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# Antimicrobial Activity of Water Chestnut Peels against Food Borne Pathogens

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**Abstract—***Water chestnuts (WCN) (Trapa natans), is a plant, mostly found in shallow lake, ponds and river banks. It bears a starchy fruit. The objective of this study is to investigate the peel of this fruit for antibiogram analysis against food borne pathogens. In general the peels are considered as garbage material in the societies but they can be a good source of antimicrobials. The pathogens used for the study are Bacillus subtilis (BS), Klepsiella planticola (KP), Micrococcus luteus (ML), Staphylococcus aureus (SA), Escherichia coli (EC), Pseudomonas aeroginosa (PA). The solvents used were ethanol, methanol, chloroform and water. The activity against pathogens was shown by water, ethanol, and methanol. The in vitro activity was performed by using Agar well diffusion method. The ethanolic extract of 50mg/ml was most effective against Pseudomonas aeroginosa and Micrococcus luteus (29mm, 29mm) respectively while the 100mg/ concentration showed highest activity against Staphylococcus aureus (28mm). The methanolic extracts of 50mg/ml were most effective against Micrococcus luteus, Pseudomonas aeroginosa, Klepsiella planticola (29mm, 28mm, and 25 mm). The 100 mg/ml concentration of methanolic extract was effective against Pseudomonas aeroginosa, Staphylococcus aureus, and Escherichia coli (29mm, 29mm, and 25 mm). The water extracts were more effective than other extracts and their activity was most effective against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Micrococcus luteus, Klepsiella planticola,, Pseudomonas aeroginosa (29mm, 23mm, 23mm, 22mm, 25mm, 26 mm ) respectively with concentration 50mg/ml. The chloroform extract was ineffective against all microbes. By this study it can be concluded that peels can be a novel source of antimicrobials and can be used as phytomedicine.*

**Keywords***: Antimicrobial activity, Trapa natans peels, Ethanolic extract, Methanolic extract, Water extract, Agar well diffusion.*

## 1. INTRODUCTION

The use of antibiotics to treat diseases that are caused due to food pathogens has initiated a rapid development and evaluation of antibiotic resistance of pathogens in human [6]. An antimicrobial is a substance that inhibits the growth of microorganisms or kills it. Microorganisms are inhibited by two mode of action i.e. Microcidal and microbistatic , Microcidal means killing of bacteria while the latter one means inhibiting the growth of microbes by ceasing the metabolic activities [4]. Nowadays the microbes are becoming resistant to the synthetic antibiotics especially food borne pathogens of animal origin. Certain classes of bacteria like enterococci have shown resistance to antibiotic vancomycin [5]. The plants and its metabolic products can be used to treat and cure the diseases caused by pathogens by killing or inhibiting the growth of microbes. The plants consist of secondary metabolites or sometimes produce them as a defense mechanism against pathogen. These metabolites act as antimicrobials for the enemy that can be a microbe or any other plant. Every part of plant consists of these antimicrobial agents whether it is a stem, root or fruit. This paper focuses on fruit peels as a novel source of natural antibiotic that shows antimicrobial activity against food borne pathogens.

## 2. HOW PATHOGENS BECOME DRUG RESISTANT?

Three mechanisms are considered to be most occurred in microbes that make them resistant to drugs:

1. Inactivation of antimicrobial agent by enzymes present in microbes
2. Modification or some changes in the target site that reduces the ability of drug to bind to the target site.
3. Barriers at the membrane blocks or reduces the entry of antimicrobial agents [7, 8].

## 3. PEELS CAN BE CONSIDERED AS A NOVEL SOURCE OF ANTIMICROBIAL AGENT

Number of researches on peels of fruits and vegetables shows that peels is rich source of antimicrobial agents. Most of the peels of fruits and vegetables are thrown in the garbage. These peels can be used to produce antimicrobics that can be used to inhibit the growth of microorganisms. These waste products of plants were used as fertilizers till date but using them as a novel source of phyto medicine is gaining popularity. They are economically very attractive and useful is eco-friendly and have the capability to produce antimicrobial agents that prevent the growth of pathogens which cause diseases. This investigation focuses on the antimicrobial activity of four different fruit peels that are commonly available in India and

their results indicate that these peels can be used in pharmaceutical industries.

## 4. MATERIAL AND METHODS

Collection and Processing of Sample: The fruits were collected from the local market of Lucknow (U.P.). The fruits were washed properly in running tap water and were rinsed two times with distilled water. The fruits were peeled off and the peels were sun- dried separately for 5 days. After 5 days the peels were grinded in a mixer to make a fine powder that can be used for preparing extract.

## 5. PREPARATION OF EXTRACT

The extract was prepared in four different solvents viz. ethanol, methanol, water and chloroform separately. 5g of powdered peel was mixed with the solvent (50ml) in a conical flask and the flasks were kept in rotary shaker for 72 hr at room temperature. After 72 h the extract was filtered using moist muslin cloth. The residue obtained was reextracted and kept on shaker for 24 h [1]. The supernatant obtained was dried in water bath and the residue was stored in Dimethyl sulphoxide (DMSO) for further activity.

## 6. MICROBIAL STRAIN

The Microbes used were food pathogens that are responsible for causing diseases. 6 strains were used namely: Bacillus subtilis, Klebsiella planticola, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa.

## 7. MICROBIAL CULTURE MEDIA

Bacterial strains were first inoculated in nutrient broth for 37ºC for 24h. Cultures were then spread on solid media nutrient agar.

The composition of nutrient agar media for 100 ml is:

- Peptone- 0.5g
- Yeast extract-1g
- Sodium chloride- 0.5g
- Agar- 2g

This composition was followed throughout the research.

## 8. ANTIBACTERIAL ACTIVITY ASSAY

The method used to determine the antibacterial activity was agar well diffusion technique [2]. The microbe to be tested was inoculated (20µl) on sterile plates of solid media and was spread using spreader. Four wells on each plate were made using sterile borer (8mm) and 50µl of the prepared extract was filled in the well using micropipette of 50µl. The concentration of the extract was 100mg/ml and 50mg/ml respectively done on separate plate. Controls were prepared using solid media plates containing microbes and well containing DMSO. The plates were incubated at 37ºC for 24h and next day the zone of inhibition was calculated in millimeters (mm) [3].

## 9. RESULT AND DISCUSSION

The concentration of 50mg/ml of all the extracts except chloroform showed antibacterial activity against all the food pathogens tested. The 50mg/ml concentration of ethanolic extract showed best results against all pathogens but the mostly inhibited pathogen was *Pseudomonas aeroginosa* (29mm), *Micrococcus luteus* (29mm) *Bacillus subtitils* (28 mm), BS respectively. While the 100mg/ml concentration of ethanolic extract was most effective against *Staphylococcus aureus* (28mm). The methanolic extract with 50mg/ml concentration showed maximum activity against ML (29mm) and showed least activity against *Klebseilla planticola* (14 mm). The 100mg/ml concentration of methanolic extract showed maximum activity against *Pseudomonas aeroginosa* (29 mm) and *Staphylococcus aureus* (28mm). The Water extract with 50mg/ml concentration was effective against all microorganisms *Staphylococcus aureus* (29mm), *Pseudomonas aeroginosa* (26 mm) while the 100mg/ml concentration showed maximum activity against *Micrococcus luteus* (28 mm). The chloroform extract of WCN was not all effective against any microbe. The maximum activity compared to all the extracts was found to be of ethanolic extract of WCN. It shows that these peels good source of antimicrobial agents. These peels are easy to obtain as they are thrown as garbage and are very cost effective and can be a cheap alternative to antibiotics. These peels can be considered as a novel source and can be effectively used as phytomedicine. The results reported by Buragohaein et al, 2014 showed maximum result of zone of inhibition of ethanolic extract of WCN peel against *M. smegmatis* [11]. The water extracts of peel of their research did not show any results. Parekh et al reported that their best antimicrobial activity of water chest nut fruit of two different varieties were with extract of 1, 4 dioxan and least effective extract was petroleum ether [11]. Razvy in 2011 reported the antimicrobial activity of two different varieties of with 600g of sample fruit and the result showed that the methanolic extract was most effective against *Bacillus subtilis(*31 mm*)* and green variety was effective against *Staphylococcus aureus* and *Shigella sonnei* [9]. .

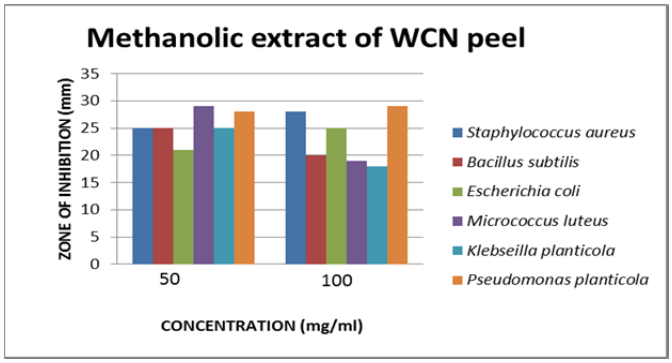


**Fig. 1: Zone of inhibition of methanolic extract of WCN peel**

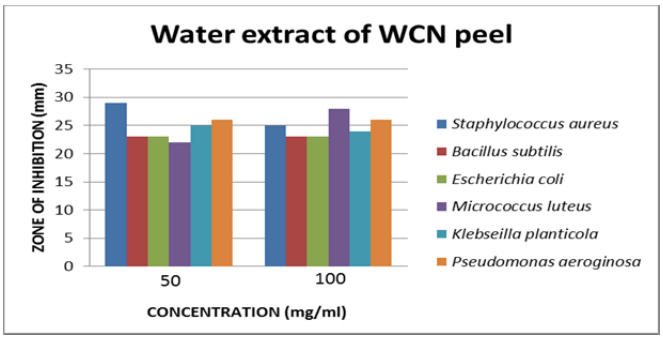


**Fig. 2: Zone of inhibition of Water extract of WCN peel**

**Table 1: Zone of inhibition**

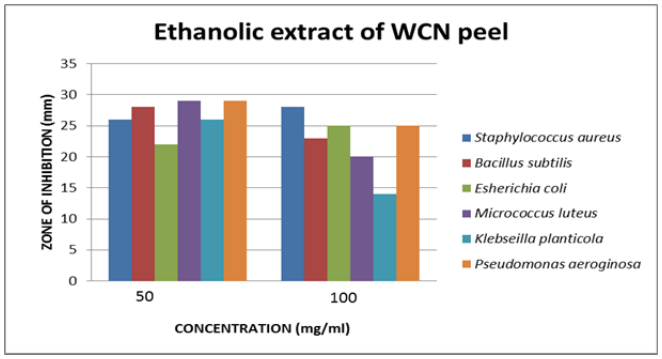


**Fig. 3: Zone of inhibiotion of Ethanolic extract of WCN peel**

| Extract Type(Solvent) | Extract Concentration (mg/ml) | Zone of Inhibtion (in mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Microorganisms | | | | | |
| | | SA | BS | EC | ML | KP | PA |
| ETHANOL | 50 | 26 | 28 | 22 | 29 | 26 | 29 |
| | 100 | 28 | 23 | 25 | 20 | 14 | 25 |
| METHANOL | 50 | 25 | 25 | 21 | 29 | 25 | 28 |
| | 100 | 28 | 20 | 25 | 19 | 18 | 29 |
| WATER | 50 | 29 | 23 | 23 | 22 | 25 | 26 |
| | 100 | 25 | 23 | 23 | 28 | 24 | 26 |
| CHLOROFORM | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 100 | 0 | 0 | 0 | 0 | 0 | 0 |

**Fig. 4: Pictures of zone of inhibition of all the microbes in 50mg/ml and 100 mg/ml concentration respectively.**

## 10. ACKNOWLEDGEMENT

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