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Antibacterial Efficacy of Medicinal Plant Extracts and Silver Nanoparticles against *Pseudomonas fluorescens* and *Proteus mirabilis*

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Abstract: This study evaluated the antibacterial properties of acetone extracts from medicinal plants-- *Murraya koenigii, Moringa oleifera, Curcuma aromatica,* and *Piper betle*—against pathogenic bacteria *Pseudomonas fluorescens*-103 and *Proteus mirabilis*-1429T. Antibiotic sensitivity tests showed significant lytic activity for both strains against Streptomycin and Azithromycin, with *P. fluorescens*-103 exhibiting high resistance to Co-trimoxazole. The acetone extract of *M. koenigii* effectively inhibited both bacteria, with zones of 28 mm. Silver nanoparticle extracts from *C. aromatica* and *P. betle* also showed activity against *P. fluorescens*-103 and *P. mirabilis*-1429T, respectively. UV-Vis analysis confirmed the presence of silver nanoparticles, while FTIR spectroscopy revealed distinct absorbance bands for all plants. These findings suggest that silver nanoparticles may be a promising, non-toxic alternative for managing pathogenic diseases.

Keywords: Silver nanoparticles, Antibacterial activity, Human pathogen, FTIR Spectroscopy, UV-Vis analysis, *Pseudomonas fluorescens*-103, *Proteus mirabilis*-1429T

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Introduction

The utilization of synthesized silver nanoparticles (AgNPs) is experiencing a significant surge globally across various sectors, including medicine, electronics, the food industry, and agriculture, owing to their distinctive properties. These nanoparticles exhibit a range of valuable characteristics, such as catalytic, optical, electronic, antimicrobial, antiviral, antiplasmodial, insecticidal, magnetic, and mosquito larvicidal effects (Santhosh *et al.*, 2015). With a high surface volume fraction, metal nanoparticles, combined with eco-friendly and economically viable plant extracts, present a promising solution. Among the various nanomaterials, biosynthesized silver

nanoparticles are particularly favored for their strong antimicrobial properties. The leaf extract of *Murraya koenigii* has been employed for the synthesis of AgNPs and tested against three strains of human pathogenic bacteria (Bonde *et al.*, 2012).

Silver nanoparticles have garnered significant attention in recent years due to their exceptional bactericidal properties, which have been shown to be effective against a wide range of micro-organisms, including bacteria, fungi, and viruses (Siddiqi and Husen, 2016). This remarkable efficacy positions silver nanoparticles as one of the most promising alternatives to traditional antibiotics, particularly in an era where antibiotic resistance is becoming a critical global health concern. Their small size, typically in the nanometer range, allows for a high surface area-to-volume ratio, which enhances their reactivity and interaction with microbial cells (Sarah *et al.*, 2012). For instance, silver nanoparticles are being investigated for their roles in electronics, where they can be used in conductive inks and components; in catalysis, where they can serve as effective catalysts for chemical reactions; in pharmaceuticals, where they can enhance drug delivery systems; and in microbial control, where they can be employed in coatings and disinfectants to inhibit microbial growth (Zaheer and Rafiuddin, 2012).

Recent studies have highlighted the potential of using plant extracts to synthesize silver nanoparticles with specific shapes and sizes. For example, extracts from plants such as *Cucurbita maxima* (pumpkin), *Moringa oleifera* (drumstick tree), and *Acorus calamus* (sweet flag) have been utilized to produce cuboidal silver nanoparticles ranging from 30 to 70 nanometers in size. These nanoparticles have demonstrated potent anticancer activity, particularly against epidermoid A431 carcinoma cells, indicating their potential as a novel therapeutic approach in cancer treatment (Nayak *et al.*, 2015; Kumkoon *et al.*, 2023).

Pathogenic bacteria significantly contribute to human morbidity and mortality, and the rise of resistance to new antibacterial agents is a global concern. Malaria, caused by the *Plasmodium* protozoan, is a critical infectious disease, with resistance to nearly all antimalarial drugs posing major control challenges (WHO, 2011). However, recent developments in plant-derived antimalarial drugs show promise, as many effective treatments originate from medicinal plants (Mishra *et al.,* 2009). In Malaysia, *Piper betle* leaves are traditionally used as an antimalarial remedy in rural communities (Al-Adhroey *et al.*, 2011).

Drug resistance in various bacterial species poses a significant challenge, as they possess genetic mechanisms to acquire and transmit resistance, making them ineffective as treatments. This issue affects individuals of all ages and is particularly concerning for hospitalized patients with weakened immune systems and the rise of multi-resistant strains (Gyles, 2011). Bacterial infections impact both human health and aquatic life, with increased antibiotic use linked to severe infections and the development of resistance (Kandhasamy and Arunachalam, 2008; Said *et al.,* 2024). The declining effectiveness of antibiotics highlights the urgent need for new therapeutic alternatives, with around 2,500 novel metabolites identified from marine organisms, including seaweeds (Arul Senthil *et al.*, 2008).

Herbal medicines in Ayurveda remain a primary health care source for many globally (Gratus *et al.*, 2009), as recognized by the World Health Organization, particularly in countries like India. Asian herbal remedies interact with both humans and their environment (Sasidharan, 2011; Ohiduzzaman *et al.*, 2024). Despite the introduction of new antibiotics, microbial resistance has increased significantly (D'Costa *et al.*, 2011; Menichetti *et al.*, 2023). This study evaluates the antibacterial efficacy of acetone leaf extracts from medicinal plants and silver nanoparticles against *Pseudomonas fluorescens*-103 and *Proteus mirabilis*-1429^{T} using the agar

well diffusion method to determine the minimum inhibitory concentration.

Materials and Methods

Collection and processing of plants material

Fresh leaves and tubers of *Murraya koenigii*, *Moringa oleifera*, *Curcuma aromatica*, and *Piper betle* were collected from the Botanical Garden at Vivekananda College in Tiruvedakam West, Madurai, Tamil Nadu. They were rinsed with tap water, shade-dried for a week, and then ground into a fine powder using mechanical shearing and sieving. The powders were stored in airtight containers at room temperature (28±2°C) until needed.

Preparation of acetone leaves extract

A fine powder from each leaf type was extracted using a Soxhlet apparatus with 25 g of powder and 300 ml of acetone (boiling point 55.50-56.50°C) (Satyavani *et al*., 2011). The extracts were evaporated in petri dishes, stored in airtight containers in a cool place, and later combined with acetone for further analysis (Okogun, 2000).

Synthesis and purification of silver nanoparticles

10 ml of each extract were mixed with 90 ml of a 1 mM silver nitrate solution in a 250 ml conical flask at 37°C, observing a color change from colorless to dark brown. After the color change, the solution was centrifuged at 5,000 rpm for 20 min, repeated three times with deionized water to remove plant metabolites. The deionized pellet was then filtered through a 0.45 µm millipore filter, and the products were lyophilized for later characterization..

Characterization of synthesized silver nanoparticles

(i) *UV-Vis spectroscopy analysis*

The aliquots reaction mixtures were subjected to the measurement of absorbance by UV-Vis spectroscopy at the wavelength of 200-800 nm in ELICO spectrophotometer at a resolution of 1 nm for detection of silver nanoparticles.

(ii) *Fourier Transform Infrared Radiation spectroscopy analysis*

The residual silver nitrate solution was centrifuged at 10,000 rpm for 10 min and the pellet was obtained and washed with 1 ml of deionized water twice to remove free proteins and other compounds. The purified suspension was freeze dried to obtain dried powder. Lastly, the dried silver nanoparticles were analyzed by FTIR spectroscopy in the range of 500-4000 cm^{-1} at a resolution of 4 cm^{-1} (Alagappa University, Karikudi).

Collection of microbial type strains

Human pathogenic Gram negative bacterial type strains, *Pseudomonas fluorescens*-103 and *Proteus mirabilis*-1429^{T} were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The purchased strains were cultured and maintained at 37°C and stored at -20°C.

Antibiotic sensitivity assay for type strains

The antibacterial potency of synthesized silver nanoparticles solution was tested against the *P. fluorescens* and *P. mirabilis* by the zone of inhibition (ZoI) and minimum inhibitory concentration (MIC). Find out the most effective inhibitory antibiotic among the selected common antibiotics such as Chloramphenical (CHL), Ampicillin (AMP), Tetracycline (TET), Gentamycin (GEN), Kanamycin (KAN), Co-Trimoxazole (CTZ), Amikacin (AMI) and Streptomycin (STR) using standard antibiotic discs (HiMedia Laboratories Pvt. Ltd. India) (Seidler *et al*., 1969).

Antibacterial susceptibility test with acetone leaves extract

The antibacterial properties of plant leaf extracts using acetone were evaluated via the well diffusion method (Bauer *et al*., 1966). Nutrient agar plates were inoculated with a 12 h culture of pathogenic bacteria, and 10 mm wells were created using a cork borer. Each well received 100 µl of acetone leaf extract, and the plates were incubated at 37°C for 24 h. After incubation, the zones of inhibition around the wells were measured in millimeters to assess the extracts' antibacterial effects.

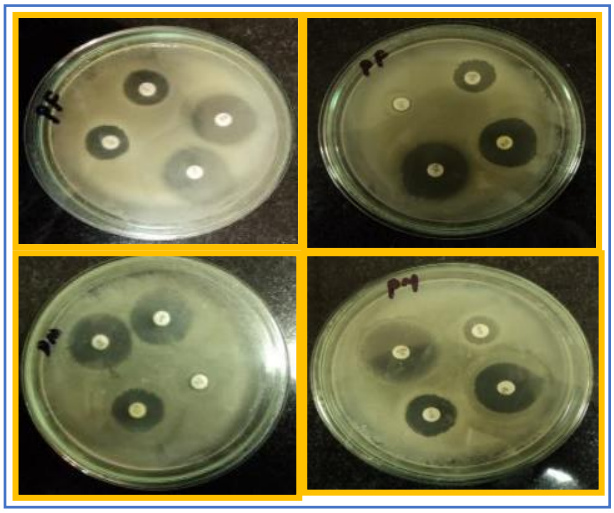

Fig. 1: Antibiotic sensitivity assay against *P. fluorescens* (a-b) and *P. mirabilis* (c-d).

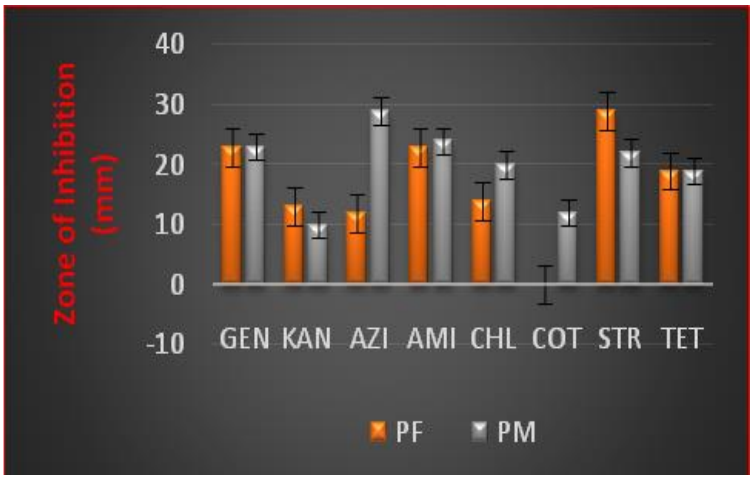

Fig. 2: Antibiotic sensitivity assay comparison with chemical antibiotics and bacterial strains.

Antibacterial susceptibility test with silver nanoparticles leaves extract

The antibacterial properties of plant leaf extracts with silver nanoparticles were studied using the well diffusion method (Satyavani *et al.*, 2011). Nutrient agar plates were inoculated with a 12 h culture of pathogenic bacteria, and 10 mm wells were created using a cork borer. Each well received 100 µl of the silver nanoparticle-synthesized leaf extracts. The plates were incubated at 37°C for 24 h, after which the zones of inhibition around the wells were measured in millimeters to assess the extracts' inhibitory effects.

Results and Discussion

Antibiotic sensitivity assay with chemical antibiotics

Bacterial growth (Optical density value) curve was measured in triplicate at 480 nm using *Pseudomonas fluorescens*-103 and *Proteus mirabilis*-1429^T. Antibiotic sensitivity assay results for the pathogenic type strains such as *P. fluorescens*-103 and *P. mirabilis*-1429^T are shown in Figures 1 and 2. Adzitey *et al.* (2015) reported that 56 water samples of *Escherichia coli* bacteria were isolated and screened against nine different antibiotics. Overall, 37.90% of the *Escherichia coli* isolates were resistant, 12.90% were intermediate and 49.21% were susceptible. Resistance to

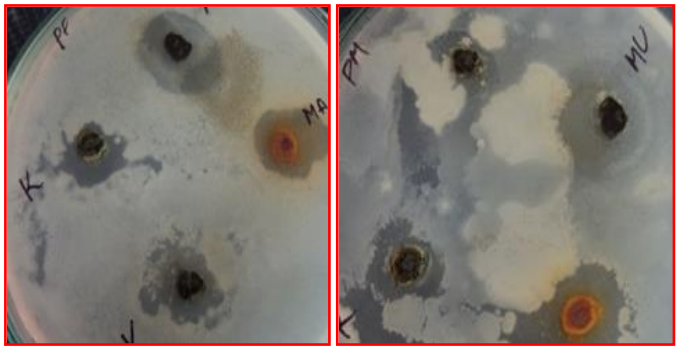

Fig. 3: Antibacterial activity with acetone leaf extract against *P. fluorescens* and *P. mirabilis.*

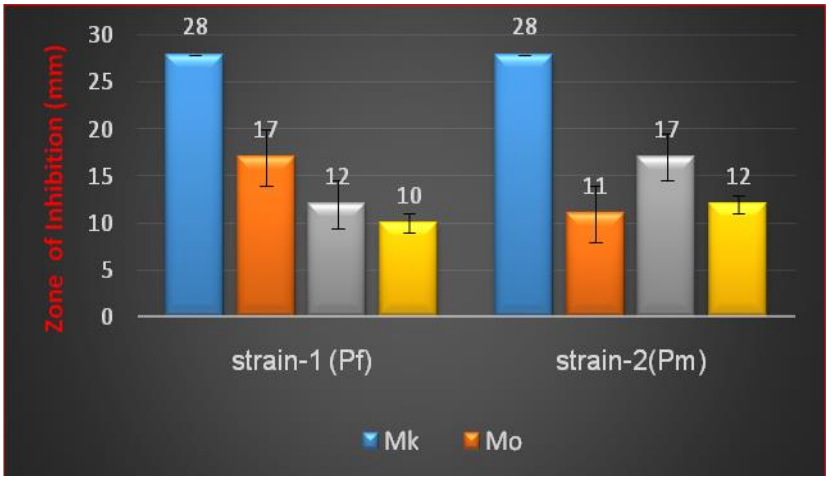

Fig. 4: Antibacterial activity comparison with acetone leaf extracts and bacterial strains

vancomycin (94.64%) and erythromycin (85.71%) was high. Susceptibility to ciprofloxacin (94.64%), gentamicin (91.07%) and ceftrioxine (89.29%) was also high.

Antibacterial susceptibility assay with acetone leaves extract

The antibacterial susceptibility test of leaves extracts of *M. koenigii*, *M. oleifera*, *C. aromatica* and *P. betle* were performed against human pathogenic type strain *P. fluorescens*-103 and *P. mirabilis*-1429^T. The acetone leaf extract (100 µl) of *M. koenigii* showed significant activity against human pathogenic strain of *P. fluorescens*-103 (28 ±0.1 mm) and *P. mirabilis*-1429^T (28 ±0.2 mm). Sathiyavathi *et al.* (2011) reported that antimicrobial activity of *Syzygium cumini* of ethanolic (18 mm, 15 mm) and methanolic (16 mm, 13 mm) leaf extract showed better activity against *S. aureus* and *C. albicans* rather than chloroform and petroleum ether.

Moderate activity such as *M. oleifera* (17±0.2 mm), *C. aromatica* (12±0.2 mm) and *P. betle* (10±0.5 mm); and *C. aromatic* (17±0.2 mm), *P. betle* (12±0.2 mm) and *M. oleifera* (11±0.1 mm) were observed against human pathogenic strains of *P. fluorescens*-103 and *P. mirabilis*-1429^T, respectively (Figs. 3, 4). Earlier studies have reported that the acetone and aqueous crude extracts of *S. birrea* at different concentrations (100 and 50 mg/ml) showed the antimicrobial activity against *S. pyogenes* and *P. shigelloides* which which were the most susceptible (21–38 mm) organisms to all extracts while *Salmonella typhimurium* was the least susceptible with partial zone of inhibition (Tanih and Ndip, 2012; Parvathalu *et al.*, 2023).

Fig. 5: Synthesis of Silver nanoparticles.

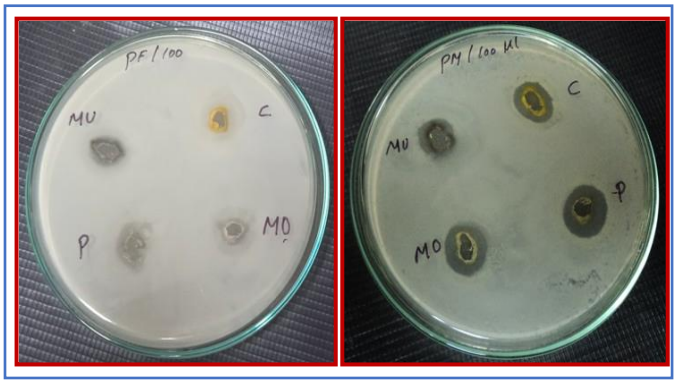

Fig. 6: Antibacterial activity of silver nanoparticles against (a) *P. fluorescens* and (b) *P. mirabilis.*

Silver nitrate reduction

The change in colour was noted by visual observation in the silver nitrate solution incubated with *M. koenigii, M. oleifera, C. aromatica* and *P. betle* (Fig. 5). The colour of the extracts was changed to reddish brown in one hour at 37˚C due to the formation of silver nanoparticles. Change of colour was due to excitation of surface plasmon vibrations in silver nanoparticles (Bonde *et al*., 2012). Moreover, similar results showed the synthesis of silver nanoparticles using leaves of *M. koenigii* and their antibacterial activity.

Antibacterial susceptibility assay using silver nanoparticles

Silver has been used for many years for its antimicrobial properties. Alexander the great used a silver vessel to store drinking water (Silver *et al*., 2006). However, the formulation of silver has changed during antiquity from bulk silver to ionic silver (Kwakye-Awuah *et al*., 2008). In this study, the antibacterial susceptibility test of *M. koenigii*, *M. oleifera*, *C. aromatica* and *P. betle* were performed against human pathogenic type strain *P. fluorescens*-103 and *P. mirabilis*-1429^T. The silver nanoparticles extract of *C. aromatica* (100 µl) showed maximum sensitivity against *P. fluorescens*-103 (14±0.1 mm) whereas *P. betle* showed maximum sensitivity against *P. mirabilis*-1429^T (14 ± 0.05 mm). In addition, the moderate sensitivity against *M. koenigii* (10±0.1 mm), *M. oleifera* (9±0.1 mm), and *P. betle* (9±0.1 mm) and *M. oleifera* (12±0.2 mm), *M. koenigii* (11±0.3 mm), *C. aromatica* (11±0.1 mm) against *P. mirabilis*-1429^T were observed against human pathogenic strains of *P. fluorescens*-103 and *P. mirabilis*-1429^T, respectively (Figs. 6, 7; Table 1). The antibacterial activities of silver nanoparticles were investigated against pathogenic bacteria of *S. aureus* and *E. coli* strains using a disc diffusion

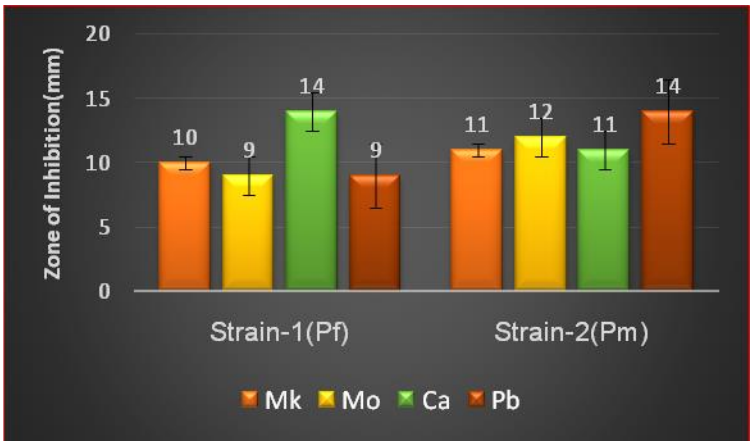

Fig. 7: Antibacterial activity comparison with silver nanoparticles extract with bacterial strains.

Table 1: Comparison of chemical antibiotics, acetone leaf extracts and silver nanoparticles

S. No.	Name of the bacterial strain	Chemical antibiotics (mm)								Acetone leaf extracts (mm)				Silver nanoparticles (mm)			
		GEN	KAN	AZI	AMI	CHL	COT	STR	TET	Mk	Mo	Ca	Pb	Mk	Mo	Ca	Pb
1	***P. florescence***	**23** ± 0.2	**13**± 0.2	**12** ± 0.2	**23** ± 0.2	**14** ± 0.05	**Nil**	**29** ± 0.1	**19**± 0.5	**28**± 0.1	**17** ± 0.2	**12** ± 0.2	**10** ± 0.5	**10** ± 0.1	**9** ± 0.1	**14**± 0.1	**9**± 0.1
2	***P. mirabilis***	**23** ± 0.1	**10** ± 0.2	**29**± 0.1	**24** ± 0.1	**20** ± 0.2	**12** ± 0.2	**22** ± 0.2	**19**± 0.1	**28**± 0.2	**11** ± 0.1	**17** ± 0.2	**12**± 0.2	**11**± 0.3	**12** ± 0.2	**11** ± 0.1	**14** ± 0.05

Mk-*M. koenigii,* Mo- *M. oleifera,* Ca- *C. aromatica,* and Pb- *P. betle*

method. Against bacterial strains of *S. Aureus*, the MIC value was found to be 20 µl/5ml (v/v), or 0.72 µg/1ml (w/v) while the strains of *E. coli* were in the range of 30 µl/5ml or 1.08 µg/1ml (w/v) (Deb, 2014). The antimicrobial properties of silver nanoparticles encourage its use in a large number of biomedical and environmental applications as well as in a growing list of cosmetics, clothing and numerous consumer products (Jain *et al*., 2011). In this study, the silver nanoparticles results showed an excellent zone of inhibition in 1 mM concentration when compared to standard chemical antibiotics and selected four acetone leaf extracts (Fig. 8; Table 1).

UV-VIS spectrum of silver nanoparticles

M. koenigii, *M. oleifera*, *C. aromatica* and *P. betle* leaf extracts were subjected to synthesis of silver nanoparticles and visible colour change indicates the formation of nanoparticles, which is confirmed by UV-Visible spectra of silver nanoparticles. UV–Vis spectrum results showed the highest absorption peak at 338 nm (*M. koenigii*), 340 nm (*M. oleifera*), 338 nm (*C. aromatica*) and 340 nm (*P. betle*) which showed the localized surface plasmon resonance characteristics of silver nanoparticles. It increasing reaction time from 30 min to 120 min. It took 2 h to complete the reaction to stable nanoparticles (Fig. 9).

Fourier Transform Infrared Radiation spectroscopy analysis

In FTIR analysis, peaks correspond to different functional groups. Among these, the absorption peak at 1626 cm^{-1} can be assigned to C=C stretching, 1452 cm^{-1} corresponds to C=H, and the

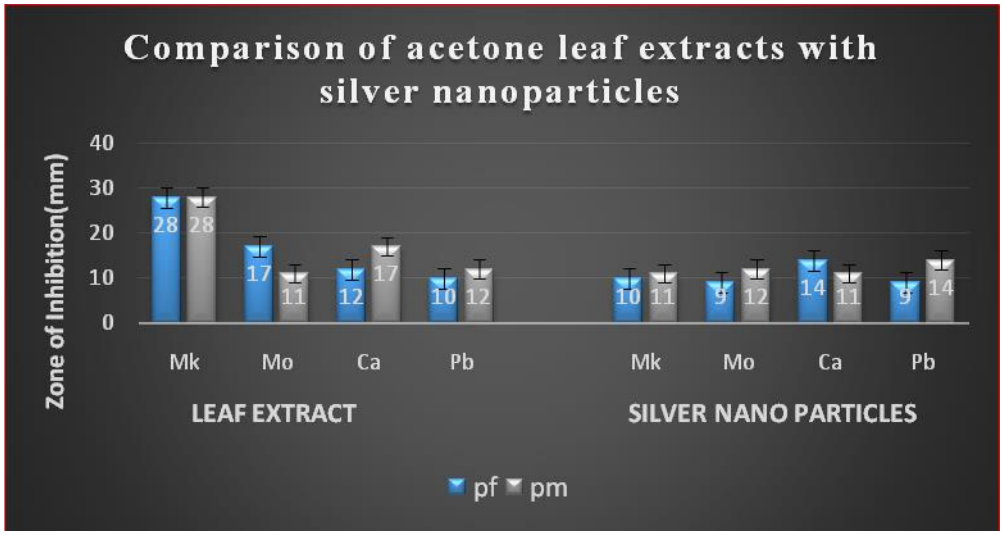

Fig. 8: Comparison acetone leaf extracts with of silver nanoparticles.

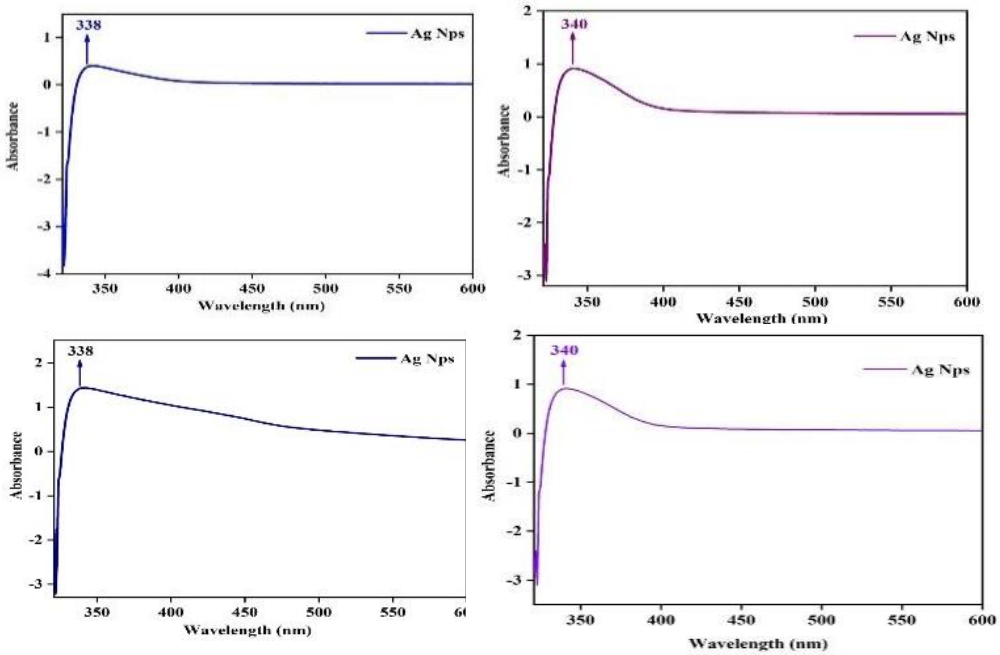

Fig. 9: UV-Vis spectra of silver nanoparticles synthesized by treating *M. koenigii*, *M. oleifera*, *C. aromatica*, and *P. betle* leaves extract with 1mM silver nitrate solution.

absorption at 1146 cm^{-1} due to C–H stretching. The absorption peak at 1037 cm^{-1} might be due to C–N stretch. The absorption spectra of control might be attributed to the functional group such as the benzene ring, C–O–C bond, and aromatic C–H stretching. These findings are supported by other researchers (Sav *et al.*, 2012). The FTIR spectrum revealed that the absorbance bands associated with inorganic silver nitrate and synthesized AgNPs by *M. koenigii*, *M. oleifera*, *C. aromatica* and *P. betle*. The FTIR results of all four samples showed characteristic absorption peaks at 3390.06 cm^{-1}, 2196.23 cm^{-1}, 1630.19 cm^{-1}, 1451.11 cm^{-1}, 1385.25 cm^{-1}, and 730.70 cm^{-1} for the silver nanoparticles (AgNPs) synthesized using *Murraya koenigii*, *Moringa oleifera*, *Curcuma aromatica*, and *Piper betle* extracts. A broad absorption band around 3200–3600 cm^{-1} was observed, characteristic of O–H stretching vibrations, indicating the presence of alcohols or

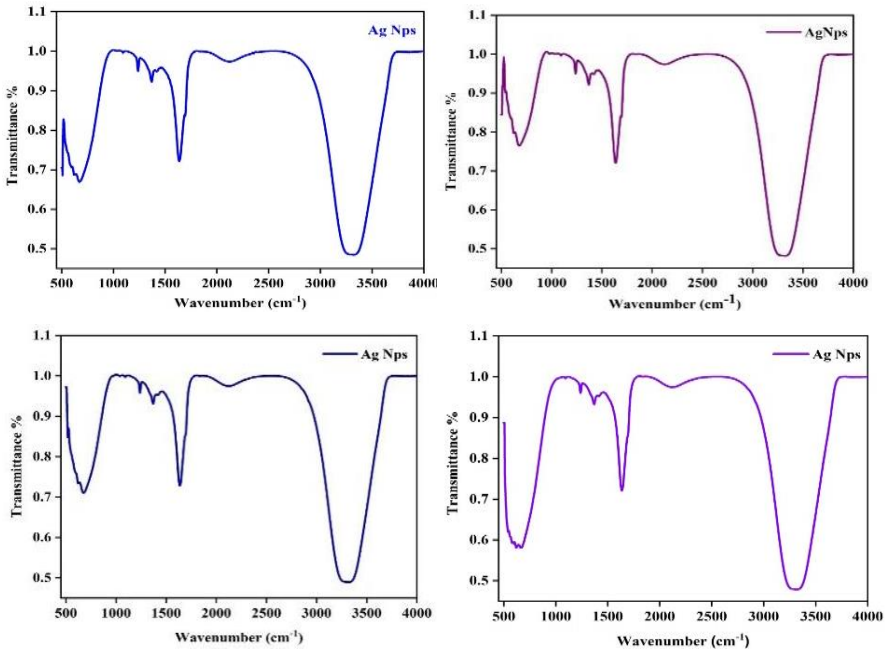

Fig. 10: FTIR spectra silver nanoparticles synthesized using *M. koenigii*, *M. oleifera*, *C. aromatica*, and *P. betle* leaves extract with 1mM silver nitrate solution.

phenols. A sharp peak in this region may also correspond to N–H stretching, suggesting the presence of amines or amides. The peaks between 2150–2950 cm^{-1} are typically associated with C–H stretching vibrations of alkyl groups, although the peak at 2196.23 cm^{-1} may also suggest the presence of C≡C or C≡N stretching (alkyne or nitrile functional groups). A prominent peak near 1630.19 cm^{-1} corresponds to C=C stretching (from alkenes or aromatic rings) or amide C=O stretching, depending on the presence of nitrogen-containing compounds. The absorption band at 1451.11 cm^{-1} may be attributed to C–H bending or C–O stretching, indicating the presence of alcohols, esters, or ethers (Fig. 10).

A similar report shows that the FTIR spectrum of AgNPs revealed the existence of carboxylic groups. The strong O-H stretch of carboxylic bands at the region of 3390.06 cm^{-1} and carboxylic stretching bands at the region of 1630.19 cm^{-1} were noted. The peaks illustrated is a region of 1451.11 cm^{-1} are endorsed via the alkene group. The alkanes stretching bands were noted in the region of 2196.23 cm^{-1} in the FTIR. Moreover, the clear and broad absorbance bands were observed at 3418 cm^{-1}, 2366 cm^{-1}, 1640 cm^{-1} and 1370 cm^{-1}; 3450 cm^{-1}, 1648 cm^{-1}, 662 cm^{-1} and 3423 cm^{-1}, 1645 cm^{-1}, 606 cm^{-1} for the AgNPs synthesised by *C .maxima*, *M. oleifera* and *A. calamus*, respectively. A very intense broad band was observed at around 3450 and 3423 cm^{-1} for all the AgNPs corresponding to the OH stretching, which gives an indication of the presence of polyphenols (Nayak *et al*., 2015).

Conclusion

The leaf extracts *of M. koenigii*, *M. oleifera*, *C. aromatica*, and *P. betle* are rich in phenolic compounds, flavonoids, alkaloids, and saponins, which are important for silver nanoparticle (AgNP) biosynthesis. *C. aromatica*'s AgNP extract showed the highest efficacy against *P. fluorescens*-103, while *P. betle* was effective against *P. mirabilis*-1429^{T}. The synthesized AgNPs exhibited superior activity at 1 mM compared to standard antibiotics and the leaf extracts. These plant extracts demonstrate significant antibacterial properties, indicating their potential as a foundation for new pharmaceuticals. They are

recommended as a promising, non-toxic, and eco-friendly alternative for managing pathogens linked to blood, lung, and urinary tract infections (UTIs).

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the PG & Research Department of Zoology, VHNSN College, Virudhunagar, Tamil Nadu, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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