Research Article

Pharmacognostic and phytochemical investigation of *Cissus repanda* Vahl leaves

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Abstract

Objective: To evaluate pharmacognostic and phytochemical analysis of leaves of *Cissus repanda*, including macroscopic, microscopic and physicochemical parameters. **Material and Methods**: Macroscopic and microscopic properties of dried leaves were investigated. Preliminary phytochemical analysis of various extracts and fluorescent analysis of dried leaf powder was performed using various chemicals. Physicochemical parameters were evaluated as per WHO guidelines. **Results**: Organoleptic parameters *Cissus repanda* leaves like colour, shape, size etc were noted. Transverse section of fresh leaves revealed the presence of collenchyma, non-lignified xylem and phloem cells and Calcium oxalate crystals in mesophyll cells. Whereas, powder microscopy showed the presence of Trichome, xylem parenchyma cells, lignified cells, crystals of calcium oxalate and Helical Xylem Vessel. Phytochemical screening of ethanolic extract of *Cissus repanda* leaves showed presence of alkaloids, flavonoids, glycoside, steroids and carbohydrates. Hydro-alcoholic extract showed the presence of glycoside, flavonoids, tannin and steroid while its aqueous extract showed presence of glycoside, tannins and flavonoid. Physicochemical parameters viz. ash value, extractive value, moisture content and fluorescent analysis of leaf powder were determined. **Conclusion**: The present study revealed pharmacognostic and phytochemical parameters of *Cissus repanda* leaves which would be beneficial for its standardization and future scope of the work.

Keywords: *Cissus repanda*, pharmacognostic study, phytochemical analysis

Introduction

Since long, plants have been used for medicinal purposes across a globe. Indian system of medicine is based on effective use of plants for the therapeutic purposes. Large numbers of plants are well documented in ancient medical literature of India. *Cissus repanda* belonging to family vitaceae is one of the reported Indian medicinal plants. It is a large climber, with soft, very porous wood with corky bark. 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 Kuman to Arunachal Pradesh, Tripura, Assam, Bihar, Orissa, Madhya Pradesh, and Western Ghats region up to 1350 meters height. Therapeutic potential of *Cissus repanda* has been well known to traditional system and widely used in folk medicine (Harisha et al., 2010a). Its roots and powder has been traditionally used in the form paste for cuts, wounds and bone fractures (Harisha et al., 2010b). Some plants belonging to *Cissus* genus have been reported to possess anti-inflammatory activity[3]. The dichloromethane and methanol extracts of *Cissus repanda* inhibit HSV at various stages in the viral multination cycle i.e. attachment, penetration and replication (Kumar et al., 2013; Nikomtat 2008; Harisha et al., 2012; Salave et al., 2011; Dhivya and Kalaichelvi, 2016). It is well reported that leaves of the *Cissus repanda* contain Sterols, Quinones, and Phenolic compounds, Anthocyanins, Saponins and flavonoids (Ansarali, 2016). In spite of presence of vital chemicals, Cissus leaves are quite unexplored. Till today, there are no reports of pharmacognostic and phytochemical analysis of *Cissus repanda* leaves.

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Considering the therapeutic potential and prospective utility of the *Cissus repanda* leaves in near future, its pharmacognostic and phytochemical studies were planned. Moreover, this is the first report of the above mentioned studies with respect to *Cissus repanda* leaves.

Material 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 Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad and voucher specimen of the plant (No PARC/2012/1299) was deposited for future reference.

Morphological evaluation

The morphological studies shows the important characteristics of plant, the structure of the leaves, the hairy surface of the leaves, the typical tongue sensation and the odour of plant. Fresh leaves of *Cissus repanda* were evaluated for its morphological features like shape, size, color, odour and type. These parameters were evaluated as per standard WHO guidelines.

Microscopical characterization

Transverse sections (TS) of the *Cissus repanda* leaves were made using sharp section cutter. TS were mounted on glass slide using suitable media and observed under a photographic microscope (DMWB1-223ASC). Transverse sections of leaves were studied for different microscopic characters like epidermis, xylem, phloem, parenchymatous cells, starch grains, calcium crystals etc (Sass, 1940; WHO, 1998). Photographs of observed microscopic characteristics of leaves were captured and stored for further referencing.

Powder microscopy

Preliminary examination and behavior of the powder with different chemical reagents was carried out and microscopical examination performed after treatment with different reagents like Phloroglucinol, Conc. HCL, Ruthenium red, Acetic acid and iodine solution. These reagents used for detection of various microscopic components like xylem, phloem, calcium oxalate crystals, starch grain etc. *Cissus repanda* leaves were dried at 50ºC using Tray dryer (S.B. Panchal, Mumbai). Dried leaves were powdered using a lab grinder (Bajaj) and used further for microscopic evaluation. Initially powder was treated with different chemical reagents as shown in table 1.

Table 1. Different chemical reagents used for powder microscopy of *Cissus repanda* leaves.

Sr. No.	Reagents	Importance
1	Phloroglucinol	Identification of xylem and phloem
2	Conc. H_2SO_4 (60%) & Dil. acetic acid	Identification of Calcium oxalate
3	Iodine solution	Identification of starch grains
4	Ruthenium red	Identification of mucilage

Physicochemical analysis

Physicochemical parameters of *Cissus repanda* leaf powder such as extractive values, ash values and loss on drying were determined according to the official methods prescribed in Indian pharmacopeia and the WHO guidelines on quality control methods for medicinal plants materials (WHO, 1998). Extractive values of *Cissus repanda* leaf powder were determined by using different solvents viz. Water, ethanol, ethyl acetate, chloroform and acetone. Five gram dried powder of leaves were placed in 5 glass-stopper conical flasks containing different solvents. All the flasks were placed in a water bath shaker for 6 hours with frequent shaking, and allowed to stand for 18 hours. After 18 hours each extract containing different solvents were filtered and 25 ml of filtrate from each conical flask was dried at 105°C for 6 hours and extractable matter of air-dried material was calculated. The total Ash value was determined by taking ground *Cissus repanda* leaf powder, approximately 2.0 gm was weighed in a previously ignited and tarred crucible (usually of platinum or silica) and it was ignited by gradually increasing the heat to 500-600°C until it was white which indicate the absence of carbon. It was cooled in desiccators and weighed. The content of total ash in mg per gm of air-dried material was calculated. The Acid insoluble ash was determined by adding 25.0 ml of hydrochloric acid (~70g/l) in to crucible containing 2.0gm of total ash. The crucible was covered and boiled for 5 min. The watch-glass was rinsed with 5.0 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter to the original crucible was dried on a hot-plate and ignited to constant weight. The residues were allowed to cool in suitable desiccators for 30 minutes and then weighed. The content of acid-insoluble ash in mg per gm of air-dried material was calculated. The water soluble ash was determined by adding 25.0 ml of water in to the crucible containing 2.0gm of total ash and it was boiled for 5 minutes. Insoluble matter was collected on an ash less filter-paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The content of water-soluble ash in mg per gm of air-dried material was calculated by subtracting the weight of this residue in mg from the weight of total ash. The water soluble ash value was calculated. Loss on drying study was

carried out by using hot air oven. The suitable crucible was taken and empty crucible weight was noted. After that 1 gm of extract was taken and allowed for 2 hours at 105 °C for drying. The following equation was used for measurement of loss on drying. The loss on drying was calculated.

Fluorescence analysis

Fluorescent characteristics of the plant powder as such and after treating them with chemical reagents were observed in daylight as well as under UV radiation. Fluorescent analyses of all the plant powders were carried out according to the methods of Chase and Pratt and Kokoshi (Chase and Pratt, 1949; Kokoshi, 1958). Behavior of powdered plant materials with different chemical reagents was carried out as described by Kay and Johansen (Kay et al., 1938; Johansen, 1940).

Extraction process

The extraction of dried powder of the *Cissus repanda* leaf was performed using maceration and soxhlet extraction process. Accurately weighed, 20 gm of dried leaf powder was subjected to macerate by using different solvent media like 100 ml of Water, Ethanol: Water (50:50) and 100 ml of ethanol. This maceration process was carried out for 24 hrs. Similarly, 20 gm of leaf powder was placed in thimble (Borosil, Mumbai, MH, India), which was inserted into a Soxhlet apparatus and extracted with 200 ml ethanol. This Soxhlet extraction process was carried out for 6 hrs. After completion of maceration and Soxhlet extraction process the solvent was evaporated on rotary vacuum evaporator at a temperature 50°C to obtain ethanolic, hydro-alcoholic and aqueous extract from maceration and ethanolic extract from Soxhlet extraction process. These extracts were stored in vacuum desiccators for further preliminary phytochemical analysis.

Preliminary phytochemical screening of extracts

Extracts of *Cissus repanda* 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. This examination was done using standard procedure (Wagner and Bladt, 1996).

Results

Morphological evaluation

Leaves are green in color, simple, broadly ovate, 12-20 cm in diameter, repeatedly toothed, and tomatoes beneath less above, base deeply cordate, petiole 15-20 cm long, stipules oblong and tendrils dichotomous opposite to leaf. Inflorescence lax, umbellate branched (Figure 1).

Microscopical characterization

Transverse section of *Cissus repanda* leaves shows single layer of epidermis. It also shows the presence of collenchyma, non-lignified xylem and phloem cells. Calcium oxalate crystals are also visible in mesophyll cell as shown in figure 2.

Figure 1. *Cissus repanda* plant

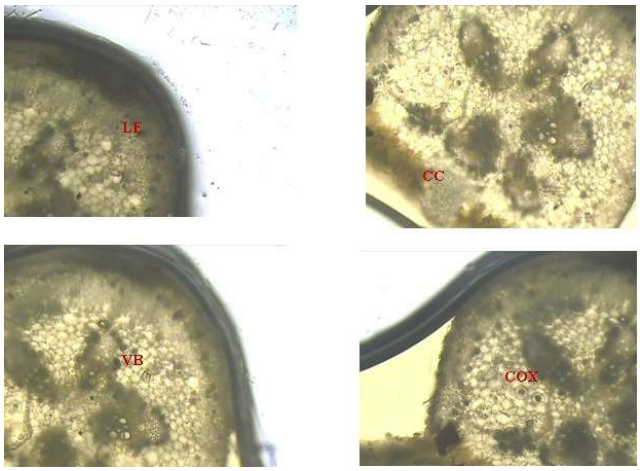

Figure 2. Microscopical analysis of *Cissus repanda* leaves. Transverse section of leaves of *Cissus repanda*: **LE**- Lower Epidermis; **CC**- Collenchyma; **VB**- Vascular bundles; **COX**- Calcium Oxalate Crystals.

Powder microscopy

Powder analysis plays a significant role in identification of crude drug. These characters will help in the identification of right variety and search for adulterants. The micro chemical test of *Cissus repanda* leaf powder reveals the presence of Trichome, xylem parenchyma cells, lignified cells, crystals of calcium oxalate and Helical Xylem Vessel as shown in figure 3.

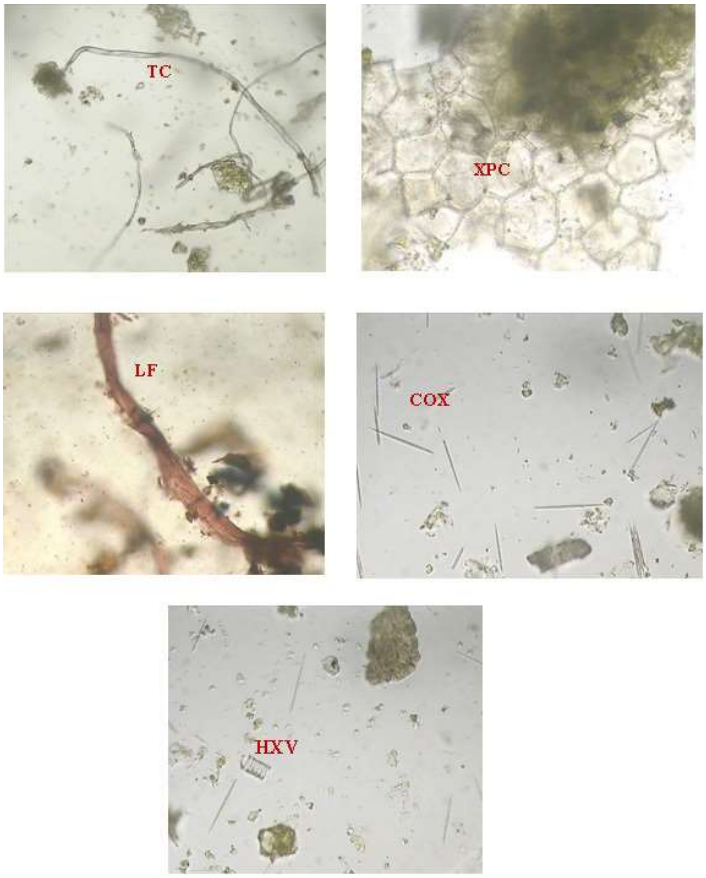

Figure 3. Powder microscopy analysis of *Cissus repanda* leaves: **TC-** Trichome; **XPC-**Xylem Parenchyma Cells; **LF-**Lignified Fiber; **COX-** Calcium Oxalate Crystal; **HXV-**Helical Xylem Vessel**.**

Physicochemical evaluation

Results of various physicochemical parameters viz. Ash, extractive values and loss on drying are summarized in table 2.

Fluorescence analysis

The fluorescent analysis of powdered drug plays an important role in the determination of the quality and purity of the drug. In fluorescence analysis, behavior of powdered plant materials with different chemical reagents was observed. Fluorescence is exhibited by various chemical constituents present in the dried plant material. The leaf powder of the plant samples was extracted in methanol, iodine, H_2SO_4, $FeCl_3$, Hcl, NaOH and H_2O. The fluorescence analysis was observed under ordinary visible light and also under UV light (245 nm). The fluorescence analysis of leaf powder of *Cissus repanda* showed green color under UV light when treated with NaOH, methanol and water, as well as, when the powder is used as such. Greenish black color was

Table 2. Physicochemical analysis of *Cissus repanda* leaves.

Sr. No	Parameters	Values (%w/w)
1	**Ash values**	
	Total ash	12.5%
	Water soluble ash	0.7%
	Acid insoluble ash	1.3%
2	**Extractive values**	
	Water soluble extractives	5.5%
	Alcohol soluble extractives	3.0%
	Ethyl acetate soluble extractives	1.5%
	Chloroform soluble extractives	1.4%
	Acetone soluble extractives	1.0%
3	**Loss on drying**	24.3%

Table 3. Fluorescence analysis of powder of *Cissus repanda* leaves

Particulars	White light	UV light
Powder	Green	Green
Powder +aqueous sodium hydroxide	Florescent Green	Yellowish green
Powder + hydrochloric acid	Florescent Green	Florescent Green
Powder + 50% sulphuric acid	Brown	Florescent Green
Powder + methanol	Florescent Green	Yellowish green
Powder + iodine	Brown	Greenish black
Powder + 5% ferric chloride	Greenish black	Greenish black
Powder + sodium hydroxide + methanol	Yellowish green	Green

Table 4. Preliminary phytochemical analysis of *Cissus repanda* leaves

Sr. No	Constituents	Test/Reagent	Ethanol extract	Hydro-alcoholic extract	Aqueous extract
1	Carbohydrates	Molish's test	+	-	-
2	Flavonoids	Shinoda test	-	+	+
3	Tannins & phenolic compounds	Lead Acetate	+	+	+
		FeCl3	-	-	-
4	Phytosterols	Salkowskii test	+	+	-
5	Alkaloids	Dragendorff's test	+	-	-
		Hager's test	+	-	-
		Mayer's test	+	-	-
6	Glycosides	Borntrager's test	+	-	-
		Keller killani test	-	+	+
		Saponin foam test	-	-	-
7	Amino acids	Ninhydrin test	-	-	-
8	Proteins	Biuret test	-	-	-

observed under visible light when the leaf powder was treated with $FeCl_3$, as well as, the powder without any chemical treatment. Florescent green color was observed in sodium hydroxide, sulphuric acid and methanol under white light and UV light. Various colors like florescent green, yellowish green and brown were also observed under different light conditions. The fluorescence analysis results of *Cissus repanda* leaf powder are depicted in table 3.

Preliminary phytochemical screening of extracts

Phytochemicals play an important role in the treatment of different types of diseases and disorders and are still used in both traditional and modern medicine. Many of the secondary metabolites isolated from plants are used in pharmaceutical industry. Ethanolic extract of *Cissus repanda* leaves showed presence of alkaloids, flavonoids, glycoside, steroids, and carbohydrates. Hydroalcoholic extract shows the presence of glycoside, flavonoids, tannin and steroid while its aqueous extract showed presence of glycoside, tannins and flavonoid. Results are depicted in table 4.

Discussion

Authentication is the primary step towards ensuring starting material. Authentication and standardization are important parameters for determination of quality of crude drugs. For this purpose pharmacognostic evaluation is the first step. Macroscopical characters of the leaves of a plant can be considered as a major identification parameter. Microscopical study of the leaf sample shows the presence of collenchyma, non-lignified xylem and phloem cells and Calcium oxalate crystals in mesophyll cells. Powder analysis of dried leaf powder reveals the presence of Trichome, xylem parenchyma cells, lignified cells, crystals of calcium oxalate and Helical Xylem Vessel. Physicochemical parameters are important for determination of purity and quality of crude drugs. The extractive value of water was highest, followed by alcohol. The ash value represents inorganic components and impurities if any present along with herbal drug. The preliminary phytochemical investigation of different solvent extracts viz. ethanol, water: ethanol and water were examined and it shows the presence of alkaloid, glycosides, flavonoids, steroids, saponins, tannins.

Conclusion

This is the first report of pharmacognostic and phytochemical investigation of *Cissus repanda* leaves. The results obtained during present study facilitate to authenticate the medicinally important plant *Cissus repanda*. Pharmacognostic parameters determined in present study may be useful for establishing the pharmacopeia standards for *Cissus repanda* plant. Preliminary phytochemical analysis may be useful for further phytochemical study and isolation of therapeutically important phytomarkers.

Abbreviations

HSV: Herpes simplex; **WHO:** World health organization; **Hcl:** Hydrochloric acid; **UV:** Ultraviolet**;** H_2SO_4**:** Sulphuric acid; **FeCl3:** Ferric chloride; **NaOH:** Sodium hydroxide; H_2O: water.

Conflicts of interest: Not declared.

References

Ansarali S, Selvarasuvasuki M, Ganapathymuru GL. 2016. Review on Phytochemical and Pharmacological activities of the genus *Cissus* Linn. International Journal of Pharmaceutical Research, 8(4):1-7

Chase CR, Pratt RJ. 1949. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Journal of the American Pharmacists Association, 38:324-331.

Dhivya SM, Kalaichelvi K. 2016. Ethnomedicinal plants used to treat skin disease and poisonous bites by the tribals of karamadai range, western ghats, tamilnadu, india. International Journal of Plant, Animal and Environmental Sciences, 6:53-58.

Harisha CR, Acharya R, Shukla VJ, Chauhan MG. 2010a. Pharmacognostical evaluation of stem of *Cissus repanda* vahl A folk medicine. International Journal of Research in Ayurveda & Pharmacy, 1:13-18.

Harisha CR, Acharya R, Shukla VJ, Chauhan MG. 2010b. Pharmacognostical evaluation on roots of *Cissus repanda* Vahl A folk medicine. Pharmacognosy Journal, 2:17-20.

Harisha CR, Acharya R, Shukla VJ, Chauhan MG. 2012. Development of Random Amplified Polymorphic DNA markers for authentification of *Cissus repanda* vahl. An International Quarterly Journal of Research in Ayurveda, 33:279-83.

Johansen DA. 1940. Plant microtechnique. Mac Graw hill, New York, 182-203.

Kay AL. 1938. The Microscopic study of a drug. Bailere Tindell and Cox, London, 16-21.

Khandelwal KR. 2005. Practical Pharmacognosy, Techniques and Experiments. 13th Edition. Nirali Prakashan, Pune, India.

Kokoshi CJ, Kokoshi RJ, Sharma FJ. 1958. Fluorescence of powdered vegetable drugs under UV radiations. Sci. Journal of the American Pharmacists Association, 48(10):715-717.

Kumar V, Satyanarayana T, Mathew A, Chandrasekhar S, Rajendra G. 2013. In-vivo Anti-inflammatory activity of methanolic extract of *Cissus repanda.* Indian Journal of Research in Pharmacy and Biotechnology, 1:2320–3471.

Nikomtat J, Thongwai N, Saisamorn S, Tragoolpua Y. 2008. Anti-Viral Activity of *Cissus repanda* Vahl. Plant Extract on Herpes Simplex Virus. Journal of Microbiology Research, 3:588-594.

Quality Control Methods for Medicinal Plant Materials WHO, 1998.

Salave AP, Reddy PG, Diwakar PG. 2011. Some unreported ethnobotanical uses from Karanji Ghat areas of Patharditahasil in Ahmednagar district (M.S.) India. International Journal of Applied Biology and Pharmaceutical Technology, 2:240-45.

Sass JE. 1940. Elements of Botanical Micro technique. New York: McGraw-Hill Book Co.

Wagner H, Bladt S. 1996. Plant Drug Analysis. 2nd Edition. Heidelberg, New York, Springer-Verlag Berlin.