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RESEARCH ARTICLE

Evaluation of the Antibacterial Efficacy and Phytochemical Composition of *Petalium Murex* Leaf Extract

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ABSTRACT

The current study aims to assess the antibacterial efficacy of various extracts derived from *Petalium murex* leaves against three bacterial species: *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*. The ethanol extract of *Bacillus subtilis* had the maximum efficacy, whereas the water extract showed the lowest efficacy against *Escherichia coli* bacterial strains. There were alkaloids, carbohydrates, saponins, flavonoids, amino acids, steroids, and phenolic compounds in the water, chloroform, and ethanol extracts of *Petalium murex*. The ethanol extract exhibited the presence of alkaloids, saponins, amino acids, terpenoids, and phenolic compounds. The chloroform extracts exhibited the existence of carbohydrates, saponins, flavonoids, terpenoids, and tannins.

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Introduction

Medicinal plants have a crucial role in promoting the well-being of individuals and communities. The therapeutic efficacy of these plants resides in the identical chemical compounds that elicit a distinct physiological response in the human body (Vaou et al., 2021). Alkaloids, tannins, flavonoids, and phenolic chemicals are the key bioactive ingredients found in plants (Roy et al., 2022). Plant-derived compounds have recently garnered significant attention due to their multifaceted uses (Samtiya et al., 2021). Approximately 14–8% of higher plant species are utilized for medical purposes, and about 74% of pharmacologically active compounds generated from plants were identified through investigations into the traditional medicinal usage of these plants (Prasathkumar et al., 2021). The process of hunting for safe and effective antimicrobial medicines to treat a wide range of bacterial illnesses has been ongoing in recent years (Salam et al., 2023). This demand has intensified in recent times due to the advent of numerous antimicrobial-resistant pathogens. The global fascination with medicinal plants demonstrates the acknowledgement of the credibility of numerous traditional assertions regarding the efficacy of natural substances in healthcare (Sofowora et al., 2013). Traditional remedies derived from medicinal plants have been employed for millennia across many regions of the globe to cure a wide range of human ailments (Petrovska, 2012; Prasathkumar et al., 2021). Therefore, scientists have recently focused on exploring safer phytomedicines and biologically active compounds derived from plant species often used in herbal remedies. These compounds should have a therapeutic index that is considered acceptable for the creation of new pharmaceuticals.

Escherichia coli are the primary factor contributing to newborn mortality and diarrheal illnesses. In instances of severe diarrhea, the patient may experience bloody diarrhea, which can pose a life-threatening risk (Levine et al., 2020). *E. coli*, which is also a causative agent of urinary tract infection, has developed resistance to the medication that has traditionally been used to treat it (O'Ryan et al., 2011).

Staphylococcus aureus exhibits resistance to antibiotics like penicillin and methicillin. This bacterium is capable of causing severe diseases like sepsis and endocarditis (Guo et al., 2020). Consequently, biochemical screening studies offer a potential alternative for producing drugs from secondary metabolites found in medicinal plants. This approach is advantageous due to its reduced side effects and lower cost.

Bacillus subtilis, sometimes referred to as the hay bacillus or grass bacillus, is a type of bacterium that is gram-positive and catalase-positive. It is commonly found in soil as well as in the gastrointestinal tracts of ruminants and humans (Errington & Aart, 2020). *B. subtilis* gained global popularity before the advent of antibiotics since it was used as an immunostimulatory agent to assist in the treatment of gastrointestinal and urinary tract illnesses (Su et al., 2020; Zhang et al., 2021).

Pedalium murex, a member of the Pedaliaceae family, has long been employed for its analgesic, anti-hypertensive, and diuretic properties. Moreover, it is renowned in the realm of traditional medicine due to its significance in the management of urinary tract infections (Patel et al., 2011). In Chinese traditional medicine, it has been utilized to treat respiratory tract infections, mastitis, and several types of eye infections (Ramadevi et al., 2020). This study analyzes the antibacterial activity of *Pedalium murex* against *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*. Additionally, it examines the phytochemical composition of the leaf extract, including carbohydrates, saponins, flavonoids, amino acids, steroids, phenolic compounds, alkaloids, terpenoids, and tannins.

Material and Methods

Collection and Preparation of Samples

Pedalium murex was obtained from Kamachipettai, located in the Cuddalore district of Tamil Nadu (Figure 1). The *Pedalium murex* leaves were rinsed with water to eliminate any dust particles. Subsequently, the specimens were meticulously dehydrated in a sheltered location and subsequently pulverized to create a refined powder, which was then employed in the production of various extracts. The plant material, which had been dried and turned into powder, weighing 10g, was extracted in a step-by-step manner using 100 ml of ethanol, water, and chloroform (Abubakar & Haque, 2020). This extraction process was carried out using a Soxhlet extractor until a complete extraction was achieved, typically taking 10–12 cycles. The extraction was performed at a temperature that did not surpass the boiling point.

Fig 1. *Pedalium murex* was collected from Cuddalore district.

Chloroform Extract

In the process of chloroform extraction, a quantity of 10 grams of powder that had been dried in the air was added to 100 milliliters of chloroform extract. The mixture was then heated for duration of 6 hours and subsequently filtered. The resulting liquid was concentrated by heating it in a water bath at boiling temperature. This concentrated liquid was then utilized for subsequent analysis (Abubakar & Haque, 2020).

Aqueous extract

To do aqueous extraction, 10g of powder that had been dried in air was combined with 100 ml of distilled water in a 1:0 ratio. The mixture was then heated for 6 hours and subsequently filtered. The resulting filtrate was condensed using a boiling water bath and utilized for subsequent analysis (Jancikova & Jablonsky, 2023).

Ethanol extract

In the process of ethanol extraction, a quantity of 10 grams of powder that had been dried in the air was combined with 100 milliliters of ethanol extract. This mixture was then heated for duration of 6 hours and subsequently filtered. The resulting liquid was condensed using a boiling water bath and utilized for subsequent analysis (Abubakar & Haque, 2020).

Analysis of phytochemicals

The primary phytochemical was evaluated using established qualitative procedures (Bakir Çilesizoğlu et al., 2022; Dubale et al., 2023). Tests were conducted on both extracts to determine the content of reducing sugar, alkaloids, tannins, terpenoids, saponins, flavonoids, and amino acids. The method employed is briefly discussed below.

Alkaloids

The 5ml extract was combined with Wagner's reagent. An alkaloid is present when a reddish-brown precipitate forms.

Carbohydrates

5ml of the filtrate was combined with 5 ml of Fehling's solution (A and B) in a test tube and then subjected to heat. The presence of carbohydrates is shown by the formation of a red precipitate.

Saponins (Foam Test)

5 ml of distilled water was added to 0.5 ml of the extract in a test tube and forcefully shaken. The solution was noticed for the production of persistent foam. The presence of saponins is indicated by the development of emulsions when froth olive oil is mixed.

Amino Acid

The filtrate (2 ml) was treated with 2–5 drops of ninhydrin solution, placed in a boiling water bath for 1-2 minutes, and monitored for the production of purple color.

Flavonoids

5 ml of sodium hydroxide was introduced into 5 ml of the extract. The formation of a rich yellow hue that diminishes when a few drops of weak sulphuric acid are introduced suggests the presence of flavonoids.

Steroids

The powder was solubilized in 2ml of chloroform in a desiccated test tube. 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid were introduced. The solution got red, then blue, and finally became bluish, which shows the presence of steroids.

Terpenoids

Terpenoids (Salkowski's Test) Extract (0.5 ml) was added to 2 ml of chloroform. 3 ml of concentrated sulphuric acid was gently poured into the walls of the test tube to form a layer. The reddish-brown color seen close to the contact points to the presence of terpenoids.

Phenolic

A phenolic compound was added to a mixture of 1 ml of extract, 2 ml of distilled water, and a small amount of 10% ferric chloride. The appearance of a blue or green color suggests the presence of phenols.

Tannins (Ferric Chloride Test)

The extract was diluted with 10 ml of water and subsequently heated in a test tube. Once the solution was filtered, a small amount of 0.1 ferric chloride was added. The development of a precipitate that ranged in color from yellow to red allowed researchers to identify the presence of tannins.

Antimicrobial Effectiveness

Microorganisms utilized for antibacterial activity

The microorganisms were obtained from the Pondicherry Centre for Biological Science in Pondicherry. The antibacterial activity was screened using the following standard strains: *Escherichia coli* (gram negative), *Staphylococcus aureus* (gram positive), and *Bacillus subtilis* (gram positive).

Assessing Antibacterial Activity Using Disc Diffusion Methods

The bacteriostatic properties of the compounds were assessed using the disc diffusion method, following the protocol outlined by Bauer Kirby (Sharma et al., 2018). The Mueller-Hinton agar ingredients were measured and dissolved in water during the preparation process. The mixture was heated over a water bath until the agar was completely dissolved. Subsequently, the item was subjected to sterilization in an autoclave under a pressure of 15 pounds per square inch and a temperature of 121 degrees Celsius for duration of 15 minutes. The aseptic condition was maintained while pouring the sterilized medium (20 ml) into sterilized petri dishes. The dishes were then allowed to harden on a flat surface. A 0.1ml aliquot of a 24 hour culture of the test pathogen was evenly distributed on the surface of a nutrient agar plate. The antibiotic disc, with a diameter of 4mm, was impregnated with a medical solvent extract. The disc was loaded with the extract at equal distances, with a concentration of 10µ. The plates were then left at room temperature for 30 minutes, allowing for the diffusion of the extract into the medium. The antibacterial properties of the different solvent extracts obtained from the leaves of *Pedalium murex*.

Results and Discussion

The aqueous extract of *Pedalium murex* exhibited the presence of alkaloids, carbohydrates, saponins, flavonoids, amino acids, steroids, and phenolic compounds. The ethanol extract exhibited the presence of alkaloids, saponins, amino acids, terpenoids, and phenolic compounds. The chloroform extracts exhibited the existence of carbohydrates, saponins, flavonoids, terpenoids, and tannins as summarized in Table 1.

Table 1. Preliminary phytochemical analysis from the *Pedalium murex* of different solvent extracts

S.No.	Phytochemical	Solvents Extract		
		Water	Chloroform	Ethanol
1.	Alkaloids	+	-	+
2.	Carbohydrate	+	-	+
3.	Saponins	+	+	+
4.	Flavonoids	+	-	+
5.	Amino acid	+	+	_
6.	Steroids	+	_	+
7.	Terpenoids	-	+	+
8.	Phenolic	+	-	+
9.	Tannins	-	+	+

-ve = negative (-); +ve = positive (+)

The ethanol extract exhibited the highest level of inhibition against *Staphylococcus aureus* compared to the chloroform and water extracts. The ethanol extract of *Pedalium murex* exhibits the strongest inhibition of *Bacillus subtilis*. Next, the chloroform and water are extracted (Table 2). The amount of inhibition is higher in the ethanol extract compared to the chloroform and water extracts as represented in Fig 2.

Table 1. Antibacterial activity of *Pedalium murex* leaves of various solvent extract

S.No.	Organisms	Solvents		
		Water	Chloroform	Ethanol
1.	*E.coli*	5	6	10
2.	*S. aureus*	6	8	11
3.	*B. subtilis*	8	7	10

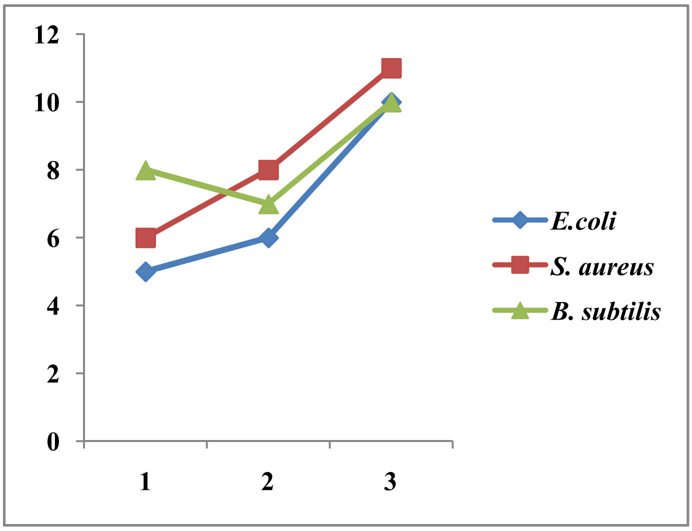

Fig 2. Graphical representation of antibacterial activity of *Pedalium murex* against microorganisms

A greater abundance of natural antibacterial substances has been documented in marine environments compared to terrestrial ones. Marine organisms, including marine algae, provide a valuable source of distinct natural compounds that possess pharmacological and biological activity (Karthikeyan et al., 2022). Macroalgae, often known as seaweeds, hold a unique position among marine creatures due to their potential as a source of medicinal chemicals (El-Beltagi et al., 2022; Leandro et al., 2020). Seaweeds have been identified as promising reservoirs of antibiotic compounds. The production of several metabolites from seaweed serves as evidence for the existence of antibacterial substances (Remya et al., 2022; Srinivasan et al., 2021). Various bioactive chemicals have been extracted from macroalgae, including antibacterial agents (Silva et al., 2020).

The issue of antibiotic resistance and the presence of multi-resistant bacteria provide a significant challenge in both clinical and public health settings since they are often resistant to treatment and can be extremely difficult, and in some cases, impossible to manage (Ventola, 2015). The current investigation involved the examination of plant extracts, which yielded the identification of phytochemicals that are recognized for their medicinal and physiological properties. For instance, tannins are polyphenolic chemicals that form complexes with proline-rich proteins, disrupting protein synthesis. Additionally, tannins have been demonstrated to possess antimicrobial properties.

Plants produce flavonoids, which are polyphenolic chemicals, in response to microbial infections. This aspect has been widely explored, and it has been established that flavonoids have antibacterial activity against a wide range of microbes in laboratory conditions (Roy et al., 2022). Their capability has been attributed to their interaction with extracellular soluble proteins and bacterial cell walls. Terpenoids, primarily valued for their aromatic properties, have also demonstrated potential as antibacterial agents. Saponins, a type of glycoside, have been discovered to possess inhibitory properties against gram-positive bacteria, specifically *S. aureus* (Górniak et al., 2019). The phytochemical study demonstrated that both the water and ethanolic extracts include chemical components with proven antibacterial properties, which could explain the results obtained from the antibacterial analysis.

The test organisms utilized in this investigation are linked to several manifestations of human illnesses. *Klebsiella pneumoniae* is the primary cause of neonatal nosocomial infection, as determined from a clinical perspective (Cruz & Cruz, 2021). *E. coli* is responsible for septicemias and can invade various parts of the body, such as the gall bladder, meninges, surgical wounds, skin lesions, and the lungs. This is particularly common in people who are weakened or have a compromised immune system (Peek et al., 2018). The prevalence of *Salmonella typhimurium* infection is a significant public health challenge in developing nations and remains a persistent worry for the food business (Popa & Papa, 2021).

The phytochemical research identified several bioactive chemicals in the *Pedalium murex* leaf extract that are responsible for its antibacterial properties against all kinds of bacteria. These compounds include carbohydrates, saponins, flavonoids, amino acids, steroids, phenolic compounds, alkaloids, terpenoids, and tannins. The discovery of antibiotic resistance in microorganisms has posed a significant threat to human health. Recently, this problem has become increasingly apparent. Given that the majority of organisms display varying levels of resistance to routinely accessible antibacterial and chemotherapeutic agents, In addition, there is a lack of access to potent medications that have not yet encountered antimicrobial resistance. These therapies are either unavailable or prohibitively expensive, making them unaffordable for the majority of people in developing countries, including India. Therefore, there is an urgent need for alternative treatments for various diseases. Given that other components demonstrate multifaceted pharmacological effects, it is likely that the leaf extract will also possess some of these capabilities in the future, which could be beneficial to humanity.

Conclusion

The current screening results demonstrated that the ethanol extract displayed the most potent antibacterial activity, whereas the chloroform and water extracts exhibited the least activity. The test organisms utilized in this investigation are linked to diverse manifestations of bacteria that are harmful to humans. The antibacterial activity of *Pedalium murex* against human infections was demonstrated to be effective.

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Conflict of interest

All authors declare that there is no conflict of interest in this work.

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