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Evaluation and phytochemical screening and antibacterial activity of *Ficus dalhousiae* Miq

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ABSTRACT



The aim of the present study was to isolate the Extract from the leaves of *Ficus dalhousiae* Miq and subsequently evaluate their antibacterial and antifungal activity. The crude various extracts of the plant n-Hexane, Chloroform, Ethyl acetate, Methanol extract was obtained by using continuous soxhlation technique using soxhlet apparatus. The antibacterial activity of plant extract were carried using cup plate method against three bacterial species *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* using agar diffusion method. those are compared with standard reference drug Ciprofloxacin. This study confirmed that bark extracts have more active constituents compare to leaf extracts. by pharmacological evaluation of *Ficus dalhousiae* Miq. Various extracts, most of them are capable of showing moderate antibacterial activity.

Keywords: *Ficus dalhousiae* Miq, Agar diffusion Method, Anti bacterial.

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INTRODUCTION

Compounds which consists of biological activities are derived from natural sources among them plants are as of natural products. which are been used by human societies since from several years based on this over the past half century pharmaceutical industry provided considerable value in particular, the therapeutic areas of infectious diseases and oncology have benefitted from numerous drug classes derived from natural product sources^[1].

Ficus, the genus consists of over 800 species and is one of about 40 genera of the mulberry family. The *Ficus* species of greatest commercial importance is *Ficus carica* L. Other notable species of *Ficus* are *Ficus*

religiosa L. (the Bo tree which sheltered the Buddha as he divined the "Truths"), *Ficus elastic* Roxb. Ex. Hornem. (The rubber tree). *Ficus benghalensis* L. (the banyan tree) and *Ficus racemosa* L. (syn. glomerata, the giant cluster tree). All *Ficus* spp. possess latex-like material within their vasculatures, affording protection and self-healing from physical assaults.

Ficus dalhousiae Miq which belongs to the family Moraceae is commonly known as kal-aal or somavalika or pei-aal. The plant grows in Kerala^[2-6]. According to the Ayurvedic literature of India, *Ficus* has been explored for its various medicinal properties viz. haemostatic, anti-inflammatory, antiseptic, diarrhea, dysentery, skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia and deficient lactation^[7].



Figure 1: *Ficus dalhousiae* Miq

Phytosterols are a large group of compounds that are exclusively found in plants. They are structurally related to cholesterol^[8]. These are important

nutrients present in vegetable oils and products made from them, nuts, cereal products, vegetables, fruits and berries have been classified as richest or significantly rich source^[9].

Among various phytosterols, β -sitosterol, β -stigmasterol and its glucosidic derivatives occupy a unique position as they are considered as good biomarkers due to their biological activity^[10].

Based on the many research articles activity of *Ficus* was been listed out in their research works *Ficus benghalensis* shows anti-oxidant activity^[4]. *Ficus carica linn* shows hepatoprotective activity. *Ficus racemosa, ficus religiosa* and *ficus benghalensis* shows various treatments such as diabetes, skin diseases, ulcers^[5]. *Ficus religiosa* shows anti helminthic activity^[6]. *Ficus benghalensis* of areal roots shows anti diabetic effect^[7].

Plant profile: *Ficus dalhousiae* Miq.

Taxonomy^[1, 2]

Domain: Eukaryota,

Kingdom: Planate

Subkingdom: Viridiaeplantae

Phylum: Tracheophyta

Subphylum: Euphylllophytina

Infraphylum: Radiatopses

Class: Spermatopsida

Subclass: Rosidae

Superorder: Urticanae

Order: Rosales

Family: Urticaceae

Sub family: Moraceae

Tribe: Ficeae

Genus: *Ficus*

Specific epithet: *dalhousiae* - Miq.

Botanical name: *Ficus dalhousiae* Miq.

Vernacular Names^[3]

Ayurvedic: Soma-valka (doubtful synonym).

Telugu: Peddakulammarri

English: Dalhousie

Siddha/Tamil :Kal Aal, Pei Aal

Malayalam: kallaal

Organoleptic characters: Color grey, Taste: no specific taste, odour: No characteristic, Texture: The surface is irregular fissured, nature: Brittle and fibrous and solubility by visual examination of the crude plant material observation with the help of sensory organs.

Physico chemical constants: The physico-chemical evaluation of a crude drug involves the determination of identity, purity and quality. Purity depends upon the absence of foreign matter whether organic or inorganic, while quality refers essentially to the concentration of the active constituents in the drug that makes it valuable to medicine. The following standardization parameters were evaluated to obtain the qualitative information about the purity and quality of *Ficus dalhousiae* Miq.

Table 1: List of activity done on *Ficus dalhousiae* Miq

Sl. No	Plant parts	Activity
Deepa, P., Sowndhararajan, K., et al., 2018	Leaves	antidiabetic effect ^[18]
Sirisha, N et al., 2010	Stem	Antioxidant effect ^[19]
Ghuri, S. S et al., 2014	Leaves	anti-pyretic effect ^[17]
Singh, K., et al., 2012	Root	antifertility ^[8]
Srivastava, S. P et al., 2016	Root	anti-inflammatory activity ^[15]
Ghuri, S. S., et al., 2014	Leaves	antihyperglycaemic activity ^[16]
N. Danamurthy et al., 2015	Root	gastroprotective activity ^[14]
Mohammed Atif et al., 2015	Leaves	Hepatoprotective activity ^[13]
Kuete, V et al., 2009	Bark	Antimicrobial activity ^[20]

MATERIALS AND METHODOLOGY

Plant Material: The leaves of *Ficus dalhousiae* Miq plant belonging to the family Moraceae were collected from Tirumala hills, Tirupati, India. In the month of December 2012, it was identified and authenticated (Reg.No. PARC/2013/21/01.; 22/01/2013). The taxonomical identification and authentication was done by Prof. P. Jayaraman, Ph.D., Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai-45.

Spectroscopic Investigation: Analytical thin layer chromatography was performed on pre-coated silica gel plates. Visualization of the spots on TLC plates was achieved either by exposure to iodine vapours or UV light or dipping in anisaldehyde followed by heating the plate under a stream of hot air. Column chromatography was performed using silica gel and the column was eluted with ethyl acetate - n-hexane as solvent system.

Extraction and Isolation: The leaves of *Ficus dalhousiae* Miq were washed and dried. Weighed leaves were grinded into a fine powder. The petroleum ether and methanolic extracts of the leaves were prepared by soxhlation. In this extraction process 200 gms of dried powder was extracted with 1800ml of petroleum ether and methanol separately at 30-50°C. Phytochemical screening of extract

showed the presence of different chemical constituents.

Thin layer chromatography of the crude extract was carried out using Silica gel G (Silica gel + CaSO₄) coated TLC plates. n-Hexane: Chloroform: Ethylacetate: Methanol (1:1:1:1), (1:1:2:2), (1:2:2:1), (2:2:1:1) was used as the solvent. plant extract was taken and dissolved in a minimum quantity of chloroform, ethyl acetate and adsorbed on silica gel. The slurry formed was allowed to dry. TLC procedure was done under laboratory conditions of 25 ± 5°C and 50% relative humidity. After development, the plate was removed and dried and spots were visualized in Iodine chamber. The results of TLC showed the presence of compound, which were further isolated using column chromatography.

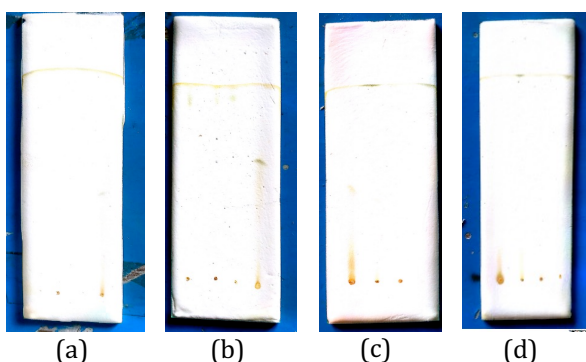


Figure 2: TLC Leaf extraction of in different ratio
(a)Ratio: 1:1:1:1; (b)Ratio : 1:1:1:2; (c)Ratio : 1:2:2:1;
(d) Ratio : 2:2:1:1

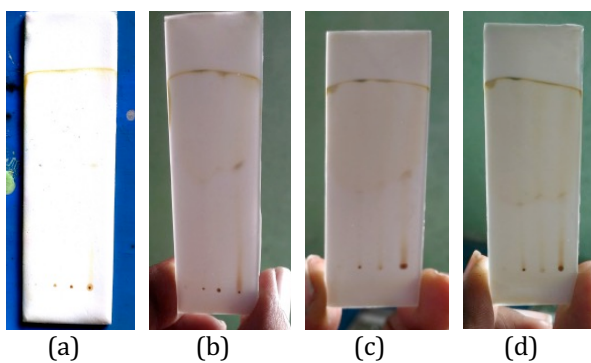


Figure 3: TLC bark extraction of in different ratio
(a)Ratio: 1:1:1:1; (b)Ratio : 1:1:1:2; (c)Ratio : 1:2:2:1;
(d) Ratio : 2:2:1:1

Determination of ash value: The ash content of raw material was established by taking the residue remaining after incineration. The ash of any plant drug is poised of their non-volatile inorganic mixtures. This value varies with in fairly extensive limits and is therefore an important parameter for the purpose of evaluation of crude drugs. In certain drugs, the percentage variation of the weight of ash from sample to sample is very small and any marked difference displays a change in quality. Hence ash value determination provides origin of judging the identity and cleanliness of a drug and gives information relative to its adulteration with

inorganic matter. Some standard ash values have been established for a number of official drugs usually these standards set a maximum limit on the total ash or on the acid insoluble ash permitted. The acid insoluble ash is the part of the total ash, which is insoluble in dilute hydrochloric acid. The ash value can be estimated by various methods to measure the total ash, acid insoluble ash, water soluble ash.

Determination of total ash: About 2 grams of powdered drug was accurately weighed in silica crucible which was already ignited and weighed. On the bottom of the crucible the powdered drug was made as a fine layer and the crucible was incinerated at a temperature not more than 450°C to remove carbon. The crucible was cooled and weighed. The same method was repeated until a constant weight was obtained. The percentage of total ash was calculated with reference to the air dried drug. The ash values of the plant *Ficus dalhousiae* Miq.

Determination of water soluble ash: Water soluble ash is that part of the total ash content which is soluble in water. It is good measure of either previous extraction of the water soluble salts in the drug or incorrect preparation. Thus it is the difference in weight between the total ash and the residue obtained after treatment of total ash with water. As mentioned in IP 1996 for the estimated the water soluble ash, the ash was boiled as mentioned before for 5 minutes with 25 ml of water. The insoluble matter was collected in a Gooch crucible or an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not more than 450 °C. Subtracted the weight of the insoluble matter from the weight of the ash; the difference of weight implies the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried powdered drug.

Determination of acid insoluble ash: The ash obtained as mentioned in the estimation of total ash was boiled with 25 ml of hydrochloric acid for 5 minutes. The Ash less filter paper was taken and the insoluble ash was collected in it. Then it was washed with hot water. Then it was transferred into already weighed silica crucible, ignited, cooled and weighed. The method was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug. The results were recorded.

Extraction value: Extractive values of raw material are valuable for their evaluation particularly when the constituents of a drug cannot be estimated by any other means. These values indicate the nature of the constituents present in a raw material. The plant *Ficus dalhousiae* Miq. was made to coarse powder then it was macerated with 100 ml of solvent in a closed flask for 24 hours. Shaking the flask routinely during the first 6 hours and permitting to stand for 18 hours. Filtered it and care was taken against loss of the solvent. 25ml of the filtrate was evaporated to

dryness in a flat bottomed deep dish dried at 105°C and weighed. The percentage of respective soluble extraction with reference to the air dried drug was calculated.

Loss on drying: Loss on drying is termed as the measure of loss in percentage w/w resulting from water and volatile matter of any kind that can be driven off under a specified condition.

A glass stopper, deep weighing bottle was weighed accurately and the quantity of the sample as definite was transferred to the bottle covered and weighed. The sample was disseminated evenly and the bottle was placed in the drying chamber. The sample was then dried for a specific period of time, and the bottle was detached from the chamber and allowed to cool at room temperature in desiccators before weighed.

Phytochemical analysis: Photochemical constituents have played a major role as basic source for the establishment of several pharmaceutical industries. Many medicinal plants occurring in India are yet to be subjected to various chemical investigations, which may help in the discovery of several new drugs. To investigate such chemical constituents from plants, photochemical screening is required. Broadly, chemical constituents in plants may be divided into major groups viz., primary and secondary chemical constituents. Primary constituents are the basic metabolites of plants such as carbohydrates, proteins, lipids, cellulose and chlorophyll which are distributed in almost all the plants. Secondary chemical constituents are selective and vary considerably from plant to plant and even within the species or varieties of same genus. Secondary chemical constituents are chiefly responsible for the biological activities of plants or drugs. The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of the various constituents.

The physico chemical analysis of the *Ficus dalhousie* Miq. was carried out as described in the materials and methods using the powder extract. The results of basic constants of *Ficus dalhousie* Miq. are described in the table.

Table 2: Physico chemical constants of *Ficus dalhousie* Miq

Sl.NO	Parameters	Results	
		Bark	Leaf
1.	Total ash	93%	47%
2.	Water soluble	10.215%	9.005%
3.	Acid in soluble ash	9.832%	8.903%

Identification Tests

Test for Alkaloids: Small portions of solvent-free methanol extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents.

Mayer's test: Filtrate was treated with potassium mercuric iodide (Mayer's reagent) and the formation

of cream coloured precipitate was indicated for the presence of alkaloids.

Dragendorff's test: Filtrate was treated with potassium bismuth iodide (Dragendorff's reagent) and the formation of reddish brown precipitate was observed for the presence of alkaloids.

Wagner's test: Filtrate was treated with solution of iodine in potassium iodide (Wagner's reagent) and the formation of brown precipitate was observed for the presence of alkaloids.

Hager's test: Filtrate was treated with a saturated solution of picric acid (Hager's reagent) and the formation of yellow precipitate was observed for the presence of alkaloids.

Test for Carbohydrates and Glycosides

Small quantity of methanolic extract was dissolved separately in distilled water and filtered. The filtrate was subjected to various tests to detect the presence of different carbohydrates.

Molisch's test: Filtrate was treated with alcoholic solution of α -Naphthol and a few drops of conc. sulphuric acid were added through the sides of the test tube. The formation of violet ring at the junction of the liquids was observed for the presence of carbohydrates.

Fehling's test: Filtrate was treated with few ml of dilute hydrochloric acid and heated on a water bath for 30 minutes. After hydrolysis the solutions were neutralized with sodium hydroxide solution. To the neutralized solutions, equal quantities of Fehling's A and Fehling's B solutions were added and heated on a water bath for a few minutes. Formation of red-orange precipitate was observed for the presence of reducing sugars.

Benedict's test: Filtrate was treated with 5ml of Benedict's reagent and heated on a water bath for a few minutes. The formation of red-orange precipitate was observed for the presence of reducing sugars.

Another small portion of extract was hydrolysed with dilute hydrochloric acid for a few hrs (2 to 4 h) in a water bath and subjected to various tests to detect the presence of different glycosides.

Liebermann-Bur chard's test: Hydrolysates was treated with a few drops of acetic anhydride, boiled and cooled. Few drops of sulphuric acid were added through the sides of the test tube. Formation of a brown ring at the junction of two liquids and green color in the upper layer indicates the presence of glycosides.

Test for Phytosterols

Methanolic extract was refluxed separately with solution of alcoholic potassium hydroxide till complete saponification took place. Saponified

Table 3: extractions of *Ficus dalhousie* Miq

S.no	Plant part used	Plant name	Method	Solvents used	Quantity used (in g)	% of extraction value
1	Bark	<i>Ficus dalhousiae</i> Miq.	Cold maceration	n-Hexane	4	4.75
				Chloroform	2.37	3.79
				Ethyl acetate	2.17	1.38
				Methanol	1.043	55
2	Leaves	<i>Ficus dalhousiae</i> Miq.	Cold maceration	n-Hexane	4	8.75
				Chloroform	2.25	4.88
				Ethyl acetate	2.08	24
				Methanol	0.899	22.02

Table 4: Phyto chemical constants of *Ficus Dalhousie* Miq

Plant part used	Phyto chemical constant	N-hexane	Chloroform	Ethylacetate	Methanol
Bark	Alkaloids	--	+	--	+
	Carbohydrates	--	--	--	--
	Flavanoids	--	+	--	+
	Steroids& terpinoids	+	+	--	+
Leaves	Alkaloids	--	+	--	+
	Carbohydrates	--	--	--	--
	Flavanoids	--	+	--	+
	Steroids& terpinoids	+	+	--	+

Table 5: Zone of inhibition of EFM

Stains	Organisms used	Control	Standard drugs	Zone of inhibition		
				Activity of Extraction	Concentration 500(µg/ml)	Standard
Bacteria	<i>Staphylococcus aureus</i>	R	Ciproflaxacin	3B	7	36
	<i>Bacillus subtilis</i>	R		1B	6	36
				2B	9	
	<i>Escherichia coli</i>	R		3L	2	38
				3B	7	

Table 6: Rf values of Leaf and bark extractions

Plant part used	Solvents Ratio	Rf values			
		N-hexane	Chloroform	Ethylacetate	Methanol
BARK	1:1:1:1	-	-	-	0.45
	1:1:1:2	-	0.93	0.9	0.61
	1:2:2:1	-	-	0.41	0.5
	2:2:1:1	-	-	-	-
LEAF	1:1:1:1	-	-	-	0.57
	1:1:1:2	-	0.52	0.60	0.66
	1:2:2:1	-	0.44	0.46	0.54
	2:2:1:1	-	0.44	0.42	0.38

mixture were diluted with distilled water and extracted with solvent ether. Ethereal extract was evaporated to dryness and the residue subjected to Liebermann-Burckhardt's test.

Liebermann-Burckhardt's test:

Ethereal residue was treated with a few drops of acetic anhydride; boiled, cooled, and 1 ml of sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two liquids and green color in the upper layer indicates the presence of steroids and triterpenoids.

Test for Saponins

Foam test: About 1 ml of methanol extract were diluted separately with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of any froth above the surface was observed for the presence of saponins.

Test for of Phenolic compounds and Tannins

Small quantities of alcohol and aqueous extracts were diluted separately in water and were tested for the presence of phenolic compounds and tannins.

Ferric chloride test: To the test solutions, a few drops of 5% ferric chloride solution were added. Formation of a bluish-black or greenish-black colour was observed for the presence of phenolic compounds and tannins.

Gelatin test: To the test solutions a few drops of 1% gelatin solution in 10% sodium chloride were added. Formation of white precipitate was observed for the presence of tannins.

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Formation of white precipitate was observed for the presence of tannins. Formation of a yellow precipitate was observed for the presence of flavonoids.

Aqueous bromine test: To the test solution, a few drops of aqueous bromine solution were added. Formation of a yellow precipitate was observed for the presence of tannins.

Test for Proteins and Free Amino Acids

Small quantities of alcohol and aqueous extracts were diluted separately in water and tested for the presence of proteins and free amino acids by subjecting the extracts to various tests.

Millon's test: To 2 ml of the test solutions, 2 ml of Millon's reagent were added and heated. Formation of white precipitate that gradually turns red was observed for the presence of proteins and amino acids.

Biuret test: To the test solutions, a few drops of 0.7% copper sulphate solution were added. Formation of a purplish violet colour was observed for the presence of amino acids.

Ninhydrin test: To the test solutions, a few drops of ninhydrin solution were added in a water bath. Formation of a bluish colour was observed for the presence of amino acids.

Test for Gums & Mucilage

About 10 ml of methanol extract was added to 25 ml of absolute ethanol with constant stirring. Precipitate was examined for its swelling properties and for the presence of carbohydrates.

Test for Flavanoids

Shinoda test: To the test solution, a few fragments of magnesium metal were added along with concentrated hydrochloric acid, and heated. Formation of magenta colour was observed for the presence of flavanoids.

Alkaline reagent test: To the test solution a few drops of sodium hydroxide solution was added. Formation of an intense yellow colour that turns less intense on addition of acid was observed for the presence of flavanoids.

RESULTS AND DISCUSSION

In vitro antibacterial activity: Methicillin, Macrolides like Erythromycin, Ciprofloxacin, Rifampicin, Novobiocin, and Vancomycin are clinical antibiotics. Even though they are having good activity against gram positive and gram negative bacteria, have some unwanted effects like hypersensitivity like skin rashes, headache, dizziness, less frequently convulsions, skin eruptions, fever and gastrointestinal disturbances. Moreover the above said antibiotics have less penetrating capacity across the wall of gram negative organisms. So, they are not potent against gram negative organisms. For that, research works are carried out to improve their activity. In that way the title compounds are used to screen the anti-bacterial activity against both type of bacterial organisms. Drugs like Ketoconazole, Minocyclone, Clotrimazole are used as anti-fungal agents.

Preliminary anti-bacterial activity

In the present study, the agar diffusion method is used to evaluate the anti-bacterial activity of various extract of *Ficus dalhousiae* Miq.

Organisms used: *Staphylococci*, *Salmonella*, *Bacillus*, *E. Coli*

The antibacterial activity of the different extracts of *Ficus dalhousiae* Miq. was studied using diffusion method. The extract was used in the concentration of 500µg/disc using a methanol as solvent. 10µg/disc was used as a standard. The diameter of the zone of inhibition were measured.

Agar diffusion method: Media Used: Peptone-10 g, NaCl-10g and Yeast extract 5g, Agar 20g in 1000 ml of distilled water Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10⁻⁴ cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound and antibiotic at different concentrations. All the plates were incubated at 37°C for 24 h and the diameter of inhibition zone were noted. Sensitive: Strains amenable to treatment in normal dosage. Intermediate: Strains amenable to treat when large doses are used. Resistant: Strains unaffected even by high concentration of drug.

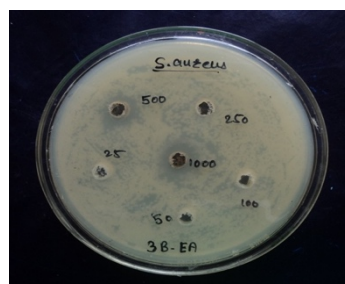


Figure 4: *Staphylococcus aureus* Ethyl acetate Bark Extraction

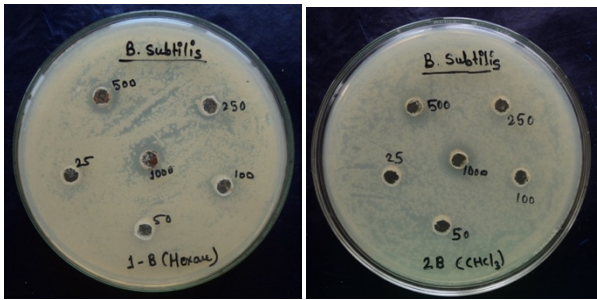


Figure 5: Bacillus Subtilis n-Hexane and Chloroform Bark extract

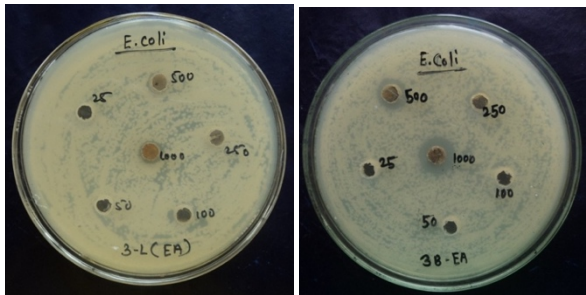


Figure 6: Escherichia Coli Ethyl acetate Leaf and Bark extract

CONCLUSION

For approval of their medicinal activity there is a need to evaluate all of *Ficus species*. In our study by extracting leaf and bark of *Ficus dalhousea* Miq using n-Hexane, Chloroform, Ethyl acetate, Methanol as solvents and conducting antibacterial activity tests, 3B, 1B, 2B, 3L proved to be moderately active but not effective compare to standard drug ciprofloxacin. This study confirmed that bark extracts have more active constituents compare to leaf extracts. There is still so such work awaiting for enthusiastic scientists to explore its remaining activities in future. Thus we can conclude that by pharmacological evaluation of *Ficus dalhousea* Miq. Various extracts, most of them are capable of showing moderate antibacterial activity.

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DISCLOSURES

Name: B. Syed salman

Contribution: This author helped write the manuscript. B. Syed salman has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.

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