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EVALUATION AND ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANT EXTRACTS (AEGLE MARMELOUS) AGAINST MULTI - DRUG RESISTANT KLEBSIELLA PNEUMONIAE

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ABSTRACT

The current study was evaluation of antibacterial activity of medicinal plant extracts (Aegle marmelos) against multidrug resistant K. pneumoniae. The medicinal plant (A. marmelos) leaves and fruit extract prepared by using aqueous acetone, butanol, chloroform, ethanol. Further carried out by phytochemical screening test, antibiotic susceptibility test (multidrug resistance test), agar well diffusion method, Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC). From this study, it was found that the very effectiveness of ethanol and butanol extracts against *K. pneumoniae*. A less effectiveness of chloroform, acetone and aqueous extracts of leaves and fruits. Due to the presence of various compounds that are essential for good health, it can also be used to improve the health status of society. The extracts showed significantly high antibacterial activity against the microorganisms. Further, the broad spectrum activity of ethanol and butanol extracts proves to be encouraging in the development of novel antimicrobial formulations in the near future.

KEYWORDS: *Klebsiella pneumoniae, Aegle marmelos,* Antibacteriall activity, Broad spectrum.

INTRODUCTION

Traditionally used medicinal plant product has known for their therapeutic properties. Nowadays, an increasing number of infectious agents are becoming more resistant to commercial antimicrobial compounds". The importance of plants in medicine remains even of greater global shift to obtain drugs, as a result of which attention has been given to the medicinal value of herbal remedies for safety efficacy and economy **(Musa A. M *et al*., 2008).**

Antimicrobial activity has become critically important in the present day due to the increasing incidence of infectious diseases and the rapid emergence of antimicrobial-resistant microorganisms. Microorganisms such as bacteria, fungi, and viruses are responsible for a wide range of infections affecting human health, and antimicrobial agents play a vital role in controlling and treating these infections. However, the misuse and overuse of antibiotics have led to the development of resistant strains, reducing the effectiveness of existing drugs **(Davies *et al.*, 2010).**

India is widely known as the botanical garden of the world since it is the largest producer of medicinal herbs. Medicinal plants act as an indigenous source of new compounds possessing therapeutic value and can also be used in drug development. 80% of the population of developing countries depend on traditional medicines, mostly natural plant products, for their primary health care needs as estimated by WHO **(G. Vines ., 2004).**

DRUG RESISTANCE OF KLEBSILLA PNEUMONIAE

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated bacterium belonging to the family *Enterobacteriaceae*. It is a major cause of pneumonia, urinary tract infections, bloodstream infections, and wound infections, particularly in immunocompromised patients. **(Ray ., 2004).**

The widespread and irrational use of antibiotics has led to the emergence of multi drug resistant strains of *K. pneumoniae,* including Amoxicillin, Ampicillin, Doxycline, Gentamycin, Kanamycin, penecillin, polymyxin B, Steptomycin, Tetracycline, Vancomycin. Which pose serious challenges in clinical treatment.

Presumptive *Klebsiella spp*. Isolates were obtained from urine samples of patiences who claimed fever, pneumonia and urinary tract infection (UTI). All presumptive *Klebsiella sp.* isolates were collected in chromogenic media plates, as blue-purple mucoid colony. After collection of all the isolates, were labeled, sub cultured and stored at -20°C for further use **(Podschun *et al* ., 1998).**

Klebsiella pneumoniae is classified as one of the ESKAPE pathogens, together with *Enterococcus faecium, Staphylococcus aureus, Acinetobacter bauman-nii, Pseudomonas aeruginosa, and Enterobacter spp.* This bacterium is widely distributed in nature, thriving in environments such as soil and shallow water bodies. Asymptomatically colonizes mucosal surfaces in the human body, particularly the gastrointestinal tract **(Paczosa *et al.*, 2016).**

MULTDRUG RESISTANCE FOR K. PNEUMONIAE

The *Klebsiella* spp. Isolates showed variable result in their antibiotic sensitivity pattern against 10 commercial antibiotic discs tested. Most of the isolates exhibited resistant to multiple commercial antibiotics and refereed as “Multidrug – resistant organisms” (MDROs). Antibiotics often used against diseases caused by *Klebsiella* spp **(Mishra *et* al., 2009).**

Antibiotics are often used against diseases caused by *Klebsiella sp.* But these pathogens are becoming increasingly antibiotic-resistant, so that many are now labeled as "Multi drug resistant (MDR) *Klebsiella sp*. **(Yan et al., 2001).**

The blessed nature of Bael plant can be recognized by analyzing its therapeutic uses, such as for treatment of Swollen Joints, High Blood Pressure, Asthma, Fractures, Dyspepsia, Healing of Wounds, Stomach pain, Vomiting, Brain Typhoid Troubles during Pregnancy, Diabetics, Anaemia, Jaundice, Diarrhoea and Healthy Mind **(T Gohil *et al.*, 2010).**

The Bael fruit pulp contains many functional, biological and pharmacological active compounds such as alkaloids, coumarins, flavonoids, carotenoids, terpenoids, phenolics and antioxidants which helps us in protecting against various chronic diseases **(P Anprung *et al.*, 2008).**

AEGLE MARMELOS

Aegle marmelous is a native plant of India. *A. marmelous* belongs to *Rutaceae* family and commonly known as wood apple, Bael and Bilipatra in Gujarati, Gold apple in english. A. marmelous is also known as Mahaphala or Great fruit.

(Aegle mar*melos, corr. (Fam. Rutaceae)* is commonly known as "*Bael*" in Bengal and *Vilvam* in Tamil is distributed throughout India in dry forests, and also cultivated The leaves are trifoliate, flowers are greenish white, bark is yellowish brown in colour and fruits are green in colour with orange brown sweet gummy (pulps. The extracts of the various parts of the plant is used for controlling diarrhoea, dysentery, dyspepsia, stomach pain, seminal weakness, uropathy, vomiting, diabetes, snake bite, fish poisoning and some antiviral activities **(Krithikar and Basu, 1999).**

Aegle marmelos (Bael) is one of them which play a vital role in day to day usage. Bael (*Aegle marmelos*) is one of the most important tree species used in various indigenous systems of medicine in India, China, Burma and Sri Lanka. Bael (*Aegle marmelos* (Linn), family *Rutaceae*, also known as Bale fruit tree, is a moderate sized, slender, aromatic tree, 6.0-7.5 m in height, and 90 to 120 cm in girth, with a somewhat fluted bole of 3.0- 4.5 meter growing wild throughout the deciduous forests of India **(Kirtikar et al., 1984).**

Aegle marmelos has also been widely investigated for its phytochemical constituents. The root, stem and leaves have been shown to contain tannins, alkaloids, sterols, coumarin, phenyl ethyl cinnamides and aromatic components **(Puksasook et al., 2008)**.

Every part of *Aegle marmelos* plant such as its fruits, stem, bark, and leaves possesses medicinal property and is used for treating various eye and skin infections. Leaf is considered to be one of the highest accumulatory parts of the plant containing bioactive compounds which are synthesized as secondary metabolites. The present study was, therefore, aimed at evaluating the phytochemical potential and antibacterial activity of *Aegle marmelos* aqueous, ethanol, butanol, chloroform, acetone leaf and fruit extracts **(Kingston et al., 2009).**

In addition it has antiproliferative and antioxidant activities. The leaf extracts significantly inhibits the dermatophytic fungi like *Trichophyton mentagrophytes T. rubrum, Microsporum canis, M.gypseum and Epidermophyton floccosum*. Various phytochemical constituents have been isolated from the different extracts of the leaves ie, alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids and steroids, in addition to coumarins, polysaccharides, seed oil and carotenoids from different parts of the tree. **(Dhankhar et al., 2011).**

AEGEL MARMELOS AGAINST *K. PNEUMONIAE*

Aegle marmelos is a very well-known and popular medicinal plant and possess antidiarrheal, antimicrobial, and antiviral, radio protective, anticancer. Chemo preventive, antipyretic, ulcer healing, antigenotoxic, diuretic, antifertility and anti-inflammatory properties **(Rahman et al., 2014).**

Klebsiella pneumoniae is a Gram-negative, opportunistic pathogen responsible for serious infections such as pneumonia, urinary tract infections, septicemia, and wound infections. In recent years, the emergence of multidrug-

resistant (MDR) *K. pneumoniae* has become a major global health concern, limiting the effectiveness of commonly used antibiotics and increasing morbidity and mortality rates.

Medicinal plants are gaining attention as alternative antimicrobial agents due to their bioactive phytochemicals, safety, and cost-effectiveness. *Aegle marmelos* (L.) Correa, commonly known as Bael, is a traditional medicinal plant widely used in Ayurveda and Siddha systems of medicine. Different parts of the plant, especially leaves and fruits, are rich in phytochemicals such as flavonoids, tannins, phenols, alkaloids, saponins, terpenoids, glycosides, and coumarins, which are known for their antimicrobial properties.

The effectiveness of *Aegle marmelos* against MDR *K. pneumoniae* highlights its potential as a natural antimicrobial agent and a promising source for the development of novel plant-based therapeutics. Therefore, studying the antibacterial activity of *Aegle marmelos* leaf and fruit extracts against *K. pneumoniae* is of great importance in addressing the growing problem of antibiotic resistance.

MATERIALS AND METHODS

COLLECTION OF SAMPLE

The samples are collected from the Hospital laboratories, Thiruvannamalai. The collected sample was inoculated into nutrient broth. After incubation observe the microbial growth, then morphological characters identified by gram staining.

IDENTIFICATION OF MICROORGANISMS FROM SELECTIVE

The nutrient broth culture was inoculated into different selective medium like Klebsiella blue agar (KBA), MacConkey agar. The specific bacteria identified by biochemical tests like Indole, Methyl red, Voges Proskauer, Citrate utilization, Triple sugar iron agar test, Urease test, Catalase and Oxidase test.

Collection of Plant Materials

Aegle Marmelos leaves and fruit were collected from the Thirvannamalai. The fresh plant materials were collected and used for the extraction process.

Sterilization of Plant Materials

Surface cleaning: The fresh leaves and fruit were thoroughly washed with tap water to remove visible dust and debris. Then washed with sterile distilled water.

Surface sterilization: Immerse the plant leaf and fruit in a disinfectant solution such as 70% ethanol for 1–2 minutes to remove microbial contamination. Again wash with distilled water.

Preparation of Plant Extraction Method

Drying: The washed plant leaves and fruit were placed on sterile absorbent paper in a sterile area. The materials are dried under shade at room temperature for 5–10 days. Every day, turn the plants to ensure even drying.

Grinding: The dried plant materials are ground with a sterile electric blender or sterile mortar and pestle to fine powder and stored in sterile airtight containers at room temperature.

PREPARATION OF EXTRACTION

One gram of powdered material was weighed on an electric balance and transferred to sterile tubes. Add 9 ml of concentrated ethanol, mixed by using vortex mixture. Then add 1 ml of distilled water and shake well. Incubate the extract for 24–48 hours at room temperature. This method were followed to remaining extract like aqueous, chloroform, acetone, butanol.

Filtration: Filter the extracts using a 0.45 µm syringe filter and store the extract in a sterile containers in the refrigerator.

SCREENING OF PHYTOCHEMICALS ANALYSIS TEST

In order to identify different classes of active chemical elements in the collected plant extracts, a qualitative phytochemical analysis were performed by using phytochemical tests like Alkaloids test, Coumarins test, Flavonoids test, Phenols test, saponins test, sterols test, Tannin test, Terpenoids test, Triterpenoids test, Quinones test.

Antibiotic Susceptibility Test

AGAR WELL DIFFUSION METHOD

The prepared, 9.5g of Muller Hinton Agar with 250ml of the distilled water and sterilized properly then the media poured into the petriplate and allowed solidified. A swab full culture of *K. Pneumonia* was taken from the mentioned culture and swabbed on the Mueller agar plate .The well were made by using sterilized 1 ml tips. The plant extract and fruit extract were loaded into the well plate and incubated at 37°C for 24 hours and observed the inhibition of zone formation.

Minimum Inhibitory Concentration (MIC)

The turbidity method or tube dilution method was used for the determination of Minimum Inhibitory Concentration (MIC). The extracts were taken at different concentrations of 5, 10, 15, 20, and 25 mg/ml in test tubes containing 1 ml sterile Mueller–Hinton broth. Then 0.1 ml of bacterial suspension was inoculated into the test tubes. A control tube was maintained. All the tubes were then incubated at 37°C for 24 hours and examined for growth by observing turbidity.

Minimum Bactericidal concentration (MBC)

A loopful of broth was collected, from the each tube of MIC, that are used for determination of MBC .The broth was inoculated into a sterile nutrient agar by streaking. The Inoculated plates were incubated at 37°c for 24 hours. After incubation the concentration at which there was no visible growth was noted as the minimum bacterial Concentration.

RESULT AND DISCUSSION

Based on the morphological shape and biochemical test results, the organism identified as *Klebsiella pneumoniae*. The present study was carried out on aqueous, acetone, butanol, chloroform, and ethanol extracts of *Aegle marmelos* to investigate the presence of medically important phytochemicals in the leaves and fruit extracts. Both the extracts revealed the presence of various phytochemicals such as alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids.

The quantitative phytochemical estimation specifies that the leaves and fruit contain a significant amount of alkaloids, flavonoids, phenolics, saponins, and tannin content In the present study, it was revealed that the selected multidrug resistance antibiotics against *K. pneumoniae*. The antibiotic susceptibility test showed that maximum resistance (no

zone formation) was observed for Ampicillin, Amoxicillin, Penicillin, and Vancomycin against *K. pneumoniae*. Table 1 shows the Antibiotic Susceptible test.

In the present study, 85 % of *Klebsiella spp*. Isolates were resistant to polymyxin B and streptomycin followed by 80% to vancomycin and kanamycin. 55-60% of Klebsiella spp. Isolates were resistance to Amoxicillin, Ampicillin and penicillin. The antibacterial screening was done using the agar well diffusion method. The antibacterial activity of this variety using aqueous, acetone, butanol, chloroform, and ethanol as solvents has been investigated. Table 2 shows the Antimicrobial activity of plant extract.

The high antibacterial activity in the ethanol and butanol extracts may be due to the presence of alkaloids, coumarins, flavonoids, glycosides, phlobatannins, phenols, saponins, steroids, tannins, terpenoids, triterpenoids, and quinones. These bioactive components act as medicinal value. This result showed the more susceptible by *K. pneumoniae*.

The antimicrobial MIC studies were carried out by the broth dilution method. MIC activity using five extract solvents like aqueous, acetone, butanol, chloroform, and ethanol of both leaves and fruit was analyzed (Table 3&4).

The inhibitory effect of phytochemical extracts of leaves and fruit of Aegle marmelos in ethanol and butanol extracts of leaves and fruits were found to be effective against K. pneumoniae. The chloroform, acetone, and aqueous extracts of leaves and fruits were found to be less effective when compared to commercial antibiotics.

The Minimal Bactericidal Concentration (MBC) result shows no growth on agar plates. It represents the lowest concentration of antimicrobial agent required to kill a specific bacteria. (Table 5&6)

When the MIC determination mentioned above has been carried out on a bacterial isolate in broth. The tubes may be sub cultured on to Nutrient agar plate, to determine the MBC of dung required of to kill 99.9% of the test organisms *K. pneumonia*.

Further, the broad spectrum activity of ethanol and butanol proved to be encouraging in the development of antimicrobial formulations and valuable drugs.

Table 1: Antibiotic Susceptibility Test of Commercial Antibiotics.

S. NO	ANTIBIOTICS	*K. PNEUMONIAE*
1.	Ampicillin	No zone
2.	Amoxicillin	No zone
3.	Doxycline hydrochloride	12mm (R)
4.	Gentamycin	14mm (I)
5.	Kanamycin	13mm (I)
6.	Penicillin	No zone
7.	Polymiyxi B	10mm (R)
8.	Streptomycin	12mm (I)
9.	Tetracycline	12mm (I)
10.	Vancomycin	No zone

(R) – Resistant (I) - Intermediate

Table 2: Antibacterial Activity of A. Marmelos Leaf And Fruit Extract By Agar Well Diffusion Method Against K.Pneumoniae.

		ZONE OF INHIBITION	
S. NO	EXTRACT	A. MARMELOS LEAF	A. MARMELOS FRUIT
1.	Aqueous	16mm	18mm
2.	Acetone	20mm	23mm
3.	Chloroform	22mm	25mm
4.	Butanol	25mm	27mm
5.	Ethanol	26mm	29mm

Table 3: Antibacterial Activity of A.Marmelos Leaf Extract By Minimum Inhibitory Concentration Against K.Pneumoniae.

S. NO	CONCENTRATION (µl)	ACETONE	BUTANOL	CHLOROFORM	AQUEOUS	ETHANOL
1	5	7.9	8.9	7.8	6.7	9.5
2	10	7.6	8.6	7.7	6.5	9.2
3	15	7.4	8.2	7.5	6.4	9.0
4	20	7.3	7.6	7.2	6.3	8.7
5	25	7.1	7.2	7.0	6.2	8.3

Table 4: Antibacterial Activity of A.Marmelos Fruit Extract By Minimum Inhibitory Concentration Against K.Pneumoniae

S. NO	CONCENT RATION(µl)	ACETONE	BUTANOL	CHLOROFORM	AQUEOUS	ETHANOL
1.	5	7.8	8.8	7.7	6.9	9.8
2.	10	7.7	8.5	7.5	6.7	9.4
3.	15	7.5	8.4	7.3	6.4	9.2
4.	20	7.2	8.2	7.2	6.3	8.8
5.	25	7.0	7.1	7.0	6.2	8.4

Table 5: Minimal Bactericidal Concentration -Leaf Extract.

S. NO	ORGANISMS	MEDIA		LEAF EXTRACT			
1.	*K. pneumonia*	Nutrient Agar (NA)	LAE	LBE	LCE	LDE	LEE
			04	-	-	01	-

Table 6: Minimal Bactericidal Concentration- Fruit Extract.

S. NO	ORGANISMS	MEDIA		FRUIT EXTRACT			
1.	*K. pneumonia*	Nutrient Agar (NA)	FAE	FBE	FCE	FDE	FEE
			06	_	_	03	_

CONCLUSION

In this study, it can be concluded that A. marmelos can be used as a source for producing antimicrobial substances, which is very effective against the clinical enteric pathogen K. pneumoniae. This present study evaluated the presence of phytoconstituents such as alkaloids, terpenoids, saponins, tannins, flavonoids and steroids. These compounds exhibited a zone of inhibition against K. pneumoniae. From this study, it was found that the very effectiveness of ethanol and butanol extracts against *K. pneumoniae*. A less effectiveness of chloroform, acetone and aqueous extracts of leaves and fruits.

Almost every part of this plant is used to cure a variety of illnesses, including the leaf and fruit, which have great potential. From the above research, it can be concluded that this plant has immense potential to be used in the area of

pharmacology and as a prospective source of valuable drugs. Due to the presence of various compounds that are essential for good health, it can also be used to improve the health status of society. The extracts showed significantly high antibacterial activity against the microorganisms. Further, the broad spectrum activity of ethanol and butanol extracts proves to be encouraging in the development of novel antimicrobial formulations in the near future.

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