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Effect of cultural condition and solvent extraction on pharmacognostical assessment and identification of scopolamine content in different parts of *Datura metel* Linn. through HPTLC analysis

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Abstract

Datura metel Linn. (Family: Solanaceae) is a common road side weed that widely distributed throughout India. The plant is commonly known as throne apple. The present study is aimed at comparative pharmacognostical studies in terms of macroscopic and quantitative microscopy on different solvent (chloroform, methanol and water) extracted leaves, stem and root parts of *D. metel*, procured from Bangalore soil zone, Karnataka, India. Initially, the soil parameters are checked for presence of various metals and other physicochemical properties. The results revealed the soil is sandy loam with the pH of 7.80, organic carbon content 0.32%, electrical conductivity (EC) was 14.20 $mScm^{-1}$ and the soil redox potential was 16.20 mV. The quality parameter includes physicochemical and phytochemical evaluation of the powder as well as the extract was determined as per the standard method. Macroscopical and microscopical evaluation of leaf, stem and root gave special identification characters. Phytochemical investigation reveals the presence of alkaloids, carbohydrate, protein, phytosterols and diterpenes. Thereafter, presence of scopolamine was identified with HPTLC method and percentage of scopolamine resulted higher of 0.78 in methanol leaf extract. This may be due to the soil nature of the Bangalore zone and the effect of solvent where active constituents are soluble maximum to get more yield.

Key words : *Datura metel* Linn., extracts, proximate analysis, HPTLC

1. Introduction

Medicinal plants are continued to provide useful tools for treating various diseases. The practices of traditional medicine are based on traditional belief and observations, which predate the development and discovery of newer medicine and moreover due to minimal side effects, the prime interest towards herbal field is explored worldwide.

So, before establishment of any therapeutic efficacy of the plant species, preliminary standardization, *i.e.*, procurement of plant species from soil zone and soil behavior are most essential and based on that chemical constituent, plant growth rate, different activities will vary. Many literatures revealed that cultural condition is the most important factor for any plants to establish any strong result likely, Orhan *et al.* (2013) reported antioxidant activity varies with the same *Centella asiatica* plant collected from Turkey and India, furthermore, the author also reported varied amount of constituents present in the same plant. Effect of agropractice is also plays a significant role for enhancing constituent present and its effect on therapeutic activities in medicinal plants (Nepovim *et al.*, 1998; Das and Dang, 2010; Das *et al.*, 2012). Thereafter, Street (2012) concluded that various medicinal plants can accumulate heavy metals from soil and environments. For example, medicinal plants like *Senecio coronatus* (Thunb.) Harv. (Asteraceae), *Datura metal* L. (Solanaceae), *Datura innoxia, Helichrysum candolleanum*, H. Buek (Asteraceae), *Blepharis diversispina* (Nees) C. B. Clarke (Acanthaceae) and *Rauwolfia vomitoria* (Apocynaceae) are accumulator of Ni, Co and Ni, Zn, Cu, Ni and Fe, respectively (Kelly *et al.*, 2002; Bhattacharjee *et al.*, 2004; Nkoane *et al.*, 2005; Okem *et al.*, 2014). Therefore, consumption of heavy metals beyond limits containing medicinal plants or plant products may invite countless health implications (Street, 2012). Besides, solvent system plays a major role in extraction of plant constituents and showed significant medicinal activities as per the research evident (Sammaiah *et al.*, 2006; Das *et al.*, 2009; Babu *et al.*, 2015). Oflate, *Datura metel* Linn. (Solanaceae) is a wild subglabrous spreading herb plant, which grows throughout India. The plant is perennial, herbaceous and about 1.5 m in height. The leaves are simple, alternate, dark green and ovate in shape. The fruits are round with thorns (Prasanna and Yuwvaranni, 2014). These characters revealed the pharmacognostic summery for identification of the plant species. Moreover, this plant is considered as a poisonous plant when taken in large doses (causes delirium, coma and even death) due to presence of high content of alkaloids but in low doses it acts as therapeutic agent (Donatus and Ephraim, 2009) and the activities depend on various phytoconstituents that present in the whole plant like alkaloids, flavonoids, phenols, tannins, saponins and sterols. Specifically constituents like hyoscyamine and scopolamines are majorly reported by several researchers (Chopra *et al.*, 1986; Oliver-Bever, 1986). Steroidal constituent, *i.e.*,

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daturasterol from the leaves (Ali and Shuaib, 1996), hyocine, hyoscyamine from root were isolated specifically. Seeds and leaves contain scopolamine. Datura herb contains 0.23% of total alkaloid among which hyoscine is the main alkaloid while L-hyoscyamine and atropine are present in very less quantities (Kokate *et al*., 2008). These constituents are resulted significant ethanopharmacological activities like in the treatment of anodyne, antispasmodic, epilepsy, hemorrhoids, skin ulcers, wounds, used to treat laryngitis and treacheries (Dabur *et al*., 2004). Further, it is also used to treat impotence, asthma, skin diseases and control fever and as a drug for criminal purposes (Parrotta, 2001). Antimicrobial activity, antioxidant activity and anti-inflammatory activity was also reported for this same species (Harbone, 1999; Okwu and Igara, 2009; De Britto and Gracelin, 2011; Alabri *et al*., 2014) with the same plant species. Recently seed extract was used for the sedative activity (Babalola *et al*., 2015). Overall summery revealed that pharmacognostical and phytochemical studies are the main important tools for the preliminary standardization of plant materials to distinguish it from its related species, isolation of constituents and screening of various pharmacological activities, ensuring the quality of the market formulations (Chidambaram and Aruna, 2013). Based on that, the present comparative study was carried out to determine the effect of soil health and other cultural conditions on Datura plant vis-a-vis in relation to identify the plant species by pharmacognostical and physicochemical estimations for the various extracted parts and presence of the constituent (scopolamine) through HPTLC method was identified in the plant parts with various solvents which has scantly scientific evidences from Indian originated Datura plant.

2. Materials and Methods

2.1 Collection of samples

The different parts of *D. metel* Linn. like leaves, stem and roots were collected from cultivated land of Indian Institute of Horticultural Research (IIHR), Bangalore, India and authenticated by Dr. T. N. Shivananda, Principal Scientist, Medicinal and Aromatic Plants Division, IIHR, Bangalore. The parts of plants were kept in department of Pharmacognosy, Al-Ameen College of Pharmacy, Bangalore, as herbarium for future references (Voucher No: 208).

2.2 Soil analysis

Soil pH was measured with a glass electrode using a 1:1 sample/ water ratio and electrical conductivity (EC) was measured with a meter and probe as using a 1:5 sample/water ratio (McLean, 1982). The soil redox potential (Eh) was measured using a standard Pt electrode (HORIBA redox potential meter, Japan). Mechanical analysis was carried out by International pipette method (Piper, 1950) to determine the percentages of sand, silt and clay from soils. Determinations of cation exchange capacity (CEC) were made in $BaCl_2$ by the Gilman method (Rhoades, 1982) and total organic carbon was determined by the Walkley and Black wet dichromate oxidation method (Nelson and Sommers, 1982). PO_4^{3-} was determined colorimetrically (Varian Cary 50 Bio spectrophotometer, Australia) according to the method described by Peachey *et al*. (1973). Extracted heavy metal concentrations were determined using atomic absorption spectrophotometer (AAS; Perkin Elmer model: AAnalyst 100; Australia). Assessment of potentially phytoavailable metals (Cd, Cr, Cu, Fe, Ni, Pb, and Zn) in soils was conducted using the DTPA/TEA method, developed by Lindsay and Norvell (1978).

2.3 Pharmacognostical evaluation

The plant parts were subjected to powder microscopical examination as per the standard methods (Kokate, 1996). Preliminary chemical tests were carried out separately for all the extracts to determine the presence of various chemical constituents (Harbone, 1994). Quantitative standards like moisture content, total ash value, acid insoluble extractive values and water soluble extractive values were determined as per the standard methods (IP-1996; WHO, 1998) and results were tabulated in the result section.

2.4 Extraction

Three solvents, *viz*., chloroform, methanol and water were used for extraction of different parts of the above said plant. The extraction was done using soxhlet apparatus.

100 g of powdered plant parts was subjected to Soxhlet extraction with three solvent such as chloroform, methanol and water for 6 h. separately. Each time before extraction with next solvent, the powdered material was air dried. All the extracts were concentrated by using rotary vacuum evaporator at 45°C and further weighed. The percentage of different extractive values was calculated with respect to air-dried substance and results are tabulated as calculated by the following formula:

$$\% \text{ of Yield} = \frac{W_1}{W_2} \times 100$$

where, W_1 is the weight of the extract after solvent evaporation, W_2 is the weight of the plant powder.

2.5 HPTLC method

HPTLC method was developed for evaluation of scopolamine content in *D. metel* Linn. The following conditions were selected for the chromatography.

Instrument: HPTLC method was developed for evaluation of scopolamine in *D. metel* Linn.

Stationary phase: Merck TLC plates silica gel 60 F 254 (10×10cm).

Mobile phase: Various combinations of mobile phase were used to standardize the HPTLC system for scopolamine content, *viz*., chloroform: methanol: dietylamine (8.5:1.5:0.1), chloroform: acetone: dietylamine (5:4:1), chloroform: methanol: 25% ammonia (85;15:0.7), ethylacetate: methanol: water (95:4:1), chloroform: methanol: formic acid (8.2:2.0:0.1) and chloroform: methanol (80:20).

Preparation of standard solution: 10 mg of standard scopolamine was dissolved in 10 ml methanol to give a concentration of 1 mg / ml.

Preparation of sample solution: 100 mg of chloroform leave, methanolic leave, methanolic stem, and methanolic root extracts of Datura samples were dissolved in 10 ml of methanol and filtered through Whatman No.1 filter paper.

Standard and sample application: For quantification of each extracts, scopolamine active extracts 10 µl of all sample solutions were spotted along with 1-10 µl of the standard solution. The chromatograms were developed and scanned at 191 nm. The amount of scopolamine present in each extracts was calculated by comparing the peak area of standard and respective samples.

Development and scanning: The spotted plates were individually developed up to 85 mm in a previously saturated chamber. The developed plate was scanned in the range of 190- 400 nm. The nm at which the peak obtained maximum height and area was considered as max. Chromatogram was scanned at max to compare the peak area of sample and standard.

Derivatization: 100 ml modified dragondroff's reagent was used as spraying reagent.

2.6 Statistical analysis

All the data were replicated thrice and statistically determined Mean ± SEM of mean and the graphs were prepared by Microsoft excel 2010. A linear correlation coefficient analysis was performed in order to determine relationship among different parameters evaluated from leaf, stem and root extracts of Datura plant and correlation between content of phytoconstituents and extract. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

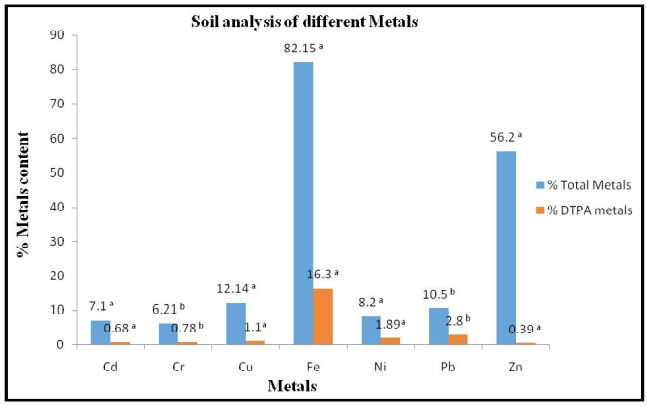
3.1 Soil analysis

Before collection of the plant samples, the soil nature of the Bangalore zone was analyzed as per the above procedure, described in the earlier section and the result was tabulated in Table 1 and Graph 1.

Table 1: Preliminary soil sample analysis

Soil parameter	Report
pH	7.80±0.02
EC (1:5) ($mScm^{-1}$)	14.20±0.16
Eh (1:1) (mV)	16.20±0.20
Organic C (%)	0.32±0.01
PO_4^{3-} (mg kg^{-1})	7.20±0.13
Sand (%)	62.03±0.54
Silt (%)	16.67±0.22
Clay (%)	21.30±0.28
Texture	Sandy loam
CEC (cmol kg^{-1})	13.20±0.12

- Values represent mean of three replications ± $SE_{\bar{X}}$



Graph 1 : Soil analysis of report of Bangalore zone

- Values represent mean of three replications ± $SE_{\bar{X}}$, same letter(s) in a graph represent non-significant difference between the metals.

3.2 Pharmacognostical evaluation

3.2.1 *Microscopic characters*

3.2.1.1 *Leaf powder*

The leaf shows uniformly thick and prominent vein lets forming well defined vein islets and vein- terminations. The islets (areoles) are wide and variable in shape. Smaller islets are squarish and larger ones are rectangular to polyhedral. Vein-terminations are invariably present in all islets. Majority of the terminations are simple, long, slightly curved. Calcium oxalate drases are abundant in the vein islets. Some nonglandular, unisarriate, unbranched trichomes are observed in the powder (Figure 1). They have thick walls with watery surface with 150 µm long and 20 µm wide at the base. The trichomes have tapering tip and curved shape.

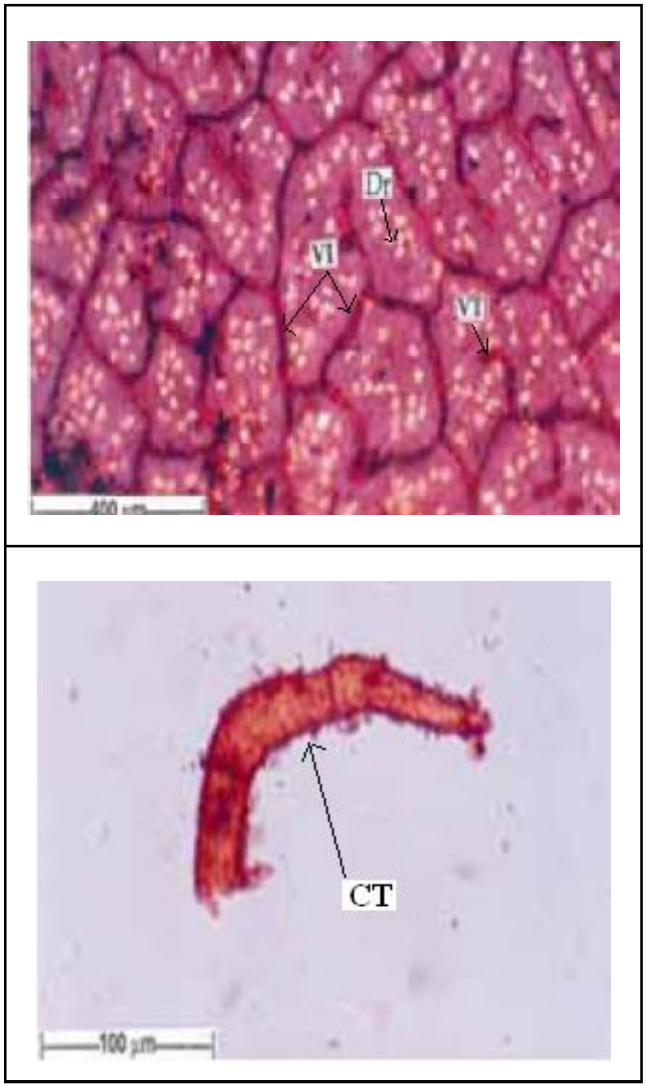


Figure 1: VT= Vein termination; VI= vein lets; CT= Curved trichome; Dr=Drases

3.2.1.2 *Stem powder*

Vessel elements and fibers are common elements in the powder. They are wide, short, cylindrical with long or short tails at both ends. They have wide, elliptical, multisaviate lateral wall pits. The vessel elements are 350-520 m long and 90-110 m wide. Fibers of two types are seen in the powder, some are wide and thin walled and some are narrow and thick walled. The narrow fibers are longer (900 m) and wider ones are (600-700 m) long. Tracheids which are similar to fibers, lent with district lateral wall pits, these fibers like tracheids are called fore tracheids. Long, wide uniformly thick-walled, darkly staining canals are frequently seen in the powder. They are probably secretary tubes or canals. Parenchyma cells and the form flaricks are very common in the powder. They are thin walled with simple pits (Figure 2).

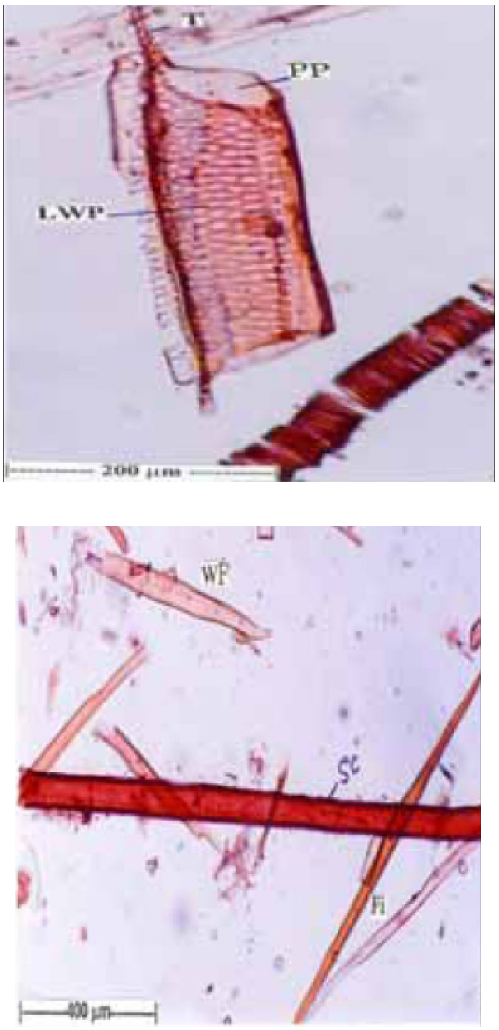


Figure 2 : Fi = fiber; PP = perforation plate; LWP = lateral wall pits; T = tail; XP = xylem parenchyma; Sc = secretory canal; WF = wide fiber

3.2.1.3 *Root powder*

The coarse root powder showed the presence of (i) Vessel elements: The vessels elements are shorts, cylindrical with shorts tails at both ends or at one end. The vessels elements have simple, circular, oblique perforation plate at both ends. Lateral wall pits are elliptical, crowded and well developed. The vessel elements are 270 m long and 50 m wide. (ii) Fibers: They are more abundant in the powder. They are of two types of fibers are observed, *viz*., narrow, thick walled, long and gradually repining fibers that are 750 m long and 20 m wide and wide fibers, they are wide in the middle and abruptly tapering at the ends. Their walls are 700-800 m long and 40 m wide. (iii) Sclereids: They are frequent in the powder. They are rectangular, triangular of square shaped. They have thick lignified walls and wide lumen (Figure 3).

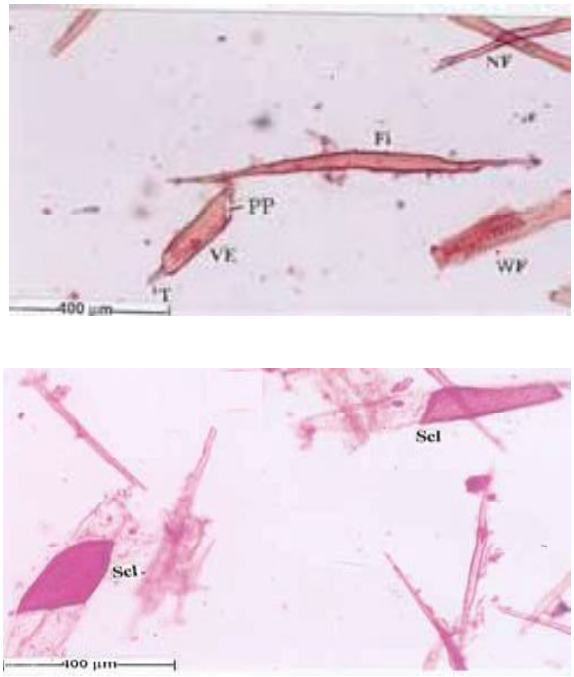


Figure 3: Fi = fiber; VE = vessel elements; PP = perforation plate; NF = narrow fiber; Scl = selereid; WF = wide fiber

3.3 Proximate analysis

All the plant parts were subjected to evaluate moisture content, total ash, acid insoluble ash, alcohol soluble extractive values and water-soluble extractive value as per the method describe above. The results were tabulated in Table 2 and Graph 2.

Graph 2 represented the R^2 values for all the parameters from different parts of plants. Leaf sample showed comparatively higher R^2 value of 0.320 than stem and root part. This may be because of storage of all the nutrients and other metal ions which enhances the result of various proximate analyses.

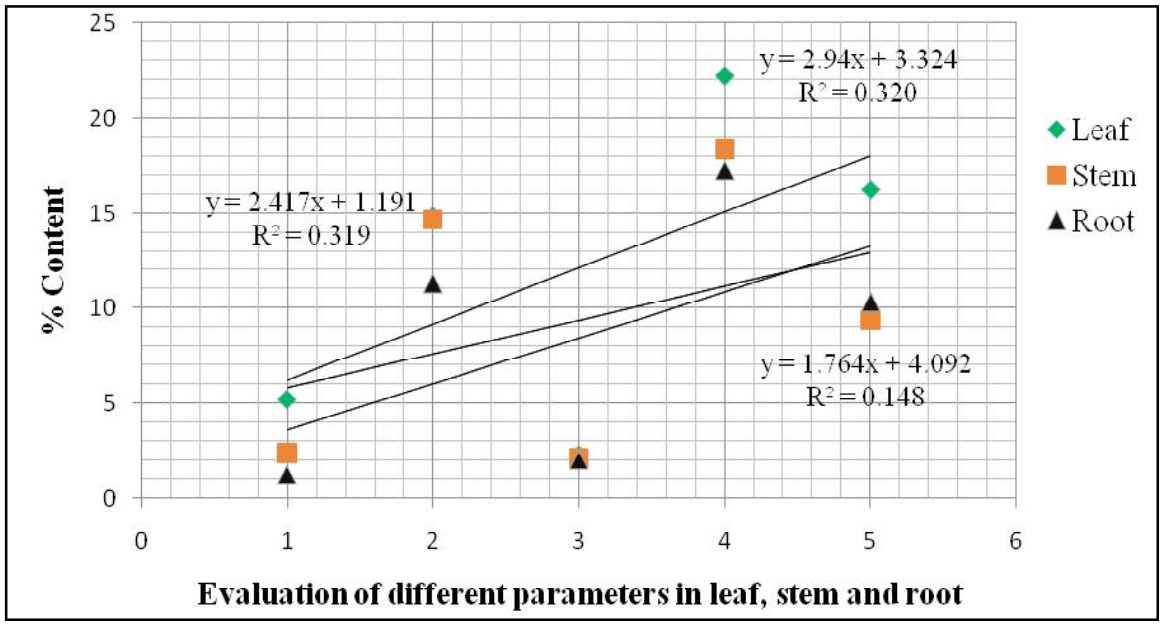
3.4 Yield of extracts

Various solvents, *viz*., chloroform, methanol and water were used for the extraction of plant constituents from leaves, stem and root parts of Datura plant and resulted varied percentage of practical yield which was calculated as per the formula described in the earlier section and the results were tabulated in Graph 3. Methanol leaf extract showed highest percentage of yield of 20.46 ± 0.02*, followed by stem methanol extract (13.27 ± 0.24).

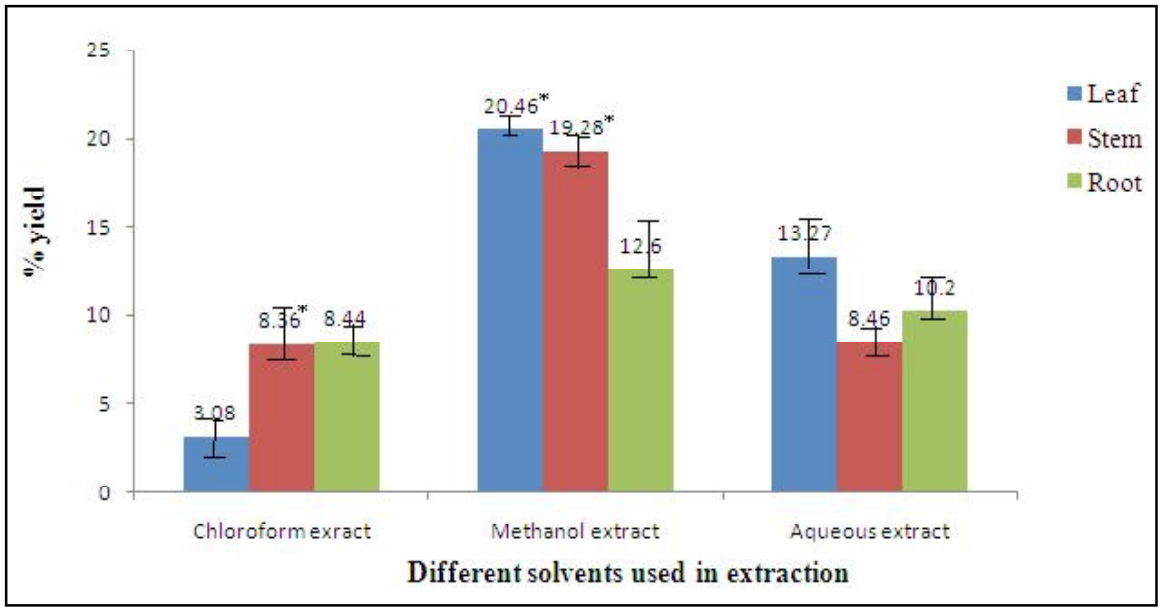
Table 2: Proximate analysis of the leaf, stem and root parts of *D. metel* Linn.

Plant parts	Moisture content (%)	Total Ash (%)	Acid insoluble ash (%)	% alcohol soluble extractive	% water soluble extractive
Leaf	5.20 ± 0.04*	14.82 ± 0.01*	2.30 ± 0.01*	22.18 ± 0.01**	16.22 ± 0.11*
Stem	2.42 ± 0.02*	14.64± 0.03*	2.12± 0.20*	18.36 ± 0.02*	9.38 ± 0.03
Root	1.24 ± 0.16	11.31 ± 0.11*	2.04 ± 0.11	17.28 ± 0.23	10.34 ± 0.02

- Values are triplicates and calculated Mean $\pm SE_{\bar{X}}$. (*) = $p<0.05$; (**) = $p<0.01$



Graph 2: Determination of moisture content, total ash, acid in soluble ash and extractive values in different parts of Datura plant



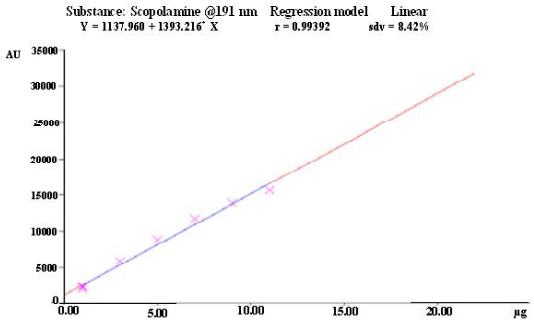
Graph 3: Percentage yields from various parts of Datura plant using different solvents

- All the values were calculated Mean $\pm SE_{\bar{X}}$ (n=3); (*) significant among the extracts.

These correlations showed that solvents played major role for optimization of yield with respect to solubility of the constituents. Earlier reports also revealed the same research (Wang *et al*., 2004; Maji *et al*., 2010; Varughese and Tripathi, 2013; Dasola *et al*., 2014; Das and Tribedi, 2015). Further, all extracts were preliminary evaluated by chemical test for the presence of chemical constituents. Chloroform extract of leaves samples showed the presence of alkaloids, phytosterols, diterpenes whereas methanol and water extracts showed the presence of carbohydrates, proteins and diterpenes. Likely, the stem extracted with chloroform showed the presence of alkaloids, carbohydrate and methanol and water extracts of stem shows the presence of glycosides, phytosterols, flavonoids, proteins and diterpenes. Thereafter, chloroform root extracts showed the presence of alkaloids, methanol root extract showed presence of alkaloids, proteins whereas aqueous extract showed presence of alkaloids, proteins and carbohydrate which were correlated with the earlier reports (Banso and Adeyemo, 2006).

3.5 HPTLC analysis

Among all the above the applied solvent systems, the mobile phase chloroform and methanol (8:2) gave good resolution with Rf value of 0.70. Further, the developed method was quantified in term of limit of detection, limit of quantification and linearity. The limit of detection of scopolamine was found to be 300 ng and the limit of quantification was found to be 900 ng after tabulated linearity graph of scopolamine (1-11 µg). The regression value was found to be 0.99392 % and the standard deviation was found to be 8.42 % and Y value was 1137.960 + 1393.216* X. Concentration and peak area of standard scopolamine obtained by linearity are shown in HPTLC chromatogram and standard scopolamine is shown in Graph 4 and Figure 4.



Graph 4: Linearity curve for standard scopolamine

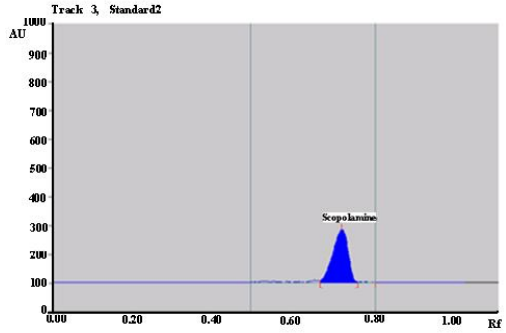
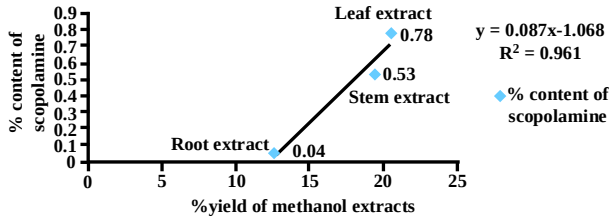


Figure 4: Detection of standard scopolamine in HPTLC at Rf 0.70

Based on standard curve, all the different extracts were applied and calculated the percentage content of the same. HPTLC figure showed that maximum amount of active constituent, *i.e*., scopolamine content observed with methanol extract of the Datura leaf part (0.78%), followed by aqueous leaf (0.58%) and methanolic stem extract (0.27%), whereas chloroform extract showed very less quantities of the same but was also showed higher in leaf (0.03%). Interestingly, the content of scopolamine was positively correlated with the percentage yield when coefficient of correlation was statistically analyzed (Graph 5) and resulted R^2 = 0.961. Das and Dang (2010) have reported the same results which also showed positive in this investigation. This may be due to scopolamine which is highly soluble in methanol. Furthermore, elemental analysis of soil samples revealed the content of Fe is very high and may be accumulation of all the minerals and other nutrients are more in leaf and, hence, leaf sample gave comparatively better results. Earlier report revealed the same concept where leaf sample accumulate maximum mineral content which resulted maximum yield (Fayed, 2010). HPTLC track of methanol extract of leaf was depicted in Figure 5 and HPTLC plate was shown for all the extracts along with standard scopolamine (Plate 1). The scopolamine content (% of dry weight) of leaves was estimated 0.190%, reported by Hiraoka *et al*. (1996) and Issaravanich *et al*. (2013) reported leaf of *D. metel* content 0.56 mg/g (dry wt. basis) scopolamine while estimated by HPLC method. But our results showed 0.78% scopolamine content when estimated by HPTLC (after method validation) which is definitely the impact of cultural condition and soil minerals content. These minerals were accumulated in the leaf through solublized form. From the amount of active constituent present, the quality of the drug can be determined.



Graph 5: Correlation graph between extract and % content of scopolamine

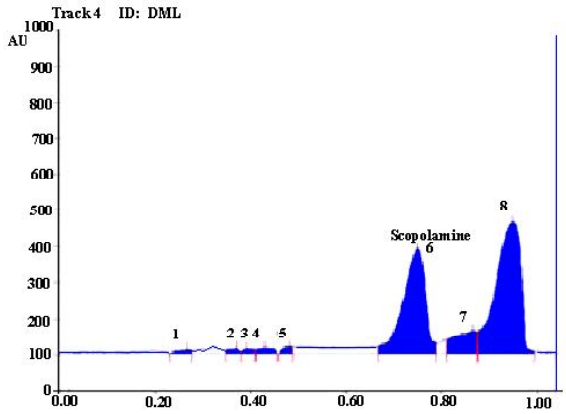
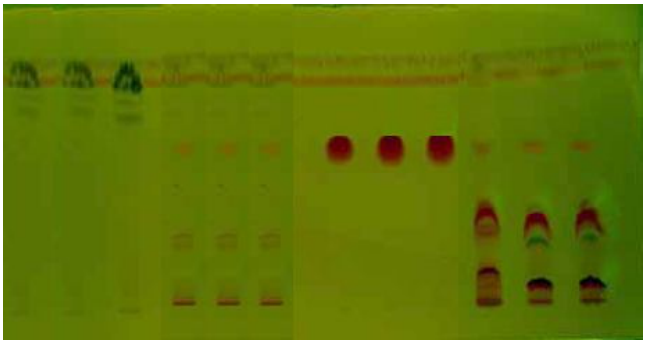


Figure 5: HPTLC chromatogram of datura methanolic leaves (DML) extract

Plate 1: HPTLC plate for all the extracts along with standard scopolamine sample



1 2 3 4 5 6 7 8 9 10 11 12

Track : 1 - 3 Chloroform extract of leaf, stem and root
Track : 4 - 6 Aqueous extract of leaf, stem and root
Track : 7 - 9 Standard scopolamine
Track : 10 - 12 Methanol extract of leaf, stem and root

4. Conclusion

The present study endow that the *D. metel* collected from Bangalore soil zone is healthy and significantly revealed the presence of high content of scopolamine when compared with stem and root part. Thereafter, methanol leaf extract showed superior results in all aspect compared to chloroform and aqueous extracts. Study confirmed with HPTLC chromatography when the same extract showed significant positive result between percentage yield and the scopolamine content. The overall results clearly stated and indicated that cultural condition, soil properties should consider for any study with the medicinal plants. Furthermore, solvent selection will also play a major role for the maximum yield with phytoconstituents as same was reported in this manuscript.

Conflict of interest

We declare that we have no conflict of interest.

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