ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 3, pp: (430-437), Month: July - September 2018, Available at: www.researchpublish.com

Phytochemical Screening of Two Selected Members in Caesalpiniaceae

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Abstract: **The preliminary phytochemical investigation of ethanolic extracts of *Humboldtia vahliana* Wt. And *Saraca asoca* (Roxb.) de Wilde highlights the importance of these selected plants in the preparation of various herbal formulations for treating different ailments. Moreover it also shows great potential in antimicrobial as well as antifungal activities. The present study may also provides an additional clues for the identification and characterization of potential phytochemical compounds for developing new drugs in future.**

***Keywords*: Phytochemical screening, *Humboldtia vahliana* Wt., *Saraca asoca* (Roxb.) de Wilde.**

1. INTRODUCTION

The medicinal value of plants lies in the chemical compounds that produce a definite physiological action on the human body. The most important of these bio-active compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds. Plants contain thousands of chemical constituents that interact in complex ways. In some all the constituents interact in complex ways to produce the therapeutic effect of the medicine [1]. The phytochemical investigation of a plant involves authentication and extraction of the plant material, separation and isolation of the plant constituents, characterization of the isolated compounds and investigation of the biosynthetic pathways to particular compounds and quantitative evaluations [2].

Many plants are well known for their medicinal effects. Plants are rich sources of potent drugs to cure mankind of diseases. Man has always relied entirely on plants to treat all kinds of diseases [3]. Today herbal remedies are coming back into prominence, when compared to modern medicine [4]. A large variety of chemical compounds are found in plants. The biosynthetic pathways responsible for these compounds also differ from one taxonomic group to another. Phytochemical analysis helps the separation and identification of chemical contents or active principle responsible for the clinical effectiveness. The present study is mainly focused on preliminary phytochemical two selected members with special reference to antimicrobial activity [5].

2. MATERIALS AND METHODS

2.1 TAXONOMIC DESCRIPTION OF SELECTED MEMBERS OF CAESALPINIACEAE:

Humboldtia vahliana Wight, Ic. tt. 1607,1608. 1850; Hook. f., Fl. Brit. India 2: 274. 1878; Gamble, Fl. Pres. Madras 411(291). 1919 (Caesalpiniaceae) Local Name: *Adimundan* [6], [7]. **(Fig. 1).**

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Fig 1: Image of *Humboldtia vahliana* Wight

Trees, to 18 m high, bark 5-6 mm thick, dark brown mottled with white; blaze pink; internodes of the branchlets solid, terete, glabrous; branchlets sometimes zig-zag. Leaves paripinnate, alternate; stipules 1.5-3 x 2.2 cm, lateral, persistent, ovate-cordate, parallel veined, glandular, glabrous, appendages reniform, divergently veined, glandular, glabrous; rachis 10-16.5 cm, stout, obscurely winged, pulvinate, tomentose or not; leaflets 4-8, opposite, estipellate; petiole 7-10 mm long, stout, pubescent or not; lamina 10-25 x 3-6.4 cm, oblong-lanceolate, base obtuse, apex acuminate, coriaceous, glabrous; lateral nerves 8-14 pairs, pinnate, arched, prominent; intercostae reticulate, prominent, shallow depressed glands beneath. Flowers bisexual, white, 27-30 mm long, in many flowered, axillary brown velvety racemes; pedicel 5-6 mm long; bracts lanceolate, acute, villous on both surfaces, persistent; bracteoles 2, connate by base, obovate, rounded at apex, fugacious; calyx tube 8-10 mm long, lined by the disc, obconical, brown villous; lobes 4, obovate-oblong, rounded at apex, slightly concave; petals 5, 10-12 x 4 mm, obovate, shortly clawed, broadly rounded at apex, glabrous; stamens 5, perfect, alternating with 5 minute staminodes; filaments 10 mm long, red, piloseupto the middle; anthers versatile; ovary 4-5 mm long, half inferior, obliquely linear, ovules 3-4; style 10-15 mm long, filiform, pilose at base, reddish; stigma capitate. Fruit a pod, 15-20 x 3.7-6 cm, elliptic, compressed, sutures thick valves prominently veined, brown villous; seeds 3-4, almost orbicular, glabrous.

Fl. & Fr.: Feb.-Apl.

Habitat: Along river banks in semi-evergreen and evergreen forests.

Distribution: Southern Western Ghats.

Saraca asoca (Roxb.) de Wilde, Blumea 15: 393. 1968. *Jonesia asoca* Roxb., Asiat. Res. 4: 365. 1799. *Saraca indica* sensu Bedd., Fl. Sylv. t. 57. 1870; Hook. f., Fl. Brit. India 2: 271. 1878, non L. 1769; Gamble, Fl. Pres. Madras 409(289). 1919 (Caesalpiniaceae) Local Name: *Ashokam* [6], [7]. **(Fig. 2).**

Fig 2: Image of *Saraca asoca* (Roxb.) de Wild.

Trees, to 10 m high, bark 2-3 mm thick, surface brown or brownish-black, lenticellate. Leaves paripinnate, alternate; leaflets 6-12, opposite, 7-28.5 x 2-8.5 cm, narrowly oblong, oblong-ovate or ovate-lanceolate, round, cuneate or acute, apex acute or acuminate, base obtuse, margin entire, glabrous, coriaceous; stipules 7-20 mm long, intra petiolar, scarious, ovate, connate; rachis 4-25 cm long, slender, pulvinate, glabrous; petiole 2-10 mm long, stout, glabrous; lateral nerves 10-15 pairs, pinnate, arched towards the margin, slender, faint, intercostae reticulate, faint. Flowers bisexual, yellow-orange or red, in dense sessile paniculate corymbs, axillary to leaves or leaf scars; bracts ovate, small deciduous; bracteoles 4 mm long oblong-spathulate, ciliolate, coloured, subpersistent. Calyx 4 cm long, petalloid, cylindric, enclosing a lobed disc; lobes 4, ovate-oblong, unequal, spreading, imbricate. Petals 0.Stamens 7 or 8, much exserted, free; filaments long, filiform, coloured, glabrous; anthers versatile. Ovary half inferior, stipitate, the stipe adnate below to one side of the disc, pubescent; style incurved, glabrous, filiform; stigma small, capitate; ovules many. Fruit a pod 10-5 x 2-5 cm, flat, oblong, coriaceous or almost woody, tapering at both ends; continuous within; seeds 2-8, 3.8 cm long, ovoid, slightly compressed.

Fl. & Fr.: Feb.-Aug.

Habitat: Evergreen forests, also grown as ornamental tree in the plains

Distribution: India and Myanmar.

2.2 PRELIMINARY PHYTOCHEMICAL ANALYSIS:

Sample preparation:

The plant species were collected, identified and authenticated. The leaves and bark of plant materials were collected separately and washed with tap water to remove any foreign material and dried under shade and then it was powdered separately using grinder. These were stored in well-closed containers for further phytochemical characterization.

2.3 QUALITATIVE ANALYSIS:

Preliminary phytochemical analyses of the methanolic and ethanolic extract of plant parts selected species were screened. The presence of protein, carbohydrate, saponine, flavonoid, terpinoid, tannin and phenols were identified [8], [9].

Test for Alkaloid:

The extracts of two selected plants were dissolved individually in dilute HCl and filtered. Then the filtrates were separately treated with Mayer's reagent to test for the presence of alkaloid.

Test for Carbohydrate:

The plant extracts were dissolved individually in 5ml distilled water and filtered. Then the filtrates were separately treated with Benedict's reagent and Fehling's reagent to test for the presence of carbohydrate.

Test for Saponin:

The plant extracts were shaken vigorously with 5ml of water in a test tube for few minutes following a process known as Foam test.

Test for Terpenoid:

In this test, plant extracts were treated with chloroform and filtered were treated with few drops of concentrated H_2SO_4 and allowed to stand and the result was observed.

Test for Phenol:

It was done by treating the extracts with 5ml $FeCl_2$ solution.

Test for Tannins:

It was done by gelatin test, in which extracts were treated with 5ml of 1% of gelatin solution containing NaCl and results were observed.

Test for Flavonoids:

It was done by alkaline reagent test, and lead acetate test. In Alkaline test, extracts were treated with 5ml of NaOH solution and observed. While in Lead acetate test, the extracts were treated with 5ml of lead acetate solution and observed.

Test for Protein:

It was done by Xanthoproteic test and Ninhydrin test. In Xanthoproteic testextracts were treated with 2 drops of concentrated HNO_3. But in Ninhydrin test, ninhydrin was dissolved in acetone, and the extract treated with it.

2.4 QUANTITATIVE ANALYSIS:

Determination of Total Carbohydrate By Anthrone Method:

Procedure: Weigh 100mg of the sample into a boiling tube. Hydrolyse by keeping it in a boiling water bath for 3 houres with 5ml of 2.5 N HCl and cool to room temperature. Neutralise it with solid Sodium carbonate until the effervescence ceases and make up the volume to 100ml and centrifuge, then Collect the supernatant and take 0.5 and 1 ml aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8, and 1 ml of the working standard. 0 serve as blank, making up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water, then add 4 ml of anthrone reagent and heat for eight minutes in a boiling water bath, cool it rapidly and read the green to dark green colour at 630 nm. Draw standard graph by plotting concentration of the standard on the X axis versus absorbance on the Y axis, from this graph calculate the amount of carbohydrate present in the sample tube [10].

Estimation of Protein by Lowry's Method:

Procedure: Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500mg sample grind well in 5 – 10 ml of the buffer. Centrifuge and use the supernatant for protein estimation.Pipette out 0.2, 0.4 , 0.6 , 0.8 , and 1ml of the working standard into a series of test tubes. Pipette out 0.1ml and 0.2ml of the sample extract in two other test tubes and make up the volume to 1ml in all the test tubes. A tube with 1ml of water serve as blank.Add 5ml of reagent C to each tube to including the blank, then add 0.5ml of reagent D , mix well and incubate at roomtemperature in the dark for 30 minutes. Blue colour is developed.Take the readings at 660nm.Draw the standard graph and calculate the amount of protein in the sample [11].

Estimation of Phenol:

Procedure: Weigh exactly 0.5 to 1.0g of the sample and grind it with a pestle and mortar in 10 times volume of 80% ethanol**.** Centrifuge the homogenate at 10,000rpm for 20 min, Save the supernatant. Re-extract the residue with five times the volume of 80% ethanol, centrifuge and pool the supernatants, then evaporate the supernatant to drynessand dissolve the residue in a known volume of distilled water (5 ml)**,** pipette out different aliquots (0.2-2mL) into test tubes also add 0.5mL folin –ciocalteau reagent, after 3 minutes, add 2mL of 20 % Na_2CO_3 solution to each test tube and mix thoroughly. Place the tubes in a boiling water for exactly one minute, cool and measure the absorbance at 650 nm against a reagent blank. Prepare a standard curve using different concentrations of catchpole sample [11].

Estimation of Alkaloid:

Procedure: 1g of sample weighed and put in a test tube and macerated with 20% sulphuric acid and 10ml of ethanol for 10 minutes.Then these tubes were allows to stand for an hour with intermitted shaking and centrifuge for 5 minutes.0.5 ml of the supernatant was transferred into test tube, 2.5 ml 60% sulphuric acid was added and two were mixed. 2.5ml of 0.5% formaldehyde was subsequently added and the test tubes were allowed to stand for 3 hours. Take absorbance at 565nm sample [12].

Screening for antibacterial activity:

The potential crude extracts and fractions of leaf and bark of selected plants were screened for antibacterial activity. The microorganisms selected are *E. coli*, *Bacillus*, *Staphylococcus*, *Proteus* and *Klebsiella.*

Procedure: Muller Hinton agar is considered basic solid medium for the cultivation of microorganisms. 1000 ml distilled water was taken in the conical flask and 38g Muller Hinton agar is dissolved in it. The mouth of the conical flask was closed with a cotton plug. It is sterilized by autoclaving at 121^0C under 15 lb/inch^2 for 15 minutes.

The plates when half set were incubated with bacteria using sterile cotton swabs. Sterile paper discs are prepared using Whatman No:1filterpaper. Disc are dipped in plant extracts were placed above the inoculated media. Ethanol extract is used in this study. All the procedure was done in aseptic conditions provided by a laminar air flow chamber. The plates were incubated in inverted position at 370^0 C for 24 hours in an incubator. Then observe the inhibition zone.

ISSN 2348-313X (Print)
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3. RESULTS AND DISCUSSION

3.1 Preliminary phytochemical investigation:

The preliminary phytochemical investigation of ethanol extracts of leaf of *Humboldtia vahliana* Wt. showed that it contains Alkaloids, Carbohydrate, Saponin, Protein, Phenol. Flavonoid, Terpinoid, Tannins were not detected in the ethanol extract of *Humboldtia* leaf. In Methanol extract of *Humboldtia* leaf Saponin, Carbohydrate, Alkaloid, Protein, Phenol were detected. Flavonoids, Terpinoids, Tannins were not detected. Ethanol extract of bark of *Humboldtia* contains Alkaloids, Carbohydrates, Saponins, Flavonoids, Terpinoids, Tannins, Protein, Phenol and the methnol extract of *Humboldtia* bark contains same secondary metabolites as in the ehanol extract of bark.

The ethanol extract of leaf of *Saraca asoca* contains Alkaloid, Carbohydrate, Saponin, Protein, Phenol. Flavonoid, Terpinoid,Tannin were not detected.In methanol extract of *Saraca* leaf shows Saponin, Carbohydates, Alkaloid, Protein, Phenol were detected. Flavonoid, Terpinoid, Tannin are not detected. Ethanol extract of *Saraca* bark contains Saponin, Flavonoid, Terpinoid, Cabohydrate, Alkaloid, Tannin, Protein and phenol. The methanol extract of *Saraca* bark contains same secondary metabolites as in the ethanol extract of bark (Table 1 & 2).

Table 1: Screening tests for secondary metabolites in ethanol extracts of leaf and bark of *Humboldtia vahliana* Wt. and *Saraca asoca* (Roxb.) de Wild.

SI.NO	Secondary metabolites	Name of the test	H.L.	H.B.	S.L.	S.B.
1	Alkaloid	a) Mayer's test	+	+	+	+
2	Carbohydrate	a) Benedicts test b) Fehling's test	+ -	+ -	+ -	+ -
3	Saponin	a) Foam test	+	+	+	+
4	Flavonoid	a) Lead acetate test	-	+	-	+
5	Terpinoid	a) Salkowski test	-	+	-	+
6	Tannin	a) Gelatine test	-	+	-	+
7	Protein	a) Xanthoproteic test	+	+	+	+
8	Phenol	a) Ferric chloride test	+	+	+	+

(+) present, (-) absent; H.L. – *Humboldtia* Leaf, H.B. – *Humboldtia* Bark;

S.L. –*Saraca* Leaf, S.L. – *Saraca* Bark

Table 2: Screening tests for secondary metabolites in methnol extracts of leaf and bark of *Humboldtia vahliana* Wt. and *Saraca asoca* (Roxb.) de Wild.

SI.NO	Secondary metabolites	Name of the test	H.L.	H.B.	S.L.	S.B.
1	Alkaloid	a) Mayer's test	+	+	+	+
2	Carbohydrate	a) Benedicts test b) Fehling's test	+ -	+ +	+ +	+ +
3	Saponin	a) Foam test	+	+	+	+
4	Flavonoid	a) Lead acetate test	-	+	-	+
5	Terpinoid	a) Salkowski test	-	+	-	+
6	Tannin	a) Gelatine test	-	+	-	+
7	Protein	a) Xanthoproteic test	+	+	+	+
8	Phenol	a) Ferric chloride test	+	+	+	+

(+) present, (-) absent; H.L. – *Humboldtia* Leaf, H.B. – *Humboldtia* Bark;

S.L. –*Saraca* Leaf, S.L. – *Saraca* Bark

Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti- inflammatory effects [12]. Tannins play a major role as antihaemorrhagic agent and has been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties [13]. Glycosides, flavonoids, tannins and alkaloids have hypoglycemicactivities [14]. Steroids, saponins showed the analgesic properties [15]. It has been reported that saponins possess hypocholesterolemic and antidiabetic properties [16]. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primaryantioxidants of free radical scavengers [17].

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3.2 Quantitative estimation of phytoconstituents:

Various chemical components like carbohydrate, protein, phenol, flavonoids, terpinoids, tannins, saponins, alkaloids are present in the leaf and bark of *Humboldtia* and *Saraca*. Comparing *Humboldtia* leaf and *Saraca*leaf, the amount of protein higher in *Saraca* leaf. While in the case of bark of each plant the amount of protein higher in *Humboldtia* bark. The amount of total carbohydrate higher in both leaf and bark of *Humboldtia* than*Saraca*. The amount of alkaloid same in both *Saraca* leaf and *Humboldtia* bark and alkaloid content higher in *Saraca* bark than *Humboltia* leaf. In *Humboltia* leaf and bark the amount of phenol higher than *Saraca*.

3.3 Antimicrobial activity:

The extract of leaf and bark of *Humboldtia* and *Saraca*, prepared in ethanol and each extract tested for it's antibacterial activity against five strains of pathogenic bacteria like, *E.coli, Klebsiella, Staphylococcus, Bacillus* and *Proteus*. After 24 hours incubation *Humboldtia* leaf shows more resistance to *E.coli.* Similarly *Bacillus* is highly sensitive (2.3 c.m.) to *Saraca* bark extract. *Staphylococcus* survive in both *Humboldtia* and *Saraca* leaf extract and at the same time it is sensitive to the bark extract of both plants. *Saraca* bark is more resistant to both *Proteus* and *Klebsiella* (Table 3).

Humboldtia and *Saraca*s shows antimicrobial property due the presence of high amount of phytoconstituents. Plant based products have been effectively proven for their utilization as source for antimicrobial compounds. Only the leaves of both plants can't resist the growth of *Staphylococcus.* The extracts of selected plant parts are used in the treatment of infectious disease caused by *E.coli, Bacillus, Staphylococcus, Proteus, and Klebsiella* sps. (Fig. 3 & 4)

Table 3: Inhibition zone on the ethanol extract of leaf and bark of *Humboldtia vahliana* Wt. and *Saraca asoca* (Roxb.) de Wild.

Sl.No	Name of microorganism	Inhibition zone in cm			
		H.L.	H.B.	S.L.	S.B.
1.	*E. coli*	1.9	1.1	1.3	1
2.	*Bacillus*	1.2	1.4	1.3	2.3
3.	*Staphylococcus*	-	1.8	-	0.9
4.	*Proteus*	1.1	1.3	1.1	1.5
5.	*Klebsiella*	1.3	1.1	1.4	1.5

H.L. – *Humboldtia* Leaf, H.B. – *Humboldtia* Bark; S.L. –*Saraca* Leaf, S.L. – *Saraca* Bark

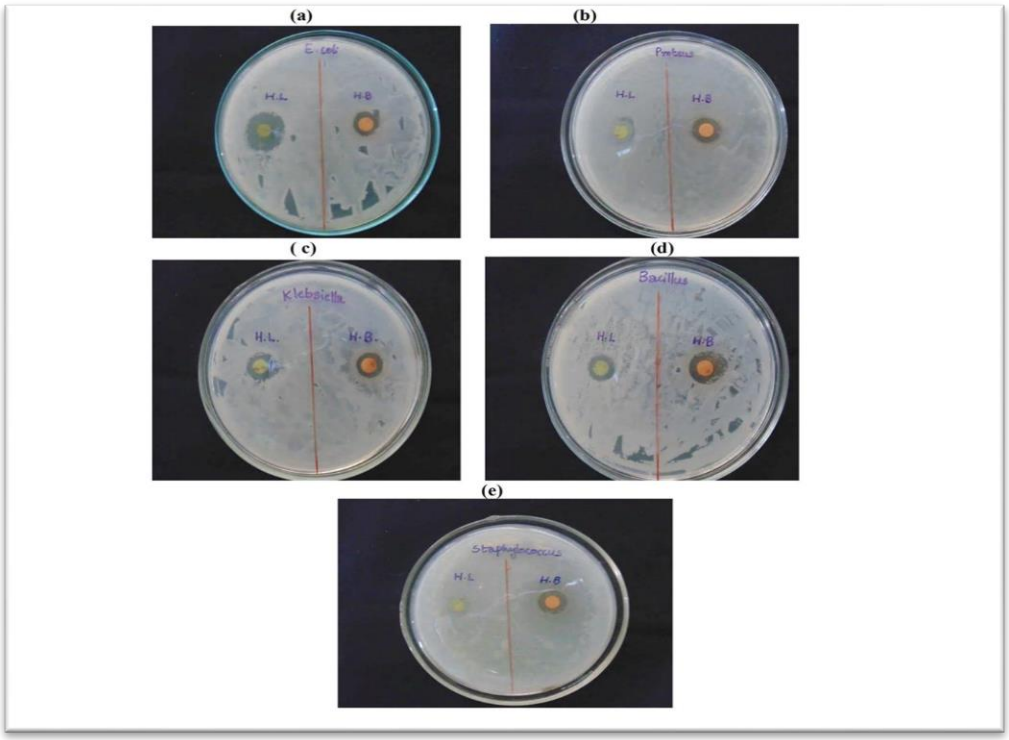

Fig 3: Antibacterial activity of *Humboldtia vahliana* Wt.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
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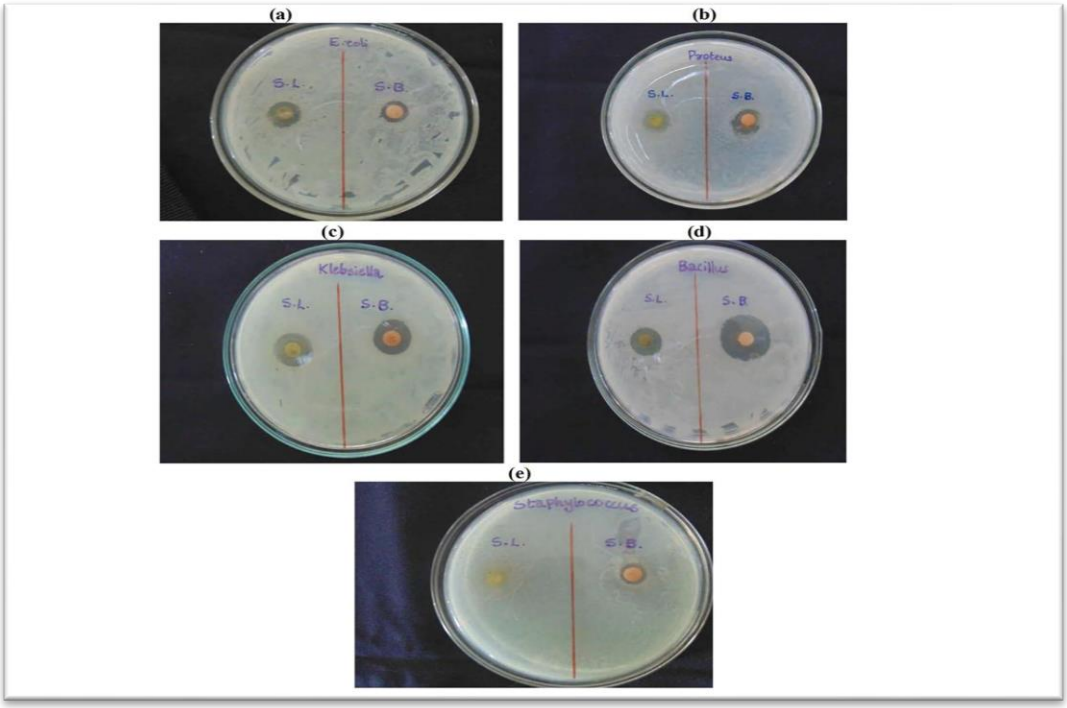

Fig 4: Antibacterial activity of *Saraca asoca* (Roxb.) de Wild.

Antifungal activity: The extract of leaf and bark of *Humboldtia* and *Saraca*, prepared in ethanol and each extract tested for it's antifungal activity against a single strain of fungus; *Aspergillus* sps. After six days of incubation, compared to control leaf and bark extract of *Humboldtia* and also the bark extract of *Saraca* strongly resist the growth of fungal mycelium. The leaf extract of *Saraca* is less resistant to fungus (Fig. 5).

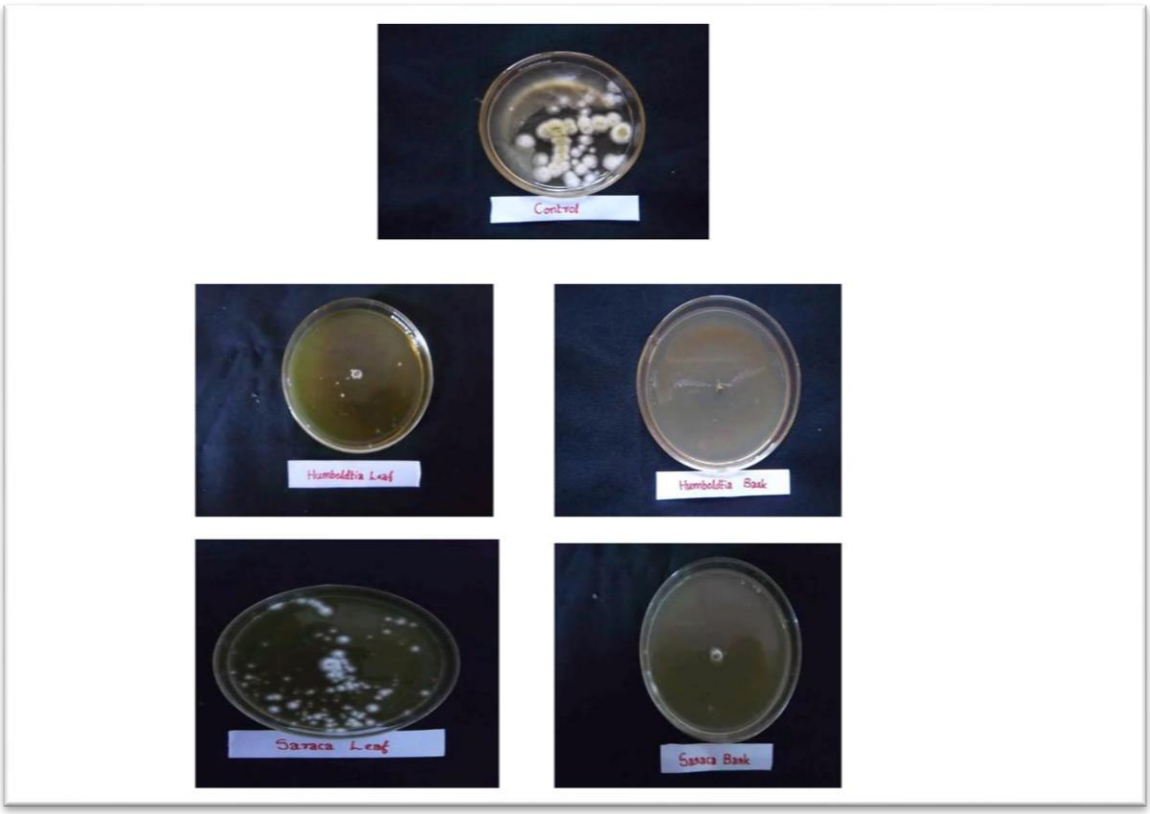

Fig 5: Antifungal activity of *Humboldtia vahliana* Wt. & *Saraca asoca* (Roxb.) de Wild.

4. CONCLUSION

The results of present investigation clearly indicates that the antibacterial and antifungal activity vary with the part of the plants, taxonomy and phytochemistry are important tools for identify the relationship of plants. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs.The antibacterial and antifungal activity were screened in ethanol extract of leaf and bark of both plants and it reveals that both plants has great potential in antimicrobial as well as antifungal activities.

Thus it is to conclude from the above results, *Humboldtia* is equally or more potential than *Saraca*, with respect to their phytochemical, antimicrobial and antifungal properties. *Humboldtia* is listed as endangered, there is an urgent need to preserve and popularize *Humboldtia vahliana*. It recommended for further study to explore it's potential medicinal uses. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. Thus we hope that the important phytochemical properties revealed through the present study may give additional clues to the identification and characterisation of new phytochemical compounds from these potential medicinal plants.

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