

Influence of Demographic Location and Solvent Extraction on Pharmacognostical Assessment and Identification of Conessine Content in Different Parts of *Holarrhena antidysentrica* Through HPTLC Analysis

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ABSTRACT

Objective: 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 HA, procured from the Bangalore soil zone, Karnataka, India. **Methods:** Initially the soil parameters are checked for the presence of various metals and other physicochemical properties. Microscopy and macroscopic analysis were performed to under the arrangement of anatomical structures of cells and tissues. Thereafter HPTLC study was performed to determine the presence of conessine in various parts of Kurchi. **Results and discussion:** The results revealed the soil is sandy loam with the pH of 7.40, organic carbon content 0.30%, electrical conductivity (EC) was 13.14 mS cm⁻¹ and the soil redox potential was 17.80 mV. Macroscopical and microscopical evaluation of leaf, stem and root gave special identification characters. Phytochemical investigation reveals the presence of alkaloids, carbohydrate, protein, glycoside, saponin, phytosterols and diterpenes. Thereafter, presence of conessine was identified by HPTLC at 192 nm using mobile phase Toluene, Ethylacetate and Diethylamine (6:3:1) and percentage of conessine resulted higher of 0.51 in methanol bark extract followed by 0.48% in the methanol root extract. This may be due to the soil nature of Bangalore zone and the effect of solvent where the active constituents are soluble maximized to get more yield. **Conclusion:** Pharmacognostical parameters and conessine content in different parts of *Holarrhena antidysentrica* through HPTLC was revealed that was dependent on various factors.

Key words: Extracts, *Holarrhena antidysentrica*, HPTLC, preliminary evaluation, solvents.

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INTRODUCTION

India is the major source of herbal plants because most of the medicinal and aromatic plants are cultivated throughout plain land of India. Apart from that still many medicinal plants are unknown that are naturally growing in the dense forest region of the Himalaya, Western Ghat and Eastern Ghat areas, Northern East part of India. Recently those unknown plants are recognized slowly and mass cultivated for their beneficial activities and supplied to the industries as a source of raw materials. Hence India is the diverse land

of agriculture where any kind of plants can be cultivated as per Good Agricultural Practices. The soil nature of different state gave suitability of the plant growth as well as also gives impact on their phytoconstituents. Many literature surveys showed the impact of geographic variation on plant cultivation as well as better biomass content viz. Lane and Jarvis,¹ reported changes in climate will modify the geography of crop suitability, Kutama *et al.*,² reported an effect of geographical locations and soil characters on



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growth and yield of Cotton (*G. hirsutum*) in Kano State, Nigeria. Significant effect of geographic locations on the types and levels of phytochemicals in various plants was described by Khan *et al.*³ Literatures revealed biological properties affect the efficacy of the raw plant materials as well as the quality of finished products.^{4,5} Furthermore solvent extraction is one of the major concerns that improve herb extract ratio along with high solubility of the phytoconstituents. Sultana *et al.*,⁶ reported radical scavenging activity of medicinal plant extracts dependent on extraction solvent and the technique of extraction. In another research Dent *et al.*⁷ reported an effect of extraction solvents and other parameters of the composition and mass fraction of polyphenols in *Salvia officinalis* L. Extracts. Hence, these two parameters i.e. geographical location and solvent selection for extraction are the prime focus before further investigation of any plant materials.

Oflate HA is a plant which is commonly known as bitter oleander and locally known as “inderjotulkh” or “kurchi”. It is a tropical Asian plant found in the Himalayan and sub-Himalayan tracts at an altitude of 1200 m.⁸ The whole part (leaf, bark, seed and root) of this plant is known to be medicinally useful due to the presence of important bioactive compounds. The main bioactive compounds are alkaloids which are mainly present in the bark, which is varied from 1.1 to 4.7%. The main constituents are alkaloids drugs like Conessine (O-free alkaloid, 0.4%), holarrhenine, holafrine (O-containing alkaloids) and few aminodeoxyglycoesteroids like holarsine B, holantosines E and F from leaves, holacine holacimine from bark. Other alkaloids are kurchine, kurchicine, holarrhimine, conarrhimine, conaine, conessimine, isoconessimine, conimine, holacetin and conkurchine are also present.⁹ Apart from that it has tannins, resins and fat.¹⁰ The plant has been used for long time traditional for a wide range of health problems viz. diarrhea, dysentery, constipation, flatulence and urethrosis.^{11,12} Various research information evident of its carminative, antispasmodic, astringent, anthelmintic, lithotriptic, diuretic, cardiosuppressant and antihypertensive properties.¹³⁻¹⁵ Eventhough standardization is most important to complete understanding about the plant. There are scanty documents and quality control parameters on this plant. It is prime importance to make an effort towards preliminary standardization of this HA plant as the source of a raw material. Effect of cultivation zone, agronomic practice, soil nature is the main and important parameters to know about the raw materials of a plant and these are the basic steps of standardization that are ignored. Further pharmacognostical and phytochemical studies of plant materials are the

initial parameters to distinguish from its related species. Based on that the present comparative study was carried out to determine the effect of soil health and other cultural conditions on HA plant vis-a-vis in relation to identify the plant species by pharmacognostical and physicochemical estimations for the various solvent extracted parts and presence of the constituent (Conessine) through HPTLC method was identified which has scanty scientific evidences from the Indian originated HA plant. Hence the present study was undertaken to investigate and to fulfill the primary steps of Standardization.

MATERIALS AND METHODS

Study area and collection of samples

Bangalore in the state of Karnataka (Latitude 12°58'38 N and Longitude 77°35'14 E) in India was selected for present study zone (Figure 1). Bangalore (officially known as Bengaluru) has a tropical savanna climate with distinct wet and dry seasons. Temperature of Bangalore has a relatively narrow range between 15.4 °C and 36 °C with an average rainfall about 144 mm for the month of June to August.

The different parts of HA (leaves, stem and roots) were collected from cultivated land of Indian Institute of Horticultural Research (IIHR), Bangalore, India and authenticated by Dr. Shivananda T. N, Principal Scientist, Medicinal and Aromatic Plants Division, IIHR, Bangalore. The parts of plants were kept in the department of Pharmacognosy, Al-Ameen College of Pharmacy, Bangalore, as herbarium for future references (Voucher No: 209).

Soil analysis

Soil sample was collected from the IIHR farm. Soil pH was measured with a glass electrode using an equal ratio of sample and water and electrical conductivity (EC) was measured with conductometer using sample and water with a ratio of 1:5.¹⁶ The soil redox potential (Eh) was measured using a standard platinum electrode (HORIBA redox potential meter, Japan). Mechanical analysis was carried out by the International pipette method¹⁷ to determine the percentages of sand, silt and clay from soils. Determinations of cation exchange capacity (CEC) were made in BaCl₂ by the Gilman method¹⁸ and total organic carbon was determined by the wet dichromate oxidation method.¹⁹ PO₄³⁻ was determined colorimetrically (Varian Cary 50 Bio spectrophotometer, Australia), method described by Peachey *et al.* (1973).²⁰ Atomic absorption spectrophotometer (AAS; Perkin Elmer model: AAnalyst 100; Australia) was used to estimate heavy metal content in the extracts. Assess-

ment of few heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in soils was measured using the DTPA/TEA method, developed by Lindsay and Norvell (1978).²¹

Pharmacognostical evaluation

The plant parts were subjected to Powder microscopical examination as per the standard methods followed by the Kokate, 1996.²² Preliminary chemical tests were carried out separately for all the extracts to determine the group of chemical constituents (Harbone, 1994).²³ Quantitative standards like moisture content, total ash value, acid insoluble extractive values and water soluble extractive values were determined as per the standard methods described in Indian Pharmacopoeia, 1996²⁴ and results were tabulated in the result section.

Extraction

Collected all the parts of HA were subjected to extraction using chloroform, methanol and aqueous solvents. Chloroform and methanol extraction were carried out using the soxhletation method whereas aqueous extraction was carried out using the hot maceration method.

250 g of powdered plant parts was subjected to Soxhlet extraction with two solvent such as chloroform, methanol for 6 h. separately. After completion of each extraction, the sample was air dried. Thereafter hot maceration was carried out using aqueous solvent for 8 hrs. All the extracts were concentrated by using a rotary vacuum evaporator at 45°C and further weighed. The percentage of different extractive values was calculated with respect to air-dried substance and percentage of yield was calculated as follows:

$$\% \text{ yield} = W_1 / W_2 \times 100$$

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

HPTLC method

HPTLC method was developed for evaluation of conessine content in HA. The following conditions were selected for the chromatography.

Instrument: HPTLC method was standardized for determination of conessine content in HA plant parts.

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

Mobile phase: Various combinations of mobile phase were selected to standardize the HPTLC system for conessine content likely, n-Butanol: acetic acid: water (4:1:5), Butanol: alcohol: water (20:10:10), chloroform: benzene (5:5), Hexane: Ethylacetate: ethanol: water (2:1:2:1), Cyclohexane: Chloroform: Diethylamine (7:2:1), chloroform: methanol (80:20), Diethylamine: Chloroform: 10% ammonia (3:1:1), Toluene: Ethylacetate: Diethylamine

(6:3:1). The solvent system was optimized and selected based on good resolution, sharp and well-defined peak.

Preparation of standard solution: 10 mg of standard conessine (98% pure) 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 kurchi sample were dissolved in 10 ml of methanol and filtered through Whatman No.1 filter paper.

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

Chromatogram development: The plates were 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 maximum 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.

Statistical analysis

All the data were replicated thrice and statistically determined Mean \pm 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 HA plant and correlation between content of phytoconstituents and extract. Differences were considered significant at $p < 0.05$.

RESULTS

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 Figure 2.

Pharmacognostical evaluation

Microscopic characters

Leaf powder

Small fragments of the lamina were observed in the powder. These fragments were cleaned with chloralhydrate and made transparent to study the venation pattern and vein islets / vein terminations. The lamina

Table 1: Preliminary soil sample analysis (n=3)

Soil parameter	Report
pH	7.40±0.12
EC (1:5) (mScm ⁻¹)	13.14±0.06
Eh (1:1) (mV)	17.80±0.12
Organic C (%)	0.30±0.11
CEC (cmol kg ⁻¹)	12.24±0.02
PO ₄ ³⁻ (mg kg ⁻¹)	6.81±0.11
Sand (%)	65.11±0.14
Silt (%)	14.65±0.20
Clay (%)	20.24±0.21
Texture	Sandy loam

Values represent mean ± SEM

shows a reticulate pattern of venation form district and wide Vein-islets which are squarish, triangular or rectangular. Almost all the islets have well developed branched vein terminations (Figure 3a). They branch repeatedly twice or thrice forming syodial dendroid appearance. Under polarized light microscope fairly large calcium oxalate crystals were seen in the intercostals (vein islet or areole) region as well as along the Veins. The crystals in the areoles are drases or sperocrystals; those along the Veins are prismatic type. The drases are 30-40 μm in diameter. The prismatic crystals are 10 μm thick (Figure 3b). Fragments of the epidermal layer of upper and lower sides are observed which are hexagonal to polyhedral; the antindinal walls are thick and straight. The lower epidermis contains paracytic type stomata. The epidermal cells have thin, wavy antindinal walls. The upper epidermis is apostomatic (without stomata) (Figure 3c).

Stem bark powder

Microscopy of bark powder revealed the presence of shows selereids and crystals. The Selereids are rectangular with thick walls and wide lumen (Figure 4a). They have wide, canal – like simple pits in the lignified walls. They 40-50 μm long and 15 μm wide. Calcium oxalate crystals are abundant with rhomboidal, cubical to rectangular shapes (Figure 4b). The rhomboidal crystals are 20- 30 μm; cuboidal crystals are 40×40 μm; rectangular crystals are 10×20 μm.

Root powder

Microscopy of root- powder showed the presence of fibers, sclereids, vessels, tracheids and starch grains. The fibers are libriform type, with thick, lignified walls, long tapering ends and less prominent pits (Figure 5a). They are 700 μm to 1 mm long and 30 μm thick. The fibers are either Straight or wavy. Sclereids are short, wide,

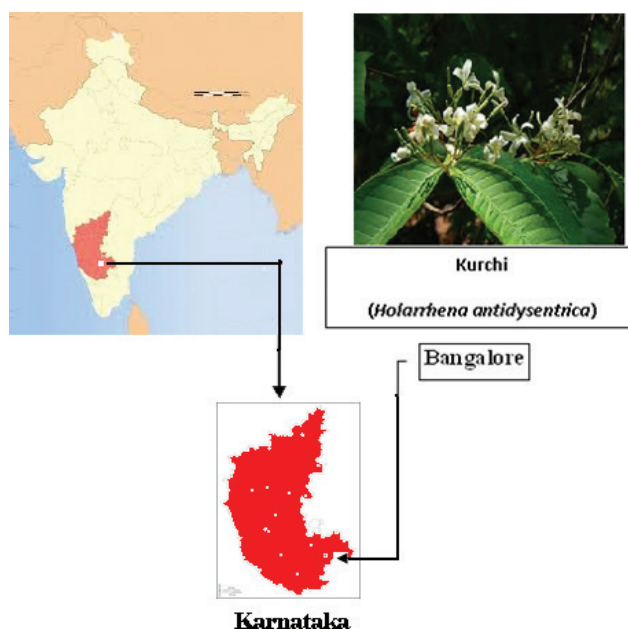


Figure 1: The map showing the locations of HA growing regions from where soil samples and leaf samples were collected in the states of Karnataka, India.

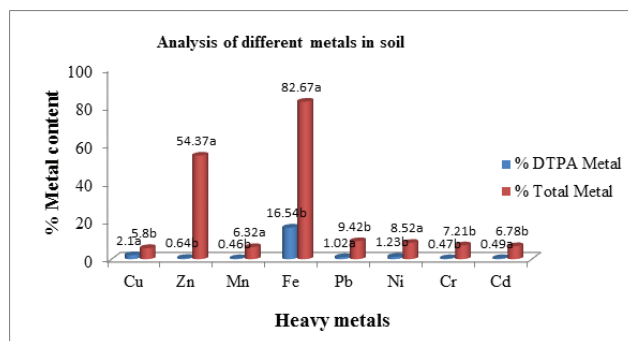


Figure 2: Soil analysis of report of Bangalore zone
Values represent mean of three replications ± SEM , same letter(s) in a graph represent non-significant difference between the metals.

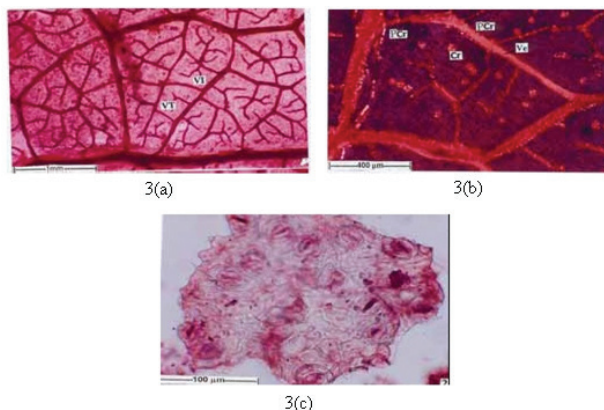


Figure 3: Microscopy of HA leaf
3(a)= VI =Vein-islets; VT = vein termination; 3(b)= Cr= crystal; Pcr = Prismatic crystal; Ve=Vein; 3(c)= Paracytic stomata in epidermis cell

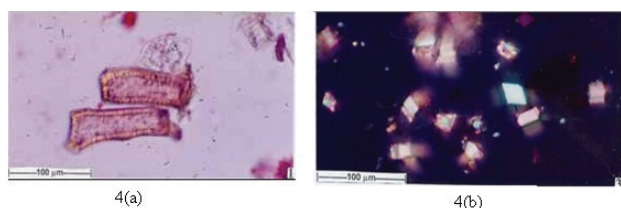


Figure 4: Microscopy of HA Stem bark powder
4(a)= Selereids; 4(b)= Calcium oxalate crystals

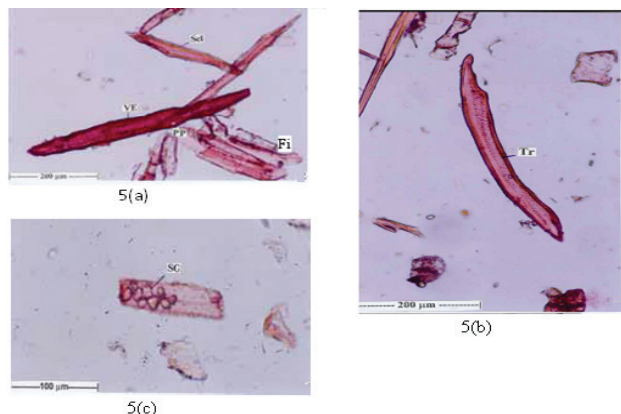


Figure 5: Microscopy of HA root powder
5(a) Fi = fibres VE = vessels elements; PP =perforation plate;
Scl = sclereid;
5(b) = Tracheid; 5(c)= Starch grains

thick walled cells with wide lumen and narrow, canals like simple pits (Figure 5a). They are Straight or lobed. They are 300µm long and 30 µm wide. Vessel elements are long, wide cylindrical cells with blunt ends. They are up to 550µm long and 50 µm wide. Tracheids are more frequent and are similar to the vessel elements, but the tracheids do not have perforations. They are long and cylindrical, thick walled and have dense pits in the lateral walls. They are 450 µm long and 30-40 µm wide (Figure 5b). Starch grains with parenchyma cells are seen in large numbers in the powder. The parenchyma cells are rectangular and thin walled, measuring 25 × 100 µm. Starch grains are elliptical triangular or Spherical. They are 8- 12 µm wide (Figure 5c).

Proximate analysis

All the plant parts viz.leaf, stem bark and roots were subjected to evaluate moisture content, total ash, acid insoluble ash, alcohol soluble extractive values and water-soluble extractive value separately. Leaves showed higher percentage of alcohol soluble extractive (16.8 ± 0.01**) followed by root part (10.4 ± 0.02**) where as leaf sample showed higher percentage of total ash content (13.7 ± 0.01) and acid insoluble ash (4.9 ± 0.10*) than others (Table 2).

Yield of extracts

Various solvents, viz., chloroform, methanol and water were used for the extraction of plant constituents from the leaves, stem and root parts of HA 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 1. The methanol stem bark extract showed highest percentage of yield of 14.80 ± 0.02* followed by a root methanol extract (12.60 ± 0.21).

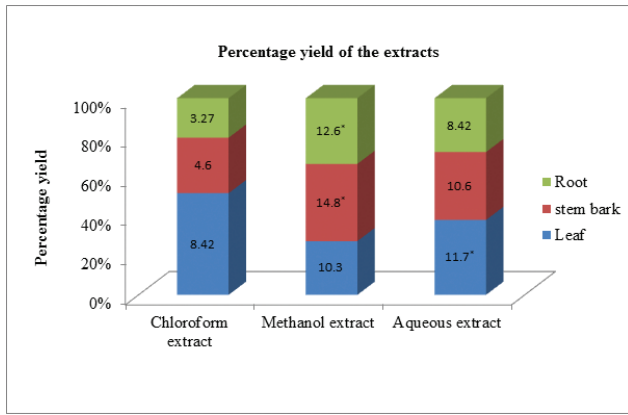
Preliminary phytoconstituents were identified by chemical tests. Methanolic leave extracts showed the presence of alkaloids, carbohydrate, saponins, phytosterols, flavanoids, protiens and diterpenes and an aqueous extract showed carbohydrate, saponin, phytosterol, flavonoids and proteins but chloroform extract not responded any of the reactions. Thereafter chloroform and methanol stem bark extracts showed presence of alkaloids, carbohydrates, glycosides, phytosterol, steriods, phenols, fats and oil, whereas an aqueous extract showed the presence of carbohydrate, glycosides, saponnins, fats, resins and phenols. Further more phytosterol, fats, oils and resins were present in the chloroform root extract, alkaloids, carbohydrate, saponins, resins and flavonoids were presence of methanol extract and carbohydrate, glycosides, saponins, phytosterols, resins and flavonoids were present the in aueous root extract.

HPTLC analysis

Standardized mobile phase Toluene: Ethylacetate : Diethylamine (6:3:1) gave good resolution with Rf value of 0.74. Further, the developed method was quantified in term of the limit of detection, limit of quantification

Table 2: Proximate analysis of leaf, stem bark and root of HA plant					
Plant parts	Moisture content (%)	Total Ash (%)	Acid insoluble ash (%)	% alcohol soluble extractive	% water soluble extractive
Leaf	2.6 ± 0.12	13.7 ± 0.01	4.9 ± 0.10*	16.8 ± 0.01**	8.6 ± 0.10
Stembark	2.4 ± 0.14	11.8 ± 0.01*	1.6 ± 0.14	8.4 ± 0.12*	12.0 ± 0.01*
Root	2.8 ± 0.11	8.3 ± 0.11*	1.9 ± 0.02	10.4 ± 0.02**	6.4 ± 0.13

Values are triplicates and calculated Mean ±SEM . (*) = p<0.05; (**) = p<0.01



Graph 1: Percentage yield of various parts of HA plant

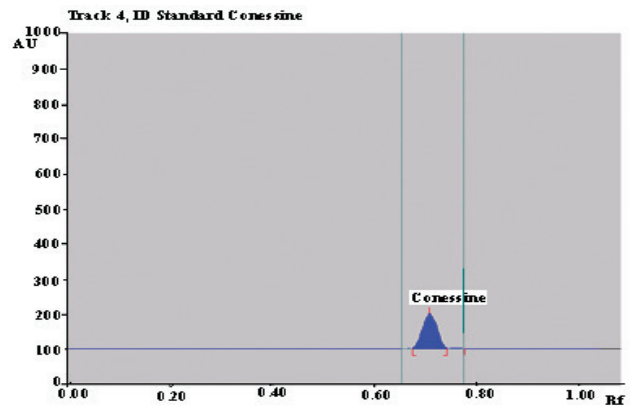
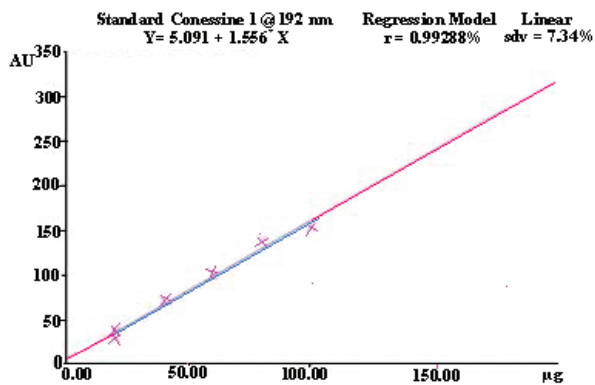


Figure 6a : HPTLC chromatogram of Standard Conessine



Graph 2: Linearity curve for standard conessine at 192 nm

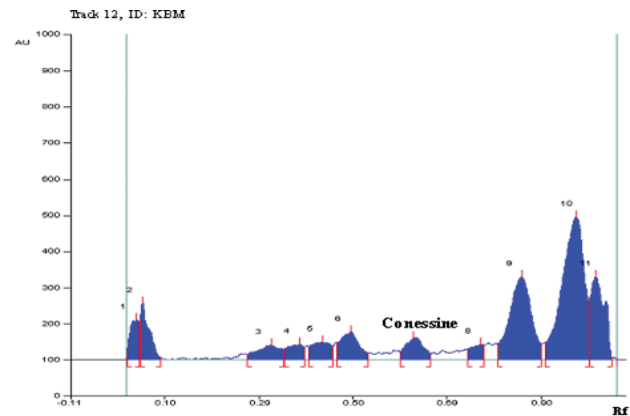
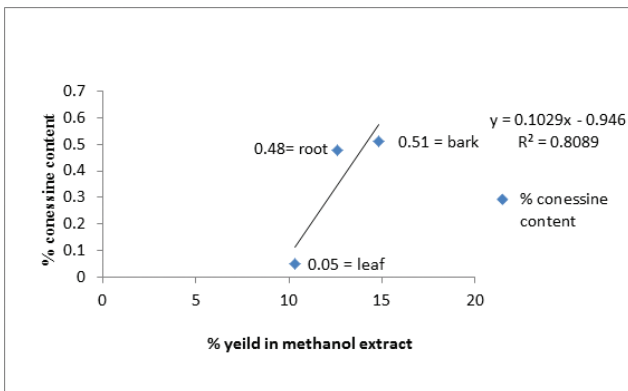


Figure 6b: HPTLC chromatogram of KRM (Kurchi methanolic stem bark extract)



Graph 3: Correlation curve between yield of methanolic extract and percentage conessine content (Leaf, bark and root) in HA plant

and linearity. The limit of detection of conessine was found to be 10 µg and the limit of quantification was found to be 30 µg after tabulating linearity graph of conessine (20-100 µg). The regression value was found to be 0.99288% and the standard deviation was found to be 7.74% and Y values was 5.091 +1.556* X. Concentration and peak area of standard conessine obtained by

linearity are shown in HPTLC chromatogram (Graph 2 and Figure 6a) along with the methanol extract of stem bark extract (Figure 6b). Based on standard curve, all the different extracts were applied and calculated the percentage content of the same. HPTLC figure showed that the maximum amount of active constituents, i.e., conessine content observed with the methanol extract of bark (0.51%), followed by a methanolic root extract (0.48%) and leaf chloroform extract (0.31%) whereas the leaf methanol extract showed conessine content 0.05%. Interestingly, the content of conessine was positively correlated with the percentage yield when the coefficient of correlation was statistically analyzed (Graph 3) and resulted $R^2 = 0.808$.

DISCUSSION

HA plant is native to Tropical countries, especially in India. The plant is tolerant of diversified types of soils. The economic parts of the plant are leaves, roots, seeds and stem bark. The plant plays an important role in the tribal and herbal medicinal system with its valuable therapeutic efficacy due to the presence of many important

alkaloidal constituents, especially conessine and is one of the major plants with number of Ayurvedic formulations. But geographical location and soil nature plays a vital role for the presence of various chemical constituents in plants that are evident of many research publications.^{25,26} Furthermore the geographical reports revealed the Bangalore soil is nearly neutral to slightly alkaline (pH 6.5-7.5) and the pH of the soil greatly varied with the climatic conditions as a result variation in content of plant constituents in the plant.^{27,28} The present study also showed the same results where the contents of the extracts varied with the cultural conditions.²⁹ Macro (N, P, K) and micronutrients (Fe, Mn, Cu and Zn) are the essential elements for healthy plant growth but at the same time non essential heavy elements (Ni, Co, Cr, Pb, As) are also playing an important physiological role in humans and animals. Nutrient solubility is highly pH dependent; a pH range from 5.5 to 7.0 is suitable for high bioavailability of most nutrients essential for plant growth and development.³⁰ Hence there is a basic need to study their content in the plant and the correlation with the various therapeutic activities. Micronutrients are significantly affected by soil pH, decreasing with increasing soil pH. Fe is one of the most essential micro elements for all organisms and its deficiency is one of the major factors affecting quality yields of the plants. Generally its deficiency is associated with high soil alkalinity or in poorly aerated or compacted soils which reduce Fe uptake by plants³¹ and hence the present study was undertaken to know about the role of elements and their interrelationship between the metals and the content of phytoconstituents. Thereafter, pH level above 7.0, the soil organic matter content and Zn deficiency gradually resulted more and resulted negative impact on plant growth. Same correlation followed in our study where results revealed Zn availability was lesser than Fe which was pH dependent. Furthermore Cu is required for the protein components of several enzymes³² but in excess quantities, it is highly toxic to plant growth, resulting in complete inhibition of growth.³³ Our present study also resulted the less content of Cu in the raw HA plant sample than Fe and Zn which may reduce the accumulation of toxic substances like Ni, Cd, Cr and Pb which are well known heavy metals in soils for their cumulative accumulation and adverse effect on plant health.

Macroscopy and Microscopy are the preliminary step for the standardization and based on that arrangement of anatomical structures are identified which further revealed the plant species identification. These studies showed the various anatomical alignment and presence of the cells and tissues which are clearly depicted in herbal monograph and was confirmed as a HA plant.³⁴

The results of proximate analysis indicated variation in moisture content, ash values and extractive values. The variations may be attributed to the geographical locations which are also reported earlier.³⁵ However all the values of proximate analysis were found to be within the stated limits of standard monographs.³⁶ Thereafter various extractive values were determined with solvents like alcohol and water to reveal the solubility of the different parts of HA plants because these are useful to evaluate the presence of soluble chemical constituents in particular solvent.³⁷ Our present investigation resulted less extractive of bark in alcoholic solvent than aqueous solvent which followed the same trend reported by Mahato and Mehta, (2015).⁹ Many literatures revealed that yield of the extracts depends on the geographical location and types of solvent used for the extraction.^{38,39} Our results also followed the same trend where methanol solvent gave a higher percentage of yield with presence of alkaloids, carbohydrate, steroids and flavonoids and the results are correlated with earlier reports.⁴⁰

HPTLC (High performance thin layer chromatography) is widely used chromatographic method for analysis of herbal drugs and their standardization. It is simple, reliable and sensitive method for detection and quantification of phytoconstituents present in crude drugs and their related formulations (single and poly formulations). The method is much suitable for analysis of poly phytoconstituents present in herbal drugs through fingerprinting method. Hence the method was selected to present study for detection of conessine content in HA plant parts and revealed higher percentage content of conessine in methanolic stem bark extract followed by root methanol extract. Earlier report revealed HPTLC method for stem bark and seed extracts for the presence of steroidal alkaloid with the mobile phase toluene, ethyl acetate and diethylamine (6.8: 2.5: 1) and the R_f were 0.78 and 0.71 for bark and 0.75 for seed extract.⁹ Whereas the present study showed, with the same mobile phase at a ratio of 6:3:1 respectively, methanol stem bark gave good resolution with R_f value of 0.74 which was nearer to the earlier report.

CONCLUSION

The present study clearly stated that the HA plant parts collected from a Bangalore soil zone is healthy and significantly revealed the presence of high content of conessine in methanolic stem bark and the same also (stem bark extract) showed superior results in all aspects compared to chloroform and aqueous extracts. Detail elemental analysis study of raw plant materials showed the less accumulation of toxic metals which

were favourable for further investigation. Thereafter, HPTLC chromatography confirmed significant positive result between percentage yield and the conessine content for the different extracts and was proved that geographical location and soil nature, have the impact on plant cultivation and vis a vis solvent plays an important role for a percentage yield containing phytoconstituents which has positive correlation with the percentage yield.

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CONFLICT OF INTEREST

Authors are declared as no conflict of Interest.

ABBREVIATION USED

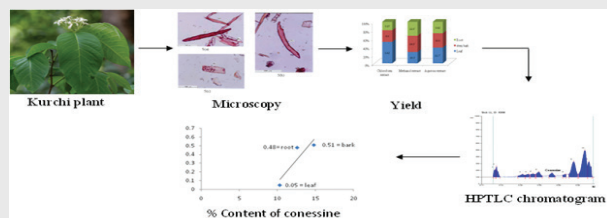
HPTLC: High Performance Thin Layer Chromatography; **HA:** *Holarrhena antidysenterica*; **DTPA:** Diethylenetriamine-penta-acetic acid; **EC:** Electrical conductivity; **CEC:** Cation exchange capacity; **Eh:** Reduction potential.

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PICTORIAL ABSTRACT



SUMMARY

- Kurchi plant has collected from Bangalore soil zone
- Soil analysis was performed for detection of metal ion content
- All the parts of Kurchi were subjected to extraction using chloroform, methanol and aqueous solvents.
- Microscopic evaluation and proximate analysis were carried out.
- The methanol stem bark extract showed highest percentage of yield.
- HPTLC chromatogram resulted the maximum amount of conessine content for the methanol extract of bark (0.51%).

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