Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
JMPS 2019; 7(4): 81-89

Received: 01-05-2019
Accepted: 03-06-2019

Aastha Jain
UG Student, Department of Bioscience, Softvision College, Indore, Madhya Pradesh, India

Amitesh Joshi
UG Student, Department of Bioscience, Softvision College, Indore, Madhya Pradesh, India

Jigyasa Joshi
UG Student, Department of Bioscience, Softvision College, Indore, Madhya Pradesh, India

Muskan Tatawat
UG Student, Department of Bioscience, Softvision College, Indore, Madhya Pradesh, India

Saima Saeed
UG Student, Department of Bioscience, Softvision College, Indore, Madhya Pradesh, India

Surbhi Telang
UG Student, Department of Bioscience, Softvision College, Indore, Madhya Pradesh, India

Yukti Choubey
UG Student, Department of Bioscience, Softvision College, Indore, Madhya Pradesh, India

Payal Puri
Assistant Professor of Bioscience, Softvision College, Indore, Madhya Pradesh, India

Correspondence
Payal Puri
Assistant Professor of Bioscience, Softvision College, Indore, Madhya Pradesh, India

Comparative study of phytochemical screening and antibacterial activity of four medicinal plants

Aastha Jain, Amitesh Joshi, Jigyasa Joshi, Muskan Tatawat, Saima Saeed, Surbhi Telang, Yukti Choubey and Payal Puri

Abstract
Objective: The present study was aimed to reveal the phytochemicals, anti bacterial activity of four Indian medicinal plants viz. *Berberis aristata, Cedrus deodara*, *Vitex negundo and Tinospora cordifolia*.
Methods: The qualitative phytochemical screening was carried out by standard biochemical assays. Thin layer chromatography of plant extracts was done by using various solvent systems under visible and uv light for characterization. Agar well diffusion method was performed to evaluate anti bacterial activity of ethanolic extract of plants against Gram positive and Gram negative bacteria.
Results: The preliminary phytochemical screening of all the four selected medicinal plants reflected the presence of alkaloids, glycosides, saponins, phenols, tannins, phytosterols, flavonoids, phenols, terpenoids, glycosides, proteins and carbohydrates. TLC profiling of ethanolic extract of *T. cordifolia* and *B. aristata* showed presence of flavonoids (with Rf values of 0.12, 0.21, 0.36 and 0.52) and alkaloids (with Rf values of 0.3, 0.52, 0.63 and 0.64). Large zone of inhibition (12 mm) was observed with ethanolic extract of *C. deodar* against *E. coli*.
Conclusion: The study depicts that maximum numbers of phytochemicals are present in *T. cordiofolia* and *B. aristata. C. deodar* shows the highest anti bacterial activity. The identified phytoconstituents can be used in modern medicine after pharmacological evaluation.

Keywords: Phytochemical, medicinal, UV, anti bacterial, TLC and R_f.

1. Introduction
Plants have been used as an important therapeutic aid in alleviating different ailments since ancient times. 80% of the world populations, particularly in the third world are fully dependent on medicinal plants for meeting their health care needs [1]. India is a country rich in indigenous herbal resources which grow on their varied topography and under changing agro-climatic conditions permitting the growth of almost 20,000 plant species, of which about 2,500 are of medicinal value [2, 3]. Many pharmaceutical companies are now showing interest in plant-derived drugs mainly due to the current widespread belief that 'Green Medicine' is safe and more dependable than the costly synthetic drugs, which have adverse side effects. Since the last decade, the rise in the failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. With the advancement of modern medicinal technology, it is now easier to identify specific botanical constituents and assess their potential antimicrobial activity.
Tinospora cordifolia, also known as guduchi in Sanskrit, giloy in Hindi is a bulky, smooth, climbing deciduous shrub lacking bristles. It is also referred to as "*amrita"* due to its ability to impart youthfulness, vitality and longevity [4]. It is well known in modern medicine for its adaptogenic, immunomodulatory and anti-oxidant activities [5]. *T. cordifolia* is also known to have anti-inflammatory, anti-arthritic, anti-allergic properties. *Cedrus deodara* is a large evergreen, dioecious. This tree is also known as deodar, cedar, devdar etc. It is useful in inflammations, dyspepsia, insomnia, cough, fever, urinary discharges, ozoena, bronchitis, itching, leucoderma, plies, disorders of the mind, diseases of the skin and of the blood [6].
Berberis aristata occupies significant position as a medicinal plant, commonly known as "Daruhaldi and chitra" is spinous shrub native to northern Himalaya region. The plant is used traditionally in inflammation, wound healing, and affection of eyes [7]. *Vitex negundo* commonly known as 'nirgundi' belonging to family Verbenaceae. Although, all parts of *V. negundo* are used as medicine in the indigenous system of medicine, the leaves are the most

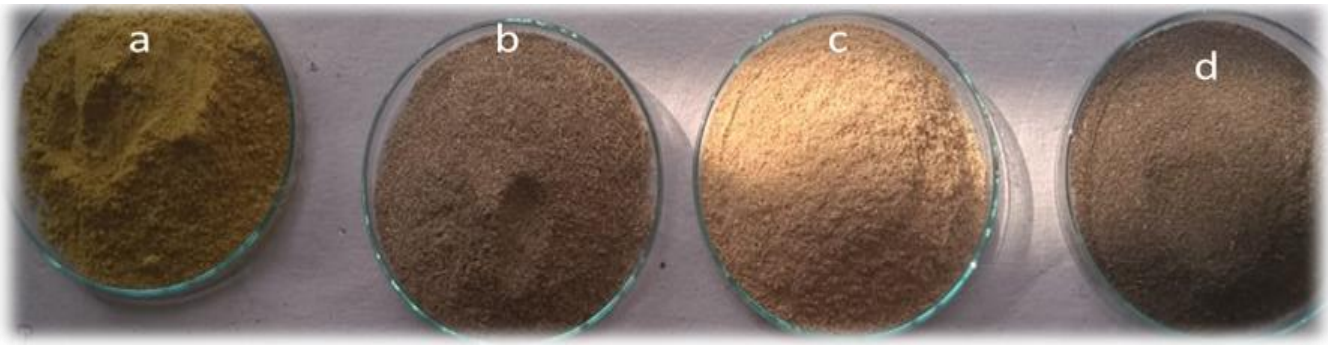
potent for medicinal use. It is used for treatment of inflammation, eye-disease, toothache, leucoderma, ulcers, cancers, rheumatoid arthritis, gonorrhoea, sinuses, bronchitis and as tonics, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal agents [8].

The present study deals with the screening based on phytochemical and antibacterial tests of four medicinal plants viz, *Tinospora cordipholia, Berberis aristata, Vitex negundo, Cedrus deodara* for identifying their chemical constituents. Many studies conducted on these four medicinal plant extracts individually reveal the presence of various secondary metabolites of therapeutic significance and potent anti microbial activity [9-14]. TLC profiling of these plant extracts in different solvent system has been reported which could explain the biological activity of the phytochemicals [15-18]. Antioxidant activity of these plants has been also studied which is responsible for the various pharmacological activities possessed by them. However, the comparative analysis of these four medicinal plants is required in order to correlate their activity and thus to evaluate the benefits of their application in the development of new drugs.

Materials and Methods

Collection of Plant Materials: The dried stem of *Tinospora cordifolia* and *Berberis aristata*, the stem woods of *Cedrus deodara*, leaves of *Vitex negundo*, were collected from different Ayurveda shops (SEVA Aushadhi and Gangaram Mohanlal) from RAJWADA(local market of Indore) where the head Vaidyarajji identified and validated them on 15th January 2019.

Preparation of powder: Stem and leaves collected were ground into a coarse powder and used for further investigations (Figure-1). Extractions were carried out using five solvents viz., Water, Ethanol, Methanol, Chloroform and Hexane.



a) *Berberies aristata* b) *Tinospora cordiopholia* c) *Cedrus deodara* d) *Vitex negundo*

Fig 1: Finely ground powder of plants for extract preparation

Procedure of extract preparation for screening: The extracts of samples were prepared by soaking 20 grams of dried powder in 80-130ml of different selected solvents in a 100ml of conical flask for 12 hours. Later, the mixture was filtered through Whatman filter paper no. 42. Collected filtrates were used for phytochemical screening by following the standard process.

Preliminary phytochemical screening:

The different qualitative chemical tests were performed for establishing the profile of given extracts to detect various phytoconstituents present in them [19-21]. The biochemical screening assays performed are as follows (Table 1):

Test for alkaloids

Wagner's test: 1-2 ml of different extracts was treated with few drops of Wagner's reagent. Formation of reddish-brown precipitate indicates the presence of alkaloids.

Test for flavonoids

i) Shinoda test: Different extracts were treated with few magnesium turnings, ethanol and then drop wise addition of concentrated hydrochloric acid. After few minutes, appearance of orange, pink, red or occasionally purple colour indicates the presence of flavonoids.

ii) Alkaline reagent test: 2-3 ml of different extracts was treated with 2 ml of 10%sodium hydroxide solution. Appearance of deep yellow colour which turned colourless after addition of few drops of dilute acid indicates the presence of flavonoids.

Test for terpenoids: a) Salkowski test: 2 ml of each of the extract was treated with 1 ml of chloroform and then few drops of concentrated H_2SO_4 were carefully added to form a layer. A reddish-brown coloration at the interface indicates the presence of terpenoids.

Test for glycosides

1. **Keller-Kellani test:** 5 ml of different extracts was treated with 2 ml glacial acetic acid and 1 ml of 5% ferric chloride. After gentle heating transfer it to a test tube containing 2 ml of conc. H_2SO4. Appearance of reddish-brown color at junction of two liquid and bluish green colour of acetic acid layer indicates the presence of cardiac glycosides.
2. **Baljet's test:** 2-3 ml of different extracts was treated with2-3 drops of Baljet's reagent. Appearance of yellow to orange color indicated the presence of cardiac glycosides.

Test for saponins

1. **Foam Test:** 2 ml of extract was diluted with5 ml distilled water and it was shaken for 5 minutes. Stable layer of foam indicates the presence of Saponin.
2. **Haemolysis Test:** Add few drops of extract to one drop of blood placed on glass slide. Appearance of haemolytic zone indicates the presence of saponins.

Test for tannins

1. **Braymer's test:** 1 ml of different extracts was treated with 2 ml of 5% ferric chloride solution. Appearance of blue-black color indicates the presence of tannins.

2. **Potassium dichromate test:** To 1 ml of different extracts 10% potassium dichromate solution was added. Appearance of yellow precipitate indicates the presence of tannins.

Test for phenols

1. **Elagic Acid Test:** 1-2 ml of extract was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turns muddy or Niger brown which shows the presence of phenols.
2. **Ferric chloride test:** 1-2 ml of different extracts was treated with 1 ml of 5% ferric chloride solution. Appearance of blue-black color indicates the presence of phenolic compounds.

Test for steroids

1. **Salkowski test:** 1 ml of different extracts was treated with 1 ml of chloroform and concentrated sulphuric acid was added along the side of the test tube and shaken well. Chloroform layer appears red and acid layer showed greenish yellow colour.

Test for carbohydrates

1. **Molisch's test:** 2-3 ml of different extracts was treated with few drops of Molisch's reagent and then 1 ml of concentrated sulphuric acid was added along the side of test tube. Formation of violet color ring at the junction indicates the presence of carbohydrates.
2. **Fehling's Test:** 1 ml of each Fehling's A and Fehling's B solutions were added to equal volume of different extracts. The mixtures were heated for 5-10 minutes on boiling water bath. Appearance of first yellow, then red precipitate indicates the presence of reducing sugar.
3. **Benedict's Test:** 1-2 ml of extract was treated with few drops of Benedict Reagent. The mixture was heated for 5 minutes in boiling water bath. Appearance of red-orange color indicates the presence of reducing sugar.

Test for proteins

1. **Biuret Test:** 2-3 ml of different extracts was treated with equal amount of Biuret Reagent. Emergence of violet or pink color shows the presence of proteins.
2. **Ninhydrin Test:** 2-3 ml of different extracts was heated with few drops of 1% Ninhydrin reagent in boiling water bath for ten minutes. Emergence of purple or bluish color showed the presence of proteins.
3. **Xanthoproteic Test:** To 1 mL extract add 1 mL of conc. HNO_3. A white precipitate is formed upon boiling for half a minute which turns into lemon yellow. Cool it under tap water and then add 1 mL of 40% NaOH. The yellow color changes to orange which indicates the presence of proteins.

Test for Lipids

Sudan Test: Take 1 ml of chloroform in a test tube and add 0.5 ml of extract drop by drop till the sample fully dissolves. To this add one drop of Sudan dye. Appearance of red color indicates the presence of lipids.

Table 1: Qualitative analysis of phytochemicals

S. No	Metabolite	Test	Procedure	Observation
1	Carbohydrates	i. Molisch's Test	2-3 ml extract + 2-3 drops of Molisch Reagent + 1 ml conc. H2SO4 along the walls of TT	Violet ring appears at the junction
		ii. Fehling's Test	1 ml extract + 1 ml (Fehling A + Fehling B) + place in boiling water bath	Red precipitate is formed
		iii. Benedict's Test	1-2 ml extract + 2-3 drops of Benedict Reagent + place in boiling water bath	Red orange colour appears
2	Proteins	i. Ninhydrin Test	2 ml extract + 2-5 drops of 1% ninhydrin reagent + place in boiling water bath for 10 minutes	Purple or blue colour appears
		ii. Biuret Test	2-3 ml extract + 2-3 ml Biuret Reagent	Violet or purple colour appears
		iii. Xanthoproteic Test	1 ml extract +1 ml conc. HNO3 + heat. Cool it + add 1 ml 40% NaOH	Yellow colour appears Yellow colour changes to orange
3	Lipids	Sudan Test	1 ml extract + 1 ml chloroform + 2-3 drop of sudan dye	Red colour appears
4	Alkaloids	Wagner's Test	2- 3 ml extract + few drops of Wagner's reagent	Reddish-brown precipitate forms
5	Flavonoids	i. Shinoda Test	2-3 ml extract + small piece of Mg metal + add Conc. HCl dropwise	Pink/ reddish/orange precipitate forms
		ii. Alkaline reagent Test	2-3 ml extract + 2 ml 40% NaOH	Deep yellow colour appears
6	Terpenoids	Salkowsky's Test	2 ml extract + 1 ml chloroform + few drops of conc, H2SO4	Reddish-brown colouration appears at the interface
7	Glycosides	i. Kellarkialliani Test	5 ml extract + 2 ml glacial acetic acid + 1 ml 5% $FeCl_3$ + heat carefully then cool +transfer it to a TT containing 2 ml conc. H2SO4	Reddish- brown and greenish- blue ring appears at the junctions
		ii. Baljet's Test	2-3 ml extract + 2-3 ml Baljet's reagent	Orange-yellow colour precipitation forms
8	Saponins	i. Foam Test	2 ml extract + 5 ml D/W + shake TT	Stable foam
9	Tannins	i. Braymer's Test	1 ml extract + 2 ml of 10% FeCl3	Dark blue colour appears
		ii. Potassium dichromate Test	1 ml extract + 10% K2Cr2O7	Yellow precipitate appears
10	Phenols	i. Elagic test	1-2 ml extract + 3-4 drops of 5% glacial acetic acid + 3-5 drops of 5% NaNO2	Muddy brown colour appears
		ii. Ferric chloride Test	1-2 ml extract + 1 ml of 5 % FeCl3	Deep blue colour appears
11	Steroids	Salkowsky's Test	1 ml extract + 1 ml chloroform +1 ml conc. H2SO4 along the sides of TT	Chloroform layer appears red and acid layer shows greenish yellow colour.

Thin Layer Chromatographic studies (TLC)

Thin layer chromatography was carried out on silica gel G (400 mesh size) plates made manually in laboratory. The samples were loaded 2 cm above from the bottom of the plates with the help of micropipettes to uniformly apply the samples and allowed to dry. The plates were developed in a chromatography chamber using different solvent systems according to the extract: solvent system 1) chloroform: ethyl acetate: methanol: distilled water (15: 80: 40: 10) and 2) chloroform: methanol (15: 1).

The solvent system used for specific detection of flavonoids and alkaloids are ethyl acetate: formic acid: water (40: 5: 5) and methanol: ammonia (20: 3) respectively. The spray reagents as boric acid and oxalic acid mixture for flavonoids and Mayer's reagent for alkaloids were used specifically for identification. The plates were air dried and then kept in hot air oven at 100 °C for 5-6 minutes [22, 23] and then were observed and visualized under visible light and longer region of ultraviolet light at 240-260 nm followed by spraying with 10% H_2SO_4 and then again the plates were visualized under UV at 240-260 nm. The retention factor (R_f values) for each active compound was calculated for visible and UV light.

Antimicrobial Activity

Extraction of plant material for antimicrobial activity: Heartwood of *Cedrus deodara*, stem of *Tinospora cordifolia* and B*erberis aristata* and leaves of *Vitex negundo* were collected dried, grounded and extracts using different solvents were prepared. The above prepared extracts were used in definite concentration for the following experiment.

Test microorganisms and microbial culture: One gram positive (*Bacillus* sp.) and one gram negative bacterial (*E. coli*) culture was used. The extracts were tested for their effects on these microorganisms. Antimicrobial activity of different plant extracts was determined by agar well diffusion method [24, 25]. 0.1 ml of freshly grown culture of test organisms was spread on surface of sterile Muller Hinton agar plates. Wells were made by gel puncture into agar surface. 4 wells were made on each plate and 20 microliters of ethanolic and aqueous extracts of plants were added on these wells separately. The plates were allowed to stand for some time for diffusion of solution and incubated for 24 hours at 37 °C. The diameter of zone of inhibition observed after incubation was measured.

Results and Discussion

Percentage Yield and Color of Plant Extract: The percentage yield and color of each fraction is presented in Table 2.

Table 2: Percentage Yield & Color of plant extracts

S.no.	Plant Extract	Color & %age Yield	*Tinospora cordiofolia*	*Cedrus deodara*	*Vitex negundo*	*Berberis aristata*
1.	Ethanol	Color	Olive green	Yellow ochre	Dark green	Orange
		Percentage Yield	51.25%	28%	47.5%	52.5%
2.	Methanol	Color	Olive green	Brownish orange	Greenish black	Reddish orange
		Percentage Yield	36.25%	38%	47.5%	47.5%
3.	Chloroform	Color	Dark Olive green	Brownish yellow	Greenish black	Yellow orange
		Percentage Yield	40%	34.5%	48.75%	51.25%
4.	Hexane	Color	Light Olive green	Fade yellow	Pale yellow	Lemon yellow
		Percentage Yield	53.75%	35%	50%	53.75%
5.	Aqueous	Color	Chocolate brown	Muddy brown	Dark brown	Saffron
		Percentage Yield	34%	25.38%	46.1%	39%

Phytochemical Screening

The phytochemical analysis of different extracts of *Tinospora cordipholia, Berberis aristata, Vitex negundo, Cedrus deodara* revealed the presence of various secondary metabolites which contribute significantly towards hypoglycaemic, anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities. All the four selected medicinal plants have shown to possess alkaloids; flavonoids, tannins, terpenoids, saponins, phenols etc. in different extracts (Table 3). The colored reactions for each chemical test are presented in Figure-2. These results are in confirmation with earlier studies done for each of the four medicinal plants separately [9-14].

Flavonoids have extensive biological properties that promote human health and aid in reduction of risk of diseases due to their antioxidant, anticancer, anti-inflammatory and anti microbial properties [26]. Tannins are basically cytotoxic agents. They act as free radical scavengers thus can be useful in treatment of various degenerative diseases like cancer, atherosclerosis, and aging process [27]. Alkaloids are being used in life saving drugs for some critical disorders like cancer, heart failure, blood pressure due to their wide range of pharmacological activities [28]. Saponins have been considered as bioactive antibacterial agent but also act as anti-tumour agents by inducing apoptosis [29].

These phytochemicals are present in enough quantity in all other screened plants except *Cedrus deodara*, indicating thereby its low medicinal value in comparison to other screened plants. But surprisingly it contains large amount of saponins and numerous studies [30] have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells which is supposed to be having one of the good medicinal value out of the four investigated plants in traditional system of medicine.

Tinospora cordipholia contains good amount of alkaloids, flavonoids, glycosides (comparatively best), terpenoids, phenols, sterols and tannins. But lesser amounts of saponins and tannins, similarly *Vitex negundo* too have all these phytochemicals but have lesser amounts of saponins and phenols. Apart from these *Berberis aristata* too have these phytochemicals but not in very potent quantities. Though, majority of analysed natural products are found to be absent in *Cedrus deodara* except phenols and saponins which are present in significant amounts. Also proteins and carbohydrates have been found to be present in all the plants in aqueous extract. Also significant results have been given by ethanolic and methanolic extracts of all the plants as compared to the extracts of hexane and chloroform.

Table 3: Comparative phytochemical study of all four plants in different fractions.

Sr. no	Plants	*Cedrus deodara*					*Tinospora cordipholia*					*Berberies aristata*					*Vitex negundo*				
S/N	Tests	Ethanol	Methanol	Chloroform	Hexane	Aqueous	Ethanol	Methanol	Chloroform	Hexane	Aqueous	Ethanol	Methanol	Chloroform	Hexane	Aqueous	Ethanol	Methanol	Chloroform	Hexane	Aqueous
1.	Protein					+					+					+					+
2.	Carbohydrate					+					+					+					+
3.	Lipid	+	+	+	+	-	+	+	+	+	-					-					+
4.	Alkaloid	+	+	+	++		+	+	+	+		+	++	+	++		++	+	+	++	
5.	Flavonoids	+	+	+	-		++	++	++	+		+	+	++	++		++	++	+	+	
6.	Terpenoids	+	+	+	+		++	++	+	++		+	+	++	++		++	++	+	+	
7.	Glycosides	++	+	+	+		++	++	++	+		++	++	++	++		++	++	++	+	
8.	Saponins	++	++	++	++		+	+	+	+		-	+	-	+		+	-	-	-	
9.	Tannins	++	+	-	-		++	+	-	+		+	++	-	-		++	+	+	-	
10.	Phenols	++	++	+	-		+	++	-	-		-	-	+	-		-	-	-	-	
11.	Phytosterols	+	+	+	+		++	++	++	++		++	+	++	++		++	++	+	+	

Present + Strongly present ++ Absent-

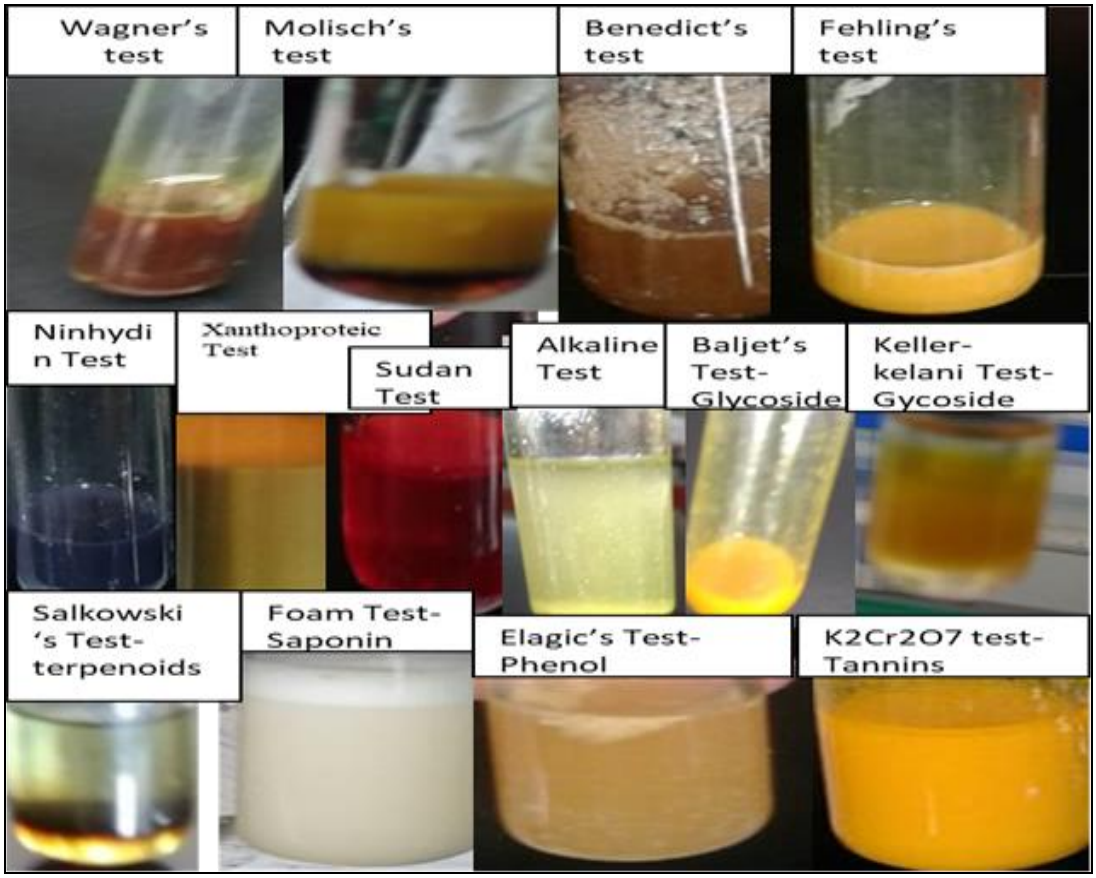


Fig 2: Colored Reactions for Phytochemical Assays

Thin Layer Chromatographic Studies

TLC profiling of extracts gave an idea about the presence of various phytochemicals. Different R_f values of various phytochemicals provided valuable clue regarding their polarity and selection of solvents for further isolation of phytochemicals [31, 32]. The significant results have been given by *Berberis aristata and Tinospora cordipholia* for the presence of alkaloids, flavonoids and other phytochemicals. The presence of promising spots observed under UV radiations by the use of spray reagent to observe fluorescence has been shown in Figure-3-6 and comparative analysis is shown in Table 4-6. The maximum numbers of spots have been observed for ethanolic and methanolic extract of *Tinospora cordipholia and Berberis aristata.*

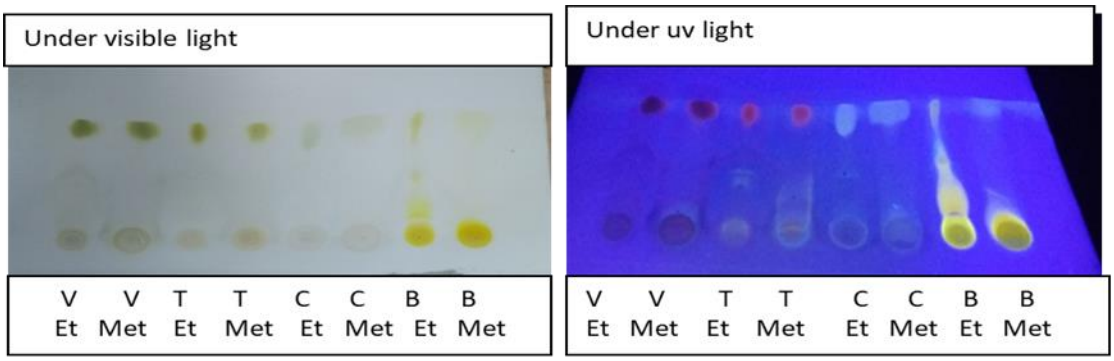


Fig 3: TLC profile of Ethanolic (Et) and Methanolic (Met) extracts of all four plants in solvent system I

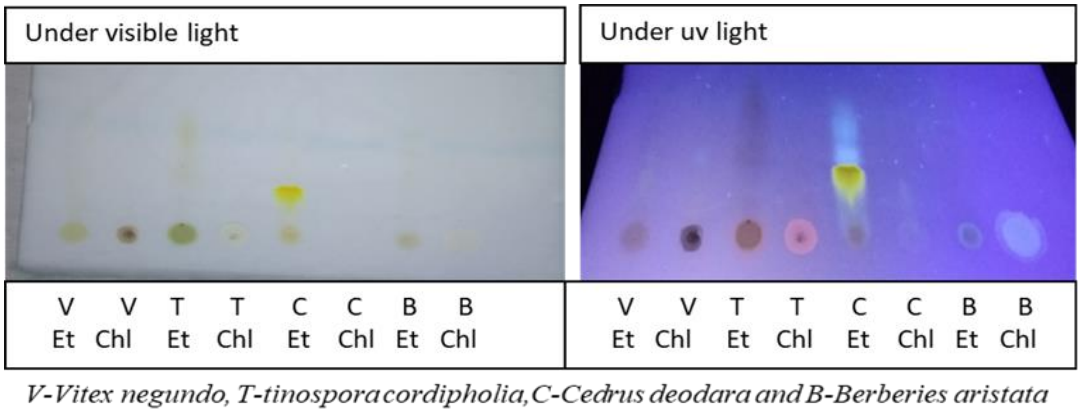


Fig 4: TLC profile of ethanolic (Eth) and chloroform (Chl) extracts of all four plants

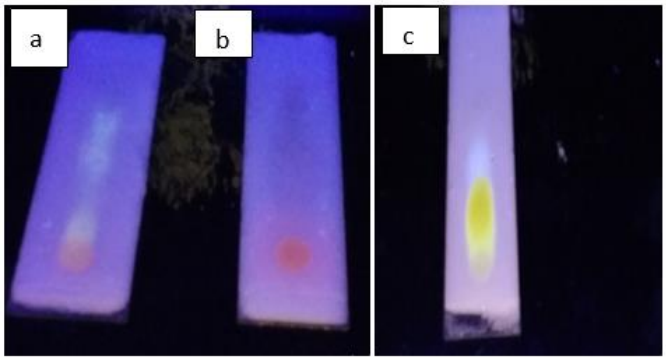


Fig 5: TLC profile of *Tinospora cordiopholia* (a), *Vitex negundo* (b) and *Berberies aristata* (c) for separation of flavonoids.

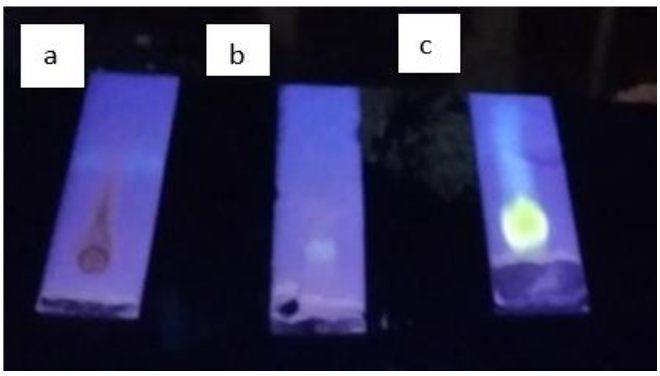


Fig 6: TLC profile of *(a) Vitex negundo, (b) Tinospora cordiopholia* (c) and *Berberies aristata* for separation of alkaloids.

Table 4: TLC of ethanolic and chloroform extract of four different plants using solvent system 1 and 2

S. No	Fraction	Plant	SS1 (CHL: EA: M: D/W)		SS2 (CHL: MET)	
			No of spots	Rf	No of spots	Rf
1	Ethanol	*Tinospoa*	2-visible Uv-1	0.06,0.2 0.017	1-visible 3-uv	0.47
		Cedrus	2-visible	0.12,0.17	1-visible 1-uv	0.44
		Vitex	2-visible 1-uv	0.06,0.2 0.114	4-visible 1-uv	0.176,0.23 0.32,0.48
		Berberies	3-visible 1-uv	0.09,0.18,0.2 0.2	3-visible	0.056,0.256 0.488
2	Methanol	*Tinospoa*	2-visible 2-uv	0.1,0.23 0.15,0.27	1-visible 2-uv	0.46
		Cedrus	3-visible 1-uv	0.07,0.15,0.25 0.24	1-visibe	0.49
		Vitex	1-visible	0.31	1-visible 2-uv	0.48
		Berberies	3-visible 1-uv	0.06,0.1,0.23 0.2	1-visible	0.48
3	Chloroform	*Tinospoa*	1-uv v	0.270.20.270		
		Cedrus	1-uv	0.25		
		Vitex	1-uv	0.19		

		Berberies	1-visible 1-uv	0.07 0.19	
4	Hexane	*Tinospoa*	1-uv	0.1	
		Cedrus			
		Vitex			
		Berberies	1-uv	0.06	

Table 5: TLC for flavonoids

Sample	Fraction	Solvent system	Spray reagent	UV visualization
Vitex negundo	Ethanol	Ethyl acetate: formic acid: water	Boric acid+oxalic acid	-
Tinospora cordiofolia				3spots i)0.12 ii)0.36 iii)0.53
Bberberis aristata				1spot i)0.21
Cedrus dedara				-

Table 6: TLC for alkaloids

Plant	Fraction	Solvent system	Spray reagent	UV visualization Spot and Rf
Vitex negundo	Chloroform	Met: ammonia	Mayer's reagent	1 spot i)0.52
Tinospora cordiofolia	Ethanol			1 spot i)0.64
Berberis aristata	Methanol			3spots i)0.30 ii)0.52 iii)0.63

Antimicrobial Activity

The antibacterial activity of the extracts of *Cedrus deodara*, *Vitex negundo* showed significant reduction in bacterial growth in terms of zone of inhibition (Figure-7). In present study, the ethanolic extract of *Cedrus deodara* and *Vitex negundo* showed antibacterial activity against test Gram negative organism as evident by a prominent zone of inhibition (1.2 cm) on agar plate followed by *Vitex negundo* (0.5 cm). The ethanolic extract of four different medicinal plants did not show any inhibitory action against Gram positive organism (Table 7). Comparatively *Cedrus deodara* was found to be efficient against *E. coli*, whereas *Tinospora cordifolia* was found to be less efficient. The antibacterial property of *Cedrus deodara* and *Vitex negundo* may be due to the presence of different bioactive antimicrobial compounds.

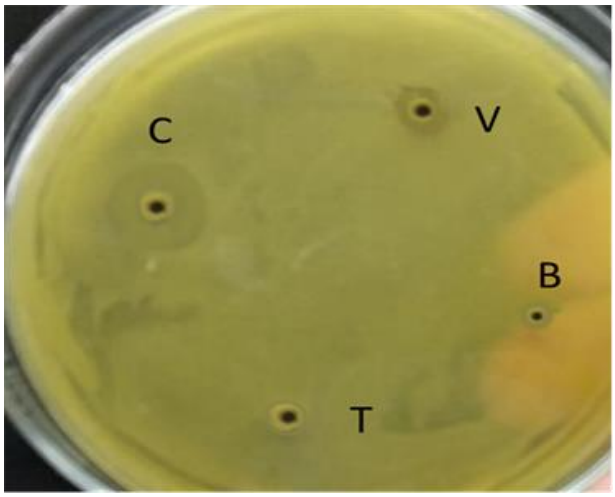


Fig 7: Antimicrobial activity in ethanolic fraction of four different plants.

E. coli streaked plate. (a) C - *Cedrus deodara*, (b) V- *Vitex negundo*, (c) B- *Berberies aristata* and (d) *Tinospora cordiopholia*.

Table 7: Antibacterial activity of ethanolic extract of different plants against selected bacteria

Plant Fraction in ethanolic extract	Muller hinton agar media (GRAM +VE)	Muller hinton agar media (GRAM-VE) Diameter	
Tinospora cordiofolia	No inhibition	Zone of inhibition	0.2 cm
Cedrus deodara		Zone of inhibition	1.2 cm
Vitex negundo		Zone of inhibition	0.5cm
Berberis aristata		Zone of inhibition	0.3cm

Conclusion

The preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without side effects that are often associated with synthetic drugs.

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. The phytochemical screening of four selected medicinal plants clearly reveals that the maximum classes of bioactive constituents are present in ethanolic extract of *Tinospora cordipholia* and then in *Berberis aristata* as compared to other two selected plant extracts. These findings suggested that stem of *Tinospora cordifolia* and B*erberis aristata* could be a potential source of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases. The heartwood of cedrus deodara can provide lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of infections caused by the organisms. Further purification, quantification and antioxidant potential of the active compounds would be our priority in future studies. Both *in vitro* and *in vivo* are recommended for their therapeutic application in modern medicine.

Acknowledgement

The authors are thankful to Prof. & Head, Department of Bioscience and Softvision College, Indore (M.P.), India for providing us lab facilities and financial support in conducting this research work.

References

1. World Health Organization. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram. 1993; 28:13-14.
2. Pandey M, Debnath M, Gupta S. Review on Phytomedicine: An ancient approach turning into future. Potential source of therapeutics, Journal of Pharmacognosy and Phytotherapy. 2011; 3(3):27-37.
3. Farnsworth NR, Fabricant DS. The value of plants used in Traditional Medicine for Drug Discovery. Environ Health Perspective. 2001; 109(1):69-75.
4. Kokate CK *et al*. Pharmacognosy, Nirali Prakashan, Pune. 2009; 8(102-8):103.
5. Sarma DNK, Kosa RL. Indian drugs. 1993, 30-35.
6. Singh PL, Tripathi K, Yadav RB, Yadav KN. Devadaru (*Cedrus deodara* (Roxb.) Loud.): A critical review on the medicinal plant. IJAPR. 2014; 2(1):1-10
7. Sharma K *et al*., Berberis aristata: A review. IRJAP. 2011; 2(2):383-388.
8. Thamaraiselvi S *et al*. Studies of chemical and medicinal value of *Vitex negundo* Linn. I.J.A.B.R. 2012; 2(2):298-301.
9. Madhavi A, Vijaylaxami A, Narasimha V. Preliminary phytochemical analysis of Guduchi C *Tinospora cordiopholia* (WILLD) MIERS leaf in different solvent extracts. International Ayurvedic Medical Journal. 2017; 5(5):1500-1505.
10. Jeychandran RT, Xavier TF, Anand SP. Antimicrobial activity of stem extracts of *Tinospora cordifolia* (Wild.) Mierr ex Hook. F. and Thoms, Ancient Sci. Life. 2003; 23(1):40-43
11. Jain *et al*. Preliminary phytochemical, pharmacognostical and physico-chemical evaluation of *Cedrus deodara* heartwood. Journal of Pharmacognosy and Phytochemistry. 2014; 3(1):91-95.
12. Yasmeen F, Ray T, Babar M, Ali S, Andleeb S. *Cedrus deodara* and *Zanthoxylum armatum* (Timur) evaluated as antimicrobial and antioxidant agents. J Pharm. Sci. Pharmacol. 2015, 110-118.
13. Malik *et al*. *In vitro* antimicrobial activity and preliminary phytochemical analysis of *Berberis aristata*. International Journal of Ethnobiology & Ethno. medicine. 2017; 4(1):1-6.
14. Kurapatti Priyadarshini *et al*. Phytochemical analysis and anti-bacterial activity of *Vitex negundo* leaf extracts against clinically isolated bacterial pathogens. Int. J Pharm. Sci. Rev. Res. 2017; 46(1):33, 183-187.
15. Meena *et al*. *Tinospora cordifolia*: Its Bioactivities and Evaluation of Physicochemical Properties. International Journal of Pharmacognosy and Phytochemical Research. 2010; 2(2):50-55.
16. Tanwar S, Jain J, Verma S, Solanki D. Standardization and phytochemical evaluation of *Tinospora cordifolia* (willd.) Miers. (Menispermaceae). International Journal of Pharmacy and Pharmaceutical Sciences. 2012; 1(4):219-223.
17. Mehra R, Naved T, Arora M, Madan S. Standardization and evaluation of formulation parameters of *Tinospora cordifolia* tablet. Journal of Advanced Pharmacy, Education and Research. 2013; 3:440- 450.
18. Wagner H, Bladt S. Plant drug analysis: a thin layer chromatography atlas. Edn 2, Springer, 1996.
19. Trease GE, Evans WC. Pharmacognosy. Edn 15, Saunders Publishers, London, 2002, 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.
20. Sofowora A. Screening plants for bioactive agents. In: Medicinal plants and Traditional Medicines in Africa. Edn 2, Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd. 1993, 134-156.
21. Harborne JB. Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis, Edn. 2, Chapman and Hall, London, New York, 1973, 49-188.
22. Geiss F. Fundamentals of thin layer planar chromatography. Heidelberg: Huthig, 1987; VI:IX.
23. Touchstone JC. Practice of thin layer chromatography. Edn 3, New York, Wiley, 1992, 9.
24. Murray at al. Manual of Clinical Microbiology. Edn 6., ASM Press, Washington DC, 1995, 15-18.
25. Olurinola PF. A laboratory manual of pharmaceutical microbiology, Idu, Abuja, Nigeria, 1996, 69-105.
26. Kumar S, Pander AK. Chemistry and biological activities of flavonoids: An overview. Sci. World J. 2013, 1-16.
27. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: A review. Crit. Rev. Food Sci Nutr. 1998; 38(6):421-464.
28. Kaur R, Arora S. Alkaloids-Important therapeutic secondary metabolites of plant origin. J Crit Rev. 2015; 2(3):1-8.
29. Desai SD, Desai GD, Kaur H. Saponins and their biological activities. Pharma Times. 2009; 41(3):13-16.
30. Inalegwu B, Sodipo OA. Phytochemical screening and haemolytic activities of crude and purified saponins of aqueous and methanolic extracts of leaves of *Tephrosia vogelii* Hook. F. Asain Journal of Plant Science and Research. 2013; 3(5):7-11.
31. Biradar RS, Rachetti DB. Extraction of some secondary

mretabolites and thin layer chromatography from different parts of *Centella asiatica* L. American Journal of Life Sciences. 2013; 1(6):243-247.
32. Kasture AV, Mahadik KR, Wadodkar SG, More HN. A textbook of pharmaceutical analysis, Instrumental methods. Edn Nirali Prakashan, Pune. 2005; 9(II):1-30.