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Research Article

ANTIMICROBIAL AND PHYTOCHEMICAL EVALUATION OF *CISSUS QUADRANGULARIS* L.

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ABSTRACT

The present investigation involves antimicrobial and phytochemical evaluation of *Cissus quadrangularis* L. The antibacterial activity of *Cissus quadrangularis* was performed using disk diffusion method. The Results of study proved prompt efficacy of herbal extract against *S. aureus* and *E. coli*. The concentration dependent antibacterial activity of extract was observed against both organisms. Study also involves phytochemical investigation of herbal extract using HPTLC, IR and UV-Visible spectrophotometer. The result of study indicated that the methanolic extract possessed most potent antibacterial activity as compared to other extract. The antibacterial activity increases with the concentration and results indicated that the diameters of zone inhibition of the extract were comparable with the standard drug. The antimicrobial potential of plant extract may be attributed to the presence of specific phytoconstituents.

KEYWORDS: *Cissus quadrangularis*, Herbs, Antimicrobial, HPTLC.

INTRODUCTION

Cissus quadrangularis is plant belongs from *Vitaceae* family which is an edible plant occurs in Malaya, Java, India, West Africa and Thailand.[1] It is used for strengthening of bone, reduces pain and help to repair bone fractures therefore also called "*Hadjod*".[2] Plant also used for the management of gout, tumors, piles, leucorrhoea and ulcers.[3] The plant also evaluated for its anti-inflammatory and antioxidant potential by various researchers. The constituent of plant considered responsible for antimicrobial potential therefore present investigation involve antimicrobial evaluation of *Cissus quadrangularis* along with its phytochemical investigation using chromatographic and spectro-photometric methods.[1-3]

Herbs and herbal products used extensively now a day's as medicine but the quality concern is major issue regarding the natural products. The quality and composition of herbs and herbal formulation may be ensured using various techniques of standardization which also confirm presence and amount of active principles/marker of formulation. The traditional approaches of standardization were lacking accuracy and precision therefore modern analytical techniques such as HPLC, HPTLC and UV-Visible Spectroscopy came in practice for the standardization of herbs and herbal products. The high performance thin layer chromatography (HPTLC) play vital role towards the analysis of compounds obtained from natural origin. This technique used for fingerprinting and quantification of marker compounds in herbs.[4-6]

Figure 1: *Cissus quadrangularis* Plant

Materials and Methods

Plant materials were collected and shade dried then homogenized to fine powder and stored in airtight bottles for further use.

Preparation of Plant Extract

Air dried powder was mixed with petroleum ether (100ml) in a cotton wool plugged container followed by frequent shaking. The supernatant was discarded and solvent allowed evaporating, the finally obtained dry powder was mixed with 100ml of methanol and rotated again to obtained methanolic extract. The extracts were centrifuged at 5000rpm and supernatant was collected, solvent evaporated and dry extract was stored in airtight bottles. The same procedure was repeated for chloroform and water extract. Petroleum ether extract (PE), methanolic extract (ME), chloroform extract (CE) and water extract (WE) were use further for antimicrobial evaluation.[7, 8]

Antibacterial Activity

Microbes were grown in broth 37°C for 24h. 100µl of the inoculums was added to each plate containing agar. Four different concentrations of the sample were used for antimicrobial activities. The sterile filter paper disks (6mm in diameter) were saturated with 50µl of each sample concentration. The plates were incubated at 37°C for 24h and the diameters of inhibitory zones were measured. The assay was carried out three times. Disks containing different concentrations of antibiotics were used as reference to compare the sensitivity of each tested bacterial species.[9-11]

HPTLC analysis

HPTLC systems equipped with Linomat-5 applicator with WinCATS-4 software were used. All the solvents used for HPTLC analysis were of selective grade. The dried methanolic extract was found to be most potent antimicrobial agent therefore it was subjected to further analysis. Extract was dissolved in methanol and used for HPTLC analysis. Mobile phase was selected by trial and error basis using various combinations of different solvent according to their polarity using precoated Silica Gel G-60 plate as stationary phase. The samples (10µl) spotted in the bands of width 8mm with a Camag microlitre syringe on pre-coated silica gel glass plate. The sample loaded plate was kept in TLC twin trough developing chamber with mobile phase methanol: ethyl acetate (6.5: 3.5) and the plate was developed up to the optimum level. Linear ascending development was carried out and the developed plate was dried to evaporate solvents from the plate. The plate was scanned by UV Densitometric scanning on Camag scanner.[12]

Ultraviolet visible absorption (UV)

The methanol extract of plant was analyzed in UV-Visible range between 200-780nm using UV-Visible Spectrophotometer (UV-1800, Shimadzu).

Infra-red spectroscopy (IR)

Under the influence of IR radiations the molecules shows various mode of vibration, which gives different absorption spectrum selective to the functional group present in molecule. The spectrum of compound is unique feature of molecular framework. The IR spectra of methanol extract of *Cissus quadrangularis* was scanned on FT-IR spectrophotometer over the frequency range from 4000-400 cm^{-1}.

Results and Discussion

The yield and appearance of different extract of *Cissus quadrangularis* were reported in table 1. The high yield of methanolic extract indicated presence of polar component in plant. The antibacterial activity of compound performed to evaluate its antimicrobial potential using two different microbial strains i.e., *S.aureus* and *E.coli.* Results of antibacterial study showed that the tested compound inhibited the growth of *E. coli* and *S. aureus* significantly. The result of study indicated that the methanolic extract possessed most potent antibacterial activity as compared to other extract (figure 1). The antibacterial activity increases with the concentration as mentioned in figure 2 and results indicated that the diameters of zone inhibition of the extract were comparable with the standard drug. The antimicrobial potential of plant extract may be attributed to the presence of specific phyto-constituents.

Table 1: % Yield and colour of different extract

S. No.	Extract	% Yield	Colour
1	Petroleum Ether Extract	45	Dark yellow
2	Methanolic Extract	69	Brown
3	Chloroform Extract	57	Gray
4	Water Extract	62	Reddish Brown

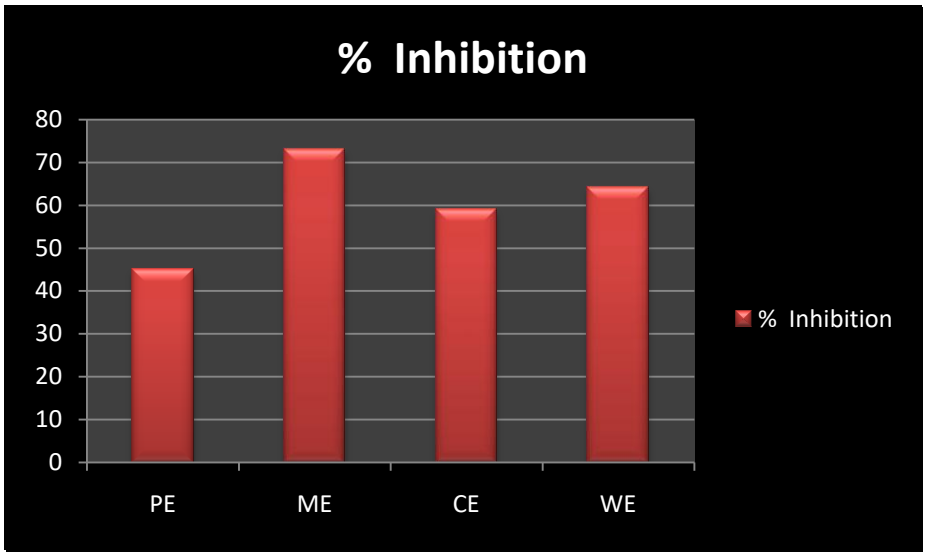

Figure 2: Comparative antimicrobial potential (% zone of inhibition) of petroleum ether extract (PE), methanolic extract (ME), chloroform extract (CE) and water extract (WE)

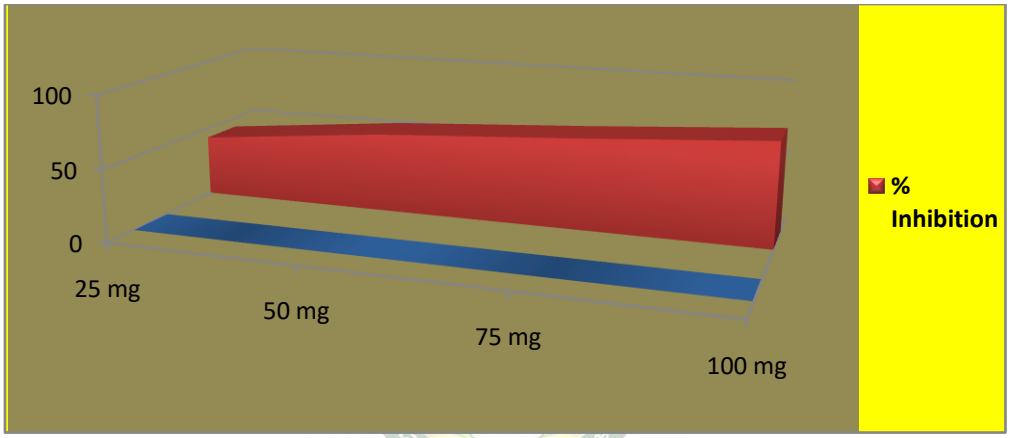

Figure 3: Antimicrobial potential (% zone of inhibition) of different concentration of methanolic extract (ME)

UV-Visible spectra of extract are shown in figure 3. The UV spectrum of *Cissus quadrangularis* showed absorption maxima at 273nm and 255nm.

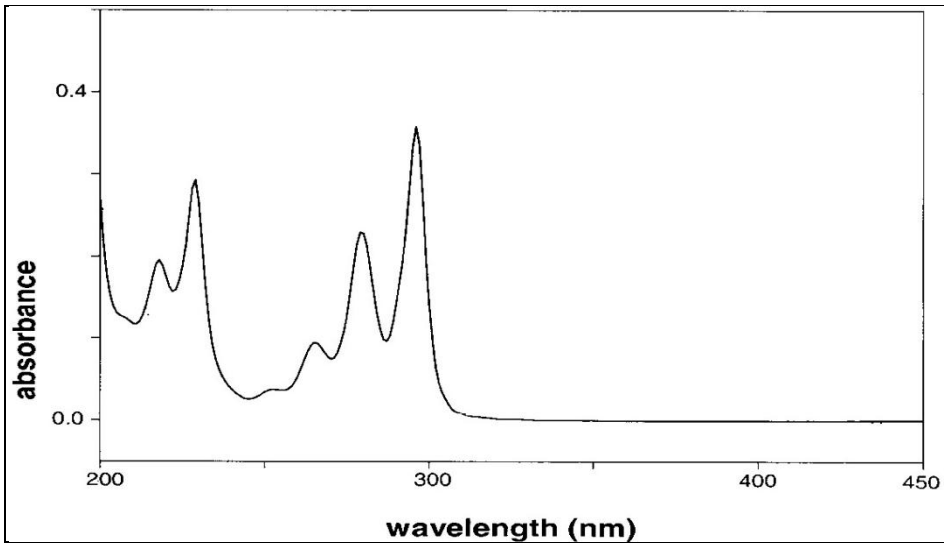

Figure 4: UV-Visible spectra of extract

IR spectra of plant extract are shown in figure 4. The infrared region approximately 4000–400 cm^{-1} was used to study the different modes of vibration spectrum. Study confirmed presence of aromatic, carbonyl, C-H stretching and bending vibration along with characteristic peaks of alcoholic and amino group.

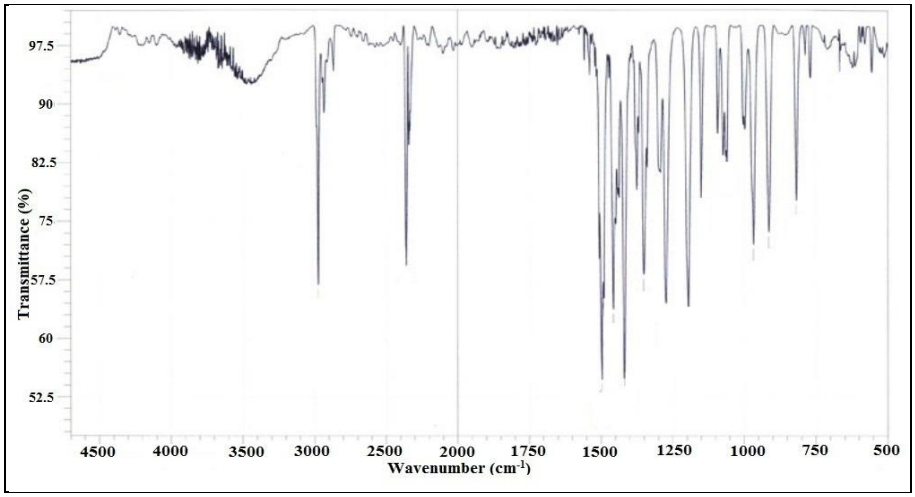

Figure 5: IR spectra of plant

High Performance Thin Layer Chromatography (HPTLC) is one of the efficient techniques for separation and identification of plant material which gives better precision and accuracy. The HPTLC fingerprinting of study are shown in figure 5 & 6. Various characteristics peaks were observed in HPTLC fingerprint of extract.

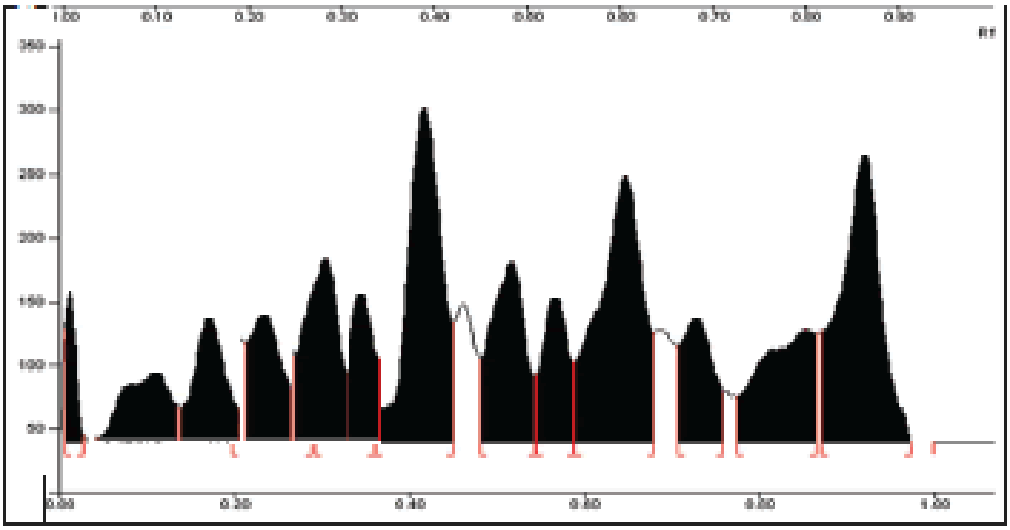

Figure 6: Densitometric chromatogram of Methanolic Extract

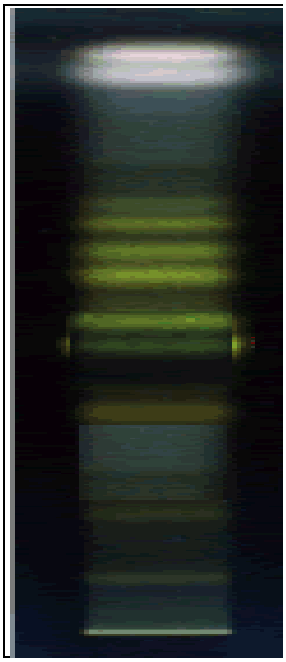

Figure 7: HPTLC Chromatogram of Methanolic Extract

CONCLUSION

The plant *Cissus quadrangularis* was evaluated for quality parameters using instrumental techniques of analysis. The results of IR and HPTLC may be used further for identification of plant material as fingerprinting tool. Study also involves antimicrobial evaluation of *Cissus quadrangularis* L. using disk diffusion method. The study proved prompt efficacy of herbal extract against *S.aureus* and *E.coli*. The concentration dependent antibacterial activity of extract was observed against both organisms.

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