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D Packiyalakshmi
Department of Botany, Sri Kaliswari College (Autonomous), Sivakasi, Virudhunagar District, Tamil Nadu, India

P Athilakshmi
Department of Botany, Sri Kaliswari College (Autonomous), Sivakasi, Virudhunagar District, Tamil Nadu, India

S Gayathri
Department of Botany, Sri Kaliswari College (Autonomous), Sivakasi, Virudhunagar District, Tamil Nadu, India

P Karthiga
Department of Botany, Sri Kaliswari College (Autonomous), Sivakasi, Virudhunagar District, Tamil Nadu, India

R Thiri Bhuvaneswari
Department of Botany, Sri Kaliswari College (Autonomous), Sivakasi, Virudhunagar District, Tamil Nadu, India

G Manikandan
Department of Botany, Sri Kaliswari College (Autonomous), Sivakasi, Virudhunagar District, Tamil Nadu, India

Correspondence
G Manikandan
Department of Botany, Sri Kaliswari College (Autonomous), Sivakasi, Virudhunagar District, Tamil Nadu, India

Antimicrobial potential of different solvents leaf extract of *Millettia peguensis* against selected pathogens

D Packiyalakshmi, P Athilakshmi, S Gayathri, P Karthiga, R Thiri Bhuvaneswari and G Manikandan

Abstract
Millettia peguensis is commonly known as Moulmein rosewood, is a legume tree species in the genus *Millettia* belongs to the family Fabaceae. It is native to Lower Burma and Siam. This is a relatively rare tree as compared to Pongam that is very similar looking, but more common in India. The powdered leaf was successively extracted with Hexane, Chloroform and Petroleum ether through soxhlet apparatus. Antimicrobial activity was studied using agar well diffusion method at various concentrations (100mg/ml, 150mg/ml and 200mg/ml) of extracts. These extracts inhibited severely on the growth of microorganisms and implied antimicrobial activity on Gram-positive bacteria; *Bacillus cereus*, *Staphylococcus aureus* and *Micrococcus mucilaginosus*, gram-negative bacteria; *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella terrigena*, Fungi; *Candida albicans*, *Candida glabrata* and *Candida* sp. Petroleum ether leaf extracts were effectively tested against microorganisms and also compared with standard antibiotic (Amphicilin; 500mg/ml). The maximum antimicrobial activities were obtained with maximum zone of inhibition in petroleum ether (200mg/ml) extract followed by chloroform and hexane. The results indicated that the leaf extracts might be utilized as natural drug for the treatment of several infectious diseases by microorganisms.

Keywords: *Millettia peguensis*, Antimicrobial, Amphicilin, Petroleum ether

Introduction
Plants have been an important source of medicine for thousands of years. Use of plants for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. Every culture on earth has relied on the vast variety of natural chemistries' found in plants for their therapeutic properties. Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body.

Medicinal plant are still major parts of traditional medicinal systems in developing countries many infections disease are known to be treated with herbal remedies throughout the history of mankind. Even today plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries [1]. Medicinal plants which from the backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plant as potential source of new compounds of therapeutic value and as source of new compounds in drug development. In many parts of the world medicinal plants are used for antibacterial, antifungal and antiviral activities a plant derived drugs serve as a prototype to develop more affective and loss toxic medicinal. Infections disease is the number one among all causes of death, accounting approximately one-lady all deaths throughout the world. About 50-75% of hospital deaths are reported due to infections disease. These numbers are still increasing due to development of resistance in microorganisms to the existing first line drug [2]. Scientists from divergent fields are investigating plants with a new age for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agent with general as well as specific activity.

The use of medicinal plants and they play a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms.

Medicinal plant possess many bioactive compounds including phenolic and polyphenolic compounds which play key function in detoxification of stress induced by free radicals and exhibit antimicrobial activities[3]. In ancient times, people used spices and herbs in their food as flavoring agents. These can also be used locally as food preservatives and in folk medicine [4]. Tribal people of many part of India such as Madhya Pradesh, Uttrakhand used the different parts of plant extracts for fever, diarrhoea, dysentery, bone fracture, tonic and vermifuge [5, 6].

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500 - 1600 B.C and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing [7].

The screening of plants usually involves several approach; ethno botanical approach is one of the common methods that are employed in choosing the plant for pharmacological study. Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In vitro* studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many plant extracts has been previously reviewed and classified as strong, medium or weak [8].

Many studies have been undertaken with the aim of determining the antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both topical and systemic microbial infections as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistant [9, 10].

The antimicrobial activity of plant extracts is due to different chemical agents in the extract. These compounds are usually the secondary metabolites, which function to attract beneficial and repel harmful organisms, serve as phytoprotectants and respond to environmental changes in plants. In humans, however the compounds have beneficial effects [11].

Millettia peguensis belongs to the family Fabaceae and it is commonly called as Moulmein Rosewood is a small deciduous tree, planted mostly for ornamental purposes. It is really beautiful when in full bloom. It blooms with racemes of mauve pea-like flowers. Leaves are pinnate and leaflets oval in shape. The tree may be confused with the Pongam Tree as the flowers appear the same. However, Pongam flowers are more whitish compared to Moulminein Rosewood flowers. Looking just at the buds, one might confuse it with Mexican Lilac, however, Moulminein Rosewood has more drooping clusters like Amaltas. This species is native to Lower Burma and Siam but it is cultivated in Burma, India and Pakistan. The species of genus *Millettia* such as *M. conrauai* is well known for its insecticidal, molluscicidal and pisticicidal activities. In some parts of Africa especially in Cameroon the plants of genus *Millettia* are used by different communities as a potent inhibitor of intestinal parasites in children's as well as colic besides oral treatment for boils.

Materials and Methods

Collection and Processing of plant materials

The fresh leaves of *Millettia peguensis* were collected from the campus of The Gandhigram Rural Institute-Deemed University. The collected leaves were washed thoroughly under running tap water and air dried at room temperature for one month. The dried leaves were then crushed into fine powder by using electric blender. The powdered samples were kept in sealed air tight containers until further use.

Preparation of Extracts

50g of dried leaf powder of *Millettia peguensis* was successively extracted with 250 ml each of organic solvents in the following sequence, *viz*., Hexane, Chloroform and Petroleum ether by using Soxhlet apparatus. The extraction procedures were continued for 3-4 hours at 60 ^{0}C. After complete extraction, respective solvent extracts were evaporated under reduced pressure and the dried extracts thus obtained were stored in air tight vials at 4 °C for further study.

Preparation of the Inoculums

The microorganisms used for the study were collected from the co-culture collections of laboratory, Department of Biology, The Gandhigram Rural Institute- Deemed University, Gandhigram, Dindigul. Gram-positive bacteria; *Bacillus cereus*, *Staphylococcus aureus* and *Micrococcus mucilaginosus*, gram-negative bacteria; *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella terrigena*, Fungi; *Candida albicans*, *Candida glabrata* and *Candida* sp. were used for the study. The young bacterial and fungal strains were maintained in nutrient agar medium. The strains were sub-cultured bimonthly and the cultured strains were allowed to grow for one week and stored at 5 ^{0}C for further analysis.

Determination of antimicrobial activities

Agar- well diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were spreaded with the help of L-rod inside the laminar air flow chamber with 8 hours old broth culture of respective bacteria and fungi. Wells with 6 mm diameter and about 2 cm a part were made in a medium of petriplate each using sterile cork borer. Stock solution of each plant extract was prepared at a concentration of 100, 150 and 200 mg/ml from different solvents viz. Hexane, Chloroform and Petroleum ether. The extracts were added with the help of sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Standard antibiotic, Ampicillin was (100 mg/ ml) selected to serve as positive control. Control experiments comprising inoculums without plant extract were set up and the plates were incubated at 37 °C for 18-24 h for bacterial pathogens and 28 °C for 48 hours for fungal pathogens. The diameter of the inhibition zone (mm) was measured by Antibiotic scale (Himedia) and the activity index was also calculated for triplicates of each experiment.

Results

Antimicrobial activity

In the present study, antimicrobial activities of Hexane, Chloroform and Petroleum ether leaf extracts of *Millettia peguensis* against selected microorganism such as gram positive, gram negative bacteria and fungi were comparable to

the standard antibiotic ampicillin by agar well diffusion method. The results clearly showed the antibiotic sensitivity against the gram positive, gram negative and fungi microorganisms. The all microorganism like gram positive bacteria such as *Bacillus cereus, Staphylococcus aureus, Micrococcus mucilaginosus* and gram negative bacteria such as *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherchia coli, Klebsiella terrigena* and fungi like *Candida albicans, Candida glabrata and Candida* sp. were also screened against the different extracts. The clear inhibition zones were found at 24h after incubation at 37 °C. In addition, the inhibition zones formed by standard antibiotics (Ampicillin) and those of negative controls were recorded. Among these different concentration (100mg/ml, 150mg/ml and 200mg/ml) of the Hexane, Chloroform and Petroleum ether leaf extracts of *Millettia peguensis* exhibited antimicrobial activity against all the selected microorganisms. Among these three concentrations (100 mg/ml, 150 mg/ml and 200 mg/ml) of Hexane, Chloroform and Petroleum ether extract the higher concentration (200 mg/ml) showed higher inhibitory activity against all the tested microorganisms.

The hexane leaf extract (200 mg/ml) of *Millettia peguensis* showed (Table 1; Fig 1-10) higher zone of inhibition (12-15mm) against *Klebsiella terrigena* (15.5±1.50mm) followed by *Pseudomonas aeruginosa* (15±1.50mm), *Candida* sp. (15±1.20mm), *Escherichia coli* (15±1.05mm), *Klebsiella pneumonia* (14±1.50mm), *Staphylococcus aureus* (14±1.40mm), *Micrococcus mucilaginosus* (14±0.80mm), *Candida albicans* (14±0.53mm), *Bacillus cereus* (13±1.03mm) and *Candida glabrata* (12±1.80mm).

The Chloroform leaf extract of *Millettia peguensis* showed maximum inhibition against *Pseudomonas aeruginosa* (17.5±1mm), *Candida albicans* (16±2.16mm), *Klebsiella terrigena* (16.5±0.57mm), *Klebsiella pneumoniae* (15.75±0.5mm), *Bacillus cereus* (15±1.62mm), *Candida* sp. (14.75±0.95mm), *Escherichia coli* (14.5±1.29mm), *Candida glabrata* (13.75±1.5mm), *Micrococcus mucilaginosus* (13.5±0.57mm) and *Staphylococcus aureus* (12±1.5) at 200mg/ml concentration showed (Table 2; Fig 1-10).

The Petroleum ether leaf extract of *Millettia peguensis* showed maximum inhibition showed (Table 3; Fig 1-10) against *Escherichia coli* (17.25±1.25mm), *Candida albicans* (17.25±0.95), *Klebsiella pneumoniae* (17±1.63mm), *Candida glabrata* (17±0.81mm), *Bacillus cereus* (16.5±1.30mm), *Candida* sp. (16.5±1.00mm), *Micrococcus mucilaginosus* (15.75±1.70mm), *Staphylococcus aureus* (15±1.63mm), *Klebsiella terrigena* (15±1.63mm) and *Pseudomonas aeruginosa* (15±1.41mm) at 200mg/ml concentration. The Petroleum ether leaf extract of this plant showed very high activity of zone of inhibition compare than the other solvents followed by Chloroform and Hexane extracts against selected human pathogenic microorganisms.

Discussions

Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. Evaluating the *in vitro* antimicrobial activity of plant (*Allium sativum*, *Zingiber officinale*, *Caryophyllus aromaticus*, *Cymbopogon citratus*, *Mikania glomerata* and *Psidium guajava*) extracts against Gram-positive and Gram-negative bacterial strains isolated from human infections[12].

In the present study confirmed the Hexane, Chloroform and Petroleum ether leaf extracts of *Millettia peguensis* was exhibit antimicrobial activity against all the selected micro organisms. The Petroleum ether extract showed very high activity of zone of inhibition compare than the other solvents. Results obtained from the current study were in conformity with earlier work of Mohammed *et al.* [13]. investigated the antimicrobial property of aqueous and Petroleum ether leaf extracts of *Jatropha curcas* against some gram positive micro-organisms: *Staphylococcus aureus, Bacillus subtilis* and some gram negative micro-organisms: *Escherichia coli, Salmonella typhi* using antibiotics; Gentamycin as control. The disc diffusion techniques was used to test the sensitivity of the micro-organism to the extracts of *Jatropha curcas* the results obtained show mean zones of inhibition between (19 + 0.6mm) to (30 + 0.3mm) for aqueous extract and (24 + 0.5mm) to (35 + 0.8mm) for petroleum ether extract. Micro-organisms showed sensitivity in the following order: *E.coli*; (17 + 0.3mm) and (25 + 0.8mm), *S.aureus*; (26 + 0.2mm) and (28 + 0.6mm), *B.subtilis*; (16 + 0.1mm) and (20 + 0.7mm), and *S.typhi* (25 + 0.2mm) and (27 + 0.6mm) for aqueous and petroleum ether extracts respectively.

The antibacterial activity of methanol extract, petroleum ether, chloroform and ethyl acetate fractions from the root bark of Akanda (*Calotropis gigantea*) were investigated by Ashraful *et al.* [14]. The antimicrobial activities of *Curcuma aromatica* plant rhizome extracts were evaluated. Petroleum ether and chloroform extracts of field-grown rhizome showed potential antimicrobial properties against several human pathogenic bacteria including *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella sonne* and *Shigella dysenteriae* with a minimum inhibitory concentration (MIC) ranging from 0.03 to 0.5 mg/mL [15].

Mohammed *et al.* [13] reported that the levels of inhibition observed ranged between 19mm – 30mm Gentamycin, 16mm – 26mm for aqueous extract and 20 – 28mm for petroleum ether extracts of *Jastropha curcas*. For *E.coli*, the petroleum ether extract shows greater inhibition (27mm) than aqueous (25mm) and antibiotics (22mm) (Gentamycin). Comparison of aqueous and petroleum ether extracts of *Jatropha curcas* against microorganisms showed that petroleum ether extract have greater inhibitory potency.

Arora *et al.* [16] evaluated the *in vitro* antibacterial activities of *Withania somnifera* extracts. The results showed that methanol extract of leaves showed higher activity against *Sallmonella typhimurtum* than *Escherichia coli* while in roots *Escherichia coli* more activity than *Sallmonella typhimurtum.* Mathabe *et al.* [17] reported that methanol, ethanol, acetone and hot water extracts from different plant parts (leaves, roots, bark and stem rhizome) of *Indigofera daleoides, Punica granatum, Syzygium cordatum, Gymnosporia senegalensis, Ozoroa insignis, Elephantorrhiza elephantina, Elephantorrhiza burkei, Ximenia caffra, Schotia brachypetala* and *Spirostachys africana.* Several medicinal plants showed remarkable antibacterial activity against *Vibro cholera, Escherichia coli* and *Staphylococcus aureus, Shigella sp.* and *Salmonella typhi*.

Conclusion

The *Millettia peguensis* an important medicinal tree species suggests that the determination of antimicrobial activity. In, the present study the Petroleum ether leaves extract of *Millettia peguensis* clearly showed strong antimicrobial activity against selected pathogens. The results indicated that the petroleum ether leaf extracts might be utilized as natural drug for the treatment of several infectious diseases by microorganisms.

Table 1: Antimicrobial activity of Hexane leaf extract of *Millettia peguensis* against selected human pathogens

S. No	Name of the Microorganisms	Zone of inhibition (mm)				
		Different concentration (mg/ml)				Antibiotic
		200	150	100	Control	500mg/ml
1	*Bacillus cereus*	13±1.03	11±1.25	9±0.75	6±0.81	20±0.95
2	*Klebsiella pneumoniae*	14±1.50	12±1.75	10.25±1.30	7±0.57	20±0.57
3	*Candida albicans*	14±0.53	10±0.90	8.75±0.90	6±0.81	21±0.50
4	*Pseudomonas aeruginosa*	15±1.50	13±1.30	10±1.23	5±0.95	21±0.81
5	*Staphylococcus aureus*	14±1.40	11±1.30	9.25±1.35	5±0.81	21±0.95
6	*Escherichia coli*	15±1.05	13±1.50	10±1.30	5±0.57	21±0.81
7	*Candida glabrata*	12±1.80	10.25±0.90	8±0.95	6±0.81	21±0.81
8	*Micrococcus mucilaginosus*	14±0.80	12±0.90	10±1.20	5±0.50	21±0.57
9	*Klebsiella terrigena*	15.5±1.50	13±1.10	11±0.90	5±0.81	21±1.50
10	*Candida* sp.	15±1.20	12±0.80	10±1.50	6±0.57	20±1.29

Table 2: Antimicrobial activity of Chloroform leaf extract of *Millettia peguensis* against selected human pathogens

S. No	Name of the Microorganisms	Zone of inhibition (mm)				
		Different concentration (mg/ml)				Antibiotic
		200	150	100	control	500mg/ml
1	*Bacillus cereus*	15±1.62	11.75±1.70	10.5±0.57	6±0.50	20±0.95
2	*Klebsiella pneumoniae*	15.75±0.5	13±0.81	11.75±0.95	7±0.81	20±0.57
3	*Candida albicans*	16±2.16	11.75±0.95	9.25±0.5	5±0.95	21±0.50
4	*Pseudomonas aeruginosa*	17.5±1	12.75±1.25	10.75±0.95	6±0.95	21±0.81
5	*Staphylococcus aureus*	12±1.5	9.75±0.95	8.25±1.5	5±0.57	21±0.95
6	*Escherichia coli*	14.5±1.29	12.5±1.29	9.75±0.95	5±0.50	21±0.81
7	*Candida glabrata*	13.75±1.5	11.25±1.25	9±0.81	5±1.29	21±0.81
8	*Micrococcus mucilaginosus*	13.5±0.57	10.5±0.57	8±1.15	4±0.81	21±0.57
9	*Klebsiella terrigena*	16.5±0.57	11.5±1.29	12±0.81	6±0.57	21±1.50
10	*Candida* sp.	14.75±0.95	11.5±1.29	9.5±0.57	5±0.95	20±1.29

Table 3: Antimicrobial activity of Petroleum ether leaf extract of *Millettia peguensis* against selected human pathogens

S. No	Name of the Microorganisms	Zone of inhibition (mm)				
		Different concentration (mg/ml)				Antibiotic
		200	150	100	Control	500mg/ml
1	*Bacillus cereus*	16.5±1.3	11.5±0.57	9.5±1.29	5±0.50	20±0.95
2	*Klebsiella pneumoniae*	17±1.63	14.5±1.29	11.5±1.29	3±0.81	20±0.57
3	*Candida albicans*	17.25±0.95	15±0.81	13±0.82	5±0.57	21±0.50
4	*Pseudomonas aeruginosa*	15±1.41	12.75±1.25	10.75±0.95	5±0.57	21±0.81
5	*Staphylococcus aureus*	15±1.63	11.5±1.29	9.75±0.95	5±0.95	21±0.95
6	*Escherichia coli*	17.25±1.25	14.75±1.25	11.5±1.29	7±0.57	21±0.81
7	*Candida glabrata*	17±0.81	14.75±0.70	12±1.15	5±1.15	21±0.81
8	*Micrococcus mucilaginosus*	15.75±1.70	13.25±1.70	10.75±2.21	5±0.50	21±0.57
9	*Klebsiella terrigena*	16±1.63	12±1.63	10.25±1.70	6±0.50	21±1.50
10	*Candida* sp.	16.5±1	14±1.41	11.25±1.89	4±0.81	20±1.29

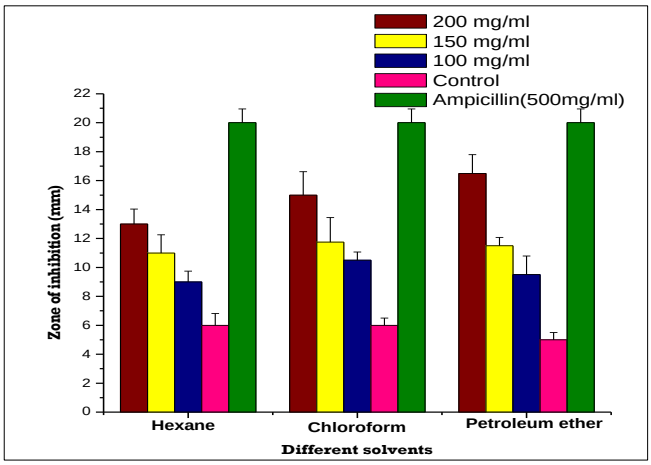

Fig 1: Comparative study on antimicrobial activity of different solvents leaf extracts of *Millettia peguensis against Bacillus cereus*

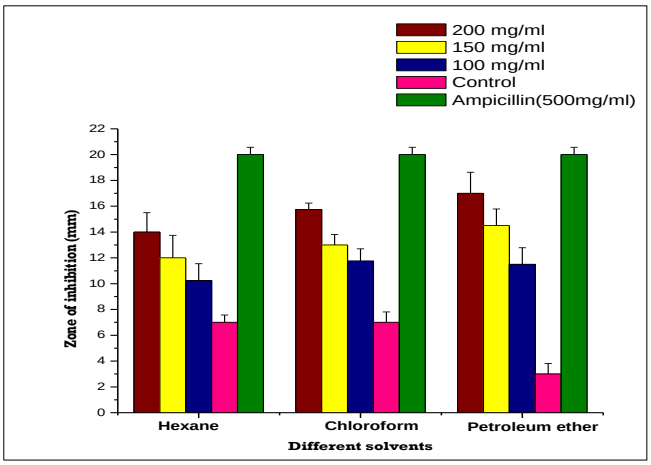

Fig 2: Comparative study on antimicrobial activity of different solvents leaf extracts of *Millettia peguensis against Klebsilla pneumonia*

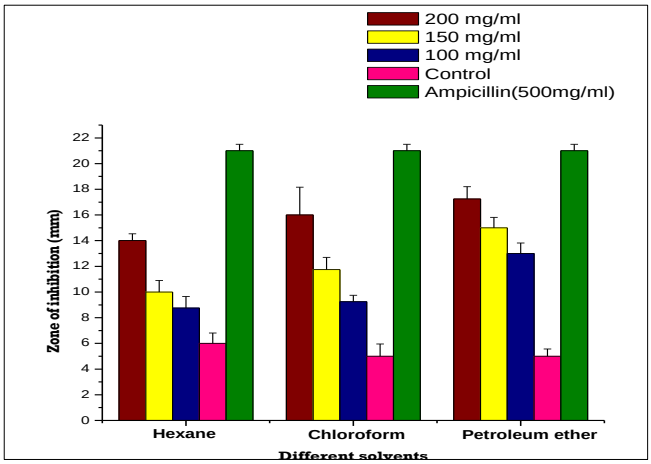

Fig 3: Comparative study on antimicrobial activity of different solvents leaf extracts of *Millettia peguensis against Candida albicans*

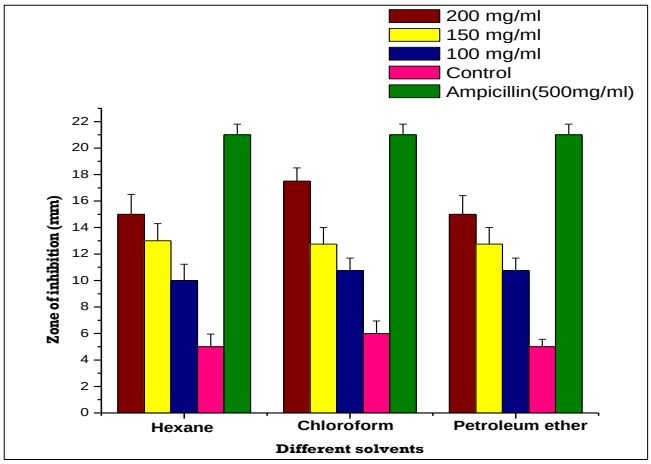

Fig 4: Comparative study on antimicrobial activity of different solvents leaf extracts of *Millettia peguensis against Pseudomonas aeruginosa*

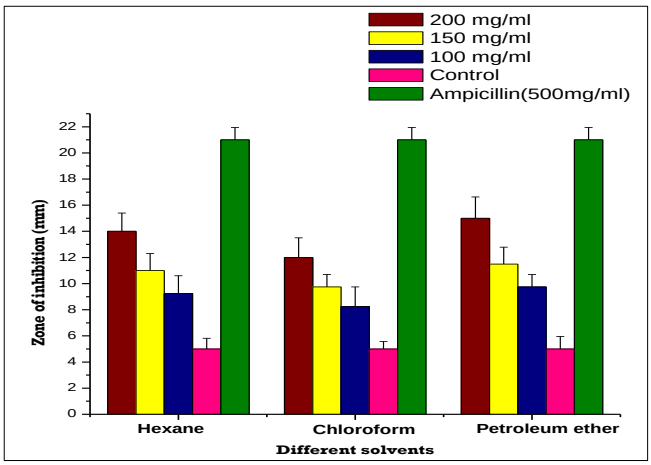

Fig 5: Comparative study on antimicrobial activity of different solvents leaf extracts of *Millettia peguensis against Staphylcoccus aureus*

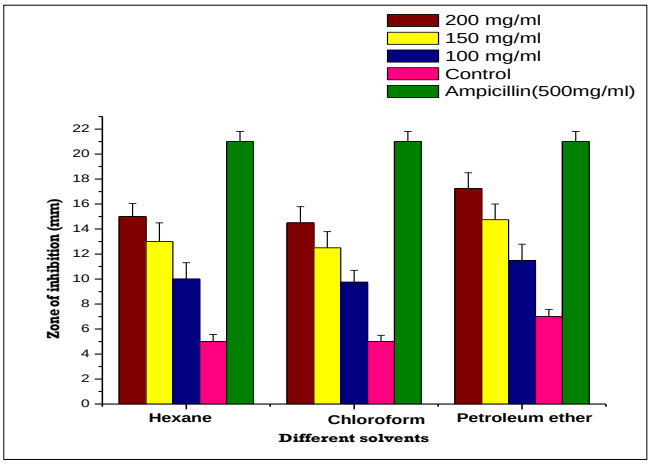

Fig 6: Comparative study on antimicrobial activity of different solvents leaf extracts of *Millettia peguensis against Escherichia coli*

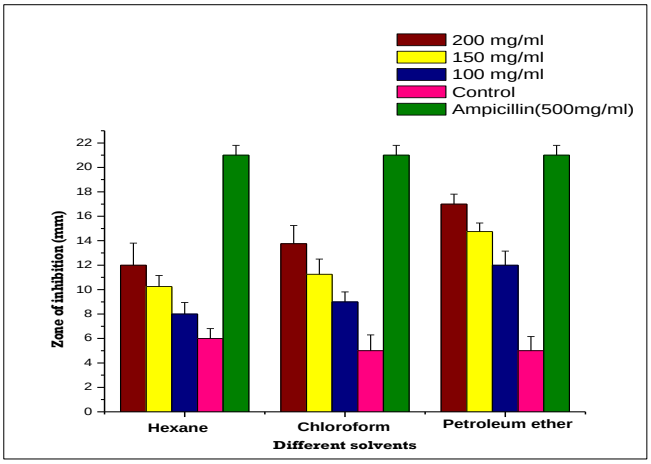

Fig 7: Comparative study on antimicrobial activity of different solvents leaf extracts of *Millettia peguensis against Candida glabrata*

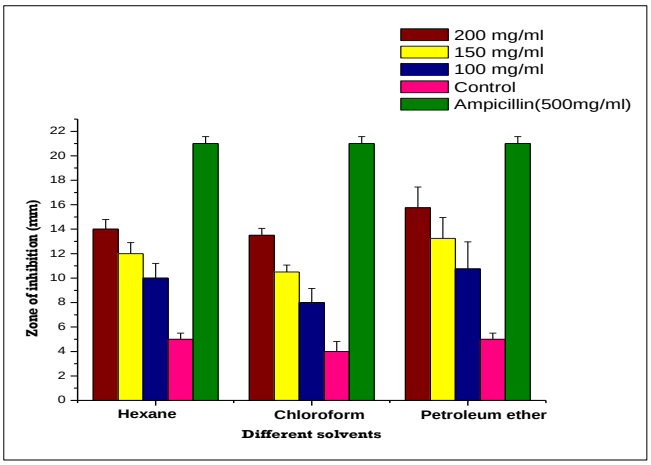

Fig 8: Comparative study on antimicrobial activity of different solvents of leaf extracts of *Millettia peguensis against Micrococcus mucilaginosus*

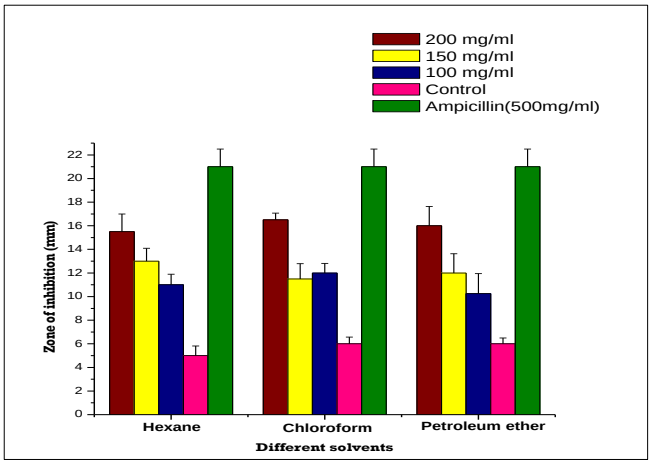

Fig 9: Comparactive study on antimicrobial activity of different solvents leaf extracts of *Millettia peguensis against Klebsiella terrigena*

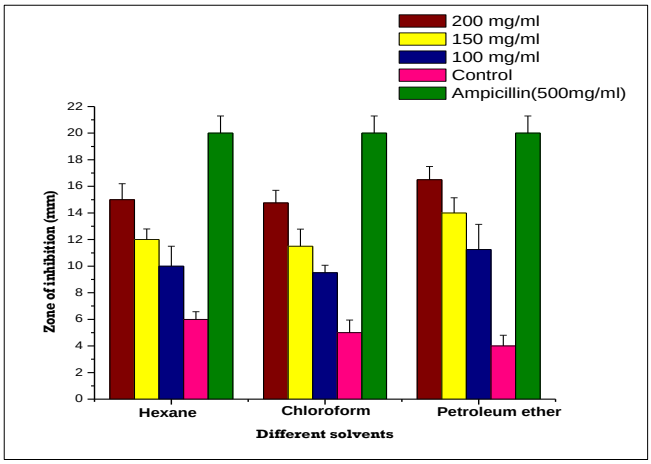

Fig 10: Comparactive study on antimicrobial activity of different solvents leaf extracts of *Millettia peguensis against Candida* sp.

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