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Review Article

Investigating the Bioactive Natural Compounds Produced by Klebsiella Pneumoniae and their Characterization as Antimicrobial Secondary Metabolites Mass Spectrometry Coupled with Gas Chromatography (GC-MS)

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Abstract: The secondary metabolites produced by microbes have a unique structure and a low molecular mass. Various biological functions, such as antibacterial agents, are displayed by the structurally varied metabolites. Klebsiella pneumoniae methanolic extract contained 39 bioactive components. GC-MS was used to identify the Tricyclo[4.3.1.1(3.8)] in Klebsiella pneumoniae. such as undecan-1-amine, 3-methylbenzaldehyde semicarbazone, carboxaldehyde, 1-methyloxime, (Z)-(+) 1,5,5-Trimethyl-6-methylene-cyclohexene, Hydroxyphenylbutylamine, 4, 5, Pamomycin is a The chemical compound 9-borabicyclo[3.31] Benzenemethanol, 2-(2-aminopropoxy), nonane, 9-mercapto- and that is 3-methyl, The solvent acetamide, An N-(6-acetylaminobenzothiazol-2-yl) • N-(2,5-Dicyano-3,4-dihydro-2H-pyrrol-2-yl)-acetamide • 4-(2,5-Dihydro-3-methoxyphenyl)butylamine 3,5-Dioxatricyclo [4.3.1.0(2,4)] December 7th, phenylmethyl ester, eicosanoic acid, 3-cyclohex-3-enylpropionic acid The compound 3,7-dialzabicyclo[3.3.1] thiocarbamate, nonane, 9,9-dimethyl- 1-(2-Furyl)-S-methyl-N-(2-methyl-3-oxobutyl)-, dl-homocysteine 1,7-Dioxa-10-thia-4,13-diazacyclopentadeca-5,9,12-trione is the synthetic name for pyridine. 1,-(β-d-Arabinofuranosyl) and 5,7-decadiyn-1,12-diol -4-O-difluoromethyluracil, Blood sugar, the pyrrolo compound [1.2-a] 1,4-dione of pyrazine, hexahydro- However, butyl undecyl ester, phthalic acid, and 12-methyl-oxa-cyclododecan-2-one. These compounds include 9,12,15-octadecatrienoic acid, 1,2,4-trioxolane-2-octanoic acid, and 2,3-bis(acetyloxy)propyl ester. ester of 5-octyl-methyl Octahydrochromen-2-one, 12-dimethylamino-10-oxododecanoic acid, L-aspartic acid, N-glycyl-,2H-oxicecin-2-one, 3,4,7,8,91,10-hexahydro-4-hydroxy-10-meth, The compound thiazolo[4,5-d] 2-amino-4-(2-ph)-dione, 3,6,12-trimethyl-1,4,7,10,13,16-hexaaza-cyclooctadecane, 2-amino-4-(2-ph)-dione, Dec-9-en-6-oxo-1-ylamide 3-(2-methylpropyl)-2-lodohiistidine, 2,5-diperazinedione The compound is 9-octadecenamide and a tetrahedronate ring structure with three methyl groups attached. A extremely active species of crustacean, Citrullus colocynthis, measured 6.39±0.27 mm. The findings from the study on the anti-fungal activity of Morganella morganii demonstrated that the volatile chemicals effectively inhibited the development of Aspergillus terreus.(0.23±5.613) Clinical pathogens chosen for their antimicrobial properties, include Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus pneumonia, and Escherichia coli The results for bacteria (Metabolites Produced by Klebsiella pneumoniae) were 4.09±0.013, 2.99±0.300, 4.37±0.200, 3.22±0.210, and 4.00±0.203 respectively. For bacteria (Streptomycin antibiotics), the results were 1.08±0.200, 0.97±0.116, 2.08±0.233, 3.04±0.261, and 0.98±0.166. For bacteria (Kanamycin antibiotics), the results were 1.02±0.180, 1.00±0.190, 2.08±0.236, 1.00±0.100, and 1.82±0.200, respectively. The bacterium Klebsiella pneumoniae produces a wide variety of biologically active secondary metabolites. Klebsiella pneumoniae compound purification has potential applications in the pharmaceutical industry due to the importance of bioactive compounds in the development of medications for the treatment of numerous ailments.

Keywords: Secondary Metabolites, Klebsiella Pneumonia, Antibacterial Activity, GC/MS.

INTRODUCTION

The rod-shaped, Gram-negative, nonmotile, encapsulated, facultative anaerobic bacterium Klebsiella pneumoniae ferments lactose. According to references [1-3], it grows on MacConkey agar as a mucoid lactose fermenter. Klebsiella bacteria usually display two kinds of cell surface antigens. Of the nine different types of lipopolysaccharide (LPS), the first is known as O antigen [4]. The second one is the capsular polysaccharide known as K antigen, which comes in over eighty different types [5-7]. Both are essential for serogrouping and play a role in pathogenicity. K. is of agricultural relevance due to its nitrogen-fixation system, which has been extensively investigated, and it is a free-living diazotroph. pneumococcal infections have been found to enhance agricultural production [8]. Outside of healthcare settings, bronchopneumonia and bronchitis caused by Klebsiella bacteria account for the vast majority of cases of pneumonia. Lung abscesses, cavitations, empyemas, and pleural adhesions are more common in these individuals. Even when treated with antibiotics, the mortality rate is close to 50%. When combined, drinking and bacteremia can cause a death rate close to 100% [9]. Despite being a typical part of the gut, skin, and mouth flora [1], aspiration (inhalation) can induce deadly alterations to the lungs of humans and animals, particularly to the alveoli, leading to bloody sputum. It is the most important Klebsiella bacterium in the clinical context, belonging to the Enterobacteriaceae family. Mr. K. of oxytoca and K. Clinical specimens from humans have also shown signs of rhinoscleromatis. Klebsiella species have emerged as major players in the nosocomial infection epidemic in the last several years. The urinary tract, the lower biliary tract, and surgical incision sites are among the organs that can get infected with Klebsiella, along with pneumonia [10]. A variety of clinical conditions can manifest, including but not limited to: pneumonia, thrombophlebitis, UTI, cholecystitis, diarrhea, URTI, wound infection, osteomyelitis, meningitis, bacteremia, and septicemia. Contamination of the device becomes a problem for patients having invasive devices in their bodies. This is especially true for devices used in newborn wards, respiratory support equipment, and urine catheters [11–14]. Another risk factor for nosocomial infection with Klebsiella bacteria is the use of antibiotics. Bacterial infection in the bloodstream can cause sepsis and septic shock. These were the aims of the research: Analysis of secondary metabolites generated by Klebsiella pneumoniae Gas Chromatography-Mass Spectrometry (GC-MS). Klebsiella pneumoniae secondary metabolite evaluation for antibacterial activity.

Infectious Bacteria

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. Although there is ongoing disagreement over its higher-level classification, one taxonomic arrangement places it in the order Enterobacterales, class Gammaproteobacteria, phylum Pseudomonadota. Based on comparative genomic investigations, Adeolu *et al*., (2016) revised the description and family members [2-5].

Many well-known diseases, including Salmonella, Escherichia coli, Klebsiella, and Shigella, are members of the Enterobacteriaceae family, which also includes numerous innocuous symbionts. The bacteria Enterobacter and Citrobacter are also members of this family and can cause diseases. Since many members of the family Enterobacteriaceae reside in animal intestines, the common names for these bacteria are enterobacteria or "enteric bacteria" [citation needed].

Germs That Cause Illness

Bacteria that are harmful to humans are the ones discussed in this article [1]. Bacteria that can cause disease are known as pathogenic bacteria. The vast majority of bacterial species are completely benign and even helpful, but a small number of them are known to cause infectious illnesses. While the number of these harmful species in humans is believed to be around a hundred, the number of species found in the digestive tract is in the thousands.

Numerous bacterial species are constantly interacting with the human body. Some of these species, known as commensals, are helpful and live on the skin and mucous membranes. Other species, known as saprophytes, are soil-dwellers and feed on decomposing organic materials. The nutrients found in bodily fluids, such as blood and tissue fluids, are enough to support the growth of numerous bacteria. A natural immunity, also known as an innate resistance, is one of the body's defense mechanisms that helps it withstand the invasion of its tissues by microbes.

Because of their unique adaptations and abilities, pathogenic bacteria are able to evade the body's typical defenses and penetrate environments like the bloodstream, where bacteria are not typically present. While some infections limit themselves to penetrating the epithelium, skin, or mucous membranes on the surface, the majority of pathogens penetrate deeper into tissues, where they proliferate via the lymphatic and blood streams. Only in extremely unusual circumstances can a pathogenic microorganism infect a perfectly healthy human being. Typically, infection happens when the body's defense mechanisms are compromised due to factors like local trauma, intoxication, chills, exhaustion, or malnutrition. It is often crucial to distinguish between colonization, in which the bacteria are not doing any damage, and infection. Death toll (A) and number of YLLs (B) worldwide in 2019, broken down by pathogen and GBD super-region [3]. One of the leading causes of death worldwide is tuberculosis, which in 2019 claimed the lives of 1.4 million people, primarily in sub-Saharan Africa. Other important diseases caused by pathogenic bacteria include pneumonia (Staphylococcus, Streptococcus, and Pseudomonas), and food poisoning (Shigella, Campylobacter, and Salmonella). Infectious diseases caused by pathogenic microorganisms include leprosy, syphilis, diphtheria, tetanus, and typhoid fever.

High infant mortality rates in developing nations can be attributed to pathogenic bacteria. According to a GBD study, which estimated the worldwide death rates from 33 different bacterial pathogens, these infections were responsible for one out of every eight deaths, or approximately 7.7 million deaths in 2019. This suggests that they could be the second leading cause of death worldwide in 2019. I have read [6, 3]. The majority of bacteria that cause disease may be cultured and identified using techniques such as Gram stain. It is common practice to test antibiotic efficacy on bacteria cultured in this manner. To prove a causal association between a bacterium and a disease, Koch's postulates are used as a standard for hitherto unknown pathogens. The Gram-negative, oxidase-negative, rod-shaped bacteria belonging to the genus Klebsiella are characterized by a conspicuous capsule made of polysaccharides [3]. Species of Klebsiella are ubiquitous in the natural world. Theoretically, this is because different sublineages improved their suitability to a given environment by acquiring niche adaptations, which include biochemical changes. Their distribution is rather wide, including in humans, as well as in water, dirt, plants, insects, and other animals.

In honor of the German-Swiss microbiologist Edwin Klebs (1834-1913), the Klebsiella bacteria bear his name. Klebsiella bacillus was for a long time known as Friedlander bacillus after Carl Friedlander, who described it. Klebsiella bacteria are common in the nasal passages, oral cavity, and digestive tracts of both humans and other animals. Klebsiella species are typically non-motile and gram-negative. They are often thinner and shorter than other members of the Enterobacteriaceae family. The cells typically measure 0.3 to 1.5 µm in width and 0.5 to 5.0 µm in length, and they have a rod-shaped appearance. You can find them on their own, in pairs, in chains, or even joined at the ends. Unlike other Enterobacteriaceae, Klebsiella does not require a specific medium for growth and can instead thrive in a standard laboratory setting. Aerobic and facultatively anaerobic organisms. Temperatures between 35 and 37 degrees Celsius and a pH of roughly 7.2 are optimal for their growth [6].

Compared to other Enterobacteriaceae bacteria, Klebsiella tend to be thicker and rounder. The most common shape for these rods is a straight one, with either rounded or slightly pointed ends. One might find them alone, in a pair, or even in a small chain. Various kinds of diplobacilli can be observed in living organisms [7]. While they do well on most laboratory media, they do best at temperatures between 35 and 37 °C and a pH of 7.2, while they are not picky about these conditions. Most strains of this facultative anaerobe species can live off of just citrate and glucose for carbon and ammonia for nitrogen [7]. Serologic identification can be done using the genus's conspicuous capsule, or slime layer; however, molecular serotyping is starting to supplant this method [8]. It is common for Klebsiella bacteria to display two distinct antigens on their cell walls. There are nine different kinds of lipopolysaccharide (LPS), one of which is O antigen. The second one is the capsular polysaccharide known as K antigen, which comes in over eighty different types. These two factors are the foundation for serogrouping and both contribute to pathogenicity [9]. Several vaccines have been developed using those two main antigenic factors [10].

Normal flora of the human nose, mouth, and intestines include Klebsiella species; nevertheless, these same bacteria can also act as opportunistic human infections. As both common bacteria and opportunistic infections, Klebsiella species are known to infect a wide range of different mammals [7, 4]. Pneumonia, UTIs, sepsis, meningitis, diarrhea, peritonitis, and infections of the soft tissues are among the many diseases that can be caused by Klebsiella germs. There is evidence that certain Klebsiella species contribute to the development of spondyloarthropathies, such as ankylosing spondylitis [7-12]. Klebsiella pneumoniae is the most common kind of Klebsiella infection in humans. Respiratory infection, then K the mineral oxytoca. Most infections include contamination of an invasive medical device, and they are more common in the very young, the very elderly, and those with serious underlying conditions like cancer [4-7]. There have been a lot of experiments in the last 40 years trying to build effective K. Vaccines against Klebsiella have been developed using novel approaches, and attempts have been made to protect against pneumoniae [13, 14]. On the other hand, the United States does not yet have a licensed Klebsiella vaccination. Mr. K. The respiratory tract and premature intensive care infections caused by pneumoniae are the most common in healthcare settings. Gram-negative bacteraemia and urinary tract infections caused by pneumoniae are the second most common. Healthcare facilities continue to face the challenge of drug-resistant bacterial isolates, which prolong patient stays, increase healthcare costs, and pose a particular threat in high-impact medical settings like intensive care units. Multidrug efflux pumps are largely believed to be responsible for this antibiotic resistance [15]. The capacity of K. The ability of pneumococcal bacteria to colonize many surfaces, including carpets, sinks, flowers, and other surfaces, in addition to the skin of both patients and healthcare workers, is a key component in the transmission of illnesses that occur in hospitals.

Enterobacter Pneumoniae

Klebsiella pneumoniae is a rod-shaped, facultative anaerobic, Gram-negative, non-motile bacteria that ferments lactose. It grows on MacConkey agar looking like a mucoid lactose fermenter. Even though it's a common part of the gut, skin, and mouth microbiome, aspiration of this germ can harm the lungs of humans and animals, particularly the alveoli, leading to sputum that looks like jelly and is red, brown, or yellow in color. It is the most important Klebsiella species in the clinical context of the Enterobacteriaceae family. Mr. K of oxytoca and K. Clinical specimens from humans have also

shown signs of rhinoscleromatis. Klebsiella species have emerged as major players in the nosocomial infection epidemic in the last several years.

It is present in soil on a natural basis, and in anaerobic circumstances, around 30% of the strains may fix nitrogen [2]. The nitrogen-fixation system of this free-living diazotroph has been extensively investigated and is of agricultural importance because K. It has been shown that pneumoniae can enhance agricultural productivity the third

Subsidiary Compounds

The total number of biological reactions that an organism performs is called its metabolism. Typically, metabolites are tiny molecules that serve as metabolic intermediates or end products. A. used the word "secondary" once. In 1891, Kossel suggests that although all cells with the ability to divide have primary metabolites, secondary metabolites are present in little amounts and do not play a crucial role in an organism's survival. Despite their origins in primary metabolism, secondary metabolites are not structurally essential to the living being. It differs from primary metabolites in that its absence does not instantly kill an organism, but it does make survival more difficult. In a phylogenetic group, it is found in ecologically disadvantaged species in both its existence and its synthesis [1].

Due to the fact that many of the intermediates involved in primary and secondary metabolism are identical, it is difficult to tell which is which when discussing metabolites [2]. Although amino acids are thought of as a primary metabolite byproduct, they are undeniably also a secondary metabolite. It has been noted that sterols are secondary metabolites that are essential to numerous cellular structures. The same metabolic pathway between primary and secondary metabolism is indicated by the mosaic form of intermediates [3]. To prevent the primary metabolic process from becoming inactive due to an excess of carbon and nitrogen, secondary metabolites provide a buffer zone. Metabolic breakdown of secondary metabolites allows the stored carbon and nitrogen to return to primary metabolites upon demand. Growth, tissue differentiation, and development of the cell or organism, as well as external stresses, impact the activities of primary and secondary metabolism, which are dynamic and delicately balanced (Figure 1) [4].

Creation of Bacterial Secondary Metabolites

Every living thing, whether it has one cell or many, undergoes metabolism at some point throughout its lifetime. There are two main categories that biological reactions fall into: catabolism and anabolism. 'Metabolites' are the byproducts of these routes that are utilized to build substrates and intermediates for other metabolic pathways. Metabolites display a number of biological characteristics that are important in the pharmacological, nutritional, and agricultural sectors. Metabolite classifications are based on functional features and metabolic pathways, and these molecules are categorized as either main or secondary metabolites. Amino acids, pyruvate, citric acid, and lactic acid are examples of basic metabolites that provide energy for cellular biochemistry and physiology. Whereas primary metabolites are required for cell proliferation, secondary metabolites help the organism endure harsh environments. Table 1 shows that the primary focus of this chapter is on bacterial secondary metabolite synthesis. These bioactive and complex compounds are synthesized by the bacteria that produce secondary metabolites throughout their late growth phase and stationary phase (Figure 1). When development conditions are restricted, environmental stress levels are high, or nutrients are depleted, the body responds by producing secondary metabolites. Many different kinds of organisms, including bacteria, fungus, plants, and marine creatures, produce secondary metabolites. Pesticides, anticancer agents, pigments, sex hormones, metal-transporting agents, antibacterial agents, immunomodulating agents, immunosuppressants, receptor agonists, and antagonists are all within these organisms' biological capabilities.

An enzyme or a set of enzymes catalyzes a process in a secondary metabolic pathway. The production of secondary metabolites involves the rerouting of intermediate or end-products from primary metabolic pathways through their respective systematic metabolic pathways (Figure 1(b)).

Compounds Produced by Bacteria as Byproducts

The secondary metabolites produced by microbes have a unique structure and a low molecular mass. Metabolites exhibit a wide range of biological activities due to their structural diversity. These include acting as antibacterial agents, enzyme inhibitors, antitumors, immune-suppressants, antiparasitic agents, herbicides, insecticides, antihelmintics, etc. During the final stages of microbial development, they are manufactured. Since the generation of secondary metabolites is typically suppressed in the logarithmic phase and stationary growth phases, it is clear that microorganisms employ specific regulatory mechanisms to regulate this process. About 40% of microbial metabolites are not amenable to chemical synthesis, and their unique molecular skeletons are not stored in chemical libraries [35].

Metabolites produced by microbes and their characteristics

- The idea and method of synthesizing natural fermentation products can be effectively used to increase its impact on the medical, agricultural, food, and environmental sectors.

- The metabolite can be used as a building block to create a desired product, which can then be further modified through chemical or biological processes.

The discovery and design of novel medications will be led by new analogs or templates in which secondary metabolites act as lead chemicals.

Uses for Secondary Metabolites Produced by Microbes

Medicines Containing Antibiotics

Initiated with the discovery of penicillin, the field of microbiology was transformed when researchers began to use microorganisms for the synthesis of secondary metabolites [5]. Novel screening and isolation methods have led to the discovery of numerous antibiotics, including β-lactam-containing compounds [36]. There are around 6,000 antibiotics known, with 4,000 of them coming from actinobacteria. The most common producers of antibiotics among prokaryotes are species of the unicellular bacterium Bacillus and Pseudomonas. The same holds true for eukaryotic organisms; second only to plants in antibiotic production are fungi. A number of myxobacteria and cyanobacteria species have recently emerged as prolific members of this esteemed group of creatures. There are 62% antibacterial derivatives in the pharmaceutical product, with 13% being sera, immunoglobulins, and vaccines, 12% being anti-HIV antivirals, 7% being antifungals, and 6% being nonHIV antivirals. The number of antibiotics exceeds 160. The most important microorganisms that contribute to the antibiotic market are Streptomyces hygroscopicus (with over 200 antibiotics), Streptomyces griseus (with 40 antibiotics), and Bacillus subtilis (with over 60 com pounds) [7].

Anticancer Medications

Over 60% of anticancer formulations are derived from natural products. Actinomycin D, anthracyclines (daunorubicin, doxorubicin, epirubicin, pirarubicin, and valrubicin), bleomy cin, mitosanes (mitomycin C), anthracenones (mithramycin, streptozotocin, and pentostatin), enediynes (calicheamicin), taxol, and epothilones are presently utilized antineoplastic molecules derived from actinobacteria [37]. The nonactinobacterial chemical known as taxol is produced by endophytic fungi Nodulisporium sylviforme and Taxomyces andreanae, as well as the plant Taxus brevifolia. It impedes the division of cancer cells that divide too quickly by interfering with microtubule break down. Breast and advanced Kaposi's sarcoma are two types of cancer that it effectively treats. Pythium, Phytophthora, and Aphanomyces are among the fungal infections that it inhibits.

Medications and Nutritional Supplements

The discovery of the fungal statins—a class of drugs that lower cholesterol—including compactin, lovastatin, pravastatin, and others—was a major accomplishment. Among A.'s products is lovastatin the ground. Medications that inhibit the immune system, including cyclosporin A, tacrolimus, sirolimus (rapamycin), and mycophenolate mofetil, are essential in human medicine. They pioneered the organ transplant area and are utilized for transplants of the heart, liver, and kidneys. Tolypocladium niveum is a fungus that produces cyclosporin A. The oldest antibiotic ever discovered, mycophenolic acid, is also produced by fungi; one of their semisynthetic products is mycophenolate mofetil. Streptomycetes produce the antibiotics sirolimus and tacrolimus [7]. Metabolites produced by probiotic bacteria have several potential health benefits, including but not limited to: preventing and treating obesity; increasing satiety; prolonging satiation; decreasing food intake; improving energy metabolism; treating and strengthening insulin sensitivity; and managing weight gain. In a typical human gastrointestinal tract, the most common helpful bacteria are Firmicutes. However, in patients with irritable bowel syndrome who experience constipation more frequently, the amount of Bacteroidetes was found to be decreased [38]. Food colorants, fish feeds, nutraceuticals, cosmetics, and antioxidants are all made from carotenoids that come from microbes. Carotene, lycopene, and Dunaliella salina are three of the most common food colorants. Fungus and Streptomyces chrestomyceticus, subsp. under the color red. An acceptable fish feed ingredient is astaxanthin, which is made from Xanthophyllomyces dendrorhous. The nutraceutical properties of astaxanthin, lutein, zeaxanthin, and canthaxanthin make them ideal antioxidants. One of the nutritional additives in infant formula is docosahexaenoic acid (DHA), which comes from the microalgae Schizochytrium spp [7].

Process Enzymes and Compounds That Block Their Activity

Detergents (34%), foods (27%), agriculture and feeds (16%), textiles (10%), leather (10%), chemicals, pulp and paper (10%), and enzymes made by microorganisms (2.3 billion USD annually) are among the enzymes' most common uses. Detergent protease subtilisin sells for $200 million a year. Two other important enzymes are penicillin amidase (60,000 tons) and glucose isomerase (100,000 tons). A total of $135 million is generated from the production of nitrilease (30,000 tons) and phytase. The conversion of D-glucose to D-fructose, an isomer, is accomplished by streptomyces glucose isomerase. This process yields 15 million tons of high fructose corn syrup annually, with a market value of $1 billion [7]. Among the most significant enzyme inhibitors, the β-lactamase inhibitor clavulanic acid is produced by Streptomyces cla vuligerus. Xanthine oxidase, glucosidases, amylases, lipases, and proteases are among the most frequent enzymes that inhibitors aim to block. Dietary starch absorption is reduced when taking amylase inhibitors, which can lead to weight loss [38].

Agricultural and Veterinary Pharmaceuticals

The agricultural and veterinary fields make extensive use of secondary metabolites, such as kasugamycin and polyoxins for biopesticide purposes, Bacillus thuringiensis crystals, nikkomy cin, and spinosyns for bioinsecticide purposes, bioherbicides (bialaphos) for bioherbicide purposes, ivermectin and doramectin for antihelmintics and endectocides against worms, lice, ticks, and mites, coccidiostats for ruminant growth promoters, and plant hormones like gibberellins for growth regulators, the most common of these applications [7].

Microbes' Ability to Produce Secondary Metabolites

1. The generation of secondary metabolites is achieved by the use of liquid fermentation batch or fed-batch culture in submerged fermentation. The inoculum is created through meticulous strain improvement of the organism that produces it. The cultures are started in shake flasks and then moved to smaller fermenters with growth media when they reach the active growth phase. Eventually, they are transferred to a bigger fermenter. The agitation and aeration rate, temperature, pH, and content of the medium are among the many variables that can be adjusted. Cephalosporin fermentations require an inducer like methionine, chlortetracycline fermentations have phosphate restrictions, and penicillin and erythromycin fermentations do not use glucose.
2. An intriguing possibility for the synthesis of secondary metabolites exists in solid-state fermentation, which is characterized as a microbial culture that grows on and within a solid matrix without the presence of free water. The kind of solid phase used to create the SSF determines whether it is a (a) solid culture of a single support-substrate phase or a (b) solid culture of two substrate-support phases [7]. One benefit of solid state fermentation over submerged fermentation is the reduced energy requirements of the process. This is because the microorganisms receive oxygen directly during the process. In many cases, sterile conditions are not necessary for the production of secondary metabolites, and the yields are substantially higher. The production times are also typically shorter [7].

MATERIALS AND METHODS

Developmental Circumstances and Metabolite Identification

This Klebsiella pneumoniae strain was collected from patients at the Maternity and Children's Hospital who had bronchitis. After 48 hours at 22°C, subcultures were harvested from the Nutrient Agar. The mixture was stirred for 10 minutes at 130 rpm after being incubated at 4ºC for 10 minutes. Using a rotary evaporator set to 45ºC, the metabolites were extracted from the liquid culture and dried to a powder .[23-14]

Prior to being used for GC-MS, the residue was diluted in 1 ml of methanol, filtered using a 0.2 µm syringe filter, and kept at 4ºC for 24 hours [24-29]. In order to determine which components were present, mass spectra were compared to those in the NIST mass spectral collection, and retention indices were compared to those found in literature or to those of real compounds.

Thermochemical and Mass Spectrometric Investigation of Bioactive Natural Chemical Components Produced by Klebsiella Pneumoniae

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. Just like in the first study, the oven temperature was programmed. The carrier gas, helium, was introduced at a flow rate of 1.0 mL/min. Through a transfer line operating at 250 °C, the GC column's effluent was injected straight into the MS source. The 230 oC ion source temperature and 70 eV ionization voltage were used. I measured a range of 41 to 450 amu. Retention times were compared to those of genuine samples from the WILEY MASS SPECTRAL DATA BASE Library [46-50], to identify the components.

Assessment of Antimicrobial Efficacy

Sterile cork-borers were used to cut five-millimeter diameter wells from the agar. Then, 25 µl of the sample solutions (Metabolites Produced by Klebsiella pneumonia) were added to the wells. Samples of the test organisms (including S. pneumoniae, E. coli, S. aureus, Proteus mirabilis, and S. epidermidis) were collected using swabs placed on Muller Hinton agar plates. Ninety microliters of fungal extracts were put into the drilled wells. The wells were bored with a diameter of half a centimeter. (Anupama *et al*., 2007) stated that after 24 hours of incubation at 37°C, the plates were analyzed. As a control solvent, methanol was utilized.

Analyzing Data

The results are shown as the mean ± SD and mean ± SE, and each assay had three replicates of the measurements. For statistical analysis of percentage inhibition, disease incidence, and disease severity in each case, the IBM SPSS 20 version statistical software program was utilized.

RESULTS AND DISCUSSION

The components in the methanolic extract of Klebsiella pneumoniae were analyzed using gas chromatography and mass spectroscopy. A phenylmethyl ester of 6,9,12-octadecatrienoic acid was identified as the peak at (Z,Z,Z). "1,4 Decadiyne," "5,7-Dodecadiyn-1,12-diol," Octadecadiynoic acid, 1-cyclopropyl-3,4-epoxyhex-5-en-1-yne, N,N-dimethyl-3-methoxy-4-methylphenethylamine The carbonitrile ethene group, Three-methyl-xylidinol and pentyl glycolate 2-(octahydro-2,3-quinoxalinediylidene)bis, 3-(1,1'-biphenyl-4-yl)butanenitrile, 4-amino-6-methoxyyaurone, ethanone 2-[1-phe, 1,1'-bis(cyclohexyl)-, 4-methoxy-4'-propyl-, [1.4]bis(chlorobenzenesulfony)-4'-carboxamide, 7-hydroxypyrrolo[2,3-d] a pyrimidin-4-amine, Vinylsulfonamide, 1-Phenyl-2-(4-methylphenyl)diazene an acid, 1, phenyl-2-(4-methylphenyl)-diazene, N-benzyl-N-ethyl-p-isopropylbenzamide di(2-methoxyethyl) ester, 1-benzylindole, isophthalic acid, sodium hydroxide, Tyramine 1-tert-butyl-3,3-bis(trifluoromethyl)diaziridine-4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene). Staphylococcus aureus, Streptococcus pneumonia, Escherichia coli, Staphylococcus epidermidis, Proteus mirabilis, and other clinical pathogens chosen for their antibacterial activity the results for bacteria (Metabolites Produced by Klebsiella pneumoniae) were 4.09±0.013, 2.99±0.300, 4.37±0.200, 3.22±0.210, and 4.00±0.203 respectively. For bacteria (Streptomycin antibiotics), the results were 1.08±0.200, 0.97±0.116, 2.08±0.233, 3.04±0.261, and 0.98±0.166 respectively. For bacteria (Kanamycin antibiotics), the results were 1.02±0.180, 1.00±0.190, 2.08±0.236, 1.00±0.100, and 1.82±0.200 respectively.

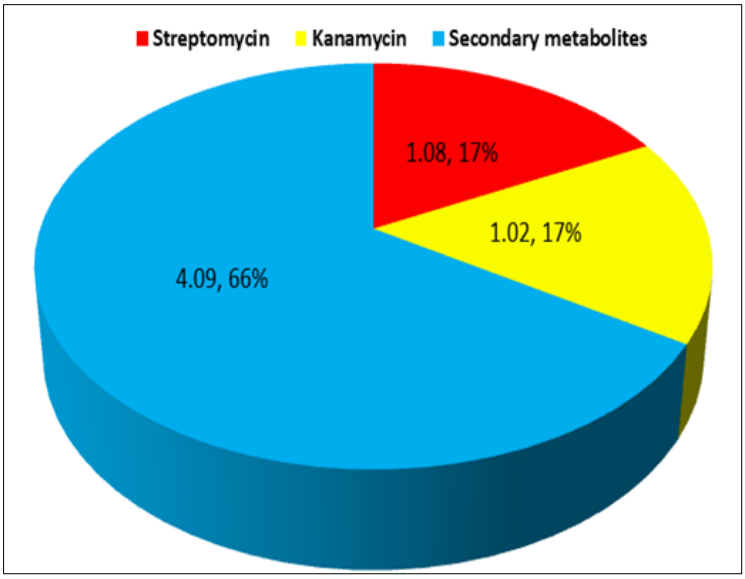

Fig. 1: Metabolite products, ststreptomycin and kanamycin as antibacterial activity against Strep pneumoniae

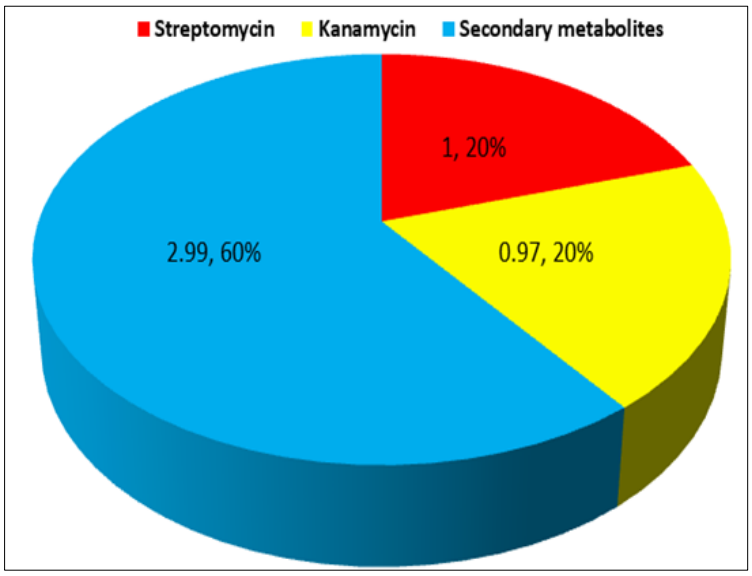

Fig. 2: Metabolite products, ststreptomycin and kanamycin as antibacterial activity against E. coli

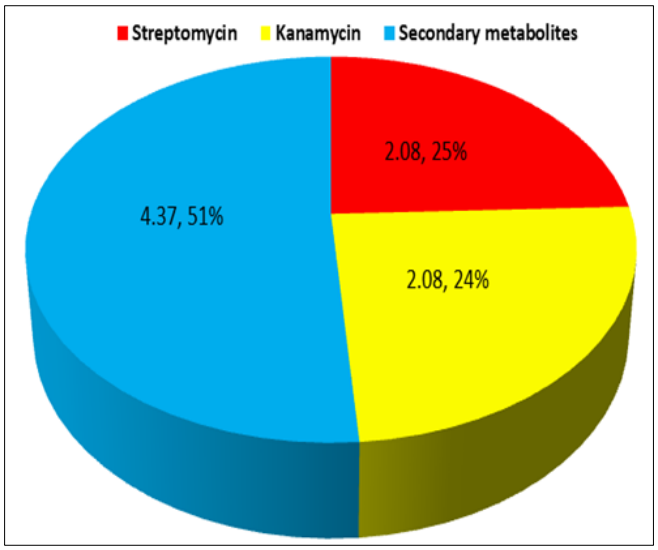

Fig. 3: Metabolite products, ststreptomycin and kanamycin as antibacterial activity against Staph aureus

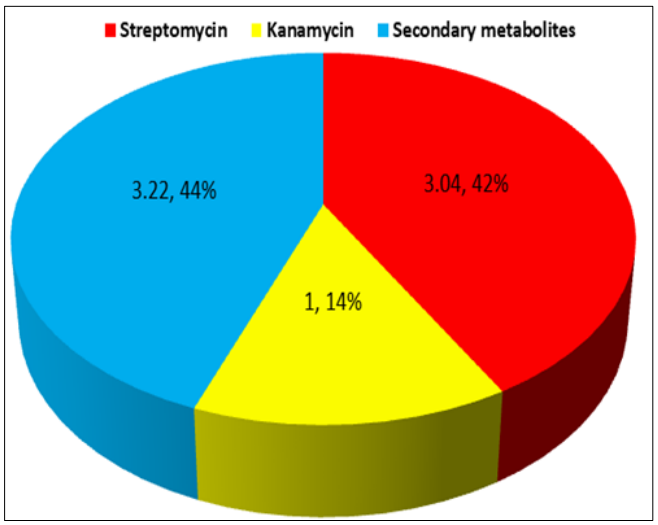

Fig. 4: Metabolite products, streptomycin and kanamycin as antibacterial activity against Proteus mirabilis.

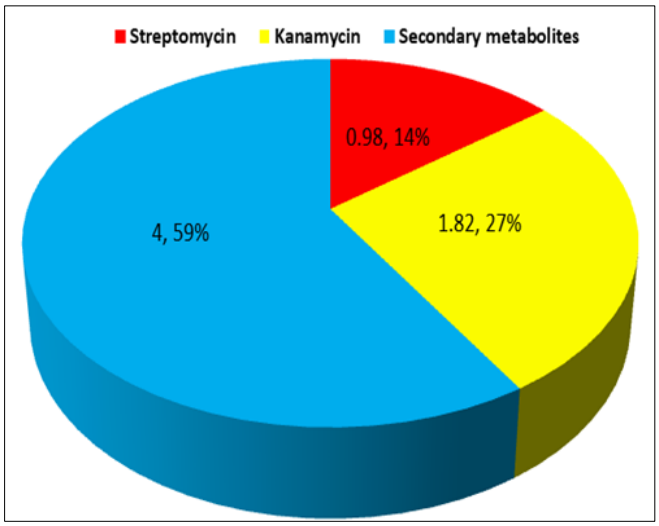

Fig. 5: Metabolite products, streptomycin and kanamycin as antibacterial activity against Staphylococcus epidermidis

Staphylococcus aureus had a maximal zone formation of 4.37±0.200 mm. The bacterium Klebsiella pneumoniae produces a wide variety of biologically active secondary metabolites. The purification of chemicals produced by Klebsiella pneumonia can be beneficial due to the importance of using bioactive compounds in pharmacy to create medications for the treatment of numerous ailments. People with compromised immune systems are more likely to get Klebsiella infections. Disabling diseases disproportionately impact men in their middle-aged and elder years. Some patients are thought to have compromised respiratory host defenses due to conditions such as diabetes, alcoholism, cancer, liver disease, COPD, glucocorticoid treatment, kidney failure, or exposure to specific occupational hazards (such papermill workers). Many of these diseases are nosocomial infections, meaning they are contracted when a patient is in the hospital for a different reason. Contact with infected tools is the second most common cause of patient infection, after feces.

CONCLUSION

In order to combat bacterial and fungal infections, cancer, and heart-related illnesses, microbes can manufacture a wide variety of pharmaceutically significant medications. Bacterial species vary greatly in their life cycles, physiological and biochemical adaptations, and the metabolic pathways they use to produce a wide array of secondary metabolites. The pharmaceutical and biotechnology sectors can benefit from advances in combinatorial biosynthesis if we can better understand the pathways and processes involved in the production of secondary metabolites. Using gas chromatography–mass spectrometry (GC–MS), 39 bioactive chemical components of Klebsiella pneumoniae were isolated from an ethanolic extract. The key platform for further phytochemical and pharmacological inquiry into the development of new possible antimicrobial compounds is the in vitro antifungal and antibacterial evaluation of secondary metabolite products of Klebsiella pneumoniae.

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