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### SYNERGISM BETWEEN AQUEOUS EXTRACT OF LEAVES OF THE *MURRAYA KOENIGII* AND NORFLOXACIN AGAINST *KLEBSIELLA* *PNEUMONIAE*

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#### ABSTRACT

*Klebsiella pneumoniae* strains is one of the UTI causing bacteria. So 32 UTI samples were collected and characterized on the basis of biochemical tests i.e. Bergey's manual of determinative bacteriology [14]. Only, 7 UTI positive *Klebsiella pneumoniae* were selected for present study. *Murraya koenigii* is medicinal plant and evaluated the antibacterial activity of aqueous extract of *M. koenigii*. Preparation of extraction of leaves of the *M. koenigii* and preparation of various treatments of *M. koenigii* and Norfloxacin were prepared by standard methods. Stock concentration of extract of the *Murraya koenigii* and Norfloxacin was 50µg/ml. 5 treatments were prepared and observed that maximum inhibition zone was observed in treatment (50% medicinal plant + 50 % antibiotics) i.e. 43 mm. Least inhibition zone has been seen in treatment 1 (100% medicinal plant) i.e. 21 mm. Our results suggested that 7 isolated strains were *Klebsiella pneumoniae* and antimicrobial study revealed that 50% aqueous extract of *Murraya koenigii* and 50% Norfloxacin showed highest antibacterial activity against *Klebsiella pneumoniae*.

**Keywords:** *Murraya koenigii*, *Klebsiella pneumoniae*, Norfloxacin, Synergism, Antimicrobial activity.

#### INTRODUCTION

Literature suggests that plants are the richest resource of drugs of traditional systems of medicine, food supplements, folk medicines, pharmaceutical intermediates [1]. Scientifically the medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis [2]. Medicinal plants are a source of great medicinal value all over the world [3]. UTI often has been defined for research purposes, as its presence is indicated by presence of at least 10<sup>8</sup> colony-forming units (CFU) per liter in freshly voided urine, although symptomatic infection can occur with 10<sup>6</sup> CFU/L [4], and some would

argue for a cutoff of 10<sup>5</sup> CFU/L in symptomatic patients when urine can be cultured without delay [5].

The genus *Klebsiella* is grouping Gram-negative opportunistic pathogens frequently isolated from bacteraemia, pneumonia, urinary tract and soft tissue infections [6]. *Klebsiella pneumoniae* strains exhibit different virulence factors such as capsular polysaccharides, type 1 and type 3 adhesins, KPF-28 fimbriae, non fimbrial adhesins CF29K [7,8,9].

*Murraya koenigii*, commonly known as curry leaf or *kari patta* in Indian dialects, belonging to Family Rutaceae [10]. The *Murraya koenigii* plant is widely used as herb, spice, condiments and also used to treat various types of

ailments in Indian traditional system. World's about 80% population relies upon herbal products, because they have been considered as safe, effective and economical [11]. The leaves of plant are use as tonic, stomachic, carminative, internally in dysentery, vomiting and plant used as antihelminthic, analgesic, cures piles, allays heat of the body [11]. The growing resistance against antibacterial agents has generated a search for alternative antimicrobial treatments. In particular, the use of topical antibiotics is under discussion since it has been suggested that such an approach induces antibiotics resistance faster than the use of oral antibiotic [12,13]. Therefore, present study, we isolated *Klebsiella pneumoniae* from urine of infected patient and characterized on the basis of biochemical tests. Further, we selected *Murraya koenigii* to investigation for antimicrobial activity against *Klebsiella pneumoniae*.

## MATERIALS AND METHODS

**Isolation of microorganism:** *Klebsiella pneumoniae* is isolated from the Urine of UTI infected persons. Pathogen was isolated on MacConkey Agar medium. The plates were incubated at 37°C for 24-48 h.

**Characterization of pathogen:** Further, strains were purified and pure isolated strains were characterized according to Bergey's Manual of determinative bacteriology [14]. Only, *Klebsiella pneumoniae* strains were selected for present study.

**Plant materials:** Dry leavies of *Murraya koenigii* was used in present study.

**Preparation of aqueous extract:** 100g dried finely powdered leaf of *Murraya koenigii* was infused in distilled water until completely exhausted. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated and dried using rotary evaporator at 60°C. The final dried samples were stored at low temperature. 50 µg per ml aqueous solution of *M. koenigii* was prepared as stock.

**Preparation of Norfloxacin solution:** Norfloxacin drug is selected as control drug. 50 µg per ml aqueous solution of norfloxacin was prepared. This stock solution was store at cool temperature and used for further study.

**Sterilization and preparation of different concentration of extract:** The dried extracts were exposed to ultra violet light (UV) rays for 24h. Dry powder extracts were initially dissolved in 1ml of dimethyl sulfoxide (DMSO). Liquid extracts were sterilized using a membrane filter (0.45-micron sterile filter). Five combination of medicinal plant and antibiotics was prepared in present study i.e. 100 % medicinal plant + 0% antibiotics (Treatment 1), 75 % medicinal plant + 25%

antibiotics (Treatment 2), 50 % medicinal plant + 50% antibiotics (Treatment 3), 25 % medicinal plant + 75% antibiotics (Treatment 4) and 0 % medicinal plant + 100% antibiotics (Treatment 5). Norfloxacin antibiotic worked as control drug.

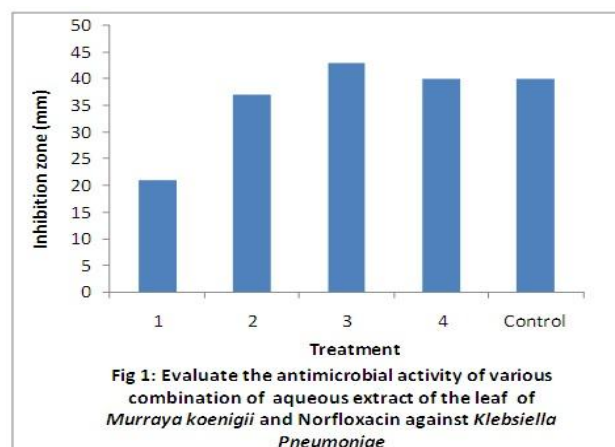
**Antibacterial activity test of various combinations:** The microorganism was activated by inoculating a loopful of the strain in muller hinton broth (30ml) and incubated on a rotary shaker. Then 0.2 ml of inoculum (inoculum size was  $10^8$  cells/ml as per McFarland standard) was inoculated into the molten Muller Hinton agar media and after proper homogenization it was poured into the sterilized Petri plate. For agar well diffusion method, a well was made in the seeded plates with the help of a sterilized cup-borer. 20µl test compound was introduced into the well and the plates were incubated at 37°C for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract [15].

## RESULTS AND DISCUSSION

Aim of present study to only isolated *Klebsiella pneumoniae*. Therefore, we selected 32 UTI patients for present study. These isolated strains were characterized on the basis of biochemical tests. *Klebsiella pneumoniae* strains did not show motility, no growth on Mannitol Salt Agar, no Gelatinase activity, no Hydrogen sulphide production (SIM Agar), no Hydrogen sulfide production (TSI), no Indol production test, no Catalase utilisation Test, no Oxidase Test. But showed growth on EMB Agar medium and MacConkey Agar medium, Citrate utilisation Test positive, Urea hydrolysis test positive. 80-90 % showed positive reaction in MR Test, VP Test and Starch hydrolysis. *K. pneumoniae* strains fermented various sugars such as Glucose, Sucrose, Mannitol, Lactose, Xylose and Maltose (Table 1). Similar observations were mentioned [14] and Cowan and Steel's manual for the identification of medical bacteria [16]. Only 7 seven *K. pneumoniae* strains were isolated and further, evaluated the impact of medicinal- Norfloxacin on growth of *K. pneumoniae*. Further, 5 treatments had prepared and antibacterial activity was analyzed. Maximum inhibition zone was observed in treatment 3 (50% medicinal plant + 50 % antibiotics) i.e. 43 mm. Least inhibition zone has been seen in treatment 1 (100% medicinal plant + 0 % antibiotics) i.e. 50 µg/ml of aqueous extract of *M. koenigii* concentration i.e. 21 mm. Further, treatment 5 (0% medicinal plant + 100 % antibiotics) i.e. 50 µg/ml norfloxacin showed 40 mm inhibition zone (Fig 1). These results suggested that aqueous extract of 5 treatments of *Murraya koenigii* and norfloxacin showed antimicrobial activity. Similarly, medicinal plants for antimicrobial activity of *Asphodelus tenuifolius* Cav., *Asparagus racemosus* Willd., *Balanites aegyptiaca* L., *Cestrum*

*diurnum* L., *Cordia dichotoma* G. Forst, *Eclipta alba* L., *Murraya koenigii* (L.) Spreng., *Pedaliium murex* L., *Ricinus communis* L. and *Trigonella foenum graecum* L. against certain pathogens and all eight medicinal plants (*A. tenuifolius*, *A. racemosus*, *B. aegyptiaca*, *E. alba*, *M. koenigii*, *P. murex*, *R. communis* and *T. foenum graecum*) showed significant antimicrobial activity ( $P < .05$ ) against most of the isolates [17]. Similarly, root extracts of *Murray koenigii* (Linn) spreng. possess antimicrobial properties against certain micro-organisms [18]. Other research supported that medicinal plants have antimicrobial activity. 30 mg/ml aqueous extract of *Tribulus terrestris* showed  $26.9 \pm 0.5$  mm inhibition zone against *E. coli* [19]. Combination of 50% antibiotics and 50% aqueous extract of medicinal plants showed more inhibition zone as compared to individual. Previously, the evidence of *in vitro* synergism of antibiotics and medicinal plants could be useful in selecting most favourable

combinations of antimicrobials for the practical therapy of serious bacterial infections [20].



**Table 1. Biochemical characterization of *Klebsiella pneumoniae***

Gram staining, Motility test, Growth on Mannitol Salt Agar, Gelatinase activity, Hydrogen sulphide production (SIM Agar), Hydrogen sulfide production (TSI), Indol production test, Catalase utilisation Test, Oxidase Test	-ve
Growth on EMB Agar medium, Growth on MacConkey Agar	+ve
Citrate utilisation Test, Urea hydrolysis test	
MR Test, VP Test, Starch hydrolysis	D
Sugar fermentation test:- Glucose, Sucrose, Mannitol, Lactose, Xylose, Maltose	+ve

D= maximum strain showed positive results

## CONCLUSION

All the biochemical tests confirmed that isolated strains were *Klebsiella pneumoniae*. Further, antimicrobial study revealed that aqueous extract of medicinal plants

showed antibacterial activity against *Klebsiella pneumoniae* and 50 % medicinal plant + 50% Norfloxacin showed highest inhibition zone against *Klebsiella pneumoniae*.

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