

## Antibacterial properties of Unripened Fruits of *Trapa natans*, *Annona squamosa*, *Ficus carica* and *Anthocephalus cadamba* against Pathogenic Gram Positive and Negative Bacteria.

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### ABSTRACT

4 gm. of the unripened ground fruits of *Ficus carica*, *Trapa natans*, *Annona squamosa* and *Anthocephalus cadamba* were weighed and homogenized with 40 ml of acetone, 70% ethanol, 80% methanol, and ethyl acetate separately in bottles. These plant extracts were evaluated for antimicrobial activity using the disk diffusion method on three reference microorganisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) which were used for antibiogram analysis of different fruits extracts.

**Keywords;** Antimicrobial activity, unripened fruits, *Ficus carica*, *Trapa natans*, *Annona squamosa*, *Anthocephalus cadamba*; acetone, 70% ethanol, 80% methanol, ethyl acetate crude extracts.

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### I. INTRODUCTION

Different types of bioactive molecules are produced by plants and these molecules are used to make different types of medicines (Sukanya J. et al., 2009)<sup>1</sup>. These bioactive molecules are phytochemicals. So, the term Phytochemical is often used to describe a diverse range of biologically active compounds found in plants. Phytochemicals are responsible for plants colour, flavour and natural protection against pest (Chede, 2013)<sup>2</sup>. Plant produce these chemicals to protect themselves but research demonstrate that they can also protect humans against diseases. Phytochemicals are not only necessary for our life but it provides the extra health benefits against pathogens. (Mathew et al., 2012)<sup>3</sup> for decreasing morbidity, mortality and health costs that are increasing because of the infectious diseases that are caused by pathogens.(Kirankumar Shiva Sharanappa et al.,2013)<sup>4</sup>. On the basis of their functions in plant metabolism, phytochemicals are divided into two groups i.e. Primary constituents and Secondary constituents. Primary constituents include common sugars, amino acids, proteins and chlorophyll while secondary constituents include alkaloids, terpenoids, steroids and flavonoids, etc. The present study revealed the antibiotic property of four fruits that are commonly not used by people of UP (India) to cure various diseases. (Thilagavathi et al., 2015)<sup>5</sup>.

Especially alkaloids (phenolic compounds) are responsible for antimicrobial activities, this is revealed by phytochemical analysis. Two types of antifungal compounds are exists in the plants. Those constitutive compounds that are in high concentration responsible for fungal infections are known as Phytoalexins (Grayer R. J. et.al.,1994)<sup>6</sup>. Phytochemicals are produced from roots, leaves, stem, flowers and fruits. Especially fruits are truly nature's great gift because they provide many nutrients and antibiotic property that essential for health and maintenance of our bodies. Now a days very few people are like to eat some wild fruits like custard apple, water caltrop, anjir, cadamba etc. Custard apple also known as Seetaphal in India, is a subtropical fruit. Water caltrop is full of great health benefit that many people don't know. It is also known as 'Singhara, an aquatic plant. In the journal of medical sciences it was found that water caltrop have a powerful antibacterial capability, thus healing the bacterial infections (Oikeh E.I. et.al.,2013)<sup>7</sup>. *Ficus carica* belongs to the family Moraceae, commonly known as 'Anjir' in India. *Ficus* have antibiotic, antipyretic, antibacterial and antifungal property. The alcoholic and aqueous extracts of cadamba (*Anthocephalus cadamba*) fruits have shown significantly higher antibacterial activity against microorganisms (Duraisamy.al.,2016)<sup>8</sup>. At present time so many

diseases are very harmful for us and for treating that disease we use medicines that are made by different-different chemicals on the basis of natural composition of antibiotic retrieve from bacteria and from different plants. These chemicals affect our other organs and their more number of side effects on our body. To get rid of these chemicals made medicines we can eat some fruits that have medicinal property and are commonly not eaten by people, which protect all these harmful diseases by only eating these fruits.

## II. METHODS AND MATERIALS:

### 2.1 Pathogenic Culture:

First of all 15 ml nutrient broth (NB) was prepared and transferred equal amount of NB in three test tubes. The bacterial pathogens *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* were cultured in this broth for antibiogram analysis of different fruits extracts.

### 2.2 Collection of fruits:

The fruits were collected from different areas of Lucknow district.

### 2.3 Preparation of fruit extract:

4 gms of the unripened ground fruits each of *Ficus carica* and *Trapa natans* were weighed and homogenized with 40 ml of acetone, 70% ethanol, 80% methanol, and ethyl acetate separately in bottles and were kept in dark at room temperature for 48 hours. Similarly, chopped unripened fruits of *Annona squamosa* and *Anthocephalus cadamba* were dissolved into above solvents and transferred these in a separate bottles and kept in dark at room temperature for 48 hours. Cover these bottles with a foil to protect the filtrate from dust and dry at 50°C in hot air oven. After drying the filtrate weighed the

bottles again. For dissolving these extracts, DMSO (act as preservative) is used.

### 2.4 Antibiogram analysis of fruit extracts:

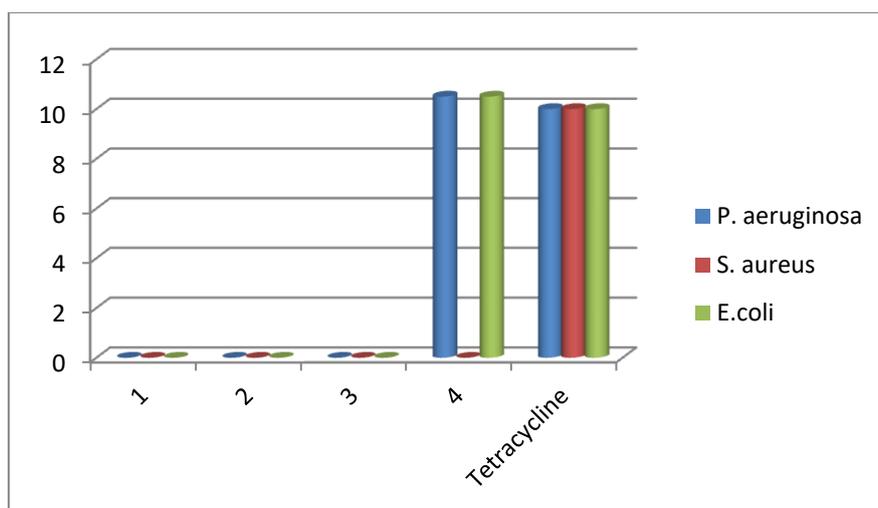
Agar Well diffusion method was used for the antimicrobial screening of the plant extracts against the test pathogens. Autoclaved (sterile) nutrient agar media was prepared and poured into sterile petriplates. Pathogenic culture was then spread on to the plates labelled as *Staphylococcus aureus* (sa), *Escherichia coli* (Ec), *Pseudomonas aeruginosa* (Pa). After 3 to 4 minutes of spreading, 4-5 wells of 8mm diameter were bored using a sterile borer. In 2 wells, 50 µl of positive control i.e. antibiotic (tetracycline 1000 µl/ml) while in other wells 50µl of each plant extract is taken separately. Bacterial plates were incubated at 37°C overnight. 1000µl/ml of tetracycline was used as standard antibiotic through out the study.

## III. RESULT AND DISCUSSION

The unripened fruit extracts of plant under study in different solvents were tested against three different pathogens showed various degree of antimicrobial activities. The Ethyl acetate extract of *Trapa natans* showed maximum sensitivity and the zone of inhibition 10.5 mm. against *P. aeruginosa* and *E. Coli*. 80% Methanol & Ethyl acetate extract of *Annona squamosa* showed maximum sensitivity and zone of inhibition 11 & 11.5 mm. respectively against all the three bacteria. Acetone & 80% of Methanol extract of *Anthocephalus cadamba* showed maximum sensitivity and zone of inhibition 13.5 mm & 12 mm respectively against *S. aureus* (shown in Table 1, 2 and 4). All extract of *Ficus carica* did not showed any zone of inhibition against *P. aeruginosa*, *E.coli*, and *S. aureus* (Table 3).

Table 1: - Analysis of antibacterial properties of unripened fruit extracts of *Trapa natans*

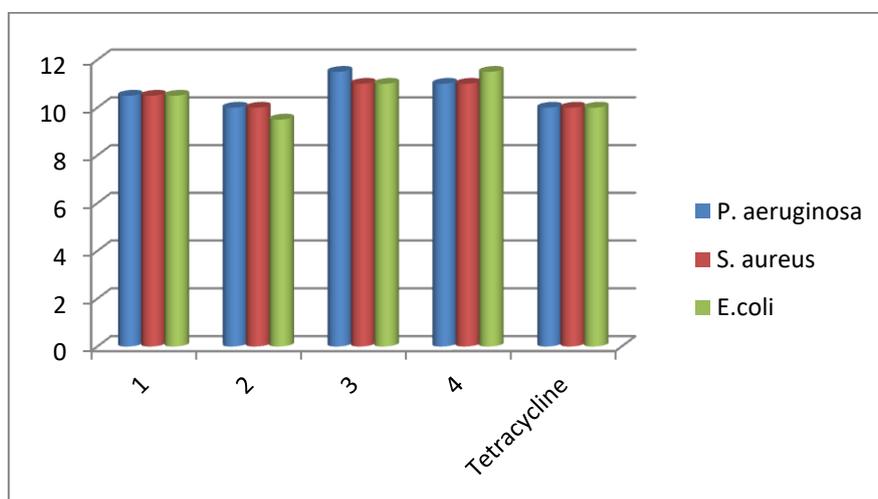
Pathogens	1. Acetone extract (50 mg/ml)	2. 70% ethanol extract (50 mg/ml)	3. 80% Methanol extract (50 mg/ml)	4. Ethyl acetate extract (50 mg/ml)	Tetracycline (1mg/ml)
<i>P.aeruginosa</i>	-	-	-	10.5	10
<i>S. aureus</i>	-	-	-	-	10
<i>E.coli</i>	-	-	-	10.5	10



**Graph 1:** - Above graph shown the result of antibacterial properties of unripened fruit extracts of *Trapa natans* (1 is Acetone extract, 2 is 70% ethanol extract, 3 is 80% Methanol extract and 4 is Ethyl acetate)

**Table 2:** - Analysis of antibacterial properties of unripened fruit extracts of *Annona squamosa*

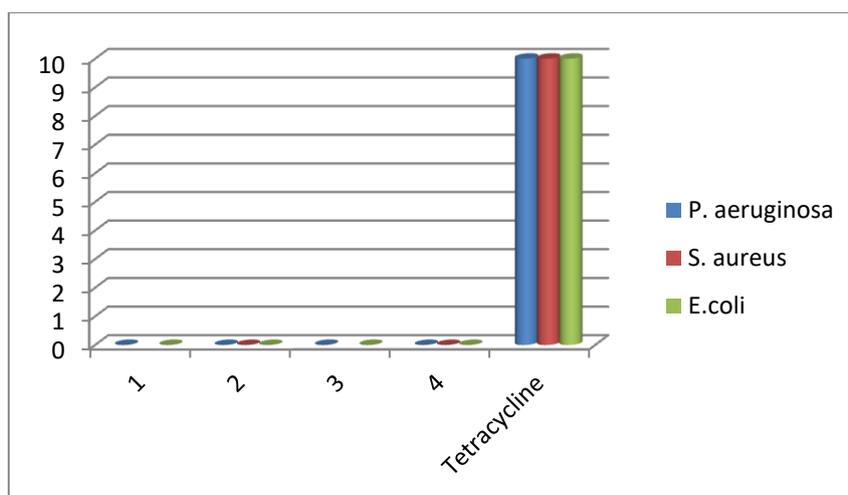
Pathogens	1. Acetone extract (50 mg/ml)	2. 70% ethanol extract (50 mg/ml)	3. 80% Methanol extract (50 mg/ml)	4. Ethyl acetate extract (50 mg/ml)	Tetracycline (1mg/ml)
<i>P. aeruginosa</i>	10.5	10	11.5	11	10
<i>S. aureus</i>	10.5	10	11	11	10
<i>E. coli</i>	10.5	9.5	11	11.5	10



**Graph 2:** - Above graph shown the result of antibacterial properties of unripened fruit extracts of *Annona squamosa* (1 is Acetone extract, 2 is 70% ethanol extract, 3 is 80% Methanol extract and 4 is Ethyl acetate)

**Table 3:** - Analysis of antibacterial properties of unripened fruit extracts of *Ficus carica*

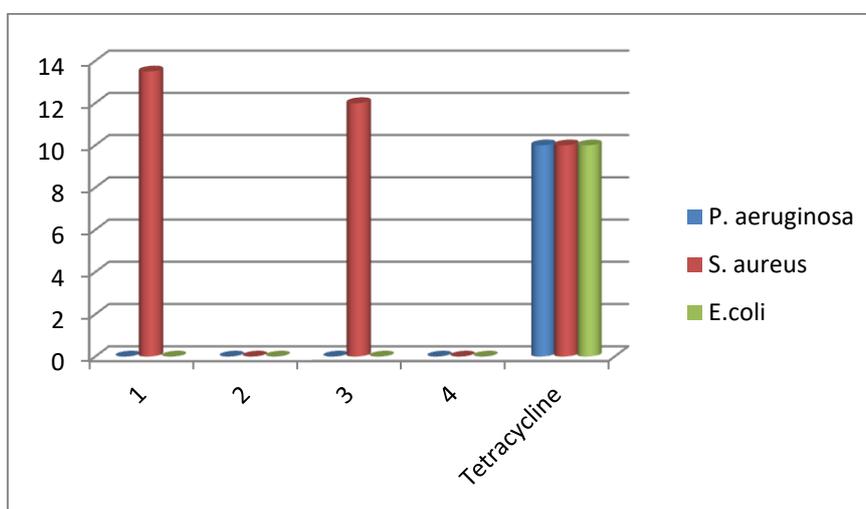
Pathogens	1. Acetone extract (50 mg/ml)	2. 70% ethanol extract (50 mg/ml)	3. 80% Methanol extract (50 mg/ml)	4. Ethyl acetate extract (50 mg/ml)	Tetracycline (1mg/ml)
<i>P. aeruginosa</i>	-	-	-	-	10
<i>S. aureus</i>	-	-	-	-	10
<i>E. coli</i>	-	-	-	-	10



**Graph 3:** - Above graph shown the result of antibacterial properties of unripened fruit extracts of *Ficus carica* (1 is Acetone extract, 2 is & 70% ethanol extract, 3 is 80%Methanol extract and 4 is Ethyl acetate)

**Table 4:** - Analysis of antibacterial properties of unripened fruit extracts of *Anthocephalus cadamba*

Pathogens	1. Acetone extract (50 mg/ml)	2. 70% ethanol extract (50 mg/ml)	3. 80% Methanol extract (50 mg/ml)	4. Ethyl acetate extract (50 mg/ml)	Tetracycline (1mg/ml)
<i>P. aeruginosa</i>	-	-	-	-	10
<i>S. aureus</i>	13.5	-	12	-	10
<i>E. coli</i>	-	-	-	-	10



**Graph 4:** - Above graph shown the result of antibacterial properties of unripened fruit extracts of *Anthocephalus cadamba* (1 is Acetone extract, 2 is 70% ethanol extract, 3 is 80%Methanol extract and 4 is Ethyl acetate)

#### IV. CONCLUSION

In this study, we found that unripened fruit also contain antibacterial properties. The unripened fruit extracts were extracted by solvent extraction method with the help of four solvents Acetone, Ethanol, Methanol and Ethyl acetate. The result showed that ethyl acetate, acetone and methanol extract of unripened fruits of *Trapa natans*, *Anthocephalus cadamba* and *Annona squamosa*

had sensitivity against *P. aeruginosa*, *E. coli* and *S. aureus* respectively. Unripened fruit of *Annona squamosa* and *Anthocephalus cadamba* are shown the better result against these bacteria.

## REFERENCES

- [1]. Sukanya et.al, Antimicrobial Activity of Leaf Extracts of Indian Medicinal Plants against Clinical and Phytopathogenic bacteria. *African journal of biotechnology*. 8(23), 2009, 6677-6682.
- [2]. Chede et.al, Phytochemical analysis of Citrus sinensis peel. *International Journal of Pharma and Biosciences*, 4(1), 2013, 339-343.
- [3]. Mathew B. B., Jatawa S. K., and Tiwaari A. Phytochemical analysis of Citrus limonum pulp and peel. *Int. J. Pharm. Pharm. Sci.* 4, 2, 2012, 69–371.
- [4]. Kirankumar Shivsharanappa et.al, Phytochemical Screening and Antimicrobial Activities of Ficus Glomerata Roxb fruit extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*. Vol.5, 2013, 0975-1491.
- [5]. Thilagavatiet.al, Preliminary Phytochemical Analysis of Screening of Different Solvents Mediated Medicinal Plant Extracts Evaluated. *International Research Journal of Pharmacy*. Vol. 6(4), 2015, 2230-8407.
- [6]. Grayer R. J. et.al, A survey of antifungal compounds from higher plants. *Photochemistry* . 37, 1994, 19-42.
- [7]. Oikeh E.I. et.al, Proximate Analysis and Citrus sinensis Fruit wastes. *The Bioscientist* .vol. 1(2), 2013, 164-170.
- [8]. DuraisamyKayaVizhiet.al, In vitro antimicrobial activity and Phytochemical analysis of fruits of Syzygium aromaticum (L.) Merr. & L.M. Perry – *An important medicinal plant*. 2016, 2230-480x.

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