Increased Bacterial Resistance

Effects of African Panaxia Extracts

Highlights

Bacteria Wound Infection in Tiko

Detection of the Inhibitory Potential

Discovering Thoughts, Inventing Future

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Abstract- *Corynebacterium striatum* is an emerging Gram-positive bacillus that presents tropism for the human microbiota; however, it has a high probability of presenting a multidrug-resistant (MDR) profile. In addition, several studies indicate its ability to cause serious infections in patients with varying levels of immune compromise. *C. striatum* samples may present different virulence mechanisms such as; disinfectant tolerance, motility, and bacterial biofilm formation. This work aims to evaluate the antimicrobial activity of the hydroalcoholic extract of *Psidium guajava* L. on MDR and MDS strains of *C. striatum* as an alternative for treatment. We used the agar disk diffusion method to evaluate the susceptibility of bacterial samples under conditions of treatment with *Psidium guajava* L.

Keywords: *corynebacterium striatum*, *psidium guajava* L., *nosocomial*, PFGE, *MDR*, MDS, antimicrobial activity, hydroalcoholic extract, myrtaceae, mueller hinton agar, quorum sensing.

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Strictly as per the compliance and regulations of:
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**Abstract** - *Corynebacterium striatum* is an emerging Gram-positive bacillus that presents tropism for the human microbiota, however, it has a high probability of presenting a multidrug-resistant (MDR) profile. In addition, several studies indicate its ability to cause serious infections in patients with varying levels of immune compromise. *C. striatum* samples may present different virulence mechanisms such as; antibiotic tolerance, motility, and bacterial biofilm formation. This work aims to evaluate the antimicrobial activity of the hydroalcoholic extract of *Psidium guajava* L. on MDR and MDS strains of *C. striatum* as an alternative for treatment. We used the agar disk diffusion method to evaluate the susceptibility of bacterial samples under conditions of treatment with *Psidium guajava* L. The results showed in the disk diffusion test bacterial strains independent of their resistance profile MDS 1987 and MDR 1961 showed sensitivity to 100% crude extract of *Psidium guajava* L. showing inhibition halos of 13mm and 14mm, respectively. In the synergism test, a better result was obtained with the MDR1961 strain, and there was no result with the MDS1987 strain. The results of this course’s conclusion research can be considered a promising alternative for treatment. We also studied the detection of the inhibitory potential of *Psidium guajava* L. in the MDR and MDS strains of *C. striatum*. We used the agar disk diffusion method to evaluate the susceptibility of bacterial samples under conditions of treatment with *Psidium guajava* L. The results showed in the disk diffusion test bacterial strains independent of their resistance profile MDS 1987 and MDR 1961 showed sensitivity to 100% crude extract of *Psidium guajava* L. showing inhibition halos of 13mm and 14mm, respectively. In the synergism test, a better result was obtained with the MDR1961 strain, and there was no result with the MDS1987 strain. The results of this course’s conclusion research can be considered a promising alternative for treatment.
source for the search for new options against bacterial multidrug resistance, giving an incentive to seek new alternatives and isolation of molecules from plants to be able to use and fight multidrug-resistant infections.

Keywords: corynebacterium striatum, psidium guajava l., nosocomial, PFGE, MDR, MDS, antimicrobial activity, hydroalcoholic extract, myrtaceae, mueller hinton agar, quorum sensing.

Resumo- A Corynebacterium striatum pertence ao gênero Corynebacterium representam um grande número de bactérias gram-positivas não formadoras de esporos, encontradas naturalmente em flora bacteriana da pele e de mucosas e encontra-se amplamente disseminadas pelo meio ambiente, onde em 2009 causou um surto nosocomial no Hospital Universitário Pedro Ernesto, e em um trabalho realizado por Baio et al. identificou por Eletroforese em gel de campo pulsado (PFGE) 10 perfis cionais de C. striatum, entre eles, o nosso trabalho utilizou os clones MDR/RJ 1987/PFGE I e MDS/RJ 1961 PFGE III. Nas bactérias existe um mecanismo que detecta a densidade de outras bactérias, chamado de quorum sensing (Q.S.), que é um sensor de densidade que está ligado a uma variedade de comportamentos fisiológicos nas bactérias, que permite que grupos de bactérias alterem o comportamento de maneiras sincrona em resposta a regulações de fatores de virulência, tolerância de desinfetante, formação de esporos, produção de toxinas, motilidade e formação de biofilme bacteriano. Este trabalho tem como objetivo avaliar a atividade antimicrobiana do extrato hidroalcóolico de Psidium guajava L., comumente conhecido como goiabeira, da família Myrtaceae, sobre cepas MDR e MDS de C. striatum e avaliar o efeito modulador do extrato sobre antibióticos convencionais, pois pode aumentar a eficácia dos agentes antimicrobianos no tratamento de infecções. Para a avaliação da atividade antimicrobiana foi utilizado o método de disco difusão em ágar Mueller Hinton (TSA). Os resultados demonstraram no teste disco difusão cepas bacterianas independente do seu perfil de resistência MDS 1987 e MDR 1961 apresentaram sensibilidade ao extrato 100% bruto de P. guajava, apresentando halos de inibição de 13mm e 14 mm, respectivamente. No teste de sinergismo obteve-se melhor resultado com a cepa MDR1961, não teve resultado com cepa MDS1987. Os resultados dessa pesquisa de conclusão de curso podem ser considerados uma fonte promissora para a busca de novas alternativa frente a multirresistência bacteriana, dando um incentivo a buscar novas alternativas e isolamento de moléculas dos vegetais para poder utilizar e combater as infecções multirresistentes.

Palavras Chaves: corynebacterium striatum, psidiumguajava l., nosocomial, PFGE, MDR, MDS, atividade antimicrobiana, extrato hidroalcoholico, myrtaceae, ágar mueller hinton, quorum sensing.

I. Introduction

The genus Corynebacterium belongs to the Actinobacteria class represents a diverse group of Gram-positive bacteria. (Ramoset al., 2014)

Bacteria of the genus Corynebacterium are gram-positive rods of aerobic or facultative anaerobic growth, immobile, incapable of forming spores, and catalase positive. The description of the appearance of Corynebacterium in direct bacterioscopy using the Gram method often appears as irregular gram-positive rods (PIBG) arranged in the form of palisades, which may have different arrangements and lengths (Potonnet et al., 2020). Currently, the genus Corynebacterium consists of 115 validly described species (Euzéby et al., 2014), and a little more than 50 species appear occasionally or rarely, causing infections in humans (Bernardet et al., 2012).

Corynebacteria are distributed in a wide range of ecological environments, such as soil, sewage and plant surfaces, some of which are pathogens for animals and humans. The best-known species of the genus is the human pathogen Corynebacterium diphtheriae, the etiologic agent of diphtheria (Jandaet al., 1998; Schroeder et al., 2012).

Corynebacterium spp. belong to the skin and mucosal microbiota and are widely disseminated in the environment. There have been increasing reports of cases of human infections caused by some species of Corynebacterium, both in industrialized and developing countries, which can lead to death in immunocompromised and immunocompetent patients (Ramoset al., 2014).

Serious infections by Corynebacterium spp. expressing a multidrug resistance (MDR) profile to antimicrobial agents is attributed to samples of Corynebacterium jeikeium, cases of infections by MDR samples of other species have been described, including Corynebacterium urealyticum, Corynebacterium amycolatum, Corynebacterium afermentans, Corynebacterium pseudodiphtheriticum and Corynebacterium striatum, especially in healthcare settings (Wanget al., 2019).

Corynebacterium striatum, a species initially considered to be part of the normal amphibiotic microbiota of human skin and nasal mucosa, has been recognized as a potentially virulent pathogen capable of causing invasive infections and nosocomial outbreaks (Wong et al., 2010; Souza et al., 2020). In recent decades, an increasing number of invasive infections caused by multidrug-resistant (MDR) and multi-sensitive (MDS) samples of C. striatum have been observed in immunocompromised and immunocompetent patients, including: pneumonia (Tarretet al., 2003; Renomet al., 2007), sepsis (Dallet et al., 1989), synovitis and septic arthritis (Scholle et al., 2007), osteomyelitis (Fernández-Ayalae et al., 2001), endocarditis, meningitis and recurrent bacteremia (Weisset al., 1996; Syed et al., 2019). C. striatum has also been recognized as an etiologic agent of liver abscesses (Stone et al., 1997), peritonitis (Bhandari et al., 1995), surgical wounds (Moore et al., 2010), keratitis (Heidemann et al., 1991) and intrauterine infections (Boltinet al., 2009). The first case of urinary infection in an immunocompetent outpatient was observed in Spain (Beteta et al., 2009).

The number of case reports of C. striatum infection has increased in several developed countries,
such as Italy, Spain, Netherlands, United States, Hong Kong and Japan (Campanile et al., 2009; Martins et al., 2009; Wong et al., 2010; Wang et al., 2019). Additionally, C. striatum has been isolated from different infections and nosocomial outbreaks in developing countries, including Brazil (Superti et al., 2009; Souza et al., 2019; Souza et al., 2020).

Epidemic outbreaks caused by MDR strains of C. striatum have been documented in patients hospitalized for long periods and, or continuously exposed to broad-spectrum antimicrobials in intensive care units (Boltin et al., 2009; Wong et al., 2010; Wang et al., 2019; Qiu et al., 2019). Using invasive medical devices and exposure to antimicrobial agents may favor respiratory tract mucosal infection the selection of MDR strains of C. striatum (Syed et al., 2019). Therefore, Gram-positive rod samples isolated from clinical material should not be simply discarded as mere contaminants, especially when obtained in pure culture from immunocompromised patients and using invasive devices (Martins et al., 2009; Wong et al., 2010; Baio et al., 2013; Souza et al., 2015).

Patients undergoing invasive medical procedures are susceptible to infections by C. striatum, because bacterial interaction with the surface of the abiotic substrate can allow colonization through the production of bacterial biofilm (Syed et al., 2019). Previous studies have also demonstrated the ability to spread C. striatum from patient to patient and through the contaminated hands of healthcare professionals (Brandenburg et al., 1996).

In a study published by Baio et al., (2013), phenotypic and genotypic characteristics of multidrug-resistant (MDR) and susceptible strains (n=14) of C. striatum isolated during an outbreak in 2009 at Hospital University Pedro Ernesto (HUPE) were described. Rio de Janeiro, Brazil. Subsequently, other strains were identified in HUPE itself and at the Hospital Municipal Jesus, revealing other multidrug-resistant pulses (Ramos et al., 2019; Souza et al., 2019; Souza et al., 2020).

The pathogen was isolated in the various sectors of the hospitals, from different anatomical sites, in adult individuals, where half the patients were 50 years of age or older. Most strains of C. striatum strains were isolated from tracheal aspirates, from patients undergoing endotracheal intubation procedures, and from blood in ICUs and surgical wards (Silva & Motta et al., 2022).

They were initially indicated by pulsed-field gel electrophoresis (PFGE- Pulsed-Field-Field Gel Electrophoresis), the presence of ten distinct clonal profiles (PFGE I, II, III and IV) with a predominance of pulse type I among, the samples. Clones I and II were isolated from tracheal secretion and blood. Type III and IV clones were isolated from urine and wound secretion, respectively. The authors identified the PFGE I, and II profiles as related clones of MDR strains. The PFGE III and IV profiles of C. striatum were identified as clones sensitive to the various drugs tested.

In bacteria, there is a mechanism that detects the density of other bacteria, called quorum sensing (Q.S.), which is a density sensor that is linked to a variety of physiological behaviors in bacteria (both Gram-negative and Gram-positive) (Zhao et al., 2020), which allows groups of bacteria to change behavior in synchronous ways in response to regulations of virulence factors, disinfectant tolerance, spore formation, toxin production, motility and bacterial biofilm formation (Mukherjee et al., 2019; Ding et al., 2020). In this system, bacteria control the behavior of the entire bacterial population to synthesize and secrete signaling molecules (called autoinducers), being able to communicate and orchestrate the structure and function of biofilms (Yu et al., 2020; Gopalakrishnan et al., 2021).

But the change in the expression and behavior of its genes only happens when the signaling (self-inducing) reaches a limited concentration, being able to have communication, and synchronize in particular behaviors on a population scale, thus gaining the ability to function as a multicellular organism (Gopalakrishnan et al., 2020).

The biofilm can be defined as a set of bacteria firmly attached to a surface, encompassed by an extracellular matrix composed of polysaccharides, proteins and nucleic acids produced by the bacteria themselves (Costerton et al., 2003). The biological cycle for the formation of a biofilm goes through 5 stages, the first being contact, where it is reversible and is maintained by non-specific physicochemical interactions; The second stage being adhesion, where there is a change from the reversible to the irreversible step; The third being the formation of small settlement, with the bacteria secreting the signaling molecules and causing all the bacteria there to create a colony that works in sync and with this colony the mature biofilm is formed; The fourth stage being maturation, where the total formation of the biofilm is completed, being surrounded by various substances and a system of exchanges of nutrients that need to come out of the biofilm; And the fifth stage is the dispersion that occurs when the environment is not more favorable and consists of the detachment in the form of cell aggregates, to colonize new habitats and restart the formation of recent biofilms (Monroe et al., 2007). During the stages of contact, adhesion and construction of small colonies, each bacterium starts to produce signaling molecules that, depending on the local stimuli and mainly on the concentration reached in the microenvironment, trigger the activation of specific genes with the change from the phenotype of planktonic bacteria to the biofilm phenotype, as illustrated in Figure 1 (Monroe et al., 2007). The extracellular envelope protects them against physical and chemical...
aggressions from the external environment, such as the action of ultraviolet rays and changes in pH and osmolarity, in addition to significantly reducing the activity of adaptive and innate mechanisms of the immune system, such as the action of phagocytic cells and opsonization of antibodies (Hoyle & Costerton 1991).

The increase in bacterial resistance to the various antimicrobial agents used in the clinic is a global Public Health problem that draws the attention of national and international government agencies such as the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC/USA), ANVISA, as well as Committees on Hospital Infections (CCIH) from various health institutions (Oliveira et al., 2009). Resistance to a particular antimicrobial may be an intrinsic property of a bacterial species or an acquired ability. To develop resistance, the bacterium must change its DNA, genetic material, which occurs in two ways: 1. induction of mutation in native DNA; 2. introduction of foreign DNA - resistance genes - that can be transferred between different genera or species of bacteria (ANVISA, 2007).

Cell membrane permeability is essential for the antibiotic to have the desired effect, be it bactericidal or bacteriostatic (Goodman & Gilman’s, 2008). Drugs can enter the bacterial cell membrane in three ways: simple diffusion through the phospholipid bilayer, by diffusion facilitated by membrane proteins, called porins, or by self-promoted uptake, where the penetration of the drug into the bacteria is related to the characteristic physical-chemistry of antibiotics, such as the polarity and sizes of molecules, modifying the liposaccharide content. Structures and amounts of porins, when they are modified, lead to bacterial resistance, as any decrease in the function and quantity of porins will lower the level of antibiotic inside the bacterial cell (Costa & Silva Junior, 2017).

Most antibiotics specifically bind to their targets with high affinity and thus prevent normal target activity. However, structural changes in the target that prevent effective binding between the target and the antibiotic confer resistance (Blair et al., 2015). Alternatively, a newly acquired gene may act to modify a target, making it less vulnerable to a particular antimicrobial. Thus, this gene carried by plasmid or transposon encodes an enzyme that inactivates targets or alters the binding of antimicrobials in order to prevent the occurrence of any inhibitory or bactericidal effect (ANVISA, 2007).

Efflux pumps are membrane proteins that export antibiotics to the extracellular environment, keeping intracellular concentrations at low levels, that code for different antibiotic transporters (Costa & Silva Junior, 2017). The enzymatic mechanism of resistance occurs due to the inactivation of the drug from the production, by the bacteria, of enzymes that degrade or inactivate the antibiotic. Involving three types of enzymatic reactions, such as hydrolysis, transfer of a chemical group or redox process (Costa & Silva Junior 2016). The classic example of this resistance mechanism is the production of β-lactamase that hydrolyzes the β-lactam ring of penicillins (Kumar & Varela 2013).

Studies described that multiple drug resistance (MDR) can be defined when gram-negative and gram-positive bacteria are resistant to three or more classes of antimicrobials. Pan-resistant bacteria (PANDR) are defined as resistant to all antimicrobial agents (Magiorakos et al., 2012).

Several studies have shown an increase in the rate of antimicrobial resistance among Corynebacterium species. Resistance to β-lactams, Clindamycin, Erythromycin, Ciprofloxacin and Gentamicin has been reported, sometimes leading to the use of Vancomycin as the drug of choice. To date, vancomycin, teicoplanin and linezolid are the most effective agents in vitro against Corynebacterium (Martins et al., 2009; Yoon et al., 2011; Reddy et al., 2012; Wang et al., 2019). Antibiotic resistance develops as a natural consequence of the ability of the bacterial population to adapt. The indiscriminate use of antibiotics increases the selective pressure and also the opportunity for the bacteria to be exposed to them. That opportunity facilitates the acquisition of resistance mechanisms (Santos, 2004).

Given the increasing reports on different bacterial genera presenting resistance to several antimicrobial agents, mainly in the last decades, concomitantly, the search for new substances with antimicrobial potential also grows exponentially (Carneiro et al., 2014).

One of these alternatives is the extract of Psidium guajava L., commonly known as guava, from the Myrtaceae family, is a plant native to tropical America (Sanchez et al., 2005), has been historically used in folk medicine, traditional for the treatment of different respiratory disorders, diabetes, hypertension, as well as analgesic, antipyretic, anti-inflammatory, healing and antimicrobial functions (Matos 2002; Fu et al., 2016). In previous studies, the biological actions of the crude extract of the leaves of P. guajava L. were proven in the treatment of diarrhea, dysentery, lung diseases, and bronchitis, other properties were also attested, giving the species antispasmodic, antimicrobial, anti-inflammatory, anticonvulsant, analgesic, antidiabetic, antihyperlipidemic and antioxidant (Souza et al., 2015).

According to Desotin 2011, the antimicrobial effect of guava essential oil was proven, by the microdilution plate method, against some Gram-positive and Gram-negative microorganisms and yeasts.

Its main constituents are tannins, flavonoids, essential oils, sesquiterpenoid alcohols, and triterpenoid acids. The parts used by the plant are the bark, shoots,
leaves, and roots. (Gondim et al., 2006; Amaral et al., 2006)

The combination of plant-derived products and conventional antimicrobial drugs is a promising strategy, as it can increase the effectiveness of antimicrobial agents in treating infections caused by multidrug-resistant microorganisms (Fernandes et al., 2012).

Therefore, we want to investigate the antibacterial action of leaves of P. guajava on the samples of C. striatum, in this way, also to evaluate its potential for synergistically modulate the action of antibiotic available for treatments against gram-positive bacteria of clinical importance.

Given the reports on different bacterial genera presenting resistance to various antimicrobial agents, mainly in the last decades, there is a need for the search for new substances with antimicrobial also increase (Carneiro et al., 2014).

About all the problems that we narrate, there is a need to seek new therapeutic alternatives to combat multidrug-resistant bacteria, where it will be necessary to have qualified pharmaceutical professionals to understand the importance of diagnosis and the functionality of antibiotics, who, together with pharmacological knowledge, can seek new ways of controlling or eliminating multidrug-resistant bacterial infections.

II. Materials and Methods

Mature leaves of P. guajava L. were collected in the lake’s region, in the city of Armação dos Búzios, in a home plantation in the Vila Caranga neighborhood, on February 20th. To avoid contamination in the material, the leaves were washed in running water and then immersed in diluted chlorine at a concentration of (1:20) for one minute, as a subsequent rinse to remove the excess. Then the leaves were left on paper towels and under protection against the sun, waiting for the leaves to dry.

To obtain the hydroalcoholic extract, 100 grams of dry material were immersed in 500 ml of 70% ethanol. The solution was stored in closed glass vials and wrapped in aluminum foil to prevent light interference. This condition was maintained for 15 days and shaken three times a day. After this interval, the solution was filtered using a funnel with hydrophilized gauze. To avoid the interference of ethanol in the test, the extract was evaporated in a water bath at 45ºC until a viscous liquid was obtained. The solution was kept in a light-free environment. (Andrade et al., 2019) being ready to perform the antimicrobial test, 1 ml of 100% extract was distributed in 6 sterile test tubes (Figure 01).

Two strains of C. striatum from a nosocomial outbreak started in 2009 and isolated from patients admitted to Hospital University Pedro Ernesto (HUPE/UEERJ) located in the metropolitan region of the state of Rio de Janeiro, Brazil, were used (Table 01).

The microorganisms are stored in Skim Milk at -70ºC, in the Bacterioteca of the Laboratory of Diphtheria, and Corynebacterioses of Clinical Importance – LDCIC – Discipline of Microbiology and Immunology – FCM/UEERJ, partner laboratory of the Faculty of the Lagos Region. Strains were thawed, reactivated and confirmed after new identification by conventional biochemical techniques and confirmed by automated methods such sequencing of 16S and rpoB genes and mass spectrometry (MALDI-TOF). Additionally, the samples were characterized by pulsed-field electrophoresis (PFGE) genetic analysis and were classified into different pulse type (Baio et al., 2013).

For this work, we selected a multi-resistant strain MDR/RJ 1987/PFGE I and another MDS/RJ 1961 PFGE III Pulse types previously characterized and identified after the outbreak.

The inoculums were prepared and standardized in sterile saline solution, comparing the turbidities with the tube n° 0.5 of the McFarland scale to obtain about 10^6 CFU/ml (Mendonça et al., 2016).

In two Petri dishes containing Mueller Hinton Agar as the culture medium, the bacterial inoculum prepared with the sterile saline solution (0.5 turbidity on the McFarland scale) was drained with sterile swabs and distributed. Uniformly over the agar surface (Silveira et al., 2009). The first plate with the MDR/RJ 1987/PFGE I strain, respectively, and the MDS/RJ 1961 PFGE III strain on the second plate. In the section for 30 seconds at a concentration of 100% of the extract in tube 1 of Figure 2 (Stieven et al., 2009). For the negative control, we used disks with saline solution and for positive control, we used vancomycin (30mcg) (Figure 2). Then, with the plates already striated and with the discs, the inverted plates were incubated at 37°C for 24 hours, after which the inhibition halos were measured, in millimeters. The result was determined by comparative descriptive statistics from the growth inhibition halos (mm) found, using a universal caliper to the halos formed (Figure 3).

To determine the modulating effect, two Mueller Hinton agar plates were used, with sterile swabs, the Mc Farland 0.5 scale inoculum was used up, and, the strains MDR/RJ 1987/ was evenly distributed on the first plate. PFGE I and on the second plate the strain MDS /RJ 1961 PFGE III, the leaves were identified with the places where the antibiotics were placed in the extracts for 30 seconds, each antibiotic in tubes 2 to 6, respectively (Figure 4), and only the antibiotics. The antibiotics Gentamicin (GEN 10), Ciprofloxacin (CIP 05), Erythromycin (ERI 15), Imipenem (IPM 10) and Ampicillin (AMP 10) were used. The result is determined by comparing the halos of pure antibiotics and antibiotics dipped in the P. guajava L extract. Then, with the plates already streaked and with the disks, the plates were...
incubated at 37°C for 24 hours, and after this period, the inhibition zones were in millimeters. (Figure 5).

III. Resultados e Discussões

Due to the abusive use of traditional antibiotics and the increasing increase in microbial resistance, clinical microbiologists have shown great interest in the investigating of plant extracts with antimicrobial potential (Volpato 2005).

The results related to the Disk Diffusion test in agar in the presence of the extract with antimicrobial expectation are described in Table 2. The bacterial strains, regardless of their resistance profile, MDS 1987 and MDR 1961, showed sensitivity to the 100% crude extract of *P. guajava*, showing inhibition halos of 13mm, and 14mm, respectively (Figure 5). Interestingly, our results corroborate the statements of Biswas et al., (2013), who showed that Gram-positive bacteria were more susceptible to an extract of *P. guajava* (Biswas et al., 2013).

Also, in the studies by Sanches et al., (2005), it was possible to verify that the ethanol-based extracts: water from leaves, stem bark and roots of *P. guajava* showed activity against *Staphylococcus aureus*, gram-positive microorganisms, as well as our samples studied from *Corynebacterium*.

For Lopes et al., (2006) the formation of inhibition halos under the microorganisms tested is due to a synergistic effect of all its constituents, phytochemical compounds: tannin, phenols, flavonoids and alkaloids (Lopes et al., 2006). A study carried out by Alves et al., (2006) showed that the extract is capable of also having antifungal properties against strains of *Candida albicans*, *Candida tropicalis*, and *Candida krusei* (Alves et al., 2006).

The results referring to the agar diffusion tests with evaluation of the bacterial potential of the hydroalcoholic extract of *P. guajava* in synergistic action showed complex and interesting results (Table 3). When the extract was synergistically exposed together with the discs containing antibiotics on the MDR 1987 sample isolated from the respiratory tract, it favored the inhibitory potential of all the antibiotics tested, since, without the action of the extract, the antibiotic discs alone were not able to inhibit the multiplication of this MDR Strain (Table 4 and Figure 5). Interestingly, demonstrating the need for more studies that can clarify several doubts about the resistance mechanisms of these *C. striatum* samples, the hydroalcoholic extract of *P. guajava*, when exposed together with antibiotics, reduced the inhibition halos of the MDS 1961/ MDS in all antibiotics, when compared to discs without the extract (Tables 3 and Figure 5).

In this evaluation, gentamicin and erythromycin were the antibiotics that were most inhibited during the synergism process, significantly reducing their effectiveness by 58% and 32%, respectively.

Table 4 shows that MDS 1961 strains did not achieve synergism. All ATM+G halos (antibiotic plus extract) decreased. A possible explanation is the presence of a secondary metabolite of the plant that caused interference in the antibiotic action and, or the possibility of the hydroalcoholic extract having diluted the antibiotic, consequently decreasing its activity and the size of the halo.

We observed better results with the MDR 1987 strain, table 4, where the bacterium was shown to be resistant to all antibiotics. Still added to the hydroalcoholic section, halos were formed, it is possible that the extract presents a certain metabolite that inhibited the mechanism of resistance of *C. striatum* MDR 1987.

Simões et al., (2018) observed that the antimicrobial action of *Psidium guajava* might be related to the inhibition of bacterial enzymes, direct action on the membrane of microorganisms, or competition for metal ions, whichessentialfor microbial metabolism. With this, it can make the synergistic interactions capable of increasing or improving the potency of antibiotics against a multidrug-resistant microorganism.

For Pereira et al., (2014), A strategy enhance the action of plant extracts, as well as to reverse the resistance of such strains to antibiotics that are already on the market, is to associate these natural products with drugs for clinical use, seeking to interactions the synergistic. Through this strategy, it can be seen in Table 4 that the synergism of the *P. guajava* extract with the antibiotics managed to inhibit and create halos of relatively positive sizes in the MDR strains, 90% of them above 20mm.

The results obtained in this research are important to show that the antimicrobial activity of the extract used against the microorganism *C. striatum* was relevant as the strains MDR 1987 since the strains tested are directly related to the occurrence of cases of nosocomial outbreaks.

IV. Conclusion

We can conclude that *C. striatum* remains an emerging and dangerous pathogen, capable of causing serious infections and promoting nosocomial outbreaks. inhibit or favor the antibiotic action of different antimicrobial agents when used in bacterial samples independent of the antimicrobial susceptibility profile. Additionally, the *P. guajava* extract also established important results in the tests combined with therapies, indicating possible selective synergism between the ATBs and the botanical extract in the Multidrug-resistant Exposures, modifying the susceptibility profile of the MDR samples, which started to show sensitivity to the tested ATBS, boosting the possibility for further studies.
that confirm the potential for selective action in MDR Selection. Given the current scenario with safe antimicrobial alternatives, and with the increase in multi-resistant microorganisms, researchers must continue the search for new therapeutic compounds, emphasizing that the extract of *P. guajava* has great projection for different treatments, including antimicrobial.

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**Figure 1**

*Legends: sterile test tubes containing 1ml of extract.*

*Source: the authors.*

**Figure 2**

*Legends: Paper discs impregnated with 100% extract and positive and negative controls on Muller Hinton agar.*

*Source: the authors*
Detection of the Inhibitory Potential of *Psidium Guajava L.* Extract in Multidrug-Resistant *Corynebacterium Striatum* Strains Isolated from Nosocomial Outbreaks

Figure 3
Legends: Antibiotics and antibiotics dipped in extract. ATM (Antimicrobial) ATM + G (Antimicrobial plus extract)
Source: the authors

Figure 4
Legends: Result of 100% gross extract obtained
Source: the authors

Figure 5
Legends: Synergism with the extract and antibiotics
Source: The Authors
Table 1: Microbiological aspects of *Corynebacterium striatum* strains used in this study previously isolated from patients of a university hospital located in the metropolitan area of Rio de Janeiro, Brazil*

<table>
<thead>
<tr>
<th>Strain/PFGE-type*</th>
<th>Clinical sites</th>
<th>Antimicrobial resistance profiles</th>
<th>Biofilm on polyurethane catheter (CFU/ml)</th>
<th>37°C</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987/I</td>
<td>BAL</td>
<td>MDR</td>
<td></td>
<td>1.4x10⁶</td>
<td>3.3x10⁶</td>
</tr>
<tr>
<td>1961/III**</td>
<td>Urine</td>
<td>MDS</td>
<td></td>
<td>1.0x10⁸</td>
<td>1.4x10⁸</td>
</tr>
</tbody>
</table>

BAL, bronchoalveolar lavage; MDR, multidrug resistant; MDS, multidrug susceptible.; *, C. striatum strains partially studied by (Baio et al., 2013); **, Analysis of complete genome sequencing with GenBank number access LAYR00000000 [15]

Table 2

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>MDS 1961</th>
<th>MDR 1987</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% crude extract obtained</td>
<td>13 mm</td>
<td>14 mm</td>
</tr>
<tr>
<td>Positive Control</td>
<td>25 mm</td>
<td>40 mm</td>
</tr>
<tr>
<td>Negative Control</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Legends: Disk diffusion halo results

Table 3

<table>
<thead>
<tr>
<th>MDS 1961</th>
<th>ATM</th>
<th>ATM + G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicine</td>
<td>35mm</td>
<td>15mm</td>
</tr>
<tr>
<td>Ampicilline</td>
<td>41mm</td>
<td>36mm</td>
</tr>
<tr>
<td>Imipenem</td>
<td>53mm</td>
<td>46mm</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>50mm</td>
<td>34mm</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>36mm</td>
<td>22mm</td>
</tr>
</tbody>
</table>

Legends: Results of disk diffusion halos of synergism in MDS

Table 4

<table>
<thead>
<tr>
<th>MDR 1987</th>
<th>ATM</th>
<th>ATM + G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicine</td>
<td>-</td>
<td>19mm</td>
</tr>
<tr>
<td>Ampicilline</td>
<td>-</td>
<td>24mm</td>
</tr>
<tr>
<td>Imipenem</td>
<td>-</td>
<td>24mm</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>-</td>
<td>23mm</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>-</td>
<td>23mm</td>
</tr>
</tbody>
</table>

Legends: Results of disk diffusion halos of synergism in MDR
Psychedelic Drugs used in the Treatment of Depression: A Literature Review


Summary: Introduction: When analyzing the main depressive conditions and their treatments, we understand that it is not so simple to take care of psychosomatic conditions, because it is a pathology that has a significant epidemiological aspect and needs a good approach, therefore, psychedelic drugs come in as an important alternative as a treatment.

Methodology: This is a literature review with research in databases, using 60 scientific articles as a basis and being filtered based on scientific impact and approach.

Results: Psychedelic agents are chemical substances that, in non-toxic doses, produce changes in conception and thinking in a state of mind, thus altering perception and reason.

GJMR-B Classification: NLMC Code: QV 77

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Psychedelic Drugs used in the Treatment of Depression: A Literature Review


Summary: Introduction: When analyzing the main depressive conditions and their treatments, we understand that it is not so simple to take care of psychosomatic conditions, because it is a pathology that has a significant epidemiological aspect and needs a good approach, therefore, psychedelic drugs come in as an important alternative as a treatment.

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Results: Psychedelic agents are chemical substances that, in non-toxic doses, produce changes in conception and thinking in a state of mind, thus altering perception and reason. There are several substances with psychedelic actions, many of which are banned and considered illegal in several countries around the world, however there are regulators that regulate various drugs and substances, and many of these deserve attention, due to the great evolution in the scientific technical environment.

Final considerations: It is important to understand the mechanism and how to analyze depressive conditions, as well as adjust changes in life, practices and quality of life.

I. Introduction

Psychiatric disorders are responsible for affecting more than 350 million people worldwide. Brazil is, according to the World Health Organization (WHO), the country with the highest number of individuals with anxiety disorders, in addition to leading the ranking in Latin America with the incidence rate of depressive disorders.

Depression is characterized as a multifactorial pathology directly affecting quality of life, in addition to generating physical and mental impacts. There are no pathognomonic symptoms of depression, but there are characteristic symptoms, these include insomnia, fatigue, decreased appetite, anhedonia, suicidal thoughts, and weight loss or gain. Depression is the disease that leads third place as a burden of disease worldwide, where the impact of mortality and morbidity of various pathologies is measured, and according to the World Health Organization, by 2030, depression will be in first place. Thus, for the diagnosis of depression and treatment, it is important to rule out other psychiatric disorders such as anxiety or bipolar disorder. The treatment for this disease is still widely studied, several classes of drugs demonstrate success in certain cases and therapeutic failure in others, thus showing the need for different therapeutic means.

Thus, new therapeutic means such as the use of psychedelics such as LSD and MDMA gain strength in the study of treatments for depression and other psychiatric disorders such as Generalized Anxiety Disorder. However, the repeated use of psychedelics can lead to the development of tolerance due to the decrease in specific receptors of these substances in neurons, and therefore it must be well indicated and monitored by specialists, with the aim of achieving beneficial therapeutic responses for the patient.

II. Methodology

This is a literature review, whose bases were taken from the SciELO and PubMed data platforms. The research period was from July 2023, meeting the inclusion criteria which were articles from the years 2000 to 2023, in Portuguese and English, online texts and in full texts. As strategies for better evaluation of the texts, the following health descriptors (DeCS) were used: "Psychedelic drugs", "Depression" and "Mental health".

III. Results and Discussion

Psychedelic agents are chemical substances that, in non-toxic doses, produce changes in thinking and thinking in a mood, thereby altering perception and reason. (VACCARINO, et al., 2006) (GOMES; MUNIZ; PAULINO, 2016; JOHNSON, RICHARDS, GRIFFITHS, 2008). There are several substances with psychedelic actions, many of which are banned and considered illegal in several countries around the world, however there are regulators that regulate various drugs and substances, and many of these deserve attention, due to the great evolution in the scientific technical environment (ESCOBAR et al. al., 2010). Psychedelics can be found naturally in fungi, animals and plants or be synthesized (CARHART-HARRIS et al., 2017).

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Psychedelics are, for the most part, substances with action capable of altering perception and consciousness in a marked and innovative way (CARHART-HARRIS et al., 2017). Based on the pharmacological profile, substances can be divided as follows: classical psychedelics (serotonin 2A [5HT-2A] receptor agonists), empathogens or entactogenics (mixed serotonin and dopamine reuptake inhibitors and releasers) dissociative anesthetic agents (serotonin and dopamine reuptake antagonists) N-methyl-D-aspartate [NMDA]) and atypical hallucinogens, which affect multiple neurotransmitter systems (GARCIA-ROMEU; KERSGAARD; ADDY, 2016).

Classic psychedelics, the class that will be discussed in this study, interact with serotonin receptors, these receptors mediate emotions and moods such as anxiety, learning memory, cognition, appetite and other biological processes. They are substances with agonist action on serotonergic 2A receptors capable of changing perception and consciousness in a marked and innovative way (CARHART-HARRIS et al., 2017). These receptors are located in the central and peripheral nervous system and thus can be used to treat diseases such as anxiety and depression (LOWE et al., 2021).

The class of psychedelic substances has a relatively high score in psychological and physiological safety if used under supervision in a monitored environment, they generally do not induce dependence or adverse effects that cannot be controlled in adequate doses and in the presence of someone who is able to offer psychological support, if necessary (KUYPERS, 2020).

The treatment performed with hallucinogens brings acute effects that are divided into two dimensions. The first is the physiological dimension which is described as the direct effect of the intervention on the brain. The second is the psychological dimension, which is described as the subjective experience reported by the individual. These acute brain effects include both direct and secondary effects. The direct effects are mediated through the serotonin receptor, while the secondary effects are mediated through the glutamate receptors (COUTO, 2017).

The use of psychedelics in mental health, and in medicine as a whole, has been portrayed in the literature for decades. Since the first trials with Lysergic Acid Diethylamine (LSD), in 1950, psychedelics had a brief contact with psychology and psychiatry, until their prohibition in the mid-60s. of mood disorders, smoking and alcoholism, prior to its ban (CARHART-HARRIS & GOODWIN, 2017). There are several substances with psychedelic actions, many of which are banned and considered illegal in several countries around the world, however there are regulators that regulate various drugs and substances, and many of these deserve attention, due to the great evolution in the scientific technical environment (ESCOBAR et al., 2010).

In Brazil, the presence of psychedelics, especially in clinical trials, occurs mainly through LSD and MDMA - synthetic substances - in addition to psilocybin, ayahuasca and ibogaine, which have their origin in nature (BRAZILIANS STUDY PSYCHEDELIC DRUGS TO TREAT DEPRESSION AND CHEMICAL DEPENDENCE - BBC, 2020). Substances of prohibited use in Brazil, according to ANVISA (2017), are substances considered prohibited, which means that despite the growing research on the use of psilocybin for pharmacological purposes, at least in Brazil there are still no cases of synthesized use mediated by law, and much less production of medicines with this substance.

There are different types of depressive disorders, with psychotic features or not, and they can be catatonic, chronic, seasonal or atypical. The depressive picture as a whole, which is characterized by: sleep disturbances, such as insomnia or hypersomnia; several awakenings during the night, causing a poor use of sleep; changes in appetite, which may include loss of appetite or excess appetite; children may not have the expected weight for the corresponding age; increased appetite is heightened by carbohydrates and sweets; decreased libido; social withdrawal; excessive crying; suicidal thoughts or behaviors; psychomotor retardation and generalized slowing, or psychomotor agitation; patients often report a feeling of heaviness in the limbs (PORTO, 1999)(IBANEZ et al., 2014).

Drug treatment constitutes the foundation of therapeutic intervention to reduce the duration and intensity of the symptoms of the current episode and, above all, to prevent its recurrence. Since adherence to treatment is one of the main causes of relapse (LAFER et al., 2000). However, the advancement of pharmacology aimed at the central nervous system in recent years has seen a remarkable development, but the use of classic drugs to treat depression remains in some patients without effect or experience withdrawal effects or adverse effects, being essential to search for new approaches to treatment of depression (GOLDBERG et al., 2020) (PAHO, 2018) (GILL et al., 2020).

Classic psychedelics, such as LSD, DMT and more specifically psilocybin, have proven to be highly efficient in the treatment of refractory depression, with improvement in symptoms, good tolerability and good adherence, since there is no need for hospitalization during treatment. Studies have shown a decrease in amygdala reactivity with psilocybin, in addition to an increase in the positive mood state, a region that becomes hyperactivated in depressive patients.

An average dose of lysergic acid diethylamide will significantly alter the user’s state of consciousness, or psilocybin (commonly known as ‘LSD’). Such a
change will be characterized by euphoria, increased capacity for introspection, visual hallucinations, synesthesia (mixtures of senses, where the experimenter can “hear a color” or “see a sound”), acceleration of thought and changes in the perception of space and time. Changes in body image and ego function may also occur (PASSIE et al., 2008; CARHART-HARRIS et al., 2016). According to Lee et al. (2012) psilocybin has the mechanism of action to decrease brain activity and connectivity. This substance, which is inactive, is metabolized to the active ingredient psilocin, which in turn activates many neurotransmitter receptors to modulate activity in excitatory and inhibitory GABA-ergic neurons. In clinical trials, there was a marked reduction in depressive symptoms in volunteers affected by major depressive disorder (MDD) resistant to antidepressant therapy in the first 5 weeks post-treatment. In this study, in which only 2 doses of psilocybin were used, 65% of patients met the parameters for remission, remaining so 3 and 6 months after administration (CARHART-HARRIS ET AL., 2018).

In another study carried out by Ibanez et al. (2014) with patients undergoing treatment for depression with prescribed pharmacology, one of the problems observed was the weariness of the patient caused by the drug treatment, which, although necessary to reduce depressive symptoms, did not always provide results that matched the expectations of patients. In addition, low adherence to pharmacotherapy and lack of knowledge regarding the therapy used by patients were also observed, factors that made treatment difficult.

Another drug being studied for the treatment of depressive disorder is methylenedioxy-methamphetamine (MDMA, popularly known as ‘Ecstasy’), a synthetic compound capable of producing a state of excitement and disinhibition, enhancing physical sensations, empathy and interpersonal proximity, not causing hallucinatory visions, like other psychedelics. In the brain, it stimulates neurons to release more neurotransmitters such as serotonin, noradrenaline and dopamine throughout the central nervous system, accelerating reasoning and intensifying emotions. Thus, changes in mood and perception are attributed to the release of dopamine and serotonin, while changes in body temperature occur through the action of all (dopamine, serotonin and noradrenaline). In addition, MDMA acts as an indirect agonist at the presynaptic serotonergic receptor, which not only increases serotonin release but also inhibits its reuptake. It also acts as an inhibitor of the enzyme monoamine oxidase (MAO), increasing the concentration and release of serotonin in the central nervous system. The action of the substance begins with 30 to 60 minutes of its use and lasts for up to 6 hours. This drug has also been shown to be quite efficient in the treatment of Generalized Anxiety Disorder (GAD). (SAIBER, 2021)

Another psychedelic under study is Ayahuasca, a tea produced with various plants originating in the Amazon and historically used in indigenous rituals. It is a drug that has in its composition dimethyltryptamine, a psychoactive, being able to increase neuroplasticity, facilitate adaptive neural architectural changes and break pathological associations, triggers and cues associated with addiction. How psychedelic images are represented in the brain and what are the neural bases of introspection and self-analysis of emotions, a process reported by users during the effect of the substance. Studies in Brazilian universities have shown positive effects of the substance in treatments for chronic depression and chemical dependency, and it is possible to observe improvement with the administration of a single session (TALIN; P; SANABRIA, E, 2017).

The drug has an anti-inflammatory effect, thus decreasing the pro-inflammatory state that patients with depressive disorder may have, increasing the effectiveness of the immune system response. People with depression often have a change in a protein called "brain-derived neurotrophic factor" (BDNF). This chemical marker is connected to neuroplasticity, that is, the ability of the neural system to promote new synapses, which also undergoes modifications with the use of the drug.

Psychedelic drugs have been researched for different treatments, such as anxiety in terminal patients, obsessive-compulsive disorder, headache, addictions and resistant depression, in addition to the debate that it raises about mystical experiences and their management in the clinic. In addition, positive effects of psilocybin were observed, which are atypical results for a pharmacotherapeutic treatment, whose effects do not last for so long (COUTO, 2017).

Also, it can be observed that, in the evaluation of the use of psychedelics in the treatment of chemical dependencies, there was a favorable result in the study aimed at smoking cessation (JOHNSON, GARCIA-ROMEU & GRIFFITHS, 2016). In addition, psilocybin has also been shown to be effective in maintaining abstinence in alcohol-dependent patients (BOGENSCHUTZ ET AL., 2015).

Repeated administration of psychedelics leads to a rapid development of tolerance known as tachyphylaxis, a phenomenon that may result from decreased expression of 5-HT2A receptors on neuron membranes. For example, the daily administration of LSD essentially leads to the complete loss of sensitivity to the effects of the drug on the fourth day of taking it (CHOLDEN; KURLAND; SAVAGE, 1955).
IV. Final Considerations

After the above, it is understood that psychosomatic illnesses have a significant epidemiological character in the population of the 21st century, in view of this, several treatment mechanisms have emerged as an important alternative since conventional treatments arise with various adversities, treatment with psychedelics comes as a strong path of treatment, with new possibilities and with less adverse effects than those presented in normal drugs, being an important way to improve the quality of life, mental health of the entire population, with the correct use and accompanied by a professional of trained health.

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Effects of African Panaxia Extracts on Staphylococcus Aureus and Klebsiella Pneumoniae from Bacteria Wound Infection in Tiko

By Augustine Eyong Bate, Junior Dinkah Libah & Walter Ojong Ebot

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Objectives: This study aimed to determine the effect of African panaxia on bacteria wound infection.

Methods: It was a Laboratory based experimental study made up of bacterial isolates from wounds, cultured on blood, EMB agar, followed by confirmatory biochemical tests and MI and MBC was done turbidity absorbance measured using spectrophotometry at 660nm. Data was analyzed using Microsoft excel 2010.

Keywords: minimum inhibitory concentration; minimum bactericidal concentration.

GJMR-B Classification: NLM: QR177
Effects of African Panaxia Extracts on Staphylococcus Aureus and Klebsiella Pneumoniae from Bacteria Wound Infection in Tiko

Augustine Eyong Bate³, Junior Dinkah Libah⁵ & Walter Ojong Ebot⁵

Abstract - Background: Wound is the disruption of cellular and anatomic continuity of living tissue produced by physical, chemical, electrical or microbial insults to the tissue. Wound healing is the dynamic process of regeneration or repair of broken tissue, due to increasing health care costs, an ageing population, and a sharp rise in the incidence of diseases such as diabetes and obesity worldwide, the present study was aimed to achieve the following objectives

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Methods: It was a Laboratory based experimental study made up of bacterial isolates from wounds, cultured on blood, EMB agar, followed by confirmatory biochemical tests and MI and MBC was done turbidity absorbance measured using spectrophotometry at 660nm. Data was analyzed using Microsoft excel 2010.

Results: Distilled water and Luke warm extracts of African panaxia exhibited inhibitory concentration at 50% and 25% on Staphylococcus aureus and Klebsiella pneumonia respectively and Ethanol extract of African panaxiaat 95% concentration exhibited bactericidal effect on Staphylococcus aureus and Klebsiella pneumonia respectively.

Conclusion: African panaxia extract from distilled water was more inhibitory than bactericidal on Staphylococcus aureus and Klebsiella pneumonia and Ethanoic extract was totally bactericidal on both Staphylococcus aureus and Klebsiella pneumonia.

Keywords: minimum inhibitory concentration; minimum bactericidal concentration.

I. Introduction

Wound is the disruption of cellular and anatomic continuity of living tissue produced by physical, chemical, electrical or microbial insults to the tissue. Wound healing is the dynamic process of regeneration or repair of broken tissue [1]. Chronic wounds are rapidly growing problem worldwide, due to increasing health care costs, an ageing population, and a sharp rise in the incidence of diseases such as diabetes and obesity [2]. The skin is under constant stress from the sun, smog, friction, tension, temperature, and other external factors. Therefore, under sufficient stress that causes injury, it results in wounds. Wounds may be classified as; open and closed, acute and chronic, avulsion and degloving, clean and contaminated, infected and colonized, laceration, incision and abrasion, puncture, penetration, and gunshot wounds. Nonetheless, they exist in various forms comprising crush injuries, ulcers, skin tears, bruises, and post-operative, which directly or indirectly affect human health conditions. If it is not treated correctly, it may ultimately lead to death. Wounds can be caused by various microorganisms such as bacteria, fungi, parasites, and viruses. Some of the commonly associated bacteria organisms include Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiellaspp., Acinetobacterspp [3]. Studies conducted in 2014 and 2021 reported a wound infection prevalence of 10% and 5.95% respectively [4, 5]. Medicinal plants and synthetic drugs have been the most valuable sources of molecules with therapeutic potential throughout the history of mankind. Folk medicine of each civilization is based on natural products and, nowadays, medicinal plants still represent an important pool for the identification of novel drug leads [6]. African panaxia being a synthesized herbal medicine is made from various herbal plants such as ginseng root, Optimum gratissimum, Panaxquiquifolium, Aloe-vera and water with active ingredients ginsenosides, methyl eugenol, saponins and salicylic acid respectively [7].

a) Rationale

The advent of the resistance that pathogenic microorganisms have developed against antibiotics has necessitated much attention to be paid on plant extracts and biologically active compounds isolated from natural plants used in herbal medicine [8]. Despite the use of various synthesized herbal plants in the treatment of bacteria wound infection, there is limited information regarding the use of African panaxia in the treatment of wound infections. Furthermore, no study has been carried out in this study area. Hence there is need to investigate on the in-vitro activity of African panaxia on bacteria wound infections.
b) Goal of study
The goal of this study was to provide base line data on the effect of African panaxia on bacteria wound infection.

c) Hypothesis
There is no significant effect of African panaxia on bacterial wound infections.

d) Objectives of study
i. General objective
The general objective of this study was to determine the effect of African panaxia on bacteria wound infection.

ii. Specific objectives
To determine the efficacy of distilled water extract of African panaxia on Staphylococcus aureus and Klebsiella species bacteria from wound infection.

To determine the efficacy of Luke warm water extract of African panaxia on Staphylococcus aureus and Klebsiella species bacteria from wound infection.

To determine the efficacy of ethanoic extract of African panaxia on Staphylococcus aureus and Klebsiella species bacteria from wound infection.

II. Materials and Methods

a) Study area and setting
This study was carried out in Tiko, in Maflekumen Medical Teaching and Research Laboratory situated in Tiko is a subdivision of Fako Division in the South West Region of Cameroon with a population of 30,000. The life style and occupation of inhabitants of Tiko including the dusty, windy and hot nature, farming, bike riding and much more favours the acquisition of wounds b humans thus making the area suitable for this study.

b) Study design and duration
This was a Laboratory based experimental study designed that was conducted from November 2022, to June 2023.

c) Specimens and sampling
This study made use of bacteria isolates from people with bacteria wound infection in Tiko community.

d) Ethical consideration
An introductory letter was obtained from MAFLEKUMEN Higher Institute of Health Sciences TIKO (APPENDIX A) and was taken to regional delegation in Buea for the approval of the project. An administrative authorization was obtained from the regional delegation (APPENDIX B) and was presented to the administration of MAFLEKUMEN. An authorisation was gotten from the MAFLEKUMEN administration to carry out the research.

e) Data collection and techniques
i. Sample collection
Bacterial isolates were obtained from Maflekumen diagnostic laboratory. Preparation of MacConkey agar, blood agar and EMB was done by weighing the powder using an electronic balance and dissolved in distilled water following the manufacturer’s instructions and was cooked to obtain the gel using a Bunsen burner and allowed to cool to 40°C. The agar was poured into petri dishes and allowed to solidify. The samples were inoculated in the plate and read after 24hrs. Presumptive identification of bacteria was done based on colony characteristics, gram reactions were recorded. Confirmatory biochemical tests were done to confirm the bacteria. For staphylococcus aureus, and Klebsiella species respectively.

ii. Catalase test
A drop of Hydrogen peroxide was placed on a slide and a colony of isolated bacteria picked and emulsified on the slide containing the hydrogen peroxide. The appearance of air bubbles indicate catalase positive.

iii. Coagulase test
A drop of normal saline was placed at both ends of the same slide, one labeled test and the other control. A colony of the isolated bacteria was emulsified on each drop of the normal saline. Serum was placed on the test path and emulsified and nothing was added on the control. The presence of coagulation indicates coagulase.

iv. Indole test
Test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water. Incubate at 35–37°C for up to 48 h. 0.5 ml of Kovac’s reagent was added and shake gently, examination for a red color in the surface layer within 10 minutes macroscopically.

v. Extraction of African panaxia (Alcohol, distilled water and Luke warm water)
African panaxia was bought from the Moghamo express in Mutengene and transported to the laboratory for sensitivity testing on the bacteria isolates. One gram of African panaxia was weighted on an electric scale balance and put in a 250 ml flask, followed by adding 100 ml of solvent (95% ethanol). The flask was then left at room temperature for two days preceding filtration funnel and Wattman No. 1 filter paper. The filtrate was concentrated under decreased pressure with an evaporator at 40°C. This crude extract was saved at 4°C until use, this extract of African panaxia was considered as the 100% concentration for ethanol extract, different stock solution for distilled water were made equally and also for look warm water respectively. Then the concentrations (100%, 75%, 50%, and 25%) were made by diluting the concentrated extract of African panaxia with appropriate volumes of sterile distilled respectively for Luke warm water. Serial dilutions were made to determine the minimum inhibitory, and bactericidal concentration respectively.

i. Different stock solutions of African panaxia were made (Absolute alcohol, Luke warm water and
distilled water, in which different volumes were used 100, 75, 50 and 25 in which the isolated species of bacteria were used to test for the minimum inhibitory concentration and minimum bactericidal concentration using dilution technique and absorbance was measured using a spectrophotometry machine at a wavelength of 660nm.

ii. A solution of the isolated bacteria was prepared and standardized by matching to the 0.5 McFarland turbidity standards using sterile saline to produce approximately $1.5 \times 10^8$ colony forming units per ml.

iii. Serial dilutions were made on the different stock solutions of *African panaxia* using four sterile dry tubes per isolate and per stock solution respectively.

iv. Two (2ml) of nutrient broth was placed in each sterile test tube, followed by adding 2ml of each stock solution in the first tube, mix well and transfer 2ml to the next tube continuously and to the fourth to remove 2ml and discard respectively. 1drop of the bacterial suspension was place in each test tube respectively.

v. They labeled test tubes were sealed and incubated at 37°C for 18 to 24hours in which the and minimum inhibitory concentration and minimum bactericidal concentration recorded by checking the turbidity of each tube and absorbance was measured using a spectrophotometer at 660nm following the control of the absorbance of 0.5 McFarland standard and Azithromycin.

f) Data analysis

Data was analyzed using Microsoft excel and the results was presented in tables and figures

III. Results and Discussion

a) Results

This chapter presents the results obtained from the effect of *African panaxia* on *Staphylococcus* and *Klebsiella* isolated from wounds. Based on extract with distilled water the concentration with 75% and 50% stocks were effective in inhibiting the growth of *Staphylococcus* and *Klebsiella* respectively. Also using a stock of 50% and 25%, it exhibited bactericidal properties on *Staphylococcus* and *Klebsiella* respectively as presented on figure 1 below.

![Figure 1: Effects of *African panaxia* on *Staphylococcus aureus* and *Klebsiella pneumoniae*](image-url)

Based on extract with luke-warm water the concentration with stocks of 100%, 75% were effective in inhibiting the growth of *Staphylococcus* without *Klebsiella* and with stocks of 50% and 25% having bactericidal activity against *Staphylococcus* with stock of 25% having inhibitory properties as presented on figure 2 below.
Figure 2: Effects of African panaxia on Staphylococcus and Klebsiella pneumoniae

Based on extract with absolute alcohol the concentration with 95% stocks were bactericidal on Staphylococcus and Klebsiella and with stocks of 75% was bactericidal and inhibitory on the growth of Staphylococcus and Klebsiella respectively as presented on figure 3 below.

Based on the inhibitory and bactericidal property of Africa panaxia on staphylococcus and Klebsiella, of all the different concentrations made with distilled water, at 25% stock concentration, the extract of African panaxia was both inhibitory and bactericidal on Staphylococcus and Klebsiella. African panaxia extract with look warm water revealed that the extract was bactericidal at 50% stock and 25% stock concentration on Staphylococcus and bacteriostatic at 25% stock concentration on Klebsiella. Finally with alcoholic extract of the African panaxia, the plant extract was bactericidal at 95% stock concentration on Staphylococcus and Klebsiella and bactericidal and bacteriostatic at 75% stock concentration on Staphylococcus and bacteriostatic on Klebsiella as presented on table 1 below.
Effects of African Panaxia Extracts on Staphylococcus Aureus and Klebsiella Pneumoniae from Bacteria Wound Infection in Tiko

Table 1: Effects of African panaxia extract on Staphylococcus and Klebsiella pneumoniae

<table>
<thead>
<tr>
<th>DILUENT</th>
<th>Stock CONC.</th>
<th>Staphylococcus</th>
<th>Klebsiella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Turbidity</td>
<td>No</td>
</tr>
<tr>
<td>Distill Water</td>
<td>100%</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>25%</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Luke Warm Water</td>
<td>100%</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>25%</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Alcohol</td>
<td>95%</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>75%</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

b) Discussion
Isolation and identification of Staphylococcus aureus and Klebsiella pneumoniae from wound infections

Infection of wounds comes from so many sources and the most common bacteria which might infect and complicate wounds include Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis and Acinetobacter baumannii. Based on the results obtained from these findings, it was revealed that the most common bacteria isolated from wounds were Staphylococcus aureus, and Klebsiella pneumonia. These findings are similar to the results obtained by Mohamed Salah et al., in 2022, who isolated Staphylococcus sp., Klebsiella sp., Pseudomonas sp., Bacillus sp., E.coli diabetic wound infections [83]. Also, Mohammed et al., in 2019, also revealed that the most common bacteria isolated from wounds were Staphylococcus aureus, and Klebsiella pneumonia. [84]. Also, these findings agree with other findings by Obi et al., in 2015 who reported that common bacteria isolates from the different types of wounds were Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis and Acinetobacter baumannii [85].

To determine the minimum inhibitory concentration of African panaxia Staphylococcus aureus and Klebsiella pneumoniae from wound infections

The minimum inhibitory concentrations were determined using extract from different concentrations such as distilled water, luke warm water and ethanoic extracts of the African panaxia. From the findings it was revealed that African panaxia extracts of distilled water and luke warm water were more inhibitory at 75%, 50% stock respectively than Bactericidal. These findings are similar to results of Korukluoglu et al. in 2010 who reported that extraction of aqueous solvent resulted in a product with greater overall antimicrobial activity than extraction with water, as aqueous extracts of all the olive oil displayed little or no antimicrobial activity against any of the bacteria tested [86]. Similarly Weerakkody et al., (2010) [5] observed that water extracts of oregano and rosemary had little or no antimicrobial activity compared to ethanol or hexane extracts. Again, Sofia et al., (2007) [6] reported that water extracts of mustard, cinnamon, garlic and clove had good inhibitory activities against E. coli and S. aureus,

To determine the minimum bactericidal concentration of African panaxia on Staphylococcus aureus and Klebsiella pneumoniae from wound infections

Comparing results found in this study with those of the literature, we notice in a previous work on antimicrobial activity of some medicinal plants from Tunisia, that methanolic extracts of C. monspeliensis leaves have shown an interesting activity against P. aeruginosa, S. aureus, E. faecalis with inhibition zones diameters of 18.0, 20.0 and 15.0 mm, respectively.[26]

Whereas, water-methanol extracts of fruit peels of pomegranate (P. granatum) have demonstrated a moderate activity when they were tested on S. aureus, P. aeruginosa and K. pneumoniae (13.0, 18.0 and 16.0 mm, respectively)[27].This activity of pomegranate peels could be attributed to tannins, for which antimicrobial activity has been demonstrated.[4]

On the other hand, the results found in the study concerning the activity of R. tripartitaerial parts are in agreement with other previous works which found significant antibacterial activity of leaves alcoholic extracts against methicillin-resistant S. aureus, 16 and no activity against E. coli and P. aeruginosa. 29 For W. frutescens, El Bouzidi et al. have reported different antibacterial activities of leaves methanolic extracts against S. aureus (11.5 mm), K. pneumoniae (18.0 mm), P. fluorescens (14.5 mm) and no activity against E. coli.30

IV. Conclusion

Based on the results obtained from the study, it could be concluded that the most common bacteria isolates obtained from this study were Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae.
pneumonia. African panaxia extract from distilled water was more inhibitory than bacteriocidal on Staphylococcus aureus and Klebsiella pneumonia and the lastly the African panaxia extract from ethanol was totally bactericidal on both Staphylococcus aureus and Klebsiella pneumonia.

V. Recommendations

From the results obtained from this study, the following recommendations can be made: Ethanoic extract of African panaxia should be used on wounds infected with Klebsiella pneumoniae and Staphylococcus aureus to obtain maximum success. Also other natural herbs should be used to determine their inhibitory and bactericidal properties on Klebsiella pneumoniae and Staphylococcus aureus.

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Relationship between Antidepressant use and Increased Bacterial Resistance

By Matheus Amorim Grigorio, Rayssa Blenda Martins, Lucca Piacesi Muniz De Melo, Victoria Carneiro Maciel, Dayane Carolini Rodrigues, Edson Brunetti Da Silva, Caio Almeida Andrade, Lorena de Sousa Moura, Juliane Alves de Mesquita, Mariana Santos Pinto, Eduarda de Oliveira, Isabella Furlan de Assis & Lucas Roberto Araújo Paiva Calabrich

Abstract- Introduction: The uncontrolled use of antidepressant drugs can be potentially harmful to individual and collective health. Analgesics are often used inappropriately and carelessly, which can result in a number of negative effects like bacterial resistance, hypersensitivity, dependence, digestive bleeding, withdrawal symptoms, and even an increased risk for certain neoplasms (Ribeiro et al., 2010). The central nervous system, as well as the respiratory and digestive systems, are the sources of the complaints that are most frequently linked to self-medication, including headache, musculoskeletal pain, fever, respiratory infections, heartburn, abdominal pain, constipation, and diarrhea (Furtado et al., 2019).

Keywords: antidepressants; bacterial resistance; drug interaction.

GJMR-B Classification: NLM: QV 744
Relationship between Antidepressant use and Increased Bacterial Resistance


Abstract: Introduction: The uncontrolled use of antidepressant drugs can be potentially harmful to individual and collective health. Analgesics are often used inappropriately and carelessly, which can result in a number of negative effects like bacterial resistance, hypersensitivity, dependence, digestive bleeding, withdrawal symptoms, and even an increased risk for certain neoplasms (Ribeiro et al., 2010). The central nervous system, as well as the respiratory and digestive systems, are the sources of the complaints that are most frequently linked to self-medication, including headache, musculoskeletal pain, fever, respiratory infections, heartburn, abdominal pain, constipation, and diarrhea (Furtado et al., 2019).

Methodology: This is a literature review whose bases were taken from the SciELO and PubMed data platforms. The research period was July 2023, meeting the inclusion criteria of articles from the years 2000 to 2023, in Portuguese and English, online texts, and full texts. As strategies for better evaluation of the texts, the following health descriptors (DeCS) were used: “Antidepressants” and “Bacterial resistance”.

Results: The choice of an antidepressant takes into account efficacy, safety, tolerability, toxicity in overdose, the previous response of the patient or a family member to a given agent, the experience of the physician in the management of a given representative, the occurrence of special situations that require antidepressants free or with a lower degree of some of the adverse effects, and cost. (MINISTÉRIO DA SAÚDE, 2012). In addition to the circumstances mentioned, another variable that proved worthy of investigation was the increase in bacterial resistance associated with antidepressant use. Globally, antibiotic resistance is a significant public health threat. An estimated 1.2 million people died as a direct result of it in 2019, and this number is expected to increase (NEWS.MED.BR, 2023). Such effects are associated with increased reactive oxygen species, enhanced stress signature responses, and stimulation of efflux pump expression. Mathematical modeling also supported the role of antidepressants in the occurrence of antibiotic-resistant mutants and persistent cells (WANG, 2023).

Conclusion: Based on the foregoing, it is clear that psychosomatic illnesses have a large epidemiological component in the population of the twenty-first century. As a result, numerous treatment options have developed, including antidepressants. Additionally, it is intriguing to highlight and develop the notion that the indiscriminate use of antidepressants ultimately has a detrimental effect on all microbiota in the body, leading to an increase in bacterial resistance. When used properly and in conjunction with a qualified health practitioner, the mental health of the entire community can be improved while also thinking about preventing potential illnesses.

Keywords: antidepressants; bacterial resistance; drug interaction.

I. Introduction

Antidepressant drug abuse has the potential to be detrimental to both an individual’s and society’s health. The improper and careless use of substances and even medications that the general public views as “simple,” like analgesics, can have a number of negative effects, including bacterial resistance, hypersensitivity, dependence, digestive bleeding, withdrawal symptoms, and even an increased risk for certain neoplasms (Ribeiro et al., 2010).

Self-medication is most frequently linked to complaints involving the central nervous system, the respiratory, and digestive systems, as well as heartburn, abdominal pain, constipation, and diarrhea (Furtado et al., 2019). These complaints include headaches, musculoskeletal pain, fever, respiratory infections, and heartburn.

Researchers are relying on “drug repositioning” as an alternative to more quickly identify effective drugs to treat infectious diseases because bacterial resistance is typically linked to the abuse of antibiotics and has alarming clinical and economic consequences for the world. (2018) (SERAFIN, MB; HORNER, R).

It’s interesting to examine the post-pandemic situation that the globe is in: the pandemic obviously made a number of psychological illnesses worse, so the negative impacts, like increasing bacterial resistance, must be discussed in scientific communities and in the general population.

II. Methods

This is a literature review whose bases were taken from the SciELO and PubMed databases. The research period was July 2023, meeting the inclusion criteria of articles from the years 2000 to 2023, in Portuguese and English, online texts, and full texts. As strategies for better evaluation of the texts, the following...
III. RESULTS AND DISCUSSION

The selection of an antidepressant considers factors like effectiveness, safety, tolerability, toxicity in overdose, prior responses of the patient or a family member to a given agent, experience of the doctor in the management of a given representative, the occurrence of special situations that require antidepressants free of or with a lower degree of some of the side effects, and cost. (2012) (MINISTÉRIO DA SAÚDE). Along with the previously described factors, the rise in bacterial resistance linked to antidepressant usage also merited further research.

Antibiotic resistance poses a serious hazard to public health on a global scale. It caused an estimated 1.2 million deaths directly in 2019, and this number is projected to rise (NEWS.MED.BR, 2023).

Such effects are linked to an increase in reactive oxygen species, improved stress signature reactions, and the induced expression of efflux pumps. A function for antidepressants in the occurrence of efflux pumps that are resistant to antibiotics and persistent cells was also supported by mathematical modeling (WANG, 2023). In bacteria grown in well-oxygenated laboratory conditions, the antidepressants caused the cells to generate reactive oxygen species—toxic molecules that activated the microbe's defense mechanisms. Most prominently, this activated the bacteria's efflux pump systems, a general expulsion system that many bacteria use to eliminate various molecules, including antibiotics. This probably explains how the bacteria could resist antibiotics without having specific resistance genes. But exposure of E. coli to antidepressants also led to an increase in the mutation rate of the microbe and the subsequent selection of various resistance genes. However, in bacteria grown under anaerobic conditions, levels of reactive oxygen species were much lower, and antibiotic resistance developed much more slowly (NEWS.MED.BR, 2023). Additionally, sertraline, at least one antidepressant, has fostered bacterial gene transfer, a technique that can hasten the emergence of resistance in a population. As a result of this transfer, resistance can move between species, including from pathogenic to harmless bacteria (NEWS.MED.BR, 2023). The antidepressant sertraline encourages plasmid conjugative transfer. One clonal line exhibits multidrug resistance and persistence, and horizontal transmission of antibiotic resistance brought on by sertraline antidepressants has been identified (WANG, 2023).

Antidepressants can influence membrane integrity and promote efflux pump expression. One important resistance mechanism is the activation of the efflux pump, which enables bacteria to control their internal environment by expelling harmful elements like antibiotics. The efflux pump may have been expressed as a result of antidepressant use, which is thought to have contributed to the development of antibiotic resistance (WANG, 2023). Antidepressants can cause chromosomal mutations and multidrug resistance. The hypothesis is that reduced susceptibility to multiple antibiotics may also be associated with mutations in the chromosome. Antidepressants may also increase persistence with antibiotics. Persisters are dormant variants of wild-type bacteria that are formed in microbial populations. Persisters are not antibiotic-resistant but exhibit high antibiotic tolerance (WANG, 2023). Antidepressants can affect the evolution of persistent and resistant bacteria. Generally, bacterial evolution through genetic mutations is a slow and incremental process. It has been found experimentally that antidepressants can significantly promote the process of antibiotic resistance and persistence (Wang, 2023).

IV. FINAL CONSIDERATION

After reading the foregoing, it is clear that psychosomatic illnesses have a substantial epidemiological component in the population of the twenty-first century. As a result, a number of treatment options have appeared, including antidepressants. Furthermore, it is intriguing to highlight and develop the hypothesis that the indiscriminate use of antidepressants ultimately has a deleterious influence on all microorganisms in the body, leading to an increase in bacterial resistance. With the proper use and in the company of a qualified health professional, thinking of preventing potential illnesses is a significant method to improve the quality of life and mental health of the entire community.

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Acknowledgments

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.
Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11"”, left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word “Abstract” in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

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The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper’s subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.

j) There should be brief acknowledgments.

k) There ought to be references in the conventional format. Global Journals recommends APA format.

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Author details
The full postal address of any related author(s) must be specified.

Abstract
The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords
A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods
Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations
Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations
Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends
Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.
Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

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Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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Tips for Writing a Good Quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

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6. **Bookmarks are useful**: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. **Revise what you wrote**: When you write anything, always read it, summarize it, and then finalize it.

8. **Make every effort**: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

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10. **Use proper verb tense**: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. **Pick a good study spot**: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. **Know what you know**: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. **Use good grammar**: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice. Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. **Arrangement of information**: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. **Never start at the last minute**: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. **Multitasking in research is not good**: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. **Never copy others’ work**: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. **Go to seminars**: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. **Refresh your mind after intervals**: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.
20. **Think technically:** Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

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22. **Report concluded results:** Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. **Upon conclusion:** Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

**Informal Guidelines of Research Paper Writing**

**Key points to remember:**
- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

**Final points:**

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

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**The discussion section:**

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

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- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

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Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.
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- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

**Approach:**

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

**Procedures (methods and materials):**

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

**Materials:**

*Materials may be reported in part of a section or else they may be recognized along with your measures.*

**Methods:**

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

**Approach:**

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

**What to keep away from:**

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.
Results:
The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:
- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:
- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:
As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:
If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:
The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

**Approach:**

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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