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Antibacterial Activity of *Costuspictus* Extracts Against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas*and *Staphylococcus* by Cup Diffusion Method

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Abstract

Costuspictus, also known as insulin plant, is a medicinal herb commonly used in traditional medicine for its potential to help manage blood sugar levels. The present study aims to investigate the antibacterial potential of different concentrations of*Costuspictus*extracts viz.,25%, 50%, 75% and 100%using different solvents viz.,Ethanol, methanol, petroleum ether, acetone and water against four prominent bacterial strains viz.,*Bacillus subtilis*, *Escherichia coli*,*Pseudomonas* and *Staphylococcus.*Ampicillin was used as standard. The extraction was carried out using the dry plant sample for 5hrs in five different solvents with increasing polarity. The extracts, in each solvent except water were obtained by using a soxhlet extractor. The water extract was obtained by using 50 g of the dry plant sample and mixing well in a blender. The extracts were then evaporated to dryness. The dry powder thus obtained was tested for antibacterial activity. 1mg of the dry sampleextract was resuspended in 1ml of the respective solvent and used as stock. The cup diffusion method was employed to assess Minimum Inhibitory Concentration and area of inhibitionon the growth of these bacteria. The study provides valuable insights into the potential application of *Costuspictus* as a natural antibacterial agent.

Keywords: Antibacterial activity, *Costuspictus, Bacillus subtilis, Escherichia coli*,*Pseudomonas*,*Staphylococcus*,Soxhlet extractor, Minimum Inhibitory Concentration

Introduction

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Pathogenic microorganisms are accountable for numerous human infections, and uncontrolled proliferation of these infectious agents can result in severe health complications.[1]To counter bacterial infections, antibiotics are employed. However, due to the growing resistance of bacterial strains to existing antibiotics,[2] the demand for novel antimicrobial solutions is ongoing. In this regard, plant extracts with established antimicrobial attributes hold substantial potential within contemporary therapy.[3] Given that antibiotics form the primary foundation for managing infectious ailments, there exists an amplified interest in exploring plants as a reservoir for human disease control.[4] The persistent development of bacterial resistance against antibiotics has prompted the quest for fresh and potent antimicrobial agents. Recently, significant attention has been directed towards identifying natural components from plant resources to replace synthetic alternatives. Natural compounds are deemed secure due to their presence in plant-based foods and are preferred over synthetic counterparts.[5]

Escherichia coli, commonly known as *E. Coli*, is a bacterium that can serve as a human pathogen. While many strains of *E. coli* are harmless and exist naturally in the human gut, certain pathogenic strains can cause various illnesses. These can range from mild gastrointestinal discomfort to severe infections, particularly in the urinary and digestive systems. Pathogenic *E. coli* strains often produce toxins that can lead to symptoms like diarrhoea, abdominal pain, and vomiting. Preventative measures include practicing proper hygiene, cooking food thoroughly, and avoiding consumption of contaminated water or undercooked meat.[6,7]*Staphylococcus* is a group of bacteria known to cause various human infections. The most notable pathogen is *Staphylococcus aureus*, which can lead to skin infections, abscesses, and even severe conditions like bloodstream infections and pneumonia. The bacterium's ability to develop antibiotic resistance poses a significant public health concern. Methicillin-resistant *Staphylococcus aureus* (MRSA) is particularly worrisome due to its resistance to many antibiotics.[8,9]*Pseudomonas* is a group of bacteria commonly found in soil and water. Some species, like *Pseudomonas aeruginosa*, can act as human pathogens. They exploit weakened immune systems and invade various body sites, causing infections like pneumonia, urinary tract infections, and sepsis. *Pseudomonas* infections are often challenging to treat due to their resistance to antibiotics and ability to form biofilms. They're particularly concerning in hospital settings, affecting immunocompromised individuals and patients with chronic conditions.[10,11]*Bacillus subtilis* is a Gram-positive bacterium known for its resilience and versatility. Commonly found in soil, it forms endospores that can survive harsh conditions. With a long history of safe use, it's employed in various industrial applications, including probiotics, enzymes, and antibiotics production. Its ability to secrete enzymes aids in breaking down organic matter. Researchers also utilize it as a model organism for studying cellular processes. *Bacillus subtilis* has a well-studied genetic system, making it useful for genetic engineering experiments. Overall, its robustness and diverse applications make it a valuable microorganism in both scientific research and industry.[12]

The escalating issue of antibiotic resistance necessitates the exploration of alternative antibacterial sources. *Costuspictus*, commonly known as insulin plant or spiral ginger, is a medicinal plant with significant potential in traditional medicine. Native to Southeast Asia, it has been used for its purported antidiabetic properties. The leaves of *Costuspictus* are the primary focus due to their observed ability to lower blood glucose levels. They contain compounds such as flavonoids, alkaloids, and terpenoids that contribute to its potential antidiabetic effects.Studies suggest that *Costuspictus* may have a role in improving insulin sensitivity, increasing glucose uptake, and reducing insulin resistance. However, while some research supports these claims, more rigorous scientific studies are needed to establish its efficacy and safety.[13,14] In the present study we subjected the *Costuspictus* leaves to solvent extraction for separating the components to the extent possible.We aimedto find out antibacterial activity of leaf extracts of *Costuspictus*and to compare antibacterial activity of ethanolic, methanolic, petroleum etheric, acetonic and aqueous extracts of *Costuspictus* on different human pathogenic bacteria.

Methodology

- Sample Collection and Preparation:

Escherichia coli, *Bacillus subtilis*, *Staphylococcus* and *Pseudomonas* were collected from the PG Department of Microbiology, Maharani's Science College for Women and maintained on nutrient agar in the culture room for studying antibacterialactivity of *Costuspictus* against these pathogens.

Fig 1: *Costuspictus* collected from Western ghats

The fresh*Costuspictus* leavescollected from Western ghats were shade dried and 50g of the dry leaf sample was used for each extraction. The extraction was carried out using the dry plant sample for 5hrs in five different solvents with increasing polarity. Ethanol, methanol, petroleum ether, acetone and aqueous extract of leaves of *Costuspictus* were used to study its antibacterial activity against human pathogenic bacteria viz., *Escherichia coli, Bacillus subtilis, Staphylococcus* and *Pseudomonas*. The extracts, in each solvent except water were obtained by using a soxhletextractor. The water extract was obtained by using 50 g of the dry plant sample and mixing well in a blender. The extracts were then evaporated to dryness. The dry powder thus obtained was tested for antibacterial activity. 1mg of the dry sample extract was resuspended in 1ml of the respective solvent and used as stock. Fromthisdilutions different concentrations viz., 25%, 50%, 75% and 100% were prepared and used to find out Minimum Inhibitory Concentration and area of inhibition.

- Antibacterial Assay

The antibacterial activity was studied using Mueller-Hinton Agarmedia (Hi-Media). It was prepared and autoclaved at 121° C and 15 lb for 20 minutes. All the glassware and other materials required were sterilized in an autoclave at 121° C and 15 lb for 20 minutes. All aseptic techniques were carried out in Laminar Air Flow bench. Study of Antibacterial activity was done

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by cup diffusion method (Perez et al., 1990).[15] The sterilized media was poured into the sterilized Petri plates. It was allowed to solidify at room temperature. 1000 µl of bacterial suspension was spread on the solidified medium using sterile glass spreader.Usingcork borer wells were made on the solidified media. Different dilutions of extracts was poured into the wells and incubated for 24hrs to 48hrs at 37° C. Ampicillin was used as standard. Respective solvents were used as control.

Results

The results demonstrated varying degrees of antibacterial activity against the tested strains. Methanolic extract showed inhibition at 25% for all the microbes studied. Maximum inhibition occurred at 100% against *Escherichia coli*. Ethanol and acetone extract also had inhibitory effect at 25%, but a significant inhibition was at 100%. Against *Pseudomonas*, ethanol, methanol and petroleum ether extract showed significant increase in the activity as the concentration was increased and at 100% maximum inhibhtion was observed. But acetone extract showed steady increase in the inhibitory activity. Ethanol extract was more effective against *Bacillus* followed by acetone extract. Against *Staphylococcus* ethanol extract showed maximum inhibition followed by methanol and acetone. The results showed that with the increase in concentration there is gradual increase in the diameter of zone of inhibition.

Table 1: Zone of inhibition exhibited by different extracts of *Costuspictus* against *E.coli*

Concentrations	25%	50%	75%	100%
Methanol	6 mm	7 mm	7 mm	8 mm
Ethanol	6 mm	6 mm	8 mm	13 mm
Petroleum ether	7 mm	8 mm	9 mm	9 mm
Acetone	6 mm	8 mm	9 mm	10 mm
Water	0 mm	0 mm	0 mm	0 mm

Table 2: Zone of inhibition exhibited by different extracts of *Costuspictus* against *Pseudomonas*

Concentration	25%	50%	75%	100%
Methanol	7 mm	8 mm	10 mm	11 mm

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Ethanol	9 mm	9 mm	10 mm	11 mm
Petroleum ether	6 mm	7 mm	7 mm	10 mm
Acetone	7 mm	7 mm	8 mm	8 mm
Water	0 mm	0 mm	0 mm	0 mm

Table 3: Zone of inhibition exhibited by different extracts of *Costuspictus* against *Bacillus subtilis*

Concentration	25%	50%	75%	100%
Methanol	5 mm	6 mm	6 mm	7 mm
Ethanol	6 mm	6 mm	8 mm	10 mm
Petroleum ether	6 mm	7 mm	7 mm	7 mm
Acetone	5 mm	6 mm	6 mm	9 mm
Water	0 mm	0 mm	0 mm	0 mm

Table 4: Zone of inhibition exhibited by different extracts of *Costuspictus* against *Staphylococcus*

Concentration	25%	50%	75%	100%
Methanol	6 mm	6 mm	8 mm	9 mm
Ethanol	7 mm	9 mm	9 mm	1.7
Petroleum ether	6 mm	6 mm	7 mm	7
Acetone	6 mm	7 mm	8 mm	9 mm
Water	0 mm	0 mm	0 mm	0 mm

Table 5: Antibacterial activity ofEthanol, methanol, petroleum ether, acetone, water and ampicillin against four prominent bacterial strains viz., *Bacillus subtilis, Escherichia coli, Pseudomonas* and *Staphylococcus.*

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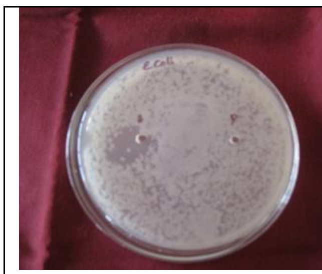
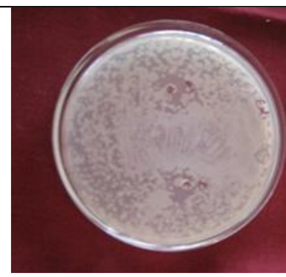
	Zone of inhibition (mm)			
Solvent	***Pseudomonas***	***Escherichia coli***	***Staphylococcus***	***Bacillus***
Methanol	3.5	0	3.5	3
Ethanol	3.5	4	4.5	3.5
Petroleum ether	0	0	4	0
Acetone	0	4.5	4.5	3.5
Water	0	0	0	0
ampicillin	18	26	24	19


Acetone and Petroleum ether


Ethanol and methanol


Water


Ampicillin

Fig 2: Antibacterial activity of positive and negative controls against *E. coli*


Acetone and Petroleum ether


Ethanol and methanol

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 Water	 Ampicillin

Fig 3: Antibacterial activity of positive and negative controls against *Pseudomonas*

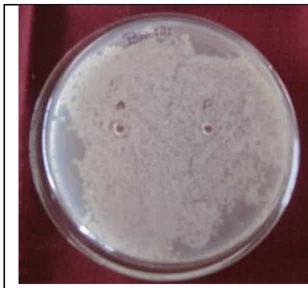
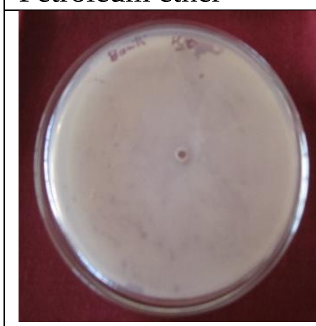
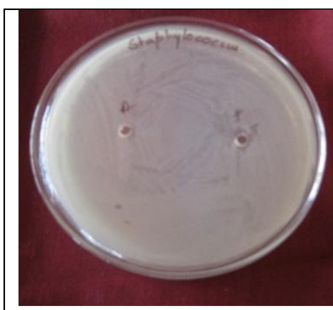
 Acetone and Petroleum ether	 Ethanol and methanol
 Water	 Ampicillin

Fig 4: Antibacterial activity of positive and negative controls against *Bacillus subtilis*

 Acetone and Petroleum ether	 Ethanol and methanol

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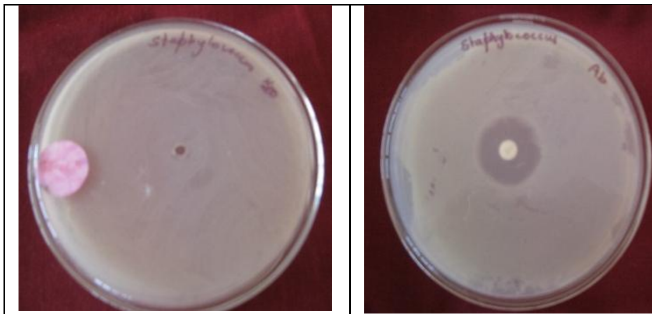
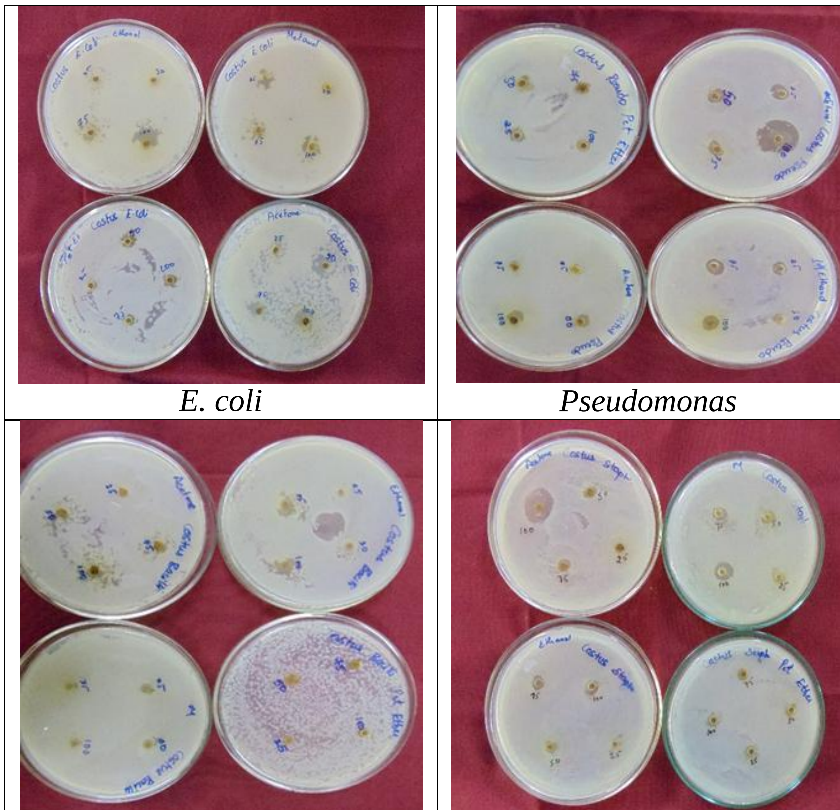


Water | Ampicillin

Fig 5: Antibacterial activity of positive and negative controls against *Staphylococcus*



E. coli | *Pseudomonas*

Bacillus subtilis | *Staphylococcus*

Fig 6: Antibacterial activity of different concentrations of ethanolic, methanolic, petroleum ether and acetonic extracts of *Costuspictus* against (a) *E. coli*, (b) *Pseudomonas*, (c) *Bacillus subtilis*, (d) *Staphylococcus*

Discussion

The methodology employed in this study involved the collection and preparation of bacterial strains and plant samples. Different solvents with increasing polarity were used to extract compounds from the *Costuspictus* leaves. The antibacterial activity was evaluated using the cup diffusion method, and the results indicated varying degrees of inhibition against the tested

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bacterial strains. Methanolic extract exhibited inhibition at 25% concentration for all microbes, with maximum inhibition against *Escherichia coli* at 100%. Ethanol and acetoneextracts also demonstrated inhibitory effects, with significant inhibition at 100%. *Pseudomonas* showed increased sensitivity with higher concentrations of ethanol, methanol, and petroleum ether extracts. Interestingly, acetone extract exhibited a steady increase in inhibitory activity. Ethanol extract was particularly effective against *Bacillus* and *Staphylococcus*, while acetone extract showed maximum inhibition against *Staphylococcus*. The study highlighted the correlation between concentration and the diameter of the zone of inhibition. These findings provide valuable insights into the antibacterial potential of *Costuspictus* extracts and underscore the importance of solvent choice in extracting bioactive compounds. This is the first report of antibacterial activity of ethanolic, methanolic, petroleum ether and acetone extract of *Costuspictus* against the bacteria studied. The plant can be used as a potent antibacterial agent. Karnataka ParisaraMahiti website has not included the plant, *Costuspictus* in its list of medicinal plants of Karnataka possessing antibacterial activity. Hence the plant can be included in the list.

Shaikh SS et al., had studied the Phytochemical, Histochemical and Antimicrobial properties of Various Solvent Extracts of *Costusspeciosus*(J. Koenig) Sm. and *Costuspictus* D. Don. Results of Phytochemical study revealed that alkaloids, flavonoids, quinones, and saponins were present in solvent extracts of both plants. Antibacterial study revealed thatinhibition zones caused by the methanol and hexane extracts showed better antibacterial activity compared to those of other extracts.[16] Similarly, in the present study Methanolic extract showed better antibacterial activity compared to other solvents. Ramya R et al., had studied the antibacterial activity of *Costuspictus* and the compounds responsible for it. Results revealed the antibacterial activity ofethanolic extracts of *Costuspictus* against*Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* and presence of active compounds such as phenols, flavonoids, tannins, terpenoids, alkaloid , steroids, glycosides and saponin in the ethanol leaf extract of *Costus pictus*.[17] Similar to that study, in the present study strong antibacterial activity of different extracts of *Costuspictus*against *Bacillus subtilis, Escherichia coli, Pseudomonas*and *Staphylococcus* was observed. From the study of Raj and Kalaivaniet al.,antibacterial activity ofaqueous leaf extract of *Costuspictus* D. Don was revealed.[18]Similar to that study, in the present study strong antibacterial activity of different extracts of *Costuspictus* against *Bacillus subtilis, Escherichia coli, Pseudomonas*and *Staphylococcus* was observed.

Conclusion

The present study revealed the antibacterial activity of ethanolic, methanolic, petroleum etheric, acetonicand aqueous extracts of *Costuspictus* on different human pathogenic bacteria viz., *Bacillus subtilis, Escherichia coli, Pseudomonas*and *Staphylococcus*. These findings provide valuable insights into the antibacterial potential of *Costuspictus* extracts and underscore the importance of solvent choice in extracting bioactive compounds. This is the first report of antibacterial activity of ethanolic, methanolic, petroleum ether and acetone extract of *Costuspictus* against the bacteria studied. The plant can be used as a potent antibacterial agent. The Further studies are required to isolate and identify the specific bioactive compounds responsible for the observed antibacterial effects. In vivo studies are essential to validate the safety and efficacy of *Costuspictus*extracts for potential therapeutic applications.

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