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Extraction and evaluation of antimicrobial activity of *Cissus quadrangularis* Linneaus

Garima Mishra, Saurabh Srivastava* and B.P. Nagori*

Swami Vivekanand College of Pharmacy, Ramnagar, Banur (PB) INDIA
**University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh (PB) INDIA*
***Lachoo Memorial College of Science and Technology, Jodhpur (RJ) INDIA*

ABSTRACT : Since ancient ages plants have served human beings as a natural source of treatments and therapies, amongst them medicinal herbs have gain attention because of its wide use and less side effects. *Cissus quadrangularis* Linneaus has been used by common man in India for promotion of fracture healing and well known as "Hadjod". Antibacterial and antifungal activity of n-hexane, chloroform, ethyl acetate, methanol and aqueous extracts of *Cissus quadrangularis* Linn. was studied. The methanolic and aqueous extracts showed remarkable inhibitory activity against various gram-positive and gram-negative bacteria where as moderate inhibitory effect against test fungal strains. The n-hexane, chloroform and ethyl acetate extract were not found to be active against any bacterial or fungal strain. The activity of methanolic and aqueous extracts was also compared with antibiotic Norfloxacin (NFX) and antifungal Ketoconazole (KTZ).

Keywords : *Cissus quadrangularis*, Antimicrobial activity, Extraction, Norfloxacin

INTRODUCTION

Cissus quadrangularis Linneaus (Vitaceae) is a common perennial climber, which is distributed throughout India particularly in tropical regions. It is commonly known as Hadjod, Asthisamhara, Harjora, Vedhari and Hadbhanga in Indian languages. In English it is called as Edible-stemmed Vine. It is prescribed both in Ayurveda as well as Unani systems for its medicinal use as an alternative, anthelmintic, anti-inflammatory, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, and in the treatment of irregular menstruation. It is very useful in asthma, fractures and back pain. The plant extracts also exhibit cardiotonic property. The *Cissus quadrangularis* Linn. Has been recognized as a rich source of carotenoids, triterpenoids and ascorbic acid and is proved to have potential for medical effects, including "Gastroprotective activity" in conjugation with NSAID therapy and in "Lipid metabolism and oxidative stress". The *Cissus quadrangularis* plant contains high amount of vitamin C, Carotene A, anabolic steroidal substances and calcium. Stem contains two asymmetric tetracyclic triterpenoids; Onocer-7-ene-3b-21a diol & Onocer-7ene 3a, 21 diol. The present research focuses on the extraction as well as evaluation of the efficacy of various extracts of *Cissus quadrangularis* against different test bacterial and fungal cultures.

MATERIAL AND METHODS

The plant was collected from Pharmanza India Herbals, Khambhat (Gujrat) and authenticated by Dr. V. Singh, Additional Director, Botanical Survey of India, Jodhpur. The plant was air dried, powdered and standardized and parameters were determined to evaluate the quality and purity of crude drug. The calculated values were compared with the standard values (as per Ayurvedic Pharmacopoeia of India). From the results, it was found that all calculated values lie within the limits and so the collected drug material was of standard quality and purity (Table-1).

Table 1 : Values of standardization parameters.

Parameter	Calculated Value (%w/w)	Standard Value (%w/w)
Total ash	4.2	NMT 5%
Water soluble extractives	24.5	NLT 23%

Preparation of Extract

Air dried powdered whole plant of *Cissus quadrangularis* Linn. was exhaustively extracted with various solvents like n-hexane, chloroform, ethyl acetate, ethanol and methanol using soxhlet apparatus. Aqueous extract was obtained by maceration. These extracts were dried and dissolved in water. The dissolution was facilitated by sufficient quantity of Dimethylsulph-oxide (DMSO). As the principal active constituents of the plant are saponins, phytosterols and phenolic compounds, which are polar in nature, so extracted best in the solvent of the highest polarity along with other polar constituents. (Table-2)

Table 2 : Yield of extracts.

Extract	Amount of powder extracted (g)	Yield (g)	Yield (%w/w)
n. Hexane	1000	4.85	0.485%
Chloroform	300	5.84	1.75%
Ethyl acetate	300	7.42	2.23%
Methanol	300	12.73	3.82%
Aqueous	300	15.0	5.00%

Determination of antimicrobial activity of extracts of *Cissus quadrangularis* Linn.

The strains were procured from Institute of Microbial Technology, Chandigarh and antimicrobial activity was performed for the different extracts against strains of *Escherichia coli*, MTCC No.443; *Bacillus subtilis*, MTCC No. 441; *Staphylococcus aureus*, MTCC No. 96, *Candida albicans* MTCC No. 227 and *Saccharomyces cerivisiae*, MTCC No. 171.

Preparation of reagents and media

Nutrient broth. Peptone (1g), beef extract 1(g) and sodium chloride (0.5g), all of bacteriological grade, were dissolved in 100 ml distilled water and the media was sterilized by autoclaving at 121°C, 15-lb/sq inch pressure for 15 min. The pH of the media was adjusted to 7.3 ± 0.1 by using 0.5 ml of N/10 NaOH.

Nutrient agar. Peptone (1g), beef extract (1g), sodium chloride (0.5g) and agar (2% w/v), all of bacteriological grade, were dissolved in 100 ml distilled water and the media was sterilized by autoclaving at 121°C, 15 lb/sq inch pressure for 15 min. The pH of the media was adjusted to 7.3 ± 0.1 by using 0.5 ml of N/10 NaOH.

Sterile water. Distilled water (100 ml) was placed in 250 ml conical flask and sterilized by autoclaving at 121°C, 15-lb/sq inch pressure for 15 min.

Preparation of innoculum

Vial containing lactose dilution (dehydrated powder) of *E.coli* MTCC No-443 was broken in aseptic conditions using sterile scalpel knife, its contents were added to 100 ml of sterile nutrient broth aseptically. The flask containing this bacterial suspension was incubated for 24 hrs at 37°C in B.O.D. incubator. After 24 hrs, turbidity was observed in the flask and this culture was used to determine number of colony forming units (CFU). Similarly inoculums were prepared for all the strains of bacteria and fungi.

Preparation of stock solution of extracts

10 mg of dry methanolic extract of *Cissus quadrangularis* Linn was dissolved in 10 ml of sterile distilled water aseptically. The solubilization was facilitated by adding 0.05 ml of DMSO. From this a 1000 µg/ml conc. of methanol extract in stock solution was obtained. Similarly stock solution for all extracts was prepared.

Standardization of innoculum

Twenty-four hrs old culture of E. coli in 100 ml of nutrient broth was serially diluted in a ten-fold dilution pattern according to the following steps :

1. 1 ml of the culture was transferred aseptically into a sterile test tube containing 9 ml of sterile water. The test tube was shaken vigorously for good mixing.
2. 1 ml of the bacterial suspension from the first tube was transferred aseptically into a second test tube containing 9 ml of sterile water. The contents were well mixed by vigorous shaking. This procedure was repeated ten times to get dilution of bacterial suspension ranging from 10^{-1} (in 1^{st} tube) to 10^{-10} (in 10^{th} test tube).
3. 0.1 ml of the culture from the test tubes no. 6, 7, 8, 9 and 10 was transferred separately on the surface of solidified sterile nutrient agar media, placed in sterile petri dishes. For each dilution, this procedure was repeated three times.
4. Petri dishes were incubated at 37°C for 24 hr. After 24 hrs the number of colony forming units (CFU) was counted on each petri dish. (Table 3)

Table 3 : Number of colony forming units.

Dilution	Petri dish No.	CFU*
10^{-6}	1	0
	2	2
	3	3
10^{-7}	1	9
	2	8
	3	4
10^{-8}	1	6
	2	7
	3	3
10^{-9}	1	8
	2	12
	3	7
10^{-10}	1	21
	2	32
	3	18

CFU* Colony Forming Units

5. Similarly all the inoculums were standardized and numbers of CFU were counted.

Preparation of stock solution of standard antibacterial drug (Norfloxacin) and standard antifungal drug (Ketoconazole)

1 mg/ml of stock solution of norfloxacin was prepared by dissolving dehydrated powder in distilled water. From the stock solution, further dilutions were made to get solutions of concentration ranging from 5 µg/ml to 200 µg/ml. While determining MIC of test extract, 5µg/ml concentration of norfloxacin was used as standard drug against the calculated CFU of given bacterial culture.

1 mg/ml of stock solution of ketoconazole was prepared by dissolving the dehydrated powder in methanol. From the stock solution, further dilutions were made to get solutions of concentration ranging from 5 µg/ml to 200 µg/ml.

Determination of MIC of various extracts against E.coli, B. subtilis, S. aureus, C. albicans and S. cerivisiae

After swabbing the surface of laminar air flow bench with 70% v/v ethanol. The petri dishes were opened near flame and suitably marked in duplicate for date of inoculation, name of organism, name of drug extract and concentration of extract used.

From the stock solution, dilutions were made directly into the petri dishes using sterile nutrient agar medium, while it was hot. The dilutions were made in such a manner so that the concentration ranging from 5µg/ml to 1000 µg/ml of nutrient media were generated. The media containing different drug concentration was allowed to solidify. When the surface of solidified media was dried completely, 0.1 ml of bacterial suspension was added on the surface of every petri dish except the petri dish marked for negative control.

Positive control was inoculated with bacteria but contained no drug. All the petri dishes with positive and negative control were transferred to incubator in inverted position. After 24-hrs of incubation at 37°C, the petri dishes were observed for the growth of bacteria. Similarly MIC was determined for all extracts against all the bacterial and fungal strains. (Table 4-8)

Table 4 : Observations of MIC of Extracts against *E. coli*.

Extract conc. (µg/ml)	Growth of *E. coli*				
	n-hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
+ ve control	+	+	+	+	+
– ve control	–	–	–	–	–
5	+	+	+	+	+
10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+
100	+	+	+	+	+
200	+	+	+	+	–
400	+	+	+	–	–
600	+	+	+	–	–
800	+	+	+	–	–
1000	+	+	+	–	–

+: growth of microorganism, -: no growth of microorganism

Table 5 : Observations of MIC of Extracts against *B. subtilis*.

Extract conc. (µg/ml)	Growth of *B. subtilis*				
	n-hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
+ ve control	+	+	+	+	+
– ve control	–	–	–	–	–
5	+	+	+	+	+
10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+
100	+	+	+	+	+
200	+	+	+	–	–
400	+	+	+	–	–
600	+	+	+	–	–
800	+	+	+	–	–
1000	+	+	+	–	–

+: growth of microorganism, -: no growth of microorganism

Table 6 : Observations of MIC of Extracts against *S. aureus*.

Extract conc. (µg/ml)	Growth of *S. aureus*				
	n-hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
+ ve control	+	+	+	+	+
– ve control	–	–	–	–	–
5	+	+	+	+	+
10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+
100	+	+	+	+	+
200	+	+	+	+	+
400	+	+	+	+	+
600	+	+	+	+	+
800	+	+	+	+	+
1000	+	+	+	+	+

+: growth of microorganism, -: no growth of microorganism

Table 7 : Observations of MIC of Extracts against *C. albicans*.

Extract conc. (µg/ml)	Growth of *C. albicans*				
	n-hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
+ ve control	+	+	+	+	+
– ve control	–	–	–	–	–
5	+	+	+	+	+
10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+
100	+	+	+	+	+
200	+	+	+	+	–
400	+	+	+	–	–
600	+	+	+	–	–
800	+	+	+	–	–
1000	+	+	+	–	–

+: growth of microorganism, -: no growth of microorganism

Table 8 : Observations of MIC of Extracts against *S. cerivisiae*.

Extract conc. (µg/ml)	Growth of *S. cerivisiae*				
	n-hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
+ ve control	+	+	+	+	+
– ve control	–	–	–	–	–
5	+	+	+	+	+
10	+	+	+	+	+
25	+	+	+	+	+
50	+	+	+	+	+
100	+	+	+	+	+
200	+	+	+	+	+
400	+	+	+	+	–
600	+	+	+	+	–
800	+	+	+	+	–
1000	+	+	+	+	–

+: growth of microorganism, -: no growth of microorganism

Determination of diameter of zone of inhibition of methanol and aqueous extract of *Cissus quadrangularis* Linn

The bacterial and fungal cultures used in this study were *Bacillus subtilis* MTCC No. 441, *Staphylococcus aureus* MTCC No. 96, *Escherichia coli* MTCC No.443, *Saccharomyces cerivisiae* MTCC No.171 and *Candida albicans* MTCC No. 227. The pure cultures of above strains were obtained as lyophilized powder. The contents of each vial were inoculated separately in 100 ml nutrient broth and incubated at 37C for 24 hrs.

The disc diffusion method was used for testing antibacterial and antifungal activity. The filter paper discs of 6 mm diameter were prepared using whatman's no.1 filter paper. These discs were sterilized by autoclaving for 20 min. at 15 lbs pressure. The discs were soaked in extract and dried in laminar air flow. Plain filter paper disc was used as negative controls while the discs soaked in standard antibiotic/antifungal solution were used as positive control. The antibiotic Norfloxacin (5 µg/ml) was used as standard for bacterial culture and Ketoconazole (5 µg/ml) for fungal culture.

The petri-dishes were sterilized in hot air oven and nutrient agar medium was sterilized by autoclaving. To this sterilized medium, 1 ml of bacterial culture was added. This media was poured in the sterile petri-dishes. The filter paper discs impregnated with plant extract were aseptically placed on the solidified agar media. The plain discs and the discs soaked in antibiotic solutions were also placed on the solidified agar media as negative and positive controls respectively. The petri dishes were suitably marked for proper orientation and future reference. The plates were incubated at 37°C for 24 hrs. for bacterial cultures and at 28°C for fungal cultures. Three replications were kept in each case. The diameter of the zone of inhibition was measured in millimeters. (Table 9, 10)

Table 9 : Diameter of zone of inhibition of methanol extract.

Strains	Diameter of zone of inhibition for ME (mm)					NFX	KTZ
	200(µg/ml)	400(µg/ml)	600(µg/ml)	800(µg/ml)	1000(µg/ml)	5(µg/ml)	5(µg/ml)
E. coli	–	14	15	17	18	21	–
B. subtilis	12	16	18	19	20	22	–
S. aureus	–	–	–	–	–	20	–
C. albicans	–	10	12	15	17	–	21
S. cerivisiae	–	–	–	–	–	–	20

Table 10 : Diameter of zone of inhibition of aqueous extract.

Strains	Diameter of zone of inhibition for AE (mm)					NFX	KTZ
	200(µg/ml)	400(µg/ml)	600(µg/ml)	800(µg/ml)	1000(µg/ml)	10(µg/ml)	10(µg/ml)
E. coli	12	15	17	19	20	21	–
B. subtilis	13	18	19	20	21	22	–
S. aureus	–	–	–	–	–	20	–
C. albicans	10	13	15	18	19	–	21
S. cerivisiae	–	12	13	16	17	–	20

* all values include 6 mm diameter of disc also, -: no inhibition, ** all values are mean of three observations

RESULTS AND DISCUSSION

Findings of complete extraction process revealed the highest yield for the aqueous extract followed by methanol, ethyl acetate, chloroform and n-hexane extracts. It has shown that as chloroform is more polar than n-hexane, so constituents of less polar nature (phytosterols) were also extracted along with flavonoids. Due to a medium polarity of ethyl acetate, nearly all the polar constituents (glycoside, saponins, tannins and flavonoids) were extracted in ethyl acetate along with phytosterols.

Thin layer chromatography of all the extracts were carried out and Rf values were calculated using the different mobile phases and the extracted compounds were identified as flavonoids, saponins, phytosterols and triterpenes.

During the standardization of innoculum it was observed that dilution of 10^{-8} was found to be suitable for calculation of CFU present in stock suspension. The present study for determination of Minimum effective concentration (MIC) has revealed that methanolic and aqueous extracts of *Cissus quadrangularis* Linn. Were effective against all strains

except *S. aureus* and *S. cerivisiae*. Only aqueous extract was found to be effective against *S. cerivisiae*. The n-hexane, chloroform and ethyl acetate, extract did not inhibit growth of any bacteria or fungus used in test.

The findings of the study for determination of zone of inhibition in diameter for methanol and aqueous extract of *Cissus quadrangularis* Linn. Has showed that aqueous extract was more active than methanol extract, as in all cases the diameter of zone of inhibition is greater for aqueous extract than methanol extract.

CONCLUSION

The plant *Cissus quadrangularis* Linn. was extracted to get various polar constituents from the crude drug and extracts were evaluated for the presence of different phytochemical constituents. The phytoconstituents were separated by TLC. Some of the phytoconstituents (tannins) and minerals (calcium and potassium) were quantitatively determined and it was found that minerals are abundantly present in the plant *Cissus quadrangularis* Linn. Antimicrobial activity of n-hexane, chloroform, ethyl acetate, methanol and aqueous extracts against *E. coli, B. subtilis, S. aureus, C. albicans* and *S.cerivisiae* strains was performed. n-Hexane, chloroform and ethyl acetate extracts were not found to be effective against any bacterial and fungal strain. Methanol and aqueous extract was found to be active against some bacterial and fungal strain.

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REFERENCES

Rostogi, R.P. and Mehrotra, B.N. (1995). *Compendium of Indian Medicinal*. Plants Vol.3, CDRI Lucknow and PID, New Delhi, 173-174.

Aslokar, L.V., Kakkar, K.K. and Chakre, O.J. (1997). *Glossary of Indian Medicinal Plants with Active Principles*. CSIR Publication, 145-150.

Metha, M., Kaur, N. and Bhutani, K.K., (2001). Determination of Marker Constituents from *Cissus quadrangularis* and their quanitation by HPLC. *Phytochemical Analysis.* **12**(2)**:** 91-5.

Nene, Y.L. and Thapliyal, P.N., (1979). *Fungicide in plant disease control*. Oxford and IBH publishing company, New Delhi. 415-416.

Dipankar, G. and Laddha, K.S., (2005). *Herbal Drug Extraction: An update*. Chemical Weekly, **2**(8)**:** 185.

Chaudhri, R.D. (1996). *Herbal Drugs Industry: A practical approach to industrial pharmacognosy*. eastern publishers, Ist edition, 373.

Harborne, J.B. (1998). *Phytochemical Methods*. third edition, Chapman and Hall publication, 291-293.

Kasture, A.V. Mahadik, K.R., Wadodkar, S.G. (2004). *Pharmaceutical Analysis*. vol. II, Nirali Prakashan, eleventh edition, 35-40.

Mukherjee, P.K. (2002). *Quality Control of Herbal Drugs*. Business Horizons Publication, first edition, 282-283.

Kokate, C.K., Purohit, A.P., Gokhale, S.B. (1999). *Pharmacognosy*. Nirali Prakashan, twelth edition, 172-173.

Prajapati, N.D., Purohit, SS., Sharma, AK. (2003). *A Hand Book of Medicinal Plants*. Agrobios publication. 145.

Ambasta, S.P. (1992). *The Useful Plants of India*. CSIR publication, 127.

Chopra, R.N., Nayar, S.N., Chopra, I.C. (1999). *Glossary of Indian Medicinal Plants*. CSIR publication, 66-67.

Sanyal, A., Ahmad, A. and Sastry, M. (2005). "Calcite growth in *Cissus quadrangularis* plant extract" *Current Science*. **89**(10)**:** 1742-1745.

Jainu, M., Devi, C.S., (2005). "Invitro and Invivo Evaluation of free radical scavenging potential of *Cissus quadrangularis"*. *African Journal of Biomedical Research*. **8**(2)**:** 95-99.

Jainu, M. and Devi, C.S. (2004). "Effect of *Cissus quadrangularis* on gastric mucosal defensive factors in experimentally induced gastric ulcer-a comparative study with Sucralfate". *Journal of Medicinal Food*. **7**(3)**:** 372-6.

Shirwaikar, A., Khan, S. and Malini, S. (2003). Antiosteoporotic effect of ethanol extract of *Cissus quadrangularis*. *Journal of Ethnopharmacology*. **89**(2)**:** 245-250.

Chidambara, Murthy, K.N., Vanitha, A., Mahadeva Swamy, M. and Ravishankar, G.A. (2003). Antioxidant activity of *Cissus quadrangularis linn. Journal of Medicinal Food*. **6**(2)**:** 99-105.

Enechi, O.C. and Odonwodo, I. (2003). An assessment of the Phytochemical and Nutrient composition of the pulverized root of *Cissus quadrangularis. Bio-Research*, **1**(1)**:** 63-68.

Deka, D.K., Lahon, LC., Saikia, J. and Mukit, A. (1994). Effect of "Cissus quadrangularis in accelerating healing process of experimentally fractured radius-ulna of dog" *Indian Journal of Pharmacology*. **26**(1)**:** 44-45.

Mehta, M., Kaur, N. and Bhutani, K. (2001). Determination of marker constituents from Cissus quadrangularis Linn and their quantitation by HPTLC and HPLC. *Phytochem Anal*. **12:** 91-105.