

# Pharmacognostic, Phytochemical and *In vitro* Biological Evaluation of *Blepharis repens* (Vahl) Roth

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## ABSTRACT

*Blepharis repens* (Vahl) Roth of Acanthaceae family, commonly known as Hadsan in Marathi and Haridachchu in Kannada, is threatened species due to exploitation from natural habitat.

**Objectives:** The objective of present study is to establish scientifically well evaluated pharmacognostic and biological efficacy parameters. **Methods:** Microscopical parameters of leaf, root and stem are evaluated. Powder microscopy, physicochemical analysis, elemental analysis, fluorescence analysis, preliminary phytochemical screening and estimation of major phytoconstituents of whole herb powder are carried out. *In vitro* biological evaluation of *Blepharisrepens* carried out for antioxidant potential by DPPH method, *in vitro* anticancer activity by SRB assay and antimicrobial efficacy by both agar well diffusion and bioautography methods. **Results:** Morphologically, roots are cylindrical and elongated, leaf has symmetric base, entire margin, acute apex while stem is cylindrical, elongated. Microscopic evaluation reveals that both leaf and stem are covered with trichomes. Leaf has unicellular uniseriate trichomes while stem has multi-cellular uniseriate covering trichomes. Physicochemical evaluation revealed that total ash, acid insoluble ash and water-soluble ash values 13.85, 7.7 and 3.1% respectively. Petroleum ether, alcohol and water extractive values found 0.84%, 1.64%, and 3.52% respectively. Loss on drying value is found 3.43%. Element analysis of whole herb confirms presence of nitrogen, sulphate and carbonate. Fluorescence tests are performed by treatment with different acids and alkalis and exposure to ultraviolet light. Phytochemical screening shows presence of carbohydrates, alkaloids, tannins, flavonoids and saponins. Estimation of phytochemicals like carbohydrate, alkaloid, tannin and flavonoid found 5.78%, 1.43%, 5.7% and 1.48% respectively. Biological evaluation reveals that plant has significant antioxidant and antimicrobial activity while plant is devoid of *in-vitro* anticancer activity against human leukemia cell line HL-60.

**Key words:** *Blepharisrepens*, Bioautography, Anti-oxidant, anti-microbial, Anti-cancer, HL-60.

## INTRODUCTION

*Blepharis repens* (Vahl) Roth of Acanthaceae family is edible herb and commonly known as Hadsan in Marathi and Haridachchu in Kannada.<sup>1,2</sup> It is commonly found in rocky regions of Tamil Nadu, Delhi, Chota-nagpur and in Sri Lanka. The whole plant is of good medicinal value and has been used as folk remedy for treating ailments like wounds, bone fracture, skin disease, urinary infections, cancer, diarrhea and leaves are useful in syphilis and dysentery.<sup>3-6</sup> Traditionally whole plant powder with milk and or wheat or black gram flour is commonly used in healing bone fracture in various parts of India. Tribal people in southern India uses leaf juice heated in gingelly oil topically for wound healing.<sup>7,8</sup> Very little information for both phytochemistry and pharmacological efficacy is available on this plant.<sup>9</sup> Pharmacologically, all parts of plant have been reported to possess antidiabetic, antihyperlipidaemic, hepatoprotective and bone and wound healing activity.<sup>10</sup>

The objective of the present study is to establish pharmacognostic and *in-vitro* biological like antioxidant, anticancer, antimicrobial efficacy parameters. The results will be helpful in

identification, preventing possible adulteration and primary evaluation as part of primary stage of quality control standardization of herbal drugs.

## MATERIALS AND METHODS

### Plant material collection, identification and authentication

Plant material was collected from surrounding open field in hill, rocky region in district Hingoli taluka Sengaoon. Identification and authentication was done by Dr. Arvind S. Dhabe, Professor, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. (Accession number is 0564).

### Plant material extraction

The plant was shade dried for 7 days and cleaned, introduced in cutter mill and powder is obtained. This powder is stored in air tight container. From that 100 gm of powder wrapped in thimble which is exhaustively extracted using petroleum ether, chloroform, methanol, water and hydro-alcohol (70%) as solvent in successive solvent extractor. Extraction continues till the solvent in sample tube became clear at required temperature to particular solvent. The extract was obtained which subjected to

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concentrate to one fourth volume under reduced pressure for further use wherever required.<sup>11</sup>

## Pharmacognostic evaluation

### Morphological, microscopical and powder microscopical evaluation

Morphological evaluation of various parts of plant like root, leaf, stem and flower was performed. Colour, odour, taste, shape is evaluated by visual observation and size is measured for width and length. For microscopical evaluation, stem, root and leaf were kept in water for some time and sections were taken with the help of blade. The sections were hydrated with glycerin and covered with cover slip and focused under digital microscope. Photographs under normal bright field at different magnifications were taken. Similarly, powder was evaluated for different microscopic parameters.<sup>12</sup>

## Physicochemical evaluation

The determination of various physicochemical constants such as ash values, extractive values, element detection and loss on drying determined as per World Health Organization guidelines. All experiments were carried out in triplicates and expressed as mean value.<sup>12-14</sup>

### Fluorescence analysis of powder drug

Powdered plant material was treated with various chemical reagents and exposed to visible, ultraviolet light (Short UV) to explore their fluorescence behavior. A small amount of powder drug was placed on a micro slide and treated with 1 N NaOH in methanol, 1 N HCl, 1 N NaOH in water, nitric acid (1:1), sulphuric acid (1:1), glacial acetic acid, 1 N potassium hydroxide and observed under UV 366 nm, UV 254 nm and in daylight under moist condition.<sup>15,16</sup>

## Phytochemical evaluation

### Preliminary phytochemical screening

Preliminary phytochemical screening of pure extract for various class of phyto-constituent such as alkaloid, glycoside, tannin, carbohydrate, saponin, protein, flavonoids, steroids, gum, mucilage and oil is completed by chemical evaluation using various reagents.<sup>13</sup> The osazone test for carbohydrate lead to formation of various shapes of crystals. Cube shape crystals may be of glucose or fructose, needle shape may be of monosaccharides and flower shape crystal may be of maltose sugar.

### Quantitative estimation of phytochemicals

Various phytochemicals present in the hydro-alcoholic (70%) extract were quantified for total carbohydrate content by anthrone method, total tannin content by Folin-Denis method, total flavonoid content by aluminium chloride method, total alkaloid content by Bromocresol-Green method using glucose, tannic acid, quercetin and atropine as standard drug respectively.<sup>13</sup>

### Thin layer chromatography analysis

TLC separation and evaluation for different phytoconstituent of four extracts is carried out using silica gel GF254 as stationary phase and different mobile phases as well as spraying reagents as summarised in Tables 1-5.<sup>13,17</sup> Extracts are dissolved in their respective solvents and then spots are applied by using microcapillary on TLC plates.

## *In-vitro* biological activity

### *In vitro* antioxidant activity by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging method

1ml of different concentration of extract solution was taken in different

**Table 1: Physicochemical standard values for *B.repens*.**

No	Parameters Evaluated	Values
1	Ash values	
	Total ash value	13.85 %
	Water soluble ash value	3.1%
	Acid insoluble ash value	7.7%
2	Extractive value	
	Petroleum ether	0.84%
	Alcohol	1.64%
	Water	3.52%
3	Loss on drying	0.43 ± 0.02%

**Table 2: Elements detection.**

No	Test	Inference
1	Calcium	+
2	Magnesium	-
3	Sodium	-
4	Potassium	-
5	Iron	-
6	Sulphate	+
7	Phosphate	-
8	Chloride	-
9	Carbonate	-
10	Nitrogen	+

Where +: Present and -: Absent

**Table 3: Preliminary Phytochemical investigation of *B.repens*.**

Sr. No.	Test	Inference	
		Aqueous extract	Alcoholic extract
1	Carbohydrates	+	+
2	Proteins	-	-
3	Alkaloids	+	+
4	Cardiac Glycoside	-	-
5	Anthraquinone Glycoside	-	-
6	Cynogenetic Glycoside	-	-
7	Coumarin Glycoside	-	-
8	Flavonoids	+	+
9	Tannins	+	+
10	Steroid or Triterpenoid	-	-
11	Saponin	+	+
12	Gum	-	-
13	Mucilage	-	-
14	Fatty Oil	-	-
15	Essential Oil	-	-

where + Present and - Absent

**Table 4: Powder drug analysis of *B.repens* with different chemicals.**

No	Test	Daylight	UV 366 nm	UV 254 nm
1	Powder + 1N NaOH in water	Green	Fluorescence Green	Herbal green
2	Powder + 1N HCl	Cream	Cream	Cream
3	Powder + 1N NaOH in methanol	Brown	Brown	Fluorescence green
4	Powder + HNO <sub>3</sub> (1:1)	Orange	Orange	Orange
5	Powder + H <sub>2</sub> SO <sub>4</sub> (1:1)	Greenish black	Green	Dark green
6	Powder + glacial acetic acid	Green (henna)	Green	Grayish green
7	Powder + 1N KOH	Brown	Brown	Fluorescence brown

**Table 5: TLC profile of extracts for different phytochemical classes.**

Phytochemical	Mobile phase:	Detection
Carbohydrate	Ethyl acetate: toluene (1:3)	10% ethanolic sulphuric acid Treatment after spraying: heated on hot plate at 110°C till color developed and intensified
Alkaloid	Methanol: Toluene: Dioxane: Ammonia (4:2:5:1)	Dragendroff's reagent
Saponin	Chloroform: Glacial acetic acid: Formic acid: Water (16:8:3:2)	Vanillin sulphuric acid. Treatment after spraying: heated on hot plate at 110°C till color developed and intensified
Flavanoid	Ethyl acetate: Formic acid: glacial acetic acid: Water (20: 2.2: 2.2: 5.2)	AlCl <sub>3</sub> Reagent Treatment after spraying: heated on hot plate at 110°C till color developed and intensified
Tannin	Ethyl acetate: acetic acid: Formic acid: Water (14: 0.4: 0.6:4)	Anisaldehyde sulphuric acid Treatment after spraying: heated on hot plate at 110°C till color developed and intensified.

Sr.No	Phytochemical class	Extract	Colour of spot	Rf value
1	Carbohydrate	Aqueous extract	Violet	0.56
		Methanolic extract	Violet, yellowish green	0.56, 0.88
		Chloroform extract	Violet	0.56
2	Alkaloid	Methanolic extract	Orange	0.87
3	Tannin	Chloroform extract	Brown	0.76
4	Saponin	Methanolic extract	Violet, Yellow, Light yellow	0.53, 0.68, 0.77
5	Flavanoid	Methanolic extract	B Brown	0.76

test tubes. To this 5 ml of methanolic solution of DPPH was added, shaken well and covered with aluminium foil, mixture was incubated at 37°C for 30 min. The absorbance was measured against methanol as blank at 517 nm. Absorbance of DPPH solution was taken as control. Percent scavenging activity was calculated by formula: Scavenging activity (%) = absorbance control - absorbance sample / absorbance control × 100. IC50 values were determined from the graphs of percentage scavenging activity against concentration of extracts. These values are defined as inhibitory concentration of the extract necessary to decrease the initial DPPH radical concentration by 50% and are expressed in µg/ml.<sup>18-21</sup>

#### *In-vitro anticancer activity by SRB assay method*

The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0 × 10<sup>5</sup> cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed once and 100 µl of different test compound concentrations were added to the cells in micro titre plates. The plates were then incubated at 37°C for 72 hours in 5% CO<sub>2</sub> incubator, microscopic examination was carried out, and observations recorded every 24 hours. After 72 hours, 25 µl of 50% trichloro acetic acid was added to the wells gently such that it forms a thin layer over the test compounds to form overall concentration 10%. The plates were incubated at 4°C for one hour. The plates were flicked and washed five times with tap water to remove traces of medium, sample and serum, and were then air-dried. The air-dried plates were stained with 100µl SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100 µl of 10mM Tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using following formula, % cell inhibition = 100 - {(At-Ab) / (Ac-Ab)} × 100.<sup>22,23</sup>

#### *In-vitro antimicrobial activity by well diffusion method*

In this method the extract diffused from well on solidified agar plate which extend such that growth of added microorganism is prevented in

circular area known as zone of inhibition. Using pipette 0.2 ml of each of the seeded broth containing 10<sup>6</sup>- 10<sup>7</sup>cfu/ml was inoculated on the plate of solidified agar and speeded uniformly by glass spreader. Then five well cut out in plate by using borer of diameter 5mm. to the five well the 0.2 ml solution of four extract and standard was added to each well. all work carried out under aseptic condition. This plate is incubated at 37°C±1°C for 24 hours. After incubation period diameter of the zone of inhibition in mm obtained around the well was measured.<sup>24-26</sup>

#### *In vitro antimicrobial activity by bioautographic method*

Different constituents of petroleum ether, chloroform, methanol and water extracts based on polarity were separated by TLC using silica gel GF254 as stationary phase. Benzene: ethanol: ammonia hydroxide (18:2:0.2) for non-polar constituents, chloroform: ethyl acetate: formic acid (5:4:1) for intermediate polar and ethyl acetate: methanol: water (40:5.4:5) for polar constituents were used as mobile phases. Bacterial culture suspended nutrient agar media is prepared and over-layed on the 48 hr. old developed TLC plates and allowed to solidify. Then plates kept in chamber and covered with lid. This overlay plate is incubated at 37°C±1°C for 24 hours. Band of inhibition is observed. Further the plates sprayed with INT dye and incubated for 24 hr. Finally growth of inhibition is visualized as yellow band on violet background.<sup>27,28</sup>

## RESULTS AND DISCUSSIONS

Pharmacognostic standardization helps for correct identification, authentication and detection of adulteration and routine quality control of crude drugs. Physicochemical numerical standards reported in this work will be useful for correct identification, doubtful specimen evaluation and compilation of a suitable monograph.

Morphological evaluation explores that fresh stem is cylindrical, brown, covered with multicellular covering trichomes and 7.5-15 cm long. Leaf is green, has symmetric base, entire margin, acute apex, oblong shape, 1.25-3 cm long and 0.5-1cm wide. Roots are cylindrical, round, long branched, 7-10 cm long and 3-5mm wide. (Figure1). Whole plant is having characteristic odor and taste.

Microscopical characteristics of stem of *B.repens* shows many layered (2-3) thin walled rounded shape cortex, multicellular uniseriate covering trichomes, single layered epidermis, xylem, phloem, thin

layered pericyclic fibres above vascular bundle. (Figure1) Microscopy of leaf shows two subsidiary cell at right angle to stoma. Hence it is cross-celled or diacytic or caryophyllus stomata. Leaf shows isobilateral lamina, unicellular trichomes, thick walled covering to vascular bundle, sclerenchymatous sheath, single layer upper epidermis, multilayered elongated and compact palisade cells, non lignified phloems, lignified xylems, multilayer loosely arranged parenchymatous cells as shown in figure 1. Root of *B.repens* shows thin walled many layered packed elongated cortex, 2-3 layered tangentially, elongated thin walled phelloderm, brown colour multi-layered elongated cork cells, xylem and phloem as shown figure 1. Microscopical examination powder of whole herb of *B.repens* shows pitted xylem vessels, sclerenchymatous fibres, parenchymatous cells, phloem fibres, multicellular uniseriate covering trichomes as shown in figure1.

Total ash value, water soluble ash value and acid insoluble ash values are determined and found 13.85 %, 3.1%, 7.7% respectively. Results reveals that plant contain significant amount of total ash and acid insoluble ash. Ash values are indication of total inorganic composition of plant drugs and particularly important in the evaluation of safety and toxicity of drugs.

Extractive values comparison confirms that plant contain more polar constituents than semi-polar and non-polar constituents as shown in Table 1. Element detection reveals that the whole plant contains calcium, sulphate, nitrogen and other elements are absent as shown in Table 2.

Preliminary phytochemical screening reveals the presence of various types of phytoconstituents like carbohydrates, alkaloids, saponins, flavonoids, tannins in *B.repens*. (Table 3) Whereas primary metabolites like protein, gum, mucilage, fatty oil and secondary metabolites like glycosides, steroids and essential oil components seems to be absent. Preliminary phytochemical screening gives idea about the qualitative nature of active phytoconstituent present in the alcoholic and aqueous extract of whole plant of *B.repens*.

Table 4 summarised results of the fluorescence analysis of *B.repens* powder under day light as well as long and short UV light (365 nm and 254 nm) after treatment with different chemicals .

Estimation of phytoconstituents in the extract (70% alcohol) is an important step prior conducting any isolation of active biochemical marker/s as it gives idea about the separable quantity of active phytoconstituents present in plant extract. From the quantitative estimation, it is observed that extract contain maximum content of

carbohydrates and tannin. The estimated values are carbohydrate 5.78%; tannin 5.7%, flavonoid 1.48%, and alkaloid 1.43% were quantified by colorimetric methods (Table 5).

Thin layer chromatography (Figure 2) confirmed presence of carbohydrates in aqueous, methanol, chloroform extracts among four extract at Rf 0.56. While presence of alkaloids observed only in methanol extract at Rf 0.87 and presence of tannins or polyphenols is observed only in chloroform extract at Rf 0.76. Saponins are confirmed only in methanolic extract having colour violet, yellow, light yellow at Rf 0.53, 0.68, 0.77 respectively and flavonoid observed at Rf 0.76.

*In vitro* antioxidant by DPPH (Table 6) reveals that both methanolic and aqueous extracts of *B.repens* possess significant free radical scavenging properties in concentration-dependent manner. Hence, it can be concluded that the *B.repens* could be further pharmaceutically exploited for antioxidant properties by *in vivo* models. Methanolic extract showed more potent antioxidant activity than aqueous extract which might be due to presence of polyphenolic compound and flavonoids.

*In vitro* anticancer activity of four extracts and alkaloid fraction of *B.repens* is performed on HL 60 cancer cell lines at ACTREC Mumbai, India. The cell viability was measured using SRB assay. As IC50 values of all extracts found above 80 µg/ml, hence it is concluded that evaluated extracts of *B.repens* do not have anticancer potential against selected leukemia cell line. Finally, it can be concluded that extracts of *B. repens* have strong antioxidant activity but no anticancer activity against human leukemia cell line HL-60.

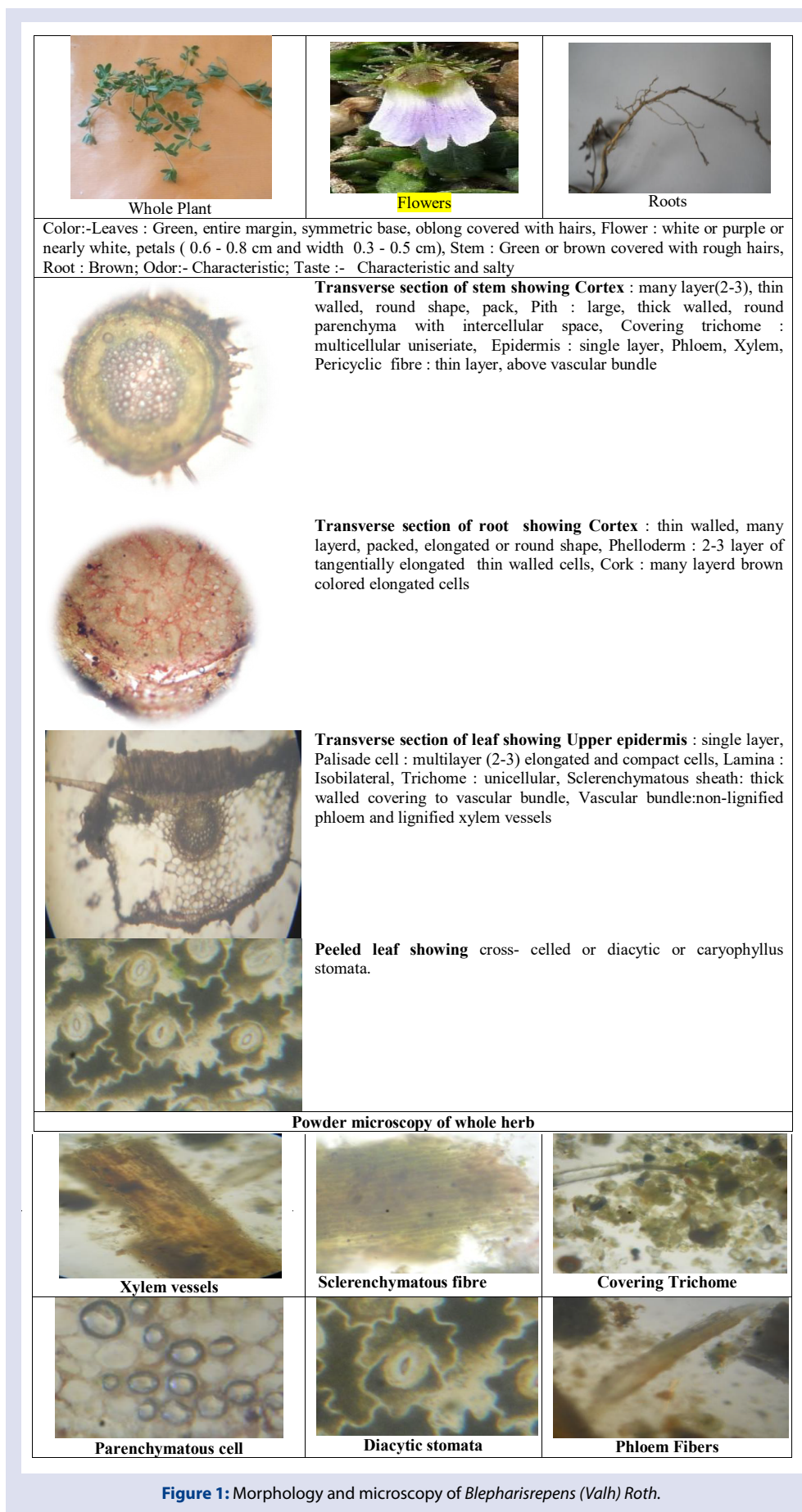
Antimicrobial activity study of methanolic, chloroform, petroleum ether extract and alkaloid fraction has shown maximum activity against *S. aureus*, medium potency for *E. coli* and very weak activity against *A. niger*. Aqueous extract showed weak activity against *E.coli* and *S.aureus* (Figure 3). All four extracts like petroleum ether, chloroform, methanol, aqueous shows potency against strain *Escherichia coli*, *Aspergillus niger*, *Staphylococcus aureus*. Chloroform and methanolic extract showed strong antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* while weak activity against *Aspergillus niger*. Bioautographic method results revealed antimicrobial potency of chloroform and methanolic extract due to presence of saponins.

Phytochemical screening by TLC revealed the presence of varied chemical components like carbohydrates, tannins, polyphenols, saponins and alkaloids in the different extracts of the plants. This is notable from the different colour changes depicted by individual

**Table 6: % Scavenging activity of different extract by DPPH method.**

Sr.no	Conc (µg/ml)	Absorbance	IC50	% Scavenging activity
Control Abs = 0.5231				
1	<b>Ascorbic acid</b>			
	100	0.0190 ± 0.0001		96.36
	250	0.0214 ± 0.0005		95.90
	500	0.0243 ± 0.0004	15564.33	95.35
2	<b>Methanolic</b>			
	100	0.1504 ± 0.0006		71.24
	250	0.2050 ± 0.0004		60.81
	500	0.2778 ± 0.0002	433.44	46.89
3	<b>Aqueous</b>			
	100	0.2290 ± 0.0004		56.22
	250	0.2789 ± 0.0003		46.68
	500	0.3799 ± 0.0003	161.450	27.37
	1000	0.4449 ± 0.0002		14.94



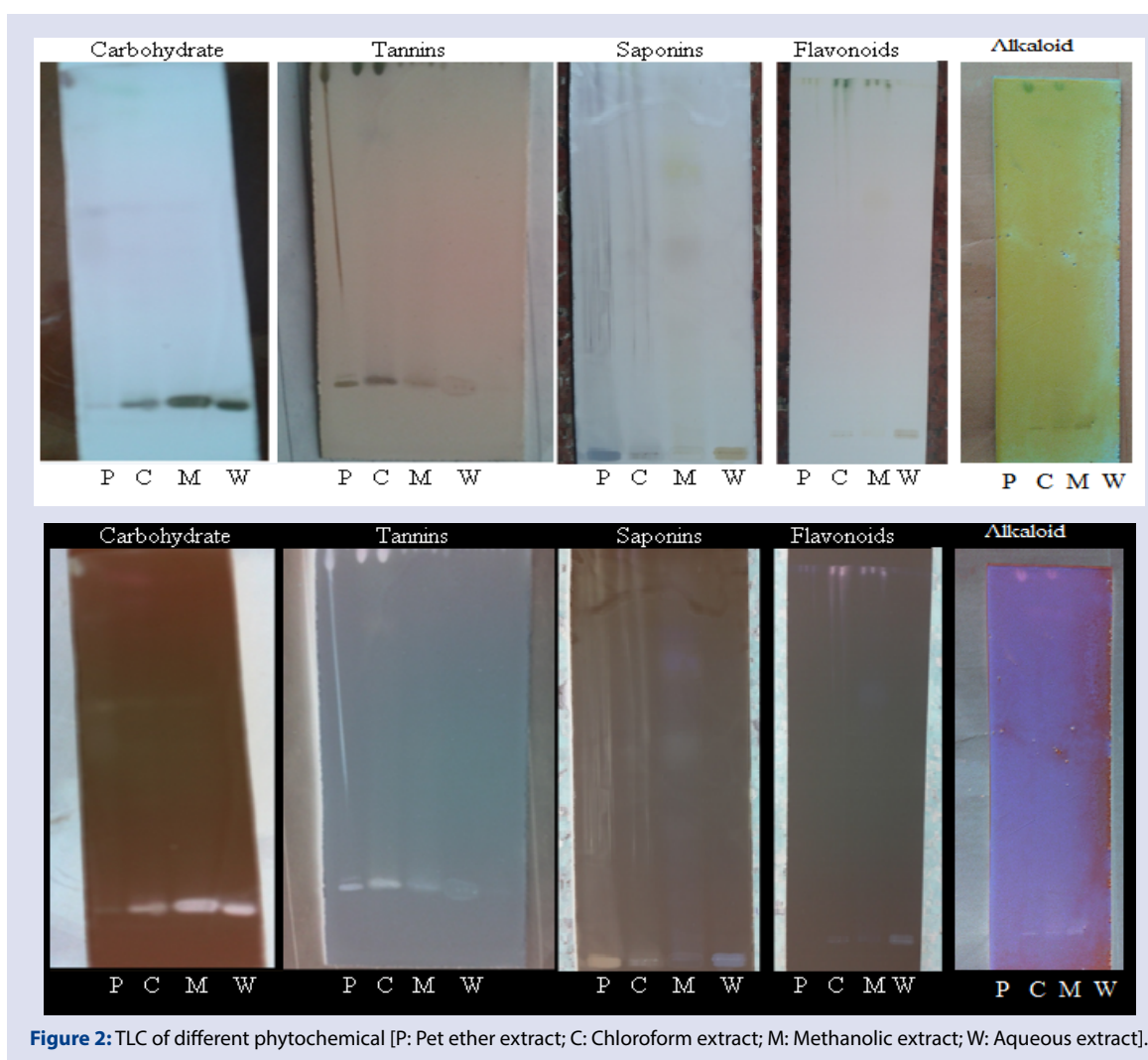


**Figure 1:** Morphology and microscopy of *Blepharisrepens* (Valh) Roth.

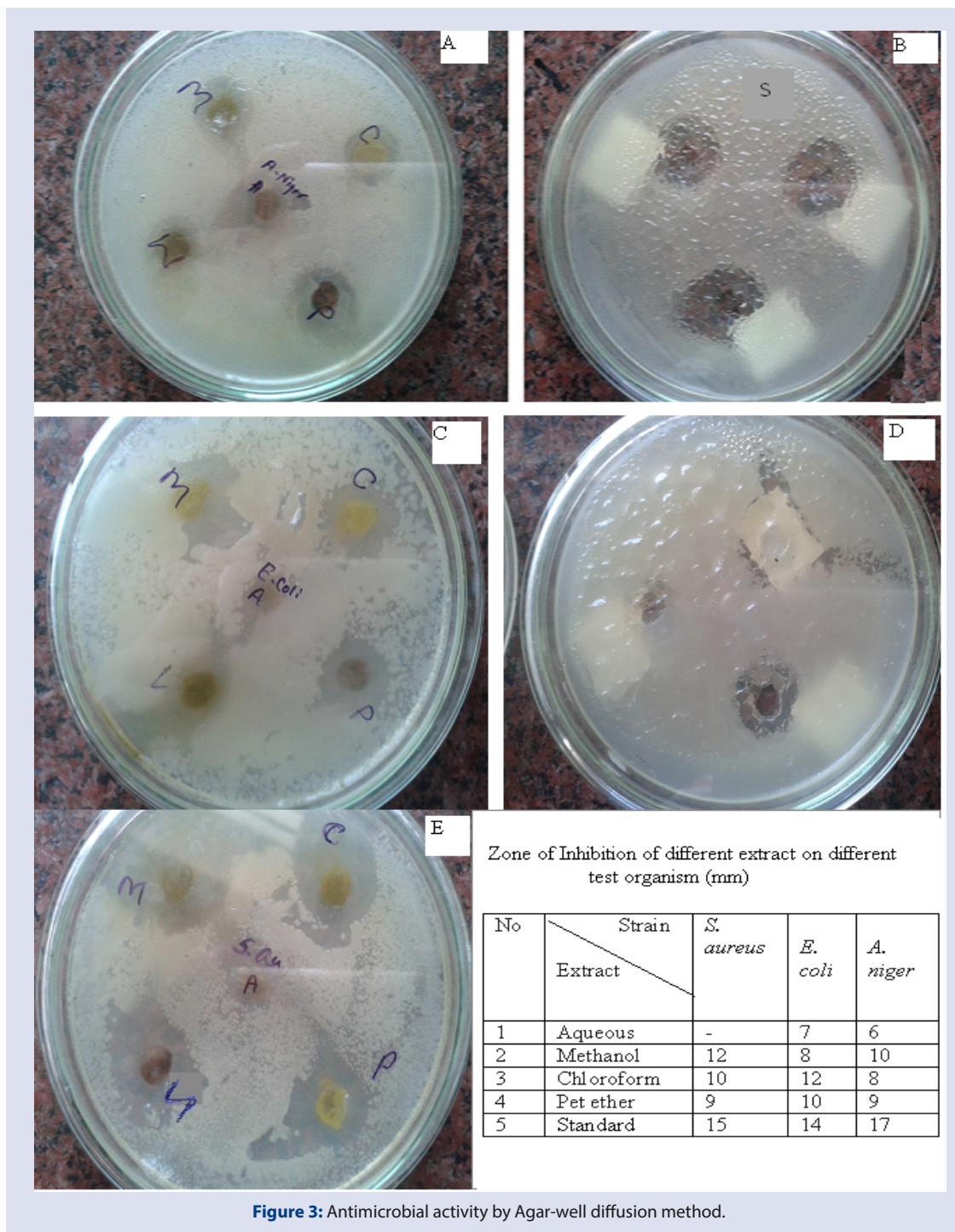
**Table 7:** % control growth of Human leukemia cell line.

Human Leukemia Cell Line HL 60 % Control Growth Drug Concentration (µg/ml)								
	Experiment 1				Experiment 2			
	10	20	40	80	10	20	40	80
B1	90.7	87.5	86.3	83.4	80.0	78.7	78.6	73.2
B2	97.6	86.7	81.9	73.9	82.8	81.7	74.7	57.2
B3	93.5	91.7	90.5	87.9	85.7	84.3	80.7	79.8
B4	97.7	94.1	91.2	90.9	90.5	87.4	86.5	84.3
B5	97.6	95.4	91.1	88.9	87.5	87.4	84.2	84.0
ADR	-4.4	-15.4	-22.2	-26.1	-14.9	-17.9	-25.2	-27.3
	Experiment 3				Average values			
	10	20	40	80	10	20	40	80
B1	89.4	86.2	84.0	74.5	86.7	84.1	82.9	77.0
B2	84.1	82.3	75.3	53.7	88.2	83.6	77.3	61.6
B3	90.9	86.4	84.5	75.4	90.0	87.4	85.2	81.0
B4	95.4	89.2	87.1	67.1	94.5	90.2	88.3	80.7
B5	90.1	85.8	84.9	61.9	91.7	89.5	86.7	78.3
ADR	-23.5	-42.6	-54.1	-84.9	-14.3	-25.3	-33.9	-46.1

Where B1 Aqueous extract, B2 Methanol extract, B3 Chloroform extract, B4 Petroleum ether extract, B5 alkaloid fraction.



**Figure 2:** TLC of different phytochemical [P: Pet ether extract; C: Chloroform extract; M: Methanolic extract; W: Aqueous extract].

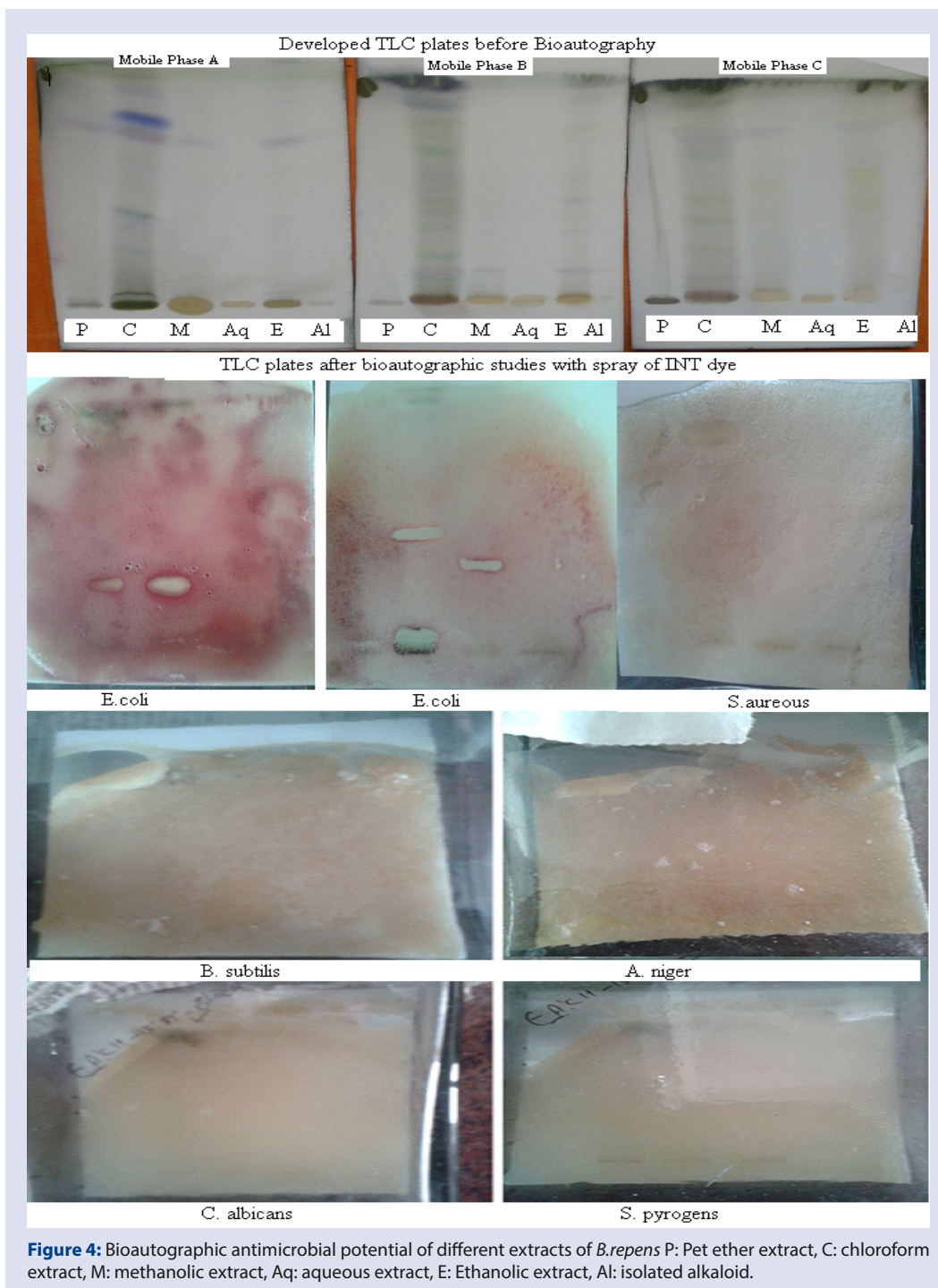


**Figure 3:** Antimicrobial activity by Agar-well diffusion method.

compounds due to their reaction with the spray reagent used (vanillin/sulphuric acid). In bioautographic method, the appearance of white areas against a purple-red background on the chromatograms denotes inhibition of growth of the bacteria or fungi (Figure 4) due to presence of compound that inhibit their growth. Actively growing microorganisms have the ability to reduce INT to a purple-red colour. In the presence of active plant compounds on the chromatograms, the growth of the organism is inhibited. Most of the antimicrobial agents detected in this study were present in extracts of relatively semi-polar solvents. Comparison of developed TLC before and after bioautography reveals that antimicrobial compounds are saponins. Plate A developed in mobile phase 2 shows band of inhibition on Rf 0.22 in both Chloroform and methanolic extract spot. Plate B developed in mobile phase 1

shows band of inhibition on Rf 0.317 and Rf 0.258 in Chloroform and methanolic extract spot (for *S.aureus*) Plate C developed in mobile phase 2 shows band of inhibition on Rf 0.9 in Chloroform extract. Thus in bioautography, it can be concluded that chloroform and methanol extract have presence of phytochemicals (saponins probably) responsible for antibacterial activity against *E.coli* and *S. aureus*. Pet. ether, aqueous, alcoholic extracts and isolated alkaloid have not shown antimicrobial activity in bioautography. But results of agar well diffusion and bioautography method shows that it might possible that all phytochemicals present in extracts have combine effect against *Aspergillus niger*, *Staphylococcus pyroginus* and *Candida albicans* hence antimicrobial potency found in agar-well diffusion method but not in bioautography.





## CONCLUSION

Folk claim of use of *B. repens* in wound healing along with secondary skin infections, skin and urinary infections, diabetes and cancer can be explored scientifically with present paper results. Antimicrobial and anti-oxidant potential is responsible for wound healing along with associated secondary infections, skin and urinary infections. Antioxidant potential is also responsible for treatment of cancer and diabetes. In present paper, *B. repens* extracts are not found anticancer against leukemia cell lines but same extracts might be useful against other cancer cell lines. Finally it can be concluded that morphological, microscopical, physicochemical, chemical and few biological parameters like antioxidant, anticancer, antimicrobial efficacy of plant *B.repens* established for the first time.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

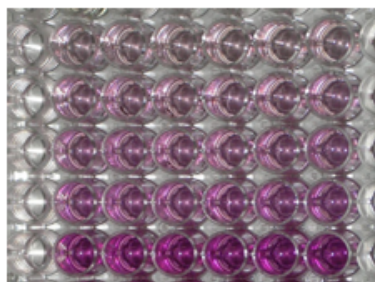
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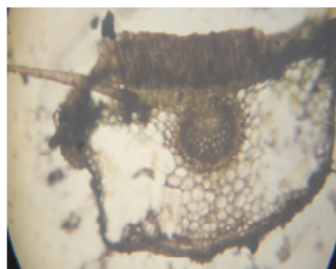


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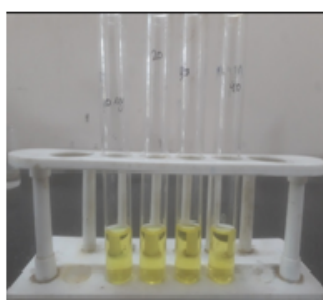
## GRAPHICAL ABSTRACT



In vitro anticancer activity by  
SRB Assay-HL-60



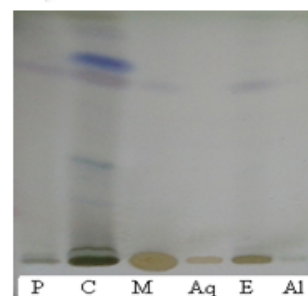
Microscopy



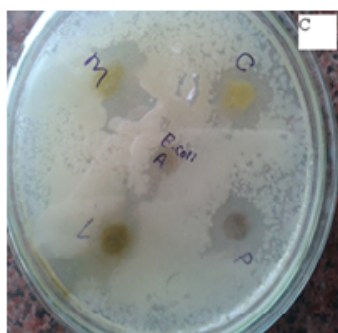
In vitro anti-oxidant activity  
DPPH Assay



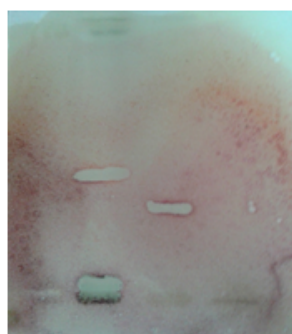
*Blepharis repens* (Vahl) Roth



TLC analysis



Antimicrobial activity  
Agar well diffusion method



Antimicrobial activity  
Bioautography

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