

## **Antibacterial activity of extracts of *Citrus*, *Allium* & *Punica* against food borne spoilage**

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### **ABSTRACT**

*The aim of the current study is to isolate the bacteria from the spoiled food, identify them on the basis of their morphology and properties & then check their inhibition from the plant extracts prepared from three plants namely Citrus limon, Allium sativum & Punica granatum. Food Poisoning is an illness which can be fatal to the one who consumes spoiled food & can also have serious consequences. The toxins produced by the microbes affect the host even after the microbes are dead. On the basis of morphology cocci & rod were present in the samples. Both gram positive & negative bacteria were identified along with some spore forming bacteria. There was a mixed population of aerobes & anaerobes. All the plant extracts showed activity against the microbes but the extract from Punica granatum were the most versatile. The concentration of the plant extracts was found to be 500mg/ml.*

**Keywords:** *Citrus limon, Allium sativum & Punica granatum*, food poisoning, plant extract, bacteria.

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### **INTRODUCTION**

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption. Food borne sickness is any illness resulting from the consumption of contaminated food, pathogenic bacteria, viruses, or parasites that infect food & we consume that spoiled food consciously or unconsciously. Food borne illness usually arises from improper handling, preparation, or food storage where hygienic approach is not followed. There is potential for a wide range of food products to become contaminated with microorganisms. Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the *Enterobacteriaceae*. Of these, *Salmonella* and *Escherichia coli* are of particular concern [1]. Other bacteria commonly responsible for food spoilage are *Bacillus cereus*, *Staphylococcus aureus*, etc. Apart from diseases caused by direct bacterial infection, some food borne illnesses are caused by exotoxins which are excreted by the cell as the bacterium grows on the food materials. Exotoxins can produce illness even when the microbes that produced them have been killed.

Thus, considering all the above facts it is important to explore remedy to illness caused by food spoilage. It is also important because we are daily exposed to such infections in our lives. We consume such food that might be unhygienic or casually prepared. Ultimately we compromise with our health. Remedy to all such disorders is not far away. As soon as we realise that nature has entitled us with solution to all of our problems. There has been a constant increase in the search of alternative and efficient compounds for food preservation aimed at a partial or total replacement of antimicrobial chemical additives [14,20]. Extract from many plants used as flavouring and seasoning agents in food and beverages have been used therapeutically for centuries [14,21]. Again the solution is looked in the natural plant resources that are present everywhere. Natural plant extracts offer us infinite potential in curing diseases caused by spoiled food. In contrast cure provided by the chemically synthesized medicines have side effects on our body. Excessive use of drugs has also caused resistance in bacteria in over a period of time [2]. These drugs also affect the natural microflora of our body which are beneficial to our metabolism. Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods [5][6]. The active ingredients of plants against microorganisms are mostly some of the secondary metabolites

(i.e. alkaloids, glycosides etc.) that are present in abundance in herbs and spices commonly used in Indian food preparations. Traditional medicines have been used for many centuries by a substantial proportion of the population of India [7] [13].

*Citrus limon* is an evergreen plant native to Asia. It is most popularly utilized for its juice, pulp & also the peels. It is used world wide in cooking dishes because its juice provides a unique sour taste. It is rich in citric acid which gives a pH of 2-3. Lemon is used to make lemonades, cocktails, beverages, etc. It is also a plant of interest because of its medicinal value [3]. It exhibits antimicrobial properties thus, its potential to inhibit microbial growth should be explored. Since it is easily available and common in use its extracts can serve as medicines.

*Allium sativum* is a plant which is native to Central Asia. It is used commonly for its pungent odour and taste. The garlic bulb is the most commonly used part of the plant. It is supposed to express antimicrobial activities [4] and thus its potential needs to be explored. Garlic and cinnamon used for food preservation because of their bacteriocin based strategies [11,12]. Since it is easily available worldwide it should be consumed in order to prevent any microbial infection [5].

*Punica granatum* is native to the area of modern day Iran and Iraq. From there it spread to Asian areas such as The Himalayas in Northern India. In the Indian subcontinent's ancient Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remedies for thousands of years [15]. The fruit and the bark of the pomegranate tree is used as a traditional remedy against diarrhoea, dysentery and intestinal parasites.

## MATERIALS AND METHODS

### COLLECTION OF SPOILED FOOD SAMPLE

Food samples were collected from the cooked food material generally prepared in Indian houses. The food samples were kept at warm & moist place to provide optimum conditions for microbes to grow. The incubation of 48 hrs was given.

### ISOLATION OF BACTERIA

Bacteria were isolated by the serial dilution method. 2gm of food sample was mixed with 10ml of distilled water. Then serial dilution of  $10^{-1}$  &  $10^{-2}$  were prepared. Now 100 $\mu$ l of solution from the two diluted test tubes were taken and spread over Nutrient Agar Media (NAM) plates. The plates were now kept in an incubator at 37°C for 24hrs.

### CHARACTERIZATION OF BACTERIAL COLONIES

It is very important to characterise the isolates to study their properties & morphology and then to develop an accurate conclusion. Three characterization tests of the isolated microbes are performed namely Grams Test (to check whether the microbes have cell wall or not), Endospore Test (to test whether the microbes produce endospores or not) & finally Catalase Test (to test whether the isolated microbes are aerobic or anaerobic).

### PRESERVATION OF BACTERIAL COLONIES

Four distinct colonies (V1, V2, V3 & V4) were observed after 24hrs of incubation on the spread plates. These colonies were picked up with the help of a sterile inoculation loop and fresh NAM plates were streaked in order to preserve the isolated colonies. Slants of the isolated colonies were also prepared for future use. In order to perform other experiments in the current research culture broths were also prepared.

### COLLECTION OF PLANT SAMPLES

*Citrus limon* leaves were collected from a local garden after proper identification. *Allium sativum* & *Punica granatum* samples were collected from the local market. Now the collected samples were brought to the laboratory, washed properly and sundried for 4 days.

### PREPERATION OF PLANT EXTRACTS

Four solvents were used to prepare plant extracts namely Methanol (85%), Propanol (85%), Acetone (85%) & Water. Each plant sample was crushed in a mortar & pestle. 3gm of each plant sample was dissolved in 30ml of the solvents thus, maintaining a ratio of 1:10. For each plant four extracts of different solvents were prepared thus in total twelve extracts were prepared. Now all the plant samples were incubated for a period of 5 days in dark. After the due time period the extracts were filtered out in already weighed petri plates. The extracts were dried in a hot air oven at 50°C. After all the samples were dried the petri plates were again weighed to find out the amount of solid extract isolated. Now finally the isolated solid extracts are dissolved in dimethyl sulfoxide (DMSO) in the twice the amount of solid extract.

## CALCULATION OF YEILD OF EXTRACTS

3gm of raw sample was taken and mixed in respective solvents. Solvents help to extract the active agents from the raw samples. It is also important to calculate the yield of amount of active agents extracted. A simple formula [16] can be used to calculate the Yield% -

$$\text{Yield\% of extract} = \frac{\text{Amount of solid extract}}{\text{Amount of raw sample used}} * 100$$

## CHECKING ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS

In order to check the antimicrobial activity of the plant extracts fresh NAM media was prepared and poured in 12 petri plates. Antimicrobial activity will be checked through well diffusion method [17]. Now with the help of a sterile borer of 8mm diameter 4 wells were created in each plate. Thus for each isolated bacteria (V1, V2, V3 & V4) four plates were prepared of respective plant extracts of *Citrus limon*, *Allium sativum* & *Punica granatum*. Each of the plate has 4 wells corresponding to 4 different types of solvents used to prepare extracts. On each plate 100µl of bacterial broth was poured & uniformly spread over it. Now in each well 50µl of plant extract was added. Now the plates are sealed and kept in incubator for 24hrs of incubation. Zone of inhibition (in mm.) will be measured to determine the antimicrobial activity of the plant extracts.

## RESULTS AND DISCUSSION

From the current study it can be concluded that a variety of bacteria infect our food materials. On the basis of their morphology and properties mostly two types of bacteria were found namely cocci and rod. Both gram positive and gram negative bacteria were found. In the study some endospore forming bacteria were also found, this means that some food spoiling bacteria can also survive harsh and extreme condition and can also cause infection when optimum condition arrive. Both aerobic and anaerobic bacteria were identified from the spoiled food.

There were four colonies isolated from the food sample V1, V2, V3 & V4. When the microbes were observed under microscope at 40X & 100X, their morphology and characterization could be predicted precisely and it is shown in **Table 1 & Table 2**.

Table.1 Showing Shapes of The Isolated Bacteria

Isolates	Shape
V1	Cocci
V2	Rod
V3	Rod
V4	Rod

Table.2 Showing the characterization results

	GRAMS TEST	ENDOSPORE TEST	CATALASE TEST
V1	Negative	Positive	Anaerobe
V2	Negative	Negative	Anaerobe
V3	Negative	Negative	Aerobe
V4	Negative	Negative	Aerobe

Three plants were used to prepare the plant extracts namely *Citrus limon*, *Allium sativum* & *Punica granatum*. Similarly four solvents were used to prepare extracts from each plant namely Methanol, Propanol, Acetone & Water. **Table 3** shows the necessary calculations in preparation of the plant extracts & **Table 4** shows the yield% of isolated plant extracts.

The zone of inhibition by respective plant extracts prepared from different solvents is shown in **Table 5, Table 6 & Table 7** respectively.

Antimicrobial activity was observed in all the three plants samples namely *Citrus limon*, *Allium sativum* & *Punica granatum*. The acetone extract of *Punica granatum* was found to be most potent against gram negative, endospore producing anaerobic cocci with a zone of inhibition of 23mm. The acetone extract of *Punica granatum* was found to be most effective against gram negative anaerobes which were rod in shape with a zone of inhibition of 22mm. The acetone extract & the propanol extract of *Punica granatum* were found to be most effective against gram negative aerobes which were rod shaped with a zone of inhibition of 22mm and 25mm respectively [18].

Table.3 Calculations For Determining Amount Of Isolated Plant Extracts

	<i>Citrus limon</i>	<i>Allium sativum</i>	<i>Punica granatum</i>
METHANOL	W(e)=47.828 W(d)=48.12 W(s)=0.292 V=0.584	W(e)=39.37 W(d)=40.041 W(s)=0.671 V=1.342	W(e)=47.432 W(d)=48.079 W(s)=0.647 V=1.294
PROPANOL	W(e)=47.708 W(d)=47.838 W(s)=0.13 V=0.26	W(e)=50.726 W(d)=50.992 W(s)=0.266 V=0.532	W(e)=37.436 W(d)=38.135 W(s)=0.699 V=1.389
ACETONE	W(e)=47.63 W(d)=47.894 W(s)=0.264 V=0.528	W(e)=29.644 W(d)=29.81 W(s)=0.166 V=0.332	W(e)=48.486 W(d)=49.341 W(s)=0.855 V=1.71
WATER	W(e)=29.429 W(d)=29.77 W(s)=0.341 V=0.682	W(e)=46.756 W(d)=48.135 W(s)=1.379 V=2.758	W(e)=46.752 W(d)=47.083 W(s)=0.331 V=0.662

In The Table,

W(e) = Weight Of Empty Petri Plate.(In gm).

W(d) = Weight Of Petri Plate After Solvent Has Dried(In gm).

W(s) = Weight Of Solid Extract Left [W(d)-W(e)] (In gm).

V = Amount of DMSO Added(In ml). DMSO is added in double the amount of solid plant extract.

Table.4 Showing The Yield% Of The Plant Extracts

	<i>Citrus limon</i>	<i>Allium sativum</i>	<i>Punica granatum</i>
METHANOL	9.73%	22.36%	21.56%
PROPANOL	4.33%	7.53%	23.3%
ACETONE	8.8%	5.53%	28.5%
WATER	11.36%	45.96%	11.03%

Table.5 Zone Of Inhibition Produced By *Citrus limon*

	METHANOL 85%	PROPANOL 85%	ACETONE 85%	WATER
V1	<b>10mm</b>	<b>12mm</b>	12mm	<b>16mm</b>
V2	-	9mm	10mm	11mm
V3	<b>10mm</b>	-	<b>14mm</b>	-
V4	<b>10mm</b>	10mm	13mm	16mm

Table.6 Zone Of Inhibition Produced By *Allium sativum*

	METHANOL 85%	PROPANOL 85%	ACETONE 85%	WATER
V1	-	-	-	-
V2	-	-	-	-
V3	<b>16mm</b>	<b>18mm</b>	18mm	<b>14mm</b>
V4	-	-	<b>24mm</b>	-

Table 7. Zone Of Inhibition Produced *Punica granatum*

	METHANOL 80%	PROPANOL 85%	ACETONE 85%	WATER
V1	<b>21mm</b>	21mm	<b>23mm</b>	18mm
V2	18mm	21mm	22mm	<b>20mm</b>
V3	16mm	20mm	22mm	18mm
V4	18mm	<b>25mm</b>	22mm	<b>20mm</b>

The water extract of *Citrus limon* gave the highest zone of inhibition 16mm [19] as compared to other of its extract (**Fig.1**). The acetone extract of *Allium sativum* gave the highest zone of inhibition of 24mm as compared to other of its extracts (**Fig.2**). The propanol extract of *Punica granatum* was the most potent with a zone of inhibition of 25mm as compared to other of its extract (**Fig.3**).

Food spoilage is a common problem faced all over the world. High temperatures, humidity, unhygienic preparation of food, lack of proper storage, etc. all contribute to food spoilage and growth of pathogenic bacteria that cause food

illness. The chemical antibiotics have side effects on host and also destroy the natural micro flora of the body. In contrast the natural plant extracts offer a very safe medical treatment with no side effects.

Figure 1-Showing Maximum Zone Of Inhibition Of Respective Extracts Of *Citrus limon*

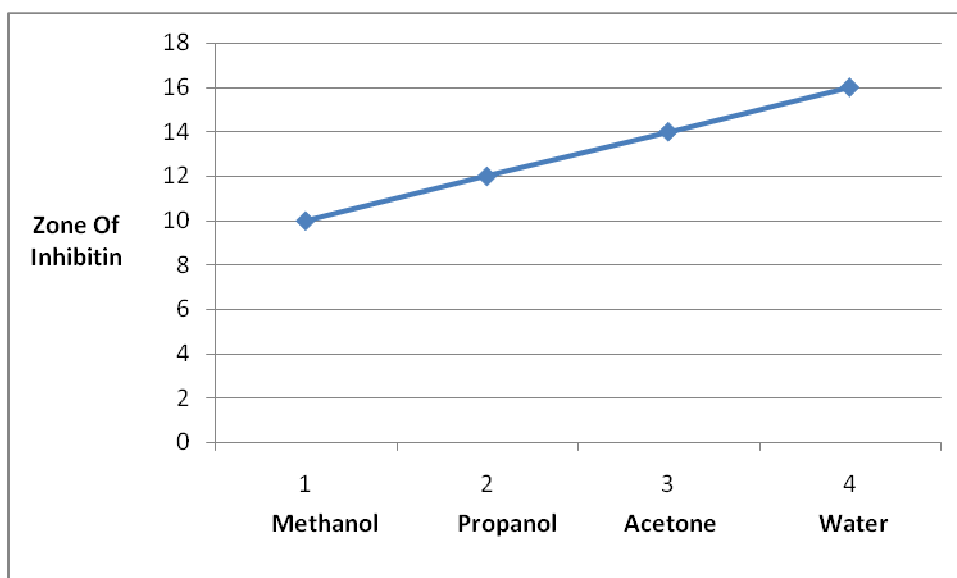
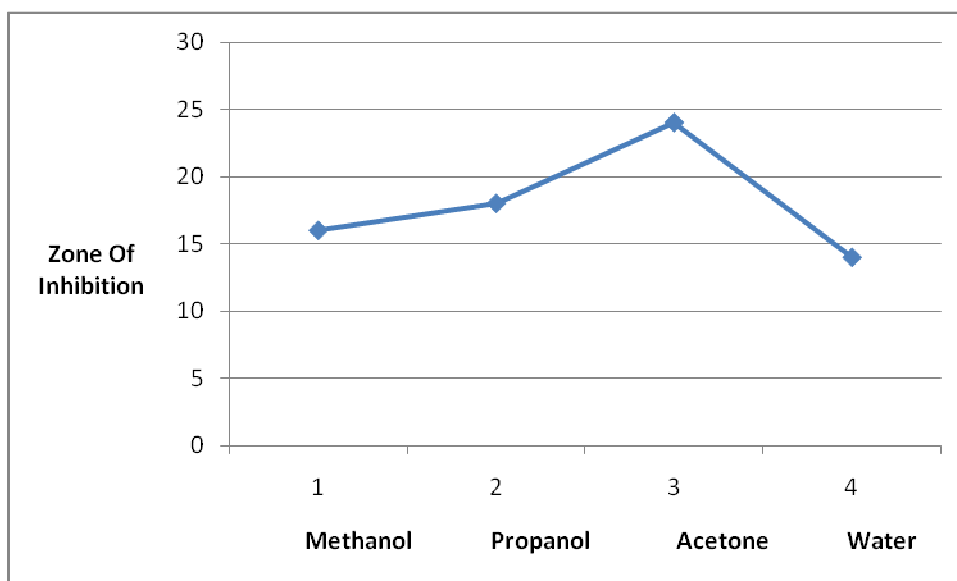


Figure 2- Showing Maximum Zone Of Inhibition Of Respective Extracts Of *Allium sativum*



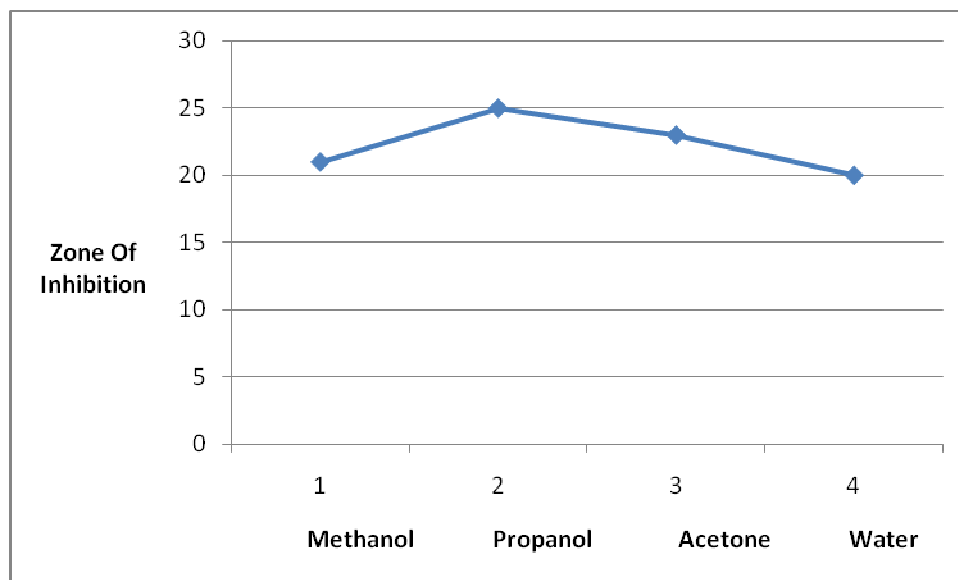
Food spoilage can occur in both canned food & house cooked food. In case of canned food chemical preservatives are added to prevent any microbial growth. These chemical preservatives can be harmful to health & can have side effects.

Garlic and cinnamon can be used as natural food preservations for fish. Garlic and cinnamon extracts can be the best replacement of chemical preservatives when their extracts are used after purification and thus the use of other harmful chemical preservatives can be minimized which will be beneficial for health [12].

Application of dried plant extracts is a safe technique of preserving canned food. It will not have any kind of side effects on the other hand it will increase the shelf life of the packed food. On the other hand food poisoning caused by food cooked in homes & not stored properly is very common because we often don't pay attention to it. Thus intake of plant extracts by people can prevent the ill effects of spoiled food. This is the easiest way to keep oneself fit in a natural way.

These plant extracts offer numerous application to us namely in food packaging, alternative medicinal therapy, in food preservation, healthy lifestyle, etc. In future a detailed study can be performed to isolate the active agents in the respective plant extracts by using high resolution techniques such as High Performance Liquid Chromatography to develop a more precise therapy to check microbial growth in context to food spoilage & others.

Figure 3- Showing Maximum Zone Of Inhibition Of Respective Extracts Of *Punica granatum*



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