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ESTIMATION OF ANTIBACTERIAL POTENTIAL OF LEAF EXTRACT OF *PORTULACA GRANDIFLORA* HOOK. AND *ALSTONIA SCHOLARIS* L.

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Abstract

Recent investigations have clearly shown that pathogenic bacteria are quickly increasing in quantity and are the root of numerous severe diseases. Equipment resembling brush sonication and alcohol wipes had been utilized for cleaning these pathogens. In the current study, *P. grandiflora* and *A. scholaris* leaves extract were used to reduce surface contamination. It had been discovered that plant leaf extracts have antibacterial qualities and can improve surface cleaning. *P. grandiflora* and *A. scholaris* plant leaves were gathered, as well as bacterial samples from the four different locations that are, washroom flush, sink, floor and door knob. Bacteria were cultivated independently for each of the four sites and the streak plate approach was used to culture them. Bacteria were studied microscopically by using gram staining technique. Under an optical microscope (Olympus CX23), the two bacteria *S. aureus* and *E. coli* were discovered. MIC technique was done to determine the concentration (of our leaf extracts) at which bacteria were eliminated or decreased. The results revealed that the MIC value against bacteria (*S. aureus*) decreased with increase in concentration of leaf extracts. MBC of both leaf extracts were also calculated. According to this study, plant extracts are able to improve surface cleaning. They can be used into soaps or detergents to enhance their cleaning capabilities.

Keywords: bacteria, contamination, leaves extract, *S. aureus*, MIC, MBC.

Introduction

Due to the shocking rise of disorders caused by pathogenic microorganisms, there is continual and crucial demands to find out natural or synthetic substance which kill and inhibit the growth of microbes (Edna *et al.*, 2015). High price of medicine, treatment and mediation have lesson the standard of living. Many nutrients like polyphenols which are micronutrients that we get through plant based food and flavonoids that are phytonutrients found in almost all fruits and vegetables can minimize the above mentioned dangers (Dhalaria *et al.*, 2020). The prevention, treatment, and diagnosis of infectious diseases are the primary objectives of control initiatives as they remain a major threat to global health. However, the growth in microbial resistance highlights the importance for innovative approaches and interdisciplinary methods to effectively combat these diseases and protect public health (Ortega *et al.*, 2020). The phenomenon of development of obstinacy has caused the microbial drug to become less effectual (Baym *et al.*, 2016).

The Portulacaceae family includes the species *Portulaca grandiflora*, often called Moss rose, Mexican rose, or Eleven o'clock. It is indigenous to southern Brazil, Argentina, and Uruguay. This plant, which is frequently grown in gardens, has a variety of medical and therapeutic applications. It is well-known for its detoxifying, sore throat-relieving, rash-soothing, antimicrobial, anti-diabetic, antioxidant, and antibacterial qualities. Additionally, it has a cytotoxic impact on cancer cells and is essential in promoting the biotransformation of L-tyrosine into LDOPA. This review sheds light on *Portulaca grandiflora's* cultivation, nutritional and chemical makeup, as well as its diverse medicinal and therapeutic properties (Mane *et al*., 2022).

A range of phytochemicals, including as glycosides, alkaloids, saponins, terpenoids, anthraquinones, reducing sugars, and steroids, are present in the *Alstonia scholaris*'s extracts. These substances show antibacterial activity against bacterial and fungal strains that are resistant to numerous drugs. The highest antibacterial activity against *Enterobacter* was demonstrated by the leaf extracts in methanol and ethyl acetate. The highest antibacterial efficacy against *Enterobacter* and Pseudomonas was demonstrated by the ethanolic extract (Altaf *et al*., 2019).

Microbial antipathy to flawless antiseptic and its quick advancement have escalated significant trouble in medicaments of transferable afflictions. Many plant chemicals in fruits, vegetables, grains, come up with advisable fitness comforts and shrink the probability of immedicable afflictions (Khameneh *et al.*, 2019). Nowadays infection that occur when a microorganism enter a person's body and cause harm and antiseptic defiance, happens when germs no longer response to antibiotic have been big dare which endanger the fitness of community. Many people died in whole world due to infection caused by microorganisms, pathogens. They can quickly multiply like bacteria (Gupta *et al.*, 2019). In recent times, several plans seemed to be adopted, to control the opposition of anti-microbial substance against bacteria. One of the approved master plan to attain above objective is the union of other particles with flop antiseptics (Brown, 2015; Rana *et al.*, 2019). These particles not containing antibiotic but have characteristics of antiseptic. They provide good chance for healing or curing (Vandevelde *et al.*, 2016). Chemical compounds produce by plants through primary and secondary metabolism have display powerful project and many scientists have used chemical compounds produce by living organism (natural product) that work opposed to bacterial animosity (Shakeri *et al.*, 2018; Bazzaz *et al.*, 2018).

In the fight against microbial illnesses, plant-derived antimicrobials offer prospective alternatives. They are effective, safe, and have a variety of modes of action, which makes them useful instruments for addressing the problems caused by infectious illnesses and antimicrobial resistance (Arip *et al.*, 2022). Exposure and spread of single bacteria, resistant to more than one anti-biotic and are capable of causing disease when enter into a body have become a major health problem. Many agents that kill or stop their growth are used against infectious diseases (Giamarellou, 2010). The configuration of living operative alloy obtain from plants that possesses therapeutic properties relay on varieties of plants, classification of soil and interconnection with pathogens (Zhao *et al.*, 2011; Morsy, 2014). Around the world, meal decomposition by pathogens or microbes largely influence fully kind of food stuff. These microbes cause decrease in quantity and quality of food even in advanced state. It has been approximate that about 40% food waste are due to microbial spoilage (Amit *et al.*, 2017).

In last few years, research proved that biological active compound, found in plants, apply their germicidal action by various measures such as rupture their plasma membrane so that cell will not be able to exchange material from surrounding, crushing the molecule produce by microorganisms, stop the working of substance that act as a catalyst in living organisms and stop the affairs by means of which microbes unalterably attached and stretch on facet and produce extracorporeal polymer that ease to fitting and manufacture array consequentials alternation in configuration (Barbieri *et al.*, 2017). Long time ago, flora have ability to repair the disorders caused by organisms by natural processes due to specialized compound present in plants like alkaloids, sulfur containing phytochemicals, terpenoids, and polyphenols. These compounds act as antiseptic against microbes that cause disease in humans (Borges *et al.*, 2015).

In this research proposal, surface bacteria were eliminated using plant leaf extracts. From contaminated surfaces, several samples of plant leaves and bacteria were collected. To detect bacterial colonies, various biochemical assays were run. Then ethanol was used to make extracts. The MIC technique was used to determine the concentration at which plant leaf extracts prevent or slow down bacterial development.

Materials and methods

Rose moss (*Portulaca grandiflora*) leaves and blackboard tree (*Alstonia scholaris)* leaves were collected from Jinnah Bagh, and then submitted to LCWU to get their voucher numbers that were *Portulaca grandiflora* (Voucher no. 0340) and *Alstonia scholaris* (Voucher no. 0215). The experiment was complete randomize designed (CRD). These leaves were thoroughly washed and remove if there were any debris, then dried for about 2 to 3 months under strong sunlight by spreading them under the region where direct sunlight were captured. Selection of solvents for the extraction relay on the species of plants, section of plants, used for extraction and the presence of solvents. Usually alcohol and water solvents are used for this purpose (Pandey & Tripathi, 2014; Sasidharan *et al.*, 2011; Altemimi *et al.*, 2017). Ethanol was used to make plant leaves extract. Dried leaves were taken in bottom of mortar, take the pestle and use it to grind into powder form. Then weighed about 68 mg leaf powder in 10 ml ethanol. Difference in MIC under different concentrations was noticed, the powder was weighed again about 145 mg per 10 ml ethanol. Dark green color mixture was observed. Same procedure were carried for both plant species and incubated for 20 to 30 minutes to homogenize the mixture.

By using transport swab, samples were taken from four different locations which were washroom flush, door knob, basin and sink. Samples were put in 5ml nutrient broth for enrichment purpose and then incubated at 37ºC for about 24 hours. Different colonies were formed in different petri dishes which were then cultured in broth. Cultured bacteria were used and a colony were picked from cultured plate, then added to nutrient agar by streak plate method (Jiang *et al.*, 2016) and identified by gram staining technique (Becerra *et al.*, 2016), under microscope (Olympus CX23). *S. aureus* and *E. coli* were identified and only *S. aureus* was further used to study antibacterial potential of leaves extract of *P. grandiflora* and *A. scholaris*. ATCC29213 strain of *S. aureus* was used for MIC. Antimicrobial assay were done by using broth dilution method (Giner *et al*., 2012). MBC were also calculated by noticing the number of well present exact before the cloudy well.

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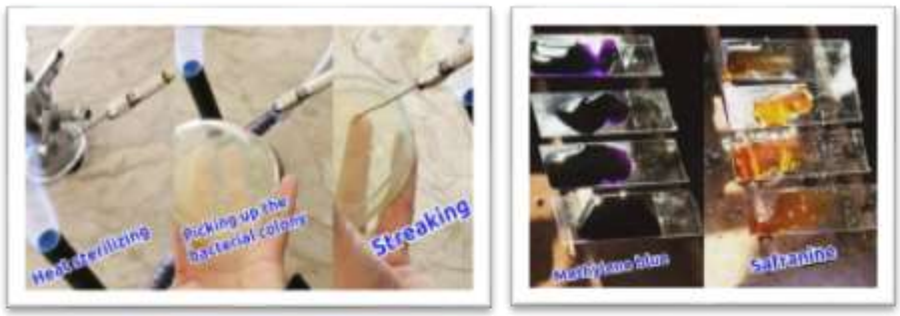

Figure 1: Streak plate and gram staining for identification of bacteria

Results

Two bacteria, *S. aureus* and *E. coli*, were detected by biochemical assays. Gram staining allowed the identification of several dark blue, spherical-shaped bacteria as *S. aureus* and rod-shaped, pink as *E. coli*. Additionally, the antibacterial effectiveness of our plant leaf extract using *S. aureus* were solely tested.

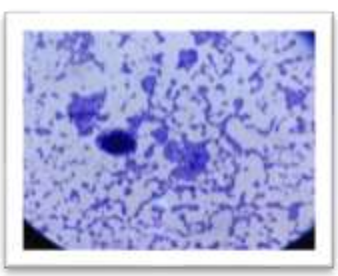

Figure 2: Microscopic study of *S. aureus*

For all four samples, the following plant leaf extract concentrations were used: 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625, 0.1953125, and 0.0765625 to the 12th well, with the exception of the 11th well (positive control). The foggy look at any number of wells was detected after 24 hours of incubation. The MIC value of that plant against *S. aureus* was presented in the well directly before the well that seemed murky. The MIC value of the plant against the bacteria dropped with an increase in extract concentration. On a plate with 96 wells, MBC values were also noted.

Cloudy appearance for washbasin sample were observed at 5th well, so MIC of *A. scholaris*, under low concentration, was 12.5 mg/l by viewing values in table 4.1. By observing flush sample, cloudy appearance was observed at 4th well, so MIC for flush sample is observed at 25 mg/l. Noticing basin sample, cloudy appearance was observed at 5th well, so MIC for basin sample is observed at 12.5 mg/l. Variation in data was seen when a different extract concentration (145 mg/10 ml) was employed. Table 1: displayed the MIC and MBC values for *A. scholaris*, with the results demonstrating that the MIC value for *S. aureus* dropped as extract concentration increased. Similar to the MBC scenario, it too rose with an increase in MIC, but MBC value was larger than MIC.

Using the same method that was previously used for *A. scholaris*, *P. grandiflora* extract was also found to be effective against *S. aureus*. It was noted that both plants' MIC values varied. By comparing the values of the two extracts, it was found that *A. scholaris* had higher MIC values than *P. grandiflora*. Consequently, *P. grandiflora* was superior to *A. scholaris* at eliminating bacteria from surfaces.

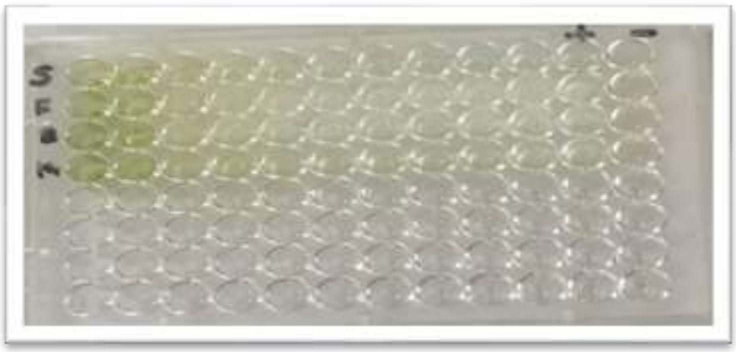

Figure 3: MIC of A. scholaris, at concentration of 68mg/10ml against S. aureus.

Table 1: MIC values observed from 96 wells plate of *A. scholaris*.

Alstonia scholaris				
Sample sources	***Concentrations used***			
	68 mg/10ml		**145mg/10ml**	
	MIC	**MBC**	**MIC**	**MBC**
Sink	12.5	25	6.25	12.5
Flush	25	50	12.5	25
Basin	12.5	25	6.25	12.5
Knob	6.25	12.5	3.125	6.25

Figure 4: MIC of *P. grandiflora*, at concentration of 68 mg/10ml against S. aureus.

Table 2: MIC values observed from 96 wells plate of *P. grandiflora*.

P. grandiflora				
Sample sources	***Concentrations used***			
	68mg/10ml		**145mg/10ml**	
	MIC	**MBC**	**MIC**	**MBC**
Sink	12.5	25	6.25	12.5
Flush	6.25	12.5	3.125	6.25
Basin	6.25	12.5	3.125	6.25
Knob	6.25	12.5	3.125	6.25

Discussions

In the fight against diseases that are resistant to antibiotics, the creation of novel antimicrobial agents, particularly those derived from natural sources like plant extracts, is a potential strategy. To assure both safety and efficacy, the process from early in vitro research to clinical application is complicated and necessitates extensive testing and validation (Liu *et al.*, 2023).

In antibacterial analysis, minimum application that stop the extension of reveal microbes known to be MIC point. It is widely recorded procedure that demonstrate the capability of antibacterial agent. Leaf extracts of *Portulaca* genus like *P. oleracea* had been studied against bacteria like *Staphylococcus aureus* and other pathogens, the results from that work indicates that *P. oleracea* prevail bacteria, ineffective by antibiotics. Highest MIC of *P. oleracea* leaves was 200 ppm and the lowest MIC was 50 ppm (Mousavi *et al.*, 2015). The MBC of *P. grandiflora* was identified by determining the lowest antibacterial agent concentration that lowered the initial bacterial inoculum's viability by a predetermined reduction (Sporna-Kucab *et al.*, 2022).

By spectrophotometry of *P. grandiflora herba*, phytochemicals like polysaccharides, flavonoids, carotenoids, reducing agents, sterols and polyphenol acids were recognized (Anghel *et al.*, 2013). Results of this research stipulated the presence of antimicrobial possessions in leaf extracts of *P. grandiflora* and *A. scholaris*. Leaves of *P. grandiflora* showed highest MIC 50 µl/ml and lower MIC 12.5 µl/ml, at the concentration of 68 mg/10ml against *S. aureus*. On increase in concentration of extract, MIC decreased from 50 to 12.5 µl/ml and from 12.5 to 6.25 µl/ml. Similarly, leaf extracts of *A. scholaris* showed highest MIC 50 µl/ml and lower MIC 12.5 µl/ml, at the concentration of 68 mg/10ml. On increase in concentration of *A, scholaris* extract, MIC decreased from 50 to 12.5 µl/ml and from 12.5 to 6.25 µl/ml, at higher concentration which were 145 mg/10ml against *S. aureus*. The minimum bactericidal concentration (MBC) were the lowest concentration of an antibacterial agent required to kill a bacterium, somewhat extended period, such as 18 hours to 24 hours, under a specific set of conditions. The MBC was identified by determining the lowest antibacterial agent concentration that lowered the initial bacterial inoculum's viability by a predetermined reduction.

The isopropanol fraction of *A. scholaris* has the highest antibacterial, antifungal, antiviral, and anti-mycobacterial activity. The leaves and bark of this plant are abundant in phytochemicals with antimicrobial effects against human diseases. *A. scholaris* has been shown to have antimicrobial activity against a number of human pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Mycobacterium tuberculosis*, as well as against drug-resistant strains of bacteria (Bagheri *et al.*, 2020).

With reference to past experiments, it has been observed that extracts of many plants were used in the preparation of different types of drugs to treat many diseases. On the basis of current studies, *P. grandiflora* and *A. scholaris* were used against the disease causing microbes. A wide range of disease causing bacteria like *S. aureus*, *E. coli* and many others can be killed by using the extracts of these plants. The extracts of both plants had bacteriostatic and bactericidal potential. Many plant extracts like *Zingiver officinales*, *Thymus vulgaris* etc. were used to treat disorders, caused by pathogens. Plant extracts of many flora can be used against diseases, caused by eating contaminated eatables. Extract of plants may also use to conserve fooders. *Cuminum cyminum* is commonly used as medicine, to cure various diseases and their solutions may be effective against some bacteria like *S. aureus*. Phytochemicals of *P. granatum*, *S. aromaticum* were very essential extracts, used against the diseases that were caused by eating contaminated food. Food may be contaminated by pathogens like *S. aureus* and *P. aeruginosa* (Mostafa *et al.*, 2017). It was observed that many plant extracts crucially damage plasma membrane of disease causing bacteria that was displayed by reducing in potential of hydrogen. It were also caused change in plasma membrane potential that make it more negative (Gonelimali *et al.*, 2018).

Conclusions and recommendation

From the above observation it was concluded that species of *P. grandiflora* and *A. scholaris* can be used to improve the surface cleaning against some pathogenic bacteria like *S. aureus.* By comparing both species, *P. grandiflora* is more effective in cleansing by calculating minimal inhibitory concentration. Both the above species can be used as disinfectants to enhance their capability to remove the pathogenic microbes from the surfaces like floor, tables etc. In this way, plant extracts can be used in our daily routine as a cheap and effective source of killing germs that may cause diseases.

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Conflict of interest

There are no conflict of interest related to this article.

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