Quantification of Phytochemicals and HPTLC Finger Printing of Stem and Leaf Extracts of Tabernaemotana divaricata.

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Abstract: - HPTLC is an analytical technique used for the qualitative and quantitative evaluation of polyherbal formulations. Tabernaemotana divaricata an important medicinal plant wide medicinal value is frequently used in a large no of traditional herbal preparations. For HPTLC stationary phase was silica gel 60 F254 plate. The mobile phase consisted of Toluene: Ethylacetate: Formic acid (7:2:0.5). In the present study the Preliminary Phytochemical screening of Tabernaemotana divaricata stem and leaf extraction has been done to identify the chemical constituents and HPTLC fingerprinting of Tabernaemotana divaricata stem and leaf tracts has been performed which may be used as marks for quality evaluation and standardization of the drug.

Key Word: - Phytochemical , Screening, HPTLC finger printing, Tabernaemotana divaricata

Introduction:-

It is an evergreen glabrous and dichotomously branched about 2-3 m high shrub and commonly called Pinwheel flower, Crape jasmine , East India rose bay and Nero’s crown (Dastur et.al 1962). Ethnobotanically have been known to possess antimicrobial , anthelmintic, antioxidant , curative properties against nervous disorders, skin problems, respiratory and eyesailments,veneral diseases, diabetes , chronic bronchitis , snake bite and cardiotonic ailments.(Ignacimutu et.al 2006 and Sathishkumar et.al 2012). Rootsare emmenagogue aphrodisiac tonic ,puragative , astrigent to the bowels and tonic to the brains , lever and spleen , and useful in paralysis.

Rahman et.al (2011) observed the antibacterial activity of leaf T.divaricata and Sathishkumar (2013) observed the phytochemicals in the leaves and flower of T. heyneana.
Chemical constituents

Kidwai and Krishna Murti (1963), reported the presence of a bacteriolytic enzyme from the latex of the plant. Besides, the latex also contains proteins, amino acids and galactose. The seeds contain citric, oleic and palmitic acids. The flowers contain dregamine, 20-epiervatamine, abernaemontanine, voaphylline, hydroxynodolenine, Janetine, hecubine, and kaempferol. The leaves contain α-amyrin, lupeol and their acetate, β-sitosterol, coronaridineapparicine, ervaticine (2-acyl indolederivative), ervatinine, hyderabadine, lahoricine, mehranine, stafpinine, flavonol glycosides, isoovoacristine, voaristine, voharine and a dimeric alkaloid, conophylline. The stem bark contains coronaridine \((C_{21}H_{26}N_2O_2)\), ibogamine, isovocangine, voacangine, 19–epi–voacangine, 11-methoxy-N-Me-dihydropericyclivine and an isomer of voacamine. The root bark yields ibogamine, coronaridine–hydroxyindolenine, 5-oxo-, 6-oxo-, 5-hydroxy--oxo- and(+)–19-hydroxy-coronaridines, pseudovobpraicine, aurantiamide acetate, bensoic acid, campesterol and cycloartenol. Besides, olivacine, heyneamine, 19 S – heyneaine-hydroxyindolenine, 3-oxo-, 19-oxo- and 19 (-2 ketopropyl) – coronaridines,3-oxo-voacangine-voacangine-hydroxyindolenine, voacristine \((C_{22}H_{28}N_2O_4)\) - hydroxyindolenina, caoutochuc, glycoflavone,leucoanthocyanins, resin and sugars have also been reported from the plant (Chatterjee and Pakrashi, 1955).

Presence of phenolics like coniferyl and sinapohyl alcohols and sterols like campesterol and stigmastanol and iriddoids, loganin , olivacine , tabernaemontanine , jecubine and janetine , trytophan and tryptamine ( Rastog and Mehrotra 1990., Ghani 2003).

Folkloric use

Chopra et al. (1958) considered the root as acrid, bitter and anodyne. The root bark is used as anthelmintic and wood is as a refrigerant. The milky juice of the leaves is applied to wounds to prevent inflammation and in the disease of eyes (Chatterjee and Pakrashi, 1995). Besides, the juice of the flowers mixed with oil is used to alleviate burning sensation and is also to cure eye sores and skin diseases.

In Thailand, the plant is used as an emetic. Theroots, leaves and flowers are all used in the treatment of snake and scorpion poisoning. The roots are used in modern medicine to treat hypertension, headache and scabies. (St. Louis 1994)

Method and materials: - The plant material was collected from Roorkee district of Haridwar, Uttarkahand The samples were washed thoroughly to remove dirt particles present on the surface. The samples were then sun dried and cut into small pieces and plant identified with the help of Duthie flora (1903-1929) and Maheshwari (1962).

Qualitative phytochemical analysis

The crude powder of different flowers was subjected to qualitative phytochemical analysis describe by Gibbs, 1974; Johansen, 1940; Harborne, 1973; Paris, 1963; and Peach and Tracey , 1955.

- **Raphides:** Hand sections of young shoot and petiole were cut treated with a saturated aqueous solution of cupric acetate. The crystals of calcium oxalate dissolved and oxalic acid so
released diffused into the intercellular spaces and yellow crystals of cupric oxalate appeared (Johansen, 1940).

- **Flavonoids:** Alkaline reagent test was performed for checking the presence of flavonoids. The crude powder of flower was treated with a few drops of diluted sodium hydroxide (NaOH) separately. Formation of intense yellow colour which turned colourless on addition of a few drops of diluted HCl indicated the presence of flavonoids.

- **Tannins:** The crude powder of flower was treated with alcoholic ferric chloride (FeCl₃) reagent. Blue colour indicated the presence of tannins.

- **Saponins:** The presence of saponins was determined by Frothing test. The crude powder of flower was vigorously shaken with distilled water and was allowed to stand for 10 minutes and classified for saponin content as follows: no froth indicates absence of saponins and stable froth of more than 1.5 cm indicated the presence of saponins.

- **Steroids:** Liebermann-Burchard reaction was performed for checking the presence of steroids. A chloroformic solution of the crude powder of flower was treated with acetic anhydride and a few drops of concentrated H₂SO₄ were added down the sides of the test tube. A blue green ring indicated the presence of steroids.

- **Cardiac glycosides:** Keller-kiliian test was performed for checking the presence of cardiac glycosides. The crude powder of flower was treated with 1.0 ml mixture of 5% FeCl₃ and glacial acetic acid. To this solution, a few drops of concentrated H₂SO₄ were added. Appearance of greenish blue colour within few minutes indicated the presence of cardiac glycosides.

- **Alkaloids:** The crude powder of flower was dissolved in 2 N HCl. The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with a few drops of Mayer’s reagent; one portion was treated with equal amount of Dragendorff’s reagent and the other portion was treated with equal amount of Wagner’s reagent. The creamish precipitate, orange precipitate and brown precipitate indicate the presence of respective alkaloids.

### Table: 1 preliminary phytochemical screeing of Methanolic extracts of *T.divaricata*

<table>
<thead>
<tr>
<th>constituents</th>
<th>Test</th>
<th>Methanolic extracts</th>
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<tbody>
<tr>
<td>Alkaoids</td>
<td>Mayer reagent</td>
<td>+</td>
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<tr>
<td></td>
<td>Dragendorff reagent</td>
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<td></td>
<td>Hager reagent</td>
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<tr>
<td></td>
<td>Wagner reagent</td>
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</tr>
<tr>
<td>Glycosides</td>
<td>Keller-kiliian test</td>
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</tr>
<tr>
<td>Flavonids</td>
<td>Shinoda test B</td>
<td>+</td>
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<tr>
<td>Saponin</td>
<td>Foam test</td>
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<tr>
<td>Tannin</td>
<td>Ferric chloride test</td>
<td>+</td>
</tr>
<tr>
<td>Raphids</td>
<td>Calcium carbonate</td>
<td>+</td>
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<td>Steriods</td>
<td>Liebermann-Burchard</td>
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**HPTLC PROFILE:** Preparation of extract 1 gm of powdered plant material was extracted in soxhlet apparatus with methanol, individually on a water bath, filtered and made up to 10 ml in a standard flask. The sample were spotted in the form of width 8.00 mm with a CAMAG 100 µl sample (Hamilton , Bonaduz, Switzerland ) syringe on silica gel per coated Aluminum plate 60 F-254 plates, (20cm × 10 cm with thickness , E Merk ,Darmstadt , Germany) using CAMAG Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. A constant application rate was used and the space between two bands was 8 mm. The slit dimension was kept at 6.00 mm×0.30 mm and the scanning speed was 20 mm/s. The mobile phase consisted of Toluene: Ethylacetate: Formic acid (7:2:0.5) and 10 ml of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin through glass chamber (CAMAG, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile was 20 min at room temperature 25°C. Following the development the TLC plates were dried in a current of ar with the help of an air dryer in a wooden chamber with adequate ventilation . The flow rate in laboratory was maintained.
unidirectional (Laminorflow, towards the exhaust). Densitometric scanning was performed using a CAMAG TLC scanner III in the reflectance absorbance mode at 254 nm and operated by CATS Software (V 3.15, CAMAG). Concentrations of the compound chromatographed were determined from intensity of the diffused light. Evaluation was by peak areas with linear regression.

Table No.2. HPTLC- Analyses of *T.divaricata* studied (STEM)

| S.N. | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| R.F. Value | .04 | .06 | .21 | .23 | .25 | .31 | .33 | .42 | .45 | .48 | .53 | .57 | .59 | .63 | .78 | .85 | .87 | .90 |
| *T.divaricata* stem | -   | -   | -   | -   | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |

Table No.3. HPTLC- Analyses of *T.divaricata* studied (LEAF)

<table>
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<tr>
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Table No- 4. Total HPTLC Maximum Area graph of *T.divaricata*

<table>
<thead>
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<th>Stem</th>
<th>Leaf</th>
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<tr>
<td>Total area</td>
<td>1078.2</td>
<td>1861.2</td>
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</table>

Track 1- Chromatogram and RF Values of Methanolic Extract of *T.divaricata* stem

Track 2- Chromatogram and RF Values of Methanolic Extract of *T.divaricata* leaf
Result:- Preliminary phytochemical analysis of stem and leaves extracts of *T. divaricata* using methanol solvents revealed the presence of various phytochemicals as summarized in table 1.

The results from HPTLC finger print scanned at wavelength 275 nm for methanolic extract of *Tabernaemontana divaricata* stem showed only one polyvalent phytoconstituents and corresponding ascending order Rf value start from 0.25 to 0.37 and leaf showed 3 polyvalent phytoconstituents and corresponding ascending order Rf values start from 0.17 to 0.99 in which highest concentration of the phytoconstituents was found to be 42.72% and its corresponding Rf value was found to be 0.88 respectively and was recorded in Track 1 and 2. The corresponding HPTLC chromatogram was present in Track 1 and 2.

Conclusion : - The initial study was carried out HPTLC and result show that there are many compounds are present in *Tabernaemontana divaricata*. From the HPTLC has been found that methanolic extracts contain not a single compound but mixtures of compound are present. This method is especially suitable for the fingerprinting and analysis of botanical samples and herbal formulations.

I wish to express my sincere gratitude to principal and management V.M.K.P.G. College, Manglore, Haridwar, for providing me an opportunity to do my work. I am short of words to thanks Arbo pharmaceuticals for helping me in the analytical results.

REFERENCE