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Research Article

In vitro Evaluation of Antimicrobial, Antioxidant and Cytotoxic Effect of Ethnobotanically Important Plant *Cissus repens*

[1]Nimisha Satish, [1]Angitha Balan, [1]K. Aswini, [2]R. Kavyashree, [1]Roopa Reddy, [1]S. Swetha, [1]Sandeep Suryan and [1]G.S. Nagananda

[1]Centre for Incubation, Innovation, Research and Consultancy, Jyothy Institute of Technology, Tataguni, Off Kanakapura Road, Bangalore 560082, India
[2]Department of Biotechnology, Oxford College of Science, Bangalore 560102, India

Abstract

Background and Objective: *Cissus repens* is an ethnobotanically important plant with immense medicinal value. The present investigation was designed to perform phytochemical screening and *in vitro* bio-evaluation of the hot and cold successive extracts. **Materials and Methods:** Qualitative phytochemical screening and TLC profiling revealed the presence of phenols, flavonoids and phytosterols. **Results:** Quantitative estimation showed the highest amount of phenols (92.52 μg mL^{-1}), flavonoids (40 μg mL^{-1}) and phytosterols (10.69 μg mL^{-1}) in hot ethanol extract. The extracts showed antibacterial activity against *S. typhi* (20±0.33 mm) sensitive to hot ethanol extracts and *S. aureus* (12±0.33 mm) to cold chloroform extracts. *S. marcescens* was found to be sensitive to cold chloroform extract (12±0.33 mm). The extracts exhibited antifungal activity against all the five tested strains. Highest percentage of free radical scavenging activity and IC_{50} value was observed with hot ethanol extracts for both DPPH (62.33% and 30.12 μg mL^{-1}) and ABTS$^+$ (43.96% and 58.03 μg mL^{-1}) methods. The preliminary toxicity studies of extracts on radish seed germination showed 90% lethality with hot ethanol extract at higher concentrations. The hot acetone and ethanolic extracts were tested for their cytotoxic effects on 3T3 cell lines using MTT assay. **Conclusion:** The results indicated a dose-dependent effect of the extracts on 3T3 cell lines. This study may provide a scientific basis for the use of this plant for various therapeutic applications.

Key words: *Cissus repens*, phytochemical screening, antimicrobial, free radical scavenging activity, toxicity, cytotoxicity, MTT assay

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Corresponding Author: G.S. Nagananda, Centre for Incubation, Innovation, Research and Consultancy (CIIRC), Jyothy Institute of Technology, Tataguni, Off Kanakapura Road, Bangalore 560082, India Tel: +919844485920

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Our nature has enriched botanical wealth and diverse plant types. India is one of the world's 'mega diversity' countries with over 45,000 plant species[1]. Plants have always been a source of medicine all over the world, traditional herbal medicine is based on the fact that plants contain natural substances that promote health and alleviate illness. One such group of plants that are used in all the continents and which are being evaluated for their therapeutic efficacies to treat various ailments are those belonging to the genus '*Cissus*'. It belongs to the family *Vitaceae* and has 13 genera and 800 species in the world which are distributed in tropical regions of India, Africa, Arabia, Sri Lanka and South Asia. In India 8 genera and 63 species have been identified[2].

Cissus repens is an ethnobotanically important plant widely used in folk medicines[2]. It is a woody climber with spirally arranged foliage, stalked leaves, heart-shaped-oval, the cordate base having papery to leathery leaf blades that are simple, hairless, 3.5-8.5 by 2.5-8 cm and with toothed margins[3]. The flowers are small in size and are borne on umbelliform inflorescences which are leaf opposed. Its fruits are round berries, up to 6 by 5 mm and with 1 seed each. The seed is up to 4 mm across[3]. In India, the plant is commonly found in the states of Maharashtra, Karnataka, Kerala and Tamil Nadu[4].

In the folk system of medicine the roots and stems of *Cissus repens* Lamk. are being used for treating snake bites, rheumatic pain and carbuncles and to treat nephritis, long-term coughs and diarrhoea the stems are being administered[5]. In Taiwan, *C. repens* is used for the treatment of many diseases, such as epilepsy, stroke, abscess and diabetes[6]. The study undertaken intended to provide a scientific basis for the use of this plant as traditional medicine. There were no reports of successive extraction studies on *Cissus repens*, so the investigation was started to extract and screen the phytochemical constituents of *Cissus repens* by hot and cold successive extraction and its bio-evaluation for antimicrobial, antioxidant and cytotoxic effects of the successive extracts.

MATERIALS AND METHODS

Study area: The study was carried out at the Centre for Incubation, Innovation, Research and Consultancy, Plant and Microbial Technology Lab, Bangalore, India from March, 2019-January, 2021.

Collection of plant material, preparation and successive extraction: The plant stem was collected from its natural habitat, from Kasaragod District, Kerala (Fig. 1). The plant stem was rinsed, dried and chopped into small pieces. Successive hot and cold extractions were carried out using 4 solvent systems according to their increasing polarity namely hexane, chloroform, acetone, ethanol by using the standardized previous protocols[7].

Fig. 1: *Cissus repens* from its natural habitat

Qualitative phytochemical screening: The different qualitative chemical tests were performed with the eight extracts obtained from cold and soxhlet successive extractions and its phytochemical profile were established. The following tests were performed on all the extracts and the presence of various phytoconstituents like alkaloids, phenols, phytosterols, flavonoids were detected[8,7].

Thin-layer chromatography: Thin-layer chromatography was done for the better identification of the bioactive compounds. Different solvent systems were used for the identification of flavonoids, phenols and phytosterols. For flavonoids, a mixture of butanol, acetic acid and water in 40:10:50 ratio was used whereas for phytosterols and phenols a mixture of acetonitrile and water in 90:10 ratio were used. The solvent systems were stored overnight in a closed chamber. For the sample application, the extracts were taken in a capillary tube and a series of small adjacent spots were applied to form a band on a preparative TLC plate coated with silica gel. The plates were vertically placed inside a sealed developing chamber (iodine chamber) which was previously saturated with different solvent systems. The different spots developed in each solvent system were identified utilizing a detecting agent and R_f value was correspondingly calculated.

Quantitative estimation of phytoconstituents

Estimation of phytosterols by liebermann-burchard method[7]: Different aliquots of standard cholesterol in chloroform (1 mg mL^{-1}) ranging from 0.2-1 mL were taken in a sequence of test tubes. Acetic anhydride (2 mL) and concentrated H_2SO_4 of 1-2 drops were added and volume was made up with chloroform, then incubated for 15 min in dark. 2 mL acetic anhydride and 1 mL chloroform were used to prepare the blank. The sample was then dissolved and mixed with 2 mL of acetic anhydride and 1 mL of chloroform. The absorbance was then measured at 640 nm.

Estimation of total phenols[7]: The extracts were dissolved in 5 mL of distilled water. Standard Catechol (50 µg mL^{-1}) ranging from 0.2-1 mL were aliquoted into test tubes. The volume was made up to 5 mL after adding 0.5 mL folin-Ciocalteu reagent. The solution was mixed well and after 3 min, 2 mL of 20% Na_2CO_3 solution was added and mixed well. The tubes were incubated in boiling water for a minute and subsequently cooled. The absorbance against a reagent blank was taken at 650 nm.

Estimation of total flavonoid content by aluminium chloride cethod[7]: Standard Quercetin (100 µg mL^{-1}) solution ranging from 0.2-1 mL were taken in a sequence of test tubes. Five hundred microlitres of the extract were mixed initially with 2 mL of distilled water followed by 0.15 mL of 5% $NaNO_2$ solution and incubated for 6 min. About 0.15 mL of a 10% $AlCl_3$ solution was added and incubated for 6 min. To this mixture 2 mL of 4%, NaOH solution was added. The final volume was adjusted to 5 mL instantly with distilled water and incubated at RT for 15 min. At 510 nm the absorbance was measured.

Antioxidant activity: The antioxidant activity of plant extracts was tested by DPPH and $ABTS^+$ free radical scavenging assay.

Free radical scavenging activity on 1, 1-diphenyl-2-picrylhydrazyl (DPPH): DPPH (0.1 mM) was prepared in 100% methanol which served as control and methanol as blank. Ascorbic acid (standard) and successive extracts (1 mg mL^{-1}) at various concentrations ranging from 10-50 µg mL^{-1} were taken and the volume was adjusted to 100 µL with methanol. 0.1 mM methanolic solution of DPPH of 0.5 mL was added and shaken vigorously. At 27°C the tubes were incubated for 20 min. The absorbance of the sample was measured at 517 nm. The experiment was performed in triplicate[7]. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula[9]:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}}$$

$ABTS^+$ (2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay: $ABTS^+$ radical cation was prepared by mixing ABTS (7 mM) with ammonium persulfate (2.45 mM) and used after 16 hrs of incubation at room temperature. Ascorbic acid (standard) and successive extracts (10 mg mL^{-1}) at various concentrations (200-1000 µg mL^{-1}) were pipetted out in a sequence of test-tube. Ethanol and $ABTS^+$ solution served as blank. One fifty microlitres of $ABTS^+$ solution was added and after incubation in dark for 30 min at RT, the absorbance was read at 745 nm and the experiment was performed in triplicate[7]. Radical cation decolourization activity was expressed as the inhibition percentage of cations by the sample and was calculated using the formula[9]:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}}$$

Antimicrobial activity

Antibacterial activity: Six different clinically isolated bacteria, both Gram-positive and Gram-negative namely *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus mutans*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Serratia marcescens* maintained in the culture bank of CIIRC were used for this study. Antibacterial activity was determined by the well diffusion method[10]. Mueller-Hinton agar plates were prepared aseptically and the surface of the agar was swabbed with bacterial culture. Ten wells were made in each of these plates using a sterile cork borer. The stock solution of each plant extract was prepared at a concentration of 1 mg mL^{-1}. The centre (10th) well was loaded with 100 µL ampicillin 1 mg mL^{-1} (positive control). The rest of the 8 wells were loaded with 100 µL of different successive extracts and one well with Dimethyl Sulfoxide (DMSO) which served as a negative control. The plates were incubated at 37°C for 24 hrs. The diameter of the inhibition zone was measured. The experiments were repeated in triplicates and the average values were recorded.

Antifungal activity: Six different clinically isolated fungi, namely *Trichophyton rubrum*, *Aspergillus niger*, *Candida albicans*, *Cryptococcus*, *Candida parapsilosis* maintained in

the culture bank of CIIRC were used for the study. Antifungal activity was performed by well diffusion method[10]. Mueller-Hinton agar plates were prepared aseptically and the surface of the agar was swabbed with bacterial culture. Ten wells were made in each of these plates using a sterile cork borer. The stock solution of each plant extract was prepared at a concentration of 1 mg mL^{-1}. The centre (10th) well was loaded with 100 μL clotrimazole 1 mg mL^{-1} (positive control). The rest of the 8 wells were loaded with 100 μL of different extracts and one well with Dimethyl Sulfoxide (DMSO) which served as a negative control. The plates were incubated at 37°C for 24 hrs. The diameter of the inhibition zone was measured. The experiments were repeated in triplicates and the average values were recorded.

Toxicity assay: Radish seeds were used as target species for this experiment because it has a short germination time and is sensitive to phytochemicals. About 10 radish seeds were counted and added to 8 test tubes, 7 containing 5 mL of distilled water and 100 μL of different leaf extracts. Sterile distilled water was used as a control. The seeds were soaked in the solution for 3 hrs. After incubation, it was poured to sterilise 10 cm Petri plates containing sterile filter paper (Whatman) inside the LAF. Petri plates were incubated in dim light at 23±2°C. The percentage of seed germination in different Petri plates were recorded after 5 days. This experiment was repeated three times.

Cytotoxicity assay: Cell viability of the hot ethanol and acetone extracts from *Cissus repens* were performed using NIH 3T3 mouse fibroblast cell line. Briefly, the cells were allowed to grow in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (fetal bovine serum) and incubated in a 5% CO_2 incubator at 37°C. A stock solution was adjusted to cell number 1×10 5 cells mL^{-1} and 100 μL of this cell suspension was pipetted into 96 well plates and incubated overnight. Samples were dissolved in 20 μL DMSO and the volume was adjusted to 1 mL DMEM media to prepare a stock solution. Different concentrations of the samples 10, 25 and 50 μg mL^{-1} were prepared from the stock solution. About 100 μL of every concentration were added into 96 well plates along with the control sample (buffer). The experiment was performed in triplicates. MTT assay was performed for three consecutive days after incubation. After each incubation time, 100 μL of 1 mg mL^{-1} of 3 -[4,5 dimethylthiazol-2 -yl] -2,5-diphenyltetrazolium bromide (MTT) solution was added to every well and incubated for 3 hrs till the purple formazan crystals are visible. The formazan crystals were dissolved by the addition of 100 μL DMSO after removing the MTT solution. The colour change was measured using an ELISA microplate reader at 570 nm. The results were expressed as a percentage of viable cells at each specified incubation time.

RESULTS

The purpose of the present study was to establish a scientific attestation for the use of our plant of interest *Cissus repens* as a potential source of medicine. All the above-mentioned experiments were carried out and the obtained results are depicted below.

Qualitative phytochemical screening: The different qualitative chemical tests were performed for establishing a phytochemical profile of eight extracts obtained from cold and soxhlet successive extraction. Phytochemical screening of all the extracts revealed the presence of flavonoids, phenols and phytosterols in both hot and cold extracts (Table 1).

TLC profiling: TLC analysis was carried out for all eight extracts and confirmed the presence of phenols, flavonoids and phytosterols. For phenols, single bands were observed in hot chloroform (R_f value-0.142), cold acetone (R_f value-0.842) and cold ethanol extract (R_f value-0.142) while hot acetone ($R_f$values-0.142, 0.385, 0.628) and hot ethanol (R_f values-0.157, 0.385, 0.628) displayed three bands. For phytosterols single bands were observed in hot acetone (R_f value-0.28), hot ethanol (R_f value-0.28) and cold acetone (R_f value-0.28) whereas two bands were observed in cold acetone ($R_f$values-0.28, 0.853). For flavonoids single band were visible in hot chloroform (R_f value-0.112), three bands in hot acetone (R_f values-0.112, 0.387, 0.564), cold acetone (R_f value-0.112, 0.564, 0.806) and cold ethanol (R_f value-0.112, 0.322, 0,580). Four bands were observed in hot ethanol (R_f values-0.096, 0.387, 0.564, 0.612).

Quantitative estimation of phytochemicals: The number of bioactive components, which were detected by phytochemical estimation of *Cissus repens* revealed the presence of high content of phytosterols (10.69 μg mL^{-1}), flavonoids (40 μg mL^{-1}), phenols (92.52 μg mL^{-1}) in the hot ethanol extract when compared to other extracts (Table 2).

Antioxidant activity

DPPH free radical scavenging activity assay: The free radical scavenging potential of extracts at different concentrations

Table 1: Phytochemical screening of eight extracts of *Cissus repens*

	Cold extraction				Hot extraction			
	HH	HC	HA	HE	CH	CC	CA	CE
Alkaloids								
Mayer's test	-	-	-	-	-	-	-	-
Wager's test	-	-	-	-	-	-	-	-
Hager's test	-	-	-	-	-	-	-	-
Dragendroff's test	-	-	-	-	-	-	-	-
Phytosterols								
Liebermann-burchardtest	-	+	+	+	+	++	+	+
Phenolic compounds								
Ferric chloride	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+
FCreagent	+	+	++	++	+	+	+++	+++
Flavonoids								
Aluminium chloride	-	-	++	+++	-	+	+++	+++

HH: Hot hexane, HC: Hot chloroform, HA: Hot acetone, HE: Hot ethanol, CH: Cold hexane, CC: Cold chloroform, CA: Cold acetone and CE: Cold ethanol

Table 2: Quantitative estimation of phytochemicals of *Cissus repens*

Extracts	Phytosterols (µg)	Phenols (µg)	Flavanoids (µg)
HH	2.7±0.001	9.18±0.001	25±0.002
HC	2.99±0.003	12.6±0.002	29.5±0.001
HA	4.95±0.002	76.68±0.005	32.5±0.004
HE	10.69±0.000	92.52±0.002	40±0.002
CH	0	9.72±0.002	23.5±0.003
CC	4.94±0.003	10.8±0.003	24.5±0.005
CA	5.17±0.004	37.62±0.000	25±0.000
CE	5.86±0.002	48.06±0.004	29.5±0.001

HH: Hot hexane, HC: Hot chloroform, HA: Hot acetone, HE: Hot Ethanol, CH: Cold hexane, CC: Cold chloroform, CA: Cold acetone and CE: Cold ethanol

Table 3: Percentage of scavenging activity of different extracts of *Cissus repens*

Extracts	DPPH of scavenging (%)	ABTS of scavenging (%)
STD	86.59	63.01
HH	35.52	3.36
HC	35.92	5.43
HA	55.63	37.67
HE	62.33	43.96
CH	36.46	3.53
CC	40.08	5.6
CA	40.08	15.08
CE	45.3	19.48

HH: Hot hexane, HC: Hot chloroform, HA: Hot acetone, HE: Hot ethanol, CH: Cold hexane, CC: Cold chloroform, CA: Cold acetone and CE: Cold ethanol

Table 4: IC_{50} value of eight different extracts of *Cissus repens*

Extracts	DPPH IC_{50}	ABTS IC_{50}
STD	11.339	48.383
HH	179.614	2896.5
HC	122.191	504.369
HA	37.296	68.419
HE	30.127	58.035
CH	153.908	3393.02
CC	105.54	653.66
CA	111.306	199.19
CE	68.83	141.44

HH: Hot hexane, HC: Hot chloroform, HA: Hot acetone, HE: Hot ethanol, CH: Cold hexane, CC: Cold chloroform, CA: Cold acetone and CE: Cold ethanol

was tested by the DPPH method. The highest percentage of scavenging activity and IC_{50} values were found to be 62.33% and 30.12 µg mL^{-1}, respectively for hot ethanol extract (Table 3 and 4). The standard ascorbic acid showed the percentage of scavenging activity and IC_{50} values were found to be 86.59% and 11.33 µg mL^{-1}.

$ABTS^{+}$ radical scavenging activity assay: Radical cation decolourization activity of extracts at different concentrations was tested by the ABTS method. The highest percentage of scavenging activity and IC_{50} values were found to be 43.96% and 58.03 µg mL^{-1}, respectively for hot ethanol extract (Table 3 and 4). The standard ascorbic acid showed the percentage of scavenging activity and IC_{50} values were found to be 63.01% and 48.31 µg mL^{-1}.

Antimicrobial activity

Antibacterial activity: The extracts of the investigated plant species showed antibacterial activity against three out of the six tested bacterial strains. The bacterial form *S. typhi* was found to be more sensitive to hot extracts with a maximum zone of inhibition (20±0.33 mm) in hot ethanol. *S. marcescens* was sensitive to both hot and cold extracts with the maximum zone of inhibition (16±0.00 mm) in hot ethanol. While *S. aureus* was found to be more sensitive to cold extracts with the maximum zone of inhibition in cold chloroform (12±0.33 mm) (Table 5).

Antifungal activity: The extracts of the plant species *Cissus repens* showed antifungal activity against all the tested fungal strains. The fungal form *T. rubrum* and *A. niger* were found to be sensitive to both hot and cold extracts with a maximum zone of inhibition in hot ethanol. *C. parapsilosis*, *Cryptococcus*, *C. albicans* were found sensitive only to hot ethanol with a zone of inhibition of 21±0.33, 25±0.33 and 38±0.33 mm, respectively (Table 6).

Table 5: Antibacterial activity of different extracts of *Cissus repens*

	Bacterial strains					
Extracts	*S. typhi*	*K. pneumoniae*	*S. marcescens*	*S. aureus*	*B. cereus*	*S. mutans*
STD	33±0.57	13±0.33	17±0.33	16±0.33	17±0.33	15±0.00
Control	-	-	-	-	-	-
HH	-	-	12±0.00	-	-	-
HC	18±0.66	-	13±0.00	10±0.00	-	-
HA	18±0.66	-	15±0.33	-	-	-
HE	20±0.33	-	16±0.00	11±0.00	-	-
CH	18±0.00	-	15±0.33	10±0.00	-	-
CC	17±0.00	-	15±0.33	12±0.33	-	-
CA	18±0.33	-	12±0.00	11±0.33	-	-
CE	-	-	11±0.00	11±0.00	-	-

HH: Hot hexane, HC: Hot chloroform, HA: Hot acetone, HE: Hot ethanol, CH: Cold hexane, CC: Cold chloroform, CA: Cold acetone and CE: Cold ethanol

Table 6: Antifungal activity of different extracts of *Cissus repens*

	Fungal strains				
Extracts	*T. rubrum*	*C. parapsilosis*	*A. niger*	*Cryptococcus*	*C. albicans*
STD	34±0.33	45±0.33	40±0.33	16±0.33	50±0.33
Control	-	-	-	-	-
HH	18±0.33	-	18±0.33	-	-
HC	18±0.33	-	19±0.00	-	-
HA	17±0.33	-	15±0.00	-	-
HE	19±0.66	21±0.33	21±0.33	25±0.33	38±0.33
CH	17±0.66	-	15±0.33	-	-
CC	18±0.66	-	18±0.33	-	-
CA	16±0.33	-	17±0.33	-	-
CE	16±0.33	-	16±0.33	-	-

HH: Hot hexane, HC: Hot chloroform, HA: Hot acetone, HE: Hot ethanol, CH: Cold hexane, CC: Cold chloroform, CA: Cold acetone and CE: Cold ethanol

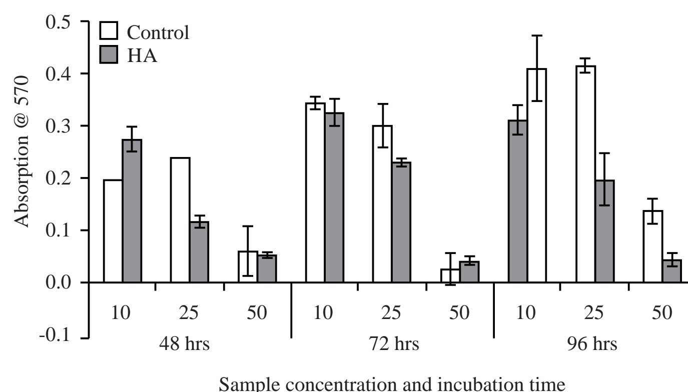

Fig. 2: Determination of cell viability (3T3 cell lines) by MTT assay using hot acetone extracts of *Cissus repens*

Raddish seed toxicity assay: Germination inhibiting properties of eight extracts from plant species *Cissus repens* were determined. Both hot and cold extracts displayed an inhibitory effect with maximum lethality percentage in hot ethanol (90%) and hot acetone (90%) (Table 7).

Cell proliferation and cytotoxicity: The acetone extracted from *Cissus repens* supported NIH_3T_3 cell growth in a dose-dependent manner over 3 days whereas the ethanol extracts had no considerable effect. Briefly, three different concentrations (10, 25 and 50 μg mL^{-1}) of extracted samples were assessed for cytotoxicity on adherent cells. It was observed that hot acetone extract exhibited cytotoxicity with increasing concentration. The values in the Y-axis in the graph (Fig. 2) represent the mean absorbance of the samples and the x-axis represents different concentrations of the acetone extracts plotted for 3 days. There are no reports on cytocompatibility studies of acetone extracts from *Cissus repens* available so far.

Table 7: Radish seed bioassay of eight different extracts of *Cissus repens*

Samples	Total number of seeds	Number of germinated seeds	Lethality (%)
Control	10	10	0
HH	10	4	60
HC	10	2	80
HA	10	1	90
HE	10	1	90
CH	10	7	30
CC	10	4	60
CA	10	3	70
CE	10	8	20

HH: Hot hexane, HC: Hot chloroform, HA: Hot acetone, HE: Hot ethanol, CH: Cold hexane, CC: Cold chloroform, CA: Cold acetone and CE: Cold ethanol

DISCUSSION

In the present investigation hot and cold successive extraction was carried out using four different solvents (hexane, chloroform, acetone, ethanol). The extracts obtained revealed the presence of phenols, flavonoids and phytosterols which agreed with the earlier finding of Sabeerali *et al.*[2] regarding the *Cissus* species. From the experimental data, it was evident that hot extraction was efficient in extracting as the extracts had a higher amount of these phytoconstituents when compared to cold extracts. The TLC profiling of different extracts displayed several bands corresponding to the bioactive components. An extra sterol band was observed in cold acetone which was absent in the hot acetone extract. This is because the bioactive component in the extract might be thermolabile, which might get denatured when extracted under heat[7].

Free radicals are produced under certain environmental circumstances and during normal cellular processes. They are set off chain reactions that can damage hundreds of molecules. Antioxidant halts this reaction and protects the cells. Some antioxidants act as free radicals themselves donate an electron, stabilize and neutralize their dangerous effect. Other antioxidants work against the molecules that act as free radicals destroying them[9]. In the study undertaken the total antioxidant activity was determined by $ABTS^+$ and DPPH assay. The extracts displayed a high amount of $ABTS^+$ scavenging activity and DPPH scavenging activity with an evident IC_{50} value which could be due to the presence of phenols and flavonoids as revealed in various *Cissus* species[2,11,12] and there were no reports specifically on *C. repens*. The DPPH involves in their hydrogen bonding ability while ABTS chemistry involves the direct generation of $ABTS^+$ radical monocation without the involvement of any intermediate radical. Hence the more scavenging activity can be directly related to their antioxidant capacity. A high amount of scavenging activity with evident IC_{50} value was observed in hot extracts, particularly in hot ethanol. When compared to cold extracts hot extracts has given out a better activity. The observed results could be due to the presence of phenolic and flavonoid compounds[13]. The IC_{50} of hot ethanol extracts were found to be best when compared to other extracts.

Antimicrobial properties of various *Cissus* species were identified[14,15] but there were no reports available on *C. repens*. From the results of the present study, it is evident that the plant extracts exhibited antibacterial effects against both Gram-negative and Gram-positive bacteria suggesting its remarkable therapeutic actions. The plant can be utilized for the treatment of diarrhoea and respiratory infections as reported by Wang *et al.*[3]. The present study agrees with the above finding as the extracts were sensitive to *S. typhi*, *S. marcescens* and *S. aureus*. The bacterial species *S. typhi* and *S. marcescens* were found more sensitive to hot ethanol extract while *S. aureus* to cold chloroform. The observation indicated that there may be some bioactive components in the cold extract too that confer antibacterial activity. The extracts possessed antifungal activity to all the five tested fungi *T. rubrum*, *A. niger*, *C. parapsilosis*, *Cryptococcus* and *C. albicans* with maximum sensitivity to hot ethanol extract. Based on the above observation it was concluded that the hot extracts especially hot ethanol had some secondary metabolites which attributes to their high antimicrobial activity. The high potency of *C. repens* against both bacteria and fungi gives a scientific basis for its uses in folk medicine in the treatment of carbuncles, long-term coughs and diarrhea[5].

Radish seed lethality is a general bioassay, which is indicative of toxicity, weedicide effects and various pharmacologic actions[16]. The extracts showed an inhibitory effect on radish seed germination when tested with higher concentrations. Whereas when tested with lower concentration there was an increase in germination rate. This could be correlated with the previous findings[11,17,18] where the dose-dependent action of plants belonging to genus *Cissus* was revealed. The above observation was given more clarity when cytotoxicity studies using MTT assay proved the same. No reports of cell culture studies on the experimental plant

Cissus repens is available to date. But the cytotoxic effect of various plants in genus *Cissus* has been well identified and recorded[19,20].

From the above observations, it is evident that plant *C. repens* is a reservoir of phytochemical components with various pharmacological actions. It could be hence utilized in the herbal medicine industry for the development of therapeutic phytomedicine.

CONCLUSION

Cissus repens have been claimed for several pharmacological activities and traditional medicinal uses. From the study, it can be concluded that it is the presence of phytoconstituents that is responsible for the actions. Hence, providing a scientific basis for the use of plant *Cissus repens* as a therapeutic agent. The plant is a boon for the herbal medicine industry as it exhibits antimicrobial, antioxidant and dose-dependent cytotoxic effects. More research is needed to isolate the constituents responsible for the particular biological actions. The present study will possibly help to bridge between traditional claims and modern therapy on *Cissus repens* and also pinpoints unexplored potential of it.

SIGNIFICANCE STATEMENT

This study discovers the possible antimicrobial, antioxidant and cytotoxicity of phytochemicals that can be beneficial for the Pharma industry in developing herbal-based drugs. This study will help the researcher to uncover the critical area of cytotoxicity that many researchers were not able to explore. Thus, a new theory on developing a novel drug for cancer may be arrived at.

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