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EVALUATION OF IN-VITRO ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT OF GREWIA FLAVESCENS JUSS (EEGF) WHOLE PLANT

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ABSTRACT
The Evaluation of Invitro Antimicrobial activity of Ethanolic Extract of *Grewia Flavescens* (EEGF) whole plant belonging to family Tiliaceae was traditionally known to be a herbal remedy for the treatment of pain, wound healing, antimicrobial, fever, urinary stones, intestinal infections but it did not claim to be a commercial drug as it did not have any scientific proof. The Present research work deals with the Antimicrobial activity which has been scientifically proven and compared with the standard drugs Ceftriaxone and Fluconazole to study the antimicrobial activity. The ethanolic extract of *Grewia Flavescens* has showed significant Antimicrobial activity. Ethanolic extract of whole plant of *Grewia Flavescens* were screened for their antimicrobial activity. The extract is tested against Gram positive organism *Staphylococcus aureus* ATCC25923, and Gram negative organisms *Salmonella tophi* ATCC-14028,*Klebsiella pneumonia* ATCC-33495 and a fungus *candida albicans* ATCC-66027. The Agar well diffusion method is used to determine the Minimum Inhibitory Concentration(MIC). All the EEGF has Significantly shown antimicrobial activity against *Klebsiella pneumoniae* and *candida albicans as* they has shown MIC value 75µg/ml whereas the MIC value of *Staphylococcus aureus* is 25µg/ml. The MIC value of 25 µg/mL for EEGF against S. aureus indicates that it is more potent than the standard drug Ceftriaxone, which has a MIC value of 50 µg/mL. Similarly, Fluconazole shows activity only against fungus Candida albicans, with a MIC value of 25 µg/Ml.

KEYWORDS: Antimicrobial activity, *Grewia Flavescens*, Ceftriaxone and Fluconazole.

INTRODUCTION
Herbs are an aromatic element with good scents and tastemakers for a variety of meals. Herbs are not only used to prepare dishes, but they are also the source of medical benefits. Herbs are used to treat and prevent diseases such as heart disease, cancer, and diabetes. In medical research that herbs can be alternative to medicine, herbal remedies, herbal nutrition, alternative pain relief, is relevant and applicable to many countries, climates and cultures throughout the world.

The major use of herbal medicines is for health promotion and therapy for chronic, as opposed to life-threatening, conditions. However, usage of traditional remedies increases when conventional medicine is ineffective in the treatment of disease, such as in advanced cancer and in the face of new infectious diseases. Furthermore, traditional medicines are widely perceived as natural and safe, that is, not toxic.

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Antimicrobial drugs interfere with the life cycle of an organism in various ways. To alter the life cycle, all antimicrobials must bind to a cellular target. Binding of the drug to its target results in alteration of the normal function of the bacterium or fungus, leading to either inhibition of growth or cell death.[1,2,3,4,5]

PLANT UNDER STUDY: *Grewia flavescens juss.*
*Grewia flavescens juss.*it is a slender climbing herb or shrub belonging to family tiliaceae in telugu language the plant is named jana, Banka jana.

Grewia flavescens juss belong to family tiliaceae, it is a slender shrub which grows along the hill sides, plains along the dry rivers.

Grewia species are valued in many cultures for their medicinal virtues. The main medicinal action appears to come from the mucilage that is found in the leaves, stems and roots, which has been shown to have soothing and healing properties. Taken internally it is often used as a remedy for diarrhoea and dysentery, for example, whilst externally it is applied to wounds, cuts, ulcers, irritations etc. The plant can be taken as a simple infusion or decoction, or it can be applied topically as a poultice of the plant, or the mucilage can be extracted from the plant, if required, by maceration and then decoction. The roots are used to treat menstrual problems, stomach problems during pregnancy and other disorders in women. The plant (part not specified) has a range of medicinal uses, including treating iImpotency, sterility and wounds.[6]

Fig. 1: *Grewia flavescens* juss whole plant.

- The traditional uses are increasingly supported by recent scientific research wherein some species of this genus have now been confirmed to possess anticancer, anti-inflammatory, antinociceptive, antioxidant, hepato protective, antidiabetic, antimicrobial, antimalarial, and sedative–hypnotic properties.
- The *Grewia flavescens* roots are useful in application of wound healing to hasten or fasten separation in the pain.
- It is very useful in heart disease, leukaemia.
- Seeds are used in chronic dysentery.
- Traditionally it is used in wound healing.[7,20]

Medicinal properties of Grewia flavescens were studied. They were anti-diabetic, anti-ulcer, anti-inflammatory, anti-urolithiasis, anti helmenthic, and larvicidal activity, and anti-oxidant properties.[8,9,10,11,,13,14,15]

Grewia flavescens is traditionally used for antimicrobial activity. *Grewia flavescens* ethanolic extract has been reported to contain 12 components which have been identified as Phenol, Acetic acid, Phytol and Di-ethyl phthalate were reported in previous studies which may be possessing anti-microbial property. As per the traditional claim and proven antioxidant activity the *Grewia flavescens whole* plant is evaluated for the antimicrobial activity. Hence the present research work was carried out on the whole plant of *Grewia flavescens juss* so, we tried to explore and expedite our research work on antimicrobial activity on the *Grewia flavescens juss* etanolic extract invitro.

GC-MS ANALYSIS OF *GREWIA FLAVESCENS JUSS* WHOLE PLANT

According to GC-MS Analysis, the 12 components were identified in the ethanolic extract of *Grewia flavescens juss* whole plant by GC-MS. The components are as follows:

S.No	Name	Molecular formula	Molecular weight	RT in minute
1.	Ethanamine, N,N-dimethyl	$C_4H_{11}N$	73.139g/mol	2.652
2.	**Acetic acid**	CH_3COOH	60.05g/mol	2.889
3.	Ethane, 1,1-diethoxy	$C_6H_{14}O_2$	118.176g/mol	4.097
4.	Pyridine	C_5H_5N	79.102g/mol	4.327
5.	**Diethyl phthalate**	$C_{12}H_{14}O_5$	222.24g/mol	17.156
6.	Ethyl. alpha-d-glucopyranoside	$C_8H_{16}O$	208.209g/mol	17.642
7.	**4-((1e)-3-hydroxy-1-propenyl)-2-methoxyphenol**	$C_{10}H_{12}O_3$	180.200g/mol	18.824
8.	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256.43g/mol	20.972
9.	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.48g/mol	21.301
10.	Desaspidinol	$C_{11}H_{14}O_4$	414.7067g/mol	21.419
11.	**Phytol**	$C_{20}H_{40}O$	296.539g/mol	22.476
12.	Octadecanoic acid	$C_{18}H_{36}O_2$	284.477g/mol	22.890

Out of 12 components which may be found by GC-MS only four components may be responsible for the antimicrobial activity whose antimicrobial articles were already reported. The four components are listed below:[12]

- Acetic acid

- Methoxy Phenol
- Phytol
- Diethyl pthalate

Acetic acid

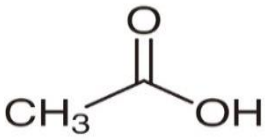


Fig. 2: Structure of Acetic acid.

The antimicrobial activity of acetic acid was investigated against bacterial that resistance to different types of antibiotics (*Streptococcus spp., Staphylococcus aureus, E. coli, Pseudomonas aeruginosa and Proteus spp.*) using the well diffusion method in at different concentration (0.5%, 1%, 1.5%, 2% and 2.5%). The mean of three replicates of the diameter of inhibition zones (in millimeters) around each well with an acetic acid solution. It was found that at all concentrations. Acetic acid was able to inhibit bacterial growth at concentrations (0.5%) to the range from (13 mm to 18 mm). The isolates studied showed sensitivity to the range (16 mm to 18 mm) at concentrations (1%), the concentrations (1.5%) to the range (20 mm to 22 mm). However, the concentrations (2%) to the range (22 mm to 27 mm) and (27 mm to 35 mm) at concentrations (2.5%). These results were agrees with Abdullah and Al-shwaikh who reported that the minimum inhibition zone of acetic acid at concentrations (1%) range between (10 mm to 15 mm), the concentration at (2%) the inhibition zone from 14 mm to 20 mm) respectively against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus spp*. Also the inhibition growth of acetic acid ranged between 0.5 and 1.0% (v/v) against (*Staphylococcus aureus* isolates I, III, *E. coli* II) was found to be the most sensitive. According to Carpenter et al Acetic acid displays residual activity to prevent the growth of pathogens. Acetic acid at the level of 0.1-0.5% in commercial significantly reduced numbers of S. aureus by 1.2-2.3 log10 CFU/ml at 10°C. Raftari et al.reported that effects of organic acid against *Staphylococcus aureus* more pronounced than *E. coli* at concentrations 1%, 1.5% and 2%. Also found bacterial growth of *Clostridium spp.* decreased more than *E. coli* in same treatment. The gram positive bacteria high sensitive to different types of antibacterial than gram negative bacteria because possess an outer membrane. The bacterial growth (*Streptococcus agalactiae*) affected by acetic acid at high concentration. Also, this bacteria sensitive to weak acids, certain lactobacilli and bacteria are can to increasing growth in low pH.[15]

METHOXY PHENOL

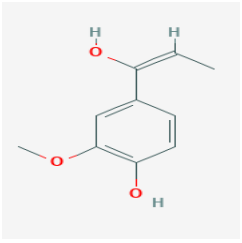


Fig. 3: Structure of Methoxy pheno.

The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins.Phenolic compounds possessing a C_3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and often cited as antimicrobial as well. Eugenol is a well-characterized representative found in clove oil. Eugenol is considered bacteriostatic against both fungi and bacteria.

Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two −OH groups, and pyrogallol has three. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity.[16]

PHYTOL

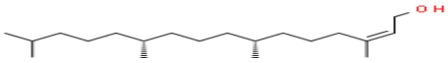


Fig. 4: Structure of phytol.

Phytol is an important member of the branched chain unsaturated terpene, and is a product of chlorophyll metabolism in the plant .The mechanism of its antimicrobial activity is not fully described. It is suggested that protein and enzyme inactivation is one of the important mechanisms for inactivation of microbes. Antimicrobial activity of phytol, its toxicity on mouse skin cells,and its stability on different surfaces were investigated and found that phytol is an approximately good antimicrobial agent. Moreover, it had no remarkable toxicity and high stability.

The MIC50 of phytol against E.coli, c.albicans and A.niger was 62.5µg/ml, and against S.aureus was>1000µg/ml.it means that S.aureus is resistant to phytol. The MIC50 of phytol against all strains was more than 1000µg/ml,i.e.none of the concentration could inhibit 90% of microbial strains.

The results revealed the toxicity of phytol was dose dependent, i.e. the minimum concentration(3.12µg/ml) caused maximum cell viability and vice versa Another finding showed that the toxicity of phytol was time

dependent. Thus, the decrease of incubation time led to an increase of cell viability.[17]

Diethyl phthalate

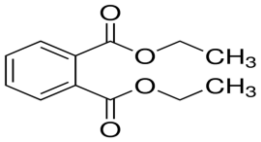


Fig. 5: Structure of Diethyl phthalate.

Diethyl phthalate could be used as a potent antimicrobial agent. Further exploration of the function of compound will facilitate a better understanding toward developing diethyl phthalate as an antimicrobial agent.[18]

AIM AND OBJECTIVE

AIM

The aim of the present study is to evaluate the antimicrobial activity of *Grewia flavescens juss* whole plant invitro.

OBJECTIVE

The objective of this study is to explore and expedite the Anti-microbial activity of *Grewia flavescens juss* whole plant ethanolic extract. As from the traditional knowledge the *Grewia flavescens* have been used extensively in traditional medicine as antimicrobial agent. However its antimicrobial potential is not yet explored scientifically.

So, The Invitro anti-microbial activity of EEGF was explored and assessed.

METHODOLOGY

Plant Collection and Identification

The crude *Grewia flavescens juss* whole plant was collected from Sri Venkateshwara University, Tirupati, Andhra Pradesh, India in the month of march 2023. The plant was authenticated by plant taxonomist Dr. K. Madhava Chetty, Assisstant Professor, Dept of Botany, Sri Venkateshwara University, Tirupati, AP, India.

The *Grewia flavescens juss* whole plant were cut into proper size and washed 3 times with drinking water then dried in shade with proper care. The dried plant material were blended into course powder and passed through sieve 60.

PREPARATION OF EXTRACT

The coarse powder 500gm of Grewia flavescens juss whole plant was subjected to maceration and transferred to stoppered flask and treated with pure ethanol 80% until the powder is fully immersed at room temperature. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours from time to time to ensure better extraction. This process is repeated for 7 days, followed by exhaustive maceration for 5 days by using solvent methanol. The solvent was decanted and filtered with filter paper and recovered with the help of rotary vacuum evaporator. The extract EEGF was dried under desicator and stored in an air tight container. The final extract was then subjected to investigate the antimicrobial activity.

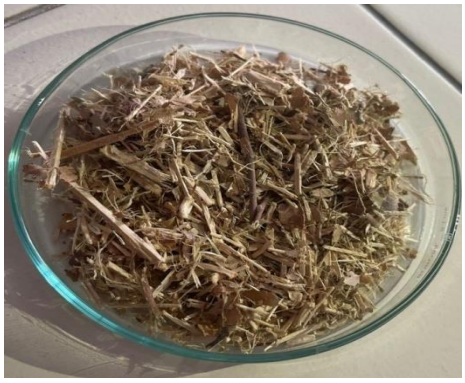


Fig 6- Dried plant of Grewia

fig 7-coarse powder of Grewia

Fig 8-Maceration process

Fig-9 Extraction of drug

Fig. 10: Ethanolic extract of *Grewia flavescens.*

ANTIMICROBIAL ACTIVITY

Bacterial and fungal strains and media

The pathogens panel consisted of *Staphylococcus aureus* (ATCC 25923), *Salmonella Typhi* (ATCC-14028), *Klebsiella pneumoniae* (ATCC-33495), *candida albicans* (ATCC-66027) are MRSA strains. These strains were procured from BEI/NARSA/ATCC (Biodefense and Emerging Infections Research Resources Repository/Network on Antimicrobial Resistance in *Staphylococcus aureus*/American Type Culture Collection, USA) and routinely cultivated on Nutrient Agar (Ref-63971) purchased from SRLChem. Prior to the experiment, a single colony was picked from plate, inoculated in Nutrient Agar supplemented broth and incubated overnight at 37 °C with shaking for 18–24 h to get the starter culture.

Procedure

Antibiotic susceptibility testing was performed on the newly synthesized compounds by calculating the Minimum Inhibitory Concentration (MIC) according to standard CLSI guidelines. MIC is defined as the minimum concentration of compound at which visible bacterial growth is inhibited. Bacterial cultures were grown in Nutrient Agar. Optical density (OD600) of the cultures was measured, followed by dilution for ~10^6 CFU/mL. This inoculum was added into a series of test wells in a microtitre plate that contained various concentrations of compound under test ranging from 75-25μg/mL. Controls i.e., cells alone and media alone (without compound+cells) and Ceftriaxone used as a reference standard in the experiment. Fluconazole used as standard for antifungal strain in the experiment and plates were incubated at 37° C for 16-18 h followed by observations of MIC values by the absence or presence of visible growth. For each compound, MIC determinations were carried independently three times using duplicate samples each time.

Bacterial Strain : *S. aureus,* ATCC-25923

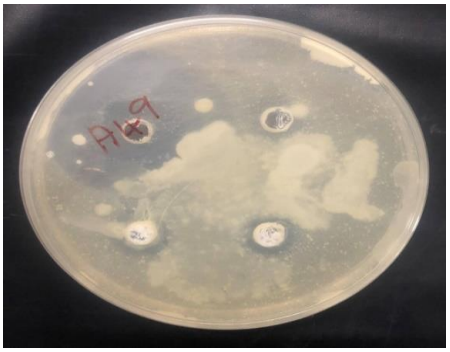


Fig 11 - 18 hrs

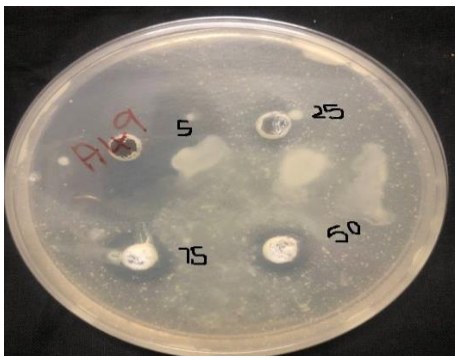


Fig 12- 18 hrs

Fungal Strain: candida albicans ATCC-66027

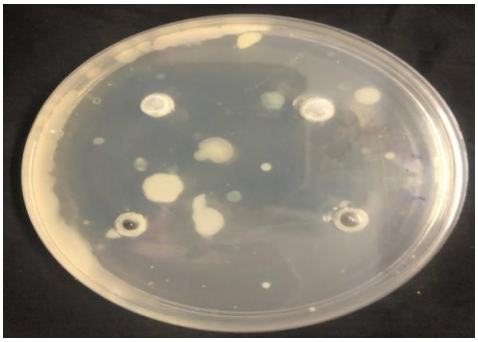


Fig. 13: Fungal strain of candida albicans.

Bacterial Strain : *Salmonella Typhi,* ATCC-14028

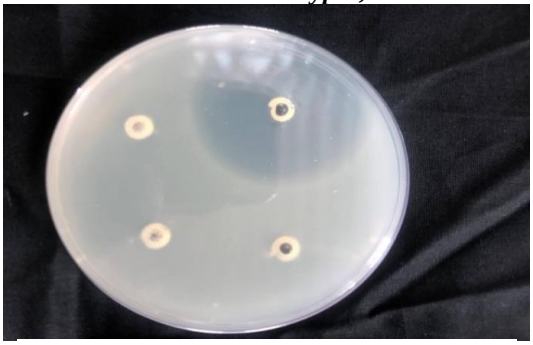


Fig. 14: Bacterial strain of salmonella typhi.

Bacterial Strain : *K. pneumoniae* ATCC-33495

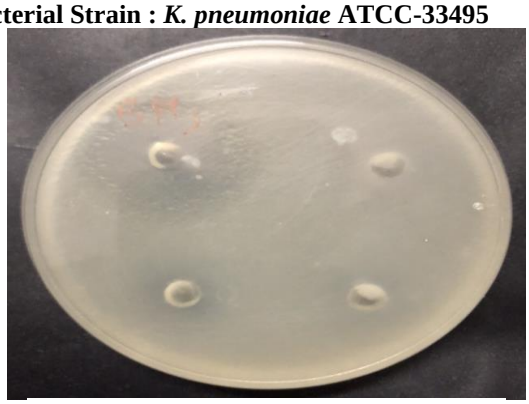


Fig. 15: Bacterial strain of K.pneumoniae.

Agar well diffusion method: The Muller-Hinton agar plates were prepared for the antibacterial activity. About 0.1 mL of the fresh 18 h old broth culture was spread on the respective media. After spreading the culture, wells of 6 mm in diameter was made at the center of the plate by using sterile cork borer. The wells were open with the help of sterile forceps. Then 100 µL of original stock solution was added by using micropipette in each well. The final concentration in the well was 1 mg/mL.The extract was allowed to diffuse; hence the prepared plates were left at room temperature for 30 min and then stored in incubator at 37ºC for 24 h.[20]

RESULTS AND DISCUSSION

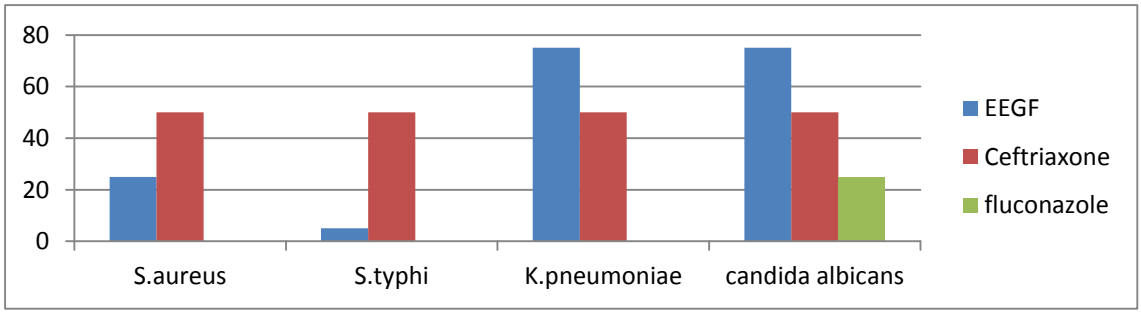
Results of Antimicrobial activity

Table 1 presents the minimum inhibitory concentration (MIC) values in micrograms per milliliter (µg/mL) of the tested compounds against a panel of bacterial and fungal strains, including S. aureus, Salmonella Typhi, K. pneumoniae, and Candida albicans. MIC values are the lowest concentration of a compound that inhibits the visible growth of a microorganism. The tested compounds include EEGF, Ceftriaxone, and Fluconazole.

Comparing the MIC values of the tested compounds with standard values is important to assess their efficacy against the target microorganisms. In general, the lower the MIC value, the more effective the compound is in inhibiting the growth of the microorganism. For example, a MIC value of 25 µg/mL for EEGF against S. aureus indicates that it is more potent than Ceftriaxone, which has a MIC value of 50 µg/mL. Similarly, Fluconazole shows activity only against Candida albicans, with a MIC value of 25 µg/mL.[19]

Table 1: MIC values (*µ*g/mL) of the tested compounds against panel of bacterial and fungal.

S.No	Compound	S. aureus ATCC 25923	Salmonella Typhi ATCC-14028	K. pneumoniae ATCC-33495	candida albicans ATCC-66027
1	**EEGF**	25	5	75	75
2	Ceftriaxone	50	50	50	50
3	fluconazole	-	-	-	25



Graphical representation of MIC values of invitro antimicrobial activity of EEGF.

DISCUSSION

Grewia flavescens juss belongs to family Tiliaceae. Traditionally the plant is known to be a herbal remedy for treating pain, wound healing, fever, dysentery, urinary stones, intestinal infections, and as a antimicrobial but did not claim to be a commercial drug as it did not have any scientific proof. In this dissertation work the evaluation of antimicrobial activity has been done.

Grewia flavescens juss is commonly called as sandpaper raisin, in Hindi it is called as khatkhati and in telugu it is called as Jana or Bankajana. It belongs to family Tiliaceae. It is an erect and woody herb found at the edges of the forest and in hilly sides commonly in tropical regions.

The collected dry plant of *Grewia flavescens juss* was authenticated by botanist Dr. K. Madhav Chetty and later subjected for extraction through cold maceration under standard laboratory conditions by using 80% ethyl alcohol and percentage yield of extract is 14.2%.

By phytochemical investigation the plant *Grewia flavescens* contain alkaloids, flavonoids, steroids, carbohydrates, tannins, saponins.

In phytochemical screening (GC-MS analysis) out of 12 chemical constituents four chemical constituents like phenol, acetic acid, phytol and diethyl pthalate may be responsible for antimicrobial activity.

The tested compounds include EEGF, Ceftriaxone, and Fluconazole. In general, the lower the MIC value, the more effective the compound is in inhibiting the growth of the microorganism. For example, a MIC value of 25 µg/mL for EEGF against S. aureus indicates that it is more potent than Ceftriaxone, which has a MIC value of 50 µg/mL. Similarly, Fluconazole shows activity only against Candida albicans, with a MIC value of 25 µg/mL.

CONCLUSION

Grewia flavescens is widely used herb in folk and Ayurvedic systems of medicines for various properties.

By phytochemical investigation the plant *Grewia flavescens* contains Phenol, Diethyl pthalate, Acetic acid, Phytol are responsible for Antimicrobial activity.

Overall, these results suggest that EEGF has potential as an anti-bacterial agent, particularly against S. aureus and K. pneumoniae, and further studies are warranted to evaluate its safety and efficacy. Ceftriaxone can be used as a standard for comparison due to its established efficacy against various bacterial infections. In summary the study provides important insights into the potential use of these compounds as anti-infective agents. Further studies are needed to evaluate their safety and efficacy in vivo and to determine their mechanism of action.

Hence, we confirm that the plant under study *Grewia flavescens juss* whole plant is a herb having alternate source of herbal antimicrobial drug and also further studies are needed to isolate a new active lead compound for suitable antimicrobial drug.

The above findings justified the traditional claim for antimicrobial activity of *Grewia flavescens juss* possess a good antimicrobial activity which is proven scientifically in a well systemic manner.

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