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Phytochemical analysis of *Tabernaemontana divaricata*

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Abstract
Objectives

1. Procurement of Raw Material: Collection of part of plant in which alkaloids are present in great concentration like roots, flowers, seeds, barks, fruits etc.
2. Extraction and Fractionation: Extraction can be done by using different techniques like Percolation and Soxhlet Extraction and Fractionation by using Column Chromatography.
3. Isolation and Characterization
4. These isolation and Characterization can be done by using TLC and Column Chromatography.
5. Standardization

The standardization of crude drug materials includes the following steps

1. Authentication (Stage of collection, Parts of the plant collected, Regional status, Botanical identity like Phytomorphology microscopically and Histological analysis, Taxonomical identity, etc.)
2. Chromatographic and spectroscopic evaluation. TLC, HPTLC, HPLC methods will provide qualitative and semi quantitative information about the main active constituents present in the crude drug as chemical markers in the TLC fingerprint evaluation of herbals (FEH). The quality of the drug can also be assessed on the basis of the chromatographic fingerprint.
3. Chromatographic analysis of herbals can be done using TLC, HPLC, HPTLC and GC, UV, Fluorimetry, GC-MS, etc.

Keywords: Phytochemical, *Tabernaemontana divaricate*, Chromatographic

Introduction

Plant Description

Leaves

- Leaves are in whorls of three, elliptic to lanceolate or obovate.
- Bright green above, pale green below.
- Tip acute or acuminate, base tapering and slender.

Flowers

- Flowers are in many flowered irregular corymbose cymes.
- Peduncles long but pedicels stout.
- White often has violet colored tinge.

Root

- Roots are tuberous with pale brown cork.

Botanical Description
Latin name *Tabernaemontana divarcata* Benth.
Family Apocynaceae
Synonyms *Chandini, crape jasmine*

Plant Taxonomy

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Gentianales
Family	Apocynaceae
Genus	*Tabernaemontana*
Species	*divarcata*

Chemical Constituents
Chemical constituents of *Tabernaemontana divarcata* are indole alkaloids. Alkaloids are the organic products of natural or synthetic origin which are basic in nature and contain one or more nitrogen atoms, normally of heterocyclic nature, and possess specific physiological actions on human or animal body, when used in small quantities.

Chemical constituents have been reported from the plant *Tabernaemontana divarcata* are as follows
Dregamine
Lahoricine
Pericyclivine
Taberhanine
Tubotaiwine
Vallcsaminc
Voafinidinc
Voafininc
Voalcninc
Vophylline
Voaphylline hydroxyindolenine

Medicinal and Traditional Uses
1. 12-hydroxy akuammicine - Uterine contraction (in vivo)
2. Apparicine - Anti-Polio III virus, Anti-microbial activity
3. Catharanthine - Anti-tumour effect
4. Conophylline - Anti-tumour effect (in vitro)
5. Coronaridine - Analgesic effect, Anti-inflammation, Hypotension
6. Dregamine - CNS stimulation, Analeptic effect (in vitro & *in vivo*)
7. Isovoacristine - Bradycardia (in vivo)
8. Tabernaemontanine - Vasodilation (in vivo)
9. Ibo gamine - CNS stimulation, Anticonvulsant effect, Anti-addictive effect
10. Isovoacangine - Negative chronotropic activity (in vitro)
11. Tabernaemontanine - Vasodilation (in vivo)
12. Voacamine - Cardiotonic effect, Anti-microbial activity
13. Voacangine - Slight CNS stimulation, Negative chronotropic activity
14. Voacristine - Weak CNS stimulation (in vivo)
15. Vobasine - Weak CNS depression (in vivo)

Herbal Medicine
Herbal medicines are the oldest remedies known to mankind. Man's dependence on plants for health care is as old as the existence of mankind on this planet.

Herbal Medicine Scenario in India
India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicine. There are very few medicinal herbs of commercial importance, which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine. There are about 9000 firms manufacturing traditional Ayurvedic medicines in India (Dubey, 2004) [4].

Herbal Medicine- Demand in All over the World
Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. In Germany and France, many herbs and herbal extracts are used as prescription drugs and their sales in the countries of European Union were around $ 6 billion in 1991 and may be over $ 20 billion now. In USA, herbal drugs are currently sold in health food stores with a turnover of about $ 4 billion in 1996 which is anticipated to double by the turn of the century. In India, the herbal drug market is about $ one billion and the export of plant-based crude drugs is around $ 80 million. Herbal medicines also find market as nutraceuticals (health foods)

Standardization
Standardization is the process of delivering a product with a specified minimum level of one or more phytoconstituents, where we can make sure about the quality of the product; broadly it covers the qualitative and quantitave part of analysis (Harborne, 1998) [6]. Standardization is adjusting the herbal drug preparation to a defined content of the active constituent. It refers to the process of delivering a product with a specified minimum level of one or more plant constituents. In some cases, it is accomplished by measuring the level of a phytoconstituent in a crude herbal extract and establishing a standard amount of that phytoconstituent for future production *viz*. vasicine in *Adhatoda zeylanica* and withaferin and withanolide in *Withania somnifera*. The concept of standardized extracts definitely provides a solid platform for scientific validation of herbals (Mukherjee, 2002) [1]

Need of Standardization
Depending upon whether the active principle of the plant is known or not, different concepts ('normalization' vs. 'standardization') have to be applied in order to establish relevant criteria for uniformity (Rudolf, 1998) [7]. Reproducible efficacy and safety of phytopharmaceuticals is based on reproducible quality. Therefore, if phytopharmaceuticals want to be regarded as rational drugs, they need to be standardized and pharmaceutical quality must be approved. The world health organization (WHO) has recognized this problem and has published guidelines to ensure the reliability and repeatability of research on herbal medicines. Standardization in medicinal plants is complicated by the complex chemical makeup of plants and the difficulty in obtaining the pure materials needed to compare and measure the amounts of any one particular compound in a plant mixture

Three attributes are desirable for standardization
Authenticity: It relates to proving that the material is true and corresponds to the right identity. It involves many parameters like gross morphology, microscopy, chemical analysis etc.

Purity: It pertains to evaluate that there are no adulterants present in the plant material. It can be evaluated by pharmacognostic studies.

Assay: This part of standardization is chemical and biological profiling by which chemical and biological effects can be assessed and curative values are established.

Quality Standards and Quality Control of Herbal Products

Proper analytical techniques

The analytical technique employed for quantitative purposes should be selective, sensitive, accurate, precise, reproducible and economical. Mainly the techniques used are HPLC, HPTLC, TLC, CC, and GC.

Selection of plants and formulations

Those plants/formulations were selected which were having established therapeutic credibility as far as the traditional system of medicines is concerned, and on which reasonable inputs in terms of modern scientific studies.

Selection of markers

Markers are defined as constituents of herbal drug or herbal formulation, which according to state of scientific knowledge may or may not contribute to the therapeutic activity but serve as an analytical purpose. Markers are suitable for identification tests and assay. They can be utilized for quantification and don't provide further knowledge about the quality of herbal drugs. They can also be used for stability studies. Stability and availability is also taken into consideration for the selection of marker compounds.

Sample preparation

Sample preparation is very important step in the analysis of any test material. The sample preparation is required not only to be simple but efficient in order to ensure maximum possible extraction of the marker and removal of interfering substances like pigments/excipients. Extraction procedures adopted in the present investigations were optimized systematically. Briefly, optimization of procedure for sample preparation involved extraction of test material with a suitable solvent for specific numbers (cycles) and periods of time, estimating the marker in the extracts thus obtained, subjecting the test material to repeated cycles of extraction using the time period yielding maximum amount of markers and finally selecting the minimum number of cycles of extraction giving maximum yield of marker.

Validation of assay

Any given assay procedure would remain incomplete without assessing its validity i.e. how accurately it is able to quantities the analyte in question. Validity of the assay was assessed by adding known amount of marker in the test sample, subjecting the mixture to optimized sample preparation procedures, estimating the content of marker and plotting the determined marker content against the content added. Slope of such a regression line gives an estimate of recovery of marker and the intercept represents the marker content in the test material.

Calibration plots

Calibration plots were prepared in order to find out the range of marker concentration, which shows a linear relation with respect to response of analytical technique. Once this range of response was established, concentrations of all test samples were so adjusted as to give the established linear range (Calixto, 2000) [5].

The present studies focus on such methods developed for the isolation of chemical markers from *Tabernaemontana divarcata* and their standardization using modern analytical techniques.

Physicochemical Investigation of Plant Material

Following parameters were used for physicochemical investigation of plant material:

Foreign matter analysis

A 100 g of the plant material was spread in a thin layer and the foreign matter was sorted into groups by visual inspection and using a hand lens. The remainder of the sample was sifted through a no. 250 sieve; dust was regarded as mineral admixture. The sorted foreign matter was weighed. The content of each group was calculated in grams per 100 g of air dried sample. The observations were recorded in Table 1.

Extractive Values

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists.

Successive extractive value

The powdered material of the drug (50 g) was packed in a Soxhlet apparatus and was subjected to successive extraction with different solvents like Petroleum ether, Ethyl acetate and methanol. Then it was filtered rapidly taking precaution against the loss of solvent. 25 ml of filtrate was evaporated to dryness in a tarred bottom china dish. It was then dried at 105 °C and weighed. The percentage of solvent soluble extractive with reference to air dried drug was calculated. The observations were recorded in Table.

$$\text{Extractive value (\%)} = \frac{\text{Wt. of extract}}{\text{Wt. of drug taken (in gms)}} \text{ x } 100$$

Loss on Drying (LOD)

2.0 gm of powder was accurately weighed in a petridish and kept in a hot-air oven maintained at 105^0C for four hours. After cooling in a desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained.

$$\text{Loss on drying (\%) (LOD)} = \frac{\text{Loss in weight}}{\text{Weight of the drug (in gms)}} \times 100$$

Ash Value

The ash remaining following ignition of medicinal plant materials is determined by 3 different methods which measure total ash, acid insoluble ash and water soluble ash.

The total ash is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash" which is derived from the plant tissue itself and "non-physiological ash" which is the residue of the extraneous matter (sand and soil) adhering to the plant surface.

1-Acid-insoluble ash
2-Water-soluble ash
a. Total ash
Accurately weighed 2g of ground dried material was taken in a previously ignited and tared crucible (usually of platinum and silica). The material was spreaded in an even layer and ignited by gradually increasing the heat to 500-600^{0}C until it was white, indicating the absence of carbon. Then it was cool in desiccators and weighed. The content of total ash was calculated in mg per g of air dried material.

$$\text{Total ash (\% w/w)} = \frac{(z - x)}{Y} \times 100$$

z = weight of the crucible + ash (after complete incineration)
x = weight of the empty crucible
y = weight of the material taken

b. Acid- insoluble ash

$$\text{Acid-insoluble ash (\% w/w)} = \frac{a}{y} \times 100$$

a = weight of the residue
y = weight of material taken

c. Water- soluble ash

$$\text{Water soluble ash (\%w/w)} = \frac{w - a}{Y} \times 100$$

w = Weight of total ash
a = Weight of the residue
y = Weight of the material taken (WHO, 1998)

d. Sulphated ash value

$$\text{Sulphated ash (\% w/w)} = \frac{(Z - X)}{Y} \times 100$$

z = weight of the crucible + ash (after complete incineration)
x = weight of the empty crucible
y = weight of the material taken

Phytochemical screening
Methanolic extract was subjected to preliminary phytochemical investigation for detection of Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, Flavonoids, Proteins and Amino acids, Saponins, Phytosterols, Acidic compounds, Resins and Reducing sugars.

Extraction, Fractionation, Isolation of Chemical Constituents and Standardization
Extract preparation
The coarsely powdered aerial roots of *Tabernaemontana divaricata* were extracted with methanol. The method of preparation is described below.

Methanolic extract
Dry Plant of *Tabernaemontana divaricata* was ground to coarse powder. The coarsely powdered aerial parts (800g) were packed in a percolator, soaked in a methanol (15litres) and kept for one week. The extract was drained, filtered and concentrated under reduced pressure using rotary film evaporator. The extraction process was repeated three times more under similar conditions. The combined extract was finally dried in vacuum desiccators and weighed.

Weight of plant material taken	:	800g
Weight of extract formed	:	32g
Extractive value	:	4.0%

Fractionation of methanolic extract
The dried methanolic extract was dissolved in (750ml) distilled water. The solution thus formed was defatted with n-Hexane (5 litres). The aqueous extract was dissolved in 10% acetic acid to adjust the pH 3.5 and extracted with chloroform three times (3×2liter) to yield alkaloid fraction(A1, 2g).The acidic aqueous filtrate thus obtained was basified to pH 9-10 by adding ammonium hydroxide and extracted with chloroform (3×2liter) to afford crude alkaloid gummy fraction(A2, 32g).

Chromatography of Acid soluble Extract
Isolation of Markers
Compounds were isolated from the ***Acid soluble*** extract by using column chromatography and fractions were monitored on TLC.

Slurry Formation
Dried ***Acid soluble*** soluble extract was taken and dissolved in the minimum quantity of chloroformlml and then adsorbed on weighed quantity of silica gel – G (60 – 120), to get free flowing material.
Weight of silica gel – G used 6.0 g
Weight of extract 2.0 g

Packing of column
A neat and dried column was taken. A cotton plug was put at the base the column. Solvent (chloroform) was poured into the column and packed with slurry of silica gel – G prepared by suspending it into the solvent. The adsorbed extract was then charged into the column.

Weight of silica gel – G used (column)	50g
Diameter of column used	2 cm
Length of column used	95 cm

Elution of the column
The column was first eluted with petroleum ether. Then column was eluted with the solvent by gradually increasing the percentage of ethyl acetate in petroleum ether.

Elution of the column

Sr. No	Fractions	Column Solvent System
1.	1 - 10	Hexane
2.	11 - 18	2% Ethyl Acetate In Hexane
3.	19 - 60	5% Ethyl Acetate
4.	61 - 74	10% Ethyl Acetate
5.	75 - 83	40% Ethyl Acetate
6.	84 - 98	100% Ethyl Acetate
7.	99 - 135	5% Methanol
8.	136 - 176	35% METHANOL
9.	177 - 229	METHANOL

Each fraction of 250 ml was collected and concentrated on rotavapour. A total of 229 fractions were collected and TLC of all 229 fractions were done using different developing solvents. The fractions were pooled on the basis of the TLC pattern shown by them.

Developing solvents used for the TLC in various proportions

1. chloroform: methanol
2. Pet ether: acetone: diethyl amine

Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with

Dragondorff'S reagent

Preparation of Dragondorff's reagent

A. Bismuth sub nitrate (0.85g) + water (20ml) + add 2 drops of conc. Nitric acid and heat till mixture become homogenous
B. Potassium iodide 8g dissolved in 20ml of water.

Mix 5ml of A & 5ml of B + 20ml acetic acid.

Fractions 29-32 eluted in 2% ethyl acetate in petroleum ether, which gives pure compound in TLC plate. Dry at rotavapour and subjected to further process and its melting point was found to be 155.5 ◦C.

Chromatography of Acid insoluble Extract

Isolation of Markers

Compounds were isolated from the *Acid insoluble extraxt* by using column chromatography and fractions were monitored on TLC.

Slurry Formation

Dried basified extract was taken and dissolved in the minimum quantity of methanol and then adsorbed on weighed quantity of silica gel – G (60 – 120), to get free flowing material.

Weight of silica gel – G used	120.0 g
Weight of extract	30.0 g

Packing of column

A neat and dried column was taken. A cotton plug was put at the base the column. Solvent (chloroform) was poured into the column and packed with slurry of silica gel – G prepared by suspending it into the solvent. The adsorbed extract was then charged into the column.

Weight of silica gel – G used (column)	450g
Diameter of column used	6 cm
Length of column used	112 cm

Elution of the column

The column was first eluted with petroleum ether. Then column was eluted with the solvent by gradually increasing the percentage of ethyl acetate in petroleum ether.

Elution of the column

Sr. No	Fractions	Column Solvent System
1.	1 - 10	Hexane
2.	11 - 18	2% Ethyl Acetate In Hexane
3.	19 - 60	5% Ethyl Acetate
4.	61 - 74	10% Ethyl Acetate
5.	75 - 83	40% Ethyl Acetate
6.	84 - 98	100% Ethyl Acetate
7.	99 - 135	5% Methanol
8.	136 - 176	35% Methanol
9.	177 - 239	Methanol

Each fraction of 250 ml was collected and concentrated on rotavapour. A total of 239 fractions were collected and TLC of all 239 fractions was done using different developing solvents. The fractions were pooled on the basis of the TLC pattern shown by them.

Developing solvents used for the TLC in various proportions

1. chloroform: methanol
2. Pet ether: acetone: diethyl amine

Visualizing agent used in TLC

The spots were visualized by spraying the chromatogram with Dragondorff'S reagent

Fractions 70- 81 eluted in 10% ethyl acetate. Which gives pure compound in TLC plate, Dry at rotavapour and subjected to further process. The melting point of the compound was found to be 131.6˚C. The compound was identified as Beta sitosterol, on the basis of TLC pattern, melting point and spectroscopic data in comparison with reported data of Beta sitosterol the literature

Chromatography of Methanolic Extract (Bark)

Isolation of Markers

Compounds were isolated from the ***Methanolic*** extract by using column chromatography and fractions were monitored on TLC.

Slurry Formation

Dried basified and centrifuged extract was taken and dissolved in the minimum quantity of methanol and then adsorbed on weighed quantity of silica gel – G (60 – 120), to get free flowing material.

Weight of silica gel – G used	200.0 g
Weight of extract	55.0 g

Packing of column

A neat and dried column was taken. A cotton plug was put at the base the column. Solvent (chloroform) was poured into the column and packed with slurry of silica gel – G prepared by suspending it into the solvent. The adsorbed extract was then charged into the column.

Weight of silica gel – G used (column)	650g
Diameter of column used	6 cm
Length of column used	115 cm

Elution of the column

The column was first eluted with petroleum ether. Then column was eluted with the solvent by gradually increasing the percentage of ethyl acetate in petroleum ether.

Elution of the column

Sr. No	Fractions	Column Solvent System
1.	1 - 6	Hexane
2.	7 - 12	5% Ethyl Acetate In Hexane
3.	13 - 23	10% Ethyl Acetate
4.	24 - 30	15% Ethyl Acetate
5.	31 - 99	20% Ethyl Acetate
6.	100 - 215	25% Ethyl Acetate
7.	216 - 235	50% Ethyl Acetate
8.	236 - 276	80% Ethyl Acetate
9.	277 - 319	Methanol

Each fraction of 250 ml was collected and concentrated on rotavapour. A total of 319 fractions were collected and TLC of all 319 fractions was done using different developing solvents. The fractions were pooled on the basis of the TLC pattern shown by them.

Developing solvents used for the TLC in various proportions

1. Chloroform: methanol
2. Pet ether: acetone: diethyl amine

Results and Discussion

In the present study, Isolation of chemical constituents and standardization of plant extracts of *Tabernaemontana divaricata* was done, *Tabernaemontana divaricata* is the well-known medicinal plant used from the ancient era to till date for their medicinal values. Standardization was done on the basis of marker compounds isolated from the plant extracts. The plant was collected and analyzed.

Foreign matter analysis

Table 1: Foreign matter analysis value of aerial parts powder of *Tabernaemontana divaricata*

S. No.	Wt. of crude Drug (g)	Wt. of drug after removal of foreign matter (g)	Wt. of foreign matter (g)	Foreign matter (% w/w)
1.	100	99.114	0.886	0.886
2.	100	99.046	0.954	0.954
Mean				0.920

Foreign matter of aerial parts powder of *Tabernaemontana divaricata* was found to be 0.920%.w/w

Loss on drying:

Table 2: Loss on drying of aerial parts powder of *Tabernaemontana divaricate*

S. No.	Wt. of Petri dish + Drug (Before drying) (g) A	Wt. of Petri dish + Drug (After drying) (g) B	A-B (g)	Loss on Drying (% w/w)
1	18.545	18.329	0.216	10.8
2.	20.488	20.264	0.224	11.2
Mean				11.0

Moisture content of aerial parts powder of *Tabernaemontana divaricata* was found to be 11.0% w/w.

Successive extractive value

Table 3: Petroleum ether extractive value (successive) of aerial parts of *Tabernaemontana divaricate*

S. No.	Wt. of drug (g)	Wt. of empty China dish (g)	Wt. of empty China dish + Wt. of extractable matter (g)	Wt. of extractable matter (g)	% Extractive value
1.	50.0	34.366	35.606	1.24	2.48
2.	50.0	37.484	38.644	1.16	2.32
Mean					2.40

Petroleum ether extractive value of aerial parts of *Tabernaemontana divaricata* (successive extractive) was found to be 2.40%w/w

Table 4: Ethyl acetate extractive value (successive) of aerial parts of *Tabernaemontana divaricate*

S. No.	Wt. of drug (g)	Wt. of empty China dish (g)	Wt. of empty China dish + Wt. of extractable matter (g)	Wt. of extractable matter (g)	% Extractive value
1.	50.0	34.366	35.600	1.234	2.468
2.	50.0	37.484	38.700	1.216	2.432
Mean					2.45

Ethyl acetate extractive value of aerial parts of *Tabernaemontana divaricata* (successive extractive) was found to be 2.45%w/w

Table 5: Methanolic extractive value (successive) of aerial parts of *Tabernaemontana divaricate*

S. No.	Wt. of drug (g)	Wt. of empty China dish (g)	Wt. of empty China dish + Wt. of extractable matter (g)	Wt. of extractable matter (g)	% Extractive value
1.	50.0	34.366	40.584	6.218	12.436
2.	50.0	37.484	43.766	6.282	12.564
Mean					12.5

Methanolic extractive value of aerial parts of *Tabernaemontana divaricata* (Successive extractive) was found to be12.5%w/w.

Ash values

Table 6: Total ash value of aerial parts of *Tabernaemontana divaricate*

S. No.	Wt. of empty crucible (g)	Wt. of crucible + Wt of crude drug (before ignition) (g)	Wt. of crucible after ignition (g)	Wt. of total ash (g)	% Total ash value
1.	26.276	28.276	26.372	0.096	4.80
2.	28.458	30.458	28.558	0.100	5.00
Mean					4.90

Total ash value of aerial parts of *Tabernaemontana divaricata* was found to be 4.90%w/w

Table 7: Acid insoluble ash value of aerial parts of *Tabernaemontana divaricate*

S. No.	Wt. of drug taken (g)	Wt. of empty crucible (g)	Wt. of crucible + acid insoluble ash (g)	Wt. of acid insoluble Ash (g)	% Acid insoluble ash value
1.	2.000	26.276	26.2893	0.0133	0.665
2.	2.000	28.458	28.4719	0.0139	0.695
Mean					0.680

Acid insoluble ash value of aerial parts of *Tabernaemontana divaricata* was found to be 0.680%w/w.

Table 8: Water soluble ash value of aerial parts of *Tabernaemontana divaricate*

S. No.	Wt. of empty crucible (g)	Wt. of total ash (g)	Wt. of water insoluble ash (g)	Wt. of water soluble ash (g)	% Water soluble ash value
1.	26.276	0.099	0.053	0.046	2.30
2.	28.458	0.097	0.047	0.050	2.50
Mean					2.40

Water soluble ash value of aerial parts of *Tabernaemontana divaricata* was found to be 2.40%w/w.

Table 9: Sulphated ash value of aerial parts of *Tabernaemontana divaricate*

S. No.	Wt. of empty crucible (g)	Wt. of crucible + Wt of crude drug (before ignition) (g)	Wt. of crucible after ignition (g)	Wt. of total ash (g)	% Total ash value
1.	26.276	28.276	26.3344	0.0584	2.92
2.	28.458	30.458	28.5196	0.0616	3.08
Mean					3.00

Sulphated ash value of aerial parts of *Tabernaemontana divaricata was* found to be 3.00%w/w

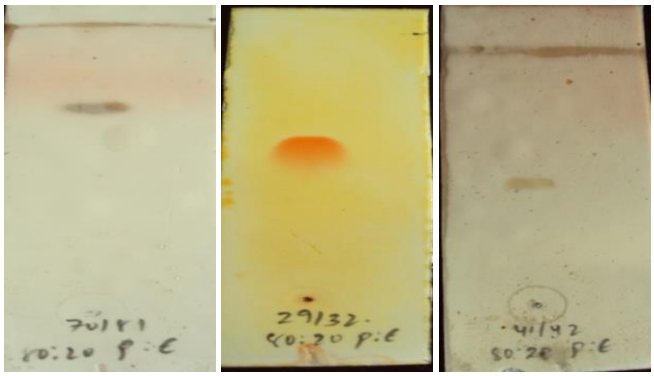
Phytochemical screening:

Table 10: Phytochemical screening of aerial parts of *Tabernaemontana divaricate*

S. No.	Constituent	Observation
1.	Alkaloids	+
2.	Carbohydrates	_
3.	Glycosides	_
4.	Phenolic Compounds and Tannins	_
5.	Flavonoids	_
6.	Proteins	+
7.	Saponins	_
8.	PhytoSterol	+
9.	Amino acids	_
10.	Fixed oils	_
11.	Resin	_
12.	Reducing sugar	_

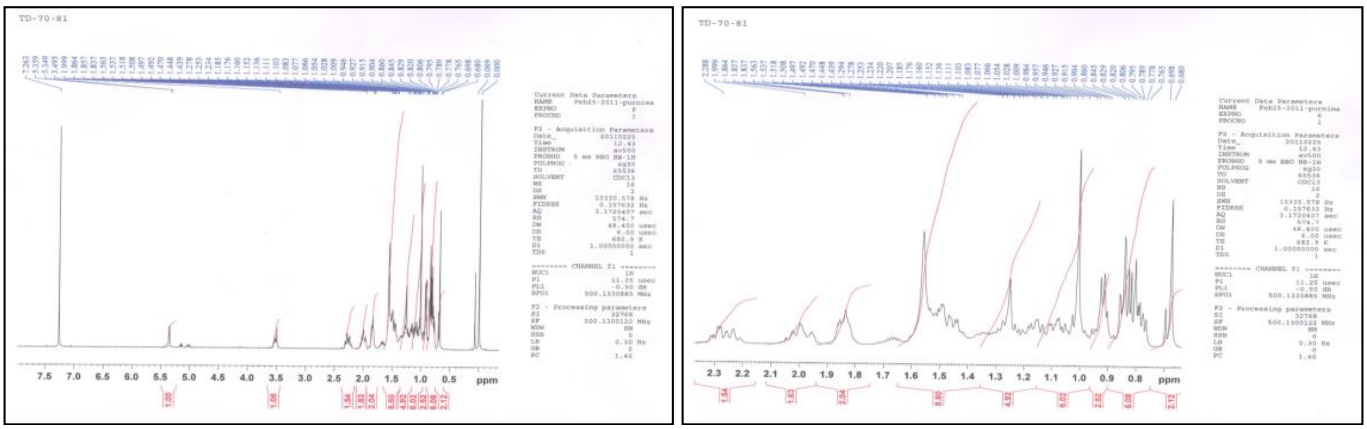
+ = Present, -- = Absent

Isolation of phytoconstituents

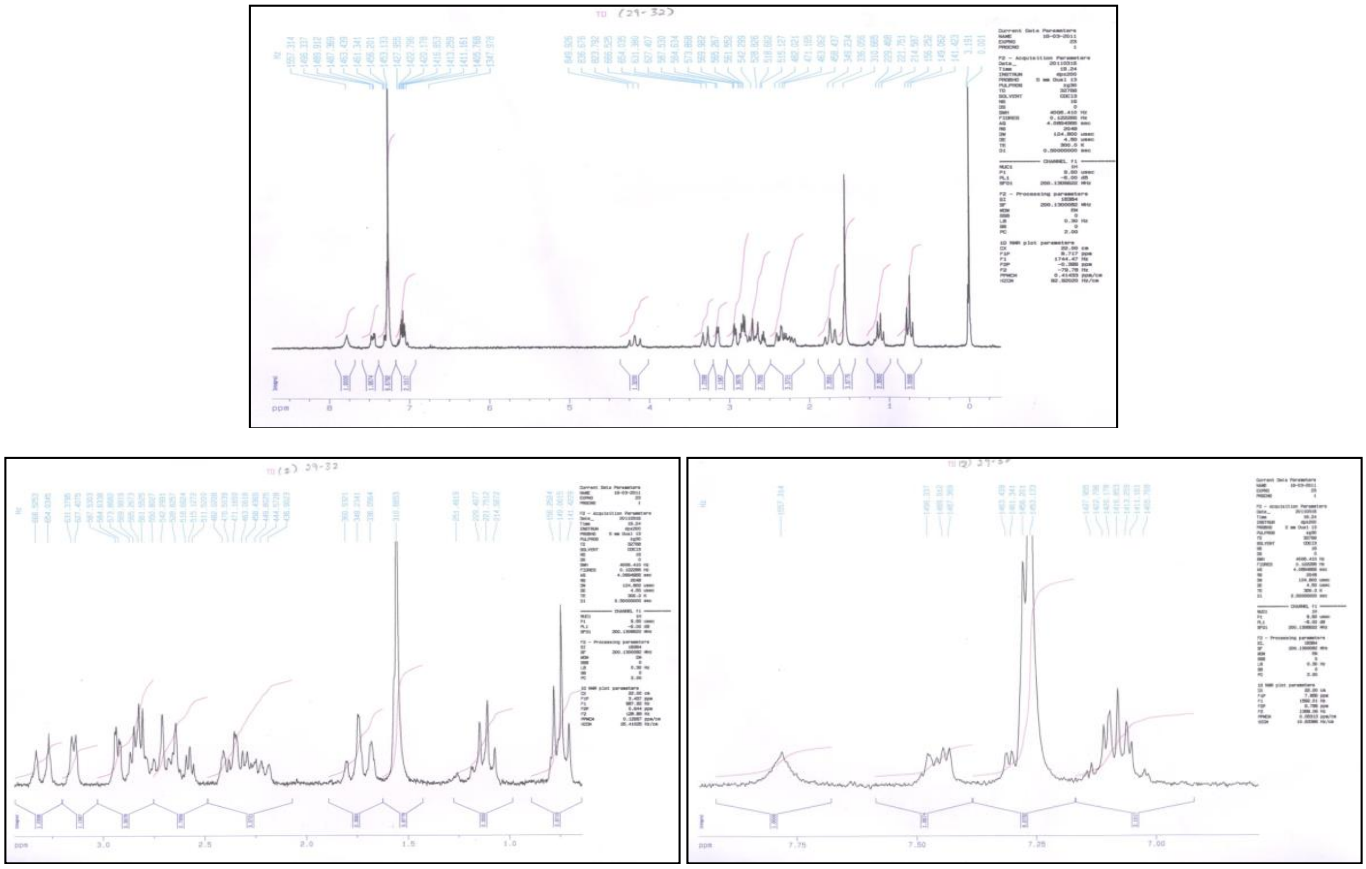


TLC Plate of β- Sitosterol, TLC Plate of Demethoxy iboluteine, TLC Plate of pentadecan – 1 – amine

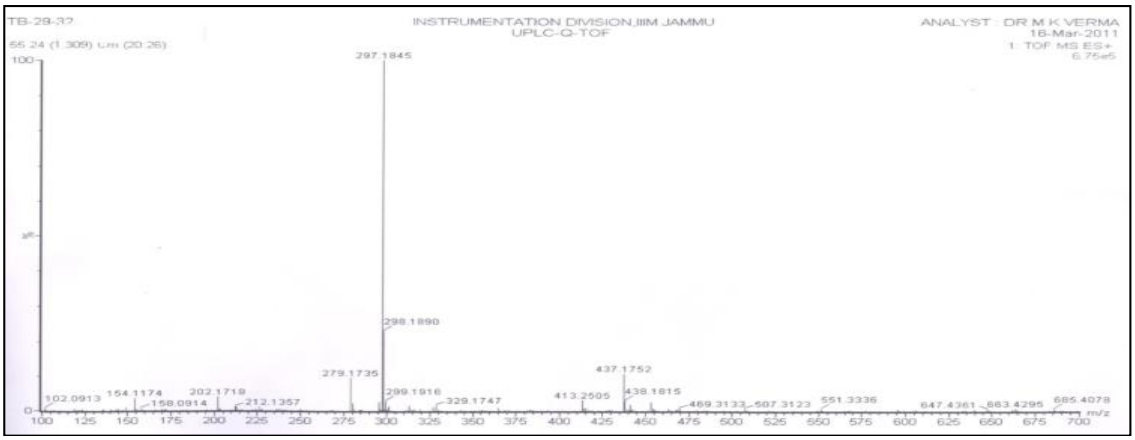
1. Standard 2. Mixture of standard and isolated compound 3. Isolated compound



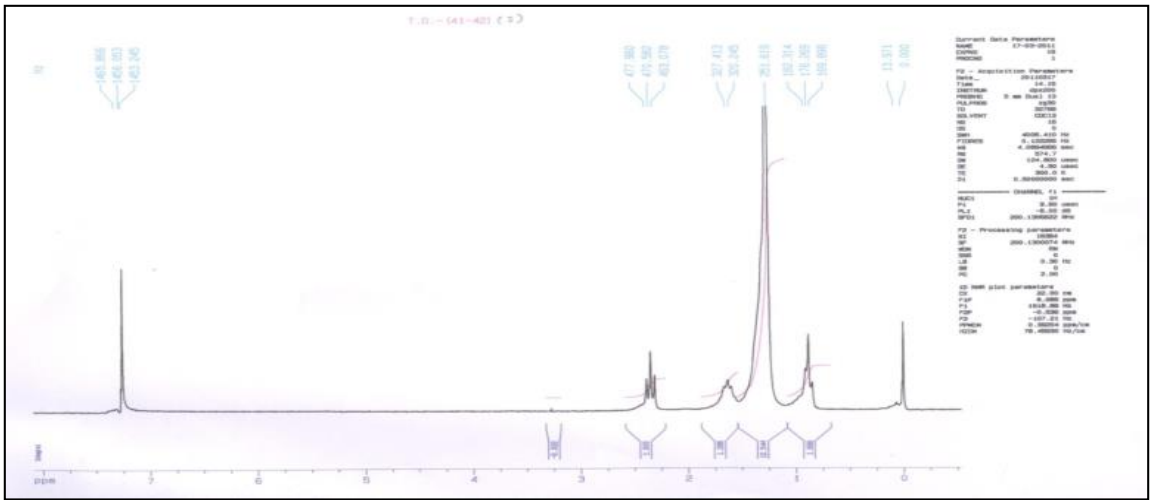
Proton NMR spectra of Beta sitosterol



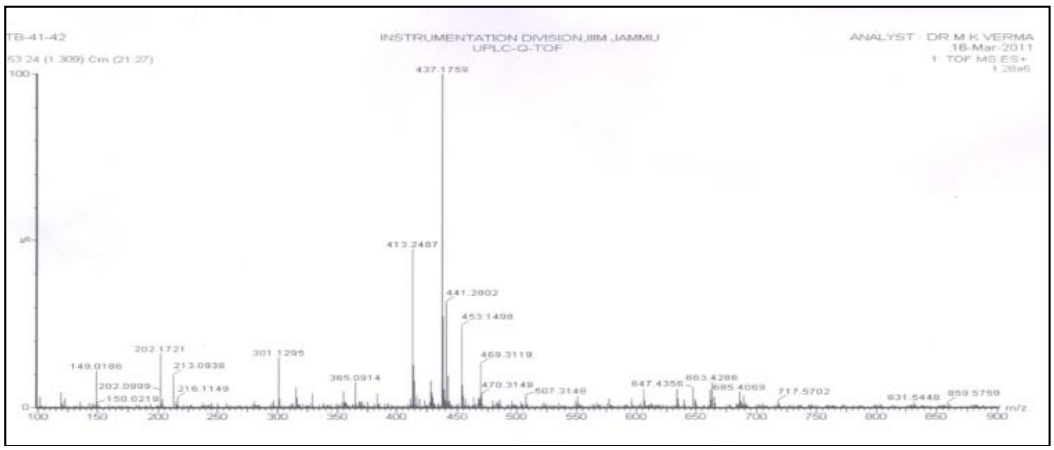
Proton NMR spectra of Demethoxy iboluteine



Mass spectra



Proton NMR spectra



Mass spectra

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