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Antiviral activity of ancient system of ayurvedic medicinal plant *Cissus quadrangularis* L. (Vitaceae)

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ABSTRACT: Partially purified methanolic extract of *Cissus quadrangularis* (belonging to Vitaceae member, South Indian medicinal plant) have been explored for antiviral activity and their phytochemical characterisation. *In vitro* antiviral activity against HSV type1 and 2, and Vero cells at non-cytotoxic concentration were determined. HSV1 and HSV2 showed more sensitivity against the partially purified compound. Phytochemical analysis showed the presence of the Steroids and Terpenoids.

KEY WORDS:
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INTRODUCTION

Cissus quadrangularis L is a medicinal plant belonging to family Vitaceae, the ancient system of medicine such as Ayurveda and used to treat various diseases and disorders. Within the past decade therapeutic options for viral infections have improved significantly, however, the emergence of resistant viruses as well. The further disposal of resistant strains is one reason for therapeutic failure (1–3). Furthermore, many of the licensed drugs are toxic as well as being expensive (4), thus the search for potential sources for the development of new drugs is very important. Based on Ayurvedic and Siddha traditional herbal medicine, several antiviral studies were performed to detect active natural products in higher plants. In these studies different viruses were included, e.g. herpes simplex virus (HSV), feline imunodificidncy virus, coxsackie virus, influenza virus parainfluenza virus, respiratory syncytial virus, etc., (5–12). *Cissus* used to treat various diseases and disorders, (13), antimicrobial, anti-osteoporotic, and antiviral activity (14–16). Very meager research has been done in this plant against antimicrobial activity especially antiviral. Hence, the current study deals with the antiviral activity of partially purified compound of methanolic extract of *Cissus quadrangularis* has been observed in two *in vitro* virus HSV types 1 and 2 and also the phytochemical characterization was carried out.

MATERIALS AND METHODS

Plant Material

Cissus quadrangularis stem were colleted from semi dry area where the plants were grown as a weed in various places in Tamil Nadu, India, which were pooled and processed for further study.

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Extraction of plant material

The shadow air dried and powdered plant materials (20g) were extracted with methanol (MeOH) (400mL) in a Soxhlet extractor for 24 hrs filtered and evaporated to dryness under reduced pressure at 40°C. The residues obtained were re-dissolved in 100% DMSO and stored at–20°C until further use.

Purification of compound

The MeOH extracts separated using TLC with the solvent hexane and ethyl acetate, in the ratio of 1:9. The separated compounds were visualized. One of the compounds were scraped and removed for cytotoxicity and antiviral activity.

HPTLC / HPLC ANALYSIS

HPTLC

Sample was prepared by diluting 10 mg of partially purified fraction with 1 ml of the solvent, about 2 µl of the sample was loaded on a TLC plate using "CAMAG Linomat 4" applicator then the plate was kept in "CAMAG Twin Trough Chamber" of separation of compounds, with mobile phase Ethyl acetate, Hexane and Acetic acid in the ratio of 7:3:0.5. After the separation was over, the TLC plates were scanned using CAMAG TLC Scanner II at 254 nm wave length.

HPLC

Partially purified compound was dissolved in acetonotrile. The sample was further separated through HPLC, C–18 (Phenomenox) analytical column using the mobile phase Acetonitrile:water in the ratio of 60:40 with the flow rate of 0.5 mL/min and detected in the UV detector at 280 nm.

Cells and Viruses

Vero cells (King Institute of Preventive Medicine, Tamil Nadu, India) were passaged in DMEM supplemented with 5% FBS. Herpes simplex virus type 1 and 2 were individually mixed with Vero cells and incubated at 36°C until the cytopathic effect develops. The cytopathic effected (virus in Vero cells) virus were estimated their $TCID_{50}$ and stored at –70°C until use.

Cytotoxicity assay

The effect of the methanolic extract on the proliferation of Vero cells was determined in 96 well tissue culture plates and incubated at 37°C in humidified atmosphere supplied with 5% CO_2. Confluent monolayers were incubated with two fold serial dilution of extracts (1:100, 200, 300, 400, 500 (100µg/mL)) in DMEM for 72 hrs. The 50% cell-inhibitory concentration (CC_{50}) was determined by tryphan blue dye uptake assay using medium as a control.

Antiviral assay

Antiviral activity was determined using dye uptake assay (17). HSV type 1 & 2 infected Vero cells were seeded in 96 well tissue culture plates at an initial cell concentration 60,000 cells/well and incubated in a humidified 5% CO_2 atmosphere and preincubated with 100µL of serial dilution of partially purified compound of MeOH extract in the medium (1:100, 200, 300, 400 (100µg/mL)) in triplicate and control virus was kept for 15 min at same condition, and incubated at 36°C for 7 days.

Phytochemical Screening

The screening of the chemical constitutents was carried out in MeOH extracts using chemical methods and TLC. Lieberman Buchard Test for Steroids, Dragendroff's test for Alkaloids, Feric solution test for Tannins, Fehling's test for Reducing sugars, Ninhydrin test for Amino acid and proteins and for Terpenoids, Ammonium heptamolybdate and cericsulphate in sulphuric acid along with sample heated at 120°C and the appearance of blue indicate the presence of terpenoids were analyzed.

Statistical Analysis

The statistical significance P value of dilution of MeOH extracts C. quadrangularis activity against HSV 1 and 2 were calculated using statistical software SPSS for Windows 10.1. P value less than 0.05 was considered to be statistically significant

RESULTS AND DISCUSSION

Partially purified compound of MeOH extracts of *C. quadrangularis* was screened for antiviral activity against HSV type 1 and 2 viruses (Fig. 1a&b). The antiviral activity was evaluated at concentrations that

TABLE 1: CYTOTOXICITY ASSAY OF PARTIALLY PURIFIED COMPOUND OF METHANOL EXTRACTS OF *C. QUADRANGULARIS* IN VERO CELLS.

Extract	CC	Wells 1:100	1:200	1:300	1:400	1:500
Methanol	0	2	0	0	0	0

CC – Cell Control; 0 – No effect; 2 – 2+ cytopathic effect

FIGURE 1a&b:

TLC purified compound of MeOH extract of *C. Quadrangularis*. Mobile phase 1:9 ratio of Hexane and Ethyl acetate. a) Separation of Crude extract compounds
b) Partially purified compound, unlabelled arrow showed the single spot.

were non-toxic for the cell system. Cytotoxicity assay of TLC purified compound of MeOH extracts showed the cytopathetic effect in 1: 200 (Table 1) dilution. In the antiviral activity of partially purified compound of MeOHextracts of C. *quadrangularis* against HSV 1 and 2 showed were inhibited statistically significant ($p<0.01$) level at 1: 400 dilution (Table 2).

Aqueous and MeOH extracts of its partner species of *Cissus subaphylla* and C. *hamaderohensis* and *Undaria pinnatifida* showed its activity against HSV (15,16,18) which confirm our experiments using partially TLC purified compound of methanol extracts of C. *quadrangularis*. This partially purified compound further studied for their purity and multiple compounds in its, 9 compounds were separated and detected in HPTLC. However, 1, 2, 3 and 9 compounds were separated distinctly where, 4, 5, 6, 7 and 8 compounds were not separated distinctly,

TABLE 2: ANTIVIRAL ACTIVITY OF PARTIALLY PURIFIED COMPOUND OF METHANOL EXTRACTS OF *C. QUADRANGULARIS* IN HSV 1 AND HSV 2 INFECTED VERO CELLS.

Extract	CC	1:100	1:200	1:300	1:400	Virus
Methanol	0	0	0	0	4**	HSV 1
	0	0	0	0	4**	HSV 2

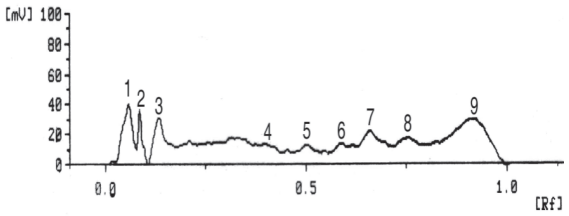
CC – Cell Control; 0 – No effect; 4 – 4+ cytopathic effect; ** Statistical significance $p<0.01$

these compounds may closely associate together (Fig. 2). In HPLC also showed 9 matching different compounds were separated. The result reveals that the partially TLC purified compound contain multiple compounds and associated together compounds (Fig. 3). Further study need to identify the single active compound against antiviral activity.

In phytochemical screening (Table 3) revealed the presence of general steroids and Terpinoids. Previously work carried out by number of investigators with these compounds posses anti-viral activity (16,19–21). Melchart, et al. (1994) (22) proved that *Echinacea* species extracts through oral treatment protects experimentally infected the Influenza–A virus in mice. The immunomodulating action may triggered by antiviral activity compounds (23).

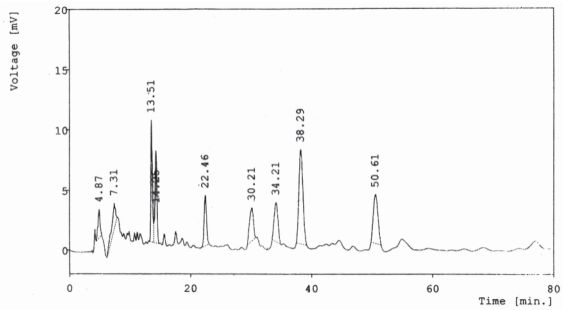
In conclusion, the present investigation showed that the partially purified compound confirms high antiviral activity. Further study has been continued of this partially purified compound, furthermore purified and to identify the active compound possessing antiviral activity in MeOH extracts and to formulate the drug against viral infection.

FIGURE 2:



HPTLC analysis of one of the partially TLC purified MeOH compound of *C. quadrangularis* using CAMAG HPTLC spectrophotometer showed 9 peaks. Mobile phase Hexane: ethyl acetate: acetic acid in the ratio of 7:3:0.5 used for separation in HPTLC silica MeRCK 60F 254 plates and detected in the wavelength of 254 nm.

FIGURE 3:



C-18 Reverse Phase HPLC analysis of one of the partially TLC purified MeOH compounds of *C. quadrangularis* showed the 9 different peaks. Mobile phase Acetonitrile and Water in the ratio of 60:40 in the flow rate of 0.5 mL/min and detected in the wavelength of 280 nm.

TABLE 3: PHYTOCHEMICAL ANALYSIS OF PARTIALLY PURIFIED COMPOUND OF METHANOL EXTRACT OF *C. QUADRANGULARIS*.

Compound	Partially purified ethanol compound	Compound	Partially purified ethanol compound
Steroids	+	Tannin	–
Terpenoids	+	Amino acid/Protein	–
Alkaloids	–	Sugar	–
Flavonoids	–		

+ Present; – Absent

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