The Antibacterial and Qualitative studies of Ajmalicine and Serpentine extracted from *Catharanthus roseus* (L.) G. Don leaves

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Research Article

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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 taken up 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 evolved by ajmalicine against *Enterococcus* which was 1.7 cm and minimum was by serpentine 0.35cm 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.

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 [1]. 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[2].

Ajmalicine and serpentine has been found to have broad application in treatment of circulatory diseases. Ajmalicine and serpentine has been found to occur in 20 species of the genus *Rauwolfia*, 4 of *Catharanthus*, 2 of *Mitragyne* and in *Paus inystalia yohirribe* and *Stemmadenia obovata* [3]. Here we propose a technique for identification and isolation of ajmalicine and serpentine from *Catharanthus roseus* (L.) G Don.

Catharanthus roseus(L.) G Don, Syst. 4:95. 1837; Sant, in Bull. Bot. Surv. India 3:15.1961. *Vinca rosea* L.syst.ed. 10.944.1759; Clark,in Hook.Fl.Brit. India 3:640.1882. 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 upto 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 Ca 2.5 Cm, long stigma cup shaped. Follicles 2 slender, cylindric straight 15–20 mm long [4]. It is an important medicinal plant of family Apocynaceae with 70 different types of alkaloids, steroids and chemotherapeutic agents which are effective as anticancerous, antimalarial and antimicrobial activity. Vinblastine and Vincristine are two important alkaloids that are being used widely to treat diabetes [5]. *Catharanthus roseus* (L.) G. Don contain terpenes and alkaloids which exhibits great pharmacological activities [6]. It has wide range of medicinal properties such as antioxidant [6] [7], anticancer [8], antidiabetic [9], antimicrobial [10], antiulcer [11], hypertensive [12] [13], wound healing [14], hypolipidemic[15] and memory enhancement[16][17].Considering the medicinal value of this plant we

have conducted present study to estimate qualitative method for isolation of phytochemicals [18] such as alkaloids [19], coumarins, anthraquinones, flavonoids[20].

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 chromophores in UV range. This technique is not time consuming and present reduced cost compared to other technique [21] .

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 [22]

Results

Phytochemical analysis revealed the presence of alkaloids and steroids in the extract (Fig. 1). When the prepared extract was taken for phytochemical analysis in steroid and alkaloid respectively. On addition of wanger's reagent the extract show brown precipitate (Fig. 1A and E) and addition of chloroform and sulphuric acid in the extract results in formation of brown ring(Fig. 1B) which reveals the presence of alkaloid and steroid respectively. The results of the study of Thin Layer Chromatography have been presented in Table 1 and 2 and Figs. 6 and 7. 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. 7. a,c,g,d(i),d(ii),d(iii),e,f), coumarins with orangish red colour (Fig. 7. b) and steroid show floroscenet blue colour (Fig. 2-(c and g),f) while in another mobile phase with chloroform: methanol (12:2) and chloroform: glacial acetic acid: methanol: water (65:32:12:8) anthroquinones show green colour (Fig. 6. A-i,A-ii,B-ii), alkaloids with florescent pink color (Fig. 6. A-iii,B-i,B-ii,B-iii) and florescent blue colour shown by steroids (Fig. 6. A-i,A-ii,Aiii,B-i,B-ii,B-iii).

The sequential extraction method was used for isolation of different fractions and identification was done on basis of Thin Layer Chromatography visualization in 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. 2) [23]. However, typical maximum values of alkaloids with aromatic rings ranges from 243 nm to 351 nm[24] with maximum absorbance at 295nm for Ajamalicine and 308 nm for Serpentine [25]. The sample number 8 showed peak at 658 nm (Fig. 3)[24].

HPLC analysis of the plant sample prepared through standard protocol according to Harborne, 1984 marks the presence of Ajmalicine and Serpentine at Retention time 8.1 and 8.6 respectively (Fig. 4-a, Table 4). The complete GCMS analysis of *Catharanthus* leaves is shown in Fig. 4-b and Table 3. Both the purified compounds were tested for antimicrobial activity against *Escherichia coli* and *Enterococcus* bacteria in two replicates. Ajmalicine showed highest zone of inhibition of 1.1 cm for at 100mg/100ml of ethanol for *Escherichia coli* (Fig. 5-a, table-3) and 1.7cm for *Enterococcus* at 75mg/100ml and 100mg/100ml of ethanol (Fig. 5-b, Table-5). While Serpentine had shown highest zone of inhibition for *Escherichia coli* 1.15 cm at 100mg/100ml of ethanol (Fig. 5-c, table-5) and for *Enterococcus* 0.7 cm at 75mg/100ml of ethanol (Fig. 5-d, Table-5).

Discussion

There is no universal method which can be applied for the extraction of secondary metabolites from *Catharanthus roseus*. However the phytochemical analysis reveals the presence of steroid and alkaloid in the extract. 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 [25]. In the present study multiple extraction method has been chosen because it shows high yield of secondary metabolites. Ferrerers *et al.* 2010, performed HPLC on methanolic extract of *Catharanthus roseus* leaves, Ajmalicine and Serpentine were obtained in small amount using methanol extraction [26]. The amount of secondary metabolites obtained using various solvents is different however we offer a technique which makes it easy to select mobile phase for qualitative estimation, this could lead 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 [27]. 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) (Fig. 8). 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 and anti diabetic properties but very little survey have been done on the antimicrobial properties. This study focus on isolation of two medicinally important chemicals serpentine and ajmalicine 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.

Methodology

Plant Material Collection and Identification -

The fresh leaves of *Catharanthus roseus* (L.) G. Don Plant was collected from garden of department of zoology, University College of Science, MLS University, Udaipur, Rajasthan, India. The plant was taxonomically identified by Prof. B.L. Yadav, Emeritus Professor, Mewar University, Gnagar, Rajasthan, India. However, the plant copy was also submitted to publicly available Herbarium at Sangam University, Bhilwara. Complete handling of plant was done according to relevant guidelines and regulations. Copy of the plant material is also submitted in herbarium of Department of Botany, Sangam University, Bhilwara accession number: SU/Herbarium/788.

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 hrs 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 Wagner's reagent were added. Presence of brown precipitate indicates the presence of alkaloids [24].

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 [24].

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 hour 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 below:

1. **For steroid isolation** - chloroform: methanol (12:2) and chloroform: glacial acetic acid: methanol: water (65:32:12:8) [18].
2. **For other phytochemicals** – toluene : acetone (8:2) , toluene : chloroform : acetone (40:35:25) and n-butanol : glacial acetic acid : water (50:10:40) [20].

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 1.5cm 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 356nm and different colour bands were obtained.

Extraction by Column Chromatography

2 grams of silica gel was added with 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 hrs 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 eppendroff 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 250-800nm 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.[23]

Extraction of pure Compounds

Extraction process using column chromatography was performed several times and the collected fraction was undergone with UV-VIS process. The samples with the range of desired compound were collected and further applied for purification by performing the same process under double column 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 identified using UV-Vis spectrophotometer. After the extraction Ajmacilin and serpentine were marked as sample no. 4 and 5 respectively.

HPLC and GCMS sample preparation:

50 grams of leaf and flower dried powdered form 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 hours. 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

Twenty five grams of nutrient agar was dissolved in one liter of distilled water and boiled. Nutrient agar was then autoclaved for 20 minutes at 121°C and left to cool at room temperature. Once the medium was cooled, it was poured into petri dish 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 minutes. Wells were punctured over the agar plates using sterile gel puncture and the isolated sample was added at various concentrations (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 hours at 37°C. The diameter of Zone of Inhibition formed were measured in cm unit and recorded.

Declarations

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Availability of data and materials:

The datasets generated in the current study are produced from various laboratories contribution. The datasets as well as concern authority detail will be made available by corresponding author on reasonable request.

Ethics and approval of consent to participate

The article does not contain any studies with human or animal subjects.

Competing interest:

The authors declare that they have no conflicts of interest with respect to work described in the manuscript.

Author's contribution:

All authors participated equally for working over this manuscript.

Consent for publication:

I give my consent for publication.

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Tables

Table 1 Details of 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) chloroform: Glacial acetic acid: methanol: water (65.32:12:8)	green(Rf value- 0.54)	Anthroquinone
			Florescent blue(Rf value- 0.81)	Steroid
			Florescent 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)	Anthroquinone
			pink(Rf value- 0.85)	Alkaloid
		Fig.-(2B-i) chloroform: Glacial acetic acid: methanol: water (65.32:12:8)	green(Rf value- 0.85)	Anthroquinone
			Florescent blue(Rf value- 0.71)	Steroid
			Florescent pink(Rf value- 0.86)	Alkaloid
3	Etnanol	Fig.-(2A-iii) Chloroform: methanol (12:2)	fluorescence blue (Rf value- 0.62)	Steroid
			green(Rf value- 0.75)	Anthroquinone
			Lightish pink(Rf value- 0.85)	Flavanoid
		Fig.-(2B-ii)	Green(Rf value- 0.54)	Anthroquinone
			Florescent blue(Rf value- 0.81)	Steroid
			Florescent pink(Rf value- 0.86)	Alkaloid

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)	fluorescence red-orange (Rf value- 0.8) Rf- 0.53)	Alkaloid Flavanoids
		Fig.-(d-i) Toluene:chloroform:acetone (40:25:35)	fluorescence red-orange(Rf value- 0.7)	Alkaloid
		Fig.-(e) n-butanol:glacial acetic acid:water (50:10:40)	fluorescence red-orange (Rf value- 0.6)	
2	DCM	Fig.-(b) Toluene:chloroform:acetone (40:25:35)	fluorescence red-orange (Rf value- 0.65) and blue dot (Rf value- 0.2) at initial point	Alkaloid
		Fig.-(d-ii) Toluene:chloroform:acetone (40:25:35)	fluorescence red-orange (Rf value- 0.83) blue dot(Rf value- 0.2) at initial point	Alkaloid
		Fig.-(f) n-butanol:glacial acetic acid:water (50:10:40)	Blue band(Rf value- 0.42) at the mid	Steroid
				Alkaloid
			red- orange(Rf value- 0.82) at ending	
3	Ethanol	Fig.-(c) n-butanol:glacial acetic acid:water (50:10:40)	blue band (Rf value- 0.6)	Steroid
			fluorescence red-orange	

	(Rf value- 0.8)	
Fig.-(d-iii) Toluene:chloroform:acetone (40:25:35)	red- orange(Rf value- 0.72)	Alkaloid
	Rf- 0.32)	Flavanoid
Fig.-(g) n-butanol:glacial acetic acid:water (50:10:40)	Florescent blue (Rf value- 0.8)	Steroid
	And blue band (Rf value- 0.6)	
		Falvanoid

Table 3
Showing complete data of GCMS

S.No.	RT Area%	NIST 11.L Database
1.	7.271	Trimethylene glycol monomethyl ether
2.	8.025	3-Furaldehyde
3.	8.489	4-Cyclopentene-1,3-dione
4.	8.905	8-Chloro-6-[2-piperidino-1-hydroxy ethyl]-2-phenylquinoline
5.	9.311	Pyridine, 3-ethyl
6.	9.349	Phenol
7.	9.639	Diglycerol
8.	9.717	Glycerin
9.	10.336	Isosorbide Dinitrate
10.	10.500	Pyridine, 3-ethyl-5-methyl
11.	10.742	1,3-Cyclohexanedione, 2-methyl- Di(pent-4-enyl)amine
12.	11.264	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-
13.	11.409	2,4,6-Cycloheptatrien-1-one Benzene
14.	11.612	Catechol
15.	11.873	Benzofuran,2,3-dihydrocatecholborane

S.No.	RT Area%	NIST 11.L Database
16.	12.057	Neopentyl 2-methylbutanoate
17.	12.395	d-Mannitol, 1,4-anhydro- D-erythro-Pentose, 2-deoxy- 2,3-Epoxybutane
18.	12.463	Cyclohexane, 1,3,5- trimethoxy-(1 .alpha.,3.alpha.,5.beta.)
19.	12.521	Propanal, 2,3-dihydroxy-
20.	12.704	1,2-Ethanediol, 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-, [S-(R*,R*)]-
21.	12.743	Erythritol
22.	13.149	Benzoic acid 2,3-dihydroxy methyl ester
23.	13.294	(3-Methyl-oxiran-2-yl)-methanol
24.	13.874	3-Deoxy-d-mannitol
25.	14.309	Diphenylmethane
26.	14.909	Ethylpareben
27.	15.576	Pyrrolidin-2,5-dione, 3-fluoro-4-methoxy-1-propyl
28.	15.876	Hex-5-ynoic acid, methyl ester
29.	16.021	4,6-Dimethyl-4-hydroxyhept-5-enoic acid lactone

S.No.	RT Area%	NIST 11.L Database
30.	16.185	Tetradecyl trifluoroacetate
31.	16.272	Benzophenone
32.	16.495	2-Amino-8-[3-d-ribofuranosyl]imidazol[1,2-a] zo[1,2-a]-s-triazin-4-one
33.	16.669	3,4-Di-O-methyl-L-arabinopyranose
34.	16.746	2,2,4-Trimethyl-3-pentanol
35.	16.852	Thiophene, tetrahydro-2-methyl
36.	16.910	alpha.-Methyl mannofuranoside
37.	17.113	Myo-inositol,4-C-methyl
38.	17.152	1,3-Dioxolane, 2-(3-methoxypropyl) -2-methyl-
39.	17.336	Methyl(methyl 4-O-methyl-.alpha.-d -mannopyranoside)uronate
40.	17.403	3-Heexanol,2,4-dimethyl
41.	17.452	4-O-methylmannose
42.	17.645	4,5-Pyrimidinediamine, 6-methyl-
43.	18.158	1,2-Benzenedicarboxylic acid, butyl octyl este
44.	18.245	1-Octadecene

S.No.	RT Area%	NIST 11.L Database
45.	18.602	Hexadecanoic acid, methyl ester
46.	18.960	n-Hexadecanoic acid
47.	19.028	Dibutyl phthalate
48.	19.211	Hexadecanoic acid, ethyl ester
49.	20.120	<div>4-Oxazolecarboxylic acid, 4,5-dihy</div> <div>dro-2-phenyl-, 1-methylethyl ester</div>
50.	20.169	<div>9,12-Octadecadienoic acid (Z,Z) methyl ester</div>
51.	20.227	<div>7,10,13-Hexadecatrienoic acid, methyl ester</div>
52.	20.323	Phytol
53.	20.391	Heptadecanoic acid,14-methyl-ester
54.	20.575	Z-6-Pentadecen-1-ol acetate
55.	20.720	Octadecanoic acid
56.	21.880	<div>(+)-trans-3,4-Dimethyl-2-phenyltetrahydro-1,4-thiazine</div>
57.	21.996	<div>Propanoic acid, 2-(benzoylamino)-3</div> <div>-phenyl-, methyl ester</div>

S.No.	RT Area%	NIST 11.L Database
58.	24.094	Bis(2-ethylhexyl) phthalate
59	25.071	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl
60	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				

Table 5 Antimicrobial activity

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

Figures

Figure 1

Phytochemical analysis in leaf extract, (C) *Catharanthus roseus* leaves extract. (A) and (E) confirm alkaloid presence by showing 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

Figure 2

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

Figure 3

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

Figure 5

(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 75 mg/100ml and 100mg/100 ml concentration.

Figure 6

Thin Layer Chromatography visualization under UV lamp at 365 nm using different plant extract and mobile phases (Table 1)

Figure 7

Thin Layer Chromatography visualization under UV lamp at 365 nm using different plant extract and mobile phases (Table 2)

Figure 8

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)

Serpentine

Ajmalicine

Figure 9

Figure 10

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