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Phytochemical analysis and Antimicrobial activity of ethanolic extract of *Cissus quadrangularis* L. against some pathogenic microorganisms

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Abstract

Plants have important medicinal properties. In traditional remedies *Cissus quadrangularis* L. has been used as a popular folk medicine.

Present investigation revealed that aqueous extract and ethanolic extract of *Cissus quadrangularis* show significant activity against pathogenic microorganisms.

The qualitative phytochemical screening was evaluated by using crude extract of the stem of plant. Antimicrobial activity of *C.quadrangularis* was studied against some pathogenic bacteria and fungi. In this study, the secondary metabolites such as steroids, tannins and flavonoids were found present in the crude extract of *C. quadrangularis*. The antimicrobial activity of ethanolic extract of plant was evaluated against some human pathogenic bacteria and fungi and the results are discussed. The ethanolic extract and aqueous extract of fresh stems exhibited antimicrobial activity against *E. coli, Psuedomonas sp., Aspergillus niger* and *Aspergillus flavus*. The study reveals the *C. quadrangularis* can be used as antimicrobial agent.

Plants have various medicinal properties. Medicinal plants are in use for thousands of years and are renowned for their effectiveness in various ailments. The medicinally usable plants were identified and extracted for biochemical profile and formulated for medicinal applications. *Cissus quadrangularis* is an important medicinal plant belonging to the family *Vitaceae*[19]. It has versatile therapeutic uses as well as pharmacological actions. The present study highlights the health promoting and therapeutic properties of *Cissus quadrangularis*[2,5,12].

In India, *C. quadrangularis* is widely used as a common food item. *Cissus quadrangularis* is one of the most common species scattered all over India particularly in tropical regions. It is known to be an ancient medicinal plant, with optimal healing in white tissue area of the body (tendon, ligament, etc.)[6]. Phytochemical analysis of *Cissus*

quadrangularis indicates the presence of carotene, phytosterol, terpenoids, β-sitosterol, δ-amyrin, δ-amyrone and calcium[4]. The presence of β-sitosterol, δ-amyrin, δ-amyrone, and flavanoids (quercetin) has also been reported, all these components have potentially different metabolic and physiologic effects[4].

Antimicrobial activity is the efficacy of the substances to inhibit the growth of microorganisms. A diverse arena of new antimicrobial agents is urgently needed to combat the diminishing efficacy of existing antibiotics. There is a need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and have fewer side effects. The effective plant constituents can combat human and plant pathogenic bacteria, fungi and viruses without toxic side effect and environmental hazards. Due to this reason, search for plant products with antimicrobial properties has intensified in recent years.

The stem of *C. quadrangularis* is also an important medicinal plant in Ayurveda as alterative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, in the treatment of irregular menstruation and asthma, in complaints of the back and spine. *C. quadrangularis* stem and leaves are used for the treatment of hemorrhoid, menstrual disorders, scurvy and as anti-oxidant, anti-flatulence, anti-bacterial, and antifungal. It is very effective in strengthening the bones and joints[3]. The plant resembles the shape of bones and joints in the body. The stem is fried in ghee and given with milk for curing fractures and osteoarthritis. The entire plant is being used in fractures, sprains, rheumatism and irregular growth of teeth, Anthrax, haematuria, elephantiasis, dislocation of hip, and various wounds[10]. It can be cooked with salt, dried and deep-fried and can be eaten as a side dish.

There is a need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and have least side effects. Ethanol extract (90%) of *C. quadrangularis* stems possess antibacterial activity against *E. coli*, and *P. aeruginosa* and mutagenicity against *Salmonella microsoma*[9]. Antimicrobial activity has also been reported from stem and root extracts of *C. quadrangularis*[13]. The alcoholic extract of aerial part was found to possess antiprotozoal activity against *Entamoeba histolytica*[16]. Alcoholic extract of the stem showed activity against *E. coli*[18], Methanol and dichloromethane extract of whole plant were screened for *in vitro* antiplasmodial activity[15].

Plant collection and Identification :

- Fresh *Cissus quadrangularis* plants were collected from a nursery, Bhopal, M.P, India.
- The plants were sent for identification to a taxonomist and compared with herbarium specimens for authentication.
- Plants were washed 3 times with tap water and then 3 times with distilled water, so as to remove all the dust particles adhering to the stem part of the plant and then surface sterilization was done by 0.1% Mercuric chloride for few seconds to decontaminate. After surface sterilization plant material was washed thoroughly with distilled water (three times).

Preparation of extract :

With the help of Soxhlet apparatus elhanolic and aqueous extracts of *C. quadrangularis* stem were obtained.

Qualitative phytochemical screening :

Ethanol extracts of *C. quadrangularis* were screened for different phytochemical constituents' viz., steroid, terpenoid, phenol, alkaloid, tannin, flavonoid, Cardiac glycosides, Anthroquinones and saponin. Phytochemical screening of the extracts was carried out by the standard methods.

Detection of carbohydrates :

Crude extracts were dissolved in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test:- Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Fehling's Test:- Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Benedict's Test:- Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars. Brown ring at the junction indicates the presence of phytosterols.

Detection of alkaloids :

Solvent free extract (50ml), was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents as follows.

Mayers test :- To a few ml of filtrate, a drop or two of Mayers reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.

Wagner's test :- To a few ml of filtrate, few drops of Wagners reagent were added by the side of the test tube. A reddish – brown precipitate confirmed the test as positive.

Hagers test :- To a few ml of filtrate 1 or 2 ml of Hagers reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

Detection of flavanoids :

Lead acetate test :- The Crude extract (50ml) was dissolved in distilled water & 3ml of 10% lead acetate solution was added. A bulky white lead precipitate indicated the test as positive.

Test for Tannins[11] *:-* About 0.5ml of extract was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue – black coloration.

Test for Saponin [15] *:-* About 2ml of the extract was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3drops of olive oil and shaken vigorously , then observed for the formation of emulsion.

Test For Steroids :- Two ml of

acetic anhydride was added to 0.5ml extract of each sample with 2ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Terpenoids :

Salkowski test :- Five ml of extract was mixed in 2ml of chloroform and concentrated, H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration at the inter face was formed to show positive results for the presence of terpenoids.

Test for Cardiac glycosides (Keller-Killani test):

Two ml of glacial acetic acid containing one drop of ferric chloride solution was added to 5 ml of the extract. To this was added another 1ml of concentrated sulphuric acid. Formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Test for Anthroquinones :

The extract (0.5 ml) was boiled with 10ml of sulphuric acid and filtered while hot. The filtrate is shaken with 5 ml of chloroform. The chloroform layer was pipetted out into another test tube & 1ml of diluted ammonia is added. The resulting solution is observed for color change.

Antimicrobial activity :

Culture and media preparation :

Antimicrobial activity was screened by disc diffusion method. The shoot extracts were tested for antimicrobial activity against microbial pathogens such as *Escherichia coli, Pseudomonas species, Aspergillus flavus* and *Aspergillus niger.*

Disc Diffusion Method :

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 psi pressure. The discs were soaked in ethanolic extract and aqueous extract, dried in laminar air flow. The discs soaked in standard antibiotic/antifungal solution were used as positive control. The antibiotic Amoxicillin (5 µg/ml) was used as standard for bacterial culture and Fluconazole (5 µg/ml) for fungal culture.

Antibacterial Assay :

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 medium was poured in the sterile Petri-dishes. The filter paper discs impregnated with plant aqueous extract and ethanol extract were aseptically placed on the solidified agar media. The discs soaked in antibiotic solutions were also placed on the solidified agar media as positive control respectively. The Petri dishes were suitably marked for proper orientation and future reference. The plates were incubated at 37±1°C for 24 hrs. for bacterial cultures and at 28±1°C for fungal cultures. Triplicates were kept in each

case. The zone of inhibition was measured from the centre of disc to the clear zone in millimeter and the results were recorded. The diameter of inhibition zones for each solvent extracts were compared with the standard antibiotic.

For antimicrobial assays, two bacterial cultures were chosen: *Pseudomonas* sp. and *Escherichia coli*. And two fungal cultures were chosen: *Aspergillus flavus* and *Aspergillus niger.*

The results of screening test revealed the presence of medicinally active compounds in the crude extract of *C. quadrangularis* From table (1), it could be seen that crude extracts revealed the presence of flavonoids, saponin, tannins, steroids, terpenoids, phenolic, Cardiac glycosides compounds etc. were present while Alkaloids and Anthroquinones were absent. The presence of alkaloids, flavonoids, saponins and tannins in *Cissus quadrangularis* were reported by earlier workers also.

Table-1. Phyto-chemical analysis of *C. quadrangularis*

S.No.	Test	Result
1.	Tanin	Positive
2.	Flavanoid	Positive
3.	Saponin	Positive
4.	Steroid	Positive
5.	Terpenoids	Positive
6.	Cardiac glycosides	positive
7.	Anthroquinones	Negative
8.	Alkaloids	Negative

Phytochemical studies of *Cissus quadrangularis* have shown the presence of various versatile constituents such as flavonoids, triterpenoids, vitamin C, stilbene derivatives and many others like resveratrol, piceatannol, pallidol perthenocissin and phytosterols. Out of which ascorbic acid, triterpene, β-sitosterol, ketosteroid, two asymmetrical tetracyclic triterpenoids and calcium were identified as major constituents of this plant[2,5,20]. The presence of medicinally active compounds like phenol, alkaloids, tannins and flavonoids were present in the ethanolic extract of *C. quadrangularis*[19].

Antimicrobial Activity :

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by very large number of researchers in different parts of the world[12].

In the present investigation antimicrobial activity of *Cissus quandragularis* was analyzed. The effect of plant extract on test pathogens is shown in Table-2 and 3.

E. coli and *Pseudomonas* species were found to be highly susceptible to Amoxicillin with zone of inhibition diameter of 23.2 mm and 22.2 mm respectively. Ethanolic extract showed maximum zone of inhibition of 20.4 mm and 16.8 mm against test pathogens whereas aqueous extract (distilled water) showed minimum zone of inhibition of 12.6mm and 14mm respectively against test pathogen.

Both *Aspergillus niger* and *Aspergillus flavus* were found to be highly susceptible to Fluconazole with zone of inhibition diameter

of 20.4 mm and 22.2 mm. Ethanolic extract showed maximum zone of inhibition of 18 mm and 17.4 mm against test pathogen where as aqueous extract showed minimum zone of inhibition of 11.2 mm and 11 mm against test pathogen. Petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of *Cissus quadrangularis* are reported to have antimicrobial activity against all tested pathogens[7].

Alcoholic extract of the stem showed activity against *E. coli*[18], Aqueous extracts showed antifungal activity in *Penicillium sps* (9.1 ±0.2), *Aspergillus flavus* (9.0 ±0.1), *Geotricum candidum* (10.2 ±0.1) N-Butanol extract confirmed activity against *Penicillium sps* (19.5 ±0.1) Acetone extract showed ineffective ness against *Penicillium sps, Aspergillus flavus, Geotricum candidum*(0) in Preliminary Phytochemical Screening[7].

Table-2. Antibacterial activity of plant extract against the test organism.

Bacteria	Diameter of zone of inhibition (mm)		
	Ethanolic extract	Aqueous extract	Amoxicillin
E. coli	20.4	12.6	23.2
Pseudomonas species	16.8	14	22.2

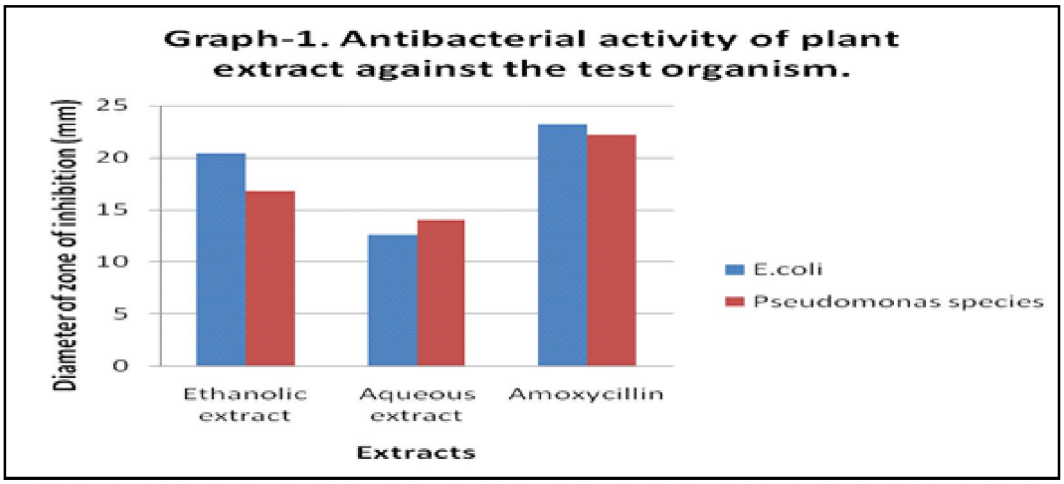
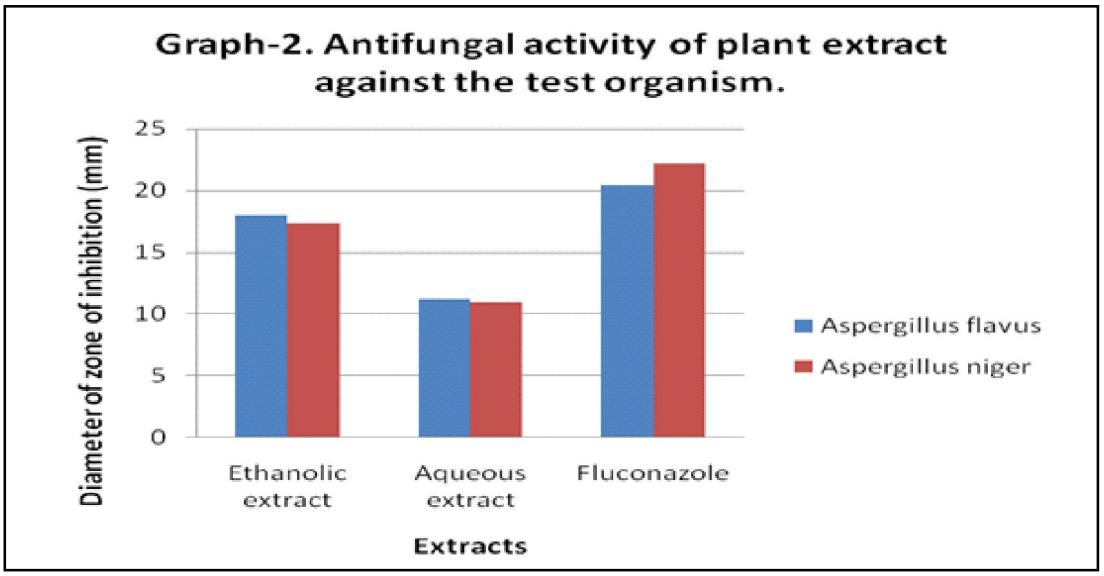


Table-3. Antifungal activity of plant extract against the test organisms

Fungi	Diameter of zone of inhibition (mm)		
	Ethanolic extract	Aqueous extract	Fluconazole
Aspergillus flavus	18	11.2	20.4
Aspergillus niger	17.4	11	22.2

Note: Data are mean of three replicates.



The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines mostly plant drugs. The experimental material selected for the study was *Cissus quadrangularis*. The present study was carried out to determine the qualitative phytochemical and anti-microbial activity of *C. quadrangularis* extracts. Results provide the information that *Cissus quadrangularis* has a large number of chemical constituents, which can be responsible for various pharmacological activities. Much more work is required in future to test the extracts of *Cissus quadrangularis* for a number of pharmacological activities before its commercialization for the human welfare and economic world.

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