extracted from leaves. 10 g of leaves in 100 mL distilled water was extracted by preparing an aqueous solution of leaves. It was carried out for half an hour at 80 °C. The extract was separated and filtered. The extracting material was applied on fibres like cotton and wool. The colouring materials from the leaves were extracted for dying of the fabric materials. The
aqueous solution of leaves was filtered and leaves were engaged out from the liquor for recycling process of extraction for the subsequent. For the application of extracted dye, the fibres like cotton and wool were selected and applied on it.

2) SECOND METHOD /OXIDATION REACTION:

In the second method of extraction of dyes the fresh leaves were selected. 10 gm of leaves were weighed for the experimental work. The leaves were used in uncrushed form. The selected uncrushed fresh leaves extracted with 100 ml of distilled water for colourants. It was kept for 10 days without any disturbance. After 10 days the pasty mass of leaves was obtained. It was separated from the solution and used to apply on fibre. It was observed that after 10 days the pasty mass produces colourant which was easily applied on cotton and wool.

3) THIRD METHOD:

It is also called as photooxidation reaction. The effect of light was observed on extraction of dye in relation time and divided into two parts. In first part 10 gm of Solanum xanthocarpum leaves crushed in 100 ml of distilled water. It was kept in earthen pot. The earthen pot was kept undisturbed for 2 hours in dark. For second part 10 gm of leaves were crushed in 100 ml of distilled water in an earthen pot. The earthen pot was kept undisturbed for 2 hours in sunlight especially between 12 noon to 2.00 pm. The extract from both beaker were filtered by using cloth to get natural dye. Change in colour and yield was observed.

Application of dyes on wool and pure cotton cloth:

Dying procedure:

The extract obtained from above green extraction methods was filtered and used for dying cotton cloth and wool. The selected materials for dying were boiled in NaOH(10%) for 10 min to remove starch from cloths. Then wool and pure cotton cloths were transferred for treatment in the dye bath for 30 min. After the processing and dye
fixation the materials were sunlight dried for 1 hour. Effect of dye without mordent on wool and cotton was also observed.

The prepared dye by green extraction method may also modified by using different metals to have its huge applications and employment. Since, India has its rich biodiversity and produced huge amount of raw material and *Solanum xanthocarpum* is waste land weed also occurs roadside. Its dye/pigments have compensation from the industries because different shades of colourants observed from its aerial part. Using different mordents dyes properties also enhance such as color fastness, washing fastness and perspiration. The obtained dye is safe and green extraction process is cost free.

**Experiment No.4**

**Azo dye preparation from leaves and fruit of solanum xanthocarpum**

**Source:** Fresh leaves and fruits were separated from plant, washed with water to remove the contamination and some foreign particles. These parts were dried for two weeks in sunlight. The fresh leaves and fruits were separated are shown in figure.
sun light dried and air dried leaves and fruits were ground in stainless steel grinder in to fine powder and stored air tight glass bottle separately to prevent moisture.

Figure 3.7A. Fresh fruits of solanum xanthocarpum

B. Fresh leaves of solanum xanthocarpum
Figure 3.7  
C. Dried fruits of Solanum xanthocarpum  
D. Powder of fruit of Solanum xanthocarpum

Figure 3.8  
A. Dried leaves of Solanum xanthocarpum  
B. Powder of leaves of Solanum xanthocarpum
Methods of preparation of azo dye:
The azo dye was prepared from leaves powder of solanum xanthocarpum was mentioned in above. The same method was applied for the preparation azo dyes from fruit powder of the solanum xanthocarpum.

Preparation of azo dye from solanum xanthocarpum fruits powder:
The azo dye was prepared by using following steps.

Preparation of Step I solution:
Azo dye was prepared with 5 gm of fruit powder. It was mixed with concentrated sulphuric acid and distilled water. This process was carried out for 10 - 15 minutes. The temperature was maintained at about 50°C in cold water bath with addition of 4 cm3 of drop wise NaNO2. The prepared solution was used in another solution which was prepared in further step. The preparation method of solution of step I was explained in flow chart.

Preparation of Step II solution: In the preparation of solution No.2 some chemicals were required like 2-napthol and 2N NaOH. The new solution was prepared by using 2.6 gm of 2-napthol and 15 cm3 of 2N NaOH. These chemicals were added in to 500 cm3 beaker and kept in cold water bath to maintain the cooling temperature. It was observed that reaction mixture attend temperature of cold water. Prepared solution from previous step was added drop wise in step II solution with constant stirring. It maintains the temperature below 10°C. After mixing both solutions HCl was added in this solution with vigorous stirring. After complete addition of both solution, shaking was continuously done till the dark red brown dye separated from the mixture. The washing of the obtained mixture was done distilled water. It was filtered and dried in air. The dried dye was used for the further characterization.

The characterization was done by TLC, IR, UV, MP and NMR and xrd. The dye in different solution was applied on fabrics like wool, cotton and paper. The obtained dye dye was applied on fibres to prove its value and strength.
5 gm fruits powder

10 cm³ conc HCl + 5 cm³ distilled water

Warm and cool at 5⁰c

Dropwise addition of NaNO₂ in 4 cm³ distilled water (cold solution)

Solution A

Flow chart 3.4: preparation of azo dye from solanum xanthocapum fruit powder (solution A)
2.6 gm 2 napthol

15 cm₃ 2N NaOH

cool at 5°C

Dropwise addition in solution A

wash with constant stirring

temp below 10°C

Addition of HCl solution with vigorously stirring

Dark black dye

Wash with distilled water and air dry

Solution B
Flow chart: Preparation of azo dye from Solanum xanthocarpum fruit powder (solution B)

Preparation of azo dye from Solanum xanthocarpum leaves powder:

The preparation was done with solution prepared by step I and step II.

**Step I:** In this experiment azo dye was prepared from the dried leaves powder. With concentrated H$_2$SO$_4$ and distilled water, 5 gm of leaves powder was heated. It was heated for 10 minutes at 50°C temperature. This solution was kept in a cold water bath for cooling. During cooling, 4 cm$^3$ of NaNO$_2$ was added in the cooling solution. After adding NaNO$_2$ in the solution and cooling, step I solution was prepared. This prepared solution was used in the next process.

**Preparation of step II solution:** After the formation of the first solution in step I, the requirement of another solution was also required. The second solution was prepared in the second step. For this preparation, 2.6 g of 2-naphthol and 15 cm$^3$ of 2N NaOH was added into a 500 cm$^3$ beaker in a cold water bath, and the reaction mixture attended the temperature of the cold water. After this step, the solution was added dropwise into the solution of step-II solution reaction mixtures by constant stirring. It maintains the temperature below 10°C. After complete addition, HCl was added in this solution with vigorous stirring and shaking till the dark red brown dye segregated from the mixture. It was washed with distilled water. After washing with distilled water, it was air-dried and further characterized. The characteristics analysis confirmed the formation of azo dye from the leaves powder of Solanum xanthocarpum. The prepared azo dye was confirm.
by UV, IR, TLC, MP, and XRD. The same dye was applied on natural and synthetic fibre to prove its utility and stability. The prepared dye was used in different solution to check and observed the various colour shades.

Application of AZO DYE synthesized from leaves of solanum xanthocarpum:

Preparation of 1% leaves dye solution: The prepared leaves dye (1g) was pasted with warm water and then 80 cm$^3$ of boiled water was mixed. After proper stirring, clear solution was formed. The obtained solution was made up 100 ml with boiled water.

\[
\begin{align*}
5 \text{ gm leaves powder} & \\
10 \text{ cm}^3 \text{ conc HCl } + 5 \text{ cm}^3 \text{ distilled water} & \\
\text{Warm and cool at } 5^0\text{c} & \\
\text{Dropwise addition of NaNO2 in 4 cm}^3 \text{ distilled water (cold solution)} & \\
\end{align*}
\]
Solution A

Flow chart 3.6  preparation of azo dye from solanum xanthocapum leaves powder
Flow chart 3.7  preparation of azo dye from solanum xanthocapum leaves powder
Experiment No.5
APPLICATION OF DYE

Application Prepared Dye on Natural Fiber:
The materials like wool, silk and paper were selected for application of extracted dye. Wool and silk fabrics were soak at 40°C in solution of H₂SO₄ (2-3%) with 10-15% glanber salt solution for 20 minutes. The dissolved 1%dye was added in this mixture. It was carried out for one hour and then washing and drying was done.

Dyeing on synthetic fiber:
The fabric like cotton and nylon were used in formic acid solution and boiled by heating. In the boiled solution ammonium acetate was added and formed dark colour was appeared. which was washed with water and air dried.

Fastness Properties:
For all the dyeing of material fastness test was carried out. The pH was maintained at about 5. It has been demonstrated to be the optimal.

Fastness of light:
The property of light fastness was tested by using xenon lamp. When dyed fibres were exposed in light of xenon lamp, the light fastness property was observed.

Fastness of washing:
The washing fastness property was observed by performing wash test. It was done with soap solution. It was prepared by 5 g/l, liquor in ratio of 50:1 for 45 minutes temperature 60°C. The changes in shade of colours were observed.

Fastness to rubbing:
This property of rubbing fastness was done according to ISO, 2001 test method. Characteristics analysis to confirm formation of Azo Dye from solanum xanthocarpum leaves prepared dye was screened by TLC, MP, UV, IR and XRD to confirm formation of resultant azo compound. The same dye was applied on natural and synthetic fibre to prove its value and strength. In industrial reaction for the preparation of dyes and pigments, azo coupling is used.