

Antimicrobial Activity of Papay (Vasconcellea Pubescens), Custard Apple (Annona Squamosa) and Tulsi (Ocimum Sanctum Linn.) Leaf Extract Against E. Coli and Pseudomonas

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Abstract: *Papaya, Custard apple and Tulsi plant have many restorative properties. The leaf extract of this plant seems to have incredible antibacterial, antiviral, and anti-inflammatory activity because it contains dynamic components such as Alkaloids, Glycosides, Tannins, Saponins, Flavonoids, Steroid compounds. Both E. Coli and Pseudomonas are gram positive and Gram-negative bacteria. They can cause Serious food poisoning and effect on human health and animals. Each Plant extract was prepared with the help of Soxhlet apparatus. Pouring method used for inoculation and well diffusion agar method was used to determine the antibacterial activity of the plant extract. New specific pathogens were plated on Mueller Hinton agar plates. The proximity of a clear restriction zone around Pseudomonas and E. coli indicates that they are valuable as antimicrobial agents. Finding of the result on E.coli all three leaves extract papaya leaves (1.4 cm.) tulsi leaves extract (2.5 cm.) and custard leaves (1.8 cm.) diameter of zones was visible and in the case of pseudomonas papaya leaves extract shows negative effects. Tulsi and custard apple shows antimicrobial activity on pseudomonas and clear zone is visible.*

Keywords: Papaya leaf extract, Custard apple leaf extract, Tulsi leaf extract, Pseudomonas, E. coli

1. Introduction

Papaya, Custard apple and Tulsi are well known medicinal plant they have many medicinal properties against bacteria and virus. The extract of papaya leaves, tulsi and custard apple (*Annona squamosa*) has excellent antibacterial, antiviral and anti-inflammatory effects because it contains many active components such as alkaloids, glycosides, tannins, saponins, flavonoids, steroid compounds. Anshu Sharma et al 2022 and Dr Kumar 2023 identified more than fifty bioactive components in papaya leaves that have been used to strengthen the immune system. According to Manoj Kumar et al. 2021 the custard apple is also used as a primary treatment for cancer and tumours, and the leaves are used as a popular dietary supplement to control diabetes and improve sleep cycles. Previous studies show that custard apple leaf extract has many antimicrobial and antifungal properties. The tulsi plant has been known for its many medicinal properties for thousands of years. Tulsi leaves, used in many forms of Ayurvedic medicine, can be useful in treating colds, headaches, stomach disorders etc.

Escherichia coli and *Pseudomonas* (*Pseudomonas aeruginosa*) are two gram-negative bacteria. *E. coli* is usually found in the intestines. Some strains are harmless, but some strains are serotyped. The serotype can grow rapidly at 7°C to 50°C and in acidic environments and is responsible for food

poisoning and food contamination. *Pseudomonas* is another Gram-negative bacterium that can cause serious disease in humans and animals. They damaged tissues and weakened the immunity of infected animals. The aim of this research is to develop value-added products and their use as health-promoting substances.

2. Materials and Methods

2.1 Collection of plant material

In January 2024, to detect Antimicrobial activity of three different plants the leaves of Tulsi, custard apple, and papaya were gathered from areas close to Jabalpur city. Before being used, the leaves were gathered in a sterile plastic bag, cleaned, sun-dried, and pulverised.

2.2 Preparation of Plant Extract

A Soxhlet device was used to prepare each plant extract. The extraction thimble is filled with a sample weighing ten grammes. Use 150 millilitres of 99.9% ethanol as the distillation flask's solvent. The device has a temperature of 80 °C. We receive 90 ml of leaf extract after 6 hours. The leaf extract should be kept in an Erlenmeyer flask. For the Tulsi and Custard apple leaf extract, repeat these steps.



(A) All three plant Extract—Papaya, Tulsi and Custard apple
(B) Culture used

2.3 Culture used

Both microorganism *Pseudomonas* and *E. coli* was used for this study. Both cultures were obtained from stock culture of the Department of Microbiology of St. Aloysius' College Jabalpur.

2.4 Inoculation and pouring

For determination of antimicrobial activity of these plant extract pour plate technique and the agar well diffusion method were used for inoculation. After inoculation various plant extracts was poured on to the plates using a micropipette. We observed the antibacterial activity of all three samples at different time intervals of 24 hours, 36 hours and 78 hours.

3. Result and Discussion

3.1 Antibacterial effect of papaya leaves on *E. coli* and *pseudomonas*

In each agar plate both pathogens poured on the surface and 2ml plant extract were filled on the well of different plates. After pouring all plates was incubated at 37°C for 24 hours. We observe the positive effect of papaya leaves on *E. coli*. The papaya leaves extract shows antibacterial effect on *E. coli* and clear zone is visible (1.4 cm.) but in the case of *pseudomonas* zone of inhibition is not visible.

S. No.	Pathogens	Plant Extract	Zone of Inhibition
1	<i>E. coli</i>	Papaya leaves extract (2ml)	Zone Visible (1.4cm.)
2	<i>Pseudomonas</i>		Zone not Visible
3	Antibiotic (Streptomycin)	Clear Zone	Clear Zone



(a) Antibacterial effect on *E. coli*



(b) Antibacterial effect on *Pseudomonas*

3.3 Antibacterial activity of Custard apple against *E. coli*

Custard apple demonstrates remarkable antimicrobial properties against both pathogens. The influence of Custard apple on *E. coli* was observed at 24-hour intervals, and the final diameter was recorded after 72 hours of incubation. The width of the inhibition zone surrounding each disk was measured at 1.8 cm. The diameter of the inhibition zone around each disk was also noted as 1.8 cm.

Table 2

S. No.		Sample A	Sample B
1	Media	Mueller Hinton Agar	Mueller Hinton Agar
2	Culture used	<i>E. coli</i>	<i>Pseudomonas</i>
3	Extract used	2ml	2ml
4	Zone of Inhibition	Zone visible (1.5cm.)	Zone visible (1.8 cm.)
5	Antibiotic (Streptomycin)	Clear Zone	Clear Zone



Effect of custard apple on Sample-A (*E. coli*)

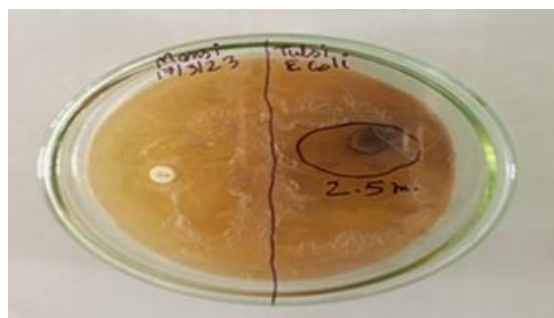


Effect of custard apple on Sample B (Pseudomonas)

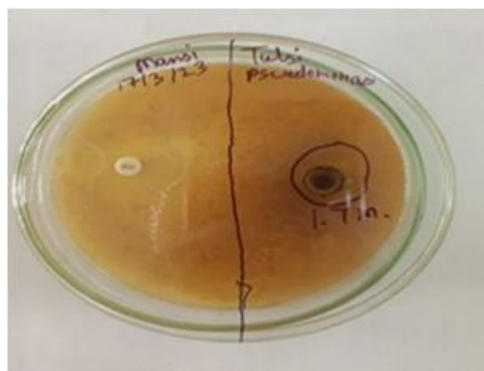
Tulsi leaves extract in E Coli and pseudomonas- We have shown that the extract of Tulsi leaves has a good effect on E. Coli bacteria, and a clear zone is visible in the other three plates.

Table-3

S. No.		Sample A-	Sample B
1.	Media	Mueller Hinton Agar	Mueller Hinton Agar
2.	Culture used	E.coli	Pseudomonas
3.	Extract used	2ml	2ml
4.	Zone of Inhibition	Zone visible (2.5c.m.)	Zone visible (1.7 cm.)
5.	Antibiotic (Streptomycin)	Clear Zone	Clear Zone



Antimicrobial activity of Tulsi on E. coli



Antimicrobial activity of Tulsi on pseudomonas

4. Discussion

The results of the current study indicate that extracts from papaya, custard apples, and Tulsi leaves exhibit significant antimicrobial properties against the tested pathogens. Notably, custard apple leaves and Tulsi demonstrated a positive effect on Pseudomonas, while the papaya extract was

unable to inhibit the growth of Pseudomonas. The presence of a clear zone of inhibition surrounding the E. coli and Pseudomonas plates suggests that these leaf extracts may serve as effective antibacterial agents. Nureen Zahra and Farheen Ansari reported an inhibitory effect of C. papaya, noting a 1.4 cm inhibition zone against E. coli. Furthermore, research by Manoj and Sushil Changan in 2021 highlighted that custard apples possess antimicrobial activity attributed to the active compound acetogenin. Acetogenin contains an active compound that interacts with both gram-negative bacteria, specifically Pseudomonas and E. coli, with the most significant zone of inhibition observed in Pseudomonas at 1.8 cm, compared to 1.5 cm for E. coli. Research conducted by Devi et al. indicates that the extract of Ocimum sanctum leaves serves as a potent antimicrobial agent, demonstrating considerable efficacy against both gram-positive and gram-negative bacteria. Furthermore, Singh et al. proposed that the elevated levels of linoleic acid present in the fixed oil of Ocimum sanctum Leaves may enhance its antibacterial properties

5. Conclusion

It can be inferred from the entire experiment that these leaves possess antibacterial properties. All of the leaf [ethanol] extracts used in this study showed the strongest antibacterial action against the two main pathogens, E. Coli and pseudomonas, which are gram-negative bacteria. The extract from tulsi leaves in our study has the strongest inhibitory impact on pseudomonas (1.8 cm) and E. coli (2.7 cm). Papaya leaf extract has strong therapeutic qualities, including antibacterial, antiviral, anticancer, hypoglycaemic, and anti-inflammatory action, according to the main findings. Present study indicate that these leaves extracts can be used as a source of antimicrobial compound for drug development, and it is suggested that tulsi leaves extract used as a coating material for fruits and vegetables for control of some bacterial and fungal spoilage.

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