

Phytochemical Investigation, TLC-HPLC Fingerprinting and Antioxidant Activity of *Cissus repanda* Roots

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ABSTRACT

Aim: To perform phytochemical screening, TLC, HPLC fingerprinting and antioxidant assay of Aqueous, Aqueous-Ethanollic and 95% Ethanollic extracts of *Cissus repanda* roots. **Methods:** Aqueous, Aqueous-Ethanollic and 95% Ethanollic extracts of *Cissus repanda* roots were subjected to preliminary phytochemical analysis. TLC patterns of all the three extracts were developed on the basis of phytochemical analysis results. All extracts were subjected to HPLC fingerprinting. The antioxidant potential of all the three extracts of *Cissus repanda* roots were evaluated by DPPH scavenging assay. **Results:** All three extracts showed the presence of various phytochemicals belonging to different chemical class viz. Alkaloid, glycosides, tannins, flavonoids, steroids and polyphenols. TLC fingerprinting of aqueous extracts showed presence of one distinct phytochemical with R_f value of 0.07 while Aqueous-Ethanollic extract showed three phytochemicals with R_f values of 0.11, 0.55, 0.95 and 95% Ethanollic extract showed two phytochemicals with R_f values of 0.16, 0.71. HPLC fingerprints of all extract showed four prominent peaks. Compounds showing peaks at 48.48 and 67.41 min. were found to be around 21.6 and 19.2 % in the extract. All the extracts were subjected to antioxidant activity. Alcoholic extract of *Cissus repanda* roots showed highest antioxidant activity of 72.02 % whereas Aqueous extract and Aqueous-Ethanollic extract showed 61.66 and 40.47 % antioxidant activity respectively. **Conclusion:** The present study provides qualitative phytochemical analysis and antioxidant potential of *Cissus repanda* root extracts. HPLC and TLC fingerprint would be useful identification and isolation of therapeutically important phytochemicals. All extracts of *Cissus repanda* root showed good antioxidant activity.

Key words: Antioxidant assay, *Cissus repanda*, HPLC, Phytochemical analysis, Thin Layer Chromatography.

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INTRODUCTION

Medicinal plants are playing an essential role to prevent various diseases. Medicinal and aromatic plants have been used for treatment of health disorders from ancient time. In developing country the herbal medicines has continuous history of long use. Medicinal plants continue to show a measure role in healthcare system for the large proportion of world's population. Side effects of several synthetic drugs, population rise, inadequate supply of drugs and prohibitive cost of treatments and development of resistance to currently

used drugs for various diseases have led to increased emphasis on the use of herbs as a source of medicines.

Cissus repanda is one of the reported Indian medicinal plants belonging to family vitaceae. It is a large climber, with corky bark and very porous wood. Stems of *Cissus repanda* yields potable water on cutting therefore it is also known as Panivel (Pani-water, Vel-creeper). *Cissus repanda* is found to be present over Tripura, Bihar, Orissa, Kuman to Arunachal Pradesh and Western Ghats region up to 1350 meters height.



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Therapeutic potential of *Cissus repanda* has been well known to traditional system and widely used in folk medicine.¹ Its roots and powder has been traditionally used in the form paste for cuts, wounds and bone fractures.² Some of the plant species belonging to *Cissus* genus have been reported to possess analgesic and anti-inflammatory activity.³ The dichloromethane and methanol extracts of *Cissus repanda* inhibit HSV at various stages in the viral multiplication cycle i.e. attachment, penetration and replication.⁴⁻⁷ It is well reported that *Cissus repanda* leaves contain flavonoids, polyphenols, sterols, quinones, saponins, anthocyanin and saponins.^{8,9} Roots of *Cissus repanda* are pharmacologically important plant parts. In spite of presence of vital chemicals, *Cissus* roots are quite unexplored. Till today, there are no reports of phytochemical analysis, TLC-HPLC fingerprinting and antioxidant activity of *Cissus repanda* roots. Present work would be the first report of phytochemical investigation, TLC and HPLC fingerprinting and the antioxidant activity of *Cissus repanda* roots. Moreover, it was envisaged that proposed work would be useful in quality assessment and identification and isolation of therapeutically important phytochemicals of *Cissus repanda* roots

MATERIALS AND METHODS

Plant material and Authentication

Cissus repanda plant material was collected from local region of Aurangabad district of Maharashtra, India. Authentication of the collected material was carried out at Botanical Survey of India, Pune wide letter no. BSI/WRC/IDEN.CER./2016/485.

Extraction process

Roots of *C. repanda* (1.5 kg × 3) were washed properly so as to remove dirt and the soil material. Roots were cut into small pieces and subjected to size reduction using industrial scale grinder (Devika, 1.5 HP 230 V). Three different types of *C. repanda* root extracts viz. Aqueous (CRF-1), Aqueous-Ethanollic (CRF-2) and 95% Ethanollic (CRF-3) extracts were prepared by cold maceration technique. Twenty gm of *Cissus* roots were cold macerated using solid to solvent ratio of 1:10 w/v. Maceration process was carried out for 24 hr. After maceration, all three extracts were filtered by and filtrate was concentrated using rotary vacuum evaporator at 50°C to obtain final extracts.

Preliminary phytochemical screening of extracts

All the three extracts were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins

and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids. Phytochemical screening was performed using standard procedure available in literature.^{10,11}

TLC Fingerprinting

TLC patterns of all the three extracts were developed on the basis of phytochemical analysis results using three different mobile phases viz. Chloroform: Methanol: Water (7:3:0.4 v/v/v), Chloroform: Methanol (8:2 v/v) and Ethyl acetate: Methanol (8:2 v/v) for CRF-1, CRF-2 and CRF-3 extracts respectively. The retention factor was calculated using

$$R_f = \frac{\text{Distance move by the substance (cm)}}{\text{Distance move by the solvent (cm)}}$$

Distance move by the substance (cm)

Distance move by the solvent (cm)

HPLC fingerprinting

Instruments

Chromatographic analysis was performed on High Performance Liquid Chromatography (Agilent Technologies) equipped with a G1329B auto sampler system and G1315F variable wavelength detector (Agilent Technologies) was used for the analysis. Mobile phase was degassed by using Ultrasonicator (PCi Analyticals). HPLC grade water was obtained from extra pure water purification system (Lab link). For weighing of chemicals, Vibra HT (Essae) analytical balance was used.

Chromatographic conditions

All the extracts of *C. repanda* viz. CRF-1, CRF-2 and CRF-3 were analyzed at 254 nm. The chromatographic separation was performed on C₁₈, 250 x 4.6 mm, 5 µm, Intersil ODS-3V (GL Sciences) having column oven temperature 40°C with the mobile phase: 1% acetic acid in water: Acetonitrile (gradient mode) at flow rate 1 ml/min with total run time of 120 min.

Antioxidant Activity of *Cissus repanda* root extracts

The antioxidant potential of all the three extracts of *Cissus repanda* roots was investigated using 2, 2-diphenyl-1-picryl-hydrazil (DPPH).^{12,13} Five mg DPPH was weighed accurately using a pre-calibrated Analytical balance (Vibra HT, Essae) and transferred to a 5 mL volumetric flask. It was dissolved in ethanol using sonication and diluted to achieve a solution with 1 mg mL⁻¹ strength (stock I). The stock I was suitably diluted with ethanol to obtain DPPH solution with 0.3 mM strength. The control reaction mixture

consisted of 100 μL of 0.3 mM DPPH solution and 3.9 ml of ethanol whereas test reaction mixture consisted of an additional 100 μL of 1 mg mL^{-1} extracts/fractions. After an incubation period of 30 min, reduction of a DPPH free radical was measured by recording the absorbance (abs) of test and control reaction mixtures at 517 nm. Percent scavenging of the DPPH free radical was measured by using the following equation:

$$\% \text{ scavenging activity} = \frac{[(\text{abs of control} - \text{abs of test}) / \text{abs of control}] \times 100}{\text{Eq. 1}}$$

The ascorbic acid was used as a reference standard in DPPH scavenging capacity assay. The antiradical activity IC_{50} , defined as the concentration of sample showing 50% DPPH radical scavenging activity was determined for ascorbic acid and extracts from *C. repanda*.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of extracts

Phytochemicals play a measure role in traditional and modern system of medicine for treatment of various disorders. Plants contain number of secondary metabolites which are used in Pharmaceutical industries. Aqueous extract of *C. repanda* roots showed presence of alkaloids, flavonoids, steroids and carbohydrates. Hydro alcoholic extract showed the presence of polyphenols, alkaloids, carbohydrates, flavonoids, steroid tannin and protein while its ethanolic extract shows the presence of flavonoids, alkaloids, tannin, steroid, polyphenols, carbohydrates and protein. Results are depicted in Table 1.

TLC profiles of CRF-1, CRF-2 and CRF-3

Thin Layer Chromatography is technique used for separation of various phytochemicals. In TLC, components are partitioned between stationery phase (usually silica gel) and a solvent (mobile phase). It can also

Sr. No.	Phytochemical Tests	CRF-1	CRF-2	CRF-3
1	Alkaloids	Present	Present	Present
2	Glycosides	Absent	Absent	Absent
3	Flavonoids	Present	Present	Present
4	Carbohydrates	Present	Present	Present
5	Steroids	Present	Present	Present
6	Polyphenols	Absent	Present	Present
7	Tannins	Absent	Present	Present
8	Proteins	Absent	Present	Present

Extract	Mobile Phase	R_f value
CRF-1	Chloroform: Methanol: Water (7:3:0.4 v/v/v)	0.07
CRF-2	Chloroform: Methanol (8:2 v/v)	0.11, 0.55, 0.95
CRF-3	Ethyl acetate: Methanol (8:2 v/v)	0.16, 0.71

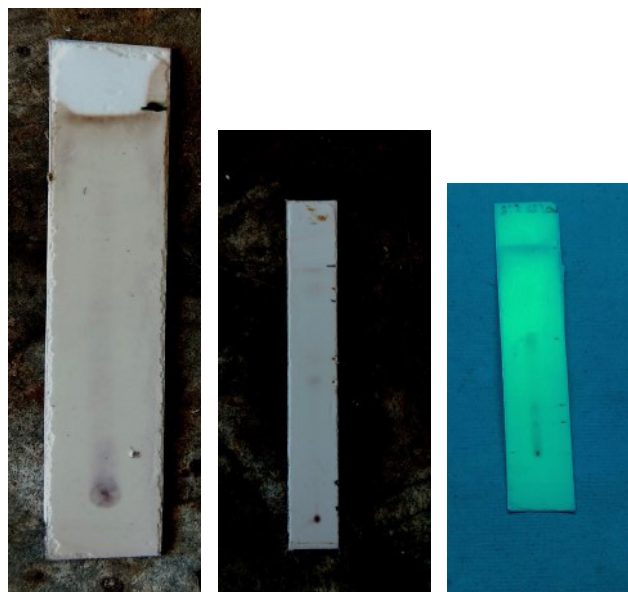


Figure 1: TLC Fingerprint of aqueous [A], Aqueous-alcoholic [B] and 95% alcoholic extract of *Cissus repanda* roots.

be used to identify compounds by comparison with known samples. The result of TLC is expressed in the form of R_f value (Retention factor). The different R_f values indicate the presence of different Phytoconstituents in the same extract. The R_f values of TLC are shown in Table 2 and photographs are shown in Figure 1.

HPLC Finger Prints of CRF-1, CRF-2 and CRF-3

HPLC analysis of CRF-1, CRF-2 and CRF-3 confirmed the presence of different phytochemicals. The HPLC fingerprints of all three extract were shown in Figure 2-4. Each extract showed around twelve peaks in HPLC representing twelve compounds, of which four peaks (compounds) were found to be very prominent. Compounds showing peaks at 48.48 and 67.41 min. were found to be around 21.6 and 19.2 % in the extract.

Evaluation of antioxidant assay

The antioxidant activity was determined by DPPH scavenging assay. The antioxidant assay of CRF-1, CRF-2 and CRF-3 showed good DPPH scavenging activity. Amongst all three extracts CRF-3 showed highest antioxidant activity followed by CRF-1 and CRF-2

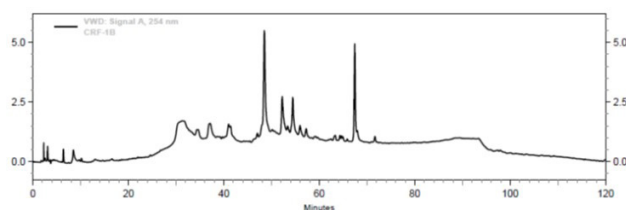


Figure 2: HPLC Fingerprint of Aqueous extract of *Cissus repanda* roots.

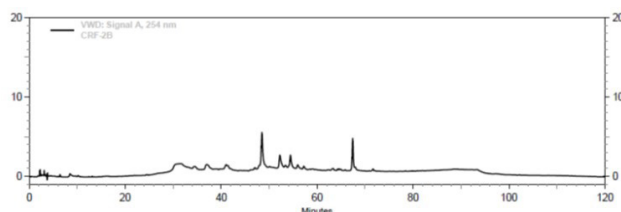


Figure 3: HPLC Fingerprint of Aqueous-alcoholic extract of *Cissus repanda* roots.

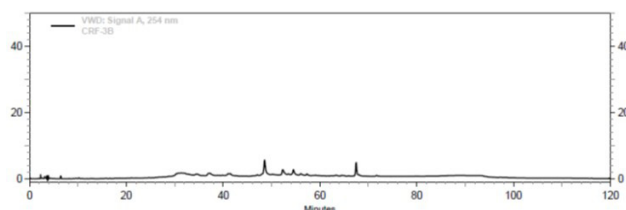


Figure 4: HPLC Fingerprint of 95% Alcoholic extract of *Cissus repanda* roots.

Table 3: DPPH scavenging of *Cissus repanda* root extracts and standard antioxidant (%).

Sample	% DPPH scavenging activity
Control	0.011
Ascorbic acid Standard	86.90
Aqueous Extract (CRF-1)	61.66
Hydro-alcoholic Extract (CRF-2)	40.47
Alcoholic Extract (CRF-3)	72.02

compared with standard antioxidant (Ascorbic acid). Results are shown in Table 3.

CONCLUSION

Phytochemical investigation of *Cissus repanda* extracts showed the presence of compounds belonging to variety of chemical classes like alkaloids, glycosides, flavonoids, tannins, steroids, carbohydrates, polyphenols and proteins. The presence of different phytochemicals was re-confirmed by TLC and HPLC fingerprints. The TLC fingerprinting showed number of spots in all three extracts. HPLC analysis confirmed the presence of different phytochemicals. All three extracts showed

good antioxidant activity by DPPH scavenging antioxidant assay. HPLC and TLC fingerprint would be useful for the quality assessment of raw material of *Cissus repanda* and also helpful in the identification and isolation of therapeutically important phytochemicals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

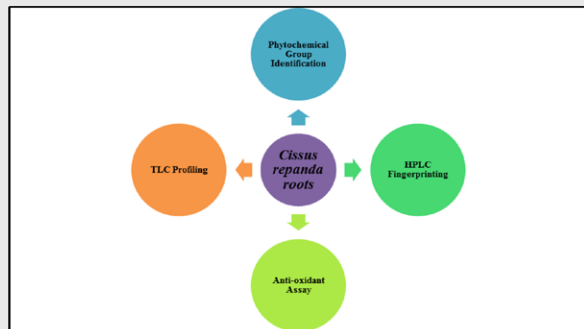
TLC: Thin Layer Chromatography; **HPLC:** High Performance Liquid Chromatography; **CRF-1:** Aqueous extract of *Cissus repanda* roots; **CRF-2:** Aqueous-Ethanollic extract of *Cissus repanda* roots; **CRF-3:** 95% Ethanollic extract of *Cissus repanda* roots.

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PICTORIAL ABSTRACT



SUMMARY

The proposed work explored the phytochemical composition of *Cissus repanda* roots along with TLC-HPLC fingerprinting and antioxidant activity. The results obtained from given research work will be useful for quality assessment of plant and identification and isolation of phytochemicals for various diseases and disorders.

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