International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 06 Issue: 09 | September - 2022 Impact Factor: 7.185 ISSN: 2582-3930

A study on micro diversity and their susceptibility for *Phyllanthus urinaria, lemon grass, Pennsylvania smartweed, Cannabis sativa* associated with motorbike helmets from 18-24yrs. riders from Meerut.

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ABSTRACT

Motorcycle helmets can serve as a potential medium for the transmission of pathogenic bacteria and fungi with serious health implications. The present study explored the microbial diversity associated with the motorcycle helmets and determines the antibiotic susceptibility of the bacterial isolates against Bhang, *Phyllanthus urinaria*, lemon grass, *Pennsylvania smartweed* extracts. A descriptive cross-sectional study was carried amongst the students of MIET at Meerut campus. Gram-negative bacteria *(Escherichia coli, Staphylococcus aureus, Streptococcus faecalis, and Pseudomonas aeruginosa*) and gram-positive bacteria *(Staphylococcus aureus, Streptococcus faecalis, Bacillus species, and Clostridium species)* were found to be inhabitants of Helmets after their morphological and biochemical identification. The results of this experiment indicate that the leaves of *Phyllanthus urinaria*, Bhang, *Pennsylvania smartweed* and Lemon Grass had potent antibacterial properties; however gram-negative bacteria were less sensitive. The findings show that methanol and acetone are superior to ether for extracting the antibacterial characteristics of lemon grass and Phyllanthus urinaria, but ether and acetone are ineffective when extracting the antibacterial activities of Bhang and *Pennsylvania smartweed*.

KEY WORDS: helmets, bacteria, medicinal plants, antimicrobial activity.

Introduction

Any human society needs a way to go around, and in most of the world, a motorcycle is among the most convenient options. Modern transportation options include motorcycles, especially for short trips. As long as there is road infrastructure, it is simple to pass frequent traffic jams and arrive at any location, which contributes to the high frequency of use and passenger flow [14]. In the case of an accident, motorcycle helmets (MCH) shield riders from severe head and brain trauma [2]. Nevertheless, the ongoing processing and use of MCH products by many groups of individuals will foster a favourable breeding environment for bacteria, mould, and other germs, and there is a chance for the transmission of infectious diseases and dangerous microbes between users [12].

The greatest organ of the human body, the skin, is constantly exposed to environmental microbes and some microbial species can easily colonise it. Bacteria on adult skin can be found in concentrations of 10^{12} cfu/ml [12]. It has been suggested that the colonisation of potentially dangerous bacteria on inanimate things including computer keyboards, stethoscopes, banknotes, and cell phones could serve as a significant source of infection [1,6,11,13].

Staphylococcus aureus, Enterobacteraerogenes, Pseudomonas aeruginosa, Staphylococcus epidermidis, Escherichia coli, Bacillus, Aspergillus, Candida, and Rhizopus were all found in earlier investigations from motorcycle helmets [7,10]. A pathological situation develops if these normally occurring spores are placed into a foreign site or an infected host [15]. Antibiotics are a crucial part of the fight against bacterial illnesses, but antibiotic-resistant bacteria pose a serious threat to public health [4,8]. Bacteria can acquire or develop intrinsic resistance to antibiotics through changes in their genetic make-up and horizontal gene transfer of mobile genetic resistance components [3]. There is mounting proof that ESBL (Extended Spectrum Beta-Lactamase) and MRSA (Methicillin-Resistant Staphylococcus aureus) create bacterial isolates in inanimate objects [5,9,13].

Cannabis sativa is an angiosperm that is a member of the *Cannabaceae* family. Specific human illnesses include allergies, burns, cuts, and wounds, inflammation, leprosy, leukoderma, scabies, smallpox, and STDs have all been treated using *Cannabis sativa*.

Linn's Phyllanthus urinaria This plant, which is also known as chamber bitter or stone-breaker, is a member of the *Phyllanthus* family of *Euphorbiacea*e. This is a widely used medicinal herb for liver, urinary, and jaundice diseases, and it's sometimes mistaken for other herbaceous *Phyllanthus* species.

The ***Polygonaceae*** (Smartweed) family includes ***Pennsylvania smartweed***. About 900 species, ranging from annual herbs to perennial trees, make up the smartweed family. The family contains rhubarb, dock, and buckwheat. The simple leaves of the genus Polygonum frequently have black spots on them. Flowers lack petals; however, they do contain sepals that resemble petals.

Cymbopogon citratus, a perennial grass in the *Poaceae* family, is grown for its flavour-enhancing, fragrant leaves and stalks. The grass has multiple stiff stems, narrow, blade-like leaves that droop toward the tips, and it grows in thick clumps. The leaves are blue-green in hue and become red in the fall. When they are broken, they release a potent lemon scent.

Methods

2.1. Study site

In order to describe the teaching staffs and students at the Meerut Institute of Engineering and Technology, Meerut, a descriptive cross-sectional study was conducted. More than 95% of the staff members in the

campus, which includes over 3000 students and 300 teaching and non-teaching staff members, use motorcycles as their primary form of transportation on a daily basis 1500 members were chosen at random for the study. The campus' Microbiology lab served as the site of laboratory experiments. Through the use of interviews, information on sociodemographic traits and personal hygiene habits was gathered.

2.2. Leaves Collection

On the campus of the MIET college, the leaves of Bhang, *Phyllanthus urinaria,* Lemon grass, and *Pennsylvania smartweed* were gathered and cut into small pieces with a knife, the leaves of *Pennsylvania smartweed, Phyllanthus urinaria*, Bhang, and other plants are then dried in an oven at 150°C for four hours or in the sun for four days. After drying, the material was sieved to a fin and ground using an electric blender before being covered in aluminium foil to prevent evaporation and light exposure for three days at room temperature. With the aid of an electric blender, the leaves were ground into a fine powder that was sieved. For later use, the leaves' powdered form is kept in a closed, sterile container. The leaves were allowed to air dry for 4 days while being exposed to the sun's heat. 75% Petroleum ether, methanol, and acetone were the three solvents utilised for the extraction. 18ml of 75% ethanol, petroleum ether, and acetone were added to exactly 10g of dried powdered pineapple peel and pulp, respectively. The combination was prepared in a volumetric flask of 250 ml. These flasks spent 72 hours on a shaker B.O.D. incubator at 70 rpm. After 72 hours, the mixture was concentrated by setting the volumetric flask on a water bath set at 40°C.

2.3. Sample Collection

With the aid of sterile cotton swabs, the sample of various microorganisms is collected from the various age groups of helmets. The agar disc diffusion method was used to test the antibacterial sensitivity. The Mueller-Hinton agar (sensitivity medium) was made by mixing 3.8g of the powder with 100 ml of distilled water, autoclaving at 121°C for 15 minutes at 15 lbs., and then pouring the mixture into sterile Petri plates until it reached a uniform thickness of about 4mm. The agar was then allowed to set at room temperature before use. To express any extra fluid, a sterile cotton swab was put into the bacterial mixture, twisted, and then crushed against the test tube wall. The Mueller-Hinton agar plate's surface was then streaked with the swab. The entire plate surface was swiped three times with the swab to ensure a confluent, uniform growth.

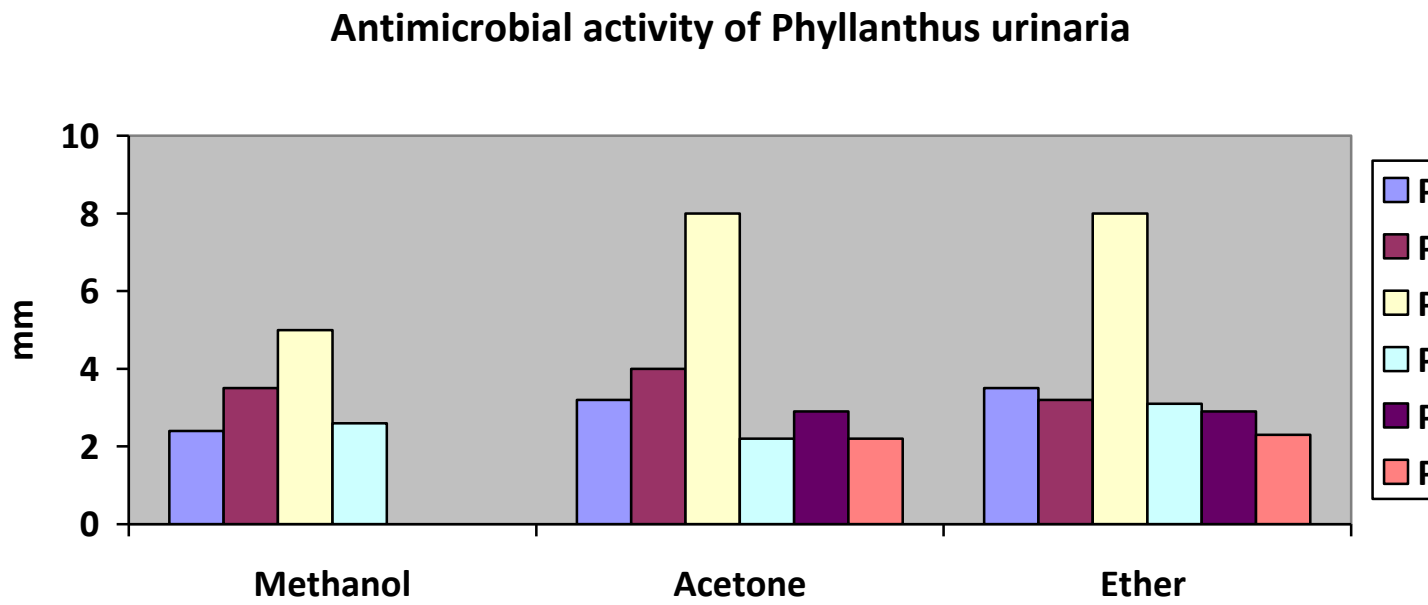
2.4. Identification of Bacteria and Fungi

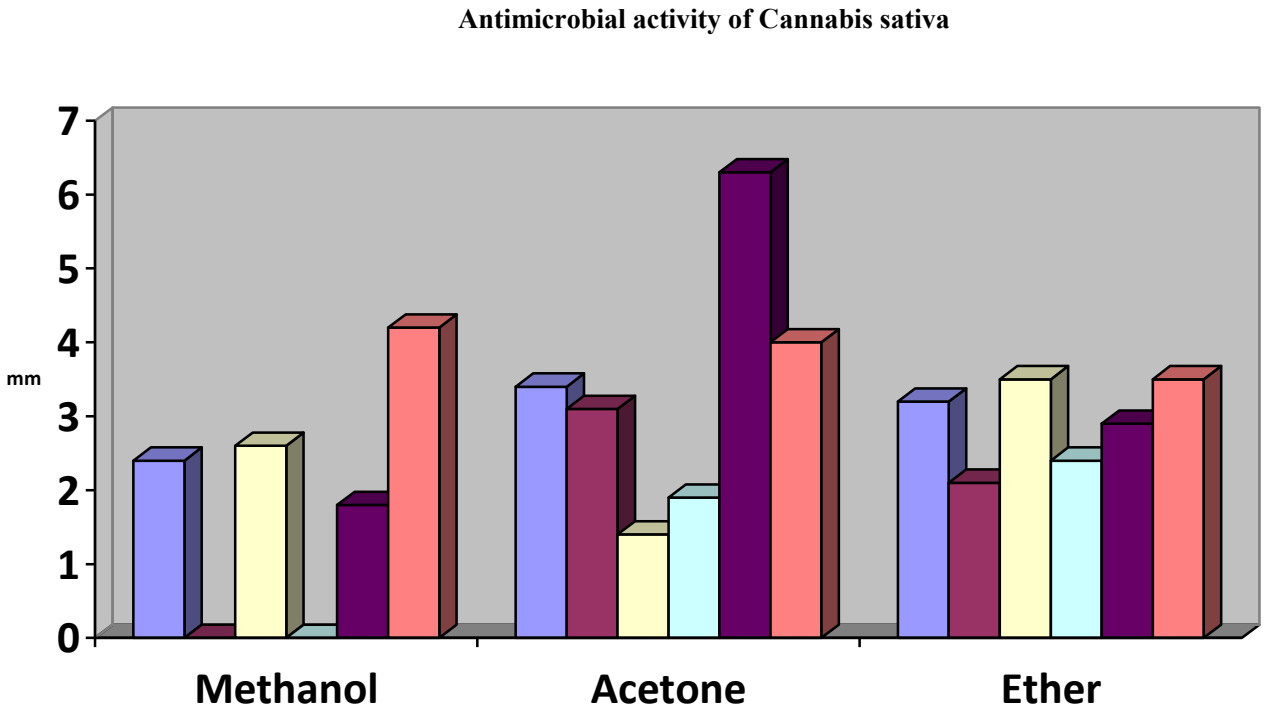
Based on their cultural, morphological, and biochemical properties, bacterial isolates were identified. As detailed by Samson and Van Reenen-Hoekstra, fungal mycelium was scraped off with the aid of an inoculating needle and teased out in a drop of lactophenol cotton blue on a clean and grease-free slide for microscopic examination. According to Samson et al., morphological and cultural traits allowed for the differentiation of fungi.

2.5. Antibiotic Susceptibility Test

Antimicrobial susceptibility testing was done using the well-diffusion method. The plant extracts were tested on Mueller Hinton II plates to detect the presence of antibacterial activity. Prior to streaking the plates with bacteria, 5 mm diameter wells were punched into the medium using a sterile borer. All plates were inoculated with the test bacterium which has been previously adjusted to the 0.5 McFarland standard solution; a sterile cotton swab was dipped into the suspension, rotated several times, and pressed firmly on the inside wall of the tube above the fluid level removing excess inoculum. The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculum with a final swab around the rim. The plates are allowed 3 to 5 min to dry the excess moisture. 70uL aliquots of each test extract was dispensed into each well after the inoculation of the plates with bacteria. The same extract was used on each plate, with a total of three plates used for each extract for selecting bacterium. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The plates are sealed with parafilm, labelled, and placed in an incubator set to 37°C. After 24 hours of incubation, each plate was examined for inhibition zones. A ruler was used to measure the inhibition zones in millimetres. Every experiment was carried out in parallel, and the results represented the average of at least three independent experiments.

RESULTS





Antimicrobial activity of extracts against different organisms against different solvent extracts of plants used in study

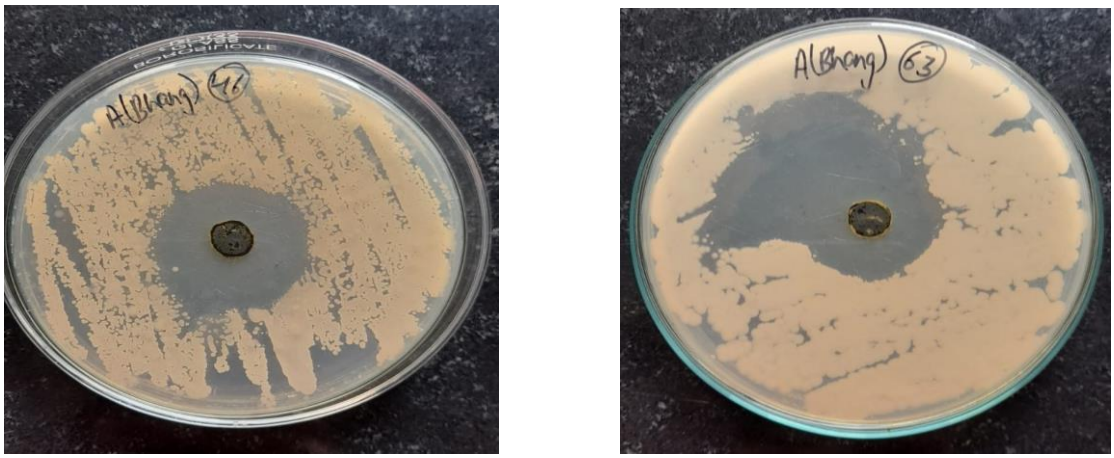


Figure showing zone of inhibition

DISCUSSION

According to earlier studies, Phytochemical investigation of various medicinal plants revealed that the leaves of *Phyllanthus urinaria*, Bhang, *Pennsylvania smartweed* and lemon grass are particularly abundant in a variety of polyphenolic chemicals (Phenol, flavonoids and tanninetc). Due to their antimicrobial properties, polyphenolic chemicals may be the most likely source of the antifungal and antibacterial effects of lemon grass, *Phyllanthus urinaria*, Bhang and other leaves. Not only that for the antibacterial function, leaves of these plants can be employed in pharmaceutical business as well as in food sector as bio preservative.

Overall findings showed that Gram negative bacteria were less sensitive to the antibacterial properties of Lemon grass, *Phyllanthus urinaria*, Bhang than Gram positive bacteria. Gram negative bacteria are less sensitive to antimicrobial substances than Gram positive bacteria, which may be because of variations in the cell walls of the two types of bacteria.

Gram negative bacteria have three different layers in their cell walls, which serve as an enclosure for some bioactive components. These comprise an inner or cytoplasmic membrane, an outer membrane made of lipopolysaccharide, and a peptidoglycan cell wall made of partly cross-linked peptide chains. Unlike gram negative bacteria, gram positive bacteria often lack this outer membrane. Certain antibiotic components and medications cannot enter the cell because the affected outer membrane serves as a barrier with a low permeability. One of the main factors causing Gram negative bacteria to exhibit higher levels of antibiotic resistance than gram positive bacteria is the existence of this outer membrane.

The importance of edible and medicinal plants to humanity is well known, since multiple discoveries have revealed that plant extracts include a variety of secondary metabolites with excellent therapeutic efficacies in addition to minerals and primary metabolites.

The study of the plant extracts found phytochemicals, which are known to have physiological and medicinal effects. For instance, tannins are polyphenolic substances that bind to proline-rich proteins and prevent protein synthesis while also possessing antimicrobial properties. According to considerable research, plants create hydroxylated polyphenolic chemicals called flavonoids in response to microbial infections, and these compounds have been shown to have antimicrobial activity in vitro against a variety of bacteria.

Their power has been linked to their capacity to assemble complexes with bacterial cell walls, soluble extracellular proteins, and both.

Although terpenoids are mostly employed for their fragrant properties, researchers have discovered that they may also be effective antibacterial agents. Glycosides called saponins have been discovered to have inhibitory effects on S. aureus, a gram-positive bacterium. As a consequence, the phytochemical characteristics showed that the methanol, ethanol, and acetone extract include chemical components that have been discovered to have antibacterial activities, which may have contributed to the findings from the antibacterial study.

CONCLUSION

The results of this experiment indicate that the leaves of *Phyllanthus urinaria*, Bhang, *Pennsylvania smartweed* and Lemon Grass had potent antibacterial properties, however gram-negative bacteria were less sensitive to the antimicrobial effects of the former two plants. The findings show that ether and acetone are superior to ether for extracting the antibacterial characteristics of lemon grass and *Phyllanthus urinaria*, but ether and methanol are ineffective when extracting the antibacterial activities of Bhang.Comparisons with relevant data from the literature show that the most varied results can be obtained from studies on antibacterial activity using various approaches. This investigation into the pharmacological effects of lemon grass, *Phyllanthus urinaria*, *Pennsylvania smartweed* and Bhang advances our understanding of the antibacterial principles and opens new doors for future research. The leaves of these plants have the potential to be a strong candidate in the quest for a natural antibacterial agent against infections and/or disorders brought on by the microbes contained in the helmets, according to the current research. This work offers new scientific understanding for exploring the pharmacological effects of Bhang, Phyllanthus urinaria, lemon grass as well as for determining the antibacterial principles. The antibacterial qualities emphasised in this work may also be explored further for producing antimicrobial medicines and goods.

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