

## Isolation of Alcoholic Extract of *Cissus Quadrangularis* and Evaluation of *In-Vitro* Anthelmintic Activity

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### ABSTRACT

The aim of the present study was the collection and isolation of plant extract of *Cissus quadrangularis* Linn root to evaluate the *in vitro* anthelmintic activity on earthworm *Pheretima posthuma* (Annelida). For the above reason alcoholic and aqueous extract of *Cissus quadrangularis* Linn root was prepared and the *in vitro* anthelmintic activity was evaluated. Both alcoholic and aqueous extract of *Cissus quadrangularis* Linn root were subjected to qualitative phytochemical tests for different constituents and it was confirmed that the plant extract contains constituents such as Phenolic compounds, tannins, proteins, saponins, steroids, carbohydrates, glycosides and triterpenoids etc. The alcoholic extract of *Cissus quadrangularis* Linn root at 100 mg/ml, caused paralysis and death at 17.00 and 30.67 minutes respectively, while, 200 mg/ml, caused paralysis and death at 8.33 and 18.50 minutes respectively against earthworm *Pheretima posthuma* when compared with the reference drug piperazine citrate which showed the same at 19.26 and 63.25 minutes, respectively.

**KEY WORDS:** Anthelmintic, earthworm, *Pheretima posthuma*, paralysis.

### INTRODUCTION

Parasitic helminthes affect animals and man causing considerably hardship and stunted growth. Most diseases caused by helminthes are of a chronic, debilitating nature, they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The prevalence of helminth disease in India is very high, especially during the wet season when infection is as high as 100% in cattle. Such high infection rates prevent them from attaining optimum productivity, especially under the traditional husbandry system. Anthelmintic or antihelminthics are drugs that expel parasitic worms (helminthes) from the body by either stunning or killing them. They may also be called as vermifuges (stunning) or vermicides (killing).

The major control strategy adopted against helminth parasites in India is the use of Anthelmintic. However, the high cost of modern anthelmintic has limited the effective control of these parasites. In some cases widespread intensive use of low quality anthelmintic has led to development of resistance and hence a reduction in the usefulness of available anthelmintic.

During the later part of 20<sup>th</sup> century herbalism has become main stream worldwide. This is due to the recognition of the value of traditional and indigenous pharmacopeias, the incorporation of some derived from these sources in to pharmaceuticals, the need to make health care affordable for all, and the perception that natural remedies are somehow safer and more efficacious than remedies that are pharmaceutically derived. For a variety of reasons more individuals nowadays prefer to take personal control over their health, not only in the prevention of disease but also to treat them. This is particularly true for a wide variety of chronic or incurable diseases (Cancer, Diabetes, Arthritis) or acute illness readily treated at home (common cold etc.). In this respect many individuals have become disenchanted with the worth of allopathic treatments and the adverse effects that can be anticipated. They are seemingly unaware of potential

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problems associated with herbal use or the fact that their limited diagnostic skills, or of those prescribing treatment for them, may prevent the detection of serious underlying conditions like malignancies.<sup>1,2</sup>

*Cissus quadrangularis* Linn is the most common species, belonging to the family Vitaceae. *Cissus quadrangularis* Linn has been used by common folk in India for promoting the fracture healing process and commonly known as "Hadjod" in Hindi or bone setter due to its bone fracture healing property.<sup>3</sup> It has been prescribed in Ayurveda as an alterative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, and in the treatment of irregular menstruation and asthma. In Cameroon, the whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of this plant are important in the management of various ailments. Earlier works on *Cissus quadrangularis* report its effectiveness on the management of obesity and complications associated with metabolic syndrome<sup>4</sup>, as well as its antioxidant and free radical scavenging activity *in vitro*.<sup>5,6</sup> Phytochemical analyses of *Cissus quadrangularis* revealed high contents of ascorbic acid, carotene, anabolic steroidal substances, and calcium. The stem contains two asymmetric tetracyclic triterpenoids, and two steroidal principles. The presence of  $\beta$ -sitosterol,  $\delta$ -amyrin,  $\delta$ -amyrone, and flavanoids (quercetin) has also been reported.<sup>7,8</sup>

The total alcoholic extract of this plant, on parenteral administration, neutralizes the anti-anabolic effects of the cortisone in healing of fractures. The extract of the plant exhibits cardiogenic and androgenic property. Alcoholic extract of the stem showed activity against *Escherichia coli*.<sup>9</sup> The leaf extract showed antifungal activity.<sup>10</sup> The plant contains various secondary metabolites as previously reported.<sup>11,12,13</sup> Since medicinal plants are also used to treat burns, swelling and malaria, one can expect that they might possess analgesic anti-inflammatory and antipyretic activities as well.

In this present study an attempt was made to isolate the alcoholic extract of *Cissus quadrangularis* Linn root and to evaluate *in vitro* anthelmintic activity.

## MATERIAL AND METHODS

### Collection and identification of plant

The fresh plants were collected in rural areas of Erode, Tamilnadu. The plant was identified by a botanist, and voucher specimen was deposited in the department of Pharmacognosy, Vels University.

### Preparation of extract

The coarse powder plant material was extracted with water and ethanol (1:1) by using Soxhlet apparatus. The solvents were removed under reduced pressure to get semisolid mass. The semisolid mass was dried and the aqueous and 50% ethanolic extract of *Cissus quadrangularis* Linn root were used for further studies.<sup>14,15</sup>

### Qualitative phytochemical Analysis

Both alcoholic and aqueous extract of *Cissus quadrangularis* Linn root were subjected to qualitative phytochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, Flavonoids, Phenolic compounds and tannins, proteins and free amino acids, saponins, steroids, and triterpenoids.<sup>16,17</sup>

### Worms collection and Authentication

Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil identified and authenticated by the entomological survey of India, Coimbatore.<sup>18,19,20</sup>

### Preparation of test sample

Samples for *in-vitro* study were prepared by dissolving crude alcoholic and aqueous extract of *Cissus quadrangularis* Linn root in 100ml of distilled water to obtain solution of 100 and 200mg/ml.

### *In-vitro* Anthelmintic Activity

The anthelmintic assay was carried out as per the method of Ajayieoba E.O., et al, with minor modifications. The assay was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.<sup>21,22,23</sup> 50 ml of each concentrations (100 and 200 mg/ml) of both extracts of *Cissus quadrangularis* Linn root were prepared in distilled water and six worms (same type) were placed in it. Time for paralysis was noted neither when no movement of any sort could be observed except the worm was shaken vigorously nor when dipped in warm water at 50 ° C (16, 17). Piperazine citrate (10mg/ml) was used as reference standard while distilled water as the control.

### Statistical Analysis

The values were expressed as mean  $\pm$  SEM. The (ANOVA) followed by Dunnet's T-test. P values <0.01 were considered significant.

**RESULT AND DISCUSSIONS**

Preliminary phytochemical screening of *Cissus quadrangularis Linn* root has shown the presence of tannin, saponins, steroids, triterpenoids, proteins, carbohydrates and glycosides as shown in Table 1.

Phytochemical analysis of the crude extract revealed the presence of tannins among other chemical constituents contained within them. Tannins were shown to produce anthelmintic activities.<sup>24</sup> From the phytochemical analysis *Cissus quadrangularis Linn* root contains tannins, which may be responsible for anthelmintic activity.

Alcoholic and aqueous extract of *Cissus quadrangularis Linn* root exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 100 and 200 mg/ml concentration. The alcoholic extract of *Cissus quadrangularis Linn* root at 100(ml), caused paralysis and death at 17.00 and 30.67 minutes respectively while, 200 mg/ml, caused paralysis and death at 8.33 and 18.50 minutes respectively against earthworm *Pheretima posthuma*.

The reference drug piperazine citrate showed the same at 19.26 and 63.25 minutes, respectively. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis<sup>25</sup> which responsible for anthelmintic activity. The results of anthelmintic activity were shown in Table 2. The alcoholic extract of *Cissus quadrangularis Linn* root not only demonstrated paralysis, but also caused death of worms at both the dose levels tested, in shorter time as compared to reference drug Piperazine citrate.

From the above study it was concluded that the plant extract of *Cissus quadrangularis Linn* root possess the anthelmintic effect and it can be further confirmed by reported anthelmintic effect of tannins that they can bind to free proteins in the gastro intestinal tract of host animal<sup>26</sup> or glycoprotein on the cuticle of the parasite<sup>27</sup> and may cause death. Since the presence of tannins in the plant extract may be the reason for paralysis and death in the earthworm *Pheretima posthuma*.

**CONCLUSION**

The present study was carried out to find out anthelmintic activity of alcoholic extract of *Cissus quadrangularis Linn* root. The phytochemical tests for different constituents confirmed that the plant extract contains phenolic compounds and tannins, proteins, saponins, steroids, carbohydrates, glycosides and triterpenoids etc. The alcoholic extract of *Cissus quadrangularis Linn* root at 100 mg/ml, caused paralysis

and death at 17.00 and 30.67 minutes respectively while, 200 mg/ml, caused paralysis and death at 8.33 and 18.50 minutes respectively against earthworm *Pheretima posthuma* when compared with the reference drug piperazine citrate which showed the same at 19.26 and 63.25 minutes, respectively. The isolated root extract was cost effective and can be best employed for the treatment of worm infestations. The study has shown that, alcoholic extract of *Cissus quadrangularis Linn* root have significantly determined anthelmintic activity and further studies are required to support the reported result.

**Table 1: Preliminary Phytochemical Screening of Ethanolic extract *Cissus quadrangularis***

S.No	Type of constituent	Result
1	Alkaloids	-
2	Phenolic compounds /Tannins	+
3	Flavonoids	-
4	Saponins	+
5	Steroids	+
6	Triterpenoids	+
7	Proteins	+
8	Carbohydrates	+
9	Glycosides	+

**Table 2: The Anthelmintic activity of alcoholic extract of *Cissus quadrangularis linn* against *Pheretima posthuma*.**

Test Substances	Concentrations (mg/ml)	Time taken for paralysis or death of <i>Pheretima posthuma</i> worms(minutes)	
		Paralysis	Death
Alcoholic extract	100	17.00 ± 0.93*	30.45 ± 0.56**
	200	8.33 ± 0.42***	18.50 ± 0.76***
Aqueous extract	100	16.33 ± 1.11*	29.00 ± 0.56***
	200	9.57±0.42 ***	27.0 ± 0.73 ***
Piperazine citrate	10	23.33 ± 0.40	49.33 ± 1.80

Values were expressed as Mean ± SEM (n = 6)  
\*p<0.05; \*\*p< 0.01; \*\*\*p<0.001 Compare Reference control

**REFERENCES**

1. Asolkar L.V, Kakkar KK, Chakre O.J. Second supplement to Glossary of Indian Medicinal Plants

- with Active Principles. *CSIR Publication*. New Delhi. 1992, 61.
2. Kritkar K.R, Basu L.M. Indian Medicinal plants. 2000, 841-43.
  3. Prasad GC, Udupa KN. *Indian Journal Med. Res.* 1964, 52, 480.
  4. Oben J, Kuate D, Agbor G, Momo C, Talla X. The use of a *Cissus quadrangularis* formulation in the management of weight loss and metabolic syndrome. *Lipids in Health and Disease*. 2006, 5(24).
  5. Gabriel Agbor A, Dieudonne Kuate, Oben E, Julius E. Medicinal plants can be good source of antioxidant: Case study of Cameroon. *Pak J Biol Sci*. 2006.
  6. Mallika J, Shyamala CSD. *In vitro* and *In vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*. *Afri J of Biomed. Res.* 2005, 8, 95-99.
  7. Mehta M, Kaur N, Bhutani K. Determination of marker constituents from *Cissus quadrangularis* Linn and their quantization by HPTLC and HPLC. *Phytochem Anal.* 2001, 12, 91-105.
  8. Jakikasem S, Limsiriwong P, Kajsongkarm T, Sontorntanasart T. Phytochemical study of *Cissus quadrangularis*. *Thai J Pharm Sci.* 2000, 24-25.
  9. George M, Venkataraman PR, Pandalai KM. *Journal Science Industries. Res.*1947, (6B), 42.
  10. Misra SS, Dixit SN. *Acta Bot. Ind.* 1949, 7,147.
  11. Sen S. *Curr. Sci.* 1966, 35,317.
  12. Sen SP. *Ind. Journal of Pharm.* 1964, 26,247.
  13. Adesanya SA, Nia R, Martin MT, Boukamcha N, Montagnae A, Pais M. *Journal of Natural Products.*1999,62,1694.
  14. Udupa K.N, Prasad G. Bio chemical Ca45 studies on effect of *Cissus quadrangularis* in fracture repair. *Ind J Med. Res.* 1964, 52, 480-487.
  15. Subbu, V.S.V. Mechanism of action of Vitis glycoside on myocardial tissue. *Ind J Med sci.* 1970, 25, 400-403.
  16. Anoop A., Jagdeesan M. Gastric and duodenal anti-ulcer and Cytoprotective effect of *Cissus quadrangularis* Linn. Variant II in rats. *Nigerian journal of Natural Products and Medicine.* 2002, 6, 1-7.
  17. Murthy K.N.C, Vsnitha A, Swamy M.M, Ravi Shankar G.A. Antioxidant and anti microbial activity of *Cissus quadrangularis* L. *J Med. Food.* 2003, 6, 99-105.
  18. Jainu M, Devi C.S.S. Potent anti-ulcerogenic activity of methonolic extracts of *Cissus quadrangularis* by anti oxidative mechanism. *J Clin Biochem and Nutri.* 2003, 34, 43-47.
  19. Jainu M., Devi C.S.S. Effect of *Cissus quadrangularis* on gastric mucosal defensive factors in experimentally induced gastric ulcer- a comparative study with sucralfate. *J Med. Food.* 2006, 7,372-376.
  20. Ajaiyeoba EO, Oncha PA, Olarenwaju OT. *In- vitro* anthelmintic properties of *Buchholzia coiaceae* and *Gynandropis gynandra* extract. *Pharm. Biol.* 2001, 39(3), 217- 20.
  21. Thorn GW, Adams RD, Braunwald E, Isselbacher KJ and Petersdorf RG. *HarrisonsPrinciples of Internal Medicine, 2<sup>nd</sup> edition*, New York. 1997,1088.
  22. Vigar Z. *Atlas of Medicinal parasitology.* 1984,216.
  23. Chatterjee KD. *Parasitology. Protozoology and Helminthology.* 1967, 168-169.
  24. Niezen JH, Waghorn GC, Charleston WA. Growth and gastrointestinal nematode parasitism in lambs grazing either Lucerne which contains condensed tannins. *J. Agri. Sci.* 1995, 125, 281- 89.
  25. Martin RJ.Y-Amino butyric acid and piperazine activated single channel currents from *Ascaris* sum body muscle. *Br.J.Pharmacol.* 1985, 84(2), 445-61.
  26. Athnasiadou S, Kyriazakis F, Jackson RL and Coop. Direct anthelmintic effects of condensed tannins towards different gastro intestinal nematodes of sheep. *In vivo* studies. *Vet. Parasitol.* 2001, 99, 19.
  27. Thompson Dp, Geary TG. The structure and function of helminth surfaces. *Biochemistry and Molecular Biology of Parasites. 1<sup>st</sup> ed.* Academic Press. 1995, 203-232.