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# Phytochemical Profiling of *Cissusquadrangularis* L. stem using HPTLC

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**Abstract:** *Medicinal plants are one of the most sensitive commodity areas of research in the world today. India is the treasure of traditional knowledge of alternative medicine in the world. The importance of medicinal plant in disease control was well explained in Vedas. Cissusquadrangularis L. has been documented in Ayurveda, as a curative medicine for various diseases made it important ancient medicinal plant for pharmacological study. The extract obtained from C.quadrangularis is a magical remedy in gout, syphilis, piles, venereal disease and leucorrhoea. Chromatographic techniques are the strong tool to reveal the constituents of medicinal plant with curative mechanism to be discovered. Among them HPLC and HPTLC are the strong tools for the phytochemical studies.*

**Keywords:** Medicinal plant, Ayurveda, HPTLC, *Cissusquadrangularis* L., Phytochemical

## I. INTRODUCTION

India is one of the greatest emporia of medicinal and aromatic plants. Among the 18 hotspots of biodiversity identified in world, and two are located in India *viz.* Western Ghats and North Eastern region, both rich in diversity pertaining to these crops. India is rich in all the three levels of biodiversity i.e. species diversity, genetic diversity, and habitat diversity. *Cissusquadrangularis* L. is one of the important medicinal plant species used in Ayurveda, or alternative system of medicine in India. *Cissus* genus showed variability at intraspecies level. Variations occur at intra and inter species level that can be studied by systematic attempts through modern approaches like anatomical, biochemical and phytochemical (fingerprinting) analysis. *Cissus* belongs to Vitaceae genus consisting of 350 species (Jakes, 1988).Distributed in tropical and lesser extent to the temperate areas of the world. The genus includes mainly woody vines and characterized by opposed inflorescence with bisexual, tetramerous flowers. The one member of genus is *Cissusquadrangularis* L. commonly abbreviated as "hadsankal." The synonym for *C. quadrangularis*L. is *Vitisquadrangularis* wall. It is an ancient medicinal plant native to hotter parts of the world *viz,* India, Sri Lanka, Tropical Africa, Thailand, Philippines and South Africa(Geissler*et al.,* 2002). Their presence is also reported on the lower slopes of the Western Ghats, and is widespread across drier areas of Arabia and Africa. The pharmacological activities of *Cissusquadrangularis* L. owing to its phytochemical constituents and toxicological studies have gained an insight into it as medicinal plant of utmost importance in bone healing and other ailments(Reddy2017 and Singh 2013).*C. quadrangularis*have been reported for its use in management of obesity and complications associated with metabolic syndrome (Oben *et al.,* 2007).Scientific studies have revealed the *Cissus*extract to possess cardiotonic and androgenic property. Its stem has been reported to have two asymmetric tetracyclic triterpenoids and two steroidal principles (Mehta *et al.,* 2007).The unique chemical constituents of *C. quadrangularis* L. novel indanes and flavonoids along with phytosterols and keto steroids have showen powerful and efficient antioxidant activity (Oben *et al.,* 2007)Phytochemical constituents of medicinal plants can be evaluated and purified using different chromatographic techniques such as HPLC and HPTLC. HPTLC is a very sensitive and reliable technique that ensures identification and potency of herbal formulations (Vijayalakshmi and Balabhaskar, 2017).

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## II. MATERIALS AND METHOD

### 2.1 Plant Sample Collection

The plant use in the study was *C.quadrangularis* L. species. Cuttings of the plant were raised and maintained in the replicated pots. Four month old replicated potted plant raised through cutting and reared under identical conditions serves as a source of young leaf tissue and stem were used in the study.

### 2.2 Phytochemical Study

For phytochemical studies sample were extracted using Direct extraction and Sequential extraction methods followed by HPTLC.

### 2.3 Direct Extraction

**A. Sample Preparation**

The samples were collected and dried in hot air oven at $45^0$Ctill constant dry weight was noted. Materials were made to fine powder using a laboratory mill.

**B. Extraction and Isolation**

The 200 mg of dried powdered sample was extracted using 100ml of solvents viz. Methanol, Hexane, Ethyl acetate and Dichloromethane. Reflux stem samples extracted in 100ml of different solvents at $100^0$C for one hour. Stem extract was cooled and filtered using whatman filter paper. 100ml of respective solvents were added and reflux was repeated for 1 hour at $100^0$C. The obtained solvent was concentrated using rotary vacuumed evaporator. The steam extracts were collected and final volume was made to 10 ml respectively. The extracts were centrifuged at 10000 rpm at $20^0$C in a micro-centrifuge and supernatant were collected for injection in HPTLC.

### 2.4 Sequential Extraction

**A. Extraction and Isolation**

The 200 mg of dried powdered sample was extracted in 100ml of hexane. Reflux stem samples extracted in 100ml of hexane at $100^0$C for one hour. Stem extract was cooled and filtered using whatman filter paper. Stem extract was placed in a vacuum evaporator for evaporation of hexane. 100 ml of di-chloro methane was added and refluxed at $100^0$C for 60 min. extract was filtered and vacuum evaporated same steps were repeated with ethyl acetate and methanol. The final methanolic extract was collected and volume was made to 10 ml using methanol and centrifuged at 10000 rpm for 10 min. at $20^0$C. Supernatant was collected for injection in HPTLC.

**B. Standardization of HPTLC Conditions**

The conditions were standardized for phytochemical profiling on HPTLC system (CAMAG, Switzerland). The performance of different eluting solvents in the separation of the phytochemical constituents was evaluated in terms of shape and resolution of the analyte peaks on pre-coated HPTLC plates. The different wavelengths were optimized for maximum peak area. Different derivatization reagents were used for the standardization of visualization of bands.

**C. Preparation of HPTLC Plates**

The pre-coated HPTLC Plates of 10x10 cm size were used for all the extracts of *Cissusquadrangularis* L. the starting line was marked at 1.0mm from bottom of plates and distance for solvent was 80mm from the bottom of plate.

**Application of HPTLC Plate**

D. The extracts were applied on plates using CAMAG (Switzerland) Linomate 5 automated spray with band applicator equipped with a 100 µl syringe and operated with the seting of eight tracks per plate, band length of 8.0 mm, application volume of 8.0 µl, distance between tracks were 10.8mm, application position was at 12.0 mm, solvent front position was at 80%, scanning speed was at 20mm/sec. and data resolving was at 100 µm/step.

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**E. Development and drying of HPTLC Plates**

The chromatograms were developed in a 10x10 cm CAMAG at room temperature using respective solvent system. The chamber was presaturated for 5 min. Plates were runedupto 80mm height in a vapor-equilibrated CAMAG HPTLC twin-trough chamber containing a saturation whattman filter paper.

Plates were marked for calculation of Rf value and mobile phase was evaporated using dryer at room temperature. These chromatograms were evaluated and scanned under different wavelength (254nm and 366nm) using a CAMAG TLC SCANNER-3.

**F. Derivatization**

The plates were poured in a glass chamber containing derivatives for few seconds. The plates were dried on hot plate and observed under UV cabin at 254 nm and 366 nm.

## III. RESULT AND DISCUSSION

Study on the percent solubility of phytochemicals from stem extract in different solvents revealed that maximum phytochemicals from stems were soluble in methanol (93.51%) followed by hexane (90.98%), dichloro methane (90.84%). However the lowest solubility of phytochemicals was showed by ethyl acetate solvent in compare to rest of solvents used (86.94%). (Table& fig.:1).

The HTPLC study showed that phytochemical profiling at 254 nm and 366 nm scanning wave lengths showed that maximum numbers of bands were detected at 366 nm. These bands represents compound having different Rf values. The bands for stem extract in different solvent were confirmed by comparing the Rf value spectra. Among all the different solvent used for phytochemical fingerprinting, maximum number of bands were obtained by methanolic stem extract in comparison to rest of the solvent at 255 nm.

However lowest number of bands were showed by hexane soluble stem extract (Table:2). The trend observed at 254 nm was not same in case of 366 nm. Maximum numbers of bands were showed by methanol and ethyl acetate solvent. The lowest numbers of bands were observed in dichloro methane (Table: 3)

**Table 1:** Percent Solubility of stem extract in different solvent

| S. No | Solvent | Percent Solubility (%) |
|---|---|---|
| 1 | Hexane- Soluble extract | 90.98 |
| 2 | Dichloro methane- Soluble extract | 90.84 |
| 3 | Ethyl Acetate- Soluble extract | 86.54 |
| 4 | Methanol- Soluble extract | 93.51 |

**Fig.1:** Percentage contribution of stem extract in different solvent

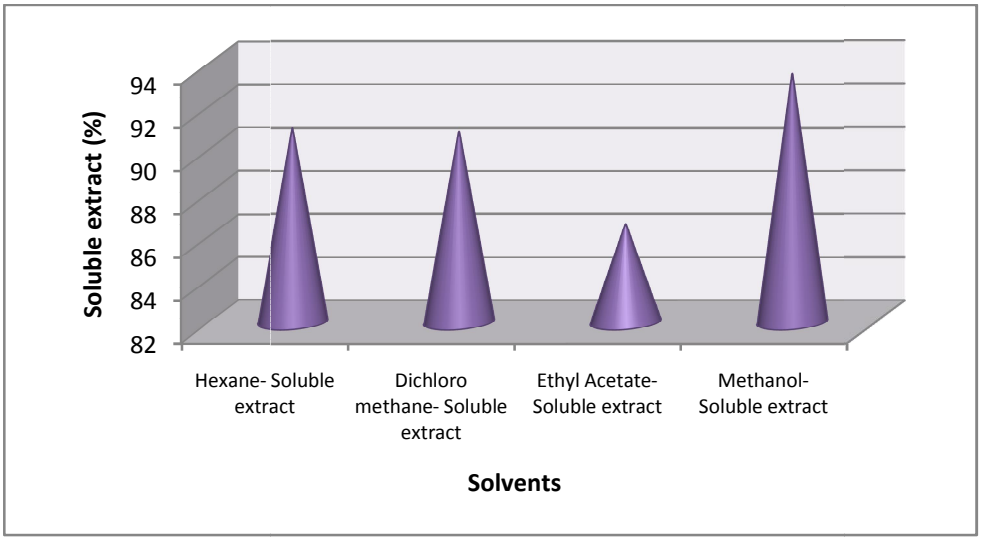

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**Table 2:** Comparison of chromatographic bands in direct extraction resolved in respective solvents extract of *Cissusquadrangularis* L. stem extract (254 nm).

| *Cissusquadrangularis* L. Stem extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Band | Hexane | | Dichloro methane | | Ethyl acetate | | Methanol | |
| | Rf | Area% | Rf | Area% | Rf | Area% | Rf | Area% |
| 1 | 0.86 | 100 | 0.6 | 12.68 | 0.61 | 14.17 | 0.32 | 3.66 |
| 2 | | | 0.69 | 4.71 | 0.85 | 61.58 | 0.39 | 6.17 |
| 3 | | | 0.84 | 59.96 | 0.9 | 24.25 | 0.49 | 7.23 |
| 4 | | | 0.89 | 22.65 | | | 0.61 | 12.68 |
| 5 | | | | | | | 0.81 | 29.12 |
| 6 | | | | | | | 0.85 | 26.6 |
| 7 | | | | | | | 0.9 | 14.53 |

**Table 3:** Comparison of chromatographic bands in direct extraction resolved in respective solvents extract of *Cissusquadrangularis* L. stem extract (366 nm).

| *Cissusquadrangularis* L. Stem extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Band | Hexane | | Dichloro methane | | Ethyl acetate | | Methanol | |
| | Rf | Area% | Rf | Area% | Rf | Area% | Rf | Area% |
| 1 | 0.41 | 4.18 | 0.4 | 9.21 | 0.41 | 6.81 | 0.32 | 15.55 |
| 2 | 0.58 | 2.81 | 0.58 | 8.91 | 0.47 | 0.76 | 0.41 | 8.46 |
| 3 | 0.69 | 14.43 | 0.68 | 14.35 | 0.56 | 7.22 | 0.49 | 3.61 |
| 4 | 0.76 | 16.90 | 0.82 | 47.62 | 0.69 | 13.69 | 0.59 | 9.14 |
| 5 | 0.83 | 31.55 | 0.88 | 19.90 | 0.76 | 20.67 | 0.70 | 13.13 |
| 6 | 0.89 | 30.13 | | | 0.83 | 26.79 | 0.83 | 47.31 |
| 7 | | | | | 0.89 | 24.00 | 0.89 | 16.80 |

## IV. CONCLUSION

*Cissusquadrangularis* L. is a plant of medicinal importance contains various phytochemicals with different medicinal properties. This can be extracted and exploited using different chromatographic techniques like HPTLC. The Rf value of the bands obtained in HPTLC study in present investigation evidenced the presence of phytochemicals in *Cissusquadrangularis* L. The extraction is optimized as different solvents were used.

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