Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Suppl 1, 2012 ISSN - 0974-2441

Research Article

EFFECT OF POLY HERBAL FORMULATION AGAINST *KLEBSIELLA PNEUMONIA* CAUSING PNEUMONIA IN CHILDREN'S

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Received: 26 September 2011, Revised and Accepted: 01 January 2012

ABSTRACT

Pneumonia is an inflammatory condition of the lung, especially of the alveoli (microscopic air sacs in the lungs) associated with fever, chest symptoms, and consolidation on a chest radiograph. While typically caused by an infection there are a number of non-infectious causes. Infectious agents include: bacteria, viruses, fungi, and parasites. Typical symptoms include cough, chest pain, fever, and difficulty breathing. With increasing use of antibiotics these bacteria have evolved itself into the way that it is becoming more and more antibiotic resistant and case of ESBL producing bacteria has been reported in genus Klebsiella. The aim of this work is based on assessing the prevalence of the bacteria *Klebsiella pneumonia* in the patients of pneumonia, and to check the activity of medicinal plants and their effect in synergism with allopathic drugs against the organism. Fruit parts and aerial parts of five medicinal plants of *Dasmodium Gangeticum, Nelumbo Nucifera, Canabis, Sesame White* and *Sesame Black* have been used in the form of three different extract i.e. *Methanolic, Ethanolic and Aqueous* and their activity was assessed.

Key words: *Klebsiella pneumonia*, Ceftazidime, Ofloxacin, Gentamicin, Ciprofloxacin.

INTRODUCTION

K. pneumoniae is a gram negative bacterium. It is facultative anaerobic. It is rod-shaped and measures 2 µm by 0.5 µm. In 1882, Friedlander C. Uber first discovered *Klebsiella* to be a pathogen that caused pneumonia (Friedlander C, 1882). Many hospital cases around the world have been linked to *K. pneumoniae*. Therefore, more studies of the strains were important and performed. The bacterium was isolated and sequenced from a patient in 2004. *K. pneumoniae* is commonly found in the gastrointestinal tract and hands of hospital personnel (Podschun, R 1998). The reason for its pathogencity is the thick capsule layer surrounding the bacterium. It is 160 nm thick of fine fibers that protrudes out from the outer membrane at right angles (Lawlor, M., 2005). Another site on the human body that this bacterium can be found is the nasopharynx. Its habitat is not limited to humans but is ubiquitous to the ecological environment. This includes surface water, sewage, and soil (Brisse, S., 2001). It is clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae (Postgate J ,1998).The detailed mechanisms of this action are not known yet, though much information about the capsules is accumulating from chemical, physiological, and immunological studies. Many types of bacteria produce extracellular polysaccharides (EPSs).*Klebsiella* are non-motile, rod-shaped, aerobic bacteria that possess a prominent polysaccharide capsule. The diseases caused by *K. pneumoniae* can result in death for patients who are immunodeficient. Differences in the diseases are determined by the different virulence factors. For example, mucoid phenotype varies as the strains for mucoid vary (Victor L. Yu, 2007). CPS and LPS O side chain are two of the most important virulence factors of *K. pneumonia* (Cortés, G.,*etal,* 2002). They serve to protect the bacterium from phagocytises by the host. Treatment is done by antibiotics such as clinafloxacin (Sylvain Brisse, *etal,* 2000). But, there is an increasing amount of antibiotic-resistance strains. *K. pneumoniae* has been considered a respiratory pathogen that causes Pneumoniae, the symptoms include: toxic presentation with sudden onset, high fever, and hemoptysis.The medicinal value of plants has assumed a more important dimension in the past few decades. This is due largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potentials (Okigbo *et al*., 2009). The use of plant extracts in traditional medicine is a worldwide practice. Medicinal plants form the basis of primary health care for majority of the people living in the rural and remote areas in Nigeria and other third world countries (Awosika, 1993).The organs or parts used in these plants vary from one plant to the other and these include the leaves, barks, roots, stems, flowers, fruits and even the seeds.

MATERIALS AND METHODS

Collection of sample

In the present investigation, the total 130 sputum samples of children (age 3-5) and swabbed sample of ventilator were collected from Civil Hospital and Puran Hospital of Paonta Valley. The sample were collected aseptically in sterile 50 ml Oakridge tubes and inoculated in nutrient broth for 24 hrs at 37^0 C. The isolates were isolated from patients who had a minimum of 5 days stay in the hospital prior to sample collection and the patient must be free of infection at the time of hospital admission**.**

Isolation and Identification of Bacterial strain

Recovered isolates were identified with Colony Characteristics, Gram Staining and Biochemical Rxn. (Holt *et.al*, 1994. Cappuccino and Sherman Microbiology (A Laboratory Manual 7th edition.).

ANTIBIOTIC SENSITIVITY TESTS

Antibiogram study of the predominant bacterial isolates against commercial antibiotics like Ceftazidime, Carbencillin, Ceftizoxime, Amikacin, Cefotaxime, Ofloxacin, Gentamicin, Amoxicillin, Ciprofloxacin, Cephalexin, Chloramphenicol, Tetracycline were used against the pneumonia causing bacteria *Klebsiella* was made by Kirby-Bauer disc diffusion method (Cappuccino 1999).

COLLECTION OF PLANT MATERIAL

Fresh fruits of *Nucifer Indicum, Desmodium Gangeticum, Sesame Indicum White seeds, Sesame Indicum Black seeds* and *Canabis* were collected from surrounding areas of Paonta Sahib (H.P.) and identified by Botanical survey of India, Dehradun. For the preparation of plant extract the fruits were dried under shade and stored into fine powder using electric blender. 50 mg of dried powder sample was taken and extracted by Soxlet apparatus using distilled water, methanol and ethanol separately. The solvents were removed under reduced pressure in a rotary evaporator until they become completely dry. Filtrates were preserved at 4^0 C.

DETERMINATION OF THE ANTIMICROBIAL ACTIVITY

From the dry filterate material, the 500mg/ml dilutions of plant paste were prepared for antibacterial assay. The modified agar well diffusion method was employed to determine the antimicrobial activity of plant extracts. Three different extractions (Aqueous, methanol and ethanol) were taken. In agar well diffusion method, 100µl of the extracts (500mg/ml) were poured in to the wells. All the agar plates incubated at 37^0C. If antimicrobial activity was

present on plates, it was indicated by an inhibition zone. The diameters of the inhibition zone were measured in millietre after 24hrs. The experiments were conducted in triplicate for each test. The mean of triplicate result were taken.

DETERMINATION OF SYNERGESTIC EFFECT OF PLANT EXTRACT AND ANTIBIOTIC DISCS

Antibacterial activity was measured using a well diffusion method according to the clinical and Laboratory Standard Institute (formerly, the NCCLS). Briefly petri plates containing approximately 25-30ml of Mueller Hinton Agar medium were inoculated using a cotton swab in with a 4-6 hour old culture of bacterial strain. Wells (6mm diameter) were punched in the agar and filled with 30µlof plant extract or antibiotics and in case of synergistic effect 30µl of plant extract in the well along with the disc were placed on the plate. Replicate of each plate was done. The plates were incubated at 37^{o}C for 18-24 hrs. The antibacterial activity was assessed by measuring the inhibition zone diameter (mm) around the well and the antibiotic discs. The average of total three replicates of the plates of each plate with antibiotic disc, plant extract and the synergetic effect of both the plant extract has been calculated. Synergism effect was considered when combinations exhibited with enlargement of combined inhibition zone size by 5mm. (National Committee for Clinical Laboratory Standards, 1993).

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC is defined as the lowest concentration of extracts that completely inhibits the growth of the microorganism in 24 hrs (Thongson C, 2004). The MIC for the extracts was determined by broth dilution method. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract. A control experiment was run in parallel to study the impact of the solvent alone (without plant extracts) on growth of the five test organisms. Methanol, Ethanol and water were diluted in a similar pattern with sterile nutrient broth followed by inoculation and incubation.

RESULTS AND DISCUSSION

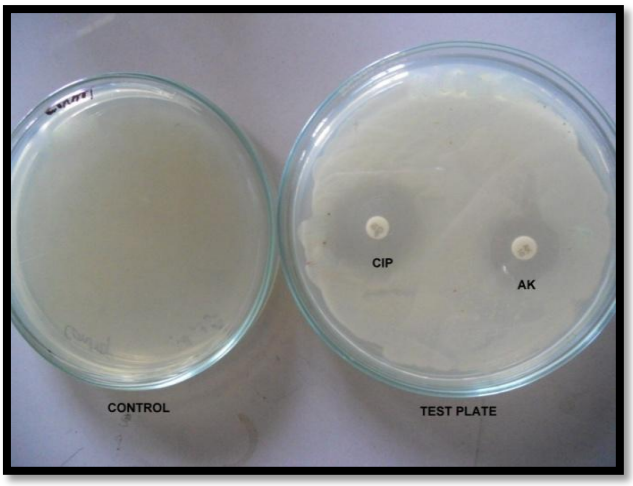
Prevalence of *Klebsiella pneumonia* the bacterium in total samples:

Total recovered isolates were 105 out of which 70 *Klebsiella Pneumonia* were isolated during the study period in 130 sputum and ventilator samples. So the prevalence of recovered isolates was 54%.The incidence of *Klebsiella Pneumonia* isolates varies according to countries, regions or even hospitals. In 50-60 years age group, the most prevalent organism was also *Klebsiella pneumoniae*; 26 out of 80 (32.5%) followed by 07 out of 38 (19%) for the age group of 30-50 years. The count of recovered isolate was negligible as the ventilator masks applied to the patient were constantly sterilized by disinfectants; hence the recovered isolates in case of ventilator were 3 out of 10 (3%).

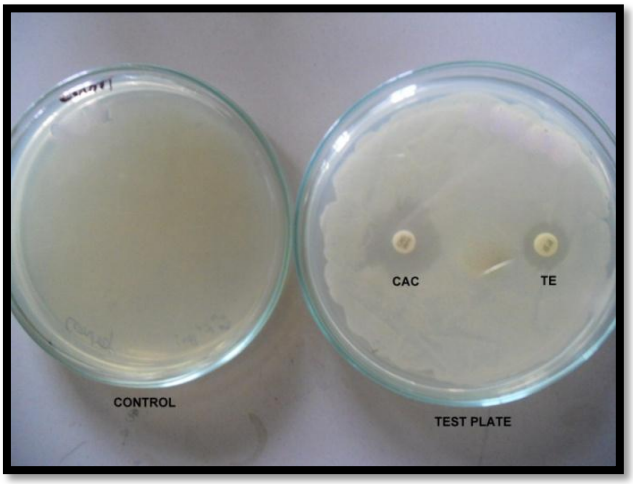
Total Samples	Recovered isolates	*Klebsiella Pneumoniae*
110	85	50

Antibiotic Sensitivity Test

The antibacterial activity of 12 commercial drugs was assayed by Kirby-Bauer disc diffusion method. Approximately 80% of the recovered isolates showed the sensitive activity against Ceftazidime, ofloxacin, gentamycin, ciprofloxacin, and resistant activity against Carbencillin, Ceftizoxime, Amikacin, Cefotaxime, Amoxicillin, Cephalexin, Chloramphenicol and Tetracycline. The diameter of inhibition zones observed in 4 drugs namelyCeftazidime(16mm),ofloxacin(20mm),gentamycin(22mm),ciprofloxacin(20mm). Whereas Carbencillin, Ceftizoxime, Amikacin, Cefotaxime, Amoxicillin, Cephalexin, Chloramphenicol and Tetracycline showed very small zone of inhibition against the growth of *Klebsiella pneumonia*, hence considered to be resistant against these drugs. The etiological picture of lactation mastitis and sensitivity of its causative agent to some antibiotics were studied.

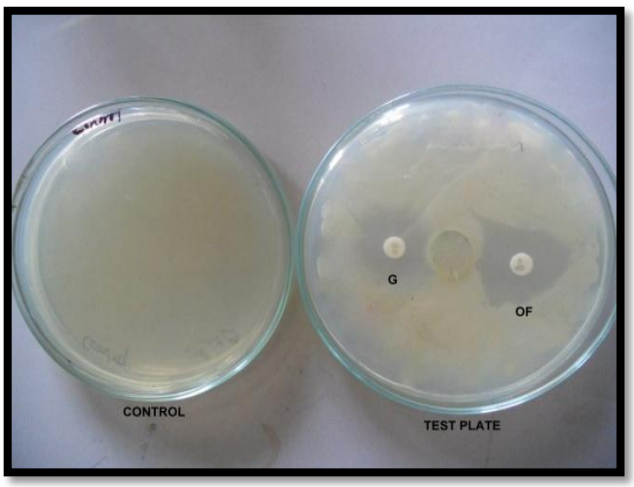


Antibacterial activity of Ciprofloxacin (CIP^{10}) [20mm] and Amikacin (AK^{10}) [12mm] against the bacterial isolate .



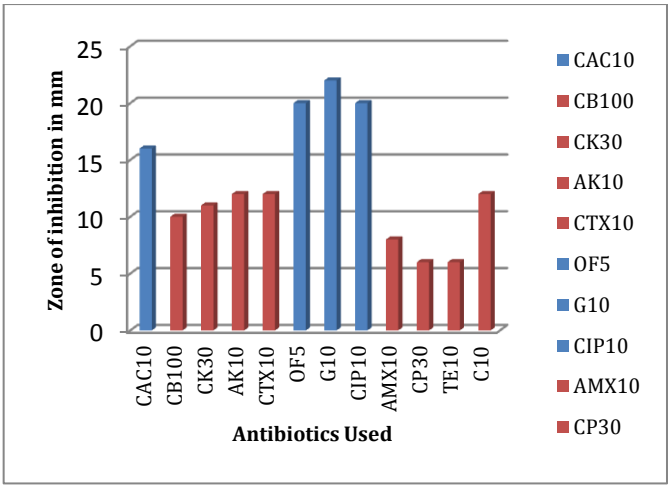
Antibacterial activity of Ceftazidime (CAC^{10}) [16mm] and Tetracycline (TE^{10}) [6mm] against the bacterial isolate.

Antibacterial activity of Gentamycin (G^{10}) [20mm] and



Ofloxacin (OF^{10}) [22mm] against the bacterial isolate

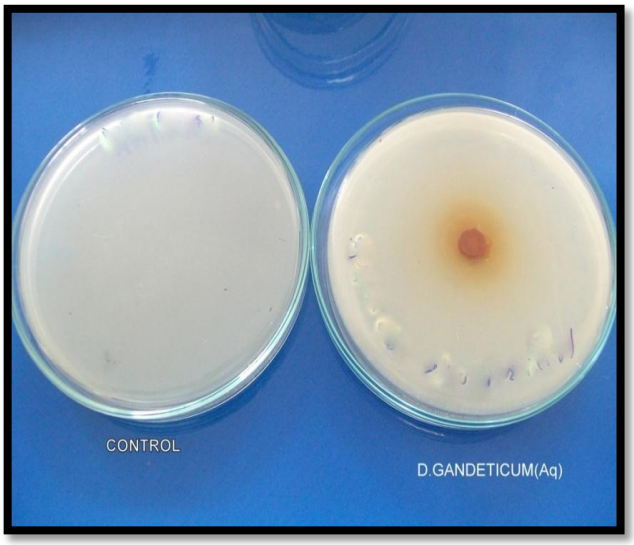
Out of 12 antibiotics used the pictures having the activity if 4 antibiotics have been shown along with the diameter of the antibiotic showing the sensitivity of bacteria against the antibiotics. The activity was assessed by applying the plot of activity of the 12 antibiotics against the number of isolates.



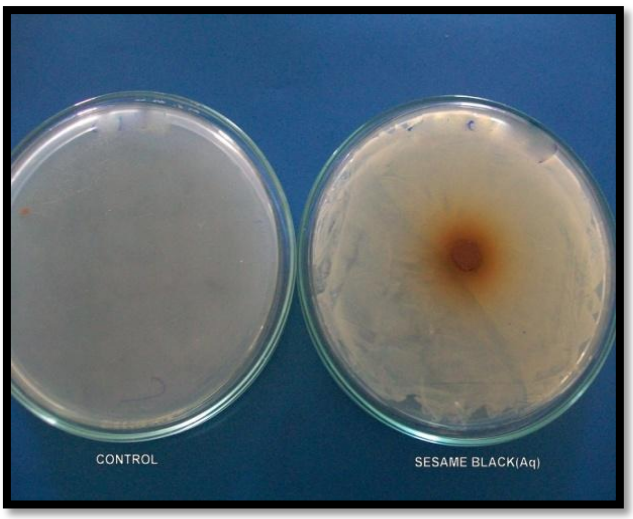
Antibiotic sensitivity of *Klebsiella pneumonia* against the disc used (Bars with red colour shows the resistance of organism towards the antibiotics and Blue colour bars shows the sensitivity of organism against the antibiotics used).

ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANT

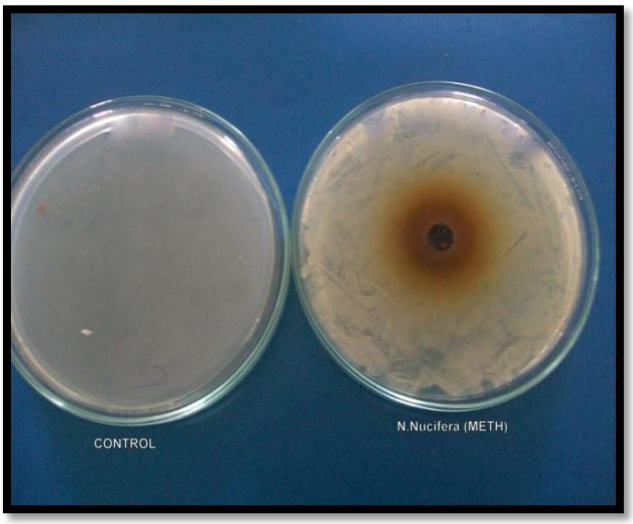
The results of antimicrobial activity of ethanol, methanol and aqueous extracts of *Dasmodium Gangeticum, Nelumbo Nucifera, Canabis, Sesame White* and *Sesame Black* by agar well diffusion method. Our study showed that aqueous extract gave maximum zone of inhibition against *Klebsiella pneumoniae* i.e., *Nelumbo Nucifera* (16mm), *Sesame White* (09mm),*Sesame Black*(12mm), *Dasmodium Gangeticum*(12mm),*Canabis*(23mm).Followed by methanolic extract *Nelumbo Nucifera* (24mm), *Sesame White* (00mm), *Sesame Black* (09mm), *Dasmodium Gangeticum* (07mm), *Canabis* (00mm). Ethanolic extract gave following zone of inhibition i.e., *Nelumbo Nucifera* (06mm), *Sesame White* (00mm), *Sesame Black* (09mm), *Dasmodium Gangeticum* (06mm), *and Canabis* (00mm). Highest mean diameter of inhibition zone was produced by the methanolic extract of *Nelumbo Nucifera* (24mm), against *Klebsiella pneumoniae* and followed by aqueous extract of *Sesame White*(09mm),*Sesame Black*(12mm), *Dasmodium Gangeticum* (12mm),and *Canabis* (23mm) extracts.



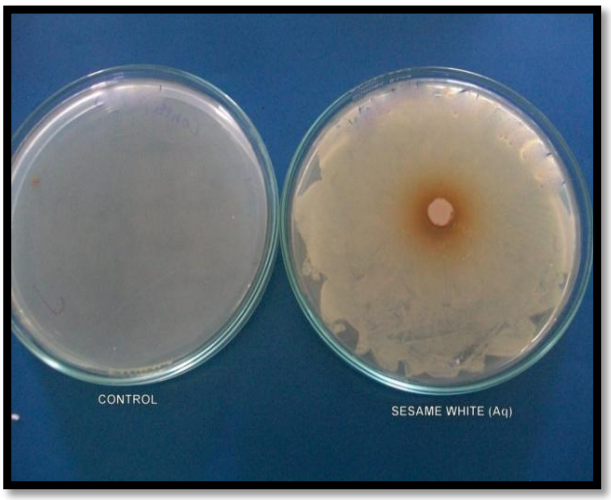
Antibacterial activity of *D. gangeticum (Aq)* against *Klebsiella pneumoniae* (12mm)



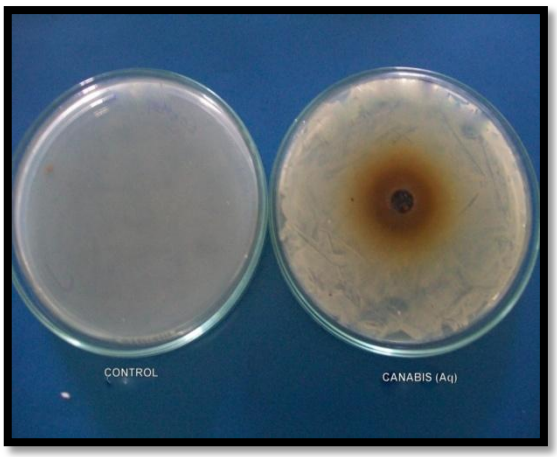
Antibacterial activity of *Sesame Black* aq. against *Klebsiella pneumonia* (12mm)



Antibacterial activity of *N.Nucifera* methanolic extract against *Klebsiella* (24mm)



Antibacterial activity of *Sesame White* aq. against *Klebsiella pneumonia* (9mm)



Antibacterial activity of *Cannabis* Aq. against *Klebsiella pneumonia* (23mm)

Table1: The antibacterial activity of plant extracts.

Plants	Aqueous extract	Methanolic extract	Ethanolic extract
N.Nucifera	16 mm	24 mm	00 mm
Sesame White	09 mm	00 mm	00 mm
Sesame Black	12 mm	09 mm	09 mm
D.Gangeticum	12 mm	07 mm	06 mm
Canabis	23 mm	00 mm	00 mm

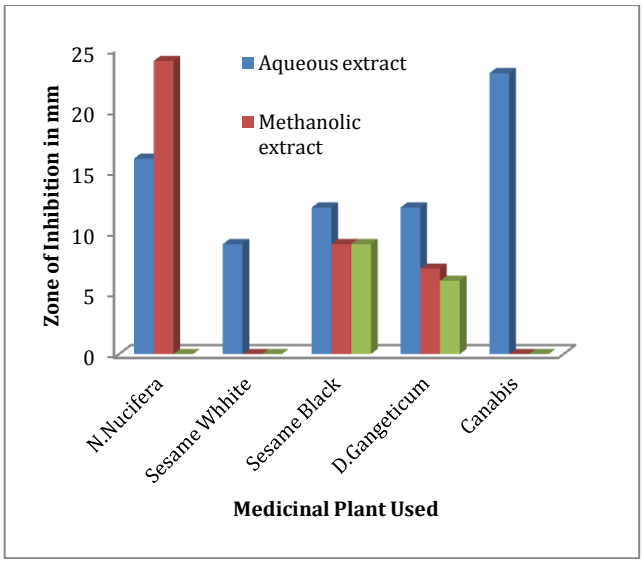
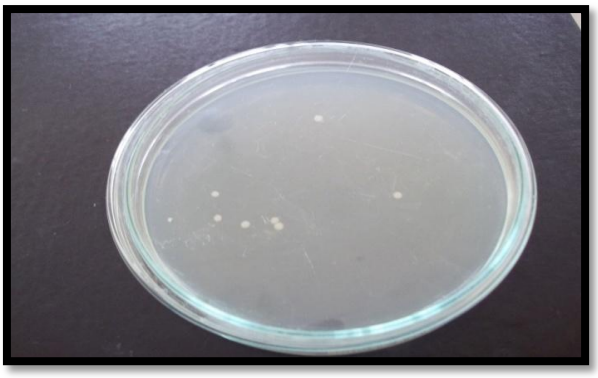


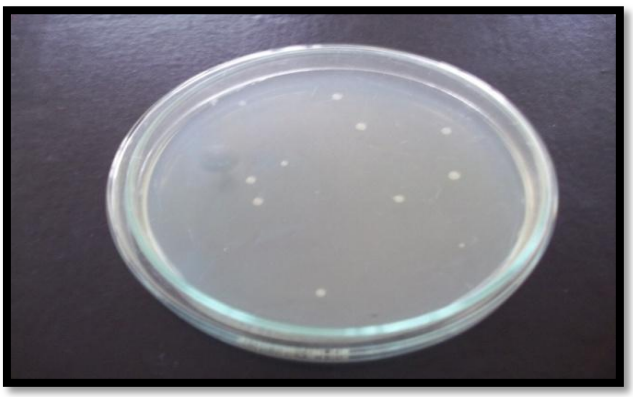
Chart 1: Antibacterial activity of plant extracts

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

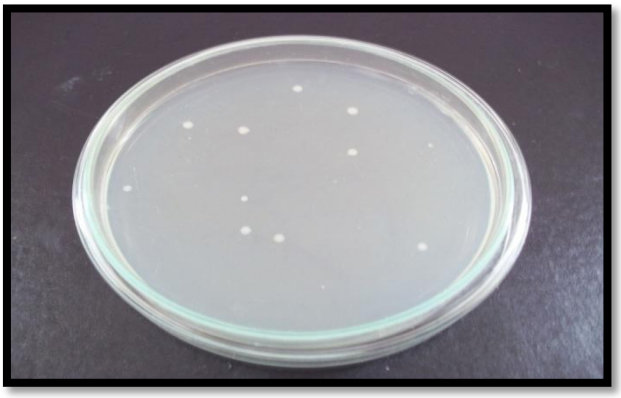
MIC is defined as the lowest concentration of extracts that completely inhibits the growth of the microorganism in 24hrs. MIC of five medicinal plants i.e. *N. Nucifera, Canabis, Sesame White, Sesame Black* and *D. Gangeticum* were observed with different extracts, namely aquoues, ethanolic and methanolic which were showing maximum activity in against the organism. The dilutions were made by taking 800µl of the plant extract with concentration of 512mg/ml, 100µl of distilled water and 100µl of broth culture thus making the total concentration of 1ml solution. The spreading of the culture on MHA plates was done after 8 hr incubation and later the plates were kept for an incubation period of 24 hrs. The MIC of the extract was determined after the colony counting on the plate was done and the count was less than 10.



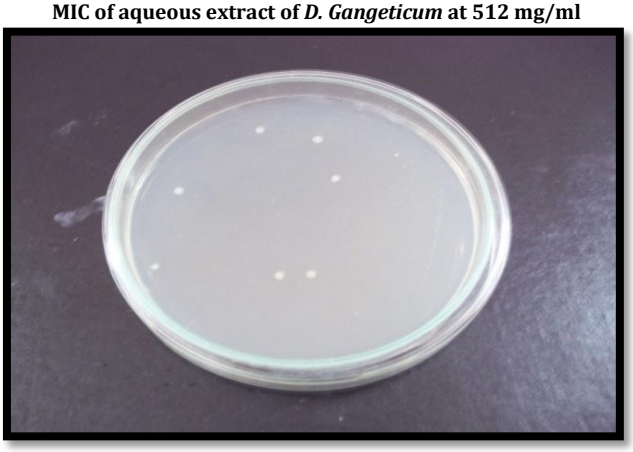
MIC of methanolic extract of *N.Nucifera* 256 mg/ml



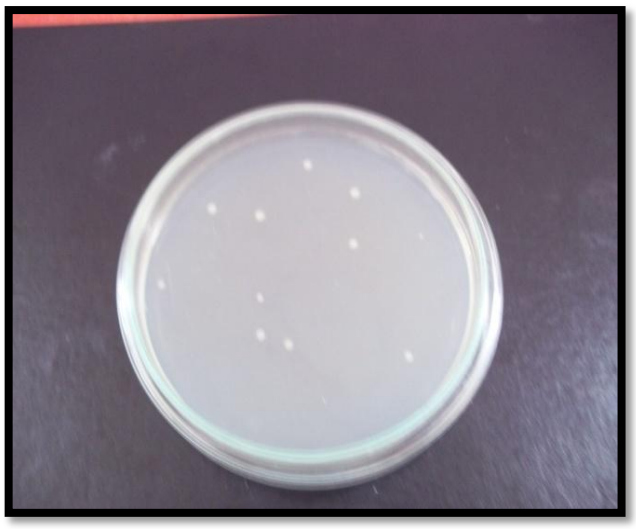
MIC of aqueous extract of *Sesame White*at 512mg/ml



MIC of aqueous extract of *D. Gangeticum* at 512 mg/ml



MIC of aqueous extract of *Cannabisat* 256 mg/ml



MIC of aqueous extract of *Sesame Black*at512mg/ml

MIC of five medicinal plants		
Plants	**Extract**	**MIC**
Nelumbo Nucifera	Methanolic	256mg/ml
Sesame White	Aqueous	512mg/ml
Desmodium Gangeticum	Aqueous	512mg/ml
Cannabis	Aqueous	256mg/ml
Sesame Black	Aqueous	512mg/ml

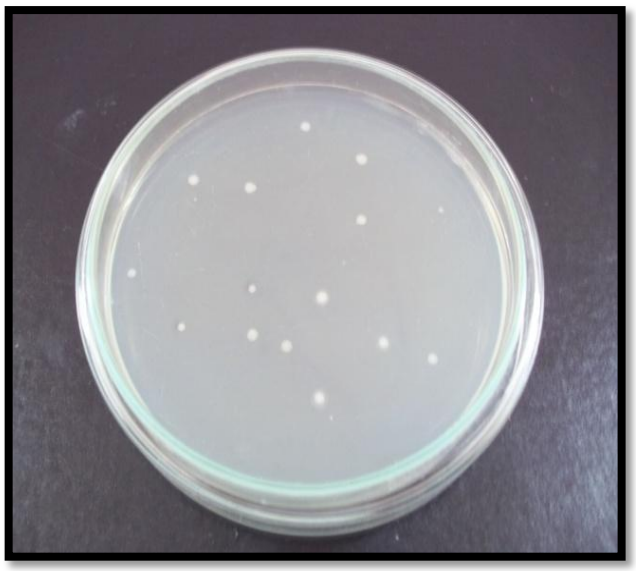
By combining the above MIC of five medicinal plants we formulated the drug against the recovered isolates causing pneumonia disease

256mg + 512 mg + 512 mg + 256 mg + 512 mg = 2048 mg

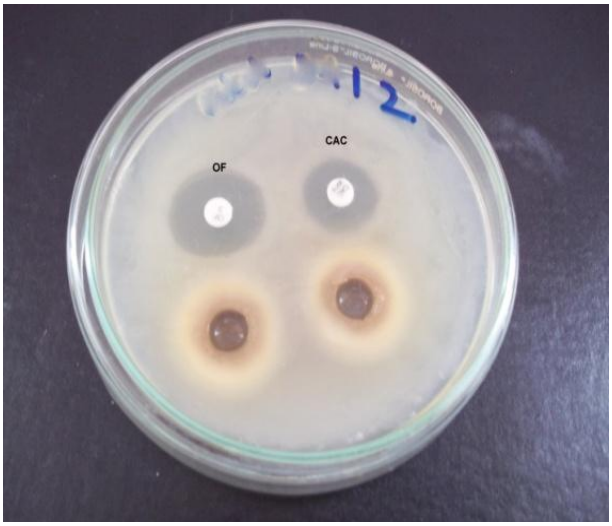
2048 mg /ml	1024 mg /ml	512 mg /ml	256 mg /ml	128 mg /ml	64 mg /ml	32 mg /ml	16 mg /ml	8 mg /ml	4 mg /ml
-	-	-	+	+	+	+	+	+	+

At the concentration of 2048 mg/ml the dilution were made in the same manner as the above method and final MIC was determined.

SYNERGIC EFFECT OF PLANT EXTRACT AND ANTIBIOTICS:

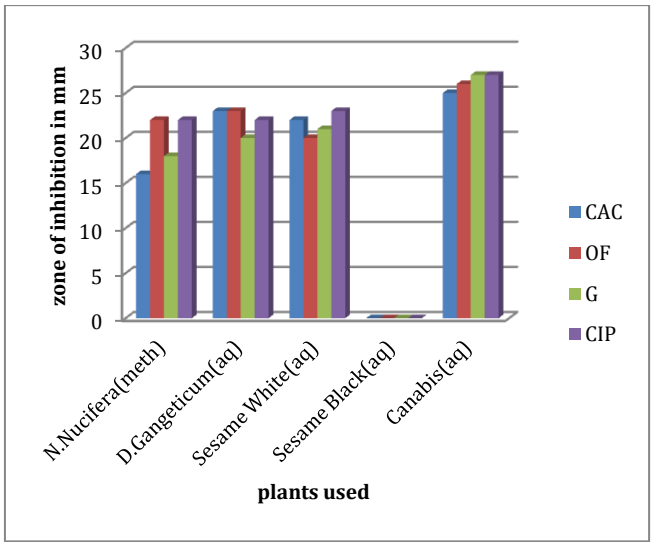


MIC 512mg/ml

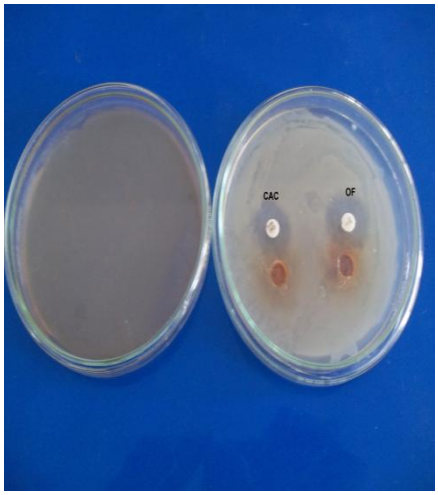


Ceftazidime(CAC^{10})[25mm],Ofloxacin(OF^{5})[26mm], with CANNABIS aq.

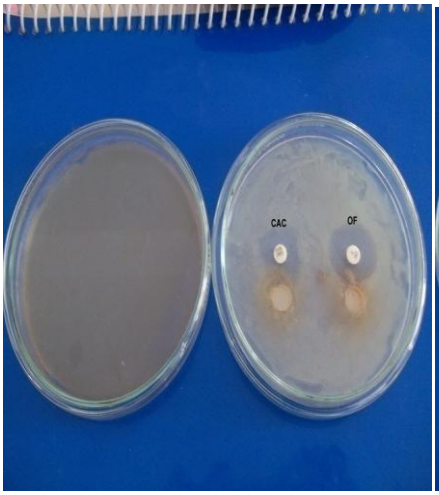
Gentamicin(G^{10})[27mm],Ciprofloxacin(CIP^{10})[27mm] with *Cannabis aq*)



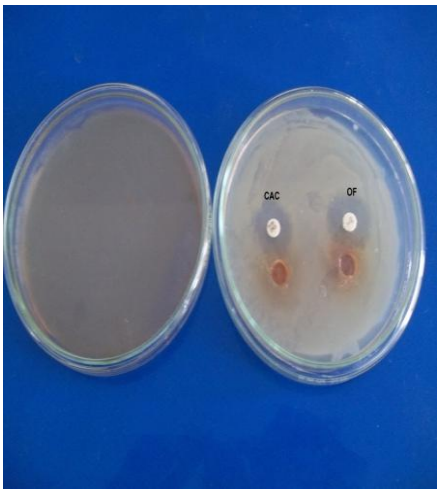
Synergic activity of plant extract and the antibiotics used.



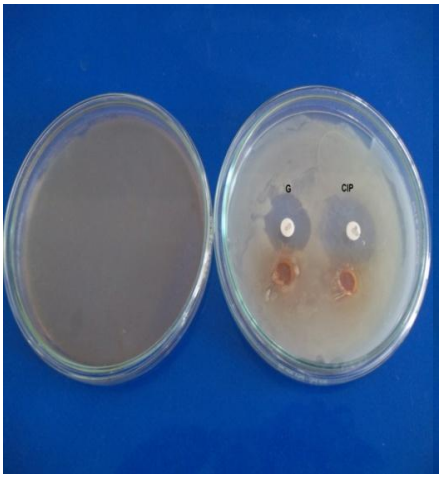
a)Ceftizidime(CAC^{10})[23mm], Ofloxacin(OF^{5})[23mm of



Ceftizidime(CAC^{10})[22mm], Ofloxacin(OF^{5})[20mm],



Ceftizidime(CAC^{10})[22mm], Ofloxacin(OF^{5})[20mm]



(b)Gentamicin(G^{10})[20mm], Ciprofloxacin(CIP^{10})[22mm] with *D.Gangeticum* (aq)

Gentamicin(G^{10})[21mm], Ciprofloxacin(CIP^{10})[23mm] with *Sesame White*(aq)

Gentamicin(G^{10})[21mm], Ciprofloxacin(CIP^{10})[23mm] with *Sesame White*(aq)

CONCLUSION

Pneumonia is the most prevalent disease and one of the reasons for high mortality rates among the infants and the people of 40-50 years of age group. The incidence of *Klebsiella pneumonia* isolates varies according to countries, regions or even hospitals. The antibacterial activity of 12 commercial drugs was assayed by Kirby-Bauer disc diffusion method. Approximately 80% of the recovered isolates showed the sensitive activity against Ceftazidime, ofloxacin, gentamycin, ciprofloxacin, and resistant activity against Carbencillin, Ceftizoxime, Amikacin, Cefotaxime, Amoxicillin, Cephalexin, Chloramphenicol and Tetracycline. The diameter of inhibition zones observed in 4 drugs namely Ceftazidime (16mm), ofloxacin (20mm), gentamycin (22mm), ciprofloxacin (20mm). The results of antimicrobial activity of ethanol, methanol and aqueous extracts of *Dasmodium Gangeticum, Nelumbo Nucifera, Canabis, Sesame White* and *Sesame Black* by agar well diffusion method. Our study showed that aqueous extract gave maximum zone of inhibition against *Klebsiella pneumonia* i.e., *Nelumbo Nucifera* (24mm), *Sesame White*(09mm), *Sesame Black* (12mm), *Dasmodium Gangeticum*(12mm), *Canabis* (23mm) against *Klebsiella pneumonia.* MIC is defined as the lowest concentration of a extracts that completely inhibits the growth of the microorganism in 24hrs. MIC of five medicinal plants i.e *N.Nucifera, Canabis, Sesame White, Sesame Black* and *D.Gangeticum* were observed with different extracts, namely aqueous, ethanolic and methanolic which came out to be 512mg/ml for the concentration of 2048mg/ml. Study of the synergic effect helped to understand the combined activity of the plant and antibiotics. Applying the plant extract and antibiotic discs collectively showed enhanced activity in case of four out of five plants. In case of *sesame black* the zone of inhibition was shown only by antibiotic discs. In our study the synergic effect was applied with sole purpose enhancing the antibacterial activity of antibiotics with help of plant extract. The results were comparable to standards fixed by National Committee for Clinical Laboratory Standards, 1993. As they were 4-5 mm more as compared to the individual activity of plants and antibiotics. *N. Nucifera, Canabis, Sesame White, Sesame Black* and *D.Gangeticum* showed maximum of 22 mm, 23 mm, 23 mm and 27 mm zone with antibiotics against organism. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. Natural products of higher plants possess a new source of antimicrobial agents with possibly novel mechanisms of action. Systematic screening of them may result in the discovery of novel active compounds.

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