CHAPTER NO. 4

RESULT AND DISCUSSION

4.1 Aim 1

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

Detection of Proteins: 1 ml sample of Solanum xanthocarpum leaves was used for detection of proteins. It was measured by Lowry method. The following result was found.

**Result:** Total amount of proteins in Solanum xanthocarpum leaves is 12.5%.

Amine Detection: Amino group detection test was carried out using leaves dried powder. The following result was observed.

**Result:**

a) It confirms that amine present in the sample.

b) When in above test NaNO₂ added oily emulsion appeared which was white in color. It indicated that secondary basic group. (-N-H) was present.

Detection of Carbohydrate:

**Result and Discussion:** The total amount of carbohydrates in Solanum xanthocarpum leaves was 16%. Carbohydrates are very important for living organism. Due to the presence of carbohydrates it was indicated that glycoalkaloids, may present in the Solanum xanthocarpum.

UV Spectrum of Extract of Solanum Xanthocarpum Leaves.

**Result:** UV Spectrum of wet extract of Solanum Xanthocarpum leaves was λ max 254nm and λ min 239.2nm.

TLC of Extract of Solanum xanthocarpum leaves.

**Result:** The Rᵢ values of sample was 0.67 in Butanol: Acetone: Water (1:3:1) system which was visualized with ninhydrin reagent.
Extraction of alkaloid from *Solanum Xanthocarpum* leaves:

i) Effect of various concentration of SDS on alkaloids:

**Result:** The total alkaloids are remains same as compared to concentration of SDS increases. It was 0.6 gm as compare to to control 0.1%(m/v) of SDS was selected for time and wave factor study.

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

**Result:** Effect of sonication on extraction of alkaloids is shown in fig. Total alkaloids are increases up to 120 min and total alkaloids are fall down after 150 min and onward hrs.

iii) Effect of varying time on extraction of alkaloids without sonication:

**Result:** Extraction of alkaloids was carried out without sonication. Total alkaloids are increases up to 120 min. Its concentration fall down after 150 min and onward hrs.

I) Solvent extraction method: It was observed

**Result and discussion:**

1) The yield of crude alkaloid by solvent extraction method is 0.78 g which is shown in figure 4.1
2) The IR of crude alkaloids is shown in figure 4.2

The IR values like 666.9 cm\(^{-1}\) shows (-N-H stretching), the IR value 1254.7 cm\(^{-1}\) shows the bending which vibrates the electrons due to hydrogen bonding, at 1522.4 cm\(^{-1}\) shows the (NH\(_3^+\)) group, 2380 cm\(^{-1}\) shows (NH stretching), 3340 cm\(^{-1}\)(NH stretching), 3700 to 3584 cm\(^{-1}\) (from superimposed OH and NH\(_3^+\) stretching bands.)

666.9 cm\(^{-1}\) region spectra indicates that - N-H wagging out of plane. 1522.4 cm\(^{-1}\) and 1254.7 cm\(^{-1}\) shows simple open secondary amides absorbs near 1540 cm\(^{-1}\) shows competition between the ring and C-O for non bonded electron pairs of nitrogen. 2380 cm\(^{-1}\) region shows that strong absorption band results from superimposed –OH and NH\(_3^+\) stretching band which characterized multiple fine structure.

3) UV of crude alkaloids was inactive because of conjugation of compound.
4) Physical constant: Melting point of crude alkaloid was observed at 202°C further heating 245°C and 262°C. There may be presence of different alkaloids. It may be confirmed in the purification chapter.

5) TLC: The Rf value of crude alkaloid is 0.52 with off-white colour in water: acetone: benzene system (1:3:1). Solvent system. All above data indicates that the possibility of solasodine, solanidine, solanine and solamargine alkaloids. It confirmed in the purification chapter.

Figure 4.1 Crude Alkaloids of *Solanum Xanthocarpum* leaves by Solvent Method
II) Soxhlet method: The alkaloids were extracted by using soxhlet method and following result was observed.

Result:

i) Yield of crude alkaloid by Soxhlet method was found 0.85g.

ii) Melting point of crude alkaloid by Soxhlet method was 240° C

iii) UV of crude alkaloid \( \lambda \) maxima. 248.4nm and \( \lambda \) minima. 225.8 nm

iv) IR (KBr): Crude alkaloids which shows characteristics peaks at 1506 cm\(^{-1}\) (aliphatic secondary amine), 1550cm\(^{-1}\) (noncyclic secondary amides), 1650 cm\(^{-1}\) (lactones)
1750 to 1700 cm\(^{-1}\) (five member ring), 3500 cm\(^{-1}\) (NH stretching vibration), 600 cm\(^{-1}\) to 800 cm\(^{-1}\) (NH wagging out of plane) (figure no. 3.5). 500 cm\(^{-1}\) shows a presence of –NH stretching, 2891 shows a –CH stretching, 1750 cm\(^{-1}\) shows C = O stretching, 1611 cm\(^{-1}\) shows C=C stretching.

**Discussion:** 10 g of dry plant material was used for Soxhlet extraction. Ethyl acetate, petroleum ether followed by diethyl ether solvent was used. Three layers were formed by addition of solvents. The layers are formed in the solution. Aqueous, organic and lipophilic layer with distinct color layer were formed. The organic layer was separated and extracted with chloroform. It was washed with distilled water to maintain the pH neutral. The drying was done with Na\(_2\)SO\(_4\) under reduced pressure. The crude alkaloid was obtained. The first layer of lipophilic shows Rf value 0.86. The green color was visualized in iodine vapours on TLC. Green color which was indicates solanine alkaloids may present in *Solanum xanthocarpum* leaves. Second layer was organic layer which shows Rf value 7.77 with pink color. For the visualization of pink colour ninhydrine reagent was used. It was indicated solasodine may present. Third layer was aqueous layer shows Rf value 0.53 with cream color spot in dragendorff reagent which indicates solamargine may be present.

From the TLC method it was expected alkaloids are Solasodine, solanine, solanidine, solamargine and solacarpine may be present in the leaves of solanum xanthocarpum. It confirmed in purification method of crude alkaloids.
**TLC:** Thin layer chromatography is suitable as rapid method for screening plant alkaloids.

<table>
<thead>
<tr>
<th>TLC by soxhlet method</th>
<th>Rf value in solvent system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soxhlet lipophilic layer</td>
<td>0.86 (green colour spot)</td>
</tr>
<tr>
<td>Soxhlet organic layer</td>
<td>0.77 (pink colour spot)</td>
</tr>
<tr>
<td>Soxhlet aqueous layer</td>
<td>0.81 (off white colour spot)</td>
</tr>
</tbody>
</table>

**Solvent system:** Water: Acetone: Butanol (1:3:1)

**Spraying reagent:** Ninhydrine reagent
Figure 4.4 IR of Crude alkaloids from *Solanum Xanthocarpum* leaves by soxhlet method
New method/Modern method for extraction:

Result: The percentage of alkaloids of solanum xanthocarpum leaves was extracted by modern method was more than soxhlet and solvent methods of extraction. It was observed that the alkaloids with modified method shows higher yield. The colour of the crude alkaloids was golden in colour and it was in powder form.

Result:
1) Yield of crude alkaloid obtained by modern method: 1.50 g. It was shown in figure 4.9.
2) UV of crude product $\lambda$ max 235 nm and $\lambda$ min 212 nm
3) Melting point of crude product 265°C
4) IR(KBr): 600-800 cm$^{-1}$ (N-H stretching), 1039 cm$^{-1}$ (C-O-C stretching), 3004 cm$^{-1}$ (aromatic CH stretching), 3400 cm$^{-1}$, 2362.4 cm$^{-1}$, 1525.8 cm$^{-1}$, 1600 cm$^{-1}$ (Benzene ring), 1290 cm$^{-1}$.
5) NMR shows triplets and quadrates shown in figure 3.11a and b.

Discussion: Isolation of alkaloids was carried out using 10 grams dry powder of Solanum Xanthocarpum leaves, 0.3% (m/v) EDTA. It was sonicated for 120 min followed by precipitated with Mayer reagent. The yield of crude alkaloid was 1.50 g. This method was one step, easily handable, more convenient and gives higher yield. The UV, melting point, and IR indicates presence of solasodine and solamargine. But peak at 3004 cm$^{-1}$ shows aromatic -CH stretching. In the spectrum band is at 1039 because of unsaturation produces absorption characteristic stretching may conclude presence of solanine and solanidine.
Figure 4.5. Total alkaloids extracted by sonication with SDS
Figure 4.6. Total alkaloids extracted by sonication without SDS
**TLC Identification:**

**Result:** The $R_f$ values of sample were 0.80 for 0.1 % (m/v), 0.81 for 0.2 % (m/v), 0.92 for 0.3% (m/v) for respectively in ninhydrin reagent. TLC plate is shown in figure 4.7.

![TLC plate](image)

**Figure 4.7** TLC of *Solanum xanthocarpum* leaves containing

a. 0.1 SDS concentration in m/v

B. 0.2 SDS concentration in m/v

C. 0.3 SDS concentration in m/v
UV Structure of sonicated sample:

**Result:** UV spectrum of crude alkaloids precipitated after sonication is shown as follows-

<table>
<thead>
<tr>
<th>Time</th>
<th>Wavelength</th>
<th>( \lambda ) Minima nm</th>
<th>Absorbance</th>
<th>( \lambda ) Maxima nm</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>210.2</td>
<td>0.249 A</td>
<td>225.8</td>
<td>0.373 A</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>213.8</td>
<td>0.877 A</td>
<td>256</td>
<td>0.558 A</td>
<td></td>
</tr>
<tr>
<td>90 min</td>
<td>235.4</td>
<td>0.828 A</td>
<td>254</td>
<td>1.163 A</td>
<td></td>
</tr>
<tr>
<td>120 min</td>
<td>225</td>
<td>0.931 A</td>
<td>244.4</td>
<td>0.558 A</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2: UV spectrum of crude alkaloids of *Solanum Xanthocarpum* leaves precipitated after sonication at various time intervals
B) Extraction of alkaloid with EDTA

i) Effect of various concentration of EDTA:

**Results:** According to figure 2.5, it was found that percentage of alkaloids were increases as the concentration of EDTA solution increases like 0.1, 0.2 and 0.3 (m/v) etc. With increasing the concentration of EDTA, it was observed that the percentage of alkaloids were also increases when edta concentration was increases in concentration of EDTA increases the percentage of total alkaloids. But in the experimental work it was found that after 0.3 % (m/v) concentration of EDTA the percentages of alkaloids formation was decreases. Therefore 0.3 % (m/v) concentration of EDTA was selected for time and wave factor study.

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

**Results:** Percentage of alkaloids precipitated according to time with sonication is shown in table no 2.2. With sonication the percentage of alkaloids were increases as compare to without sonication. It is shown in fig.4.8. The percentage of alkaloids extracted without sonication was also carried out.

iii) Effect of varying time on extraction of alkaloids (without sonication):

**Results:** The percentage of total alkaloids was increased upto 90 min after 90 min it decreased which is shown in table.4.3.
Figure 4.8 Effect of various concentration of EDTA
Figure 4.9. Total alkaloids extracted by sonication with EDTA
<table>
<thead>
<tr>
<th>Time Factor</th>
<th>Amount of alkaloids precipitated at 0.1% (m/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sonicated extract</td>
</tr>
<tr>
<td>30</td>
<td>0.338</td>
</tr>
<tr>
<td>60</td>
<td>0.528</td>
</tr>
<tr>
<td>90</td>
<td>0.665</td>
</tr>
<tr>
<td>120</td>
<td>0.702</td>
</tr>
<tr>
<td>150</td>
<td>0.700</td>
</tr>
</tbody>
</table>

Table 4.3 Percentage of alkaloids precipitate in *Solanum Xanthocarpum* leaves according to time interval.

For the precipitation of alkaloids 0.1% of EDTA solution was selected. The extraction was done with sonication and without sonication. The time factor was selected from 30 minutes to 150 minutes. It was observed that at 30, 60, 90, 120 and 150 minutes the weight were found 0.338, 0.528, 0.665, 0.702 and 0.700 respectively. At 120 minutes higher weight precipitate was found. After 120 min it was decreased. Without sonication the higher weight was found at 120 min and after it was decreased. Therefore 120 minutes time duration was fixed for the precipitation of alkaloids.
**TLC Identification:**

**Result:** The TLC was done for the determination of Rf value of samples of leaves Solanum Xanthocarpum sample. The Rf values were found 0.77 for 0.1 % (m/v) solution and 0.78 for 0.2 % (m/v), solution. For 0.3 % (m/v) of EDTA solution it was found and 0.92.

**UV Spectra:**

UV spectrum of Solanum Xanthocarpum leaves sample containing 0.3% (m/v) EDTA, sonicated at various time interval. Solanum Xanthocarpum leaves sample shows UV maxima at 224.2 nm and minima 213.2 nm when it is exposed to sonicator for 30 min. At 60 min, it was observed that UV maxima at 224 nm and minima 213 nm. UV maxima at 224.4 nm and minima 211.2 nm when it is exposed to sonicator for 90 min. While UV maxima at 221 nm and minima 215 nm when it is exposed to sonicator for 120 min. At 30 min, 60 min and 90 min the same alkaloid was isolated from Solanum Xanthocarpum leaves because UV spectrum indicates that maxima at 210 nm and minima is at 213 nm.

**Discussion:** Sonication and without sonication the experiments were carried out. At room temperature the extraction time of alkaloids was determined. Powdered leaves of Solanum Xanthocarpum was used in 0.1 % (m/v) solution of sodium dodesyl sulphate. The highest yield of alkaloid was obtained after two hours. All experiment sets were carried out triplicate and confirmed the 2 h. for sonication for the extraction of alkaloids.

The same experiment was carried out with the surfactant 0.3 % (m/v) of EDTA with sonication and without sonication of powdered leaves of Solanum Xanthocarpum at
room temperature to determine the optimum extraction time. The highest yield was obtained by this method after 1.5 to 2 hours. All experiments were triplicate and confirmed 2 hours sonication as a standard time for extraction of alkaloids.

**Comparative study of different surfactant:** The effect of EDTA and SDS on *Solanum Xanthocarpum* leaves alkaloids study was carried out. In this experiment it was observed that use of surfactant increases the quantity of alkaloids. 0.3 % concentration of surfactant was selected for experiments. Laboratory conditions and seasonal change may vary the results. The yield obtained by SDS and EDTA is shown in Table 3.4

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Time</th>
<th>Percentage of alkaloids With sonication</th>
<th>Percentage of alkaloids without sonication</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS</td>
<td>2h</td>
<td>0.690</td>
<td>0.300</td>
</tr>
<tr>
<td>EDTA</td>
<td>2h</td>
<td>0.702</td>
<td>0.449</td>
</tr>
</tbody>
</table>

Table 4.4 Comparative Studies: effect of SDS and EDTA concentration on *Solanum Xanthocarpum* leaves alkaloids.

From the Table 4.4 data indicates that, EDTA gives superior result than SDS. According to SDS study, it was observed that *Solanum Xanthocarpum* leaves samples shows four types of alkaloids. It was very difficult to isolate the alkaloids from plant material when SDS used as surfactant. SDS forms foam, interferon of phenols and chlorophyll which makes the process tedious. The color and texture of sample was dark green, sticky and thick. It required time to remove stickiness property of sample and interference of color. It was not possible to separate precipitated alkaloids in our laboratory condition so for further study was carried out by using EDTA solution. The characterization of the obtained crude dye was done by IR, UV, TLC, and NMR.
flow chart diagram of extraction of alkaloids from leaves of \textit{Solanum Xanthocarpum} by Modern method is shown in flow chart no.3

\textbf{UV/VIS Spectra:} It as recorded on uv spectrophotometer 2100 of shemadzu corp. IR and NMR of sample was carried out in IIT Pawai, Mumbai which is shown in following figure respectively. TLC of crude alkaloid sample was carried out which is shown in figure 4.7.

Figure 4.10 The crude alkaloids of \textit{Solanum Xanthocarpum} leaves by modern method.
Figure 4.11 IR of crude Alkaloids of *Solanum Xanthocarpum* leaves

by Modern Method

IR values are at 3004 cm$^{-1}$ which indicates aromatic ring and OH starching. 760 cm$^{-1}$ indicates the aromatic ring.
Figure 4.12 IR of crude Alkaloids cake of *Solanum Xanthocarpum* leaves

by Modern Method

From above IR -1526.2 cm value indicates that there is removal of NH3+ group. At -2372.6 indicates the removal of −NH stretching.
IR values of leaves powder of *Solanum Xanthocarpum* indicates the presence of −OH stretching and aromatic -NH streching. At 1615 cm$^{-1}$, NH bending indicates.
Figure 4.14  TLC of crude alkaloids of *Solanum Xanthocarpum* leaves by modern method.
This confirmation of C-O stretching vibration but slightly a coupled asymmetric vibration involved C-O-C stretching. The bond angle changes due to this. Thus ring C-H out of plane bending of aromatic molecule which couples between H and C-C bond in the ring in which hydrogen atom are attached. This interaction of coupling of stretching and bending vibration shows absorption of secondary acyclic amides. The absorption band was observed in the $1525.8 \text{ cm}^{-1}$ The crude alkaloids isolated from *Solanum Xanthocarpum* leaves. This absorption involves coupling of the N-H bending and C-H bending. NMR spectra show presences of –CH2,-CH3 groups.
Figure 4.15  NMR of crude Alkaloids of *Solanum Xanthocarpum* leaves by Modern method
Figure 4.16. NMR crude Alkaloids of *Solanum Xanthocarpum* leaves by Modern Method
Comparison of Modern Method with Solvent and Soxhlet Method:

The comparison between solvent extractions, Soxhlet extraction with modern method shown in Table 3.2 In all above methods optimum condition was constant. According to Table No. 3.2 it was observed that the new method produce good result than other two methods .It was again indicated in figure 3.12. In modern method Solanum Xanthocarpum leaves contain 1.5g of alkaloid. It takes less time, to isolate alkaloids. It gives more yields as compared to other. It is cheaper and conventional method. Spectral data -IR, UV, NMR, Physical constant of all methods are varied which conclude that different alkaloids were present in Solanum Xanthocarpum leaves IR of Soxhlet, solvent and modern method shows characteristics peaks at different wave number indicates that different functional groups present in the crude alkaloids. The functional group –NH, C-O for Soxhlet method, Aromatic and hydroxy group cyclic ether in modern method. It confirms that they all are different.

The Comparison of the obtained data from IR of crude alkaloids from leaves and remining cake was done to observe the difference. The conformation was also done from the modern or novel method which was is very suitable for the extraction of all groups. It was observed that IR peaks are common in crude and absent in cake.
<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Solvent Method</th>
<th>Soxhlet Method</th>
<th>Modern Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield of Crude Alkaloid</td>
<td>0.78 g</td>
<td>0.85 g</td>
<td>1.50 g</td>
</tr>
<tr>
<td>M.P.</td>
<td>202°C</td>
<td>223°C</td>
<td>265°C</td>
</tr>
<tr>
<td>U.V.</td>
<td>λ235 nm, λ215 nm</td>
<td>λ265 nm, λ254 nm</td>
<td>λ256 nm, λ223 nm</td>
</tr>
</tbody>
</table>

Table 4.5 Comparison between solvent extractions, Soxhlet extraction with modern method.

The comparison between solvent extractions, and Soxhlet extraction with modern method was done. The following results were obtained.

**Yield of crude alkaloids**: It was observed that the yield of crude alkaloids in solvent method was found 0.78 g, in soxhlet method yield was found 0.85 g, and in modern method the yield was found was 1.50 g. As compare to solvent and soxhlet methods, the yield in modern method was higher.

**Melting point of crude alkaloids**: In solvent method, the melting point of crude alkaloids was obtained at 202°C. In soxhlet method melting point was 223°C and in modern method melting point of crude alkaloid was 265°C.

**UV of crude alkaloids**: In Solvent method λ max 235 nm, and λ min 215 nm. In soxhlet method λ 265 nm, and λ 254 nm. In modern method λ 256 nm and, λ 223 nm
Figure 4.17 Comparison between Percentages of crude alkaloid precipitated (gm) in solvent extractions, Soxhlet extraction and modern method.
<table>
<thead>
<tr>
<th>Plant powder</th>
<th>Modern crude alkaloids</th>
<th>Modern cake</th>
</tr>
</thead>
<tbody>
<tr>
<td>708 (-NH stretching )</td>
<td>600-800 (-NH stretching )</td>
<td>-2372(-NH stretching removed removed )</td>
</tr>
<tr>
<td>1064</td>
<td>1039(C-O-C) symmetrical stretching</td>
<td>-1526(NH₃ removed )</td>
</tr>
<tr>
<td>1344(–OH stretching)</td>
<td>1290.9(C-O stretching)</td>
<td>3000CH₂,CH₃ group</td>
</tr>
<tr>
<td>1521.4</td>
<td>1525.8</td>
<td>1600-1800(NH stretching)</td>
</tr>
<tr>
<td>1550</td>
<td>1600 Benzene ring</td>
<td>3500-CH₃,NH stretching</td>
</tr>
<tr>
<td>1640</td>
<td>2362.4(sup- –OH stretching)</td>
<td>3440</td>
</tr>
<tr>
<td>2402</td>
<td>3004 arromatic</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.6 Comparison of IR of Plant powder of *Solanum Xanthocarpum* leaves crude alkaloids, and cake.

**Purification of Alkaloids: Structure elucidation of alkaloids by TLC, UV, IR and MP.**

**Result:** The Rf values of fraction three, five seven and nine are shown in table no.4.1. IR of fraction three matches with the structure of Solamargine. It shows characteristic peaks at $3400-3200$ cm$^{-1}$ (NH stretching), $1640$ cm$^{-1}$, $1610$ cm$^{-1}$, $1450$ cm$^{-1}$, $1410$ cm$^{-1}$, $1378$ cm$^{-1}$ occurs NH, -OH and conjugated C=O. The crystalline form of Solamargine and structure of Solamargine are shown in figure no 4.3 and 4.4.
Figure 4.18 TLC of fraction Three (Solamargine)

1: Solution of Fraction Three

2: Standard Solution of Solamargine
Figure no.4.19 IR of fraction three (Solamargine)
Figure 4.20 Fraction three contains needle shaped crystalline form of Solamargine

Figure no.4.21 Structure of Solamargine
Figure 4.22 Abundance and structure of fraction no.3 (Solamargine)
Solamrgine:

A glycoalkaloid like solamargine can be extracted from the solanaceae family. It has been extracted from the berries of Solanum khasianum. Now it is extracted from leaves of Solanum xanthoxcarpum. Including solamargine alkaloids and some other may be functional against infection related to HIV (McMohan et al;1995 and Sethi, 1979) and the intestinal infections related to AIDS (McDevitt et al;1996). Microbial effects found in alkaloids against Entamoeba and Giardia species (Ghoshal et al; 1996).

Fraction Five:

TLC of fraction Five: The Rf value of fraction five was 0.78. That is exactly matches with standard Solasodine, is shown in Figure.4.23

I.R. (KBr) of fraction five: IR of fractions five shows characteristics peaks at wave number in cm\(^{-1}\). They were 666.9 cm\(^{-1}\) region spectra indicates that -N-H wagging out of plane.1522.4 cm\(^{-1}\)and1254.7 cm\(^{-1}\) shows simple open secondary amide 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\(^{-1}\)and1660 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 3584cm\(^{-1}\)(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. The detailed of UV and Physical Constant are shown in table 4.1.

The solid form of Solasodine was looking very attractive. The crystals were formed in the test tube. From the above mentioned data the structure of Solasodine are determined and compare with the standard alkaloid. It is shown in figure no 4.25 and 4.26 respectively. In the thin layer chromatography for this fraction silica coated TLC was used. The Rf values of fraction five and standard soalsodine was compared. and it was found exactly same. Therefore it indicateds that solasodine was present in the fraction number five.
Figure 4.23 TLC OF FRACTION FIVE

1: Solution of Fraction five

2: Standard Solution of Solasodine
Figure 4.24 IR of fraction Five (Solasodine)

Figure 4.25 Fraction five contains solid form of Solasodine from *Solanum xanthocarpum* leaves.
Physical properties of SOLASODINE:

The molecular formula of solasodine is: C_{27}H_{29}NO_{2}

UV $\lambda_{\text{max}}$ (MeOH) were found at 207 nm and shoulders are at 208 and 240.

IR (KBr) cm$^{-1}$ values are at 1580 cm$^{-1}$, 1610 cm$^{-1}$, 1630 cm$^{-1}$, 1660 cm$^{-1}$ and 3360(N-H)

Stretching Mass m/e was showed at 409 and 2981

Melting point was found in between the range of 200-202°C and needle shaped crystals were formed by the process of hydrolysis. It is slightly soluble in water and not soluble in ether. The solubility was observed in, acetone, chloroform, benzene and methanol.

Figure 4.26 Structure of Solasodine
UV IR and TLC were done for the fractions which were derived from the silica column.

Fraction seven:

TLC:
The thin layer chromatography of fraction seven shows 0.40 Rf value. For the visualization of colour dragendorff reagent was used. The Rf value was exactly matched with standard alkaloid Solanine.

**IR of fraction seven (Solanine):** $700 \text{ cm}^{-1}$, $3500-3200 \text{ cm}^{-1}$ (-NH stretching)

**UV of fraction seven:**
UV spectra of fraction seven were $\lambda_{\text{max}}$ 235nm and $\lambda_{\text{min}}$ 205nm.
Structure of Solanine and the crystalline form of Solanine is shown in figure no.4.10 and 4.14 respectively.

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

![Figure no.4.27  Structure of Solanine](image)

**Solanine:**
Solanine is a toxic glycoalkaloid. the taste of solanine is bitter. The molecular formula of solanine is $\text{C}_{45}\text{H}_{73}\text{NO}_{15}$. The formation of these alkaloids by solanidine through a
carbohydrate side. In a variety of Solanaceae it is found in fruits and leaves of potatoes and tomatoes. By the consumption of solanine humans causes neurological disorders and gastrointestinal disorders like abdominal pain, diarrhea and vomiting. The glycoalkaloids amount is varies in potatoes because of the variety different cultivation methods and environmental conditions availability. In potatoes the 0.075 mg/g glycoalkaloid concentration is found (Fukuda Tet al; 2001) It has been reported that sometimes Solanine has been responsible for food poisonings by eating the berries of green potatoes, Solanum dulcamara and Solanum nigrum (Rocha P et al; 2002, Matsuda J et al; 1991)

**Fraction Nine:**
The molecular formula of solanidine is $\text{C}_{27}\text{H}_{43}\text{NO}$.  
**TLC:** In thin layer chromarography spot of fraction nine was used. The brown spot was visualized with iodine fumes. The Rf value was measured and found 0.98. This Rf value was exactly matched with standard alkaloid solanidine. 
**IR:** In IR determination it was observed that $1760 \text{ cm}^{-1}(\text{C}=\text{O} \text{ from COOH})$ and $1245 \text{ cm}^{-1}(\text{C-O from COOR}) 985 \text{ cm}^{-1} (\text{C-O}), 860 \text{ cm}^{-1}$ (lactum).
**UV of fraction nine:** The UV spectra of fraction nine was reported as $\lambda_{\text{max}}$ was found at 225nm and $\lambda_{\text{min}}$ was found at 218.3nm. 
After the vaporization of solvent from the test tube the needle shaped crystals of solasodine was formed.
Figure 4.28 Fraction seven contains needle shaped crystalline form of Solanidine.

Figure no. 4.29 Structure of Solanidine
Figure no. 4.30 Solid form of alkaloids

a. Fraction one contains chlorophyll/phenols
b. Fraction seven contains Solanine
c. Crude Solanine.

Discussion:
Crude alkaloid from Soxhlet method was extracted with CHCl₃ for 18 hrs. The concentrated chloroform extract was subjected to column chromatography over silica gel by eluting with solvents chloroform: Ethyl acetate: Butanol in the ratio (5:2:1) to afford thirteen fractions. Fractions were monitored by TLC using iodine vapours and ninhydrine reagent to visualize the spots. Fraction three contain 0.22 mg Solamargine alkaloids, the rf value was 0.54 with brown spot visualized by Dragendorff reagent. The UV of Solamargine was 205 nm and the melting point was 293-5° C. Fraction five visualized with off-white spot in color. Its rf value 0.78 by Dragendorff reagent. It was crystalline in nature. It is insoluble in water and soluble in hot alcohol. When TLC of it visualized in the ninhydrin reagent its rf value was 0.81 with pink color spot. UV and m.p. of fraction five were 240 nm and 202 °C. IR study of fraction five indicates
Solasodine was present which a match with standard Solasodine alkaloid was. Solid crystals of solasodine (0.26mg) was formed.

On the basis of the these information the conclusion was drawn that solasodine is hexacyclic, contains β hydroxyl group at C-3 ;a double bond between five and six carbon atom. A basic nitrogen and oxygen atom in ether linkage, forming spiroaminoketel system. From above observations it may confirms that the fraction five contains solasodine alkaloid.

TLC of fraction seven shows dark brown spots with Rf value 0.40 which is matches with standard solanine. UV spectra of fraction seven were $\lambda_{\text{max}}$ 218.3 nm. By treating with HCl it undergoes hydrolysis to solanidine which was confirmed by physical constant. Melting point of fraction seven was 290°C which exactly matches with the solanine. The melting point of fraction nine was 197.5°C and TLC shows brown spot in iodine fumes. The Rf value 0.98 which matches with standard solanidine. UV spectra of fraction nine was $\lambda_{\text{max}}$ 225nm and $\lambda_{\text{min}}$ 218.3nm. which was confirmed the expected structure.

All above data in the present study indicates that *Solanum Xanthocarpum* leaves contains four alkaloids solamargine, solasodine, solanine and solanidine etc. On the basis of the these information the conclusion was drawn that solasodine is hexacyclic, contains β hydroxyl group at C-3 ;a double bond between five and six carbon atom. A basic nitrogen and oxygen atom in ether linkage, forming spiroaminoketel system.

From above observations it may confirms that the fraction five contains solasodine alkaloid. TLC of Fraction seven shows dark brown spots with Rf value 0.40 which is matches with standard solanine. UV spectra of fraction seven were $\lambda_{\text{max}}$ 218.3 nm. When solanine treated with HCl it hydrolysis to solanidine which was confirmed by physical constant and literature review. Melting point of fraction seven was 290°C which exactly matches with the solanine. The melting point of fraction nine was 197.5°C and TLC shows brown spot in iodine fumes. The Rf value 0.98 which matches with standard solanidine. UV spectra of fraction nine was $\lambda_{\text{max}}$ 225nm and $\lambda_{\text{min}}$ 218.3nm. which was confirmed the expected structure.
All above data indicates that *Solanum Xanthocarpum* leaves contains four alkaloids solamargine, solasodine, solanine, and solanidine etc.

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

Green extraction methods were used for isolation of dyes/colourants from leaves.

In first extraction method light red colour was obtained from the leaves of *Solanum xanthocarpum*. In second method light red colour was obtained. Third method of extraction was carried out in dark and light. In sunlight shade of colour was light red and in dark, wine red colour was appeared. The second method of dye extraction uncrushed leaves were kept for 10 days to get colour of dye, gave best colouration and was found to be the best extraction method. Wool and pure cotton cloth samples showed best colouration (dark yellowish-brown and brown). The intensity of color produced on cloth and wool by dyeing without mordanting was found slightly less than that obtained for and dye used successively. Effect of dark and sunlight on formation of colour and effect of temperature on color of paper are shown in table No.4.7 and 4.8 respectively. Time factor was very important for the colour appearance. It was observed that at 20 min. no colour was observed in solution in sunlight as well as in dark. In 40 min the changes were observed in the solution. The colour was changes from colourless to light red in colour. In 60 min. light brown in dark and dark yellow in sunlight. In 80 min. the colour was observed light brown in dark and light yellow in sunlight. After 100 min the colour was appeared light brown in dark and light yellow in light. After 120 min dark brown in dark and light yellow in sunlight.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Colour in dark</th>
<th>Colour in sunlight</th>
</tr>
</thead>
<tbody>
<tr>
<td>20’</td>
<td>No colour</td>
<td>No colour</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Time (min)</td>
<td>Application of dye on paper</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>No change in colour</td>
</tr>
<tr>
<td>30</td>
<td>20</td>
<td>No change in colour</td>
</tr>
<tr>
<td>40</td>
<td>30</td>
<td>Fading in colour</td>
</tr>
</tbody>
</table>

Table 4.8 Effect of time on colour of wool and cotton
The time factor and temperature affecting on the shades of colour. It at 20 °C, after 10 minutes no change in colour observed at 30 °C, no change in colour and at 40 °C fading in colour was observed.

**Figure 4.31** A. Light red colour obtained in first extraction method. B. Effect on colour in earthen pot in presence of dark and light.

<table>
<thead>
<tr>
<th>Time</th>
<th>In dark</th>
<th>In sunlight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed A and B for 60’ min</td>
<td><img src="image1" alt="A" /></td>
<td><img src="image2" alt="B" /></td>
</tr>
</tbody>
</table>
Exposed
A and B for 120’ min

**Figure 4.32** Effect of sunlight and dark on formation of colours on Leaves of *Solanumxanthocarpum*.
<table>
<thead>
<tr>
<th>Dyeing with wool without mordant</th>
<th><img src="image1.png" alt="Image" /> <img src="image2.png" alt="Image" /> <img src="image3.png" alt="Image" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyeing with cotton without mordant</td>
<td><img src="image4.png" alt="Image" /> <img src="image5.png" alt="Image" /> <img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 4.33** Dyeing with dye in sunlight and dark on wool and cotton.

**Figure 4.34** Effect of sunlight and dark on formation of colours on Leaves of *solanum xanthocarpum*
The obtained colours from the above solution were shown separately in Figure.4.35

Figure. 4.35
Effect of sunlight and dark on formation of colours on Leaves of Solanum xanthocarpum

Now a day the demand of natural dyes is increases not only in textile industry but in leather, food, pharmaceuticals and cosmetics. The rich biodiversity of our country has provided us plenty of raw materials, yet sustainable linkage must be developed between cultivation, collection and their use. The present work shows that leave Solanum xanthocarpum can be used as dye quite efficiently and commercially. Different shades of color using different mordents and the color fastness, wash fastness properties also can be improved by different treatment procedures and so it is employed throughout India for dyeing silk and cotton fabrics on a commercial scale. Natural dye has no side effect on skin and it has safe for environment also. The green extraction process is economical, as the raw materials are available as it is waste land weed. The cost of production is also very low.
In the preparation of azo dye by using 4 gm of leaves powder 4.6 gm azo dye was obtained.

In theory the collection of various azo dyes can make beautiful rainbow of colors and in practice the colours like brown, blue, yellows, oranges and red colours are obtained. The differences in colours are occurs due to different substituents on the aromatic rings It leads the differences in the extent of π conjugation system in azo dye. Generally the fewer extensive the conjugated π system of a molecule in which the shorter wavelength of visible light will absorb.

Blue colour shows longest π system where the green, red, orange and colourless dye shows π system. The eyes recognize color because some wavelengths is absorbed by dyes and reflect others. Some compound absorbs in the green visible region the combination of the remaining wavelengths which are reflected makes the compound appear red or purple.

An azo dye synthesis of requires two organic compounds like coupling component and a diazonium salt.

It was blakish in colour. By using same method azo dye was also prepared from 4 gm of fruit powder black coloured 5.8 gm azo dye was obtained. The product by using fruit powder is more than leaves powder. It was observed that azo dye prepared from fruit and leaves of solanum xanthocarpum were appeared yellow in water. In different solution like acetone, acetic acid, ethyl alcohol etc. azo dye shows red, brown and light brown colour shades.

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>sample</th>
<th>Weight of dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 gm leaves powder</td>
<td>4.6</td>
</tr>
<tr>
<td>2</td>
<td>4 gm fruit powder</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Table 4.9 Weight of azo dye from solanum xanthocarpum laves and fruit
Figure 4.36 Azo Dyes /colourants obtained from leaves of solanum xanthocarpum:
A.: wet dye B: dried dye

Figure 4.37 Azo Dyes /colourants obtained from fruits of solanum xanthocarpum:
A.: wet dye B: dried dye
It was observed that Azo dye was prepared by using leaves of Solanum xanthocarpum was sticky in nature, and from fruit, dye was in powder form shown in above figure.

**Color occurrence in different solvents:**

It was observed the obtained dye from leaves of Solanum xanthocarpum shows different shades of colours in different solution.

**Solubility of crude dye in acetone:**

The crude dye of leaves is dissolve in acetone and shows yellow color. It was observed that yellow color was turned light green.

**Result:**

1. Leaves crude dye/colourant in acetone showed dark brown colour.
2. Leaves crude dye/colourant in acetic acid showed dark red colour.
3. Leaves crude dye/colourant in ethyl alcohol pink colour

**TLC:**

TLC of azo dye obtained from *Solanum xanthocarpum*: 1:1 ratio of 50% ethyl acetate:hexane was used for separation of dye. The rf values 0.08, 0.38, 0.86, 0.92 were obtained. The different colours like green, orange, brown and purple colour were observed.

**Melting point:** The melting point of solanum xanthocarpum leaves and fruit were found uncorrected.

Melting point was checked on 280°C to 300°C. (Uncorrected)

**Use of mordent:** The brown colour was appeared in acetic acid with the use of SnCl₂, it was appeared gray colour. It is shown in Fig. 4.39
Figure 4.38 A: Red colour obtained in Ethyl alcohol from leaves dye

B: Dark brown colour obtained in acetic acid from leaves dye

C: Yellow colour obtained in water from leaves dye

In the above picture the crude dye was in ethyl alcohol shows red colour and dark brown colour was appeared in acetic acid freenish yellow colour was found in water.

The azo dye is used for the fabric piece in the process of direct dyeing. The direct dyeing is attained with treating fabrics and diazo component a coupling component to formulate insoluble azo dye. The last color can be forbidden by the selection of the coupling components and diazo components. In the direct process of dyeing the acidic and basic groups on the dye interacts with fabrics materials and forms salts. This makes the fabrics materials ionized surface on fibres. As compare to cotton, the absorption of dyes is better in silk and wool.

Since the ancient time dyes play an important role in the history of human. Processes of dyeing are repeatedly considered as an important attribute of a meticulous culture and civilization. As the dyes are used as commercial product in the various forms like pigments, food, clothing and paints etc. Many classes of dyes are available in which azo dyes is very important class of dyes.
More than half of the dyes used in industries are azo dyes. The basic structure of azo dyes are like \( \text{Ar} - \text{N} = \text{N} - \text{Ar} \). In this structure \( \text{Ar} \) and \( \text{Ar} \) are represented as two aromatic groups.

When nitrogen-nitrogen double bond is present in a unit is called as azo dye group. On both sides of the azo group the nature of the aromatic substituent is controls the azo compounds colours and solubility of dye in water and shows finely they get binds with particular fabric.

Those compounds contains one or more azo groups in which \(-\text{N}=\text{N}-\) linkage is present in between two carbon atoms shows a various types of uses. Azobisisobutyronitrile is aliphatic azo compounds. In polymerization of alkenes to the plastics it is act as radical initiators. Aromatic azo compounds are used as acid-base indicators in food beverages, biological stains, and marketable colorants for, cosmetics clothing and plastics.

Several azo-dyes like, methyl orange, methyl red and congo red, are use as acid base indicators. It is use as indicators because of their ability to acts as weak bases and acids.

The changes in colours are occurs due to changes in degree of electrons delocalization. Due to the more delocalization the shifts the absorption max to longer wavelengths. It makes the light absorbed redder and less delocalization shifts the absorption max to shorter wavelengths.

Due to the geometrical isomerism of the azo group the changes in colour are occurred. A trans azo group to become cis. Due to UV radiation It leads to photochromism. It is light-induced reversible color change.

Some azo dyes can regress slowly to the trans isomer in the dark are used in car sunroofs and sunglasses. Some azo dyes such as sudan red and scarlet red, are used as biological dyes. As they are fat soluble and absorbed into microscope slides of fat cell tissues. Form 70 % of synthetic dyes, azo dyes are commercial colorants and used in various field.. These dyes having various advantages as compare to some other
commercial dyes which includes wide range of colours, color fastness and ability for the absorption of light.

The synthesis is very cheap because the starting material is easily available for the manufacturing of dyes. Low-priced compounds and low room temperature is required the impact of the environment is because in all reaction, water used as solvent.

The obtained dye from fruit in acetic acid shows dark brown colour on wool with the use of mordent gray colour was appered.
Figure 4.40  A. Azo dye from fruit in acetic acid shows dark brown colour on wool

B. Azo dye in acetic acid shows gray colour with mordant SnCl₂ on cotton fabric

C. Azo dye in ethyl alcohol shows pink colour on wool.

In above images, it was found that azo dye from fruit of solanum xanthocarpum shows dark brown colour in acetic acid and appeared gray in colour with mordant SnCl₂ on cotton fabric. Azo dye prepared from fruit shows pink colour on wool when mixed in ethyl alcohol.
Figure 4.41 Azo dye in different solution and applied on wool
Figure 4.42 The leaves crude dye shows different colour shades in solution.

Figure 4.43 The leaves crude dye show no colour change on oxidation.
Figure 4.44 The dye fruit shows shades of colours different solution
Figure 4.45 The dye fruit shows dark brown, dark brown and red colour shade in different solution on oxidation

**Fruit dye:**

From the above test it was observed that the fruit dye in ethyl alcohol, acetone and acetic acid gives golden yellow, golden colour and red colour respectively. After one hour, on oxidation the golden colour changes to dark brown, golden colour changes to red colour and red remains as it is. No effect of oxidation was observed.

**Leaves dye:** Dark brown colour was observed in ethyl alcohol, dark brown in acetone and dark red colour was observed in acetic acid. On oxidation no change in colour was observed. It was shown in table 4.11

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solution</th>
<th>Colour appered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td>Ethyl alcohol</td>
<td>Golden yellow</td>
</tr>
<tr>
<td>Fruit</td>
<td>Acetone</td>
<td>Golden yellow</td>
</tr>
<tr>
<td>Fruit</td>
<td>Acetic acid</td>
<td>Red</td>
</tr>
</tbody>
</table>

Table 4.10. Colour apperance of fruit dye in ethyl alcohol, acetone and acetic acid.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solution</th>
<th>Colour appered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit</td>
<td>Ethyl alcohol</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Fruit</td>
<td>Acetone</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Fruit</td>
<td>Acetic acid</td>
<td>Red</td>
</tr>
</tbody>
</table>
Table 4.11. Colour appearance of fruit dye in ethyl alcohol, acetone and acetic acid on oxidation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solution</th>
<th>Colour appeared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Ethyl alcohol</td>
<td>Dark brown</td>
</tr>
<tr>
<td>leaves</td>
<td>Aetone</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Leaves</td>
<td>Acetic acid</td>
<td>dark Red</td>
</tr>
</tbody>
</table>

Table 4.12. Colour appearance of leaves dye in ethyl alcohol, acetone and acetic acid on oxidation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solution</th>
<th>Colour appeared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>Ethyl alcohol</td>
<td>Dark brown</td>
</tr>
<tr>
<td>leaves</td>
<td>Aetone</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Leaves</td>
<td>Acetic acid</td>
<td>dark Red</td>
</tr>
</tbody>
</table>

Table 4.13 colour change on oxidation
Azo dye obtained from fruit applied on wool and paper which show different colours like dark brown and yellow colours in acetic acid. With addition of mordant SnCl2 gray colour was appeared on cotton and paper.
Figure 4.46 Fruit Crude dye applied on cotton and paper.

It was observed that azo dye from leaves in acetone form the crystals. It is shown in Figure 4.47.

Figure 4.47 Crystal formation in acetone with leaves azo dye.
The Thin layer chromatographic studies:

Chromatography is the technique of separation of components from the mixture. It distributes the mobile phase and stationary phase from each other. It is an analytical method which removes the complication of the mixture and determines the purity of individual components. Thin layer chromatographic process is depends upon chromatography of adsorption. In this process separation depends on the selective adsorption of the components from the mixture on the surface of solid. The stationary phases used were very thin in form and hold on to a suitable form of supporting material over which the mobile phase was permitted to ascend by capillary action. Conventionally analytical thin layer chromatography was found applicable in the detection and examination of compound through a separation method. Silica gel coated glass plates are used for thin layer chromatography. Aluminium foil and polyester sheets can be cut in desired shape and used for TLC.

Thin layer chromatography can be carry out by applying the spot of solute on the TLC plate. TLC plates were placed in the tank of suitable solvents combination for proper development through capillary action. The coloured spots developed on TLC. This chromatogram provide information about the number of component present in mixture. The TLC plate were observed in UV light (254nm). In UV light different colours such as green, orange and purple were appeared.

The Rf value can be calculated by using following formula:

\[
Rf = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}}
\]

The Rf value from the TLC depends on the following factors:

1. the particle size of different set of adsorbent.
2. The degree of saturation of the solvent present in tank.

3. Thickness of the layer on TLC.

4. Storage condition and activation of TLC plates

Figure 4.48 A. TLC of elutant of leaves dye, Spot were visualized showed green orange yellow and purple colour band of ethyl acetate fraction and acetone fraction.

B. TLC of elutant of fruit dye, Spot were visualized showed orange yellow and purple colour band of ethyl acetate fraction and acetone fraction.

Thin layer chromatography study of azo dyes extract was performed by using various solution. TLC determination was performed on silica gel coated aluminium plates. 1mg
of crude leaves azo dye was dissolved in ethyl acetate and acetone. These solutions were used for the further isolation. 1 mg of crude dye was dissolved in ethyl acetate and prepared solution of the sample was sucked. For this a capillary tube was used. The solution was spotted on the TLC plate nearly 1 cm above of the TLC base. The spots were equally sized as far as possible and had a diameter ranging from 2-5 mm. The development chamber or tank was lined inside previously with filter paper moistened with the mobile phase so as to saturate the atmosphere. If this kind of saturation of the atmosphere is not done, edge effect or tailing effect occurs where the solvent front in the middle of TLC plate moves faster than that of the edge. The solvent front was allowed to rise to a distance about $\frac{3}{4}$ from the base line and the plate was removed from the tank and allowed to dry in the air. Chapter 5 Extraction and Bioassay guided isolation 49 After the development of TLC plates the spots were detected by using spray reagent and UV chamber. By several trial and error methods a suitable solvent system was selected for column chromatography (Stahl, 1869; Mukherjee, 2002; Wagner 1996).
Figure 4.48 c.. TLC of elutant of leaves dye, Spot were visualized showed green orange yellow and purple colourd band of ethyl acetate fraction and acetone fraction.
Figure 4.49  Azo dye with mordant FeSO₄ and CuSO₄ shows greenish colour.

It was observed that crude dye from solanum xanthocarpum leaves with mordant FeSO₄ and CuSO₄ applied on wool. Greenish colour was observed on wool.
Figure 4.50.  
A. azo dye with mordant Sncl$_2$ shows gray colour on cotton
B. azo dye with mordant Sncl$_2$ shows gray colour on paper
Figure 4.51  

A. Azo dye from fruit in acetic acid shows dark brown colour on wool

B. Azo dye in acetic acid shows gray colour with mordant SnCl2 on cotton fabric

C. Azo dye in ethyl alcohol shows pink colour on wool.
Figure 4.52: A: dye obtained from fruit of *Solanum xanthocarpum* shows dark brick colour in ethyl alcohol

Fig: B: dye obtained from fruit of *Solanum xanthocarpum* shows faint brick colour in ethyl alcohol
Figure 4.53: A: dye obtained from leaves of Solanum xanthocarpum shows dark brown colour in acetone

Fig: B: dye obtained from fruit of Solanum xanthocarpum shows light pink colour in acetone
Figure 4.54: Dye obtained from fruit of Solanum xanthocarpum shows light pink color in acetone.

Figure 4.55:
A: Azo fruit dye in acetic acid
B: Azo fruit dye in Ethyl alcohol
C: Azo leaves dye in ethyl alcohol
D: Azo dye in acetic acid
Figure 4.56 A. Azo dye from leaves applied on paper in acetic acid and in acetone

B. Dye applied of paper with SnCl₂ mordant
TO ASSESS MOLECULAR STRUCTURE OF COLOURANTS /DYE BY USING SPECTROSCOPIC METHODS-

The above experimental data of solanum xanthocarpum have been analysed for its depth knowledge to give an idea about its nature,. It was evaluated by, IR. NMR, TLC, UV .This test has percolated the knowledge for molecular structure determination by using techniques such as NMR, FTIR ,XRD. Details of data is discussed in result and discussion section part.

Natural dyes shows dyeing as well as medicinal properties with large range .Due to the increasing awreness about the ecofriedly dyes from the plant ,the demand of natural dyes is increasing .Natural dyes are safe and nontoxic in nature.In daily life natural product are becomes very essential .Various product are obtained from the plants which are utilized for making drugs dyes and some other hearth care procutks in pharmaceutical companies.Proper and accurate methods are required for the documentation ,formulation of dyes and commercial use of medicinal plants .Now a day need of natural dyes is required in every field like textile ,paper and pharmaceutical .Formulation of dyes is beneficial for safe use .Scientific investigation and characterization are also needed for the formulation and development of dyes and drugs .

Spectral determination :Uv and visible spectra of obtained dyes are recored on uv-2100spectrophotometer.The quartz cuvette was 10mm .UV/vis spectophometer was from shemadzu company.IR and NMR of samples were carried out in Diya lab Thane
Mumbai which is shown in respective figures. TLC of crude alkaloid sample was carried out which is shown in figure.

**Result:** Yield of dye obtained from leaves of *Solanum xanthocarpum* was found 5.8 g. the weight of crude azo dye was found more than taken leaves powder.

1) The UV was carried out in double beam spectrophotometer. It was found $\lambda$ max 345 cm$^{-1}$

2) Melting points of colourants/dye: Melting point of crude dyes obtained from leaves and fruits of *Solanum xanthocarpum* were recorded in glass capillary tubes and are uncorrected.

3) TLC: The purity of sample was checked on silica TLC plates. 1:1 ratio of 50% ethyl acetate : hexane was used for separation of dye. On TLC of silica gel different colours were appeared such as green orange, brown and purple colours.

**TLC:** The Rf values were a)0.09, b)0.37, c)0.84 and d)0.91 respectively.

**IR of sample E of leaves of Solanum xanthocarpum:**

N=N stretching 1509 cm$^{-1}$. -CH, -CH$_2$, -CH$_3$ Aliphatic groups at 2917 cm$^{-1}$, 876.183 cm$^{-1}$, 904.577 cm$^{-1}$, 958 cm$^{-1}$, 843 cm$^{-1}$ $\equiv$ C–H, –NH alkenes and aromatic compounds or Aliphatic amines may be present. aromatic ring at 741 cm$^{-1}$, 469 cm$^{-1}$, $-\text{OH} – \text{NH}$ groups may present at 3200 cm$^{-1}$ -CH, -CH$_2$, -CH$_3$ aliphatic group at 2800 cm$^{-1}$ amine N-H stretch may present at 3300 cm$^{-1}$

**UV/VIS Spectra:** The UV of obtained dye was recorded on UV-2100 spectrophotometer in 10 mm quartz cuvettes (SHEMADZU.). IR and NMR of sample was carried out in Diya lab Thane Mumbai which is shown in figure respectively. TLC of crude alkaloid sample was carried out which is shown in figure.

**Result:**

1) Yield of dye obtained from fruit of *Solanum xanthocarpum* was found 4.6 g from 5 gm of fruit powder.

2) UV of azo dye from fruit was observed $\lambda$ max 348 cm$^{-1}$

3) Melting points of colourants/dye: Melting point of crude dyes obtained from fruits of *Solanum xanthocarpum* was recorded in glass capillary tubes and are uncorrected.
**TLC:** The purity of sample was checked on silica TLC plates. 1:1 ratio of 50% ethyl acetate: hexane was used for separation of dye. The rf values 0.08, 0.38, 0.86, 0.92 were obtained. Green, orange, brown and purple colour were observed.

**IR of sample F of fruit of solanum xanthocarpum:**

- OH, -NH at 3286 cm\(^{-1}\). -CH, -CH\(_2\)-, -CH\(_3\) Aliphatic groups at 2853 cm\(^{-1}\), 2923 cm\(^{-1}\), 876.183 cm\(^{-1}\), 904.577 cm\(^{-1}\), 958 cm\(^{-1}\), 843 cm\(^{-1}\) NH- alkenes and aromatic compounds or Aliphatic amines may be present. Aromatic ring at 741 cm\(^{-1}\), 479 cm\(^{-1}\), -OH –NH groups may present at 3200 cm\(^{-1}\) -CH, -CH\(_2\)-, -CH\(_3\), Aliphatic groups may present at 2800 cm\(^{-1}\). -OH -NH C-H at 3286 cm\(^{-1}\) C=C, C=N, NH at 1600 cm\(^{-1}\) and 1628 cm\(^{-1}\),
Figure 4.58 Infra-Red spectra recorded of FTIR of sample of Fruit dye from solanum xanthocarpum fruit.
FTIR of sample of E(leaves)

**Figure 4.59.** Infra-Red spectra recorded of sample of leaves dye from solanum xanthocarpum leaves.
Scanning

drive axis        = Theta-2Theta
scan range        = 10.000 - 80.000 scan mode         = Continuous Scan
scan speed        = 6.0000 (deg/min)

sampling pitch    = 0.0200 (deg)
preset time       = 0.20 (sec)

<table>
<thead>
<tr>
<th>No.</th>
<th>&lt;2Theta&gt;</th>
<th>&lt; d &gt;</th>
<th>&lt; l &gt;</th>
<th>&lt;I/Io&gt;</th>
<th>&lt;FWHM&gt;</th>
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Table 4.14  a.XRD data of leaves dye
### Table 4.14  b.XRD data of fruit dye

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Figure 4.60 XRD of leaves dye

Figure 4.61 a. XRD of leaves dye
Figure 4.61 b. XRD of fruit dye
Figure 4.62 NMR of Crude dye of leaves
Figure 4.63a. NMR of fruit azo dye
Figure 4.63 b. NMR of leaves azo dye
Structure Elucidation of Azo dye:

From leaves of solanum xanthocarpum the natural colourant was extracted. The azo dye was prepared by using leaves powder and fruit powder. The azo dye (yield: 5.8 g.; was obtained as blackish solid and gave four spots on TLC. 1:1 ratio of 50% ethyl acetate : hexane was used as mobile phase for separation of dye. The rf values 0.08, 0.38, 0.86, 0.92 were obtained. Green, orange, brown and purple spots were observed. Dye gave UV absorption at λ max 345 nm. The FT-IR spectrum gave peaks at 1509 cm⁻¹ (N=N stretching). -CH, -CH₂-, -CH₃ Aliphatic groups at 2917 cm⁻¹; 876.183 cm⁻¹, 904.577 cm⁻¹, 958 cm⁻¹, 843 cm⁻¹ =C–H, =–NH alkenes and aromatic compounds or Aliphatic amines may be present. Aromatic rings may present at 741 cm⁻¹, 469 cm⁻¹, –OH –NH groups may present at 3200 cm⁻¹ –CH, –CH₂–, –CH₃ Aliphatic groups may present at 2800 cm⁻¹ amine N-H stretch may present at 3300 cm⁻¹. 1125 cm⁻¹ corresponds C-N and. 1442 cm⁻¹ corresponds N=N.

From spectrum, it is observed that dye contains hydroxyl group –OH at (3500), amine group– NH is also observed at 2000, carbonyl group C=O (1700), and alkyl group CH (3000). The presence of these groups indicate that the dye sample contains chromophores which are color giving groups and auxochromes, (color retaining groups) responsible for good coloring characteristics on cotton fabric.