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# Using Gas Chromatography to Examine Klebsiella's Bioactive Natural Compounds and Antimicrobial Metabolites Profile

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**Abstract:** The properties of bacterial secondary metabolites are their low molecular mass and their unusual structure. Structurally different metabolites with a wide spectrum of biological activity, such as antibacterial compounds, have been found. The methanolic extract of Klebsiella pneumoniae contained 39 different bioactive compounds. GC-MS analysis of Klebsiella pneumoniae revealed the existence of the 3-Methoxybenzaldehyde semicarbazone, carboxaldehyde , 1,5,5-Trimethyl-6-methylene-cyclohexene, Paromomycin , 9-Borabicyclo[3.31]nonane , 9-mercapto- , Benzenemethanol , Acetamide , N-(6-acetylaminobenzothiazol-2-yl)-2-(adamantan, rin-6-carboxylic acid , N-(2,5-Dicyano-3,4-dihydro-2H-pyrrol-2-yl)-acetamide, 3-Cyclohex-3-enyl-propionic acid, Eicosanoic acid , 9,9-dimethyl-, Dithiocarbamate, dl-Homocysteine, 2-(2-Furyl-4-O-difluoromethyluracil, Uric acid, hexahydro-,12-Methyl-oxa-cyclododecan-2-one, Phthalic acid , butyl undecyl ester, 9,12,15-Octadecatrienoic acid , 2,3-bis(acetyloxy)propyl ester, methyl ester, 12-Dimethylamino-10-oxododecanoic acid , Octahydrochromen-2-one, N-glycyl-,2H-Oxecin-2-one , 3,4,7,8,91,10-hexahydro-4-hydroxy-10-meth, 2-amino-4-(2-ph, Dec-9-en-6-oxo-1-ylamide, 3,6,12-Trimethyl-1,4,7,10,13,16-hexaaza-cyclooctadecane, 2,5-Piperazinedione ,3,6-bis(2-methylpropyl)-, 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1. Bacterial products (bacterial metabolites produced by Klebsiella pneumoniae) exhibited antibacterial activity in clinical pathogens from 4.09±0.013 to 4.00±0.203; bacterial products of streptomycin antibiotics was 1.08±0.200 to 0.98±0.166; and drugs of Kanamycin was 1.02±0.180 to 1.82±0.200. K. pneumoniae is known to produce a number of secondary metabolites that show remarkable biological activity. The importance of bioactive chemicals in the process of producing pharmaceutical medicines, which are used to cure several diseases, makes its purification using bacteria crucial, and this is one of the potential applications.

**Keywords:** Klebsiella's Bioactive, Compounds, Metabolites Profile.

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## INTRODUCTION

Klebsiella pneumoniae is a rod-shaped, nonmotile, facultative anaerobic, Gram-negative bacteria that can ferment lactose. It is a mucoid lactose fermentor which forms on MacConkey agar, as described in the literature [1-3]. Klebsiella bacteria normally have two types of cell surface antigens. O antigen is one of nine different kinds of lipopolysaccharide (LPS) [4]. Secondly, K antigen is a capsular polysaccharide and there are approximately 80 different types of K antigen [5-7]. Both are used in serogrouping and are responsible for pathogenicity. Nitrogen fixation and the relationship between K. pneumoniae, a free-living diazotroph that has intrigued both scientists and farmers because of its potential to increase crop yields under agricultural conditions [8], led us to choose it. The majority of cases of pneumonia in the community are associated with Klebsiella bacteria either as bronchopneumonia or bronchitis. These people are at increased risk for developing pleural adhesions, empyemas, cavitations, and lung abscesses. Despite antibiotic treatment, there is 50% mortality rate. Alcoholics dying with bacteremia have more than a 100% mortality rate [9]. This bacteria, commonly found in the mouth, gut and skin of humans and animals, can cause significant damage to the lungs. Its target is the alveoli of the lungs; this can lead to red sputum. It is a bacterium of the Klebsiella spp., the most

important species in a clinical setting of Klebsiella spp.It is a Klebsiella bacterium and is the most important species from a clinical scenario of Klebsiella spp. There is evidence of K in human clinical specimens as well. oxygen and potassium. Influenza of the nasal passages. The nosocomial infection caused by Klebsiella species has recently attracted great attention in terms of public health. Klebsiella infections include lower biliary system infections, surgical incision sites infections, and urinary tract infections as well as pneumonia [10]. Infections may manifest as wound infections, osteomyelitis, meningitis, bacteremia, septicaemia, pneumonia, thrombophlebitis, UTI, cholecystitis, diarrhoea, URTI and many other clinical disorders may present. Patients undergoing any kind of intrusive device (including newborn wards, urinary catheters and respirators) have a higher risk of infection [11–14]. Prescribing antibiotics is another factor that helps spread Klebsiella bacteria in healthcare facilities. Both sepsis and septic shock can be caused by bacteria that have entered the bloodstream. A vast group of Gram-negative bacteria are known as Enterobacteriaceae. More than 100 species and 30 genera have since been added to Rahn's 1936 proposal. Beyond the family level it is somewhat controversial whether to place it in the order Enterobacterales, the class Gamma-proteobacteria, and the phylum Pseudomonadota. The Enterobacteriaceae group (enterics) comprises many common diseases (like Salmonella, Escherichia coli, Klebsiella and Shigella) as well as a lot of harmless symbionts. Other members of this family are Enterobacter and Citrobacter which are also pathogenic. The Enterobacteriaceae family, also known as "enteric bacteria" or enterobacteria, are bacteria that are commonly found in the gut. Pathogenic bacteria are germs that can make you sick. The focus of this work is on pathogenic bacteria. Most bacteria species are beneficial and harmless, and a few bacteria can cause infectious diseases. Only less than a hundred of such dangerous species are believed to exist in the midst of humans. However, there are thousands of species in the gut flora [12]. There is a big diversity of microorganisms present in the human body all the time. Some of these bacteria are found on and in the skin and mucous membranes, and provide a beneficial commensal status. The other category of bacteria, saprophytes, are generally found in places such as soil and rotting organic material. Blood and other physiological fluids have enough nutrients to sustain the growth of a wide variety of bacteria. The body's defence mechanisms, such as the immune system, help it to fight against the entry of various pathogens and prevent them to cause damage to the tissues. Harmful bacteria can outsmart the body's natural barriers and enter into bodily systems that are normally free of bacteria, including the blood stream. Rarely may a healthy individual get infected with an infectious germ; most infections occur when the body's defences are weak because of anything like a wound, drunkenness, chills, fatigue, or starvation. In many cases it is necessary to distinguish between infection and colonisation, which is not associated with dangerous bacteria [13]. Pathogenic bacteria such as those responsible for pneumonia (Staphylococcus, Streptococcus and Pseudomonas) and for food poisoning (Shigella, Campylobacter and Salmonella) are a worldwide health problem. Other diseases caused by pathogenic microbes are typhoid fever, diphtheria, syphilis, leprosy and tetanus [14]. Microorganisms that cause disease are the main cause of infant death in developing countries. In 2019, a GBD study estimated global death tolls due to 33 different bacterial infections and estimated that infections could have caused nearly 7.7 million deaths in 2019 [16, 17]. Most pathogenic bacterial cultures can be identified by use of technologies like Gramme stain. This is a common method for testing the efficacy of antibiotics against bacteria grown in this way. Koch's postulates can be used as a standard to determine the causal relationship of a bacteria to a disease when it is not known. The polysaccharide capsule is the hallmark of Klebsiella genus of rod-shaped, gram-negative, oxidase-negative bacteria. Klebsiella species are found all over nature. This is theoretical because different sublineages have adapted to the different niche, and are therefore better suited to the environment. These organisms can be found in water, soil, vegetation, insects, and even humans [19, 20].

The bacteria klebsiella was named after the German-Swiss scientist Edwin Klebs (1834-1913). Due to Carl Friedlander's description, Klebsiella bacillus was formerly known as Friedlander bacillus. Klebsiella bacteria are present in the noses, mouths and guts of many animals and people. The majority of Klebsiella species lack motile cells and are gram-negative. Are stouter and less tall than most other Enterobacteriaceae species. The common width of the rod-shaped cells is 0.3 to 1.5 microns and their common length is 0.5 to 5.0 microns. They can be found as single beads in the market or as pairs or even as a string. The bacteria Klebsiella can grow in many different settings, just as other members of the Enterobacteriaceae family do. Things which can breath air and things which can breath air and air only. A pH of about 7.2 and a temperature range of 35–37 °C are ideal for their growth. Klebsiella often have a more rounded and thicker shape than other member bacteria of the Enterobacteriaceae family [21]. They are most commonly seen as straight rods with slightly rounded or pointed ends. They may be singly, in pairs or in small numbers. It is common to find diplobacillary forms in living organisms [22]. They are not particular about the growing conditions, and will develop on standard laboratory media, but temperature of 35-37 °C and a pH of 7.2 will be optimal. Most are facultative anaerobes and grow on citrate and glucose as sources of carbon and ammonia as the source of nitrogen. Although genetic serotyping might eventually supplant the use of the distinctive slime layer and capsule for serologic identification, the current use of these may not be necessary. Klebsiella bacteria often have two different antigens on their cell walls [24]. The O antigen is one of nine different types of lipopolysaccharide (LPS).

Secondly, there are over 80 different capsular polysaccharides known as K antigen. Both are involved in serogrouping and are both involved in pathogenicity. Many immunisations have been based on these two antigenic factors. Klebsiella species are also found in the mouth, nose and intestines of healthy humans, but also can infect humans when provided with opportunity. Klebsiella bacteria may infect humans, as well as many other animals, either through natural or opportunistic routes. Klebsiella bacteria can be the cause of numerous diseases. Some of the most common are soft tissue infections, pneumonia, urinary tract infections, meningitis, peritonitis, diarrhoea and sepsis. Klebsiella species have also been associated with the onset of spondyloarthropathies, such as ankylosing spondylitis [27]. Of the two most common types of Klebsiella infection in humans, pneumococcal and oxytoca, the latter is more common. Children, elderly and individuals with serious underlying health conditions such as cancer are at higher risk of infection, most of which are due to the contamination of an invasive medical device [29]. Researchers have used innovative methods to create effective vaccinations against Klebsiella and K throughout the last 40 years bacterial pneumonias. There is, however, at present no Most Klebsiella pneumoniae-associated infections are in hospital environments, such as the neonatal intensive care unit, and in the respiratory tract. K. pneumoniae also causes the most common cases of urinary tract infection and gram-negative bacteraemia. Drug resistant bacterial isolates are a major issue in health care settings, and can result in longer hospital stays, higher health care costs, and an increased risk in high impact medical areas such as intensive care units. Multidrug efflux pumps are commonly thought to be responsible for the spread of antibiotic resistance, and that's just what Klebsiella pneumoniae, a non-motile, Gram-negative, facultative anaerobic, rod-shaped bacterium that ferments lactose, is doing. Klebsiella pneumoniae can be found on everything in a healthcare facility from carpets to sinks to flowers to even the skin of patients and staff. It grows on MacConkey agar similar to a mucoid lactose fermentor. Although it is common in the gut, the skin and mouth, it can cause damage to your lungs if you inhale it, particularly the alveoli, and produce sputum resembling jelly, which is red, brownish or yellow. This may occur to either humans or animals. In the clinical setting of the Enterobacteriaceae family, this Klebsiella species is the most important. There is evidence of K in human clinical specimens as well. oxygen and potassium. Inflammation of the nasal passages. Klebsiella species are soil bacteria that are able to fix nitrogen under anaerobic conditions, with approximately 30% of Klebsiella strains able to do this. Such nosocomial infections have become a serious health issue in recent years due to these bacteria. K. has been demonstrated to stimulate agricultural crop production and has drawn the attention of farmers because of its free living diazotrophic activity. Metabolic rate is the total of all the metabolic reactions in an organism. Metabolites are intermediate and end products in metabolism, and are in most cases very small molecules. While all functional cells contain primary metabolites, A. Meslar coined the term 'secondary' metabolites. According to Kossel's theory from 1891, these metabolites are only there and aren't essential to an organism's survival. While they are not part of the organism's primary metabolism, secondary metabolites are derived from primary metabolism. Its lack does not prove lethal in the short term to an organism, as with main metabolites, but it does make survival more difficult. It is synthesised by environmentally disadvantaged species within a certain evolutionary group [30]. It is difficult to differentiate between primary and secondary metabolisms since many of the intermediates in both types are present. Amino acids are certainly a secondary metabolite and may well be a product of the main metabolism. Although sterols are indeed necessary for the proper functioning of many cellular components, this is not always the case. For an intermediate with a mosaic shape, the metabolic pathways of primary and secondary metabolism are same, as written by [31, 32]. Secondary metabolites may switch the primary metabolism which may be inactive and may buffer surplus C and N. The stored carbon and nitrogen can be converted back into primary metabolites when they are needed through the metabolic breakdown of secondary metabolites. Primary and secondary metabolism processes are always dynamic and tightly controlled; they are controlled by both external and internal factors, including growth, tissue differentiation and development within the cell and/or organism. Metabolic processes are continuous biochemical activities which all living things undergo, from single cells to networks of cells. The biochemical reactions are mainly classified as catabolic or anabolic. The "metabolites" that are produced as a result of these routes are essential components of more complex metabolic reactions. Biologically significant molecules, known as metabolites, have a wide range of applications, including in the food industry, medicine and agriculture. The metabolites associated with specific metabolism activities are referred to as primary metabolites and other metabolites that are not associated with specific metabolism activities are termed as secondary metabolites. Amino acids, citric acid, pyruvate and lactic acid are examples of fundamental metabolites that provide energy for cell biochemistry and physiology. Primary metabolites are required for growth of cells while secondary metabolites help to survive in adverse conditions. As can be seen in Figure 1, the bacteria that produce secondary metabolites produce these complex and bioactive chemicals during their late and stationary growth phases. Secondary metabolites are produced as a response to environmental stress or nutrient limitation or to conditions of limited development. Secondary metabolites can be found in bacteria, fungus, plants and marine animals. They can produce a myriad of metabolites, such as molecules acting as sex hormones, pigments, anticancer drugs, pesticides, immunomodulating agents, immunosuppressants, agents

involved in metal transport, and receptor agonists and antagonists. A secondary metabolic pathway involves a reaction that is catalysed by an enzyme or enzymes. The metabolic pathways shown in Figure 1(b) are for the synthesis of secondary metabolites, which are side products or intermediates of the primary metabolic pathways. The goals of this research were to use gas chromatography–mass spectrometry (GC–MS) to examine secondary metabolites generated by Klebsiella pneumoniae. Characterisation of Klebsiella's secondary metabolites for their antibacterial action.

## MATERIALS AND METHODS

### Conditions for Growth and Metabolite Determination

A Klebsiella pneumoniae strain, obtained from bronchitis patients at the Maternity and Children's Hospital, was purchased. Nutrient Agar was then incubated at 22°C for 48 hours, and then subcultures were taken. After 10 minutes incubation at 4ºC, the mixture was stirred for another 10 minutes at 130 rpm. The metabolites were extracted from the liquid culture at 45ºC in a rotary evaporator and lyophilized to a powder. The residue was then diluted in 1ml of methanol, filtered through 0.2 µm syringe filter and stored at 4ºC for 24 hrs prior to GC-MS analysis. A comparison of the mass spectra with the NIST mass spectral collection and literature data or retention indices of real compounds, were used to determine which components were present.

### Multivariate Analysis of Bioactive Natural Chemical Compounds of K. Pneumoniae by Means of Gas Chromatography/Mass Spectrometry (GC/MS)

The GC-MS system, which was set up with a DB-5MS column (30 m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA), was used to perform the analysis. The oven was heated to the same temperature as used in the previous analysis. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The injection of the GC effluent was performed by a transfer line directly into the MS source at 250 °C. The ion source temperature was 230oC and the ionisation voltage was 70eV. The range measured was between 41 and 450 amu. Retention times of the components were compared with the retention times of valid samples in the WILEY MASS SPECTRAL DATA BASE Library to establish their identification.

### Antimicrobial Activity Determination

Five 1 to 2 mm diameter holes were made in the agar with a sterile cork-borer. Then, 25 µl of the sample solutions (Metabolites Produced by Klebsiella pneumonia) were poured into the holes. The germs that were tested were swabbed in plates of Muller Hinton agar. The fungal extracts were added to the bored wells. The diameter of the wells was 0.5cm. The plates were incubated at 37°C for 24 hours, and then the plates were examined. As a control solvent, methanol was utilised.

### Evaluation of Data

The data are presented as mean ± SD and mean ± SE and all the measures were repeated three times for each assay. In each case, the IBM SPSS 20 version statistical software programme was used to statistically analyse the percentage inhibition, disease incidence and disease severity.

## RESULTS AND DISCUSSION

The chemicals in the methanolic extract of Klebsiella pneumoniae were analysed using gas chromatography and mass spectroscopy. Peaks were determined to be Tricyclo[4.3.1.1(3.8)]undecan-1-amine, carboxaldehyde, 1,5,5-Trimethyl-6-methylene-cyclohexene, 4-(2,5-Dihydro-3-methoxyphenyl)butylamine, Paromomycin , 9-Borabicyclo[3.31]nonane , 9-mercapto-, Acetamide , N-(6-acetylaminobenzothiazol-2-yl)-2-(adamantan, rin-6-carboxylic acid , N-(2,5-Dicyano-3,4-dihydro-2H-pyrrol-2-yl)-acetamide, 3,10-Dioxatricyclo [4.3.1.0(2,4)]dec-7-ene, 3-Cyclohex-3-enyl-propionic acid, Eicosanoic acid ,phenylmethyl ester, 9,9-dimethyl-, Dithiocarbamate , S-methyl-,N-(2-methyl-3-oxobutyl)-, dl-Homocysteine, 2-(2-Furyl)pyridine, 5,7-Dodecadiyn-1,12-diol, 1-(β-d-Arabinofuranosyl)-4-O-difluoromethyluracil, hexahydro-,12-Methyl-oxa-cyclododecan-2-one, Phthalic acid , butyl undecyl ester, 9,12,15-Octadecatrienoic acid , 2,3-bis(acetyloxy)propyl ester, methyl ester, 12-Dimethylamino-10-oxododecanoic acid, L-Aspartic acid, N-glycyl-,2H-Oxecin-2-one, Thiazolo[4,5-d]pyrimidine-5,7(4H,6H)-dione, 2-amino-4-(2-ph, Dec-9-en-6-oxo-1-ylamide, 2-lodohiistidine, 2,5-Piperazinedione ,3,6-bis(2-methylpropyl)-, 9-Octadecenamide.

### Activity against Bacteria

The bacterial products concentrations of the antibiotics streptomycin and kanamycin ranged from 1.08±0.200 to 0.98±0.166 and 1.02±0.180 to 1.82±0.200, respectively, and of the clinical pathogens of Strep. pneumonia, E. coli, Staph. aureus, P. mirabilis and Staph. epidermidis ranged from 4.09±0.013 to 4.00±0.203.

**Table 1. Bioactive chemical compounds identified in methanolic extract of *Klebsiella pneumoniae*.**

| / Bacterial products Antibiotics | Zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | Bacteria | | | | |
| | *Streptococcus pneumonia* | *Escherichia coli* | *Staphylococcus aureus* | *Proteus mirabilis* | *Staphylococcus epidermidis* |
| **Bacterial products** | 4.09±0.013 | 2.99±0.300 | 4.37±0.200 | 3.22±0.210 | 4.00±0.203 |
| **Streptomycin** | 1.08±0.200 | 0.97±0.116 | 2.08±0.233 | 3.04±0.261 | 0.98±0.166 |
| **Kanamycin** | 1.02±0.180 | 1.00±0.190 | 2.08±0.236 | 1.00±0.100 | 1.82±0.200 |

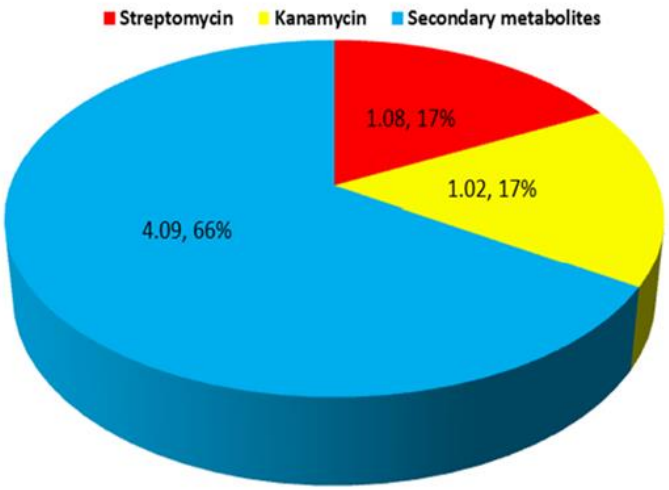



**Figure 1. Metabolite products, streptomycin and kanamycin as anti-bacterial activity against *Strep. pneumonia*.**

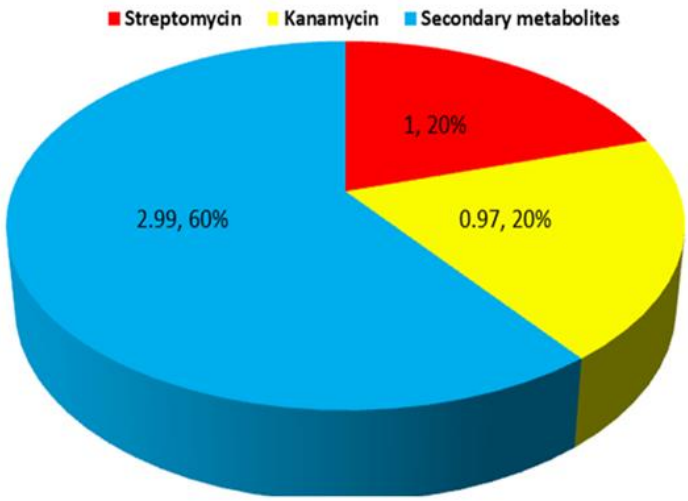



**Figure 2. Metabolite products, streptomycin and kanamycin as anti-bacterial activity against *E. coli*.**

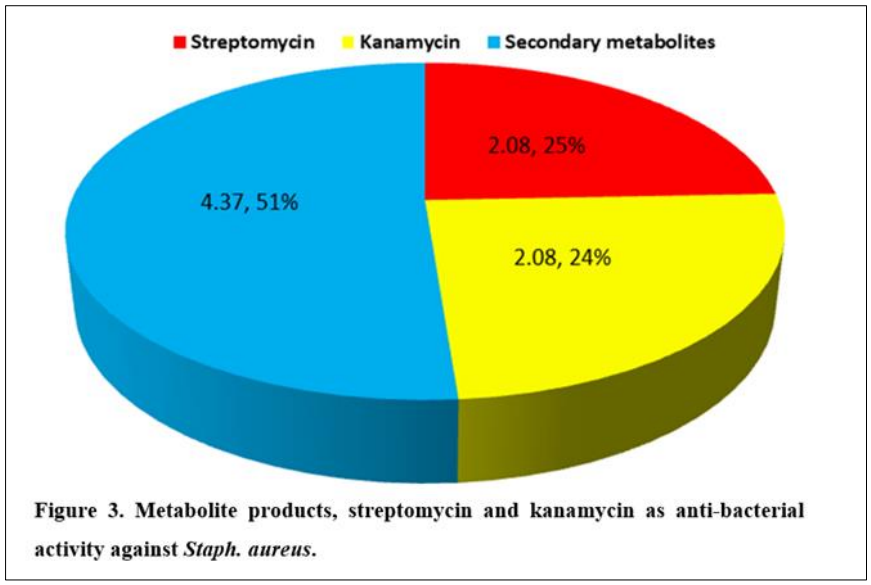



**Figure 3. Metabolite products, streptomycin and kanamycin as anti-bacterial activity against *Staph. aureus*.**

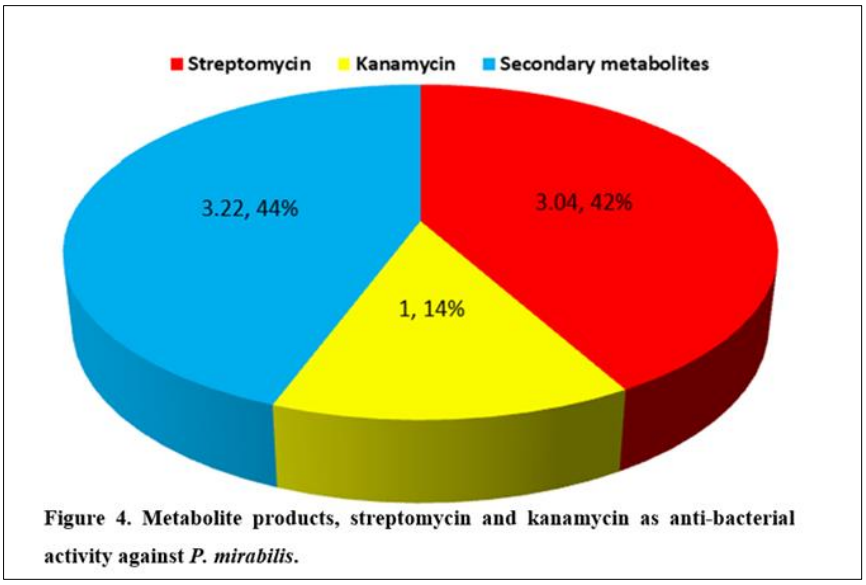



**Figure 4. Metabolite products, streptomycin and kanamycin as anti-bacterial activity against *P. mirabilis*.**

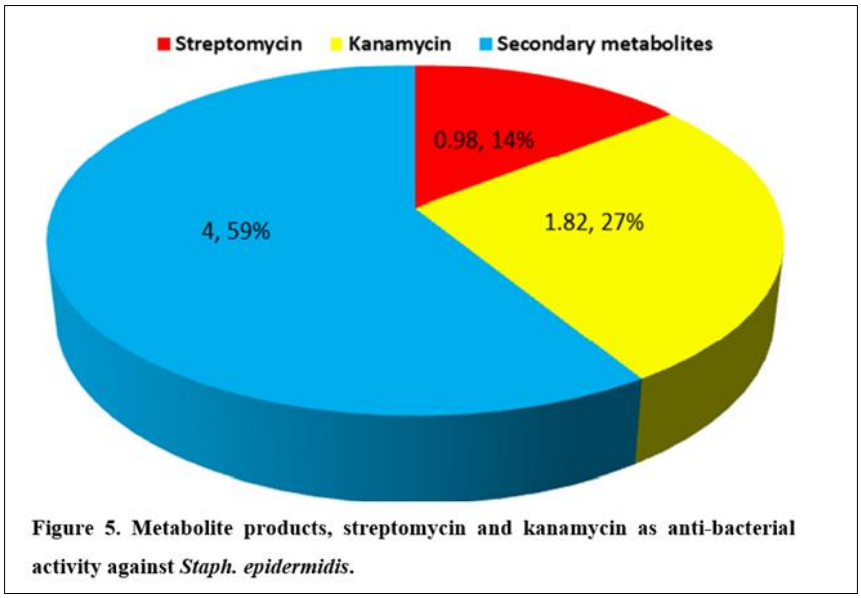



**Figure 5. Metabolite products, streptomycin and kanamycin as anti-bacterial activity against *Staph. epidermidis*.**

Table 1 shows the maximum zone development against Staphylococcus aureus, which is 4.37±0.200 mm. Numerous secondary metabolites with significant biological activity are produced by Klebsiella pneumoniae. Purifying substances made by Klebsiella pneumonia can be beneficial since bioactive molecules are used in pharmacy to make medications that treat various ailments. *Klebsiella* infections usually occur in people who are already sick due to other health conditions. Middle aged and older males are most affected by disabling diseases. The following factors are believed to weaken the host defence of the respiratory system: diabetes mellitus, alcoholism, cancer, liver disease, COPD, glucocorticoid therapy, kidney failure, and certain occupational exposures (e.g., papermill workers). A number of these diseases are nosocomial infections, acquired while the patient is hospitalized for other conditions. Infection of patients through contact with infected tools is the second most common source of infection after faeces.

## CONCLUSION

A strong feature of the microbiome is its ability to produce antibiotics and other medically important substances that would battle bacterial and fungal infections, cancer and cardiac diseases. While there are similarities between the life cycles of bacterial species, there are significant differences in the metabolic pathways they follow for the production of a broad spectrum of secondary metabolites, and in their physiological and biochemical adaptations. If pathways and processes in secondary metabolite biosynthesis can be better understood, the pharmaceutical and biotechnology industries could benefit from the advances in combinatorial biosynthesis. An ethanolic extract was used to isolate 39 bioactive chemical components of Klebsiella by gas chromatography–mass spectrometry (GC–MS). The secondary metabolite products of Klebsiella pneumoniae have been tested for antifungal and antibacterial activities in vitro. This could be beneficial for further phytochemical and pharmacological investigations to find new antimicrobial agents.

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