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INVITRO VALIDATION OF ANTIMICROBIAL ACTIVITY OF BIOACTIVE COMPOUNDS FROM THERAPEUTIC PLANTS

ABIRAMI. M[1], KAYATHIRI. S.S[1], VIDYA.P[1], FARHANA SHIREEN.A[1], RAJALAKSHMI.V[1]*

[1*]PG & Research Department of Microbiology, Dwaraka Doss Goverdhan Doss Vaishnav College, Arumbakkam, Chennai, Tamil Nadu

Email: rajalakshmi.v@dgvaishnavcollege.edu.in

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ABSTRACT

Herbal plants have historically served as vital alternative treatments, especially in developing countries, due to their natural antiseptic properties and role in traditional medicine. This research paper explores the significance of medicinal plants in drug development, noting that a substantial proportion of populations in underdeveloped regions still rely on herbal remedies. The extraction process involved the use of various solvents including methanol, chloroform, and acetone to obtain plant extracts from *C. roseus, M. oleifera, E. alba*, and *C. papaya.* The antibacterial activity of the plant extracts were evaluated using the agar well diffusion method, revealing their effectiveness against different bacterial strains. Notably, the maximum zones of inhibition varied for each plant extract, demonstrating varying levels of activity against specific bacterial cultures. Furthermore, the minimal inhibitory concentration (MIC) of the plant extracts was determined, which ranges from 100µg to 1000µg. The MIC values for the herbal extracts of *C. roseus* against *S.aureus* and *E. coli* were successfully identified, highlighting their inhibitory effects on bacterial growth. Additionally, phytochemical screening indicated the presence of bio-active compounds such as saponin, terpenoid, flavonoid, alkaloid, and oil in the plant extracts. Finally, chemical characterization of the plant extract of *C. roseus* using Fourier Transform Infrared Spectroscopy (FT-IR) revealed the presence of functional groups including alkanes, aliphatic amines, carboxylic acid, and aromatic compounds.

INTRODUCTION

Herbal plants have long been used as alternative treatments in developing countries due to their natural antiseptic properties (Gurib-Fakim, 2006). For thousands of years, they have been an essential part of traditional medicine, helping to create treatments that are still in use today. The creation of new drugs is still greatly aided by the abundant resources present in medicinal plants. Indeed, it is claimed that 70 to 95 percent of people in underdeveloped nations still receive their medical care from Ayurvedic professionals (Ranabhat *et al.*, 2023). Several investigations on the impact of plant extracts on microbes have demonstrated their promise as safe, natural substitutes for contemporary drugs.

Herbal medicines are believed to counteract disease effects within the body, with some plants shown to reduce cancer progression (Greenwell and Rahman, 2015). Their low side effects, non-toxic nature, and accessibility are the reasons behind their rising popularity. Natural chemicals derived from plants are valuable resources in the drug development process since they exhibit reduced resistance in contrast to synthesized medications (Shakya, 2016). There is a misperception that herbal compounds are completely free of negative effects, even if they are thought to be safe.

These herbal plants' antibacterial, anti-cancer, and anti-inflammatory qualities are being studied extensively. Both nationally and internationally, a lot of study is being done on these biocompounds and phytochemicals. Ancient medicine served as the foundation for modern allopathic medicine, and it is expected that many significant new treatments will be found and made available to the public in the future (Gurib-Fakim, 2006). Herbal medicine is used extensively all over the world. The global trend has shifted from synthetic to natural medications. The WHO has acknowledged the importance of herbal medicine in primary healthcare. Compared to allopathic medications, herbal remedies are more cost-efficient, have fewer adverse effects, and are more effective. Phytochemicals are secondary metabolites found in plants. Plants are shielded against microbial diseases by these substances. Alkaloids, glycosides, saponins, terpenes, carotenoids, steroids, flavonoids, and tannins are examples of phytochemicals, which are active ingredients with therapeutic qualities that are regarded as medicines or drugs. These substances are found to be significant in herbal plants and are primarily extracted from them for drug development (Shakya, 2016).

Nevertheless, many people are turning to medicinal plants for their biochemical and pharmacological properties. These include putative anti-mutagenic properties as well as anti-inflammatory, antioxidant, and anti-cancer properties (Sreeja et al., 2021). Through their bioactive components, medicinal plants including *Eclipta alba, Catharanthus roseus, Moringa oleifera, and Carica papaya* have been shown in studies to suppress cancer cell proliferation and boost overall health. These plants have a broad range of medicinal uses, including the treatment of diabetes, hypertension, microbial infections, liver protection, and more.

Eclipta alba, often called Bhringraj, has been employed for its liver-protective qualities in Ayurvedic medicine. It has ingredients like wedelolactone, which has hepatoprotective and anti-aging properties (Mithun et al., 2011). In the same way, *Catharanthus roseus* is well recognized for its anticancer

qualities; vinblastine and vincristine, for example, are chemicals found in cancer chemotherapy (Khalil, 2012)

Carica papaya is well-known for its antibacterial, antifungal, and antimalarial properties. Its leaf extracts have anticancer qualities, and it is used to treat ailments like jaundice, dengue fever, and enlarged spleen (Anibijuwon and Udeze, 2009). Another potent medicinal plant, *Moringa oleifera,* is high in vitamins A and C. Its leaves also contain substances that suppress cancer cells and heal a range of stomach issues (Sreeja et al., 2021).

Research has also demonstrated that the solvents employed during the extraction process have a major impact on the effectiveness of plant extracts. In order to increase the yield of chemical ingredients, active compounds are frequently isolated using methanol, chloroform, and acetone. It has been demonstrated that these phytochemicals, or bioactive substances, improve human health by preventing and curing a number of illnesses.

Thin layer chromatography was carried out to identify the compounds present in a given mixture and the purity of those compounds. It was one of the easiest methods for the separation of components and involved in the separation of non-volatile compounds. They're most commonly used to identify pharmaceuticals and drugs, as well as to determine the concentration of active components, auxiliary substances, and as preservatives in drugs and drug preparations, and also to control process in synthetic production processes (Lawal et al., 2019)

FTIR method was employed to obtain the infrared spectrum of the extracted plant sample. This method was one of the easiest methods and the cell wall component; functional groups were analysed (Ashokkumar and Ramaswamy, 2014).

Secondary metabolites found in medicinal plants are considered as an attractive target for screening novel drugs due to their unique structural nature, great diversity in chemical characteristics, and low toxicity. As a result, the researchers are in search of novel drugs with minimal toxicity towards the infectious disease.

MATERIALS AND METHODS:

PREPARATION OF LEAF EXTRACT:

The fresh leaves of different medicinal plant species namely *Catharanthus roseus* (Nithiyakalyani), *Carica papaya* (Papaya), *Eclipta alba* (Karusilankanni) and *Moringa oleifera* (Murungai keerai) were collected from a local market in and around Chennai. All plant materials were thoroughly washed using sterilized deionized water and blotting paper was used to remove the excess water from the plant material. The leaves were air dried at room temperature (27°C) and were made into fine powder.

The leaf powders were separately mixed with different solvents such as acetone, chloroform and methanol for 24 hrs. It was filtered using WhattmanNo.1 filter paper and was dried using a rotary vacuum evaporator.

COLLECTION OF MICROORGANISMS:

ANTIBACTERIAL ASSAY:

The antibacterial activity of the medicinal plant was determined by the well diffusion method. The Mueller Hilton agar plates were prepared against six different pathogenic bacterial cultures namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus mirabilis*, *Klebsiella pneumonia*. The 8mm wells were created and loaded with 100µl of different plant extract. The positive control was chloramphenicol (30µl) and DMSO (30µl) was used as a negative control. Similarly, the same procedure has been repeated for all the six bacterial strains and for all the different plant extracts. The plates were incubated at 37°C for 24hrs. After incubation it were observed for the zone of inhibition (Erturk, 2006).

ANTIFUNGAL ACTIVITY:

The Sabouraud dextrose agar was used as medium for the assessment of antifungal activity by agar well diffusion method About 0.1ml of fungal culture maintained from the one-hour stock culture was inoculated onto the plate by using swab technique. Using a cork borer 4 wells were made on the agar plate which were around 8mm. The wells were filled with 100µl of the different plant extract. The fluconazole solution was used as a positive control (Webster et al., 2008). The same procedure had been repeated for the other fungal culture of different plant extracts. The agar plates were incubated at 30°C for 48 hrs and the plates were observed for the zone of inhibition.

MINIMUM INHIBITORY CONCENTRATION (MIC):

Minimum Inhibitory Concentration was performed in *in vitro* technique. Luria Bertani broth was prepared and 5 ml of broth was poured into each test tube separately. As per the previous assessment the plant which had shown significant activity was subjected for MIC technique (Anibijuwon and Udeze, 2009). The plant extract used for the evaluation was *C.roseus* against *S.aureus* and *E.coli* at the concentration range of 100 µg/ml to 1000 µg/ml.

THIN LAYER CHROMATOGRAPHY:

The crude extract of *C.roseus, M.oleifera, C.papaya* and *E.alba* were subjected to Thin Layer Chromatographic analysis (Kabesh et al., 2015). The retention factor (Rf) value of the plant extract was evaluated using the formula. The compounds present in the sample were identified by comparing Rf values with the help of the standard chart.

$$RF = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent}$$

FTIR

Fourier transform infrared spectroscopy (FT-IR) was used to study the functional groups present in the extract and the pure and composite membranes (Ashokkumar and Ramaswamy, 2014).

RESULT

Extraction of the plant:

Various solvents were used to extract the plant namely methanol, chloroform and acetone. The plant *C.roseus, M. oleifera, E. alba, C. papaya* dried plant powder was weighed for 10% w/v taken and plant extract was obtained. The maximum yield of extracts for *C. roseus* was 0.46% obtained in methanol, followed by chloroform – 0.36% and acetone - 0.33%. For *M.oleifera* the maximum yield of extract 0.35% was obtained in methanol, 0.4% in acetone and chloroform - 0.34%. For *C.papaya* the maximum yield of extracts 0.46% was obtained in methanol, followed by 0.41% in chloroform and acetone - 0.33%. Finally for *E.alba* dried material was weighed and the maximum yield of extract was obtained 0.35% in methanol 0.4% in acetone and chloroform - 0.34%.

Antibacterial activity:

The antibacterial activity was carried out for all the extract by agar well diffusion method against bacterial culture and the plant extracts (acetone, methanol and chloroform) had shown the activity against different bacterial strains.

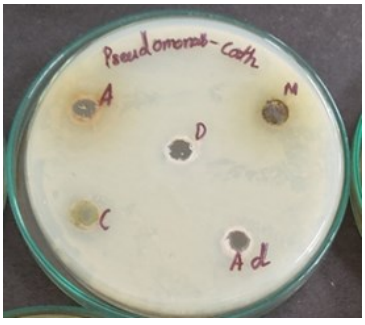
The *C. roseus* extract with the acetone, methanol and chloroform had shown activity for all the bacterial culture (Table 1). The maximum zones of inhibition against all the test organisms were observed for acetone extract in the following order: *Pseudomonas* > *Proteus* > *Klebsiella*> *E.coli* > *S. aureus*> *Bacillus*. For *E.coli, Proteus, Pseudomonas, and Klebsiella,* the chloroform extract had not shown any activity.

Table 1: Antibacterial activity of *C.roseus* extract against pathogenic bacterial organisms

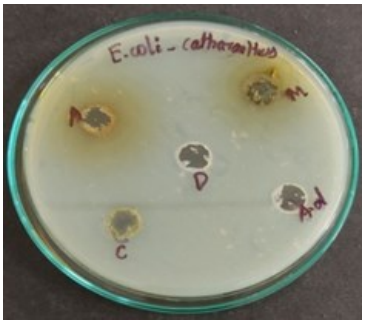
Organism	Antibiotic solution	*C. roseus*			DMSO solution
		Acetone	Methanol	Chloroform	
	Zone of Inhibition (in mm) (diameter)				

Pseudomonas	19	25	19	-	-
Bacillus	29	22	18	23	-
Proteus	-	24	20	-	-
S. aureus	33	20	-	18	-
E.coli	15	23	23	-	-
Klebsiella	-	23	30	-	-

*(-) no zone of inhibition



Pseudomonas



E.Coli

Bacillus

Klebsiella

Proteus

S.aureus

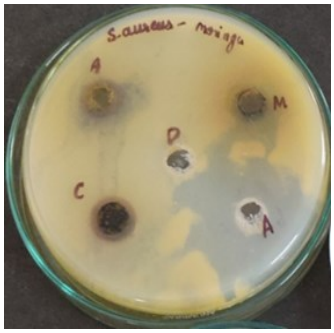
Fig 1: Antimicrobial activity of the *C.roseus* extract against bacterial culture by agar well diffusion method

The *M. oleifera* extract with the acetone, methanol and chloroform had shown activity for all the bacterial cultures The *M. oleifera* had shown maximum zone of inhibition against the test organisms in methanol extract in the following order: *E.coli* > *S. aureus* > *Proteus*> *Klebsiella* > *Pseudomonas* (Table 2). The chloroform extract does not show any antibacterial activity for *E.coli*, *Proteus*, *Pseudomonas* and *Klebsiella*. The methanolic extraction had not shown any activity for the *bacillus* and the acetone extraction had not shown any activity for the *Pseudomonas* and *E.coli*.

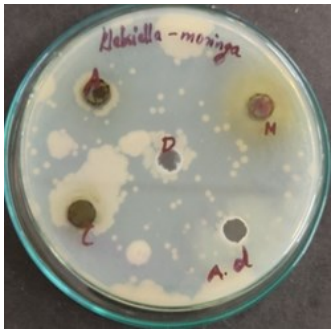
Table 2: Antibacterial activity of *M.olifera* extract against pathogenic bacterial organisms

Organism	**Antibiotic solution**	***M. olifera***			**DMSO solution**
		Acetone	**Methanol**	**Chloroform**	
	Zone of Inhibition (in mm) (diameter)				
Pseudomonas	-	-	20	-	-
Bacillus	-	23	-	14	-
Proteus	18	22	20	-	-
S. aureus	39	12	23	11	-
E.coli	16	-	25	-	-
Klebsiella	24	22	20	-	-

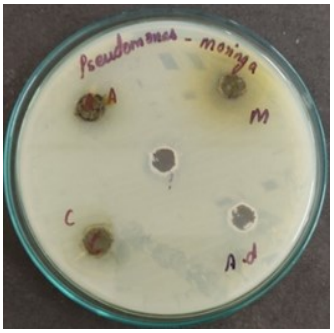
*(-) no zone of inhibition



S.aureus



Klebsiella



Pseudomonas

Proteus

Bacillus

E.coli

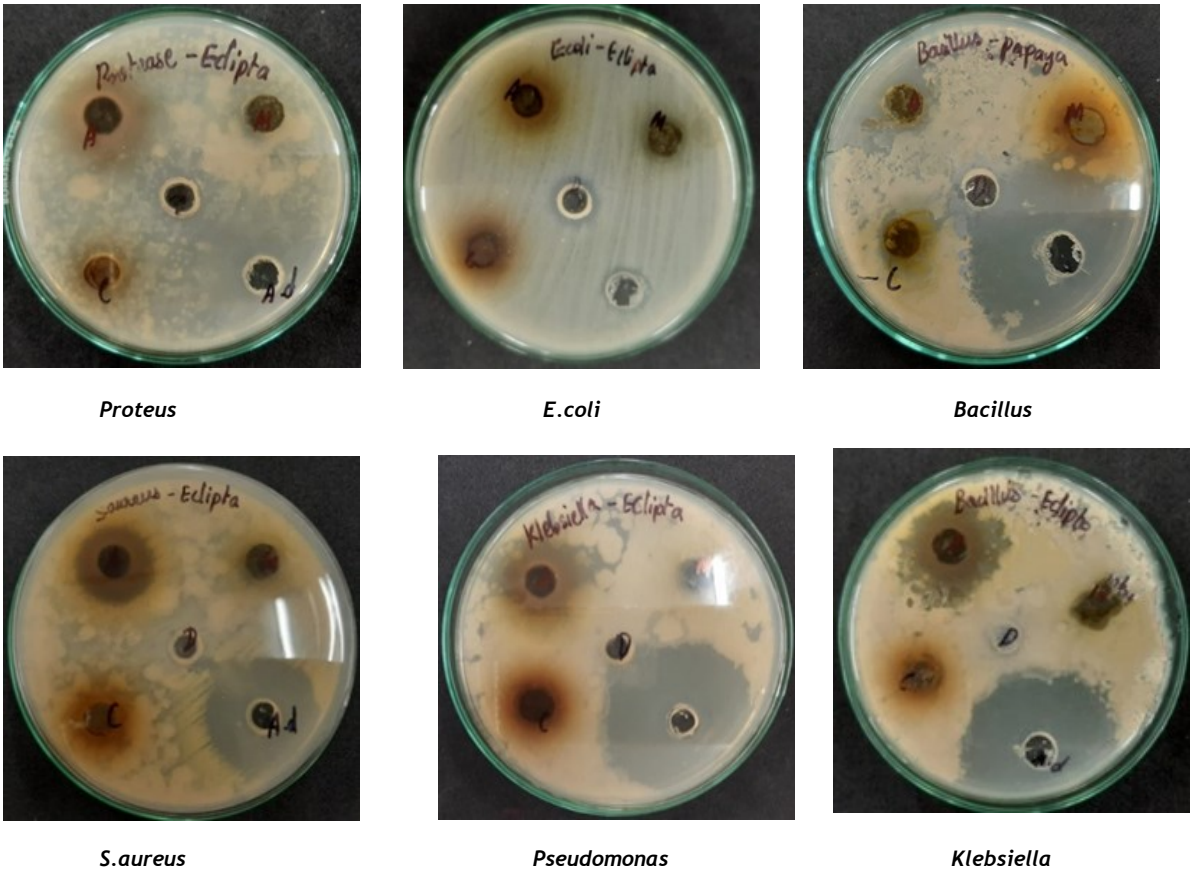
Fig 2: Antimicrobial activity of the of *M.olifera* extract against bacterial culture by agar well diffusion method

The *E.alba* extracts with the acetone, methanol and chloroform had shown activity for all the bacterial culture. The maximum zones of inhibition against the test organisms were observed for acetone extract in the following order: *Pseudomonas* > *Bacillus* > *S.aureus*> *proteus* > *Klebsiella* > *E.coli* (Table 3). The chloroform extract didn't show any activity for *E.coli*, *Proteus*, *Pseudomonas* and *Klebsiella*.

Table 3: Antibacterial activity of *E.alba* extract against pathogenic bacterial organisms

Organism	**Antibiotic solution**	***Eclipta alba***			**DMSO solution**
		Acetone	**Methanol**	**Chloroform**	
	Zone of Inhibition (in mm) (diameter)				
Pseudomonas	25	40	40	-	-
Bacillus	44	25	10	17	-
Proteus	37	15	-	10	-
S. aureus	34	21	19	15	-
E.coli	-	15	-	20	-
Klebsiella	25	20	-	15	-

*(-) no zone of inhibition



Proteus ***E.coli*** ***Bacillus***

S.aureus ***Pseudomonas*** ***Klebsiella***

Fig 3: Antimicrobial activity of the *E.alba* extract against bacterial culture by agar well diffusion method

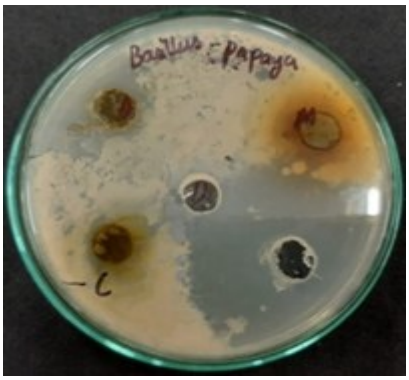
The *C.papaya* extracts with the acetone, methanol and chloroform had shown activity against the bacterial culture. The maximum zones of inhibition against the test organisms were observed in methanol extract in the following order (Table 4): *E.coli* > *Pseudomonas* > *Proteus* > *Klebsiella* > *Bacillus* (Table 5). *E.coli*, *Pseudomonas*, *Proteus* and *Klebsiella* did not show any activity against acetone extraction. Except for *Bacillus*, the chloroform extract did not show any activity for *Pseudomonas* and *S.aureus* had not shown any activity for all the extract.

The antibiotic solution namely chloramphenicol served as a positive control for all the test organisms and the zone of inhibition was observed and shown in table 1-4. The DMSO did not show any activity against the test organism.

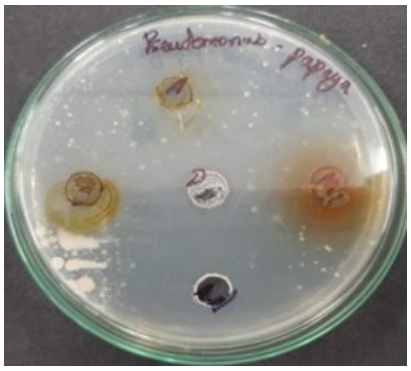
Table 4: Antibacterial activity of *C. papaya* against pathogenic bacterial culture

Organism	**Antibiotic solution**	***Carica papaya***			**DMSO solution**
		Acetone	**Methanol**	**Chloroform**	
	Zone of Inhibition (in mm) (diameter)				
Pseudomonas	-	-	20	-	-
Bacillus	44	25	10	17	-
Proteus	25	-	20	15	-
S. aureus	-	-	-	-	-
E.coli	-	-	28	15	-
Klebsiella	45	-	20	15	-

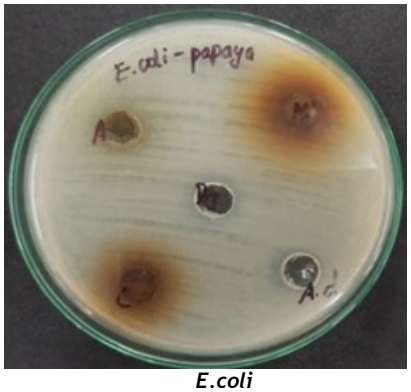
*(-) no zone of inhibition



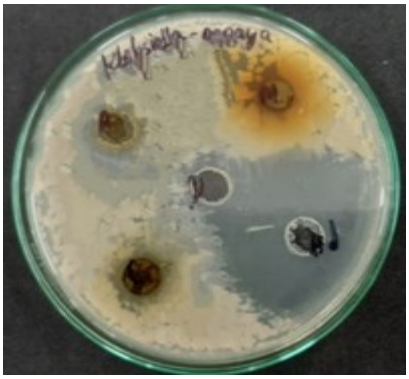
Bacillus



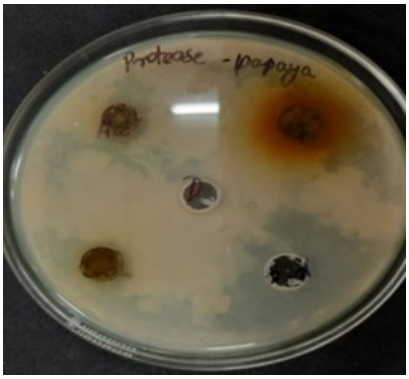
Pseudomonas



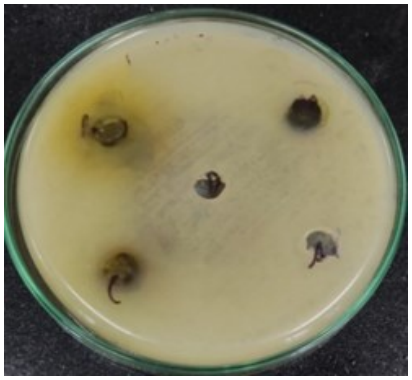
E.coli



Klebsiella



Proteus



S.aureus

Fig 4: Antimicrobial activity of the *C. papaya* extract against bacterial culture by agar well diffusion method

Minimum Inhibitory Concentration:
The plant extracts were assessed for their minimal inhibitory concentration ranges from 100µg/ml - 1000µg/ml. The concentration of the leaf extracts was increased the growth of the organisms also inhibited. The MIC technique was performed for the plant extracts and MIC value was successfully determined using the solvent extract of *C. roseus* against *S.aureus* and *E.coli* respectively. The results of MIC revealed that *E. coli* and *S. aureus* exhibited a MIC value at 1000 µg/ml and 60 µg/ml for the herbal extracts.

Table 5: Minimum inhibitory concentration of *C. roseus* extract

Organism	**Concentration of the plant extract (µg/ml)**									
	100	**200**	**300**	**400**	**500**	**600**	**700**	**800**	**900**	**1000**
S.aureus	11.2	21.5	35.7	48.9	56.3	67.7	71.5	78.2	84.0	91.1
E.coli	13.5	35.4	49.3	68.7	82.7	97.2	-	-	-	-

Phytochemical Screening:
The phytochemical screening test was evaluated for the four different plant extracts and the qualitative analysis of the sample from the plant extract had shown the presence of medically bio-active compounds. The phyto-constituents namely saponin, alkaloid and oil were present in all the four plants. Except *C.roseus* all other plant extracts had shown the absence of steroids. Terpenoids was found to be absent in the extract of *M.oleifera*

Table 6: Phyto-chemical screening of plant extract

Phytochemicals	**Plant Extract**			
	C. papaya	*E. alba*	*M. olifera*	*C. roseus*
Terpenoids	+	+	-	+
Steroids	-	-	-	+
Flavonoids	+	+	+	+
Saponin	+	+	+	+
Alkaloid	+	+	+	+
Oil	+	+	+	+

Thin Layer Chromatography:
TLC was carried out to analyse the different organic compounds and other phytochemical constituents. Different solvents play a significant role for the isolation of phytochemical compounds.

Fourier Transform Infrared Spectroscopy analysis of *C. roseus*:

FT-IR analysis revealed the presence of C–H stretch, C–C Stretch (in-ring), C–N stretch, and O-H bond which represents the functional groups such as alkanes, aliphatic amines, carboxylic acid and aromatic compounds (Table 7 and figure 5).

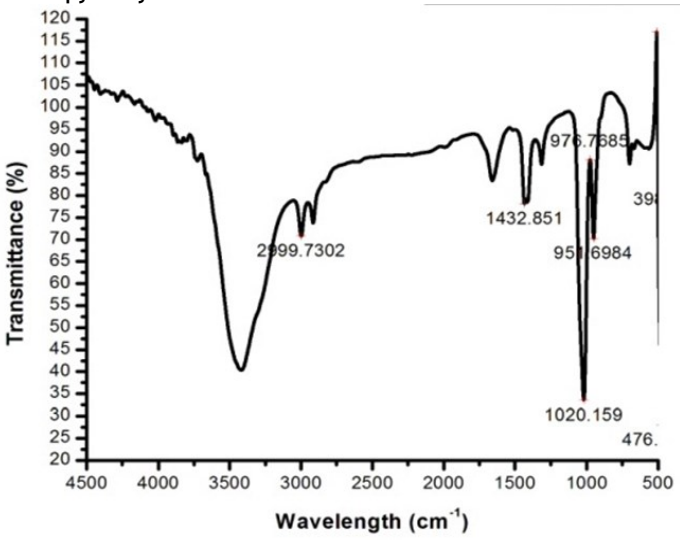


Fig 5: Chemical characterization of *C. roseus* extract

Table 7: FT-IR analysis of *C. roseus* extract

S.NO	Frequency (cm^{-1})	Range	Bond	Functional group
1.	2999	3000–2850	C–H stretch	Alkanes
2.	1432	1500–1400	C–C Stretch (in-ring)	aromatics
3.	1020	1250–1020	C–N stretch	aliphatic amines
4.	976	1000–650	=C–H bond	Alkenes
5.	951	950–910	O-H bond	carboxylic acids

DISCUSSION

Medicinal plants have been used as a system of traditional medicine for treating various diseases since prehistoric times (Jamshidi-Kia et al., 2017). They contain significantly unique properties for the presence of various bio-active novel compounds which are involved in the treatment of disorders including skin disease, rheumatism, diarrhea, hepatic disorder etc.

The obtained plant extract from different solvents had been examined for the antimicrobial activity against bacterial cultures. In order to describe the antimicrobial activity of the plant extract, various phyto-compounds have been identified from the herbal plant extract (Mihaylova et al., 2024). The efficacy of the isolated organic compound was assessed by anti-microbial activity and was subjected to the treatment of disease.

The plant extract was prepared using different polar and non-polar solvents namely methanol, chloroform and acetone. The plants namely *C.roseus, M.oleifera, C.papaya, E.alba* were subjected to the solvents extraction for the antimicrobial activity using agar well diffusion method. The antibacterial activity of the solvent extracts was identified by the size of the zone of inhibition and the plant extracts showed an efficient bactericidal activity but were not much effective against some of the test organisms such as *Pseudomonas, Protease* and *Klebsiella*. As per the standard chart, the zones of inhibition <12 are resistant, 13-17 are intermediate and >18 are sensitive to the plant extract (Rajendran et al., 2020).

The medicinal plant extract does not only alter the structures but also penetrate into the cell, leading to dehydration of proteins and enzymes. In addition, imbalance of the K^+ and H^+ ion concentration leads to death of the microorganism as reported (Farzaneh, and Carvalho, 2015).

The *E.alba* extract of both acetone and chloroform showed significant activity and for *C. papaya* the methanol and chloroform extraction had shown greater anti-bactericidal effectiveness. Both the *M. oleifera* and *C. roseus* had shown a significant zone of inhibition for the acetone and methanol extraction.

The MIC values were determined for the *C. roseus* extract against the test organism. The formation of turbidity was in the initial stage and the plant extract colour changed from green to yellow depending upon the part of the plant being used. The values are significantly less for acetone and methanol extraction of *C.roseus*. The results of MIC revealed the *E. coli* and *S. aureus* exhibited a minimum range of MIC value for the herbal extracts.

The phytochemical constituents which are present in the medicinal plants cause definite pharmacological actions to the human body and the purified compounds contain more effectiveness in respect to the inhibition of bacterial and fungal isolates (Castronovo et al., 2021). The solvents used for the phytochemical screening were methanol, acetone and

chloroform. The investigation showed that methanol extraction of *C.papaya* contains terpenoid, flavonoid, saponin, alkaloid and essential oil.

The major components of papaya leaves are the alkaloid and flavonoid. Alkaloids are generally originated from the amino acids and nitrogen is present in their structure. It is mainly present in the root, stem and seed part of the plants. The alkaloids namely carpine which are involved in the treatment of cardiovascular effects and the presence of flavonoid possess anti-bacterial, anti-fungal and anti-cancer activities. The chloroform extraction of *E. alba* contained the terpenoid, flavonoid, saponin, alkaloid and essential oil. Wedelolactone, eclalbasaponin, ursolic acid, luteolin, and apigenin present in *E.alba* are some of the substances that are responsible for the treatment of cancer, arthritis, and liver illnesses (Halder et al., 2021).

The acetone extraction of *M.oleifera* contains alkaloid, flavonoid, saponin and oil. Quercetin, kaempferol, apigenin, and myricetin are the most frequent compounds found in the genus of flavonoids. They are the naturally occurring substances in plants and they contain antioxidant properties (Pareek et al., 2023). The methanol extraction of *C.roseus* contains terpenoid, flavonoid, saponin, steroid and other essential oil. The leaves of *C. roseus* possess ursolic acid flavonoids such as apigenin, polyphenols, anthocyanins, luteolin, eugenol, thymol or sesquiterpene alcohols and showed the anti-inflammatory, anti-arthritic, anti-stress antibacterial and antipyretic activity.

Generally, in plants the distribution of phytochemical compounds varies depending upon the factors according to phylum/order/family and population within species. The phytochemicals present in the plants were useful in their antibacterial activity against the test organism and the extracted compounds contain more effectiveness in respect to the inhibition of bacterial and fungal isolates. The phytochemical constituents which are present in the medicinal plants cause definite pharmacological actions to the human body (Hussein and El-Anssary, 2019).

TLC was carried out to analyse the presence of organic compounds using the solvents namely acetone, methanol and chloroform for *E.alba*, *M.oleifera*, *C.papaya* and *C.roseus*. With the help of the standard chart the presence of the phyto-compounds was detected. FT-IR technique was helpful to determine the functional groups of the sample containing methanolic extract of *C.papaya*.

CONCLUSION

The study emphasizes the safety and efficacy of plant extracts against various diseases, particularly their anti-bacterial, anti-cancer, anti-inflammatory, and antioxidant properties. Key medicinal plants such as *Eclipta alba*, *Catharanthus roseus*, *Moringa oleifera*, and *Carica papaya* are examined for their bioactive compounds that inhibit cancer cell proliferation and promote overall health. The paper also discusses the impact of extraction solvents on the yield and potency of these compounds. Techniques like thin layer chromatography and FTIR are highlighted for their role in analyzing and purifying active ingredients. Ultimately, this review underscores the potential of secondary metabolites in medicinal plants as promising candidates for the development of novel drugs with minimal toxicity, paving the way for innovative therapeutic options in combating infectious diseases.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors for publishing this manuscript.

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