9.1. INTRODUCTION

9.1.1 Plant profile of Holarrhena antidysenterica

Holarrhena is a genus of trees or shrubs and it comprises seven or eight species which are distributed in Asia, tropical areas of Africa, Madagascar, India, Philippines and Malayan Peninsula. The common and only Indian species, H. antidysenterica, is an important plant in Indian Medicine. The seeds are called 'Indra's seeds' in Sanskrit and are said to have sprung from drops of the 'Amrita of life' which fell on the ground from the bodies of the monkeys of Lord Rama, who were then restored to life by Indra318.

Holarrhena antidysenterica.

(a) Classification:

Kingdom : Plantae – Plants
Division : Magnoliophyta
Class : Magnoliopsida
Subclass : Asteridae
Order : Gentianales
Family : Apocynaceae
Genus : Holarrhena.
Species : Holarrhena antidysenterica Wall.

(b) Vernacular names:

Sanskrit: Kutaja, Kalinga, Indrayava
Hindi : Kurchi, Kuda
Kannada : Kodsinge, Kodmurak,
Malayalam : Kutakappala
Tamil : Kutasappalai
Telugu : Kodisapala-vittulu

English : Tellicherry bark

Gujarati: Indrajav, Kado

(c) **Part used**: Stem bark, root bark and seeds.

(d) **Botanical description**: It is a small shrub or deciduous tree, up to 13 m in height, with a milky latex. The bark peels off in flakes and is grey to pale brown in colour. The leaves are shiny on the upper surface, dull and hairy on the lower, opposite, subsessile and elliptic. The flowers are white and the fruits are cylindrical, dark grey with white specks, and occur in pairs. The seeds are light brown, 0.5-1.5 cm long, with long tufts of hair.

(e) **Origin and distribution**: It is found all over India and other Asian countries up to an altitude of 1300 m, especially in the sub-Himalayan tract, in deciduous forests and open wastelands.

(f) **Traditional use**: Kutaja is primarily used for the treatment of dysentery but has several other therapeutic usages. It is particularly useful in bleeding disorders such as menorrhagia, haemorrhoids, diabetes and oedema and has been used for tumours, abscesses, aches and pains, bronchitis, colic, diarrhoea, splenitis and as a vermifuge, laxative and astringent.

![Fig. 9.1: Stem bark of Holarrhena antidysenterica](image_url)
(g) Pharmacology and Clinical Studies\textsuperscript{321-323}:

i. Antibacterial activity: The methanolic extract of stem bark was tested for antibacterial efficacy against \textit{Staphylococcus aureus}, \textit{Staphylococcus epidermidis}, \textit{Streptococcus faecalis}, \textit{Bacillus subtilis}, \textit{Escherichia coli} and \textit{Pseudomonas aeruginosa} using both the microdilution broth method as well as the disc diffusion method. The extract was active against all tested bacteria. Further studies revealed that the antibacterial activity was mainly associated with alkaloids, which showed remarkable activity against \textit{Staphylococcus aureus} (MIC = 95 \textmu g/ml). The seeds also exhibited antibacterial activity, particularly the chloroform and methanol extracts.

ii. Antidysentery and antidiarrhoeal activity: The efficacy of \textit{Holarrhena antidysenterica} in chronic and amoebic dysentery has been established. Conessine was reported to be the most effective of the alkaloids. In a small clinical trial of 25 patients suffering from diarrhoea and dysentery, relief for about 80\% was observed after 3 days of treatment with an Ayurvedic formulation. This consisted of \textit{Holarrhena antidysenterica} seeds, \textit{Berberis aristata} wood, \textit{Embelia ribes} fruits, \textit{Cyperus rotundus} bulbous roots, \textit{Aegle marmelos} fruit pulp and \textit{Butea monosperma} seeds.

iii. Immunomodulatory activity: An ethanolic extract stimulated phagocytic functions while inhibiting the humoral component of the immune system in mice.

iv. Hypoglycaemic activity\textsuperscript{320}: This has been reported in the aqueous and alcoholic extracts of the seeds of \textit{Holarrhena antidysenterica}. In a study conducted in rats in three models, a significant decrease in blood glucose level was observed both in normal and diabetic rats.
Clinical tests with conessine on patients with intestinal and hepatic amebiasis have been found to give results, comparable to those obtained with emetine.

(h) **Toxicity**\(^{321}\): Use of conessine must, however, be closely supervised, as in some cases it can produce neurological troubles like vertigo, sleeplessness, agitation, anxiety and delirium.

(i) **Phytochemistry**\(^{324, 326}\):

**Alkaloids** - O-free alkaloids (conessine, conimine), conkurchine group alkaloids (including conessidine) and O-containing alkaloids (holarrhenine, holafrine, holarrhetine) present in bark whereas leaves contain O-containing alkaloids (kurchiphyllamine and kurchiphylline). Two new aminoglycostereoids – holontosines A and B isolated from leaves (Tetrahedron 1970, 26, 1695). Three new aminodeoxyglycostereoids – holarosine B and holontosines E and F isolated from leaves (Chem. Abstr. 1973, 78, 4474 f). Two new alkaloids – holacine and holacimine isolated from bark and a new alkaloid holarricine isolated from seeds. The alkaloid content of the bark was found to be at a maximum when the plant was between 8-12 years old, and during the months of July to September.

(j) **Active Principles**: Conessine
9.1.2 Conessine as marker compound for Standardization

![Structure of Conessine](image)

**Fig. 9.2: Structure of Conessine**

**C₂₄H₄₀N₂**  
Mol. wt. 356.6

**Conessine** is a steroid alkaloid found in a number of plant species from the *Apocynaceae* family, including *Holarrhena antidysenterica*, *Holarrhena floribunda* and *Funtumia elastica*, several of which are used in traditional herbal medicine as a treatment for amoebic dysentery. It acts as a histamine antagonist, selective for the H₃ subtype. This compound is biologically active and thus it can be considered as a bioactive marker.

There are many products of *Kutja* are getting explored in market and with a good therapeutic activities. The qualitative and quantitative estimation of conessine can target to assess the authenticity and inherent quality of various plant-based formulations containing this marker.

Considering the demand of this plant product the need to assure the quality of the formulations has aroused. Thus, the objective of the present work was to develop and validate a HPTLC method for estimation of conessine in *Kutja* preparations.
9.2 ISOLATION OF CONESSINE

9.2.1 Procurement of dried stem bark of *Holarrhena antidysenterica* Wall.

Authenticated, dried and powdered stem bark of *Holarrhena antidysenterica* Wall. was provided by Piramal Life Sciences Pvt. Ltd as gift sample.

9.2.2 Extraction of dried stem bark of *Holarrhena antidysenterica* Wall.

Dried and powdered stem bark (100gm) was extracted with 2 M Hydrochloric acid over a period of 4 days. The acid soluble portion was extracted with chloroform, (5 x 10 ml) to remove neutral components. The aq. acidic layer was then made alkaline (pH 8.5) with ammonium hydroxide solution (30%) and repeatedly extracted with chloroform, (5 x 10 ml). The combined extracts were washed with water, and then evaporated to dryness. Dark brown sticky mass was obtained as crude total alkaloids with the yield 0.85% w/w.

9.2.3 Procedure for isolation of Conessine

a) Preparation of column

350 gm of silica gel (60-120 mesh) was activated for 30 mins at 105°C. Slurry of silica gel was prepared in 1000 ml petroleum ether (60-80°C) and was transferred to glass column; precaution was taken to avoid air entrapment. The length of the column was approximately kept to 70 cm.

b) Preparation of sample

The chloroform extract of *Kutja* was adsorbed on silica (approximately 10 gm) and loaded on the column in form of thin band. Petroleum ether was added slowly to the column, precaution was taken to avoid air entrapment.

c) Elution

Following combinations of solvents were used to elute the column. 100 ml of solvent each combination was used at a time to run the column.
Table 9.1: Pattern of column chromatographic elution for Conessine

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Solvent composition (%)</th>
<th>TLC studies of fractions</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petroleum Ether</td>
<td>Chloroform</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>100</td>
<td>0</td>
<td>No spot</td>
</tr>
<tr>
<td>2.</td>
<td>95</td>
<td>5</td>
<td>One Spot detected with a blackish blue color after spraying with 10% sulphuric acid alcohol, but no spot with Dragendorff’s reagent (Rf 0.93)</td>
</tr>
<tr>
<td>3.</td>
<td>90</td>
<td>10</td>
<td>No spot</td>
</tr>
<tr>
<td>4.</td>
<td>85</td>
<td>15</td>
<td>A orange spot with high intensity after spraying with Dragendorff’s reagent. (Rf 0.82) Component IV.</td>
</tr>
<tr>
<td>5.</td>
<td>80</td>
<td>20</td>
<td>No spot</td>
</tr>
</tbody>
</table>

50 ml aliquots were collected, concentrated and subjected to TLC studies. 4th fraction [eluted with Petroleum ether: Chloroform (15:85)] gave an intense orange spot at Rf 0.82 after spraying with Dragendorff reagent with mobile phase toluene: ethyl acetate: diethylamine (2.5:6.5:1) (v/v) (that matched with the reference standard, Table No 9.1). Component IV was subjected to crystallization with acetone. The component IV gave a single band in TLC
Standardization of Some Plant-Based Formulations By Modern Analytical Techniques

Standardization of formulations containing *Holarrhena antidysenterica*

studied. (Fig. 9.3). The isolated compound, component IV was compared with the standard conessine.

![TLC plate image](image)

Derivatization with Dragendorff’s reagent

**Fig. 9.3**: Video-image of TLC plates showing Component IV and Reference standard Conessine

Track 1: Component IV, Track 2: Chloroform fraction of *Kuitja*

Component IV showed orange single colored spot on TLC plate after spraying with Dragendorff reagent indicating the presence of Alkaloid.
9.2.4 Physicochemical analysis of component IV

The component IV was subjected to different physicochemical parameters such as melting point, solubility, elemental analysis. The parameters were compared with that of the reference standard. The melting point was found to be 127 °C which was matching with the standard (126 °C). Solubility was tested in different solvents such as chloroform, methanol and water. It was observed that component IV was readily soluble in methanol and chloroform. The Lassaigne’s sodium fusion test was carried out for detection of elements. Carbon, hydrogen, nitrogen are present and halogens and sulphur were found to be absent. The yield obtained was 0.97 % w/w of methanol extract of Kutja by column chromatography. The summarized data is mentioned in Table 9.2.

Table 9.2: Physicochemical analysis of component IV

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Parameters</th>
<th>Component IV</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Color</td>
<td>Pale yellow</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>2.</td>
<td>Melting point</td>
<td>127 °C</td>
<td>126 °C</td>
</tr>
<tr>
<td>3.</td>
<td>Solubility</td>
<td>Methanol and Chloroform</td>
<td>Methanol and Chloroform</td>
</tr>
<tr>
<td>4.</td>
<td>Elements present</td>
<td>C, H, N</td>
<td>C, H, N</td>
</tr>
<tr>
<td>5.</td>
<td>Yield (%w/w)</td>
<td>0.97%</td>
<td>-</td>
</tr>
</tbody>
</table>

9.2.5 Confirmation of identity of isolated compound as Conessine

The isolated component IV was confirmed to be conessine by comparing with reference standard by HPTLC and UV studies
HPTLC Studies: Standard Conessine and the isolated component IV was dissolved in chloroform and the HPTLC analysis was carried out using the following densitometric conditions:

- **Stationary phase**: Precoated plates of Silica Gel 60 GF\(_{254}\) (Merck)
- **Mobile phase**: Toluene: ethyl acetate: diethylamine (2.5:6.5:15)
- **Saturation time**: 15 min
- **Development time**: 15 min
- **Wavelength**: 247 nm
- **Lamp**: Deuterium
- **Visualizing reagent**: At visible light after spraying with Dragendorff’s reagent
- **Band width**: 7 mm
- **Length of chromatogram**: 8 cm

The isolated component IV showed a single peak at \(R_f\) 0.82 (Fig 9.4 and Fig 9.5), which perfectly matched with the reference standard of conessine. Thus component IV was confirmed to be Conessine.

UV Spectroscopy: The UV spectrum of the standard Conessine and isolated component IV was recorded in methanol.

The UV \(\lambda_{\text{max}}\) of standard conessine and also of component IV was obtained at 247 nm.

Thus from the comparison with reference standard of Conessine and from chemical and spectral studies, component IV was confirmed to be Conessine.
Standardization of formulations containing *Holarrhena antidysenterica*

**Fig. 9.4:** HPTLC chromatogram of standard Conessine

**Fig. 9.5:** HPTLC Chromatogram of isolated conessine
9.3 METHOD DEVELOPMENT

9.3.1 Preparation of standard solutions

Stock solutions of standard Conessine were prepared by dissolving 11.50 mg of Conessine in 10 ml methanol, yielding stock solution of concentration 1.15 mg ml$^{-1}$. From this 1, 3, 5, 7, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 100 µL of solution were applied using LINOMAT 5 applicator with the band width of 8 mm, which gave different concentration ranging from 1.15 to 111.5 µg / spot respectively.

9.3.2 Chromatographic conditions

The different parameters of HPTLC such as mobile phase, band width, detection wavelength were optimized.

Stationary phase: The stationary phase i.e, Silica Gel 60 GF$_{254}$ was used as stationary phase. The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. The components were well resolved in the extracts of Kutja.

Mobile phase: The reported mobile phase in Indian herbal pharmacopeia Toluene: ethyl acetate: diethylamine (2.5:6.5:1) was employed. The mobile phase used gave a well –resolved band of conessine at R$_f$ 0.82 in the extracts of Kutja. (Fig 9.5 and Fig 9.6).

Band width: Band width of three different sizes 6 mm, 7 mm, 8 mm were used. Band width of size 7 mm gave good separation without the over saturation of the spot with sample and with good number of tracks were spotted in one 20 x 10 cm TLC plate.
Detection: The UV spectrum of conessine was observed and the λmax was obtained to be 247 nm. The TLC plate was scanned at 247 nm using deuterium lamp.

The following densitometric conditions were used for HPTLC studies:

Stationary phase: Precoated plates of Silica Gel 60 GF254 (Merck)
Mobile phase: Toluene: ethyl acetate: diethylamine (2.5:6.5:1)
Saturation time: 15 min
Development time: 15 min
Wavelength: 247 nm
Lamp: Deuterium
Band width: 7 mm
Solvent front: 8 cm

Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. Densitometric scanning was performed with Camag TLC scanner III in the reflectance-absorbance mode and operated by Win CATS software (1.3.0 Camag). Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was carried out by comparing peak areas of conessine and with linear regression.

The proposed method gave very good resolution of the standard conessine (Rf value = 0.82) in extracts in Kutja and formulation as indicated in Fig. 9.6 and Fig 9.7.
Fig. 9.6: HPTLC Chromatogram of Standard Conessine using optimized parameters

Fig. 9.7: HPTLC Chromatogram of chloroform extract of Kutija using optimized parameters
9.4 METHOD VALIDATION

Validation of HPTLC method as per ICH guidelines

Analytical method validation is a process of performing several tests designed to verify that an analytical test system is suitable for its intended purpose and is capable of providing useful and valid analytical data. The developed method was validated for various parameters like linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, robustness and system suitability as per ICH guidelines.

9.4.1 Linearity:

Different concentrations of conessine were spotted and analyzed. The analysis was done in triplicate and the concentration range showing regression coefficient ($r^2$) near to one with précised value of $r^2$ in all triplicate analysis was chosen.

Linearity was evaluated in the range of 10.35 - 109.50 µg / spot for Conessine. Peak area versus concentration was subjected to least square linear regression analysis and the slope, intercept and correlation coefficient for the calibration were determined.

A linear correlation between the peak area and applied concentration was found to occur in the concentration range of 11.5 - 103.5 µg / spot of conessine. The correlation coefficient was found to be 0.9997. The peak area ($y$) is proportional to the concentration of conessine ($x$) following the regression equation $y = 53.283x + 19.85$ (Fig 9.8).
Standardization of formulations containing Holarrhena antidysenterica

232

9.4.2 Limit of detection (LOD) and limit of quantitation (LOQ):

LOD and LOQ were determined by using standard deviation method. A calibration curve was prepared using concentrations in the range of 3.45-10.35 µg/spot which is below the linearity range. Standard deviation of residuals was measured and kept in the following equation for determination of detection limit and quantitation limit. Detection limit = \(3.3 \sigma /S\) and quantitation limit = \(10 \sigma /S\) where \(\sigma\) is the residual standard deviation of a regression line and \(S\) is the slope of the calibration curve.

The experimentally derived LOD and LOQ for Conessine were determined to be 3.45 and 10.35 µg/spot respectively.

9.4.3 Precision studies

Precision of the method was evaluated by repeatability (intra-day) and instrumental precision. Each level of precision was investigated by three sequential replicates of spotting of Conessine at concentrations of 34.50, 57.50 and 80.50 µg/spot.

Precision data on repeatability (intra-day) and instrumental variation for three
Standardization of formulations containing Holarrhena antidysenterica

Different concentration levels are summarized in Table 9.3. Precision studies showed R.S.D. less than 1%, indicating a sufficient precision of the method.

**Table 9.3: Results of Precision Studies of Conessine**

<table>
<thead>
<tr>
<th>Type of Precision</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC for concentration of Conessine (µg/spot)</td>
<td>AUC for concentration of Conessine (µg/spot)</td>
</tr>
<tr>
<td>Sr. No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>34.50</td>
<td>57.50</td>
</tr>
<tr>
<td></td>
<td>1925.61</td>
<td>3341.56</td>
</tr>
<tr>
<td>2.</td>
<td>1927.28</td>
<td>3337.84</td>
</tr>
<tr>
<td></td>
<td>1971.26</td>
<td>3317.45</td>
</tr>
<tr>
<td>3.</td>
<td>1931.05</td>
<td>3355.87</td>
</tr>
<tr>
<td></td>
<td>1958.94</td>
<td>3365.28</td>
</tr>
<tr>
<td>Mean</td>
<td>1927.98</td>
<td>3345.09</td>
</tr>
<tr>
<td></td>
<td>1958.817</td>
<td>3342.673</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.41</td>
<td>0.47</td>
</tr>
</tbody>
</table>

**9.4.4 Accuracy studies**

In order to evaluate the validity of the proposed method, accuracy was evaluated through the percentage recoveries of known amounts of Conessine added to solutions of extracts and all commercial products. The analyzed samples were spiked with 80, 100 and 120 % of median concentration (57.5 µg) of Conessine standard solution. For sample preparation of formulations please refer section 9.5. A constant application volume of 10.0 µl/spot was employed for all the sample solutions.

Accuracy was calculated from the following equation:

\[
\frac{\text{[(spiked concentration} - \text{mean concentration)}/\text{spiked concentration}] \times 100}
\]

The limit described for recovery studies in ICH guidelines is 80-120%. Satisfactory recoveries were found in the range of 99.18-109.77 % of conessine. Thus, indicates that the proposed HPTLC method is accurate for
the quantification of conessine in Kutja extract, vati and capsule formulations

(Table 9.4).

**Table 9.4: Recovery studies for Conessine in formulations containing Kutja**

<table>
<thead>
<tr>
<th>10 µl of each Extract &amp; Formulation</th>
<th>Amount added in µg</th>
<th>AUC Formulation</th>
<th>Standard</th>
<th>Standard spiked formulation</th>
<th>Recovery ± S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract of Kutja</td>
<td>46</td>
<td>1955.25</td>
<td>2470.86</td>
<td>4472.58</td>
<td>101.05 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>57.5</td>
<td>1955.25</td>
<td>3083.62</td>
<td>5239.92</td>
<td>103.99 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>1955.25</td>
<td>3696.37</td>
<td>5752.21</td>
<td>101.78 ± 0.17</td>
</tr>
<tr>
<td>Kutja Vati</td>
<td>46</td>
<td>1573.31</td>
<td>2470.86</td>
<td>3793.83</td>
<td>93.81 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>57.5</td>
<td>1573.31</td>
<td>3083.62</td>
<td>4517.68</td>
<td>97.01 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>1573.31</td>
<td>3696.37</td>
<td>5105.79</td>
<td>96.89 ± 0.09</td>
</tr>
<tr>
<td>Kutja Capsules</td>
<td>46</td>
<td>1955.25</td>
<td>2470.86</td>
<td>4346.88</td>
<td>98.21 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>57.5</td>
<td>1955.25</td>
<td>3083.62</td>
<td>4992.51</td>
<td>99.08 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>1955.25</td>
<td>3696.37</td>
<td>5583.23</td>
<td>98.79 ± 0.07</td>
</tr>
</tbody>
</table>

**9.4.5. Robustness**

For the determination of the robustness of method, chromatographic parameters, such as mobile phase composition and chamber saturation time,
were intentionally varied to determine their influence on the retention factor and quantitative analysis.

The mobile phase composition was altered by ± 5 % changes in the ratio of toluene and diethylamine. The two composition of toluene were tried 2.625 (+5% of 2.5) and 2.375 (-5% of 2.5) and of diethylamine 1.05 and 0.95 (+5% and -5% of 1) was tried (Table 9.5.1).

The chamber saturation time was altered from 15 min to 30 min (Table 9.5.2). No changes were observed in retention time and area under curve along with peak shape of conessine with the changes made with mobile phase composition and chamber saturation time.

9.4.6. Stability studies

Stability of conessine in the sample solutions was evaluated to verify whether spontaneous degradation occurred within 3 days. Stability of the sample solutions was tested after 24, 48 and 72 hours after preparation and storage at 4.0°C and 25.0°C separately. Stability was assessed by comparing the chromatographic parameters of the solutions after storage with the same characteristics of freshly prepared solutions. The results were calculated as the percentage of non-degraded content of conessine standard at the 21, 48, 72 hours.

Methanol extract showed 1.89 % of degradation after 72 hrs at 4 °C and 2.99 % of degradation after 72 hrs at 25 °C. In formulations, at 4°C vati showed maximum degradation after 72 hrs of 1.12 % and of 2.02 % at 25°C. The percent of degradation after 72 hrs of capsule was 1.13 % and 1.45 % at 4 °C and 25 °C respectively Table 9.6.
Table 9.5.1: Robustness (Mobile phase variation) studies of Conessine

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Mobile phase composition (v/v)</th>
<th>R_f</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toluene Ethyl acetate Diethylamine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>2.5 6.5 1</td>
<td>0.82</td>
<td>3040.12±0.27</td>
</tr>
<tr>
<td>2.</td>
<td>2.625 6.5 0.5</td>
<td>0.82</td>
<td>3041.35±0.51</td>
</tr>
<tr>
<td>3.</td>
<td>2.375 6.5 0.5</td>
<td>0.82</td>
<td>3040.93±0.17</td>
</tr>
<tr>
<td>4.</td>
<td>2.5 6.5 1.05</td>
<td>0.82</td>
<td>3041.06±0.29</td>
</tr>
<tr>
<td>5.</td>
<td>2.5 6.5 0.95</td>
<td>0.82</td>
<td>3040.59±0.08</td>
</tr>
<tr>
<td>S.D.</td>
<td>- - -</td>
<td>0.0</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Table 9.5.2: Robustness (Chamber saturation time variation) studies of Conessine

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Chamber saturation time (min)</th>
<th>R_f</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>15</td>
<td>0.82</td>
<td>3040.12±0.27</td>
</tr>
<tr>
<td>2.</td>
<td>20</td>
<td>0.82</td>
<td>3040.19±0.13</td>
</tr>
<tr>
<td>3.</td>
<td>25</td>
<td>0.82</td>
<td>3041.66±0.28</td>
</tr>
<tr>
<td>4.</td>
<td>30</td>
<td>0.82</td>
<td>3040.38±0.29</td>
</tr>
<tr>
<td>S.D.</td>
<td>-</td>
<td>0.0</td>
<td>0.72</td>
</tr>
</tbody>
</table>
Table 9.6: Stability Studies of conessine in formulations containing *Kutja*

<table>
<thead>
<tr>
<th>Extracts &amp; Formulations</th>
<th>Percentage of non-degraded conessine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature</td>
</tr>
<tr>
<td></td>
<td>4 °C</td>
</tr>
<tr>
<td></td>
<td>24 hrs</td>
</tr>
<tr>
<td>Methanol extract of <em>Kutja</em></td>
<td>99.07</td>
</tr>
<tr>
<td><em>Kutja</em> Vati</td>
<td>99.85</td>
</tr>
<tr>
<td><em>Kutja</em> Capsule</td>
<td>99.67</td>
</tr>
</tbody>
</table>
9.5 Quantitative analysis of formulations containing *Holarrhena antidysenterica* for content of Conessine by HPTLC

9.5.1 Procurement of *Kutja* formulations

Two formulations of *Kutja* namely Vati and Capsules were procured from the local market.

9.5.2 Preparation of Sample solutions

9.5.2.1 For *Holarrhena antidysenterica* Wall. Extracts

2 g of methanol extract was macerated with 50 ml of methanolic 2 M HCl in a conical flask on a mechanical shaker for 24 hrs at room temperature. The suspension of extract was filtered and extracted with chloroform (50ml). The pH of aqueous layer was adjusted to 8.5 by adding 30 % ammonium hydroxide. The alkaline aqueous solution was extracted with chloroform (50ml). Then, the chloroform layer was evaporated to dryness. 10 mg of this fraction was diluted to a 10 ml with chloroform and used for analysis.

9.5.2.2 For *Kutja vati*

2 g of powdered vati was transferred to 100 ml volumetric flask containing 50 ml of methanolic 2M HCl and the mixture was macerated on a shaker for 24 hrs at room temperature. Then the same treatment as given to above extract was applied to Vati.

9.5.2.3 For *Kutja Capsule*

Sample solutions for capsule formulation were prepared by same procedure of vati by transferring 2 g of capsule contents.

A constant application volume of 10.0 µl was applied for all the sample solutions.

The developed and validated method was applied for standardization of both
standardization of formulations containing Holarrhena antidysenterica

traditional and modern dosage forms viz. Kutja extract, Vati and Capsule.

The peak shape was not altered by other substances present in the matrix. The percent content of Conessine for all three products are indicated in Table 9.7.

Table 9.7: Percent Content of Conessine in formulations containing Kutja

<table>
<thead>
<tr>
<th>Extracts &amp; Formulations</th>
<th>Percent Content ± S.D. (%)</th>
<th>Weight of per unit</th>
<th>Content per unit of dosage form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract of Kutja</td>
<td>0.901±0.71</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kutja Vati</td>
<td>0.725±0.11</td>
<td>250 mg</td>
<td>1.813 mg/vati</td>
</tr>
<tr>
<td>Kutja Capsule</td>
<td>0.692±0.24</td>
<td>200 mg</td>
<td>1.384 mg/capsule</td>
</tr>
</tbody>
</table>

This method enabled to detect and quantify the amount of conessine as least as 0.692% in vati formulations to as high as 0.901 % in methanol extract of Kutja.

Thus, the method can be applied by the herbal manufacturers to estimate conessine in their products as quality control method for their routine analysis.