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Catharanthus roseus: Alkaloid Fraction Isolation and its Antibacterial Activity

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Abstract: *Catharanthus roseus* (L.) G. Don is an important medicinal plant through the tropical region. The present work focuses on multiple solvent extraction method for extraction of Serpentine and Ajmalicine. The purified bioactive compounds from *Catharanthus roseus* plant leaves have been evaluated for antimicrobial activity. Increasing polarity based separation is used for compound separation of bioactive compounds in column chromatography and identification has been done through spectroscopic analysis. The purified compounds were further taken for antibacterial assay. The maximum Zone of Inhibition was evoluted by ajmalicine against *Enterococcus* which was 1.7 cm and minnimum (0.35 cm) was by serpentine against *Escherichia coli*. Multiple extraction method is better approach for ajmalicine and serpentine separation using TLC and column chromatography which can be used as a potential drug against pathogenic bacteria.

Keywords: *Catharanthus roseus*, Column Chromatography, TLC, UV-Vis spectroscopy, Serpentine, Ajmalicine

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Introduction

The plants are natural source of secondary metabolites which possess therapeutic importance to animals and can be separated and identified using methods as well as solvents for extraction (Kabesh *et al.*, 2015) . In spite of ever increasing advancement in the field of medicine and molecular diagnosis, it is estimated that world's 80% population depends on plant derivatives pharmaceuticals (Cragg and Newman, 2005).

Ajmalicine and serpentine has been found to have broad application in treatment of circulatory diseases (Barik *et al.*, 2016). Ajmalicine and serpentine has been found to occur in 20 species of the genus *Rauwolfia,* 4 of *Catharanthus* (Zenk *et*

al., 1977), 2 of *Mitragyne* and in *Paus inystalia yohirribe* and *Stemmadenia obovata* (Zenk *et al.*, 1977). Here we propose a technique for identification and isolation of ajmalicine and serpentine from *Catharanthus roseus* (L.) G Don.

Catharanthus roseus (L.) G Don is Perennial under shrub, woody at base, 30-90 cm tall, young branches pubescent. Leaves oblong 2.5-6 X 1.5 -2.5 cm, entire, obtuse, base acute, petiole short, 3-10 cm long with many glands at axil. Inflorescence of axillary 1-4 flowered cymes. Flowers pink or white; pedicle 1-3 mm long, calyx tube short, lobes linear, acute, 5-7 mm long, hairy. Corolla tube up to 1 cm long sparsely pubescent above, throat densely hairy with in below the stamens, lobes oblong rounded or obvate, 20-25 mm lay spreadingly. Disc scales higher than ovary, calyx 2-3 mm long. Ovary pubescent; style Calyx 2.5 cm, long stigma cup shaped. Follicles 2 slender, cylindric straight 15-20 mm long (Nisar *et al.*, 2016). It is an important medicinal plant of family Apocynaceae with 70 different types of alkaloids, steroids and chemotherapeutic agents which are effective as anti-cancerous, anti-malarial and antimicrobial activity. Vinblastine and Vincristine (Scherf *et al.*, 2008) are two important alkaloids that are being used widely to treat diabetes (Cragg and Newman, 2005). *Catharanthus roseus* (L.) G. Don contain terpenes and alkaloids which exhibits great pharmacological activities (Rischer *et al.*, 2006). It has wide range of medicinal properties such as antioxidant (Rischer *et al.*, 2006; Espín *et al.*, 2007), anticancer (Ueda *et al.*, 2002), antidiabetic (Chattopadhyay *et al.*, 1991), antimicrobial (Patil and Ghosh, 2010), antiulcer (Nosálová *et al.*, 1993), hypertensive (Anvikar *et al.*, 2012), wound healing (Nayak *et al.*, 2007), hypolipidemic (Patel *et al.*, 2011) and memory enhancement (Mishra and Verma, 2017)). Considering the medicinal value of this plant we have conducted present study to estimate qualitative method for isolation of phytochemicals (Fazli and Hardman, 1971) such as alkaloids (Poh-Yen, 2016), coumarins, anthraquinones and flavonoids (Pascual *et al.*, 2002).

A procedure for the preparation of plant extracts was also established in this work. Selection of method of extraction was performed after a thorough survey of literature. The separation was performed on silica plates using different solvent systems, each one applied for the identification of active principles according to their polarity. Spots were visualized under short and long wavelength of ultraviolet light and the same solvent system was then used for Column Chromatography for separation of active compounds whose conformation was then done by UV VIS Spectroscopy. UV-VIS was used for qualitative analysis and for identification of certain classes of compound in both pure and biological mixture. For the qualitative analysis UV-VIS was used because aromatic molecules are powerful chromatophores in UV range. This technique is not time consuming and present reduced cost compared to other technique (Yubin *et al.*, 2014) .

Many bioactive molecules have been isolated and purified by using thin layer and column chromatography methods. Most of these methods are still being used due to their convenience, economy and availability in various mobile and stationary phases (Altemimi *et al.*, 2017)

Materials and Methods

Plant Material Collection and Identification:

The fresh leaves of *Catharanthus roseus* (L.) G. Don was collected from garden of Department of Zoology, University College of Science, MLS University, Udaipur, Rajasthan, India. The plant was taxonomically identified at the Department of Botany, University College of Science, MLS University ,Udaipur, Rajasthan, India.

Extraction:

The fresh green leaves were collected from garden, shade dried and grinded. The powdered leaves were extracted successfully using 200 ml of hexane in soxhlet apparatus for 6 h at 70°C. The same powder was used for extraction with Dichloromethane (DCM) and Ethanol sequentially.

Rotary evaporator was used for removal of extra solvent and crude extract was collected.

Phytochemical analysis for steroid and alkaloid presence in leaf extract:

Test for Alkaloids: To 2 ml of extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Wanger's reagent were added. Presence of brown precipitate indicates the presence of alkaloids (Harborne, 1984).

Test for Steroids: To 1 ml of fruit extract equal volume of chloroform is added and a few drops of concentrated sulphuric acid added. Appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids (Harborne, 1984).

Chromatography:

For separation of phytochemicals Thin Layer Chromatography (TLC) and column chromatography were used. For Thin Layer Chromatography silica gel slurry prepared in CCl_4 was employed on glass plates. Coated plates were activated by placing in hot air oven for 1 h at 120°C. Samples of crude extract were then applied to the activated plates and separation was done using different solvents. The spots were visualized using UV lamp at 365 nm. The solvent which gave better result was then used for extraction of active group by column chromatography using silica gel slurry in chloroform as stationary phase and further confirmation was then done by UV VIS.

Mobile phases used for elution are given bellow:

1. For steroid isolation - chloroform: methanol (12:2) and chloroform: glacial acetic acid: methanol: water (65:32:12:8) (Fazli and Hardman, 1971).
2. For other phytochemicals – toluene : acetone (8:2), toluene: chloroform : acetone (40:35:25) and n-butanol : glacial acetic acid : water (50:10:40) (Pascual *et al.*, 2002).

Preparation of leaf extract for phytochemical separation:

Two methods were adopted for isolation of steroids and other phytochemicals. Firstly collected plant material was degreased through hexane and sequential extraction was done with dichloromethane (DCM) and ethanol. For steroidal isolation two mobile phases viz. chloroform:methanol (12:2) and chloroform: glacial acetic acid: methanol: water (65:32:12:8) were selected. TLC and Column elution were collected and further assessed for presence of steroids.

For other phytochemical analysis toluene: acetone (8:2), toluene: chloroform: acetone (40:35:25) and n-butanol:glacial acetic acid:water (50:10:40) mobile phases were taken and various solvent systems were used for Thin layer Chromatography and column chromatography. TLC and Column elution were then assessed for presence of phytochemicals.

The hexane, DCM and ethanol extracts were added as spots using capillary at one end of the TLC plate at 1.5 cm above. Plates were allowed to air dry. The dried plates were placed in a different beaker containing mobile phase. The samples were allowed to run towards the other end of the plate. The plates were removed and allowed to air dry. Plates were then visualized under the UV light at 356 nm and different colour bands were obtained.

Extraction by Column Chromatography:

2 g of silica gel was added to 30 ml chloroform and put in the glass column. The column containing gel was washed twice with chloroform. 4 ml ethanol extract was added carefully to the top of gel and 10 ml polar solvent was added into it. The fractions were allowed to pass through gel. After 2 h 10 samples were eluted and fractions were collected at different time intervals.

Ultra Violet- Visible Spectroscopy:

JENWAY 7205 UV-Visible Spectrophotometer was used for completion of this experiment. The fractions were collected in eppendorf tubes. Absorbance spectrum was obtained for all eluted fraction using UV-VIS Solvent used for column

chromatography was used as control and spectrum was obtained between 250-800 nm for all fractions separately.

5 ml elution of column chromatography was collected in different tubes and TLC was done for different samples. The sample showing highest concentration was further taken for UV-VIS and results were recorded.

UV-VIS peaks were observed at 261 nm, 268 nm, 295 nm, 308 nm, 334 nm and 373 nm. From that 261 nm peak reciprocate flavonoids and 268 to 373 nm peaks reciprocate to alkaloids (Sangster and Stuart, 1964)

Extraction of pure Compounds:

Extraction process using coloumn chromatography was performed several time and the collected fraction was undergone with UV-VIS process. The samples with the range of desired compund was collected and further applied for purification by performing the same process under double coloumn chromatography. Fractions which were collected then run over TLC following above procedure visualized in 365 nm UV lamp. Two sharp bands were observed, these two bands were cut from plates and collected in different tubes and identified using UV-Vis spectro-photometer. After the extraction Ajmacilin and serpentine were marked as sample no. 4 and 5, respectively.

HPLC and GCMS sample preparation:

50 g of dried leaf powder was soaked in 500 ml methanol overnight. Evaporation of the methanol was done on water bath. The remaining residue was resuspended in 20 ml 0.1 N HCl and again extracted with ethyl acetate for 3 h. Ethyl acetate was evaporated and residue was resuspended in methanol and filtered for removal of residues. The samples were sent to Department of Chemistry, IIT Roorkee for HPLC analysis.

Antibacterial assay:

25 g of nutirent agar was disssolved in one liter of distilled water and boiled. Nutirent agar was then autoclaved for 20 min at 121°C and left to cool at room temperature. Once the medium was cooled, it was poured into petridish and allowed to solidify.

Agar well diffusion method was used for antibacterial assay. Overnight grown culture was spread onto 20 ml of sterile agar plates by using a sterile L shaped spreader and the surface was allowed to dry for 5 min. Wells were punctured over the agar plates using sterile gel puncture and the isolted sample was added at avrious concentration (50,75 and 100 mg/100 ml of Ethanol). 100 µl of different test sample was added to well while ethanol was used as control. The plates were incubated for 24 h at 37°C. The diameter of Zone of Inhibition formed were measued in cm unit and recorded.

Results

The results of the study of Thin Layer Chromatography have been presented in Tables 1 and 2 and Figures 1 and 2. In mobile phase of chloroform: methanol (12:2) and chloroform: glacial acetic acid: methanol: water (65:32:12:8), anthroquinones show green colour (Fig. 1), alkaloids with florescent pink color (Fig.1) and florescent blue colour shown by steroids (Fig.1).

The UV analysis of bands at 365 nm of TLC with mobile phase toluene: acetone (8:2), toluene: chloroform: acetone (40:35:25) and n-butanol: glacial acetic acid: water (50:10:40) showed alkaloids with florescent pink color (Fig. 2), coumarins with orangish red colour (Fig. 2) and steroid show floroscenet blue colour (Fig. 2).

The graphical representation of various mobile phases on phytoche,micals has been represented in Figure 3 which clearly states that alkaloid is isolated in almost all mobile phases. The alkaloid phase of the selected plant contains ajmalicine and sepentine (Fig. 4) with rich medical values.

Phytochemical analysis revealed the presence of alkaloids and steroids in the extract (Fig 5) when the prepared extract was taken for phytochemical analysis in steroid and alkaloid respectively. On addition of Wanger's reagent the

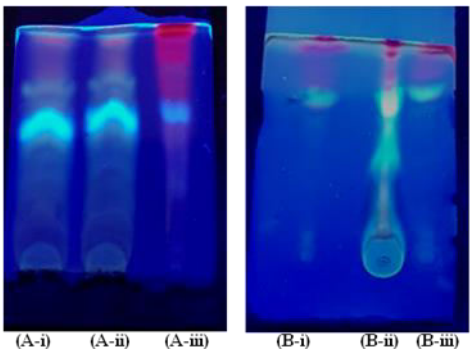


Fig. 1: Thin Layer Chromatography visualization under UV lamp at 365 nm using different plant extract and mobile phases.

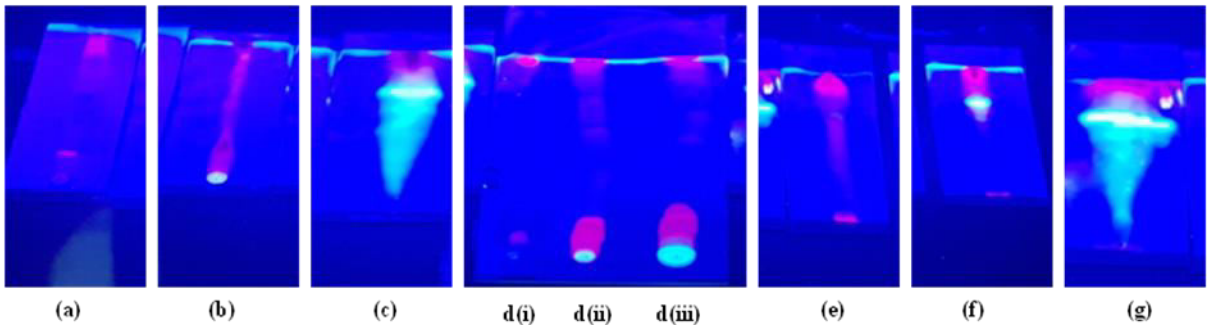


Fig. 2: Thin Layer Chromatography visualization under UV lamp at 365 nm using different plant extract and mobile phases.

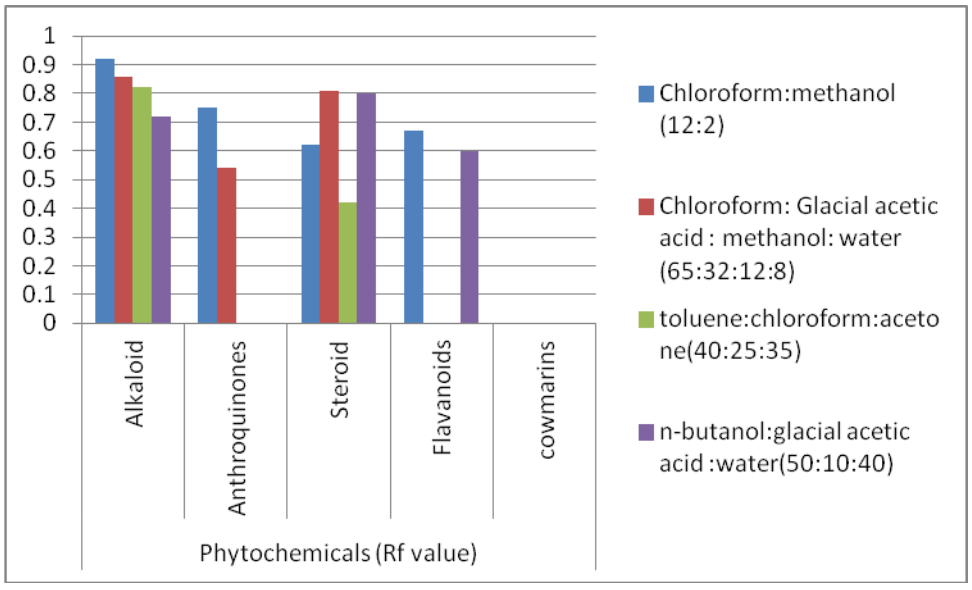
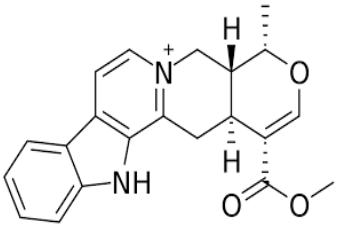


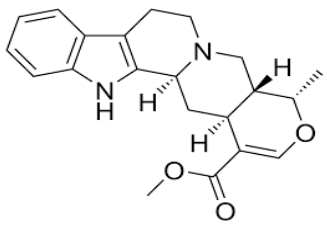
Fig. 3: Phytochemical analysis using various solvent systems highest Rf value shown by alkaloids in solvent system chloroform:methanol (12:2) while lowest by steroids in toluene:chloroform:acetone (40:25:35).

Table 1: Plant extract in different solvents and their phytochemical analysis

S. No.	Plant extract	Solvent for TLC	Band under 365nm UV	Phytochemical
1	Hexane	Fig.- (2A-i) Chloroform: methanol (12:2)	Light blue (Rf value- 0.62)	Steroid
			Reddish orange(Rf value- 0.67)	Flavanoid
			Florescent pink(Rf value- 0.92)	Alkaloid
		Fig.-(2B-iii) ıloroform: Glacial acetic acid: methanol: water (65.32:12:8)	green(Rf value- 0.54)	Anthroquin one
			Florescent blue(Rf value- 0.81)	Steroid
			Floroscent pink(Rf value- 0.86)	Alkaloid
2	DCM	Fig.-(2A-ii) Chloroform: methanol (12:2)	Florescent Blue (Rf value- 0.62)	Steroid
			Lightish green(Rf value- 0.75)	Anthroquin one
			pink(Rf value- 0.85)	Alkaloid
		Fig.-(2B-i) chloroform: Glacial acetic acid: methanol: water (65.32:12:8)	green(Rf value- 0.85)	Anthroquin one
			Florescent blue(Rf value- 0.71)	Steroid
			Florescent pink(Rf value- 0.86)	Alkaloid
3	Etnanol	Fig.-(2A-iii) Chloroform: methanol (12:2)	florescence blue (Rf value- 0.62)	Steroid
			green(Rf value- 0.75)	Anthroquin one
			Lightish pink(Rf value- 0.85)	Falvanoid
		Fig.-(2B-ii)	Green(Rf value- 0.54)	Anthroquin one
			Florescent blue(Rf value- 0.81)	Steroid
			Florescent pink(Rf value- 0.86)	Alkaloid



Serpentine



Ajmalicine

Fig. 4: Chemical structure of Serpentine and Ajmalicine.

Table 2: Details of plant extract in different solvents and their phytochemical analysis

S. No.	Plant extract	Solvent for TLC	Band under 365nm UV	Phytochemical present
1	Hexane	Fig.- (A)	florescence red-orange (Rf value- 0.8)	Alkaloid
				Flavanoids
		Fig.-(d-i) Toluene:chloroform:acetone (40:25:35)	Rf- 0.53) florescence red-orange(Rf value- 0.7)	Alkaloid
		Fig.-(e) n-butanol:glacial acetic acid:water (50:10:40)	florescence red-orange (Rf value- 0.6)	
2	DCM	Fig.-(b) Toluene:chloroform:acetone (40:25:35)	florescence red-orange (Rf value- 0.65) and	Alkaloid
		Fig.-(d-ii) Toluene:chloroform:acetone (40:25:35)	blue dot (Rf value- 0.2) at intial point	
			florescence red-orange (Rf value- 0.83)	Alkaloid
			blue dot(Rf value- 0.2) at initial point	
		Fig.-(f) n-butanol:glacial acetic acid:water (50:10:40)	Blue band(Rf value- 0.42) at the mid	Steroid
	Etnanol	Fig.-(c) n-butanol:glacial acetic acid:water (50:10:40)	red- orange(Rf value- 0.82) at ending	Alkaloid
			blue band (Rf value- 0.6)	Steroid
			florescence red-orange (Rf value- 0.8)	Alkaloid
		Fig.-(d-iii) Toluene:chloroform:acetone (40:25:35)	red- orange(Rf value- 0.72)	Flavanoid
		Fig.-(g) n-butanol:glacial aceti acid:water (50:10:40)	Rf- 0.32) Florescent blue (Rf value- 0.8)	Steroid
			And blue band (Rf value- 0.6)	Flavanoid

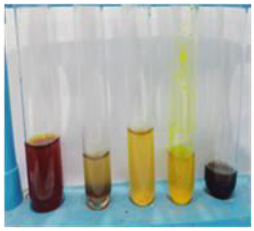


Fig. 5: Phytochemical analysis in leaf extract, (C) *Catharanthua roseus* leaves extract. (A) and (E) confirm alkalaoid presence by showng brown color precipitate on addition of wanger's reagent. (B) confirms the presence of steroids by showing brown color ring on addition of a few drops of chloroform and sulfuric acid.

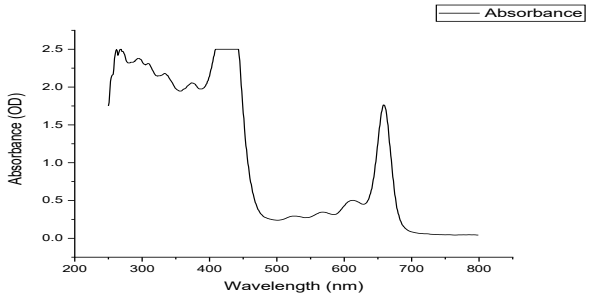


Fig. 6: UV VIS spectrum of sample No. 7 eluted form ethanol extract using n-butanol: glacialacetic acid: water (50:10:40) showing peak at 261 nm,268 nm,295 nm,308 nm,334 nm,373 nm and 658 nm.

extract show brown precipitate (Fig. 5) and addition of chloroform and sulphuric acid in the extract results in formation of brown ring which reveals the presence of alkaloid and steroid, respectively.

The sequential extraction method was used for isolation of different fractions and identification was done on basis of Thin Layer Chromatography visualization at 365 nm UV lamp, based on which furthur isolation was done in column chromatography of ethanol extract with mobile phase n-butanol: glacial acetic acid: water (50:10:40) was done and 10 samples were eluted. Among 10 eluted samples, sample No.7 showed peak at 261 nm, 268 nm, 295 nm, 308 nm, 334 nm,373 nm and 658 nm, alkaloids show its peak between 218 to 390 nm (Fig.6) (Sangster and Stuart, 1964). However, typical maximum values of alkaloids with aromatic rings ranges from 243 nm to 351 nm (Harborne, 1984) with maximum absorbance at 295 nm for Ajamalicine and 308 nm for Serpentine (Hisiger and Jolicoeur, 2007). The sample number 8 showed peak at 658 nm (Fig. 7) (Harborne, 1984).

Complete HPLC and GCMS analysis of the alkaloid fraction is been done which confirms the presence of over 100 types of molecules (Fig. 8). Complete GCMS analysis of the extract is summerized in Tables 3 and 4. After the complete analysis two compounds were isolted from extract i.e. ajmalicine and serpentine. Both the purified compounds were tested for antimicrobial activity against *Escherichia coli* and *Enterococcus* bacteria in two replica. Ajmalicine showed highest zone of inhibition of 1.1 cm for at 100 mg/100 ml of ethanol for *Escherichia coli* (Fig. 9a) and 1.7 cm for *Enterococcus* at 75 mg/100 ml and 100 mg/100 ml of ethanol (Fig. 9b, Table 3). Serpentine had shown highest zone of Inhibition for *Escherichia coli* (1.15 cm) at 100 mg/100 ml of ethanol (Fig. 9c) and for *Enterococcus* 0.7 cm at 75 mg/100 ml of ethanol (Fig. 9d). The comaprative analysis of the zone of inhibition has been represented in Figure 10 and Table 5.

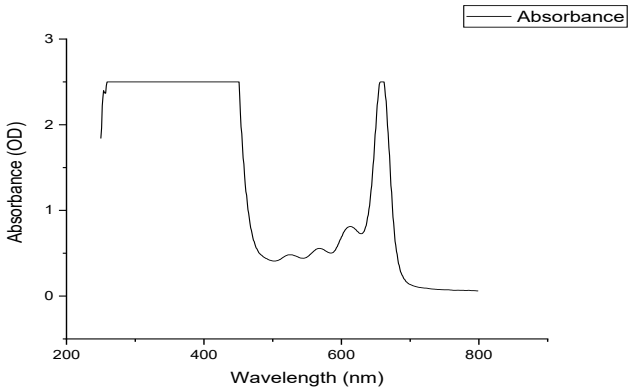
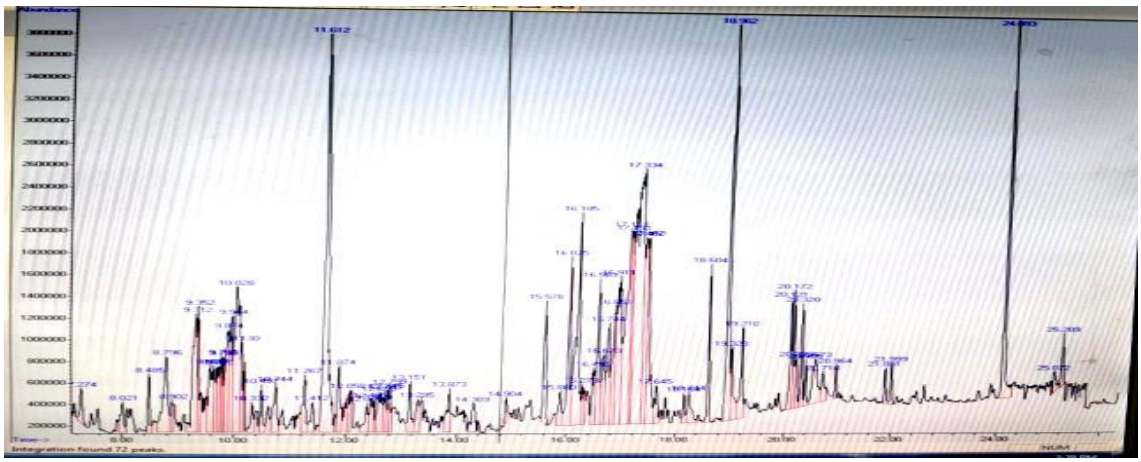
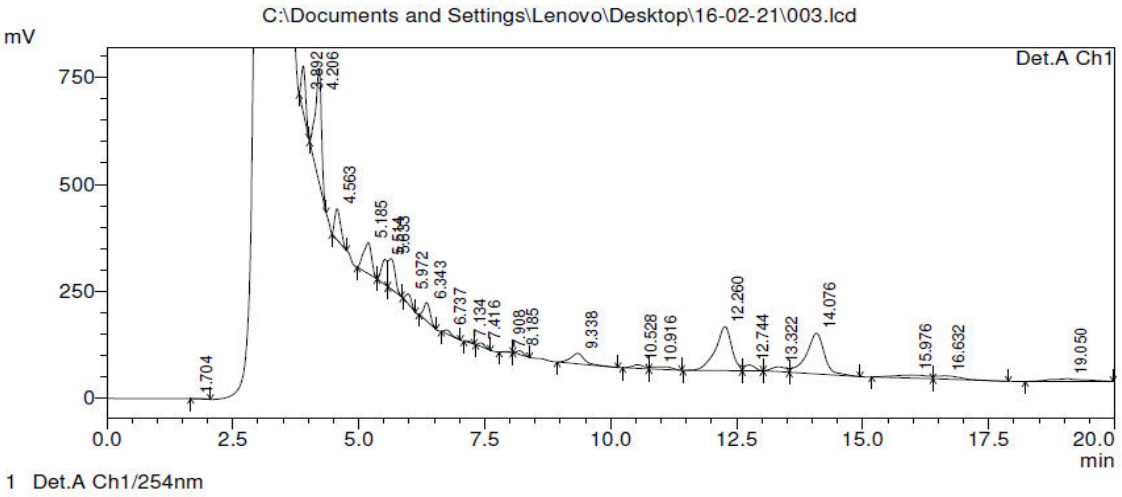


Fig. 7: UV VIS spectrum of sample No. 8 eluted form ethanol extract using n-butanol: glacial acetic acid: water (50:10:40) showing peak at 658 nm only.



(A)



(B)

Fig.8: Graphical representation of HPLC (A) and GCMS (B) analysis of *Catharanthus roseus* leaves

Table 3: Table showing complete GCMS analysis

1.	11.612	Catechol
2.	11.873	Benzofuran,2,3-dihydrocatecholborane
3.	12.057	Neopentyl 2-methylbutanoate
4.	12.395	d-Mannitol, 1,4-anhydro- D-erythro-Pentose, 2-deoxy- 2,3-Epoxybutane
5.	12.463	Cyclohexane, 1,3,5- trimethoxy-,(1 .alpha.,3.alpha.,5.beta.)
6.	12.521	Propanal, 2,3-dihydroxy-
7.	12.704	1,2-Ethanediol, 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-, [S-(R*,R*)]-
8.	12.743	Erythritol
9.	13.149	Benzoic acid 2,3-dihydroxy methyl ester
10.	13.294	(3-Methyl-oxiran-2-yl)-methanol
11.	13.874	3-Deoxy-d-mannitol
12.	14.309	Diphenylmethane
13.	14.909	Ethylpareben
14.	15.576	Pyrrolidin-2,5-dione, 3-fluoro-4-methoxy-1-propyl
15.	15.876	Hex-5-ynoic acid, methyl ester
16.	16.021	4,6-Dimethyl-4-hydroxyhept-5-enoic acid lactone
17.	16.185	Tetradecyl trifluoroacetate
18.	16.272	Benzophenone
19.	16.495	2-Amino-8-[3-d-ribofuranosyl]imidazol[1,2-a zo[1,2-a]-s-triazin-4-one
20.	16.669	3,4-Di-O-methyl-L-arabinopyranose
21.	16.746	2,2,4-Trimethyl-3-pentanol
22.	16.852	Thiophene, tetrahydro-2-methyl
23.	16.910	alpha.-Methyl mannofuranoside
24.	17.113	Myo-inositol,4-C-methyl
25.	17.152	1,3-Dioxolane, 2-(3-methoxypropyl) -2-methyl-
26.	17.336	Methyl(methyl 4-O-methyl-.alpha.-d -mannopyranoside)uronate
27.	17.403	3-Heexanol,2,4-dimethyl

28.	17.452	4-O-methylmannose
29.	17.645	4,5-Pyrimidinediamine, 6-methyl-
30.	18.158	1,2-Benzenedicarboxylic acid, butyl octyl este
31.	18.245	1-Octadecene
32.	18.602	Hexadecanoic acid, methyl ester
33.	18.960	n-Hexadecanoic acid
34.	19.028	Dibutyl phthalate
35.	19.211	Hexadecanoic acid, ethyl ester
36.	20.120	4-Oxazolecarboxylic acid, 4,5-dihy dro-2-phenyl-, 1-methylethyl ester
37.	20.169	9,12-Octadecadienoic acid (Z,Z) methyl ester
38.	20.227	7,10,13-Hexadecatrienoic acid, methyl ester
39.	20.323	Phytol
40.	20.391	Heptadecanoic acid,14-methyl-ester
41.	20.575	Z-6-Pentadecen-1-ol acetate
42.	20.720	Octadecanoic acid
43.	21.880	(+)-trans-3,4-Dimethyl-2-phenyltetrahydro-1,4-thiazine
44.	21.996	Propanoic acid, 2-(benzoylamino)-3 -phenyl-, methyl ester
45.	24.094	Bis(2-ethylhexyl) phthalate
46.	25.071	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl
47.	25.206	Methyltris(trimethylsiloxy)silane

Table:4. Showing HPLC analysis

Reference	Compound	Retention time	Cloumn	Moile phase	Detector	Elution
Bhadra *et al.* (1993)	Tryptoamine	3.5	Bondpack C_{18} 10 µm	Acetonitrile:o.1 % TFA in water	Ch1/254 nm	Isocratic 1 ml min^{-1}
	Secologanin	4.0				
	Vindolin	4.9				
	Ajmalicine	8.1				
	Serpentin	8.6				
	Catharanthine	9.3				
	Vincristine	12.5				
	Taberosinine	13.5				

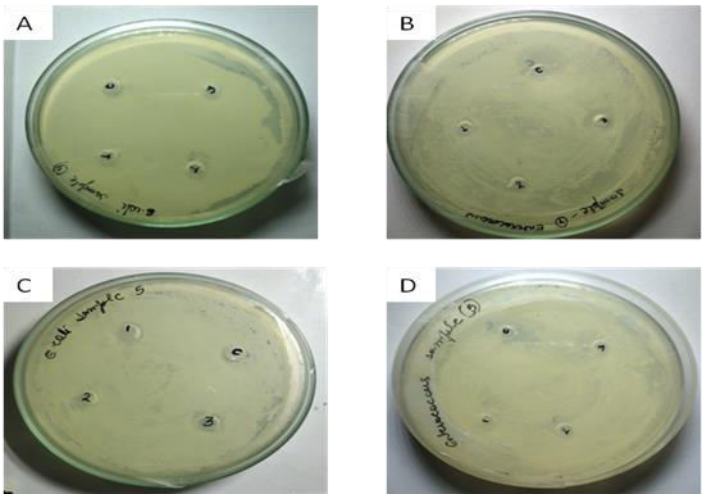


Fig. .9: (a) and (b) zone of inhibition of *Escherichia coli* and *Enterococcus* against Ajmalicine (sample 4) highest being 1.15 cm at 100 mg/100ml concentration; (c) and (d) zone of inhibition of *Escherichia coli* and *Enterococcus* against srpentine (sample 5) highest being 1.7 at 7. mg/100 ml and 100 mg/100 ml concentration.

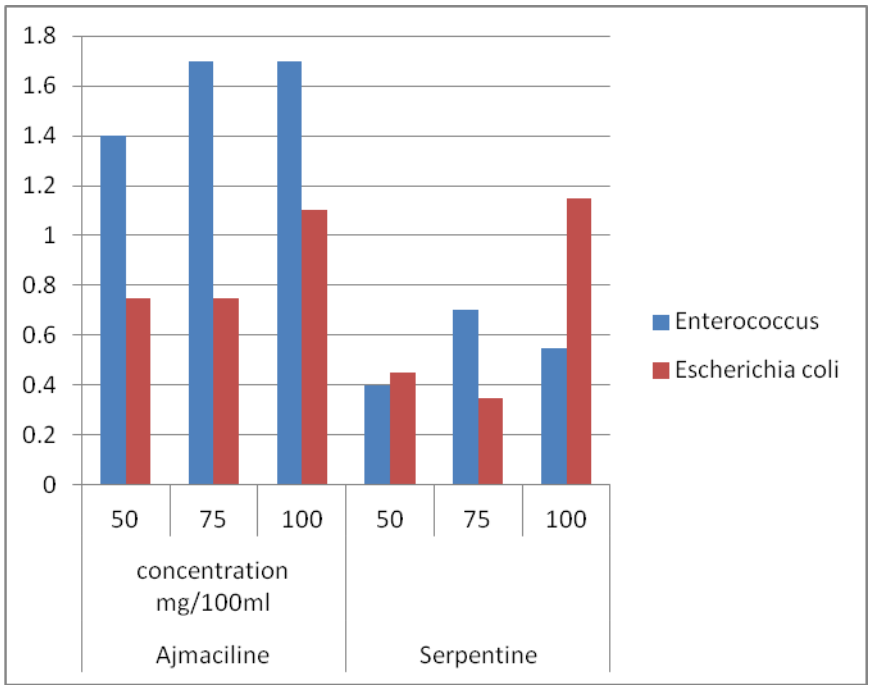


Fig. 10: Antimalarial activity by ajmalicine and serpentine against *Enterococcus* and *Escherichia coli* where Maximum Zone of Inhibition was evoluted by ajmalicine against *Enterococcus* which was 1.7 cm and minnimum was by sepentine 0.35 cm against *Escherichia coli*.

Table 5: Antimicrobial activity

Samples	Antibacterial Activity (Zone of Inhibition in cm)	
	Escherichia coli	*Enterococcus*
Ajmalicine	07±0.36	1.6 ±0.17
Serpentine	0.6±0.4	0.55 ±0.15

Discussion

There is no universal method which can be applied for the extraction of secondary metabolites from *Catharanthus roseus* (Kumar *et al.*, 2022). However, the phytochemical analysis reveals the presence of steroid and alkaloid in the extract (Uddin *et al.*, 2022). Tikhomiroff and Jolicoeur (2002) reported low extraction yields when using a single methanol extraction step for intracellular serpentine, vincristine, vindoline, catharanthine, tabersonine and tryptophan while a relatively high yield was shown by multiple successive extraction of plant parts using different solvents (Hisiger and Jolicoeur, 2007). In the present study multiple extraction method has been chosen because it shows high yield of secondary metabolites. HPLC on methanolic extract of *Catharanthus roseus* leaves, Ajmalicine and Serpentine were obtained in small amount using methanol extraction (Cheng *et al.*, 2010).The amount of secondary metabolites obtained using various solvents is different (Zheng and Wu, 2004). However, we offer a technique which makes it easy to select mobile phase for qualitative estimation, this could led to further quantification of experiments for quantitative isolation using HPLC as Digvijay *et al*. (2004), conducted studies on the simultaneous quantification of alkaloids in leaves but the amount of serpentine and ajmalicine obtained were 0.0034% and 0.29% only. However, in referred work single extraction method was used with methanol while in our study multiple extraction steps were used for better result.

In the result stated above it is clear that alkaloid show higher mobility in chloroform: methanol (12:2) and n-butanol:glacial acetic acid:water (50:10:40) while steroids show higher mobility in chloroform : glacial acetic acid : methanol:water (65:32:12:6) and toluene: chloroform: acetone (40:25:35). From the data depicted above it could be concluded that n-butanol:glacial acetic acid:water (50:10:40) is better mobile phase for alkaloid isolation.

From literature survey we came to know that this plant has been mostly studied with respect to its anti cancerous (Taher *et al.*, 2019) and anti diabetic properties (Singh *et al.*, 2001) but very little survey have been done on the antimicrobial properties (Patil and Ghosh, 2010). This study focus on isolation of two medicinally important chemicals serpentine (Elizabeth, 2001) and ajmalicine (Wenkert *et al.*, 1961) from *Catharanthus roseus* plant leaves and its antimicrobial property. The significance of study is that these compounds can be used as substitutes for antibiotic therapy and the procedure used for the study can provide a rapid and economic way for extraction of various phytochemicals and their identification with a wide choice of solvents for better yield.

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