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SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC ANALYSIS OF *CISSUS QUADRANGULARIS* FROM VIDISHA REGION

Biological Science

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ABSTRACT

In present study extract of Cissus quadrangularis is studied by spectrophotometric and chromatographic techniques. The crude extract was subjected to phytochemical screening using petroleum ether, ethyl acetate and methanol as solvent. Terpenoids, steroids, alkaloids, saponins, carbohydrates, tannins and phenolic compounds were found in petroleum ether extract. Alkaloids, flavanoids, saponins, tannins and phenolic compounds were found in ethyl acetate extract and terpenoids steroids, flavonoids, proteins, amino acids, alkaloid, saponin, tannins, phenolic compound were present in methanol extract. HPLC analysis were quantified at 254nm which shows presence of flavonoids. Spectral analysis also shows the presence of flavonoids and phenolic compounds.

KEYWORDS

HPLC – High performance liquid chromatography, nm- nanometer.

Introduction

Many medicinal aspects are being discovered for the treatment of many diseases, wide herbal formulations are being used. Plant material is extracted at different levels to get desired product as per requirement. *Cissus quadrangularis* is being studied for the presence of biochemical. It belongs to family vitaceae known commonly as 'hadjod'. As name suggest (had + jod) means that heals fractured bones. It is a herb reaching the height of 1.5 m and has quadrangular sectioned branches, grows natively in hot deccan peninsula also on the slopes of western slopes and dry areas of Arabia, Africa, India, Srilanka, Malaysia and Thailand(Udupa *et al.,*1970) and used as food in southern India(Chidambaram *et al.,*2003).

The stem and leaves are used for the treatment of hemorrhoid, menstrual disorder, scurvy and as antioxidants, antiflatulence, antibacterial and antifungal activity(Neha agrawal and Richa jain, 2013). Every plant have their unique chemical composition i.e. their flavanoid, terpenoids, triterpenes, saponins, alkaloids and many more (Jainu and Devi, 2004). Each of this constituents work for different kind of disease.

Material and methods

Plant material *Cissus quadrangularis* was collected in autumn season ie October – November. Whole plant is washed with distill water. It is then allowed to air dry at room temperature. It took few months to dry because the plant is fleshy. Dried plant is powdered and sieved, packed in polythene bags for further use.

For our work 200gm of powdered material is taken and 500ml of different solvent were taken mainly petroleum ether, ethyl acetate, methanol, respective extract were obtained by cold percolation.

These extracts were subjected to thin layer chromatography for separation by solvents. Column chromatography was done for purification of different fraction. The n butanol soluble and ethyl acetate soluble part were subjected to silica gel column chromatography for isolation of phytoconstituents. The n butanol soluble part was eluted gradiently with chloroform, chloroform :methanol mixtures (95:5, 90:10, 80:20, 50) and methanol. Ethyl acetate: Methanol extract fraction on TLC show single spot and named F1. Ethyl acetate soluble extract is chloroform, chloroform: ethyl acetate (50:50), ethyl acetate, ethyl acetate: methanol (90:10) and methanol. Fraction eluted with $CHCl_3$: MeOH (50:50) is designated F2. and spectral analysis for the required stiudy.

Observation

Crude Petroleum ether extract, ethyl acetate extract and methanol extract of plant obtained after column chromatography was further studied by phytochemical method.

Table1: Phytochemical Screening of crude extracts of Petroleum ether, Ethyl acetate and methanol from *Cissus quadrangularis*

S. No.	Tests	Observation for extracts		
		Pet. Ether	Ethyl acetate	Methanol
1	Test for carbohydrates			
	Fehling's Test	_	+.	+
2	Test for Alkaloid	_	_	+
	Wagner's test	_	_	+
3	Test for Flavonoids			
	Shinoda test	_	+	+
	Alkaline reagent test	_	+	_
4	Test for Terpenoids			
	Salkowski test	+	_	_
5	Test for Saponins	_	+	_
	Foam test		+	
	Test for proteins	_	+	+

Phytochemical screening can be performed with the appropriate tests to get an idea regarding the type of phytochemicals existing in the extract mixture or fraction. After assuring the pharmacologically active of crude methanol extract of *Cissus quadrangularis*. The fractions of successive methanol extract were screened for preliminary phytochemical analysis and revealed the presence of flavonoids, carbohydrates, saponins, sterols, tannins, amino acids and proteins as shown in (Table 2). Saponins, sterols, terpenoids and carbohydrates were found present in n-butanol soluble fraction, while as ethyl acetate soluble fraction showed only the presence of flavonoids.

Table 2: Phytochemical investigation of petroleum ether, Methanolic extract of *Cissus quadrangularis* and its isolated fractions.

Phytochemical Screening			Observations			
S.No.	Phytochemical Constituent	Tests	PEE	METP	EAF	nBF
01.	Alkaloids	Mayer's reagent Test	_	_	_	_
		Wagner's reagent Test	_	_	_	_
		Hager's reagent Test	_	_	_	_
		Tannic acid Test	_	_	_	_
02.	Flavonoids	Alkaline reagent Test	_	+	+	_
		Zinc HCl Test	_	_	+	_
		Shinod's Test	_	+	+	_
03.	Carbohydrates	Molish's Tests	+	+	_	+
		Pentose Test	_	_	_	+
04.	Saponins	Froth formation Test	_	+	_	+
		Heamolytic Test	_	+	_	+
05.	Tanins	$FeCl_3$ Test	_	+	_	+

		Lead acetate Test	_	+	_	+
06.	**Fats**	Saponification Test	_	_	_	_
07.	**Aminoacids**	Ninhydrine Test	+	+	_	+
08.	**Anthraquinoneglycoside**	Borntrager's Test	_	_	_	_
09.	**Sterols/Steroids**	Salkowaski Test	_	_	_	+
10.	**Terpenoids**	Liebermann –Burchard Test	_	_	_	+
11.	**Proteins**	Biuret Test	_	+	_	_
		Xanthoproteic Test	_	_	_	_

PE-Petroleum ether extract; METP- Methanolic extract; EAF- Ethyl acetate raction; nBF- n butanol Fraction; (+) Present; (-) Absent.

Total phenolic content (TPC)

TPC in ethyl acetate extract was analyzed using spectrophotometer by Folin-Ciocalteu method using gallic acid as standard. Similarly concentration of 0 .5- 1mg/ml of plant extract was also prepared in methanol. 0.5ml of each extract sample was taken, mixed with 2.5 ml of (a 10 fold) dilute folin Ciocalteu reagent and 2ml of 7.5% sodium carbonate solution.

Total flavanoid determination

The methanolic extract (0.5 ml of 200mg/ml FW) was diluted with 4 ml double distilled water. Diluted extracts of fruits were mixed with 5% (0.3 ml) NaNO2. 10% aluminum chloride was then added with reaction mixture. Absorbance was measured at 510nm in spectrophotometer.

Chromatographic resolutions

10gm of ethyl acetate extract was suspended in water, extracted successively with ethyl acetate and n butanol (6×300 ml each) and then resulting solutions were concentrated to provide ethyl acetate (5gm), n butanol (4gm). The solvent systems used for separation ; n butanol: acetic acid: water (4:1:5) and chloroform: methanol (90:10) were found to be most appropriate for ethyl acetate and n butanol fractions respectively. Ethyl acetate spotted plate was sprayed with anisaldehyde sulphuric acid and heated at 110^{0}C for 5 minutes.TLC plates were developed and observed under uv lights.

The n butanol soluble part (5gm) and ethyl acetate soluble part (5gm) were subjected separately to silica gel (60-120 mesh) column (60cm x 4.5 cm) chromatography for the isolation of phytoconstituents (Harborne *et al,* 1984).

Fluorescence Study

Many herbs show fluorescence when the cut surface or powder is exposed to UV light and this can be useful in their identification. Fluorescence study is an essential parameter for first line standardization of crude drug. The fluorescence character of the plant powders (40 mesh) was studied both in Visible (254) nm and UV light (365 nm and 400-800 nm). Fluorescence analysis of the powered drug of *Cissus quadrangularis* is treated with various chemicals exhibit various colours in the UV light. When the powdered drug was treated with water it showed a clear green colour. But while on with 1N NaOH, CH_3OH: NaOH (1:1), Ethanol, Sulphuric acid it showed yellow colour at 254 nm and 400-800 nm UV light, but at 365 nm all showed dark yellow colour(Table 3).

Similarly the fluorescence studies on the alcoholic crude extract of *Cissus quadrangularis* clearly depicted that no change in fluorescence or color occurs at 254 nm and 365 nm UV light treated with different chemicals. Color change was only found at 400-800 nm UV light with different chemical

Table 3: The fluorescence behavior of the Methanolic Extract of *Cissus quadrangularis.*

S. No.	Treatment	Observation		
		Visible 254 nm	Short UV 365 nm	Long UV 400-800 nm
01.	Water	Light Green	Grey Black	Light Green
02.	Methanol	Light Green	Grey Black	Light Green
03.	1N NaOH	Light Green	Grey Black	Light Green
04.	CH_3OH:NaOH (1:1)	Light Green	Grey Black	Light Brown
05.	Ethanol	Light Green	Grey Black	Light Green
06.	Sulphuric acid (66%)	Light Green	Grey Black	Light Brown
07.	Conc. Sulphuric acid	Light Green	Grey Black	Light Brown
08.	Nitric acid	Light Green	Grey Black	Light Green
09.	Hydrochloric acid	Light Green	Grey Black	Yellow Green
10.	Acetone	Light Green	Grey Black	Yellow Green

Table4: Observations of TLC of crude fractions of n butanol and ethyl acetate from Successive methanolic extract.

Fractions	Solvent System	Detecting reagents	No.of Spots	Rf Value (x100)
n-butanol	Chloroform: methanol (90:10)	Sprayed with vanillin sulphuric acid and heated at 100°C for 5 minutes	05	33, 57, 62, 66, 89
Ethyl acetate	n butanol: acetic acid: water (4:1:5)	Sprayed with anisaldehyde sulphuric acid and heated at 110°C for 5 minutes Sprayed with $AlCl_3$ in methanol and observed under UV light	06 04	23, 46, 60, 72, 85, 95 47, 64, 87, 95

In the present study after conducting thin layer chromatography, the ethyl acetate fraction gave five spots with Rf values of 0.4, 0.45 and 0.48 indicate the presence of phenolic compounds (Thorat, *et. al.*, 2009) 0.56 and 0.557 which may be due to the presence of flavanoids. (Dalala *et. al*., 2010).

Total five fractions after column chromatography out of which, two of n-butanol soluble fractions two of ethyl acetate soluble fraction and ruminant water soluble fraction or aqueous fraction were isolated in significant quantities and were evaluated for antioxidant activity to check the significance of the fractions for their bioactivity as a potential antioxidant.

Table 5 : Separation of bioactive constituents from n-butanol fraction.

S. No.	Eluting Solvent & Composition	Collected Isolates	Compounds	Detection for Single spot to pool the same fractions	Final Fractions % Yield (w/w of crude extract)
01.	**$CHCl_3$:MeOH (100:0)**	**1-17**	**No Residue after evaporation**	**On TLC plates using chloroform: methanol (90:10v/v) as mobile phase & iodine vapors as detecting agent.**	**–**
02.	**$CHCl_3$:MeOH (95:5)**	**18-47**	**Fr-I (R_f=0.37)**		**BF-I (5.5% w/w, 0.275 gm)**
03.	**$CHCl_3$:MeOH (90:10)**	**48-59**	**Very less quantity was isolated**		**-**
04.	**$CHCl_3$:MeOH (80:20)**	**60-70**	**Fr-II (R_f=0.67)**		**BF-II (12% w/w, 0.6 gm)**
05.	**$CHCl_3$:MeOH (50:50)**	**71-122**	**No Residue after evaporation**		**-**

Table 6 : Separation of bioactive constituents from ethyl acetate fraction.

S. No.	Eluting Solvent & Composition	Collected Isolates	Compounds	Detection for Single spot to pool the same fractions	Final Fractions % Yield (w/w of crude extract)

01.	**$CHCl_3$ (100)**	**1-10**	**No Residue after evaporation**	**On TLC plates using n butanol: acetic acid: water (4:1:5) using anisaldehyde sulphuric acid reagent and for 5 minutes.**	
02.	**$CHCl_3$: Ethyl acetate (50:50)**	**11-30**	**No Residue after evaporation**		
03.	**Ethyl acetate (100)**	**31-45**	**Fr-III (R_f = 0.64)**		**EF-I (27% w/w, 1.3**
04.	**Ethyl acetate: MeOH (90:10)**	**46-60**	**Very less quantity was isolated**		
05.					
06.	**Ethyl acetate :MeOH (0:100)**	**76-95**	**No Residue after evaporation**		

After studying all the observation it can be concluded that the ethyl acetate fractions from both the chromatography suggest, the presence of flavanoids and phenolic compound in the respective plant extract.

Spectral Analysis

UV spectrum of the purified isolated Compound-I has characteristic bands: I at λ = 362 nm, and band II at λ = 247 nm, which shows the presence of flavanoids.

IR spectra (in KBr) showed absorption bands at 932cm-1(-CH=CH-trans), 1095cm-1(-CO-CO, ether), 1165cm-1 (-C-O-ester), 1565 and 1611cm-1 (-C=C- , conjugated polyene), and 3382cm-1 (-OH) shows the presence of flavanoids.

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