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Evaluation of Antimicrobial activity of *Tribulus terrestris* extract against Multi resistant bacteria

A Graduated Research Report Submitted for Partial Fulfillment of Bachelor's Degree in Medical Laboratory Field

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TABLE OF USED ABBREVIATIONS

TT	*Tribulus terrestris*
Dr.	Doctor
T. terrestris	*Tribulus terrestris*
EG	Ethylene glycol
HPLC	high-performance liquid chromatography
S. aureus	*Staphylococcus aureus*
MRSA	Methicillin-Resistant *Staphylococcus aureus*
SCCmec	Staphylococcal chromosomal cassette mec
PBP-2a	penicillin-binding protein 2a
IV	intravenous
TSST-1	toxic shock syndrome toxin 1
PVL	Panton-Valentine leukocidin
P.aeruginosa	*Pseudomonas aeruginosa*
ICUs	infections in critical care units
HAP	hospital-acquired pneumonia

HCAP	healthcare-associated pneumonia
VAP	ventilator-associated pneumonia
GI	gastrointestinal
K. pneumonia	*Klebsiella pneumonia*
LPS	lipopolysaccharide
E. coli	*Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
EAEC	Enteroaggregative *Escherichia coli*
EHEC/STEC	Entrohemorrhagic *Escherichia coli*
CFU	Colony forming unit
CDC	Ceters disease control
PCR	Polymerase chain reaction
HUS	hemolytic uremic syndrome
EIEC	Enteroinvasive *Escherichia coli*
cAMP	cyclic adenosine monophosphate
MAHA	microangiopathic hemolytic anemia
UTI	urinary tract infection
CAP	Urethra acquired pneumonia
IDSA	Infectious Diseases Society of America
ISTM	International Society of Travel Medicine
D.W	Distel water
g	Gram
ml	Milliliter
mm	millimeter
mg	Milli gram
µg	microgram

Abstract

Background: Currently, it is helpful to develop new and efficient antibacterial medications due of rising antibiotic resistance organisms. Employing medicinal plants for natural therapy of diseases caused by bacterial origin has generally been considered.

Objective: This study seeks to evaluate the antibacterial efficacy of ethanolic extracts of Tribulus terrestris against four pathogenic bacteria in vitro.

Methods: In this experimental work, the antibacterial effects of a Tribulus terrestris extract evoked by ethanolic solvent on *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* were investigated using the disc diffusion assay.

Results: Tribulus terrestris ethanol extract has potential antibacterial activities against gram positive *S. aureus* and gram negative *P. aeruginosa*. The diameter of inhibition zones ranged from 15 to 23 mm. Tribulus terrestris extract showed non antibacterial activity to *E. coli* and *K. pneumonia.*

Conclusions: In conclusion, plant extracts are of good value as natural antimicrobials and can be used as alternative sources of antibacterial compounds.

Chapter 1: Introduction

Introduction

Tribulus terrestris (TT) (Zygophyllaceae) is an useful herb well-known for its use in traditional medicine in several regions of the world due to its high concentration of components with biological significance, such as saponins, flavonoids, alkaloids, glycosides, phytosteroids (Sun, 2002). Many studies have showed that the main active constituents of the plant steroidal saponins, which act as a natural testosterone and highly active surface substances (Kostova, 2005). Indeed, many pharmaceutical preparations and dietary supplements that include the saponin fraction of TT. are presently on sale extensively (Kostova, 2005).

It is an annual creeping herb that can be found worldwide in Mediterranean, subtropical, and desert temperature zones, including India, China, the Southern United States, Mexico, Spain, and Bulgaria (WC, 2002).

Many beneficial actions have reports for the dry fruits extract of TT. In complementary medicine, TT used as palliative, tonic, erotogenic, astringent, diuretic, stomachic, antihypertensive, Antiurolithiatic, and urinary disinfectant. TT has been used for centuries in India, South Africa, Turkey, Bulgaria and in traditional Chinese medicine to treat impotence, venereal diseases, eye trouble, edema, abdominal distension, emission, and morbid leukorrhea, and sexual dysfunction (Abirami, 2011).

Medicine is increasingly receptive of the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective and because of the rapid rate of plant species extinction. There is a feeling among natural-products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably. as the traditional systems are shown, *Tribulus terrestris* has been used from the ancient times as antimicrobial agents. In recent years, the antibacterial l activities of *Tribulus terrestris* extracts were confirmed against a large number of bacteria (Ahmed A. Hussain) .

Ethylene glycol (EG) is a key organic compound and chemical intermediate used in several industrial processes (e.g. energy, plastics, automobiles, and chemicals). Certainly, due to its exceptional properties and multipurpose commercial applications, a variety of chemical reactions (*e.g.*, catalytic and non-catalytic) have been explored for the synthesis of Ethylene glycol, especially by reaction mechanisms derived from biomass-based resources and fossil fuels (such petroleum, gas, and coal) (Yue, 2012).

The aim of this work was to study the biological activity of the Yemen herb *Tribulus* terrestris as antibacterial.

Chapter 2: Literature review

2.1.plant:

The genus *Tribulus*, belonging to family *Zygophyllaceae*, comprises about 20 species in the world, of which three species, *viz. Tribulus cistoides*, *Tribulus terrestris*, and *Tribulus alatus*, are of common occurrence in India Among them, *T. terrestris* (TT) is a well-patronized medicinal herb by Ayurvedic seers as well as by modern herbalists (Saurabh Chhatre, 2014).

2.1.1 Taxonomical classification:

- Kingdom: Plantae
- Division: Phanerogams
- Subdivision: Angiospermae
- Class: Dicotyledonae
- Subclass: Polypetalae
- Series: Disciflorae
- Order: Giraniales
- Family: *Zygophyllaceae*
- Genus: *Tribulus*
- Species: *terrestris Linn*. (M. Akram1, 2011)

2.1.2 Description:

It is a tap rooted herbaceous perennial plant that grows as a summer annual in colder climates. The stems radiate from the crown to a diameter of about 10 cm to over 1 m, often branching. They are usually prostrate, forming flat patches, though they may grow more upwards in shade or among taller plants. The leaves are pinnately compound with leaflets less than a quarter-inch long. The flowers are 4 to10 mm wide, with five lemon-yellow petals. A week after each flower blooms, it is followed by a fruit that easily falls apart into four or five single-seeded nut lets. The nutlets or "seeds" are hard and bear two to three sharp spines, 10 mm long and 4 to 6 mm broad point-to point. It is a trailing and spreading herb, densely covered with minute hair. Leaves compound, in opposite pairs, leaflets 3 to 6 pair, up to 8 cm long. Flowers are usually silky, white or yellow, solitary, arises from the axils of leaves. Ovary briskly, style short and stout. Fruits are globose, spinous or tuberculate; consisting of fine hairy or nearly glabrous, often matriculate and woodi cocci, each with two pairs of hard sharp spines, one pair longer than the other. Fruit often cling to clothes and bodies of animals. Seeds are many in woodi cocci. Plant is widely distributed

in different parts of India upto 3000 m altitude. Steroidal saponin and diosgenin is isolated from this plant. It is very rich in protein and calcium. Dried fruit contain semi-drying oil, peroxides, diastase, traces of glucosides, resins, protein and large amount of inorganic matters. From the roots, stem and leaves, sitosterol and srtigmasterol were also isolated (M. Akram1, 2011).

Fig 1: *Tribulus terrestris* and its fruit with fruit powder

.

2.1.3 Properties and actions:

- Taste based on activity: sweet.
- Properties: heavy to digest.
- Potency: cooling.
- Taste after digestion based on activity: sweet.
- Pharmacological actions: nourishing, pacifies, aphrodisiac, removes urinary stone, cures bladder ailments (Saurabh Chhatre, 2014).

2.1.4 Chemical constituents:

The preliminary phytochemical study of TT revealed the presence of saponins, flavonoids, glycosides, alkaloids, and tannins. According to literature data, the saponin composition and the saponin content of TT from different geographic regions is different studied the chemistry and bioactivity of saponins in TT. They reported that furostanol and spirostanol saponins of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin,

diosgenin, chlorogenin, ruscogenin, and sarsasapogenin types are frequently found in this plant. In addition, four sulfated saponins of tigogenin and diosgenin type were also isolated. Majorly present are furostanol glycosides including protodioscin and protogracillin, of which protodioscin is the most dominant saponin and spirostanol glycosides are present in small quantities. The quantity of main flavonoids is about 1.5 times that of main saponins. This indicated that the flavonoid contents in TT should be studied, developed, and further used. Extracted kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, and tribuloside [kaempferol-3-β-d-(6″-p-coumaroyl) glucoside] from leaves as well as fruits and identified them by spectroscopic analysis. detected 18 flavonoids (caffeoyl derivatives, quercetin glycosides, including rutin and kaempferol glycosides) using high-performance liquid chromatography (HPLC) in four *Tribulus* species leaf extracts. Optimization the extraction condition using orthogonal experiment, isolated three flavonoid glycosides, viz. quercetin 3-O-glycoside, quercetin 3-O-rutinoside, and kaempferol 3-O-glycoside from the aerial parts of *T. terrestris* .

Identified flavonoids from the petroleum ether and chloroform extracts of fresh fruits of TT from India using ethyl acetate: benzene (1:9) solvent system. These flavonoids were not detected in the fruit extracts of other variety, namely T. alatus. Hence, presence of such pharmacognostic constituents can be used as a diagnostic tool in the identification of the species and study of contamination/adulteration. isolated and characterized three new compounds, terrestribisamide, 25R-spirost-4-en-3, 12-dione, and tribulusterine, together with 10 known compounds, N-pcoumaroyltyramine, terrestriamide, hecogenin, aurantiamide acetate, xanthosine, fatty acid ester, ferulic acid, vanillin, phydroxybenzoic acid, and β-sitosterol, from the dried fruits of TT. The alkaloids present are harmane and norharmane. The β-carboline alkaloid, tribulusterine, is present in minor quantities in fruits. Gas chromatography-mass spectrometry analysis of methanolic extract of the whole plant of TT revealed the presence of α-Amyrin as the major constituent and seven minor constituents, which are 3,7,11,15-tetramethyl-2-hexadecen-1-ol, n-hexadecadienoic acid, hexadecadienoic acid ethyl ester, phytol, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, and 1,2-benzenedicarboxylic acid disoctyl ester. Sterols such as β-sitosterols and stigmasterols were also found to be present. (Saurabh Chhatre, 2014)

2.1.5 Traditional pharmacological uses:

The fruits of TT have been used for tonifying the kidneys and as a diuretic and cough expectorant that improves eyesight and for the treatment of skin pruritus, headache and vertigo, and mammary duct blockage. In India, the fruits have been used in the treatment of infertility, impotence, erectile dysfunction and low libido in Ayurveda. In addition, the roots and fruits are considered to have cardiotonic properties . In Sudan, TT has been used as demulcent and in nephritis and the treatment of inflammatory disorders. In addition, it has been used for diuretic and uricosuric effects in Pakistan. Modern investigation showed that the chemical constituents steroidal saponins and flavonoids with the prominent anti-inflammatory and antiaging activities of TT were the main contributors to the traditional pharmacological activities. (Wenyi Zhu, 2017)

2.1.6 Antibacterial activity:

All parts (fruits, stems, leaves, and roots) of Turkish and Iranian TT showed antibacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, in contrast to the aerial parts of Yemeni TT which had no detectable antibacterial activity against these bacteria, while only the fruits and leaves of Indian TT were active exclusively against *E. coli* and *S. aureus*. These different results relating to the antibacterial activity of TT may be due to using different geographic sources of the plant, types of strains, and assay methods. The methanolic extract of fruits of TT was found to be most active against gram-positive and gram-negative bacteria; while moderate activity was observed in its petroleum ether extract and chloroform extract (Saurabh Chhatre, 2014).

2.2 *Staphylococcus aureus*:

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations. Infections are common both in community-acquired as well as hospital-acquired settings and treatment remains challenging to manage due to the emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant *Staphylococcus aureus*). *S. aureus* is found in the environment and is also found in normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals. *S. aureus* does not normally cause infection on healthy skin;

however, if it is allowed to enter the bloodstream or internal tissues, these bacteria may cause a variety of potentially serious infections. Transmission is typically from direct contact. However, some infections involve other transmission methods (Tracey A. Taylor, 2022).

a)Etiology

Staphylococcus aureus is Gram-positive bacteria (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters that are described as "grape-like." On media, these organisms can grow in up to 10% salt, and colonies are often golden or yellow (aureus means golden or yellow). These organisms can grow aerobically or anaerobically (facultative) and at temperatures between 18 C and 40 C. Typical biochemical identification tests include catalase positive (all pathogenic *Staphylococcus* species), coagulase positive (to distinguish *Staphylococcus aureus* from other *Staphylococcus* species), novobiocin sensitive (to distinguish from *Staphylococcus saprophyticus*), and mannitol fermentation positive (to distinguish from *Staphylococcus epidermidis*) (Rasigade JP, 2014) (FD, 1998). MRSA strains carry a *mec* gene on the bacterial chromosome, which is a component of the larger Staphylococcal chromosomal cassette *mec* (SCC*mec*) region, conferring resistance to multiple antibiotics depending on the SCC*mec* type. The *mec* gene encodes the protein PBP-2a (penicillin-binding protein 2a). PBP-2a is a penicillin-binding protein (PBP), or essential bacterial cell wall enzyme that catalyzes the production of the peptidoglycan in the bacterial cell wall. PBP-2A has a lower affinity to bind to beta-lactams (and other penicillin-derived antibiotics) when compared to other PBPs, so PBP-2A continues to catalyze the synthesis of the bacterial cell wall even in the presence of many antibiotics. As a result, *S. aureus* strains that synthesize PBP-2A can grow in the presence of many antibiotics, and these MRSA strains are resistant to many antibiotics. MRSA strains tend to be resistant to methicillin, nafcillin, oxacillin, and cephalosporins (Tracey A. Taylor, 2022).

b)Epidemiology

Staphylococcus aureus (including drug-resistant strains such as MRSA) are found on the skin and mucous membranes, and humans are the major reservoir for these organisms. It is estimated that up to half of all adults are colonized, and approximately 15% of the population persistently carry *S. aureus* in the anterior nares. Some populations tend to have higher rates of *S. aureus* colonization (up to 80%), such as health care workers, persons

who use needles on a regular basis (*i.e.*, diabetics and intravenous (IV) drug users), hospitalized patients, and immunocompromised individuals. *S. aureus* can be transmitted person-to-person by direct contact or by fomites (Tracey A. Taylor, 2022) (Rasigade JP, 2014).

c)Pathophsiology

S. aureus are one the most common bacterial infections in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections (e.g., impetigo, folliculitis, furuncles, carbuncles, cellulitis, scalded skin syndrome, and others), osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections (Tong SY, 2015). Depending on the strains involved and the site of infection, these bacteria can cause invasive infections and/or toxin-mediated diseases. The pathophysiology varies greatly depending on the type of *S. aureus* infection. Mechanisms for evasion of the host immune response include the production of an antiphagocytic capsule, sequestering of host antibodies or antigen masking by Protein A, biofilm formation, intracellular survival, and blocking chemotaxis of leukocytes. Binding of the bacteria to extracellular matrix proteins and fibronectin in infectious endocarditis is mediated by bacterial cell wall-associated proteins such as fibrinogen-binding proteins, clumping factors, and teichoic acids. Also, Staphylococcal superantigens (TSST-1 or toxic shock syndrome toxin 1) are important virulence factors in infectious endocarditis, sepsis, as well as toxic shock syndrome. Pneumonia infections are associated with the bacterial production of PVL (Panton-Valentine 18eucocidin), Protein A, and alpha-hemolysin, and infections are more common following influenza virus infection as well as a diagnosis of Cystic Fibrosis. Prosthetic device infections are often mediated by the ability of *S. aureus* strains to form biofilms as well as communicate using quorum sensing in a bacterial cell density-dependent manner (Tracey A. Taylor, 2022).

d)Treatment\ Management

Treatment of *S. aureus* infections depends largely on the type of infection as well as the presence or absence of drug resistant strains. When antimicrobial therapy is needed, the duration and mode of therapy are largely dependent on the infection type as well as other factors. In general, penicillin remains the drug of choice if isolates are sensitive (MSSA, or methicillin sensitive *S. aureus* strains) and vancomycin for MRSA strains. In some cases,

alternative therapy is necessary for addition to antimicrobial therapy. For example, fluid-replacement management is often required for toxin-mediated illness and removal of foreign devices for prosthetic value endocarditis or catheter-associated infections. Because many MRSA strains are resistant to multiple antibiotics, MRSA infections are emerging as serious pathogens in both the hospital and the community settings (Tracey A. Taylor, 2022).

2.3 *Pseudomonas aeruginosa*:

Pseudomonas aeruginosa is an aerobic , gram negative, nonfermentative rod that typically dwells in damp settings. It uses a variety of chemical substances for growth and has low nutritional needs. This metabolic adaptability reflects a larger and more complex genome compared to that of many other bacterial species, and it aids in a wide ecological dispersion and adaptation (ROSSOLINI, et al., 2005).

a)Etiology

Patients with burn wounds, cystic fibrosis, acute leukemia, organ transplants, and intravenous drug addiction are particularly susceptible to Pseudomonas infection. *P. aeruginosa* is a frequent nosocomial contaminant, and numerous elements of the hospital environment have been linked to outbreaks. Patients who stay in hospitals for a long time are frequently colonized by this organism and are more likely to get sick. Malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia are some of the most dangerous illnesses. The severity of the patient's underlying disease process affects the likelihood of recovery from a pseudomonas infection. (DRISCOLL, et al., 2007)

b)Epidemiology

Pseudomonas aeruginosa and Nosocomial Infections: When a microbiological isolate is identified, *P. aeruginosa* is a frequent cause of nosocomial infections, accounting for 11.8% to 13.8% of all nosocomial infections. *P. aeruginosa* is often responsible for an even larger percentage of nosocomial infections in critical care units (ICUs), with rates of 13.2-22.6% observed,

P. aeruginosa has been found as the second most frequent cause of hospital-acquired pneumonia (HAP), healthcare-associated pneumonia (HCAP), and ventilator-associated pneumonia (VAP), trailing only *Staphylococcus aureus* in frequency. However, trends may

differ within institutions. The most prevalent infectious isolate in HAP developing after 4 days in an ICU, in VAP following 4 days of mechanical ventilation, or in VAP following percutaneous tracheostomy has been identified as *P. aeruginosa* is listed as the most frequent cause of nosocomial pneumonia in pediatric intensive care units. (DRISCOLL, et al., 2007)

c)Pathophsiology

With the help of respiratory mucins and the glycolipid asialoGM1, *P. aeruginosa's* flagella and pili are essential for motility and respiratory infection . The interaction of bacterial adhesins with host receptors results in bacterial adherence to the respiratory epithelium, which is a necessary stage in the infection process. The single flagellum, which is required for motility, adhesion to cells, and biofilm formation, and type IV pili, which are appendages made of pilin polymers, are the main adhesins for *P. aeruginosa* infection. These pili also play important roles in biofilm formation and respiratory epithelial cell attachment . The relevant pathogenic methods of *P. aeruginosa* that it employs to assault. (DRISCOLL, et al., 2007)

d)Treatment\ Management

Since *P. aeruginosa* is a potentially fatal pathogen and the timing of chemotherapy is crucial to how the infection turns out, there are empirical regimens suitable for *P. aeruginosa.*

Combination chemotherapy with at least two different anti-pseudomonal agents is typically advised for the treatment of severe *P. aeruginosa* infections, such as endocarditis, nosocomial pneumonia, and urinary tract infections. This is except for upper tract infections complicated by abscess formation, infections in neutropenic patients, or infections whenever there is a suspicion of bacteraemia, Combination chemotherapy is primarily used to lessen the likelihood that resistant mutants would be selected during treatment and to take advantage of some medicines' potential synergistic effects. As demonstrated by in-vitro studies, aminoglycosides and –lactams remain the preferred combination, and the findings of numerous clinical studies support the superiority of similar regimens over monotherapy for the treatment of P. aeruginosa bacteremia, particularly in neutropenic patients (ROSSOLINI, et al., 2005).

2.4 *Klebsiella pneumonia*:

Klebsiella pneumoniae for the first time described as an encapsulated bacillus after isolating the bacterium from the lungs of those who had died from pneumonia. Originally named Friedlander's bacillus, it was not until 1886 when the bacterium garnered the name *Klebsiella*. *Klebsiella pneumoniae* is a gram-negative, encapsulated, non-motile bacterium found in the environment and has been associated with pneumonia in patient populations with alcohol use disorder or diabetes mellitus. The bacterium typically colonizes human mucosal surfaces of the oropharynx and gastrointestinal (GI) tract. Once the bacterium enters the body, it can display high degrees of virulence and antibiotic resistance. Today, *K. pneumoniae* is considered the most common cause of hospital-acquired pneumonia in the United States, and the organism accounts for 3% to 8% of all nosocomial bacterial infections. (Jondle CN, 2018) (Aghamohammad S, 2020)

a)Etiology

Klebsiella pneumoniae, a member of the family *Enterobacteriaceae*, is a rod-shaped, Gram-negative, lactose-fermenting bacillus with a prominent capsule. Typical *K. pneumoniae* is an opportunistic pathogen that is widely found in the mouth, skin and intestines, as well as in hospital settings and medical devices. Opportunistic *K. pneumoniae* mostly affects those with compromised immune systems or who are weakened by other infections. Colonization of the GI tract by opportunistic *K. pneumoniae* generally occurs prior to the development of nosocomial infections, and *K. pneumoniae* colonization can be further found in the urinary tract, respiratory tract and blood. *K. pneumoniae* biofilms that form on medical devices (e.g., catheters and endotracheal tubes) provide a significant source of infection in catheterized patients (Schroll C, 2010). Nosocomial infections caused by *K. pneumoniae* tend to be chronic due to the two following major reasons*: K. pneumoniae* biofilms formed in vivo protect the pathogen from attacks of the host immune responses and antibiotics and nosocomial isolates of *K. pneumoniae* often display multidrug-resistance phenotypes that are commonly caused by the presence of extended-spectrum β-lactamases or carbapenemases, making it difficult to choose appropriate antibiotics for treatment. (Li, 2014)

b)Epidemiology

Humans serve as the primary reservoir for *K. pneumoniae*. In the general community, 5% to 38% of individuals carry the organism in their stool and 1% to 6% in the nasopharynx. The main reservoirs of infection are the patient's gastrointestinal tract and the hands of hospital personnel. It can lead to a nosocomial outbreak. However, higher rates of colonization have been reported in those of Chinese ethnicity and those who experience chronic alcoholism. In hospitalized patients, the carrier rate for *K. pneumoniae* is much higher than that found in the community. In one-study, carrier rates as high as 77% can be seen in the stool of those hospitalized and are related to the number of antibiotics given (Esposito EP, 2018). causing nosocomial infections, but a subset of hypervirulent serotypes (including predominantly K1 and K2) due to increased production of CPS affect previously healthy persons to cause life-threatening invasive infections. CPS is the most important virulence factor of *K. pneumoniae*, which plays important roles in resistance to phagocytosis, suppression of early inflammatory response, resistance to antimicrobial peptides, and inhibition of DC cell maturation. Additional *K. pneumoniae* virulence factors include LPS, fimbriae, outer membrane proteins, and determinants for iron acquisition and nitrogen source utilization. (Ashurst JV, 2022)

c)Pathophsiology

Typical *Klebsiella pneumoniae* is an opportunistic pathogen, which mostly affects those with weakened immune systems and tends to cause nosocomial infections. A subset of hypervirulent *K. pneumoniae* serotypes with elevated production of capsule polysaccharide can affect previously healthy persons and cause life-threatening community acquired infections, such as pyogenic liver abscess, meningitis, necrotizing fasciitis, endophthalmitis and severe pneumonia. *K. pneumoniae* utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for iron acquisition and nitrogen source utilization, for survival and immune evasion during infection. (Schroll C, 2010)

d)Treatment\ Management

Given the low occurrence of *K. pneumoniae* in the community, the treatment of pneumonia should follow standard guidelines for antibiotic therapy. Once infection with *K. pneumoniae* is either suspected or confirmed, antibiotic treatment should be tailored to local antibiotic sensitivities.

Current regimens for community-acquired *K. pneumoniae* include a 14-day treatment with either a third or fourth generation cephalosporin as monotherapy or a respiratory quinolone as monotherapy or either of the previous regimes in conjunction with an aminoglycoside. If the patient is penicillin-allergic, then a course of aztreonam or a respiratory quinolone should be undertaken. For nosocomial infections, a carbapenem can be used as monotherapy until sensitivities are reported (Thakuria B, 2013).

2.5 *Escherichia coli*:

E. coli is a gram-negative, non-sporulating, rod-shaped, facultative anaerobic, and coliform bacterium pertaining to the genus *Escherichia* that commonly inhabits the environment, foods, and warm-blooded animals' lower gut. In the domains of biotechnology and microbiology, it is the most widely studied prokaryotic model organism. It can live for long periods of time in feces, soil, and water, and is frequently used as a water contamination indicator organism. For 2–3 days, the bacterium multiplies rapidly in fresh feces under aerobic circumstances, but its numbers gradually fall after that. (B.S. Gunashree, 2022)

a)Etiology

Escherichia coli is a type of bacteria that lives in many places in the environment, including the gastrointestinal system of humans and warm-blooded animals, where it is part of the gut microbiota. Some strains of *E. coli* can be administered as probiotics and are known to have a positive effect on host health. However, some strains can be pathogenic, causing intestinal and extraintestinal infections in humans as well as animals. *E. coli* is hence a bacterium with a wide range of different natural types of strains, each with its own set of features. Because of its unique qualities, such as simplicity of handling, availability of the entire genome sequence, and capacity to grow in both aerobic and anaerobic conditions, *E. coli* is also a popular bacterium for laboratory research and biotechnology (de la Cabada Bauche J, 2011) (Karch H, 2001).

b)Epidemiology

Escherichia coli results in intestinal illness as well as infection outside of the intestine. Intestinal illness caused by *E. coli* is caused by one of five subtypes, and they are identified according to their O and H antigens. The O antigen is determined by a repeating

polysaccharide chain present in the lipopolysaccharide)LPS(outer membrane, and the flagellum determines the H antigen. (Nataro JP, 1998)

- ETEC causes watery diarrhea in resource-limited settings and is commonly found in food and water in areas without adequate sanitation. Approximately 100,000,000 organisms must be ingested to cause illness in a healthy person. It is the single most important organism causing traveler's diarrhea. ETEC is also a significant contributor to dehydrating diarrheal illness in infants and children in resource-limited settings.
- EPEC was the first *E. coli* pathotype identified as a causative agent of watery diarrhea primarily in infants and young children in resource-limited settings and is responsible for sporadic and epidemic outbreaks. Diarrheal illness caused by EPEC is most commonly contracted through ingestion but can also be spread person-to-person. (Regua AH, 1990)
- EAEC is a causative organism of acute and chronic watery diarrhea in resource-limited and resource-rich regions and recently has been increasingly identified as a cause of traveler's diarrhea. (Huang DB, 2006)
- EHEC/STEC produces Shiga-toxin and includes serotypes O157:H7, as well as others EHEC/STEC has been responsible for large diarrheal outbreaks after ingesting contaminated produce)e.g., spinach, sprouts, lettuce, fruit(and undercooked beef. EHEC/STEC is linked to the consumption of raw dairy products. EHEC/STEC is commonly found in ground beef, which can be contaminated during processing. Vegetables are contaminated when are fertilized with manure containing EHEC/STEC, and water runoff from these crops leads to EHEC/STEC found in water systems. Relatively low inoculums)102 CFU(result in illness, facilitating the ease of transmission from the environment to humans and humans to humans. The World Health Organization reports there are roughly 2.8 million cases of EHEC/STEC infections globally as of According to the CDC, there were reportedly 3,127 cases in the United States in .2014 While the number of reported cases of O157:H7 declined in the United States in .2019 the number of non-O157:H7 EHEC/STEC cases rose by 35% compared to the ,2019 previous year. This is likely due to more readily available PCR-based assays to identify organisms that allow laboratories to distinguish *E. coli* O157:H7 from non-O157:H7 strains. EHEC/STEC infections are common across all age groups, but hemolytic uremic

syndrome)HUS(resulting from EHEC/STEC infections is most common in children less than five years old and adults greater than 60years old. (Mueller M, 2022)

- EIEC-induced diarrheal illness is uncommon due in part to the relatively large inoculum required, although recent studies suggest EIEC-induced diarrhea may be underdiagnosed. EIEC is closely related to Shigella and is contracted through ingesting undercooked meats and contaminated vegetables. (Kaper JB, 2004)

Extraintestinal illness caused by *E. coli* results from a translocation of gut bacteria into other parts of the body or the environmental spread in hospitals and long term care facilities. *E. coli* is the predominant gram-negative bacteria to cause extraintestinal illness in humans and can cause urinary tract infection, abdominal and pelvic infection, pneumonia, bacteremia, and meningitis, among others. (Mueller M, 2022)

c)Pathophsiology

Intestinal illness caused by *E. coli* results from the ingestion of bacteria and theinnate ability of *E. coli* to overcome host defenses. Gram-negative bacteria are characterized by their cell envelope, which comprises an inner cytoplasmic cell membrane, peptidoglycan cell wall, and outer membrane. The outer membrane is made of a lipid bilayer, associated proteins, and lipopolysaccharide)LPS(, resulting in a toxic reaction if lysed. Pathogenic *E. coli* strains each has distinctive virulence factors encoded on plasmids, transposons, and bacteriophages. (Mueller M, 2022)

- ETEC: Colonizing fimbriae expressed by ETEC enable the bacteria to attach to the stable -(LT) and/or heatlabile toxin -intestinal wall. Once connected, ETEC expresses a heat toxin (ST), which are secretory toxins encoded on plasmids. LT stimulates adenylate cyclase leading to increased intracellular cyclic adenosine monophosphate (cAMP) and the ls. This mechanism also inhibits subsequent chloride secretion from intestinal crypt cel intestinal villi from absorbing sodium chloride. This process leads to free water secretion into the intestinal lumen, thus producing watery diarrhea.
- EPEC to -EPEC: A bundle chment to enterocytes in the small intestine. Once bound, the outer form a localized atta membrane protein colonization factor, intimin, facilitates enhanced adherence. Intimin is an outer membrane protein colonization factor encoded on the eae gene within the locus of te effacement (LEE) chromosomal island. The LEE chromosomal island elaborates enterocy approximately 20 secretory toxins that are

injected in the enterocyte by a type III injectisomeThese toxins result in a series of events, ultimately leading to the acteristic effacement of microvilli, increased permeability of tight junctions, and char secreted -alterations in water and electrolyte secretion and absorption. EspF is a LEE testinal protein that is not involved with attaching and effacing. It appears to disrupt the in barrier function by increasing monolayer permeability via alteration of electrical resistance. protein interaction domains that may function by interacting with -EspF has several protein spG2, inhibit luminal endocytic regulation. Two other secreted proteins, EspG and E exchanger -OH/-membrane chloride absorption by decreasing surface expression of the Cl via disruption of microtubules.

- EAEC: EAEC exhibits a stacked brick pattern of adherence to epithelial cells. The virulence scriptional activator AggR which activates several virulence plasmid encodes the tran factors, although scientific understanding of this process is not complete.AggR likely III), adhesin, surface protein dispersin, and -induces aggregative adherence fimbriae (AAF/I mediated -1, ShET1, and ShET2. Dispersin likely promotes AAF-oxins Pet, EAST the enterot colonization
- EHEC/STEC: EHEC/STEC produces bloody diarrhea due to its ability to express Shiga lated to Shiga toxin 1 (Stx1) and or Shiga toxin 2 (Stx2). Stx1 and Stx2 are closely re toxin (Stx) produced by Shigella dysenteriae. EHEC/STEC, which expresses Stx2, results in bloody diarrhea and may also express Stx1, while bacteria that do not express Stx2 do not protein toxins composed of induce bloody diarrhea. The Stxs are a group of bacterial AB one A subunit and five identical B subunits capable of inhibiting protein synthesis through their ability to target eukaryotic ribosomes. The A subunit is responsible for inhibiting protein ycosphingolipid Gb3, a cellular receptor on synthesis while the B pentamer binds gl endothelial cells. The inhibition of protein synthesis results in enterocyte cell death and subsequent inflammatory colitis. The EHEC/STEC genome also encodes intimin, which is -possesses a plasmid (pO157) that expresses a poreits primary adhesin, and EHEC/STEC hemolysin. Once EHEC/STEC attach and produce -forming toxin termed EHEC localized intestinal damage, the Stx toxins enter the host and travel to target organ epithelial ells undergo similar damage as enterocytes, and as a result of cells. Glomerular epithelial c cell death, detach from the glomerular membrane. This inflammatory state results in thrombosis and activation of the coagulation cascade yielding subsequent damage, the HUS triad. EHEC/STEC is also known thrombocytopenia, anemia, and renal

for its ability to induce hemolytic uremic syndrome (HUS). HUS is characterized by the triad of microangiopathic hemolytic anemia (MAHA), thrombocytopenia, and renal insufficiency. s induce secretory diarrhea. Subsequent colonization and

- EIEC: Like EHEC, enterotoxin cell spread result in inflammatory colitis-to-invasion of colonic mucosa, replication, cell to cell spread result the in inflammatory colitis. (Kaper JB, 2004)

Extraintestinal infections caused by *E. coli* are generally the result of thetranslocation of *E. coli* outside of the intestine. The urinary tract is the most common commensal extraintestinal site of infection caused by *E. coli*. UTIs are a significant reason for ambulatory care visits in the US and is the second most common cause of hospitalization umonia.Urinary tract infections from result from bacteria ascending after pne the urethra and are more common in *E. coli* women than men, given the proximity of the urethra. -acquired pneumonia (CAP) caused by *E. coli* is common, but ventilator-Community iated pneumonia (VAP) is more common. Hospitalized patients, particularly those assoc on mechanical ventilation, are at an increased risk of aspirating gastric contents. *E. coli* -ommunitybacteremia is often the result of a primary *E. coli* infection at another site. C acquired *E. coli* bacteremia is most frequently the result of urinary tract infections in older adults, while hospitalized patients likely develop bacteremia as a result of lower respiratory tract infection. (Mueller M, 2022)

d)Treatment\ Management

Treatment is dependent on the strain, as well as the illness. Care of the patient with an intestinal disease caused by *E. coli* begins with symptomatic management. Diarrheal illness can be extremely distressing for patients. Experts recommend rehydration and antidiarrheals as the mainstays of treatment for mild disease. Oral rehydration is recommended first-line therapy for all patients with diarrheal illness when tolerated and is equally efficacious as compared to intravenous hydration is recommended when patients cannot tolerate oral intake. Distressing symptoms are treated with antimotility agents such .as bismuth-subsalicylate and loperamide. (Shane AL, 2017)

Antibiotics are not recommended as first-line treatment for diarrheal illness caused by *E. coli* for most patients due to the harmful side effects and association with antibiotic resistance. For patients with severe disease (e.g., more than six stools per day, fever,

dehydration necessitating hospitalization, diarrhea lasting more than seven days, or bloody diarrhea), antibiotics may be reasonable. Rifaximin, azithromycin, and ciprofloxacin are currently recommended by the Infectious Diseases Society of America)IDSA(and the International Society of Travel Medicine)ISTM(to treat *E. coli* diarrheal illness. For patients suspected of having EHEC/STEC, antibiotics are not recommended, especially in children and older adults, due to the increased risk of hemolytic uremic syndrome. (Mueller M, 2022)

2.6 Study Objective

Objective of this study was to evaluate, in vitro, antimicrobial activity of hydroalcoholic of *Tribulus terrestris (TT)* extract against the most common pathogenic bacterial species in Yemen.

2.7 Research Question

Are they a significant in vitro antimicrobial activity of hydroalcoholic of *Tribulus terrestris* (TT) extract against the most common pathogenic bacterial species?

Chapter 3: Materials & Methods

Materials & Methods

3.1 Collection and Extraction of Plants:

Tribulus terrestris was collected from Sana'a city, Yemen and identified by Department of Pharmacognosy at University of Science and Technology, Sana'a Yemen. Dried TT fruit, root and leave were pulverized by using an electrically operated grinder.

Powder of whole plant of TT (2000 g) was suspended in 5 liter of ethanol and was left to macerate at room temperature for 2 days. The extraction process was repeated twice to ensure complete extraction. The combined extracts was filtered and subject to remove solvent under vacuum by rotary evaporator and dried in freeze dried. (Bonakdaran, 2008)

3.2 Microbial isolated:

In this study, we used bacterial species isolated in the University of Science and Technology Hospital. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* were used as tested isolates. The bacterial isolates were cultured on nutrient agar a night before the experiment and they were incubated at 37°C. 1-2 colonies of bacteria were suspended in normal saline and their turbidity's were adjusted to 0.5 McFarland (1×10^8 CFU/ml).

3.3 Cleaning of discs:

The discs were soaked and washed twice by D.W for quarter of an hour and dried for one day.

3.4 Preparation of extract stock solution:

A stock concentration of 100 mg/mL was used for the tests, and it was made by combining 1 g of the crude extract with 10 mL of sterile distilled water. (Usman, 2005)

3.5 Impregnation of the discs:

- Sterile discs were placed in petri dishes approximately 5mm apart.
- A paper disc of 6mm diameter can absorb 50µl of solutions. The concentrations of extract solutions were expressed in µg/µl.
- The impregnated discs were dried in 37°C incubator for 18 to 24 hours and immediately used for the sensitivity test. (Bauer, A.W., Kirby, W.M)

3.6 Disc Diffusion Method:

The suspension of Gram-positive and Gram-negative bacteria was prepared and adjusted to 0.5 McFarland and, then swabbed on the surface of Mueller Hinton Agar. Following that, discs (6 mm in diameter) will be aseptically mounted on inoculated agar and incubated for 24 hours at 37°C, after being impregnated with 50µl of the 100 mg/ml of the crude extract at a concentration of 5 mg disc. As positive controls, filter paper discs (6 mm in diameter) containing the conventional antibiotics amoxicillin-clavulanate (30 µg), Levofloxine (5 µg), Ciprofloxacin (5 µg), and ceftriaxone (5 µg) were used as positive control. A transparent meter rule will be used to measure the diameter of the inhibition free zones surrounding the discs. Each extract and standard will be independently tested in triplicate and results will be expressed as mean. (Usman, 2005).

Chapter 4: Results

Results

The antimicrobial activities of the plant extract against the four bacterial species were assessed by the presence or absence of inhibition zones. The inhibition zones of plant extract tested for antibacterial activity are given in Table 1. *Tribulus terrestris* ethanol extract has potential antibacterial activities against gram positive *S. aureus* and gram negative *P. aeruginosa*. *Tribulus terrestris* extract showed non antibacterial activity to *E. coli* and *K. pneumoniae*.

Table 1: The Antibacterial Activity of *T. Terrestris* Extracts against Bacteria in

	Bactreia (Inhibition zone diameter (mm))			
	S. aureus	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
Ethanolic Extractants	15	23	0	0
Ciprofloxacin 5µg	S	S	R	R
Amoxicillin 25µg	R	-	R	-
Ceftriaxone 30µg	R	R	R	R
Levofloxacin 5µg	S	S	R	R
	R indicates resistance S indicates sensitive I indicates intermediately resistant			

Agar Diffusion Assay

The table above shows that the isolates of *Escherichia coli* and *Klebsiella pneumoniae* showed resistance to all antibiotics tested. This probably explains their resistance to plant extracts.

Chapter 5: Discussion

Discussion

The present study was carried out a preliminary to investigate antibacterial activity of *Tribulus terrestris* plant against four bacterial species in Sana'a. The results of this study showed the effectiveness of the *Tribulus terrestris* extract in high concentrations against *S. aureus*, and *P. aeruginosa* isolates, while it was ineffective against *E. coli* and *K. pneumonia*. One study indicated that experiments of this plant extract at a concentration of 5 µg/disk showed antimicrobial activities. (Zaidan, 2005)

It is reported that ethanol extract of medicinal plants lack antibacterial activities (Zaidan, 2005). This observation has also been indicated by other study showing that ethanol is not a good solvent for extraction for antimicrobial substances from medicinal plants (Ahmad, 1998). The antibacterial activities of the plant are particularly noteworthy considering the medical importance of *S. aureus*. *S. aureus* causes infections including superficial skin lesion, localized abscesses, and food poisoning.

Since ethanol extract showed potential activities to these bacteria, further evaluation on these plants are needed. The two bacterial species that were not susceptible to the plant extracts were *E. coli* and *K. pneumoniae*. These could be due to several possible reasons, the distinctive feature of gram-negative bacteria is the presence of a double membrane surrounding each bacterial cells. Although all bacteria have an inner cell membrane, gram-negative bacteria have a unique outer membrane. This outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than other gram-positive bacteria. This could be the beginning for further research on the screening approach by taking into consideration the extracts preparation and the mechanism of action. Although the nature and number of active components involved in each extract are not clear, however they are promising.

There are popular claims that describe the extract of this plant as having antimicrobial efficacy, and therefore we can say that the results of our study came in the context of these claims. This finding can form the basis for further studies to prepare and optimize preparation of the herbal extract to further evaluate them against a wider range of bacteria strains.

Chapter 6: Conclusions & Recommendations

Conclusions

In conclusion, *Tribulus Terrestris* extracts possess a spectrum of somewhat activity against a group of bacteria responsible for the most common bacterial diseases. These findings may open the possibility of finding new biologically active antibacterial compounds by other alcoholic solvents.

Recommendations

Conducting other studies using various alcoholic solvents on *Tribulus Terrestris* and other endemic plants in the Yemeni environment in order to obtain compounds with antibacterial effectiveness, especially at a time of increasing antibiotic resistance.

References:

Abirami, P. and Rajendran, A. 2011. GC-MS analysis of Tribulus terrestris. l. *Asian Journal of Plant Science & Research.* 2011.

Aghamohammad S, Badmasti F, Solgi H, Aminzadeh Z, Khodabandelo Z, Shahcheraghi F. 2020. First Report of Extended-Spectrum Betalactamase-Producing Klebsiella pneumoniae Among Fecal Carriage in Iran: High Diversity of Clonal Relatedness and Virulence Factor Profiles. *Microb Drug Resist.* 2020.

Ahmad, I., Mehmood, Z. & Mohammad, F. 1998. Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology. 1998.

Ahmed A. Hussain, Abbas A. Mohammed, Heba. H. Ibrahim, and Amir H. Abbas. 2009. Study the Biological Activities of Tribulus Terrestris Extracts . *International Journal of Chemical, Molecular, Nuclear, Materials and Metallurgical Engineering .*

Ashurst JV, Dawson A. 2022 . Klebsiella Pneumonia. *StatPearls [Internet].* 2022 .

B.S. Gunashree, M. Basavaraju and. 2022 . Escherichia coli: An Overview of Main Characteristics. 2022**.** Escherichia coli: An Overview of Main Characteristics.

Bauer, A.W., Kirby, W.M.M., Serris, J.C. & Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. . *American Journal of Clinical Pathology 45: 493-496. .* 1966.

Bei Li, Yuling Zhao, Changting Liu, Zhenhong Chen & Dongsheng Zhou. 2014. Molecular pathogenesis of Klebsiella pneumoniae. *Future Microbil.* 2014.

Blandine Rammaert, Sophie Goyet, Julien Beaut, Sopheak Hem, Vantha Te, Patrich Lorn Try, Charles Mayaud, Laurence Borand, Philippe Buchy, Bertrand Guillard and Sirenda Vong. 2012. Klebsiella pneumoniae related

communityacquired acute lower respiratory infections in Cambodia. *Clinical characteristics and treatment.* 2012.

BODEY, Gerald P., et al. 1983. Infections caused by Pseudomonas aeruginosa. *Reviews of infectious diseases.* 1983.

Bonakdaran, A. et al. 2008. Investigation of the hypoglycemic effect of Tribulus terrestris extract on diabetic rats. *Journal of Medicinal Plants.* 2008.

Chhatre, S. et al. 2014. Phytopharmacological overview of Tribulus terrestris. *Pharmacognosy reviews.* 2014.

de la Cabada Bauche J, Dupont HL. 2011. New Developments in Traveler's Diarrhea. *Gastroenterol Hepatol (N Y).* 2011.

DRISCOLL, James A., BRODY, Steven L. and KOLLEF, Marin H. 2007. The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections. *Drug.* 2007.

Esposito EP, Cervoni M, Bernardo M, Crivaro V, Cuccurullo S, Imperi F, Zarrilli R. 2018. Molecular Epidemiology and Virulence Profiles of Colistin-Resistant Klebsiella pneumoniae Blood Isolates From the Hospital Agency "Ospedale dei Colli," Naples, Italy. *Front Microbiol.* 2018.

FD, Lowy. 1998. Staphylococcus aureus infections. *Staphylococcus aureus infections.* 1998.

Huang DB, Nataro JP, DuPont HL, Kamat PP, Mhatre AD, Okhuysen PC, Chiang T. 2006. Enteroaggregative Escherichia coli is a cause of acute diarrheal illness. *Clin Infect Dis.* 2006.

Jondle CN, Gupta K, Mishra BB, Sharma J. 2018. Klebsiella pneumoniae infection of murine neutrophils impairs their efferocytic clearance by modulating cell death machinery. *PLoS Pathog.* 2018.

Kaper JB, Nataro JP, Mobley HL. 2004. Pathogenic Escherichia coli. *Nat Rev Microbiol.* 2004. Pathogenic Escherichia coli . *Nat Rev Microbiol.* 2004.

Karch H, Bielaszewska M. 2001. Sorbitol-fermenting Shiga toxin-producing Escherichia coli O157:H(-) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. *Clin Microbiol.* 2001.

Kostova, I. and Dinchev, D. 2005. Saponins in Tribulus terrestris - Chemistry and bioactivity. *Phytochemistry Reviews.* 2005.

Li, Zhao, Liu, Chen & Zhou. 2014. Molecular pathogenesis of Klebsiella pneumoniae. *Future Microbiol.* 2014.

M. Akram1, H. M. Asif2, Naveed Akhtar2, Pervaiz A. Shah3, M. Uzair, Ghazala Shaheen, Tahira Shamim, S. M. Ali Shah2 and Khalil Ahmad. 2011. Tribulus terrestris Linn.: A review article. 2011.

Mueller M, Tainter CR. 2022. Escherichia Coli. *StatPearls.* 2022.

Nataro JP, Kaper JB. 1998. Diarrheagenic Escherichia coli. *Clin Microbiol Rev.* 1998.

Rasigade JP, Vandenesch F. Staphylococcus aureus. 2014. Staphylococcus aureus: a pathogen with still unresolved issues. *Infect Genet Evol.* 2014.

Regua AH, Bravo VL, Leal MC, Lobo Leite ME. 1990. Epidemiological survey of the enteropathogenic Escherichia coli isolated from children with diarrhoea. *J Trop Pediatr.* 1990.

ROSSOLINI, G. M. and MANTENGOLI, E. 2005. Treatment and control of severe infections caused by multiresistant Pseudomonas aeruginosa. *Clinical Microbiology and infection.* 2005.

Saurabh Chhatre, Tanuja Nesari, Gauresh Somani, Divya Kanchan, and Sadhana Sathaye. 2014. Phytopharmacological overview of Tribulus terrestris. 2014.

Schroll C, Barken KB, Krogfelt KA, Struve C. 2010. Role of type 1 and type 3 fimbriae in Klebsiella pneumoniae biofilm formation. *BMC Microbiol.* 2010.

Shane AL, Mody RK, Crump JA, Tarr PI. 2017. Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea. *Clin Infect Dis.* 2017.

Sun, W. et al. 2002. A new steroidal saponin from Tribulus terrestris Linn. *Natural Product Letters.* 2002.

Thakuria B, Singh P, Agrawal S, Asthana V. 2013. Profile of infective microorganisms causing ventilator-associated pneumonia: A clinical study from resource limited intensive care unit. *J Anaesthesiol Clin Pharmacol.* 2013.

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. 2015. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev.* 2015.

Tracey A. Taylor, Chandrashekhar G. Unakal. 2022. Gram postive bacteria. 2022.
—. 2022. Staphylococcus aureus . *Statpearls.* 2022.

Usman, H. et al. 2005. Phytochemical and antimicrobial screenings of the leaf extracts of Celtis integrifolia Lam. *J. Trop. Biosc.* 2005.

WC, E. 'Trease and Evans. 2002. pharmacognosy Nottingham. *University of Nottingham.* 2002.

Wenyi Zhu, Yijie Du, Hong Meng, Yinmao Dong and Li Li. 2017. A review of traditional pharmacological uses, phytochemistry, and pharmacological activities of Tribulus terrestris. *Chemistry Central Journal.* 2017.

Yue, H. et al. 2012. Ethylene glycol: properties, synthesis, and applications. *Chemical Society Reviews.* 2012.

Zaidan, M.R.S, Noor Rain, A, Badrul, A.R, Adlin, A, Norazah, A.& Zakiah,. 2005. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Tropical Biomedicine .* 2005.

الملخص بالعربي

مقدمة: من المفيد في الوقت الحالي تطوير أدوية جديدة مضادة للجراثيم وفعالة بسبب انتشار الكائنات الدقيقة المقاومة للمضادات الحيوية. وفي هذا السياق ، يعتبر استخدام النباتات الطبية كمصادر طبيعية للمضادات الحيوية ضد البكتيريا والفطريات الممرضة ذو اهمية متزايدة.

الهدف: تهدف هذه الدراسة إلى تقييم الفعالية المضادة للبكتيريا لمستخلص نبات القطرب الإيثانولي في المختبر ضد أربعة أنواع من البكتيريا المسببة للأمراض.

الطريقة: في هذا العمل التجريبي ، تم فحص التأثيرات المضادة للبكتيريا لمستخلص نبات القطرب الإيثانولي على S. aureus و E. coli و P. aeruginosa و K. pneumoniae باستخدام الانتشار القرصي البسيط.

النتائج: يحتوي مستخلص نبات القطرب الإيثانولي على مركبات ذات فعالية مضادة للبكتيريا موجة الغرام S. aureus والبكتيريا سالبة الغرام P. aeruginosa. تراوحت اقطار مناطق التثبيط من 15 إلى 23 ملم. لم يظهر مستخلص الايثانول لهذا النبات فعالية مضادة تجاه E. coli و K. pneumoniae.

الخاتمة: تعتبر المستخلصات النباتية ذات قيمة جيدة كمضادات ميكروبات طبيعية ويمكن استخدامها كمصادر بديلة للمركبات المضادة للبكتيريا.

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تقييم نشاط المضاد للميكروبات لمستخلص تريبولوس تيريستريس ضد البكتيريا المسببة للأمراض الشائعة

بحث مقدم الى برنامج المختبرات الطبية في كلية الطب والعلوم الصحية، جامعة العلوم والتكنولوجيا، كجزء من استكمال متطلبات نيل درجة البكالوريوس في المختبرات الطبي

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