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PHYTOCHEMICAL, SPECTRAL ANALYSIS AND ANTIMICROBIAL EVALUATION OF LEAF EXTRACTS FROM *AILANTHUS EXCELSA* ROXB

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ABSTRACT

The present study was performed on *Ailanthus excelsa* Roxb. leaves to probe phytochemical constituents and characterize the bioactive compounds using spectral analysis and its antimicrobial activity. Extraction was done using the Soxhlet apparatus method with aqueous, ethanol, methanol, and chloroform. The phytochemical analysis disclosed the presence of alkaloids, flavonoids, glycosides, phenols, reducing sugars, saponins, steroids, and tannin. The UV spectroscopy studies showed significant peaks for the presence of various phytochemicals. The FTIR analysis confirms the presence of alcohols, amines, alkenes, alkanes, amine salts, carboxylic acids, ketones, aldehydes, alcohols, aromatic esters, and Fluro compounds. An antimicrobial study was carried out to evaluate the potential antimicrobial activity against four strains of bacteria (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris*) and two strains of fungi (*Aspergillus niger, Candida albicans*) of methanol and chloroform leaf extracts. The agar disc diffusion method assessed the antimicrobial activity of methanol and chloroform leaf extracts compared with standard drugs. The results showed a potential effect as the maximum zone of inhibition was 8.76 mm in methanol and 7.89 mm in chloroform extract. The present study's results reveal the presence of phytochemicals, generated FTIR spectrum profile, and antimicrobial studies for the medicinally important Ailanthus excelsa Roxb. which can be used to determine its therapeutic values for developing new drugs.

Keywords: *Ailanthus excelsa*, Phytochemical, Spectral analysis.

INTRODUCTION

Ailanthus excelsa Roxb. P1. Cor 1: 24. t. 23.1795; Bennett in Hook. f., F1. Brit. India 1: 518. 1875; Cooke, 1. Pres. Bombay 1: 205. 1958, also known as *Pongelion excelsum* (Roxb.) Pierre, commonly known as Maharukh, Varul belongs to the family Simaroubaceae. *Ailanthus excelsa* Roxb. is a moderate size to tall tree and deciduous. Bark smooth, greenish-white, or grey. Leaves are pinnate and crowded at 2-5 × 1-1.5 cm, spindle-shaped, one-seeded, membranous, prominently nerved; seeds oblong, glabrous. Plants are common in wastelands, forest fringes, etc., sometimes along roads. Flowers and fruits are observed from December to May (Patil D. A., 2003).

Ailanthus excelsa, also known as the "Indian Tree of Heaven", is significantly valued in folklore medicine as different parts of the plant, like roots, bark, and leaves, are used in the form of decoction, infusion and paste as a medicine for spermatorrhea, jaundice and fracture respectively (Tayade *et al*, 2016; S. K. Tayade and D. A. Patil, 2005). Root extract of 'Maharukh' for about 2 spoons daily, is drunk against stomach ache or 3-4 days (Ahirrao et

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al., 2007). The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma (Kirtikar and Basu, 2003; Chevallier, 1996). The leaf and stem bark extract in ethanol has been shown to inhibit implantation and cause abortion in the first stage of pregnancy. Historically, children with fevers used to sleep on a mattress made of leaves. The bark and leaves are widely used as a tonic in Bombay, particularly for women feeling weak after giving birth. The bark is also used as a bitter, refrigerant, astringent, appetizer, anthelmintic, and febrifuge in dysentery, earache, skin disease, troubles of the rectum, and fever. It is also used in gout and rheumatism. In Ayurveda, it is used to remove the bad taste of mouth. Fruits are used in diarrhea, polyurea, piles and fever. Leaves, along with twigs, are found to be suitable fodder for cattle, sheep, and goats (Ghumare P. and Dattatraya J., 2024).

MATERIALS AND METHODS

Field Work: The fresh leaves of *Ailanthus excelsa* were collected from the Taloda region, Nandurbar District. Identification of plant was performed using the Flora of Dhule and Nandurbar District. The fresh leaves were shade-dried, powdered using a mixer grinder, filtered through a 40-mesh sieve, and stored in an airtight bottle for further use. Using a Soxhlet apparatus, the powder was extracted with different solvents such as water, methanol, chloroform, and petroleum ether.

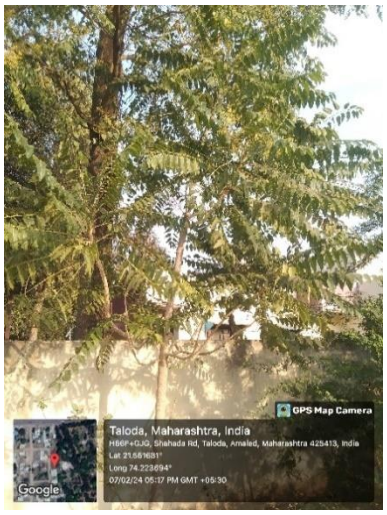

Fig 1: Habit

Fig 2: Leaves for Shade drying

Fig 3: Extraction with Soxhlet Apparatus

***Ailanthus excelsa* Roxb.**

Preliminary phytochemical analysis:

Condensed plant leaf extracts were obtained after each successive solvent extraction and qualitatively tested for the presence of various phytochemicals. The preliminary phytochemical screening was carried out using the method described by Harborne (1998),

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Kokate (1994), and other workers with suitable modifications. Following is the list of preliminary phytochemical work for solvent leaf extracts of plant studied.

Test for Alkaloids:

Dragondroff Test: Take extract and add 4-5 ml of dilute HCl and Dragondroff reagent, Orange precipitate presence of alkaloids.

Wagner's Test: Take extract and add 4-5 ml of dilute HCl, shake well and add a few drops of I_2 in KI, Brown precipitate, presence of alkaloids (Vaishali *et al.,*2013).

Test for Coumarins:

Two ml of plant extract and three ml of 10% NaOH were tested for the presence of coumarins. and the appearance of a yellow colour indicated the presence of coumarins (Dharmendra *et al.*, 2012).

Test for Flavonoids:

Shinoda Tests: Add 2-3 ml extract, few fragments of magnesium metal were added in a test tube, followed by dropwise addition of concentrate HCl. The formation of magenta color indicated the presence of flavonoids (Krishnaiah *et al.*, 2007; Dharmendra *et al.*, 2012; Yusuf *et al.*, 2014).

Alkaline Reagent Test: Extracts were treated with a few drops of sodium hydroxide solution. The formation of an intense yellow colour, which becomes colourless with the addition of dilute acid, indicates the presence of flavonoids (Dinesh Kumar *et al.*, 2010).

Test for Glycosides:

H_2SO_4 Test: Extract treated with Conc.H_2SO_4, shake and allow the content to stand for a few minutes, reddish colour, presence of glycosides.

Kellar Kilani Test: Extracts were treated with Glacial Acetic acid, cool and added 2 drops of FeCl3. Transfer the contents to a test tube having 2 ml of Conc. H_2SO_4, Reddish brown colour was observed at junction of liquids, presence of glycosides (Dharmendra *et al.*, 2012; Yusuf *et al.*, 2014).

Test for Phenols:

Ellagic acid test: Extract+3 drops of 5% $NaNH_2$ Solution, Muddy/Niger, brown precipitate, presence of phenol (Dharmendra *et al.*, 2012; Yusuf *et al.*, 2014; Kangogo *et al.*, 2014).

Phenol test: Add extract and 1ml of 5% $FeCl_3$ solution, Intense colour, presence of phenol

Test for Reducing sugars:

Dissolve extract in 0.5 ml of water and filtered. It was then boiled with Fehling's solution A and B for a few minutes. The presence of orange-red precipitate indicated the presence of reducing sugars (Yusuf *et al.*, 2014).

Test for Saponins:

A sample of the extract mix with water and shaken vigorously. The formation of a honeycomb-like froth indicated the presence of saponins (Dharmendra *et al.*, 2012; Yusuf *et al.*, 2014; Kangogo *et al.*, 2014).

Test for Steroids:

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Salkowski's test: Extract (dissolve in chloroform) + conc.H_2SO_4, and red colour indicated presence of steroids.

Liebermann Burchard's test: Extract + (dissolved in Chloroform + Conc.H_2SO_4 + Acetic acid), green colour layer indicating the presence of steroids. (Dharmendra *et al.,* 2012, Vaishali *et al.,* 2013 and Yusuf *et al.,* 2014).

Test for Tannin:

Add ferric chloride solution to a plant extract, which results in the formation of a blue-black or green-black color. (Vaishali *et al.*, 2013, Yusuf *et al.*, 2014).

Test for Triterpenes:

The Noller test: Add few drops of acetic anhydride and concentrated sulfuric acid to the plant extract. A red fluorescent color indicates the presence of triterpenes. (Vaishali *et al.*, 2013, Yusuf *et al.*, 2014, Kangogo *et al.*, 2014).

UV-Visible Spectroscopy:

UV-visible spectral Analysis for *Ailanthus excelsa* leaf extract in different solvents such as methanol and chloroform in the 200 nm to 800 nm range was performed using a Perkin Elmer Spectrophotometer to detect characteristic peaks.

Fourier Transformed Infrared Spectroscopy:

Sample Processing for FT-IR Analysis: Chloroform and Methanol leaf extract of *Ailanthus excelsa* was used for analysis. Since the ATR-FTIR technique was employed, the sample was directly placed on the ATR crystal for FTIR Spectral analysis using Bruker Routine FT-IR Spectrometer. The absorption spectrum was recorded in the spectrum within a 4000–400 cm^{-1} spectral range. Before sample measurement, a background correction was performed to ensure accurate spectral acquisition. Chloroform and methanol leaf extract samples were carefully placed on the ATR crystal, and uniform pressure was applied to ensure proper contact. Spectra were recorded at 4 cm^{-1} resolution, with 25 scans per sample for optimal signal quality. Fourier Transform Infrared Spectrophotometer (FT-IR) has been tested for the identification of functional groups present in the structure of organic constituents present in leaves of *Ailanthus excelsa*.

Antimicrobial Activity:

The antimicrobial activity was carried out for Chloroform and methanol leaf extract of *Ailanthus excelsa*. In this activity study, four reference bacterial strains, two reference fungal strains and standard microbial drug were used. The antimicrobial assay was conducted using the agar disc diffusion method (Jorgensen J. H. and Turnidge, 2007; Espinel-Ingroff and Pfaller M. A., 2007). The microbial standard strains were obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune 411008 [India]. In this study, bacterial strains such as *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2109) and *Proteus vulgaris* (NCIM 2172); and fungal strains such as *Aspergillus niger, Candida albicans* (spp) were considered for antimicrobial activity tests with standard drug Chloramphenicol and Amphotericin-B.

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The microbial strains were inoculated on sterile plates using sterile cotton swabs. A 6 mm diameter disc was made in each plate using a sterile scavenger. A sample of 40 µl was added to each disc, containing leaf extract in chloroform and methanol solvents. The plates were incubated at room temperature for 72 hours, and the inhibition zone around the colonies was measured in millimetres (mm) (Bauer *et al.*, 1996, Biswa *et al.*, 2009, Gunaselvi *et al.*, 2010).

To prepare a 50% concentration of *Ailanthus excelsa* leaf extract, different concentrations of solvents were made from the curdled Soxhlet extract then the concentration was prepared using the corresponding solvent and extract concentration. Then, 40 µl of the prepared 50% concentration leaf extract was used per well to test for antimicrobial activity.

Table 1: Concentration of *A. excelsa* leaf extract for antimicrobial assay

Leaves extract (50 µl)	**Solvent Concentration (50 µl)**	
	Chloroform	Methanol
Ailanthus excelsa Roxb.	100 µl	100 µl

RESULT AND DISCUSSIONS

Preliminary Phytochemical Analysis:

Table 2: Preliminary phytochemical test of *Ailanthus excelsa* Leaves

Sr. No.	**Phyto- constituents**	***Ailanthus excelsa* Roxb. LEAVES Extract**			
	Solvent System	**Aqueous**	**Methanol**	**Chloroform**	**Pet. Et.**
1	Alkaloids	+	+	-	-
2	Coumarins	-	-	-	-
3	Flavonoids	+	+	+	+
4	Glycosides	-	+	+	+
5	Phenols	-	+	-	-
6	Reducing sugars	+	-	-	-
7	Saponins	-	+	-	-
8	Steroids	-	+	-	+
9	Tannin	-	+	-	-
10	Triterpenes	-	-	-	-
'+' for Present, '-' for Absent, 'Pet. Et.'- Petroleum Ether					

UV visible spectral Analysis:

In 60% Conc. Chloroform leaf extract of *Ailanthus excelsa* was subjected to UV-visible analysis. During chloroform analysis, three different λ max values were obtained at 245 nm, 371 nm, and 416 nm, and its maximum was observed at 245 nm. In 60% Conc. Methanol leaves extract of *Ailanthus excelsa* was subjected to UV-visible analysis. During methanol analysis, three different λ max values were obtained at 268 nm, 336 nm and 411.50 nm and its maximum peak was observed at 268 nm.

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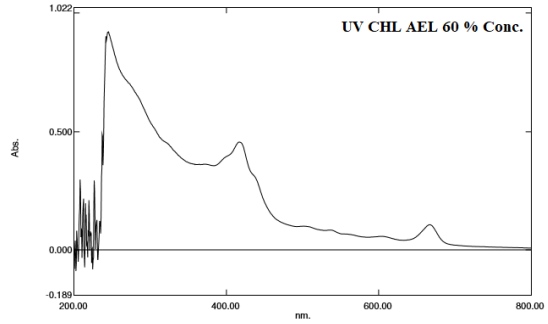
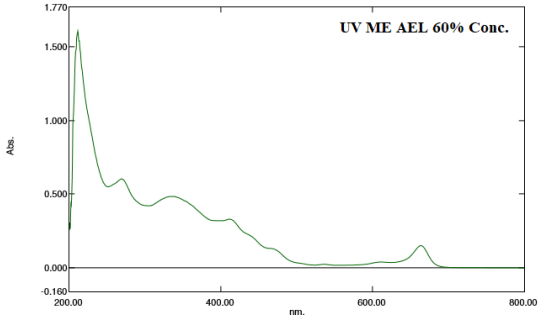


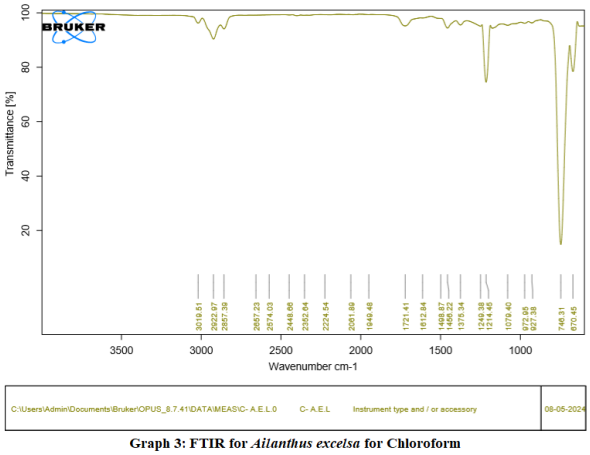


Graph 1. UV Spectra for *Ailanthus excelsa* (60 % Conc. for Chloroform) Graph 2. UV Spectra for *Ailanthus excelsa* (60 % Conc. for Methanol)

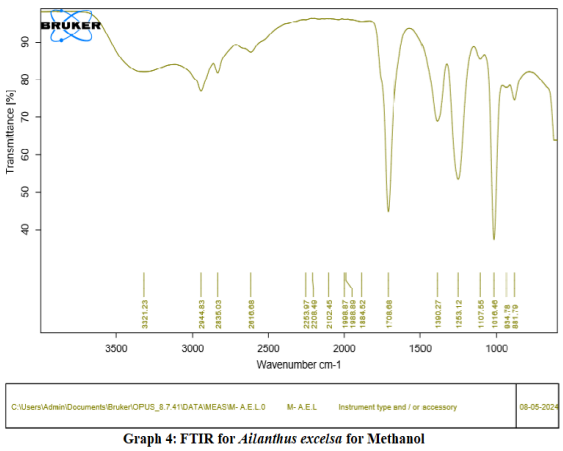
FT-IR Spectral Analysis:

FTIR spectral results for the Chloroform leaf extract of *Ailanthus excelsa* solvent show characteristic absorption bands at peak for Alcohols (C-O) at 2922.97 cm^{-1}, Amines (C-N) at 1214.45 cm^{-1}, monosubstituted and 1,2 disubstituted at 746.31 cm^{-1}, Alkenes (C-H) at 670.45 cm^{-1} like compounds in stretch between 3500 to 500 cm^{-1} in spectral search.

FTIR spectral results for the Methanol leaf extract of *Ailanthus excelsa* solvent showed a broad spectrum over 3000 cm^{-1} suggests the presence of -COOH group in the structure, characteristic absorption bands at peak for Alkanes (C-H) at 2944.83 cm^{-1}, Amine salts and Carboxylic acids (O-H) at 2835.03 cm^{-1}, Ketones (C=O) and Carboxylic acids (C=O) carbonyl group at 1708.68 cm^{-1}, Aldehydes (C=O) and Alcohols (C-O) at 1390.27 cm^{-1}, Aromatic ester at 1253.12 cm^{-1}, Amines (C-N) at 1107.55 cm^{-1}, Fluoro compound at 1016.46 cm^{-1} compounds in stretch between 3500 to 500 cm^{-1} in spectral search. (FTIR Functional Group Database Table with Search - InstaNANO. https://instanano.com/all/characterization/ftir/ftir-functional-group-search/)



Graph 3: FTIR for *Ailanthus excelsa* for Chloroform



Graph 4: FTIR for *Ailanthus excelsa* for Methanol

Antimicrobial Activity

Ailanthus excelsa was investigated to evaluate their antimicrobial activity against pathogenic microbes, including four strains of bacteria and two strains of fungus, using the agar disc diffusion method. Evaluation of antibacterial activity of plant extract was recorded

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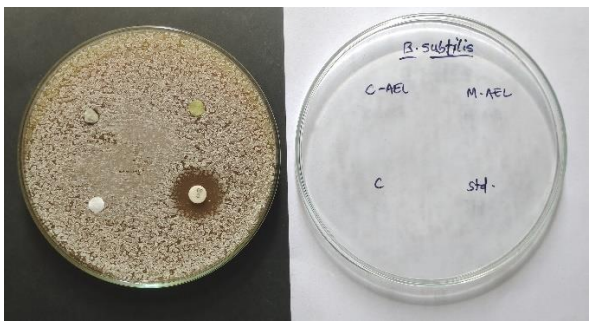
in Table 3 and illustrated in Figure 4. The result revealed that chloroform and methanol leaf extract of *Ailanthus excelsa* effectively suppress pathogenic microbial growth with variable potential. Antimicrobial activity against chloroform leaf extract was recorded. *Staphylococcus aureus* was the most susceptible strain compared to *Proteus vulgaris*, whereas a zone of inhibition was not observed against *Bacillus subtilis* and *Escherichia coli*. Antifungal activity was not observed against the chloroform leaf extract of *Ailanthus excelsa*. Methanol leaf extract was the most effective extract retarding microbial growth of tested pathogens except *Bacillus subtilis* and *Staphylococcus aureus* at 50% concentration of *Ailanthus excelsa*. Antibacterial activity against methanol leaf extract was recorded in *Escherichia coli* was the most susceptible strain compared to *Proteus vulgaris*. In contrast, zone of inhibition was not observed against *Bacillus subtilis* and *Staphylococcus aureus*. Antifungal activity against methanol leaf extract was recorded. *Candida albicans* was the most susceptible strain compared to *Aspergillus niger*. Hence, experiments were conducted to determine their antimicrobial activity.

***Ailanthus excelsa* Roxb.**		**Standard Anti-microbial with Zone of inhibition (In mm)**		**Leaves Extract with Zone of inhibition (In mm)**	
		Chloramphenicol	**Amphotericin B**	**Chloroform**	**Methanol**
Bacterial Strain	*i. Bacillus subtilis*	19.55 ± 0.5	NA	-	-
	ii. Escherichia coli	20.21 ± 0.5	NA	-	9.19 ± 0.2
	iii. Staphylococcus aureus	19.26 ± 0.5	NA	7.89 ± 0.2	-
	iv. Proteus vulgaris	14.40 ± 0.5	NA	7.52 ± 0.2	8.76 ± 0.2
Fungal Strain	*v. Aspergillus niger*	NA	32.21 ± 0.5	-	7.99 ± 0.2
	vi. Candida albicans	NA	32.49 ± 0.5	-	8.04 ± 0.2
Diameter in 'mm' calculated by Vernier Caliper '-' means no zone of inhibition, NA- Not applicable					

Table 3: Antimicrobial Activity of *Ailanthus excelsa* solvent leaf extract and standard drugs against bacterial strains and fungal strains.



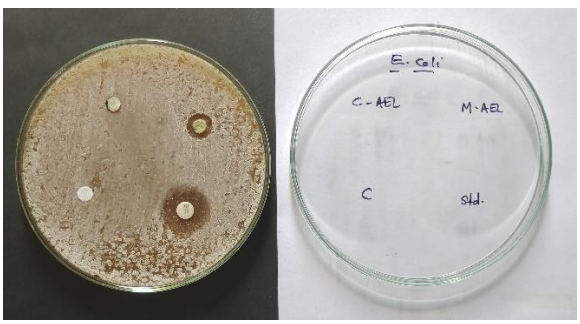


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Fig 4. (a) *Bacillus subtilis*

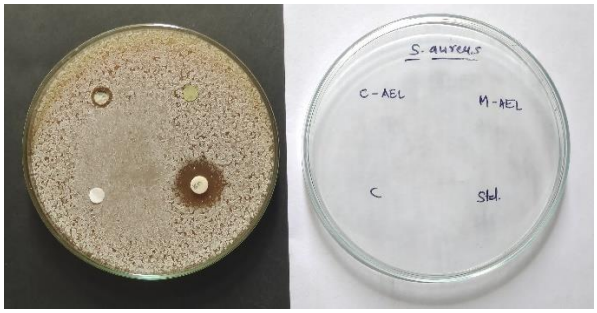

Fig 4. (b) *Escherichia coli*

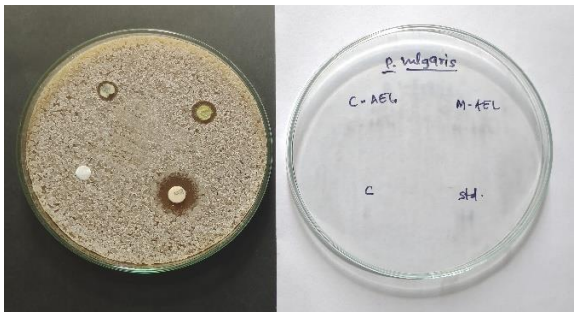

Fig 4. (c) *Staphylococcus aureus*

Fig 4 (d). *Proteus vulgaris*

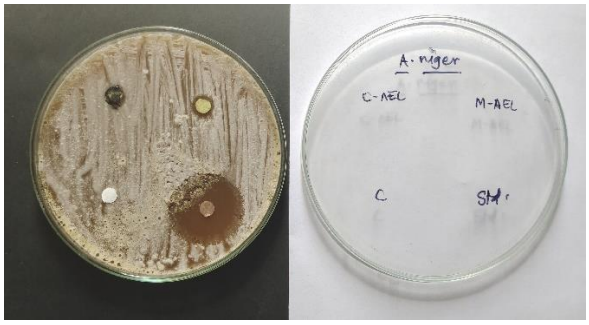

Fig 4. (e) *Aspergillus niger*

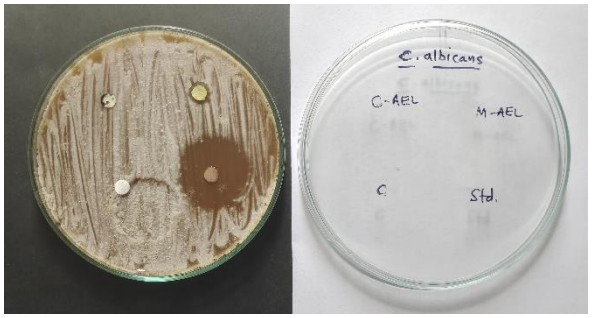

Fig 4. (f) *Candida albicans*

CONCLUSION

Ailanthus excelsa, commonly known as Maharukh, Varul, belongs to the family Simaroubaceae, is a tree of heaven, and has the most significant group of valuable metabolites. Preliminary Phytochemical screening revealed the presence of phytochemicals like alkaloids, glycosides, tannins, terpenoids, saponins, etc. UV-vis spectral studies showed remarkable peaks conveying the presence of phytochemicals. FTIR studies for chloroform leaf extract showed a characteristic absorption band at detecting the presence of alcohols, amines, and alkenes, whereas methanol leaf extract with alkanes, amine salts, carboxylic acids, ketones, aldehydes, alcohols, aromatic esters, and Fluro compounds in the stretch between 3500 to 500 cm^{-1} in spectral search. The agar disc diffusion method screened the chloroform and methanol leaf extracts for their antimicrobial activity. Methanol leaf extract was effective as an antibacterial and antifungal agent, Chloroform extract was found to be least active against bacterial strains, and no inhibition activity against fungal strains was recorded. Traditional medicine, which can serve as a novel therapeutic agent as they are effective, cost-efficient, and has fewer side effects. Standardization of herbal drugs has immense potential for serving the society, and attempts should be made to do it to have a number of traditional medicines.

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