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RESEARCH ARTICLE

Evaluation and characterization of *Vachellia leucophloea* (Roxb.) Maslin, Seigler & Ebinger using phytochemical, antioxidant, GC-MS, antibacterial, and anticancer properties

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Abstract

Medicinal plants provide a diverse spectrum of biological compounds that have significant uses in medicine and the well-being of humans. *Vachellia leucophloea* is a widely recognized medicinal plant in India with a wide range of therapeutic characteristics. This tree has been used in traditional medicine to treat numerous types of ailments since ancient times. *Vachellia leucophloea* contributes a significant part in regulating the death of cancer cells despite causing no adverse effects. In this present study, the bark of *V. leucophloea* was subjected to phytochemical analysis, total antioxidant activity by phosphor-molybdenum method, and DPPH assay to detect the radical scavenging activity, total phenol content for determining the plant's antioxidant activity. Antibacterial and antifungal activities were performed by the agar well diffusion method. UV-Vis, FTIR, GC-MS, and TLC studies were performed to confirm the presence of compounds. The extract was subjected to MIC and TVC studies in which the plant's ability to inhibit bacterial growth can be determined. DNA fragmentation assay was also analyzed by agarose gel electrophoresis. MTT assay was performed to analyze the anticancer activity against the MCF-7 Cell Line.

Keywords

antibacterial; anticancer; antioxidant; GC-MS; *Vachellia leucophloea*

Introduction

Medicinal plants have been used in the treatment of various diseases since ancient times. These plants contain various compounds which have significant therapeutical properties. The drugs being used these days contain compounds that can cure diseases and most of those compounds are present in medicinal plants naturally. Due to this ability to cure diseases, studies using these medicinal plants have been conducted to identify their pharmacological activity. World Health Organisation (WHO) has stated that nearly about 80% of the world depends on conventional medicine which contains active compounds that possess therapeutic properties (1). These plants and phytochemicals possessing antimicrobial activities can be extremely significant in medicinal practices (2). Understanding the chemical substances in plants has become vital for the development of medicinal products and scientific endorsement of traditional therapies. Continuous assessment of medicinal plant extracts for their effective biological functions has recently acquired significance in the pharmaceutical, food, and aesthetic industries (3).

Vachellia leucophloea (Roxb.) Maslin, Seigler & Ebinger belonging to the family Fabaceae have been a part of India's traditional medicinal

system. It is a huge, thorny tree that grows to a height of approximately 35m with white to yellowish-gray bark. It is commonly known as White Bark Acacia and in Tamil, it is referred to as Velvel. India, Bangladesh, Myanmar, Pakistan, Vietnam, Thailand, Sri Lanka, and Cambodia are the native countries of this tree. This tree is also utilized to produce certain chemical products, fiber, food, and drink for both humans and animals, medicine, and timber (4). Since being a part of the traditional medicinal system, different parts of this tree were used to treat numerous diseases. Certain phytochemicals are present in the seeds which add beneficial health effects besides their high nutritional value. Leaves were used for treating fever, and stomach problems, and along with cow's milk it was used for bleeding piles and to treat measles when applied throughout the body. Bark has been a significant component in Ayurvedic medicinal practices which is therapeutic against some diseases. Some compounds are present in the bark which can treat gastrointestinal and respiratory problems. Barks have been utilized for the purification of beverages and also produce a reddish-brown stain that has been used in the production of dyes. Leaves and bark were utilized against dyspepsia, and renal and cardiac edema and when applied twice daily they were used to treat Psoriasis. Varying parts of this tree were used for ophthalmia, leprosy, cancer, inflammation, etc. Both seeds and bark contain tannins and fatty oil (5,6). Tannins extracted from the bark were used as an astringent to treat diarrhoea dysentery and internal bleeding. Externally, they were used for wounds, skin problems, haemorrhoids, eye problems, and also as a mouthwash. The trunk and stem of the tree contain gum which is taken internally to treat diarrhoea and haemorrhoids. Irregular menstruation was also treated using gum and bark extracts. The barks of this tree were used as traditional medicine in Pakistan such as astringent, an anthelmintic, antipyretic, antidote for snake bites, expectorant, demulcent, and for the treatment of certain disorders including bronchitis, stomatitis, ulcers, cough, vomiting, tooth decay, etc (7). The secondary metabolites found in these plants that have medicinal properties have become a desirable target for the assessment of potential therapies against terrible diseases like cancer, since being less toxic (8).

Cancer is a deadly disease characterized by the uncontrollable proliferation of abnormal cells that are capable to invade and destroy healthy body tissue. It has the potential to develop and spread throughout the entire body. Since 2000, the most widespread forms of cancer in the world were lung, breast, and colorectum cancer. Around 2015, cancer became the second major cause of mortality worldwide contributing to about 8.8 million deaths. Globally, cancer has been responsible for one out of six deaths with a prevalence of 70% in developing countries (9). There was a growing interest in studying the anticancer activities of medicinal plants which has resulted in the discovery of different compounds from them (10). Over the past few years, there has been a rise in the research on the antioxidant activity of plant extracts, because free radicals called reactive oxygen species (ROS), have been associated with a wide range of diseases, including cardiovascular disease, stroke, arteriosclerosis and cancer (11). In this study, bark extracts of *Vachellia leucophloea* were investigated for their antioxidant, antibacterial antifungal, and anticancer activity against the MCF-7 cell line of breast cancer cells.

Materials and Methods

Sample collection and preparation

The bark samples of *Vachellia leucophloea* were collected from the garden of the Centre for Bioscience and Nanoscience Research (CBNR), Coimbatore, Tamil Nadu, India. The collected bark sample was washed with running tap water to remove the adhering dust and dirt particles. Then it was dried under sunlight and powdered to prepare the ethanolic and aqueous extract. Both extracts were prepared according to the method described by Mariswamy *et al*. (12) with slight modifications. The ethanolic and aqueous extract was prepared by dissolving 1g of the sample in 20 ml of ethanol and distilled water and placing them in an orbital shaker for proper agitation. After agitation, both extracts were filtered through Whatman No.1 filter paper to remove the extracted debris. Then the extracts were used for further characterization studies.

Phytochemical Analysis

Phytochemical constituents such as Alkaloids, Terpenoids, Phenol test, Sugars, Saponin test, Flavonoids, Quinines, Proteins, and Steroids (13) were qualitatively analyzed and identified the presence of compounds.

Antioxidant activity

Total antioxidant activity

The phosphomolybdenum technique was used to assess the total antioxidant activity of the sample. 0.5 mL of the reaction mixture 1 (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was mixed with a 0.5 ml aliquot of the sample solution. After being covered, the test tubes were incubated for 90 minutes at 50 °C in a water bath. The absorbance of the combination was measured at 695 nm when the samples were cooled. Ascorbic acid was used as the standard to calculate the total antioxidant content (mg/AAEq) (14).

DPPH assay

The radical scavenging activity of the aqueous and ethanol extracts was determined by the DPPH (1,1 -diphenyl -2-picryl-hydrazine) method. Each sample of 0.5 ml was taken to which 0.2 ml of 0.1 N DPPH was added and mixed well. The tubes were then incubated at room temperature for 5 minutes. After incubation, 0.4 ml of 50 mM Tris HCl was added to each tube and incubated at room temperature for 30 minutes. The absorbance of the solution was measured at 517 nm against a blank in a UV -Vis spectrophotometer followed by the mg/g of DPPH was calculated (15).

Total phenol content

Total phenol content was measured by the Folin-Ciocalteu

assay method. The 1ml of the extract was mixed with 0.2ml of 10% Folin-ciocalteu reagent and 1ml of the 20% Na_2CO_3 solution, the mixture was allowed to mix and kept in the water bath at 45 °C for 45 minutes. After incubation, the OD value was measured at 765nm under a spectrophotometer. Gallic acid was used as a standard to calculate the mg/g of the total phenol content (15).

Characterization of the plant compounds

UV- Vis study

The extracted sample was primarily characterized with UV-visible study using a microprocessor LT 291 spectrophotometer. The extract was scanned from the wavelength of 200 to 800 nm to identify the bioactive compounds present in the plant extract (16).

FT-IR

The FT-IR analysis was performed to identify the functional groups involved in the capping, reduction, and stability of plant extracts, which may be associated with several chemical bonds. The prepared extracts were characterized by Fourier transform infrared (FT-IR) spectroscopy (Shimadzu system FTIR mode - 100l) at the range of 400–4000 cm^{-1} (17).

Thin Layer Chromatography

To identify the presence of phytocompounds, the extracts were prepared separately and exposed to TLC (20 × 20 cm aluminium sheets coated with silica gel 60 F254). The extracts were spotted on the TLC plate and developed in Methanol: Ethyl acetate: Acetic acid: Toluene: Water at a ratio of 3:2:1:1:2 and observed in daylight and under UV light (254 and 365 nm) after drying (18).

GC-MS analysis

Bioactive compounds were studied using Agilent CH-GCMSMS-02 GC System, 7000 GC/TQ with Helium as mobile phase. The temperature was 50 °C at 1 min hold time Run time: 1 min 5 °C / min 120 °C hold 1 min Run time 16 mins10 °C / min 210 °C hold 1 min Run time 26 mins 10 ° C / min 280 °C hold 5 mins Run time 38 mins Mainly the Mass spectra were taken at 70 eV; a scan interval of 30-900 m/z, 5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative percentage of each component was calculated by comparing its average peak area to the total areas (6).

Antibacterial activity by well diffusion

The agar well diffusion method was used to study the antibacterial activity of the sample. *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa,* and *Staphylococcus aureus* (70 µl) were taken and spread on the nutrient agar plates. Followed 3 wells were made with cork borer and the samples were added (Aqueous extract, Ethanolic extract, and negative control-DMSO) to the respective wells along with positive control (Antibiotic disc - Penicillin). Finally, the petri dishes were incubated for 24 h at 37 °C. To evaluate the antibacterial activity of the samples, the diameter of the inhibition zone was measured and noted in mm (19).

Antifungal activity

The antifungal properties of plant extracts were assessed against *Aspergillus niger* and *Aspergillus flavus* on malt agar medium using a well diffusion method. After swabbing the culture wells were punched using a cork borer at a specific distance and were filled with the samples along with positive control (Fluconazole) and negative control (DMSO). The parafilm tape was used to seal the plates and incubated at 28 °C for 3 to 5 days. The antifungal activity was measured in millimeters (mm) (19).

Minimum Inhibitory Concentration and TVC

The sample was tested against the bacterial strains for antibacterial action by ELISA method. The suspension of culture (*Escherichia coli* and. *Staphylococcus aureus*) of 10 µl was added to the well containing sterilized LB broth and the sample was added (20 µl). This was incubated at 37 °C for 24 hours. The absorbance was measured at 600 nm and the sample which showed inhibition of bacterial growth was taken as MIC. For the blank reaction, the sterile broth was used in place of suspension cultures (without inoculum). In control tests, sterile broth, and bacterial suspension were used (20).

The percentage of bacterial inhibition was calculated by:

Absorbance of control OD-Absorbance of Sample OD/ Absorbance of control OD × 100.

The total viable count (TVC) was determined by the plate assay method. The sample that showed maximum inhibition in MIC was taken for Total viable count. Initially, Mueller Hinton agar was prepared and poured into the Petri plate containing 10 µl of the MIC sample (control and treated). After proper rotation the plate was allowed for solidification and incubated for 24 to 48 hours at 37°C and the bacterial growth was noted.

Anticancer activity

The effect of Ethanolic extract on the viability of MCF 7 cells was determined using an MTT assay. It is determined by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) to a blue formazan product. MCF 7 cell line was allowed to grow in the CO_2 incubator with 5% CO_2, 80% humidity, and at 37 °C for 48 hours. In a 96-well plate, different concentration of samples (5, 10, 15, 20, 25 µl) was taken and added 100µl of cell line. As a control, cell lines were used, and the plate was incubated for 24 hours in a CO_2 incubator. After incubation, the cells were washed with DMSO, and 50 µl trypsin followed by the addition of 20 µl of MTT dye. After 4 hours of incubation, OD values were taken at 570 nm using an ELISA reader (21). The percentage of cell death was calculated using the following formula;

Control OD- sample OD/ Control OD× 100

DNA Fragmentation

DNA fragmentation of the sample was studied with the MIC sample, using the Phenol-Chloroform method. After the isolation of the DNA, 1% agarose was prepared with the addition of EtBr in a gel casting tray along with comp and allowed for solidification. After solidification comp was

removed and placed in the AGE unit. The isolated sample was loaded with loading dye and run with 50V for 45 to 60 minutes, then the gel was placed in gel documentation systems with UV-Transilluminator (22) and visualized.

Results and Discussion

Phytochemical analysis

The phytochemicals present in plants play a vital role in the prevention and treatment of numerous diseases. A preliminary phytochemical screening of aqueous and ethanol extracts of *Vachellia leucophloea* bark was presented in Table 1. Saponins, Flavonoids, Quinines, and Steroids were found to be present in the aqueous extract (Fig. 1). Alkaloids, Phenols, Protein, and Steroids were found to be present in the ethanol extract (Fig.1).

A comparative study of phytochemical screening of methanolic extract of leaves and bark (23) concluded the presence of carbohydrates, saponin, flavonoids, steroids, starch, alkaloids, glycosides, terpenoids, and proteins. Another study (24) reported the presence of terpene in petroleum ether and chloroform extract and the presence of alkaloids, terpenes, flavonoids, and tannins in alcohol extract.

Antioxidant activity

Total Antioxidant activity

This study revealed that aqueous extract exhibited a high antioxidant capacity of 256 µg/ml whereas ethanol extract showed antioxidant activity of about 201 µg/ml. The previous study (25) reported that the antioxidant activity of acetone and methanol extracts was 95.8 and 90.1 g.

Table 1. Phytochemical analysis of Aqueous and Ethanol extracts of *V. leucophloea*

Phytochemical constituents	Aqueous extract	Ethanol extract
Alkaloids	-	+
Terpenoids	-	-
Phenol	-	+
Sugar	-	-
Saponins	+	-
Flavonoids	+	-
Quinines	+	-
Protein	-	+
Steroids	+	+

+ indicates the presence of compounds; -s indicates the absence of compounds.

DPPH Assay

The DPPH assay of aqueous and ethanol extract of *V. leucophloea* bark was calculated. The DPPH activity of the aqueous extract was found to be 26 µg/ml and ethanol extract was 18 µg/ml which concludes that the aqueous extract exhibits high radical scavenging activity.

A comparative study of DPPH activity (25) has reported that the scavenging activity of acetone and methanol extract was 18.6 µg/ml and 31 µg/ml respectively.

Total phenol content

The total phenolic content in the plant contributes to the plant's antioxidant activity. The total phenol content of the aqueous and ethanol extract of *V. leucophloea* bark was calculated. The high phenol content was recorded in aqueous extract which contain 192 µg/ml whereas the ethanol extract exhibited 168 µg/ml of phenol content. The previous study (25) reported that the total phenolic content of acetone and methanol extract was 78.9 g GAE/100 g extract and 76.3 g GAE/100 g extract.

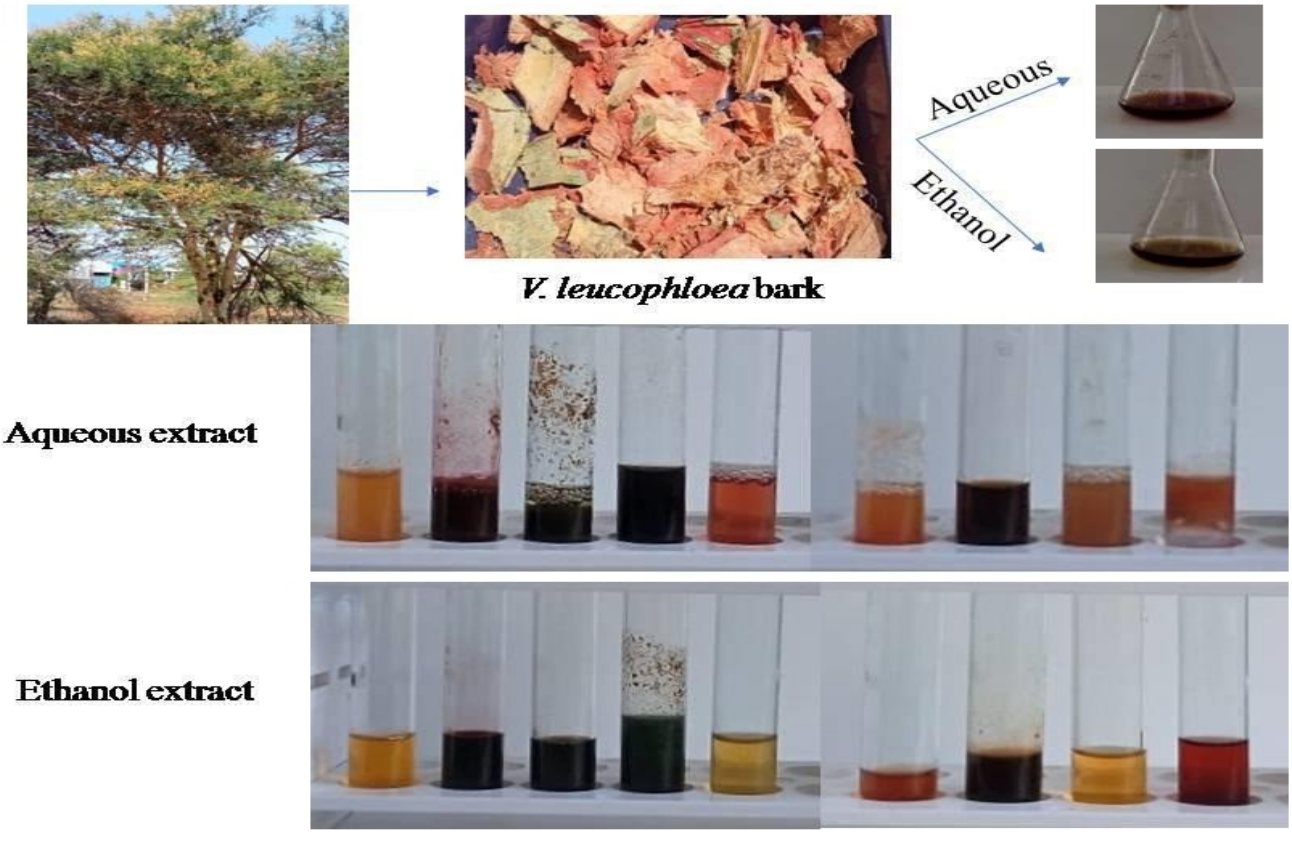

Fig. 1. Phytochemical analysis of *V. leucophloea*

Characterization studies

UV-Vis Spectrophotometer

The UV-Vis analysis of aqueous and ethanol extracts of *V. leucophloea* was analyzed by the peaks in the wavelength ranging from 200 – 800 nm. Aqueous extract exhibited peaks at 210, 215, 220, 225, 245, 265, 315,355 and 440 nm (Fig. 2). The ethanol extract exhibited peaks at 225, 230, 250, 345 and 460 nm (Fig. 3).

According to the previous study (16), the UV-Vis spectroscopy profile of acetone extract of *A. rigidula* showed peaks at 215, 235, 260, 270, 290, 310, and 325 nm with absorption of 4.523, 10.000, 10.000, 10.000, 10.000, 3.676 and 3.232 respectively.

FT-IR

FT-IR analysis of aqueous and ethanol extract of *V. leucophloea* was performed to detect the presence of functional groups. The FT-IR spectrum of aqueous extract showed major peaks at 408.91 cm^{-1}, 455.20cm^{-1}, 547.78cm^{-1}, 601.79cm^{-1}, 686.66cm^{-1}, 1527.62cm^{-1} and 1620.21cm^{-1} as shown in Fig. 4.The peak at 1620,21 cm^{-1} indicates the presence of C=C alkene group and peak at 1527.62cm^{-1} is due to NO_2 stretch. The presence of the C-Cl group and C-Br group is indicated by the peaks at 686.66cm^{-1} and 601.79cm^{-1}.

The FT-IR spectrum of ethanol extract showed major peaks at 424.34cm^{-1}, 455.20cm^{-1}, 509.21cm^{-1}, 555.50cm^{-1},

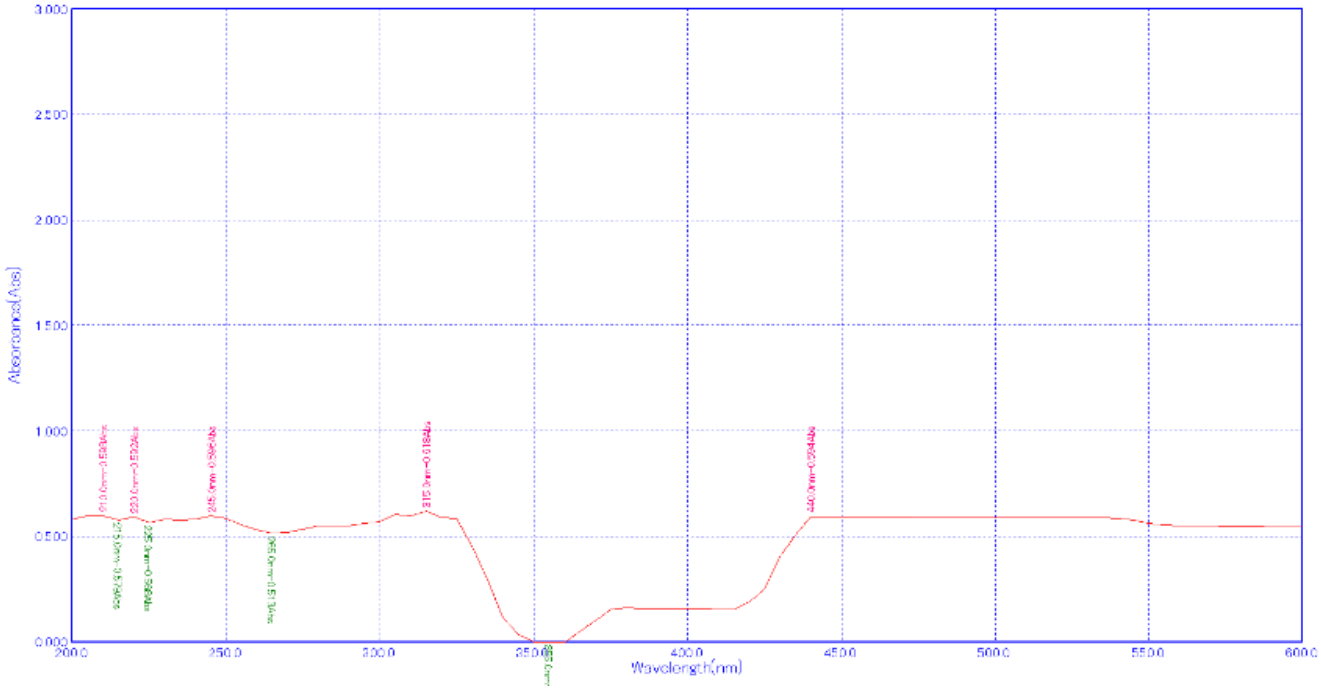

Fig. 2. UV-Vis analysis of aqueous extract of *V. leucophloea*

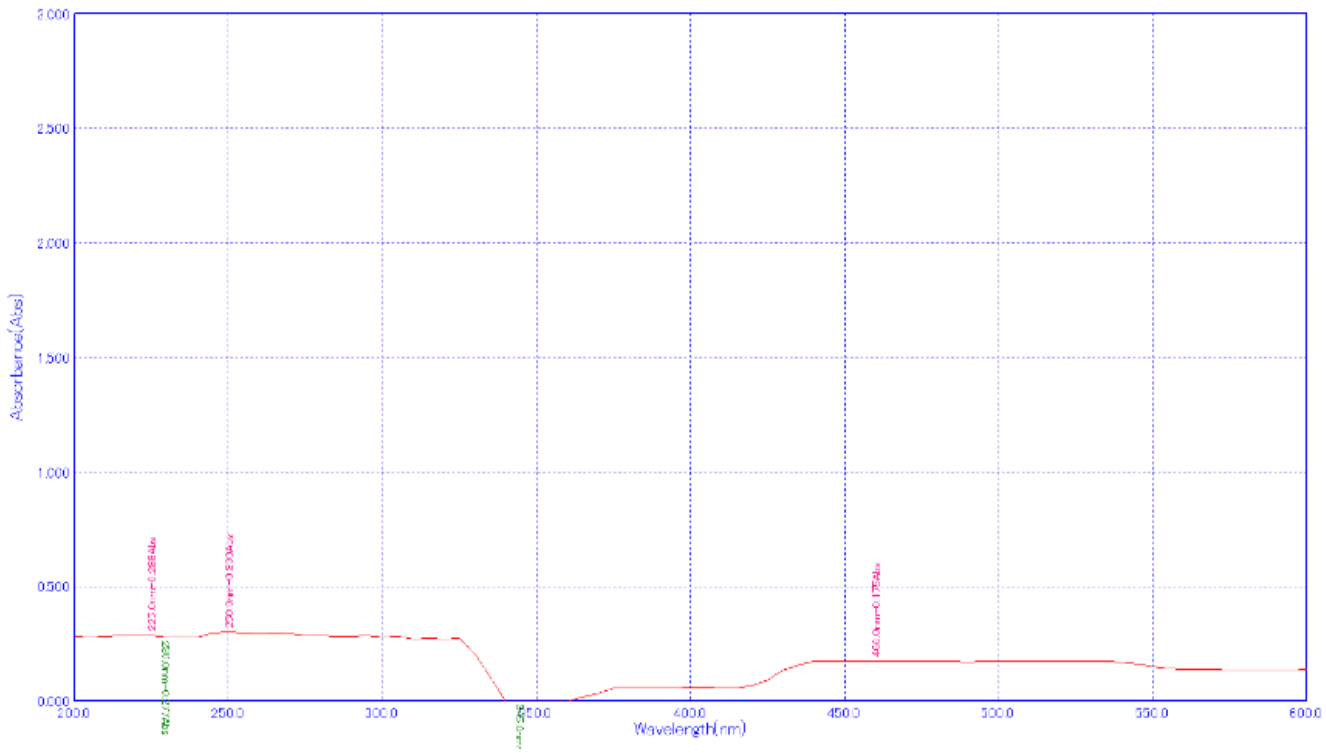

Fig. 3. UV-Vis analysis of ethanol extract of *V. leucophloea*

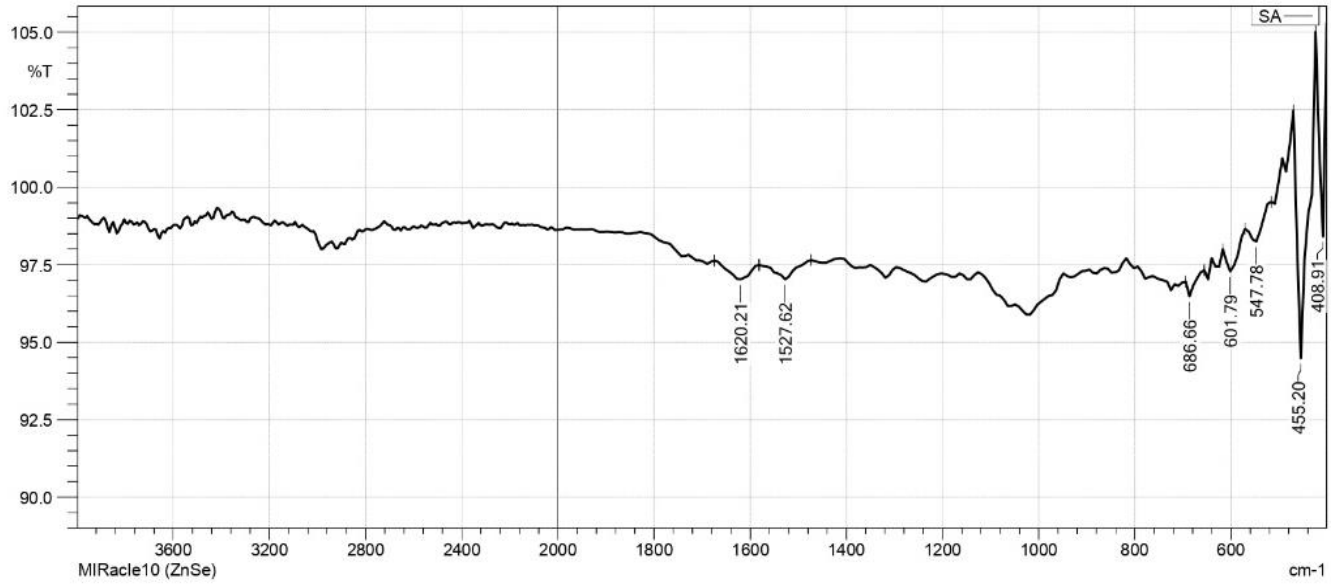

Fig. 4. FT-IR analysis of aqueous extract of *V. leucophloea*

601.79cm^{-1}, 686.66cm^{-1}, 910.40cm^{-1}, 987.55cm^{-1}, 1049.28cm^{-1}, 1234.44cm^{-1}, 1597.06cm^{-1} and 2353.16cm^{-1} as shown in Fig. 5. The peak at 1597.06cm^{-1} is due to presence of NO_2 stretch and the peak at 1234.44 cm^{-1} indicates the presence of C-O-C stretch. The peak at 1049.28 cm^{-1} indicates the presence of C-OH stretch. The presence of the C-Cl group and C-Br group is indicated by the peaks at 686.66cm^{-1} and 601.79cm^{-1}. A similar study (17) reported the presence of functional groups at different peaks.

Thin Layer Chromatography

TLC assessment provides an impressive result revealing the presence of phytochemicals. The analysis of aqueous and ethanol extract was subjected to a TLC study and spots were detected in the TLC plate (Fig. 6). Both extracts showed 2 spots for which the R_f value was calculated. The R_f values of aqueous extract were 0.781 and 0.484 whereas for ethanol extract 0.718 and 0.593. A similar study of TLC (24) has reported that the petroleum ether and chloroform extract showed nine spots whereas the ethanol extract showed four spots. Another comparative study of TLC analysis (26) reported the R_f values of petroleum ether, methanol, and chloroform extracts of bark, heartwood, and leaves.

GC-MS

The GC-MS analysis of ethanol extract of *V. leucophloea* bark revealed the presence of many compounds with different peaks (Fig. 7 and Table 2). A previous study (6) has reported that the GC-MS analysis of methanolic extract exhibited 40 peaks revealing the presence of different components. The presence of phyto components in the plant contributes to the plant's potential for pharmacological activity.

The GC-MS analysis concluded the presence of compounds with some therapeutic uses. 2-Propanol, 1-methoxy-was used in diesel engines as an antifreeze and acts as a carrier/solvent in printing/writing inks and paints and coatings. Dimethyl ether was used in some freeze spray products with propane to treat warts. Hexanoic acid,

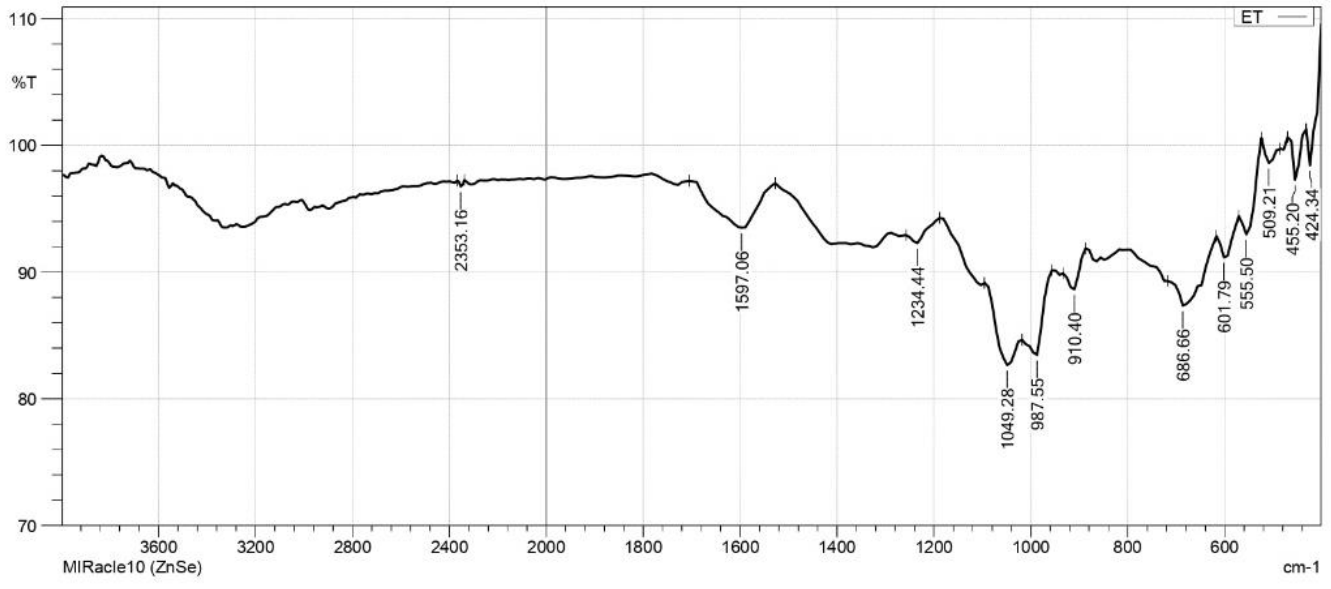

Fig. 5. FT-IR analysis of ethanol extract of *V. leucophloea*

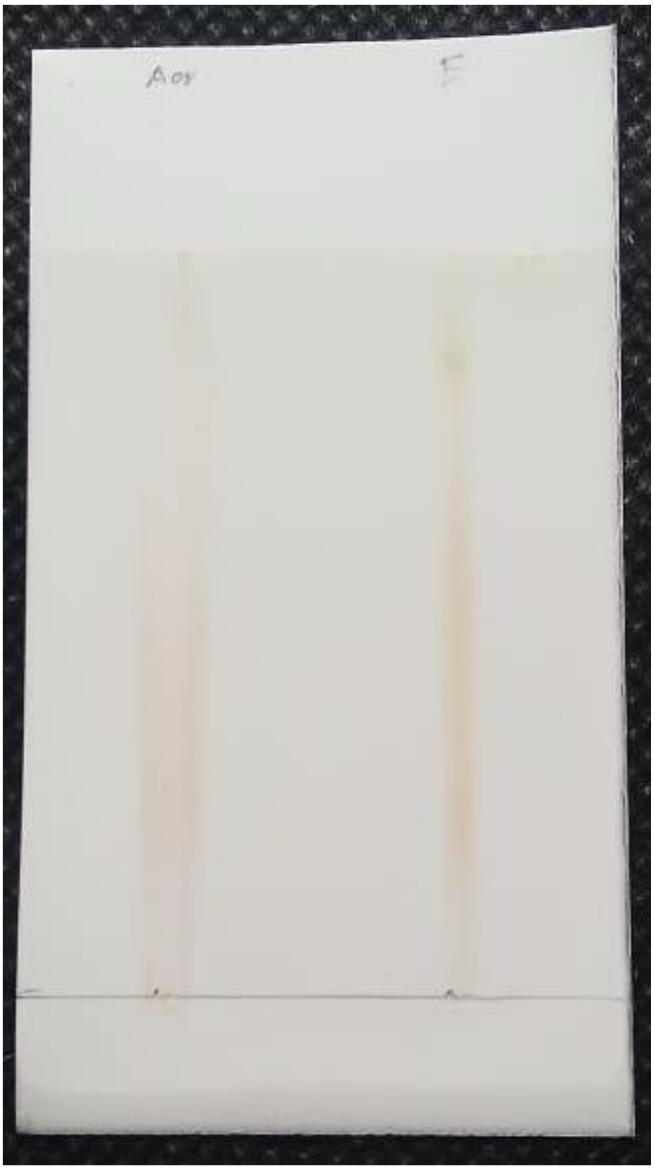

Fig. 6. TLC analysis of aqueous and ethanol extracts of *V. leucophloea*

ethyl ester was used to produce nano micelles derived from polyethylene glycol, a non-viral gene carrier. Naphthalene was utilized to generate nadoxolol drug for beta-blocking and has anti-inflammatory, antimicrobial, antiviral, antidiabetic, anti-insecticidal, antitubercular, anticancer, antihypertensive, anticonvulsant, anti-neurodegenerative, antidepressant and antipsychotic activities. Resorcinol was used to cure skin disorders like eczema, acne, seborrheic dermatitis, and psoriasis, and to treat corns, calluses, and warts. Diethyl Phthalate (DEP), Bis(2-ethylhexyl) phthalate (DEHP), Triethyl citrate, Pentanoic acid, and Ethyl oleate were used in consumer goods, including plastic packaging films, solvent for fragrances, cosmetics, a surface lubricant in food and pharmaceutical packaging gas a plasticizer, and in tubing used for medical purposes. n- Hexadecanoic acid possesses anti-inflammatory, Nematicide, Pesticide, Antiandrogenic, Hypocholesterolemic, and Hemolytic activities and is a 5-Alpha reductase inhibitor.

Oxalic acid mono-(N-dimethyl)-amide, methyl ester finds application in pharmaceutical companies. Octacosanol has beneficial effects in amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease), Parkinson's disease, and high cholesterol, and improves the health of fat cells and athletic performance. Pyrovalerone is a psychoactive drug with stimulant effects that acts as a norepinephrine-dopamine reuptake inhibitor (NDRI), and an anorectic or appetite suppressant to treat chronic fatigue or lethargy. Supraene was used in the initiation or maintenance of surgical anesthesia as an inhalation anesthetic.

Antibacterial and Antifungal activity

The antibacterial activity of aqueous and ethanol extracts of *Vachellia leucophloea* bark against *Escherichia coli, Salmonella typhi, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. The ethanol extract showed high antibacterial

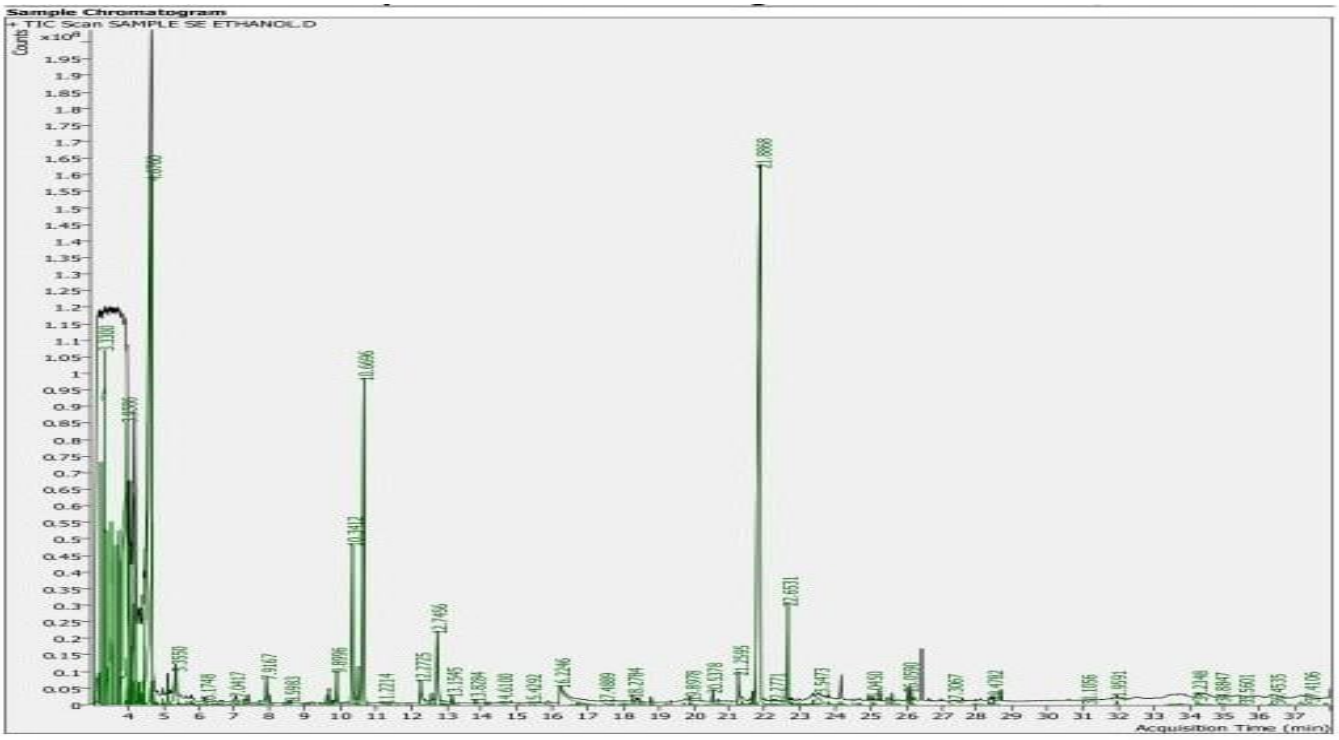

Fig. 7. GC-MS analysis of ethanol extract of *V. leucophloea*

Table 2. GC-MS analysis of ethanol extract of *V. leucophloea*

Component RT	Compound Name	CAS#	Formula	Component Area	Match Factor
3.3300	2-Propanol, 1-methoxy-	107-98-2	$C_4H_{10}O_2$	172205570.6	63.7
3.9586	Dimethyl ether	115-10-6	C_2H_6O	664428495.1	94.9
6.1748	Pentanoicacid,ethyl ester	539-82-2	$C_7H_{14}O_2$	5931595.5	86.5
7.9167	Hexanoic acid, ethyl ester	123-66-0	$C_8H_{16}O_2$	23132145.6	97.7
12.2725	Naphthalene	91-20-3	$C_{10}H_8$	31339116.0	99.0
15.4292	Pyrovalerone	3563-49-3	$C_{16}H_{23}NO$	517111.2	63.9
16.2246	Resorcinol	108-46-3	$C_6H_6O_2$	78579072.1	95.9
21.8868	Diethyl Phthalate	84-66-2	$C_{12}H_{14}O_4$	914569652.5	98.4
22.6531	Triethyl citrate	77-93-0	$C_{12}H_{20}O_7$	59668856.0	97.7
26.0590	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$	10152024.4	96.1
28.4782	Ethyl Oleate	111-62-6	$C_{20}H_{38}O_2$	3928859.8	88.0
31.9591	Bis(2-ethylhexyl) phthalate	117-81-7	$C_{24}H_{38}O_4$	2025224.2	92.2
34.2348	Supraene	7683-64-9	$C_{30}H_{50}$	2831749.4	82.5
35.5601	Oxalic acid mono-(N-dimethyl)-amide, methyl ester	1000452-65-6	$C_5H_9NO_3$	679611.8	63.9
37.4106	Octacosanol	557-61-9	$C_{28}H_{58}O$	4194169.4	69.5

activity against *S. typhi, S. aureus,* and *P. aeruginosa* compared with *E. coli.* Whereas the aqueous extract exhibited activity against *S. typhi* and *P. aeruginosa* and low zone of inhibition against *E. coli* and no zone of inhibition against *S. aureus* (Fig. 8). A comparative study (27) reported that the methanol extracts showed high activity against test organisms followed by acetone and aqueous extracts whereas hexane extract showed less antibacterial activity than others.

In this study, both aqueous and ethanol extracts of *V. leucophloea* bark exhibited a vulnerable zone of inhibition against *Aspergillus niger* and *Aspergillus flavus*. A similar study (27) has reported that the methanol extract showed high activity whereas acetone and aqueous extracts exhibited moderate activity and hexane extract showed poor activity against fungal cultures.

Minimum Inhibitory Concentration and TVC

The minimum inhibitory concentration of the samples was analyzed with the ELISA method. The MIC of a sample against *E. coli* and *S. aureus* shows 51.86 and 72.20% resistance activity. According to a previous study (28), the hexane and chloroform extracts do not show any MIC and the acetone extract showed a MIC of 600 µg/ml against *S. aureus*. The methanol extract exhibit MIC of 300 µg/ml against *E. coli* and the aqueous extract exhibit MIC of 300 µg/ml against *E. coli, B. Cereus,* and *K. aerogenes*. Another comparative study (16) reported MIC of *A. rigidula* against *P. alcalifaciens, S. aureus, Y. enterocolitica,* and *E. faecali* ranged from 37.5 to 75 mg/ml and *A. berlandieri* from 37.5 to 150 mg/ml.

From the minimum inhibitory concentration of the sample total viable count was performed as an indicator of overall microbiological quality and noted for the growth of bacterial colonies. The sample which showed more resistance against *S. aureus* was taken and it is evident from the Fig. 9. Large number of colonies was present in the control plate and a smaller number of colonies was observed in the treated plate (only 8 colonies). The comparative count clearly shows that the sample inhibits the growth of *S. aureus*.

Anticancer activity

The anticancer activity of the ethanol extract at different concentrations against MCF-7 cell lines was analyzed and the percentage of cell death was calculated. The percentage of cell death at concentrations of 5, 10, 15, 20, 25 µl was 50.58%, 55.55%, 65.11%, 67.23% and 67.84% (Fig. 10).

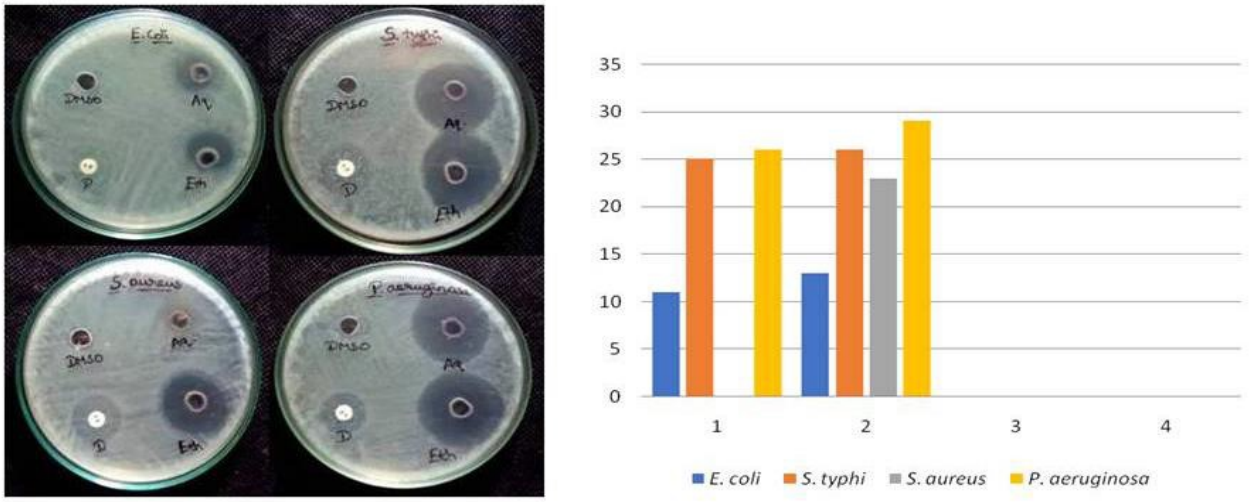

Fig. 8. Antibacterial activity of aqueous and ethanol extract of *V. leucophloea* against *E. coli, S. typhi, S. aureus* and *P. aeruginosa*

In a previous study (29), *A. hydaspica* extracts of methanol and ethyl acetate showed no cytotoxic against normal Vero cells and the ethyl acetate extract was effective against cancer cells with IC50 values of 29.9 ± 0.909 µg/ml for MDA-MB-361 cell line and 39.5 ± 0.872 µg/ml for HCC-38 cell line.

DNA Fragmentation

The fragmentation of DNA by agarose gel electrophoresis viewed under UV transilluminator was shown in Fig. 11. The bacterial DNA did not appear in the agarose gel stained with EtBr whereas a bacterial DNA smear was present in the control where the sample was not inoculated with the bacteria. This concludes that the bacterial DNA was cleaved when treated with the sample. In a previous study (30), it was reported that *A. nilotica* cleaved the bacterial DNA in a time-dependent manner confirming its antibacterial activity.

Fig. 11. DNA fragmentation: Lane1: Control DNA; Lane 2: Extract treated DNA showed fragmentation

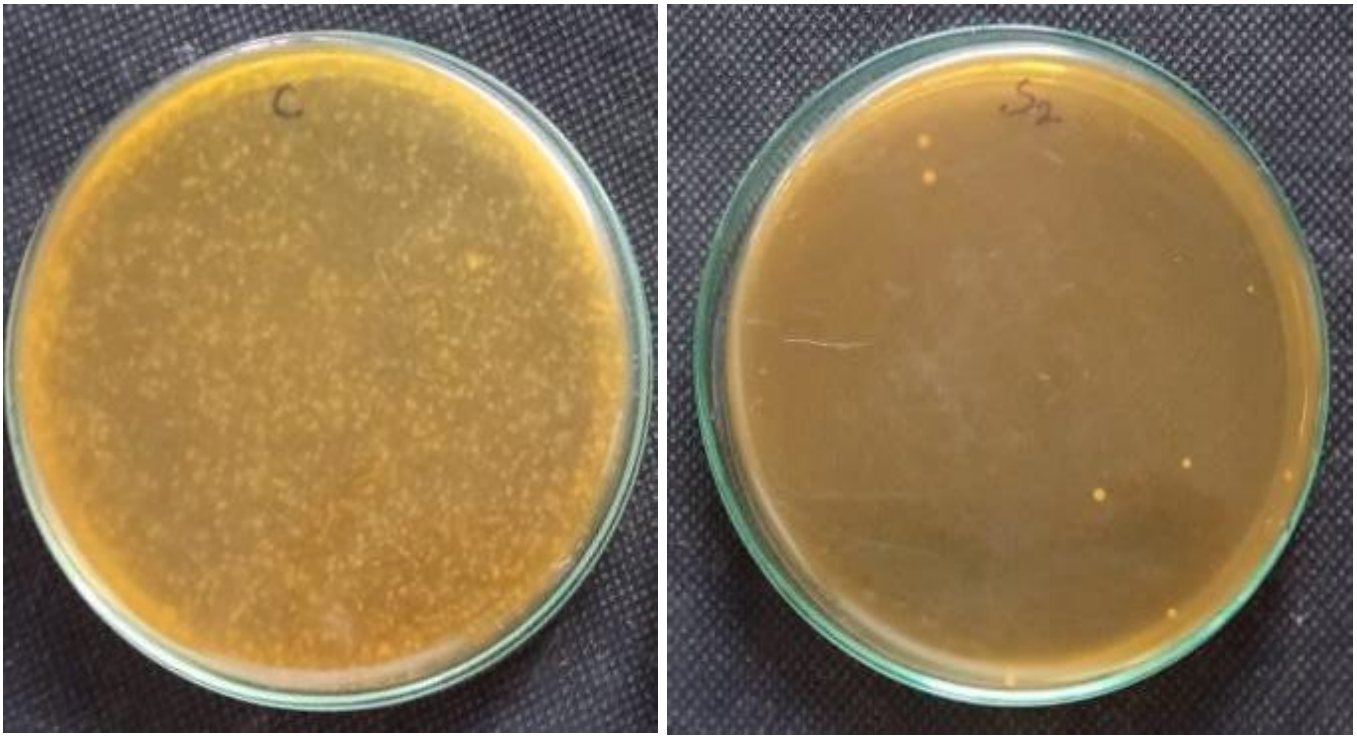

Fig. 9. Total viability count

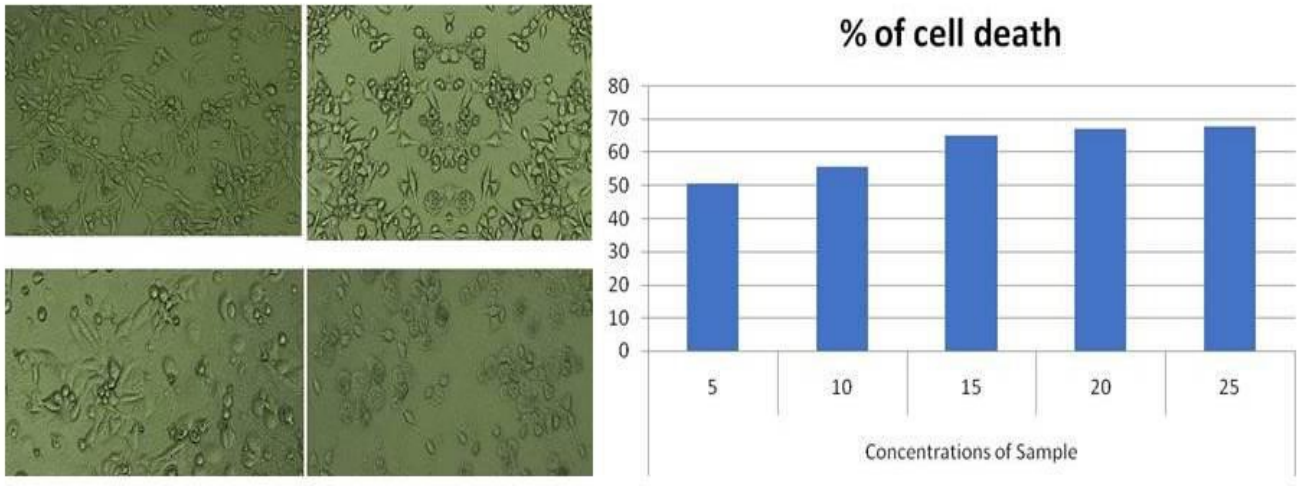

Fig. 10. Anti-cancer activity against MCF 7 Cell Line

Conclusion

In this study, aqueous and ethanol extracts of *V. leucophloea* were analyzed for their biological properties such as phytochemical constituents and antioxidant activity, followed by an antibacterial study, MIC, TVC, and DNA fragmentation study was carried out to confirm the efficacy of the plant extract. FT-IR and GC-MS analysis showed the presence of more bioactive compounds containing antibacterial and anti-cancer compounds. Anticancer activity against the MCF-7 cell line shows 67.84 % cell death which indicates its medicinal applications.

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Authors' contributions

Santhiya S collected the plant sample and performed the experiments under the guidance of Dr.RR. Dr. JJ analyzed and consolidated the results, all three authors made significant contributions in drafting the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest.

Ethical issues: None.

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