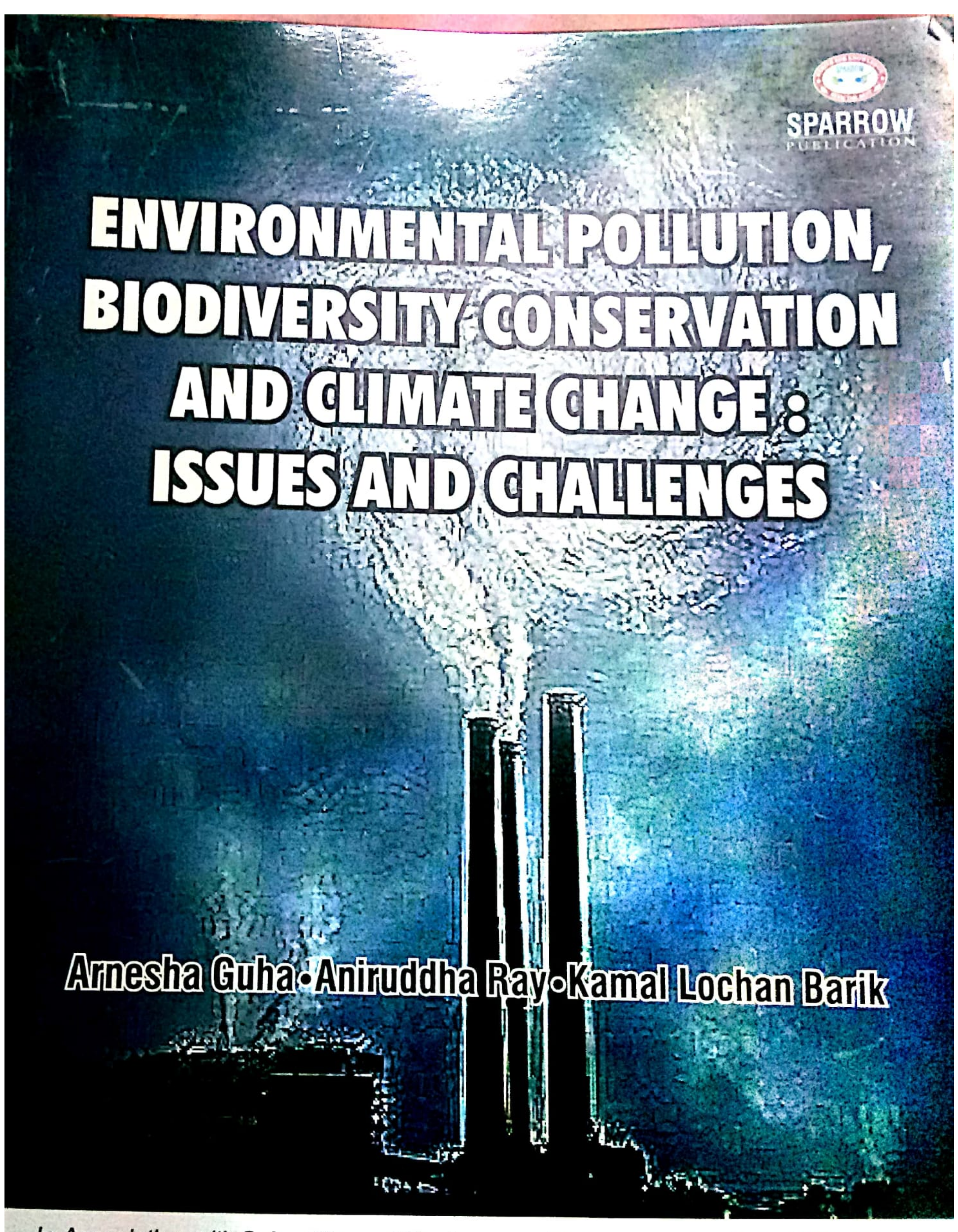

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Environmental Pollution Biodiversity Conservation and Climate Change: Issues and Challenges

Arnesha Guha
Kamal Lochan Barik
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Chapter 15

Antibacterial Activity of the Vegetable and Fruit Peel

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ABSTRACT

Fruit and vegetables wastes and their by products are formed in great amount during industrial processing and in kitchens, and this cause a serious environmental pollution and many disease causing microorganisms may form in this garbage wastes .Peels of various fruits and vegetables are generally considered as waste product and are normally thrown away by us. It was noticed after different experiments that peels of vegetables and fruits have some activities like antimicrobial , antioxidants, ant proliferative, anti-inflammatory etc, The present study was focused on the antimicrobial activity of some selected vegetable peel and fruits peels with the selected pathogens.

Keywords: Fruits peels, vegetable peels, environmental pollution, antioxidants, antimicrobial activity

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INTRODUCTION

Historically, plant have provided a source of inspiration for novel drug compounds and the use of the plant for treating various disease in an age old practice in a large part of the world especially in developing countries where there is dependence on the traditional medicine for variety of disease. In recent times there has been increasing interest in study of bioactive compounds from the peels, seeds, leaves, flowers and stem, bark due to their antioxidative, antimicrobial and other health promoting properties. Fruits and vegetables are considered as an important part of a good diet. Fruit and vegetable peels are thrown into the environment as agro waste which can be utilized as a source of antimicrobics. It will be economic; eco friendly wastes can improve the overall economics of processing units. Besides this, the problem of environmental pollution can also be reduced considerably. Fruits, especially tropics fruits have the capacity to produce a large number of bioactive phytochemicals (Nand. K., 1998). According to world health organization, 2003 medicinal plants would be the best source of variety of drugs and therefore such plants should be investigated to better understanding of their properties safety and efficiently. The use of plants and plant products as medicines could be traced as far as the beginning of human civilization. Nature has very rich botanical wealth and a large number of desire types of plants that grow in different parts of the country. Various researches have reported that the plant that grow in different parts of the country. Various researchers have reported that the plant extract have antimicrobial activity, anti-inflammatory activity, antibacterial activity and contain antifertility agents.

AIMS AND OBJECTIVE

The aim of the present study was focused on the antimicrobial activity of vegetable peel and fruit peels wastes with the selected pathogens.

MATERIALS AND METHODS

A. Miscellaneous: Petri dishes, Sterile micro tips, Micropipette, Cork borer, Cotton swabs, Mortar & pestle, Beakers, Screw cap tubes, Cotton, Conical flask, Alcohol(95%), Tissue paper, Muslin cloth, Marker, Stickers, Nutrient broth, Agar agar, Culture tubes, Cold Centrifuge. The chemicals and media were purchased from the following sources:

1. Nutrient Agar (Himedia lab Pvt Ltd)
2. Nutrient broth (Himedia lab Pvt Ltd)
3. Agar agar (Himedia lab Pvt Ltd)
4. Alcohol (95%)

B. Test organism:

i) Staphyllococcusaureus
ii) Escherichia coli
iii) Pseudomonas aeruginosa
iv) Bacillusspizizeni

C. Sample (vegetable & fruit):

1. Ridge gourd (Luffaacutangula)
2. Bitter gourd (Momordicacharantia)
3. Bottle gourd (Lagenariasiceraria)
4. Orange (citrus sinesis)
5. Pomegranate (Punicagranatum)
6. Pineapple (Ananascosus)

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Extraction of solvents

The peels were collected from the kitchen wastes and separately kept in a clean petri plate. Then the peels were thoroughly washed under running tap water for few minutes and then kept in open to be air dried. Meanwhile 95% pure alcohol was taken in a separate beaker about 30ml. Then the peels were dipped carefully in the alcohol and taken out immediately with help of a forceps and kept in a separate clean grease free petri plates wrapped with tissue paper for faster drying. Thereafter the peels were allowed to dry in the laminar air flow.

For Raw Extract: The dried peels were taken and weighed about 10g in the weighing machine and taken in a clean grease free mortar and pestle. The peels were then evenly smashed and grinded for a short period of time. When the peels were evenly grinded, 10ml of sterile double distilled water were measured using a measuring cylinder separately and was poured in the grinded mixture of peels. It's mixed well with the help of pistle.

The sterilised screw cap tube was taken and muslin cloth was held over it. The above prepared mixture was slowly poured through 8 layers of muslin cloth to obtain a clear solution of extract in the screw cap tube. Now, this obtained solution was transferred in an eppendrof tube using a micropipette followed centrifugation i.e. at 6000 RPM for about 15 min.

For Boiled Extract: To obtain the boiled extract the above written procedure is repeated that is the required peels are washed, dried, weighed in the weighing machine then smashed in the mortar and pestle, further the 10 ml of sterilized distilled water is measured and taken separately in a clean grease free beaker. Then the smashed peels were transferred in the beaker. The crushed mass was placed in the heater for about 1015 mins .When it comes to boil the beaker was removed from the heater and allowed to cool for some times. Then the crushed mass was taken in 8 layers of muslin cloth and was squeezed firmly to obtain a clear extract in the sterilized screw cap tube. Finally the extract was transferred in the eppendrof tube using micropipette and was centrifuged at 6000 rpm for about 15 mins.

Preparation of culture media

Nutrient broth was taken measured for 1000 ml of distilled water. Agar powder was taken weighted (1.5%).The nutrient broth & the agar powder were taken in conical flask along with distilled water were stirred properly. The media was then sterilized in autoclave at 121°C & 15lb/inch^2 pressure for 15 minutes. As the meida cooled to some extent it was poured in the sterilized petriplates& was allowed to solidify.

Preparation of bacterial lawn

A sterilized cotton swab was taken & dipped in the broth culture of *Staphylococcus aureus*.Then bacterial lawn was made over the agar surface of the petriplate.Thepetriplate was then kept in the refrigerator for 4 minutes to obtain a synchronous culture of test organism.

Preparation of agar cup

After getting a synchronous culture of the test organism an agar cup was made in the centre of the plate using an alcohol dipped cork borer having a diameter of 5 cm.

Filling wells with extracts

After making cups in the pre labelled petriplates,boiled & raw solvents were taken in a micropipette with sterilized micro tips & then very cautiously 0.1ml of the extract was poured drop by drop in the cups to avoid overflowing.

Incubation of the test culture

After filling the vegetable& fruit extract in the agar cup the plates were carefully put in the incubator in upright position (so that the extract do not come out of the well) at 37°C (since it is the optimum temperature for the growth of the test culture) for 24 hours.

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OBSERVATIONS AND RESULTS

After 24 hours incubation a zone of inhibition (ZI) was observed around the agar cups. The diameter of the zone of inhibition was measured and it was observed that the plate containing the raw and the boiled extract of selected vegetables and fruits peels.

Table 1: Zone of Inhibition (ZI) Chart

Name of vegetables and fruits peels	Bacterial species	Zone of inhibition(in cm)		
		(Raw extract)	(Boiled extract)	Reference(Antibiotic) Ampicillin(1mg/ml)
Luffaacutangula(Ridge gourd)	*Escherichia coli*	0.1 cm	0.0 cm	2.0cm
	Bacillus spizizenii	0.0 cm	0.0 cm	1.8cm
	Staphylococcus aureus	0.0 cm	0.0 cm	1.6cm
	Pseudomonas aeruginosa	0.0 cm	0.0 cm	2.1cm
Momordicacharantia (Bitter gourd)	*Escherichia coli*	1.1 cm	0.5 cm	2.3cm
	Bacillus spizizenii	1.5 cm	0.7 cm	1.6cm
	Staphylococcus aureus	1.6 cm	0.9 cm	1.6cm
	Pseudomonas aeruginosa	1.5 cm	1.0 cm	2.2cm
Lagenariasiceraria (Bottle gourd)	*Escherichia coli*	0.1 cm	0.0 cm	2.3cm
	Bacillus spizizenii	0.0 cm	0.0 cm	1.8cm
	Staphylococcus aureus	0.0 cm	0.0 cm	1.6cm
	Pseudomonas aeruginosa	0.0 cm	0.0 cm	2.1cm
Citrus sinensis (Orange)	*Escherichia coli*	1.1 cm	0.0 cm	2.0cm
	Bacillus spizizenii	0.9 cm	0.0 cm	1.6cm
	Staphylococcus aureus	1.0 cm	0.0 cm	1.6cm
	Pseudomonas aeruginosa	0.6 cm	0.0 cm	2.6cm
Punicagranatum (Pomegranate)	*Escherichia coli*	2.1 cm	0.3 cm	2.0cm
	Bacillus spizizenii	1.5 cm	0.6 cm	1.8cm
	Staphylococcus aureus	1.5 cm	0.1 cm	1.6cm
	Pseudomonas aeruginosa	1.5 cm	0.0 cm	2.1cm
Ananascomosus (Pineapple)	*Escherichia coli*	0.1 cm	0.0 cm	2.0cm
	Bacillus spizizenii	0.0 cm	0.0 cm	1.6cm
	Staphylococcus aureus	0.0 cm	0.0 cm	1.6cm
	Pseudomonas aeruginosa	0.0 cm	0.0 cm	2.2cm

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Second step of our project is to determine the inhibitory action of the peels extract of Momordica charantia and Punica granatumby increasing the concentration of the extract for example:

10mg/10ml, 20mg/10ml and so on.

Observations:

Test Bacterial sample:				
Escherichia coli	10g/10ml	20g/10ml	30g/10ml	40g/10ml
Momordicacharantia	1.6cm	1.4cm	1.6cm	1.7cm
Punicagranatum	2.1cm	2.15cm	2.3cm	2.4cm
Test Bacterial sample:				
Psuedomonasaeruginosa	10g/10ml	20g/10ml	30g/10ml	40g/10ml
Momordicacharantia	1.5cm	1.6cm	1.7cm	1.75cm
Punicagranatum	1.5cm	1.6cm	1.8cm	1.85cm
Test Bacterial sample:				
Bacillusspizizeni	10g/10ml	20g/10ml	30g/10ml	40g/10ml
Momordicacharantia	1.5cm	1.6cm	1.62cm	1.75cm
Punicagranatum	1.5cm	1.7cm	1.72cm	1.75cm
Test Bacterial sample :				
Staphylococcus aureus	10g/10ml	20g/10ml	30g/10ml	40g/10ml
Momordicacharantia	1.6cm	1.7cm	1.75cm	1.8cm
Punicagranatum	1.5cm	1.2cm	1.6cm	1.9cm

DISCUSSION AND CONCLUSION

The search for antimicrobials from natural resources has received much attention and efforts have been put to identify compounds that can act as suitable antimicrobial agents to replace synthetic ones. Phytochemical derived vegetable peels serves as prototype to develop less toxic and more effective medicines in controlling the growth of micro organisms. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or viruses. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial compounds. Therefore, vegetables peels which are generally considered as agro wastes are finding their way into pharmaceuticals, nutraceuticals and food supplements.

In the present investigation, extracts of Luffaacutangula, Momordicacharantia, Lagenariasiceraria, Citrus sinensis, Punicagranatum, Ananascomosus. We tested with two different kind of extract making procedure one the raw extract and boiled extract.It was seen that only in two types of peels prominent inhibition was observed i.e. in bitter gourd (Momordicacharantia) and pomegranate (Punicagranatum). Although very light inhibition was obtained in *Citrus sinensis*.

Agar cup method was used to check the antimicrobial activity of these waste peels. And antibiotics were used as control to check was whether the agar diffusion is taking place was properly or not.

Second stage of our project consist of obtaining inhibitory zone when concentration was serially increased for example 10g/10ml, 20g/10ml and so on. It was seen that the microbial activity generally increases. It was also

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observed that of the peels extract on boiling looses its antimicrobial activity, so raw extract shows maximum antimicrobial activity.

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Chapter 16

Magic of Nanobacteria on Environment

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ABSTRACT

Nanobacteria (calcifying nanoparticles or nanobes) are one of the most controversial issues in contemporary biology. Studies show accumulating evidence on association of nanobacteria with oral pathologic calcifications such as calculus, pulp stone, and salivary gland stones. Experiments have shown that nanobacteria are excreted from the body in urine and saliva, lifted from the ground by winds into the cloud, and transit between the high humidity region of the clouds and the relatively dry inter-cloud regions. Remnants of a sticky protein coating that nanobacteria make it act as an extremely efficient cloud condensation nuclei. Following condensation of cloud, nanobacteria return to the earth via rain and snow. They grow best under aerobic conditions: 5% CO_2 and 95% air. These organisms grow slowly. Doubling time of nanobacteria is about three days with the metabolic rate, which is 10,000 times slower than that in Escherichia coli. Transmission of nanobacteria via clouds is not surprising when compared with cosmic transmission of nanobacteria. A double defence with the apatite layer and an impermeable membrane combined with a very slow metabolism is a likely explanation for the resistance of nanobacteria. Recent environmental scanning electron microscope images showed a huge number of nanoparticles collected in the stratosphere via balloon. They were virtually indistinguishable from nanobacteria isolated from mammalian sources: The absolute set of seven morphologic parameters (shape,size, size distribution, interconnection, chain arrangement, conglomeration, and cracking in apparently mineral shells) was identical to the analogous structures regarded as characteristic of nanobacteria, both *in vivo* and *in vitro*. It has been shown that not only can more than 1,000 nanovesicles form on a substrate, but also larger clumps can be formed by their aggregation. The presence of this aggregation supports the hypothesis that under favorable conditions, e.g., in clouds, nanobacteria can use their slime to form larger clumps. Nanobacteria are a fairly new field of study in medicine.

Keywords: Calculus, clouds, nanobacteria, oral pathologic calcification, pulp stone, salivary gland stones

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INTRODUCTION

Nanobacteria, also known as calcifying nanoparticles (CNPs), were first described by Kajander and Cift,cio_glu in 1998. The nature of these unknown cell-culture contaminants and their possible role in human organ disease has raised a lot of attention in medical and human health research over the last decades. They are 80-500 nm in diameter, typically have coccoid, coccobacillar, or bacillar form. They have hydroxyapatite shell, cellular membranous structure, and central cavity and can form microscopic colonies. Nanobacteria divide by binary fission, fragmentation, or gemmation and can form thermo-resistant biofilms. They are Gram negative and can be stained by DNA-specific dyes. Doubling time is 3 days; their metabolism is 10,000 times slower than in *Escherichia coli* and they calcify under physiological pH. Nanobacteria have reportedly been found in animal and human blood, bile, tissue culture, wastewater, Australian sandstones, and in the stratosphere. However, studies show accumulating evidence on the association of nanobacteria with human diseases.

The disease-causing mechanisms of nanobacteria include the known effects of calcium on blood vessels, blood coagulation, and thrombus formation; elevation of intracellular (Ca^{2+}) levels and its consequences (stimulation of either apoptotic cell death or uncontrolled cell growth which could potentially contribute to tumoral growth or malignancies); induction of autoimmune diseases; inflammation; arthritis; and pathological calcification. Nanobacteria shelter themselves from the immune system and the antibodies (calcific semidormant defence) and they can live where other bacteria cannot (extremophilic defence) because of their calcific defence. They have been implicated in the formation of pathogenic calcifications, e.g., kidney stones, arterial plaque, and calcification of coronary arteries and cardiac valves.

The aim of this paper was to perform a narrative review of publications on CNPs since 1998 and highlight on the fact that Nanobacteria in clouds can spread various diseases around the world.

DETECTION AND CULTURAL METHODS

Nanobacteria, the smallest known self-replicating bacteria, are classified as Gram-negative organisms. They grow best under aerobic conditions: 5% CO_2 and 95% air. These organisms grow slowly. Doubling time of nanobacteria is about three days with the metabolic rate, which is 10,000 times slower than that in Escherichia coli. These organisms are found in biological systems, such as tissues, cells blood and urine. The best methods for detection of these organismsto include the immunodetection with nanobacteria-specific monoclonal antibodies, electron microscopy and culture techniques. Due to their very small size, 0.22 mm-pore size filters, which have the ability to disallow most common microbes, are often used to clean up fluid specimens, before the culturing of nanobacteria. Moreover, their rate of replication can be measured by particle counting and specific optical density at 650 nm.

Studies on freshly fallen snow suggest that vitality level of nanobacteria in clouds and the spreading of airborne diseases might be much more common than suspected. It has been shown that microbes can safely travel long distances in clouds. Biological particles do seem to play a very important role in generating rain, and snowfall especially at relatively warm cloud temperatures. Most of the rain-making bacteria make their living as plant pathogens, breaking the cell walls of the plants that they feed on. It is quite plausible that the organisms might be using their ice-nucleating ability to get out of the atmosphere.

These findings are not surprising. The idea that bacteria can travel long distances in clouds and then return to Earth had been developed more than one decade ago by Hamilton *et al.* Nevertheless, current models predict that the elevation of the Earth's surface temperature because of global warming is accompanied by a warming of the troposphere, and a thickening cloud cover associated with longer lasting clouds, in particular over land. These effects can have an instant impact on the vitality level of microorganisms (nanobacteria) in clouds and the spreading of airborne diseases.

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RESULTS AND DISCUSSION

Experiments have shown that nanobacteria are excreted from the body in saliva and urine, lifted from the ground by winds into the cloud, and transit between the high humidity region of the clouds and the relatively dry inter-cloud regions, leading to oscillations between a dormant state and one of activation. Remnants of a sticky protein coating that nanobacteria make act as extremely efficient cloud condensation nuclei, with a tendency to aggregate to clusters upon contact. Following condensation of cloud, nanobacteria return to the earth via rain and snow. Of more interesting thing that nanobacteria can even arrive from space via space travels or interstellar dusts or meteorites.

Recent environmental scanning electron microscopic studies showed a huge number of nanoparticles collected in the stratosphere via balloon. They were virtually indistinguishable from nanobacteria isolated from mammalian sources: The absolute set of seven morphologic parameters (shape, size, size distribution, interconnection, chain arrangement, conglomeration, and cracking in apparently mineral shells) was identical to the analogous structures regarded as characteristic of nanobacteria, both *in vivo* and *in vitro*. It has been shown that not only can more than 1,000 nanovesicles form on a substrate, but also larger clumps can be formed by their aggregation. The presence of this aggregation supports the hypothesis that under favorable conditions, e.g., in clouds, nanobacteria can use their slime to form larger clumps. Such giant hydrophilic nuclei would serve as ideal cloud condensation nuclei, with a potential to finally form larger rain drops, thus allowing nanobacteria to reach the surface of the Earth in a viable state.

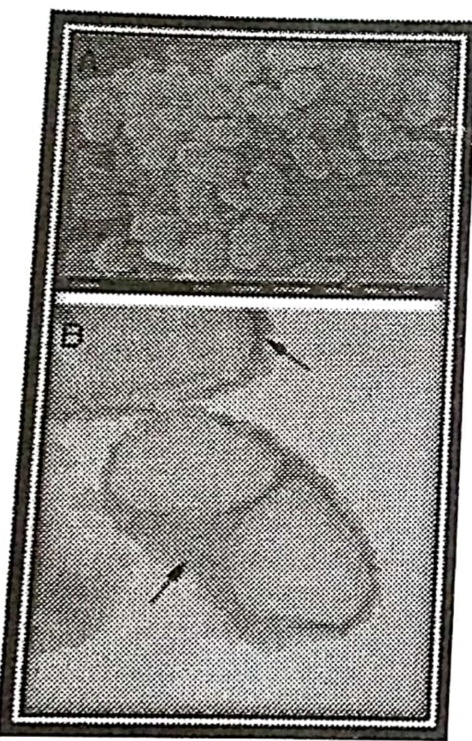

Fig. 1. Images of cultured nanobacteria, taken by (A) scanning electron microscope and (B) transmission electron microscope

Note: Arrows on (B) show an apatite envelope of apparently multiplying nanobacterium.

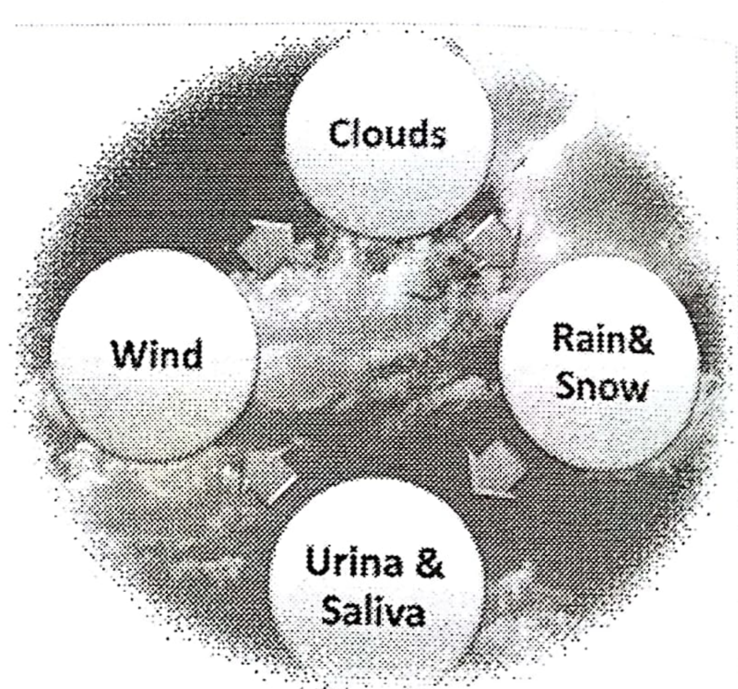

Fig. 2. Schematic diagram of circulation of nanobacteria-induced diseases via clouds. Also nanobacteria even can arrive from space via space travels or meteorites or interstellar dusts

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CONCLUSION

It is very hard to believe that airborne nanobacteria bring us oral pathologic calcifications such as calculus. How can they be alive in the stratosphere? How such organisms can tolerate high solar intensity coupled with high irradiation intensity? Transmission of nanobacteria via clouds is not as surprising as the cosmic transmission of them. These organisms can tolerate harsh conditions extremely well. The apatite mineral layer around the organism serves as a primary defence shield against various critical life-threatening conditions. A double defence with the apatite layer and an impermeable membrane combined with a slow metabolism is likely to be the reason for the resistance of nanobacteria.

As it is known, nanobacteria are a fairly new field of study in medicine. This study can bridge a gap between the etiological factors in some systemic cardio vascular and renal diseases with periodontal diseases. Also, due to their unique model of replication and metabolic nature, only specific agents can affect them. More studies need to be conducted to further understand the biological characteristics of Nanobacteria.

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Isolation, Screening, and Characterization of Cellulolytic Bacteria from Forest and Kitchen Soil of Purulia District, West Bengal

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ABSTRACT

Cellulases are inducible enzymes which are synthesized by large number of microorganisms either cell-bound or extracellular during their growth on cellulosic materials. Cellulose, a crystalline polymer of D-glucose residues connected by -1, 4 glucosidic linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature. Complete enzymatic hydrolysis of enzyme requires synergistic action of 3types of enzymes, namelycellobiohydrolase, endoglucanase or carboxyl methyl cellulase (CMCase) and glucosidases. Cellulose is commonly degraded by an enzyme called cellulose. Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria and protozoan's that catalyse the cellulolysis (or hydrolysis) of cellulose. The main aim of this paper is to isolate efficient cellulolytic bacteria found in forest soil and kitchen soil of Purulia district and to characterize their morphological and biochemical nature. For this purpose, soil samples were collected from two forest areas namely Purulia Deer Park and Balarampur Forest. Also Kitchen soil samples were collected from Household Kitchen waste (Nodiha, Purulia). Firstly, the physiochemical properties of the soil were identified and then it were used for isolation of cellulolytic bacteria. For isolation of cellulolytic bacteria, 10 g of soil were dissolved in 90ml distilled water and from it serial dilutions up to 10-5 were prepared. A 0.1ml of 10-4and 10-5 dilution were plated on the modified Cellulose Congo red agar (CCRA) medium and incubated for 5 days at 37°C .For bacterial isolates Candid powder (1 mg/ml medium) was added to the CCRA medium after autoclaving to prevent the fungal growth. The Colonies grown on CCRA media were not considered as pure even though only one type of colony appeared and exhibited the zone of clearing. These bacterial colonies were purified by taking single colony each time in a streak plate method on cellulose-Congo red agar medium repeatedly at least seven times until plate contained uniform one type of colonies. The purified colonies were checked for cellulolytic activity. Clear zones were observed around the bacterial colonies. These zones were formed due to the degradation of cellulose. This indicates the production of cellulolytic enzymes by the bacteria. Colony Characteristics of the bacteria are observed and noted. Identification of the cellulolytic bacteria was carried out on the basis of Gram Staining and various biochemical tests such as starch hydrolysis, carbohydrate fermentation, MRVP, Nitrate Reduction, Indole Production, etc. Results and conclusions were noted.

Keywords: Cellulases, Carboxy Methyl Cellulase, Cellulose Congo Red Agar Medium, MRVP

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INTRODUCTION

Cellulases are inducible enzymes which are synthesized by large number of microorganisms either cell-bound or extracellular during their growth on cellulosicmaterials. Cellulose, a crystalline polymer of D-glucose residues connected by -1, 4 glucosidic linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature. Complete enzymatic hydrolysis of enzyme requires synergistic action of 3types of enzymes, namelycellobiohydrolase, endoglucanase or carboxyl methyl cellulose (CMCase) and glucosidases. Cellulose is commonly degraded by an enzyme called cellulose. Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria and protozoan's that catalyse the cellulolysis (or hydrolysis) of cellulose.

The major industrial applications of cellulases are in textile industry for bio-polishing of fabrics and producing stonewashed look of .denims, as well as in household laundry detergents for improving fabric softness and brightness(Cavaco-Paulo, A. 1998)[1]. Application of enzymes in textile, food, detergent, leather and paper industries demands identificationof highly stable enzymes active at extreme pH and temperature. Cellulase is used in the fermentation of biomass into biofuels, fibre modification and they are even used for pharmaceutical applications (Bhat, M. K. 2000)[2]. Bacteria has high growth rate as compared to fungi has good potential to be used in cellulose production (Alexander, M. 1961)[4]. Celluloytic property of some bacterial genera such as *Cellulomonas* species, *Pseudomonas* species, *Bacillus* species and *Micrococcus* species were reported. Enzyme production is closely controlled in microorganisms and for improving its productivity these controls can be ameliorated. Cellulase yields appear to depend on a complex relationship involving a variety of factors like inoculum size, pH, and temperature, presence of inducers, medium additives, aeration, and growth time. The aim of this study was to isolation, screening and characterization of cellulose producing bacteria from forest soil.

MATERIALS AND METHODS

Materials Required:

(i) Distilled water,
(ii) Petri plates, culture tubes and test tubes
(iii) Soil sample-Forest and Kitchen soil,
(iv) Media (selective and differential)
(v) Incubator
(vi) Laminar air flow
(vii) Micro pipettes and pipettes
(viii) Micro-pipettes tips

Procedures:

a) Collection of soil sample: Soil samples were collected from two forest areas namely **Purulia Deer Park and Balarampur Forest** under the district of Purulia. Kitchen soil samples were collected from household kitchen **(Nodiha, Purulia).** These two soil samples were used for determination of physiochemical properties (Table 1). The soils were air dried and mixed thoroughly to increase homogeneity.

b) Isolation of cellulolytic bacteria: Ten grams of soil sample was dissolved in 90 ml sterile distilled water. The solution was left in uninterrupted condition for 15 minutes. Serial dilutions were prepared by transferring 1ml of supernatant to 9ml of sterile distilled. A 0.1ml of $10^{-4}$and 10^{-5} dilution were plated on the modified cellulose Congo red agar (CCRA)medium and incubated for 5 days at 37°C .For bacterial isolates **Candid powder** (1mg/ml medium) was added to the CCRA mediumafter autoclaving to prevent the fungal growth.

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c) Screening of cellulolytic bacteria: The Colonies grown on CCRA (Hendricks, Charles W., Doyle, Jack D., Bonnie, H. 1995)[1] media were not considered as pure even though only one type of colony appeared and exhibited the zone of clearing. These bacterial colonies were purified by taking single colony each time in a streak plate method on cellulose-Congo red agar medium repeatedly at least seven times until plate contained uniform one type of colonies. The purified colonies were checked for morphology (Table 2) and cellulolytic activity by the method described below:

Method to check cellulolytic activity

In this method, the bacterial colonies grown on cellulosic medium without Congo red for two days at 37°C,

Then the medium was flooded with an aqueous solution of Congo red (1mg/ml) for 15 min.

The excess Congo red solution was then poured off, and plates were further flooded with 1M Nail for 15 min.

Clear zones were observed around the bacterial colonies. These zones were formed due to the degradation of cellulose. This indicates the production of cellulolytic enzymes by the bacteria (Fig.1)

Identification of cellulolytic bacteria

Identification of cellulolytic bacteria was carried out by method as described by bellow which was based on morphological and biochemical tests:

For Forest Soil Sample and kitchen Soil Sample

A. Gram Staining

1. Bacterial smear was prepared on a clean greese free slide and was heat fixed.
2. The slide was flooded with crystal violet left for 2 minutes.
3. The slide was washed using tap waterand was air dried.
4. The slide was flooded with gram's iodine and left for 1 minute.
5. The slide was washed with 4-5 drops of 70% ethanol.
6. Safranin was added and allowed to react for 2minutes.
7. The slide was then washed with tap water and was air dried.
8. Then it was observed under 40x objectives lens.

B. Starch Hydrolysis

- Sterilized starch agar plate was prepared and the bacterial culture was streaked on them in a line streaking manner.
- The plates were incubated at 37°C for 24 hours.
- After incubation iodine solution was poured on the plates and was observed clear colourless zones around the streaking lines (Table 3).

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C. Carbohydrate Fermentation

- All fermentation tubes with their nutrient broth and Durhan's tubes were sterilized.
- All the sterilized fermentation tubes were labelled according their respective sugar and
- The tubes were inoculated according their respective microorganism labelled on that tube one uninnoculated tube of each broth were kept as negative control.
- All the tubes were inoculated at 37 degree c for 24-48 hours.
- Results were observe (Table 3)

D. Nitrate Reduction

- 10 ml of nitrate broth was dispensed into tubes.
- All the tubes were autoclave at 121.6 degree c for 15 minute.
- After autoclave, tubes were inoculated with 1 ml suspension of 24 hours active fresh culture or a deep park of bacterial colony.
- Negative control was prepared without any bacteria.
- All the tubes were incubated at 37°C for 24-48 hours and the results were observed (Table 3).

E. Indole Production

- 1% tryptophan broth was prepared and poured into respective test tubes.
- Then, autoclave it at 15lbs psi at 121°C for 15 minutes.
- After autoclaving it was taken out and cooled up to room temperature.
- The incubation was done by the given samples and then incubated for 24 hours at 37°C (Table 3)

F. Methyl Red (MR) and Vogues Proskauer (VP) Test

- Preparation of MRVP broth (pH=6.9) tubes.
- Autoclave at 15lbs at 121°C for 15 minutes and then cooled.
- Then inoculate with samples and incubated for 24 hours.
- Then the indicators were added and observations were recorded (Table 3).

G. Citrate Utilization test

- Simon's citrate agar was made by dissolving in the distilled water and autoclaved at 15lbs psi at 121°C for 15 minutes.
- A slant is made and allowed to cool down.
- Samples were streaked in agar slants and incubated at 37°C for the 24 hours.
- After incubation the observations were recorded (Table 3).

H. Catalyse Activity

- At first bacterial colony was picked from agar slant culture by a sterilized nichome wire loop and was placed on a clean glass slide.
- Then 3% H_2O_2 was added to the bacterial colony by the help of dropper.
- The colony and H_2O_2 were rubbed by the help of a sterilized nichrome wire loop and was observed for effervescence (Table 3).

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I. Oxidise Activity

- *First a filter paper* was soaked in 1% kovacs reagent.
- A well isolated colony from a fresh bacterial culture was picked up by the help of inoculating loop and was rubbed on the pre-treated filter paper.
- Rubbed filter paper was observed for colour change (Table 3).

Table 1: Observation table of physiochemical properties of soil

SOIL SAMPLES	FOREST I	FOREST II	KITCHEN I	KITCHEN II
COLOUR	Black	Reddish	Brown	Light brown
ODOUR	Normal	Normal	Bad	Foul smell
SOIL TEXTURE	Clay	Clay	Sand	Sand
PH	7.0	7.5	6.0	5.5

Table 2: Colony Morphology

COLONY CHARACTERISTICS	ISOLATE 1	ISOLATE 2
Colony shape and size	Large and irregular	Circular and pin headed
Margin	Undulating	Entire
Elevation	Convex	Convex
Colour	White	Whitish or agar type
Texture	Dull	Smooth
Cell morphology	Bacillus	Staphylococcus

Table 3: Biochemical Test

Sl. No.	Name of the Test	Isolate-1(Forest Sample)	Isolate-2(Kitchen Sample)
1	Gram Staining	Gram +ve ,rod	Gram +ve , cocci
2	Catalase Activity	+ve	+ve
3	Oxidase Activity	+ve	-ve
4	Starch Hydrolysis	+ve	+ve
5	Nitrate Reduction Test	+ve	+ve
6	Methyl Red Test	-ve	+ve
7	V-P Test	+ve	-ve
8	Citrate use	+ve	+ve
9	TSI		
	Lactose	-ve	+ve
	Dextrose	+ve	+ve
	Sucrose	+ve	+ve
	H_2S production	+ve	+ve
10	Indole Production	-ve	-ve

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J. Triple Sugar Iron Agar Test

- At TSI media was prepared and autoclaved at 15lbs at 121°C for 15 minutes.
- Then agar stab was prepared and allowed to solidify.
- A well isolated colony was touched with a sterile loop.
- Inoculate TSI by first stabbing through the centre of the medium to the bottom of the tube and then streak the surface of the slant.
- Leave the cap loose and incubate the tube at 35°C for 18 to 24 hours.
- Observations were recorded.

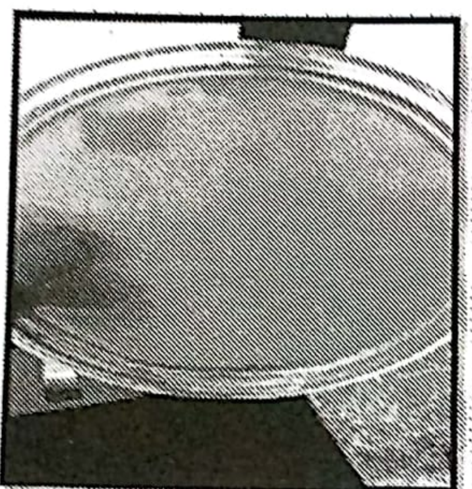

Fig. 1. Zone of clearence in cera plates of forest and kitchen soil sample

RESULTS AND CONCLUSION

The analytical results of physiochemical parameters of both kitchen and forest soil sample were present in table 1.

The soil samples underwent changes in all measured parameters of physiochemical properties in comparisons to control. The soil texture in terms of clay slit or sand is recorded. The above result indicates that the soil with plant residues had clay and slit in texture. This may due to microorganisms discharged into the soil sample through plant waste reduces the porosity of the soil resulting poor yields. All the samples were easily dissolved in water. The forest soils sample has a normal odour but the kitchen soil samples have foul smell. A total of two bacteria were isolated in these three different soil samples.

Subsequently two bacteria were isolated from the test soil by enrichment methods and morphology or biochemical of two cultures was analysed. Isolate1 was gram positive cocci and spore former, while Isolate 2 was gram negative rod shaped and non-spore former. The results of biochemical test were given in table 3.

Two isolates were identified as *Bacillus sp.* from forest soil sample and *Staphylococcus sp.* from domestic kitchen soil sample.

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Anti-Microbial Activity of Leaf Extract of Guava

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ABSTRACT

Besides the traditional methods of microbial control by chemical agents such as use of Phenols, Alcohols, Aldehydes, Heavy metals, Halogens, Quaternary Ammonium Compounds, Sterilizing Gases etc., plant organs such as leaves, roots, barks etc. or the extract being collected from them are seems to have great antimicrobial activity and are effective against a wide variety of bacteria as well as fungi. A recent research shows that leaf extract obtained from Guava (*Psidium guajava*) is effective against two gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*) and two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) which are some of food borne and spoilage bacteria. At first the guava leaves were extracted in four different solvents of increasing polarities (hexane, methanol, ethanol, and water). Effectiveness of these extracts were tested against those bacteria through a well-diffusion method employing 50 μL leaf-extract solution per well. According to the findings of the antibacterial assay, the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3 mm, and the ethanol extract had a mean zone of inhibition of 6.11 and 11.0 mm against *Bacillus cereus* and *Staphylococcus aureus*, respectively. On the basis of the present finding, guava leaf-extract might be a good candidate in the search for a natural antimicrobial agent. This study provides information about the effectiveness of natural things against microorganisms.

Keywords: Guava leaf extract, *Staphylococcus aureu, Escherichia coli, Bacillus cereus, Salmonella enteritidis*

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INTRODUCTION

Now a days a lot of attention focused on natural medicines and products. The extracts of some fruits and body parts of plants shows antimicrobial activity against gram positive as well as gram negative bacterial species. This clearly indicates that plant which manifest relatively high level of antimicrobial action may be source of some chemicals which has the capacity to inhibit the growth of foodborne pathogens. When microbial cells treated with those extracts, it can destroy the cell by irregular disruption of intercellular matrix or by rupture of cell wall and cell membranes.

Since the ancient time in our country the plant Guava (*Psidium guajava*), which is a phytotherapeutic plant used as a natural remedial ingredient belongs to family Myrtacae. Many parts of the plant have been used in traditional medicine to manage conditions like Malaria, Gastroenteritis, Vomiting, Diarrhoea, Dysentery, Wounds, Ulcers, Toothache, Coughs, Sore throat, Inflamed Gums and a numbers of other serious health disorders such as Diabetes, Hypertension and obesity.

In this study we tried three different procedures by which we can determine the ability of leaf extract of guava to eliminate the pathogenic microbial flora.

In our first procedure, we dissolved the powdered leaf with methanol and ethanol and applied it on gram positive and the gram negative bacterial population and it showed its inhibitory capability only on gram positive bacteria (*Staphylococcus aureus*) but doesn't showed any inhibitory effect on gram negative bacteria (*Escherichia coli*).

In our second procedure, we prepared different dilution of guava leaf juice and applied it on the culture of *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) and it again showed its antimicrobial activity against the gram positive one.

In our third and last procedure, we applied the boiled leaf extract of guava, on *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) and found that it showed clear zone of inhibition against *Staphylococcus aureus*.

MATERIALS AND METHODS

In our first procedure we prepared different solutions of guava leaf and applied it on the cultures of a gram positive and a gram negative bacteria.

Dry Leaf Extract

Procedure: The collected leaf samples were first washed with tap water followed by cleaning it with distilled water. Then the leaf samples were allowed to dry and placed into a mixer grinder to be turned into powder. Three solvents were prepared in increasing polarity; methanol, ethanol & boiled distilled water were used for the extraction procedure. The leaf powder then mixed with each solvent to make a 20% concentration. To avoid evaporation, we prepared the mixture in sterile screw cap tube. Then the mixtures were allowed to stand for 3 days at room temperature without being exposed to light. After 3 days of soaking in the solvent, the mixtures were transferred to Eppendorf tubes. First we centrifuged it at 1000 rpm for 15 minute. After collecting the supernatant we centrifuged it again at 4000 rpm for 5 minute. It is then applied on MH agar media to check the antibiotic sensitivity of the extract by agar cup method and incubated it for 24 hours.

Agar Cup Method: At first we prepared a bacterial lawn by using two types of bacterial culture, one is *Staphylococcus aureus* and another is *Escherichia coli* in 12 petri dishes and cut the agar cup. We applied the centrifuged, non-centrifuged and water based solution in the agar cups.

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Result: After 24 hours of incubation period we got visible clear zones on dishes. The results are as follows-

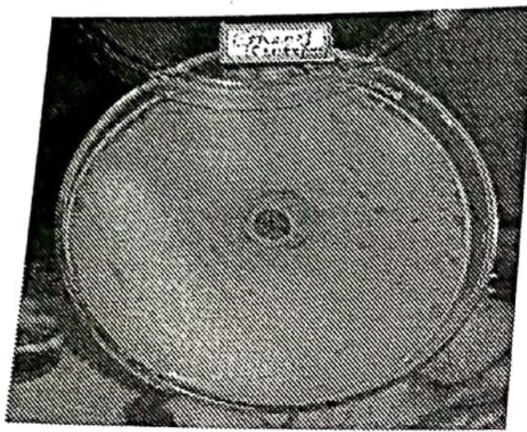

Fig. 1.1. Ethanol (centrifuged)

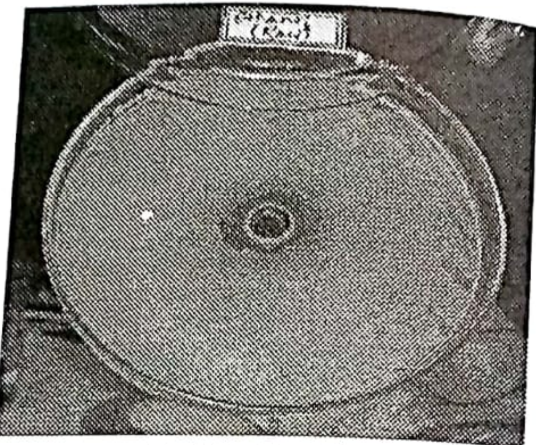

Fig. 1.2. Ethanol (raw)

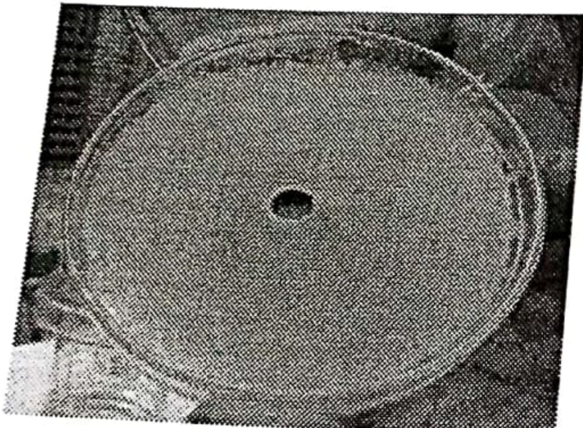

Fig. 1.3. Control

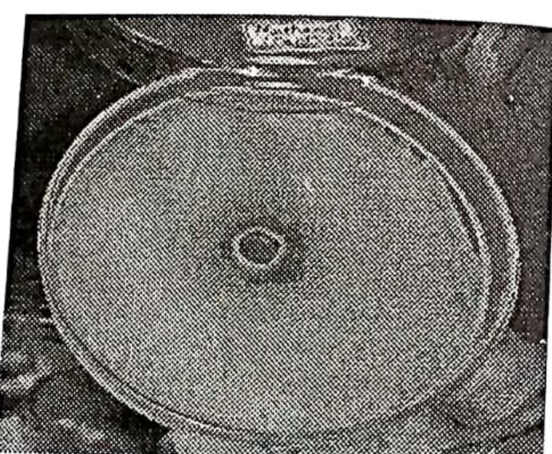

Fig. 1.4. Methanol (centrifuged)

Fig. 1.5. Methanol (raw)

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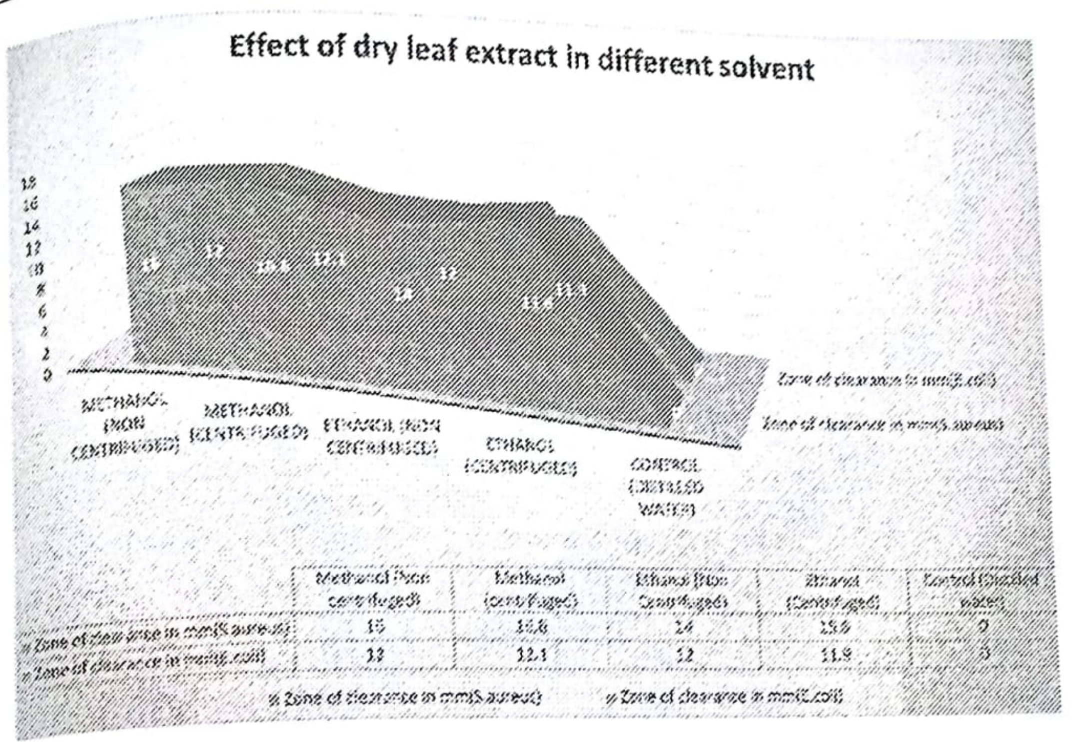

Fig. 2. Graphical representation of procedure 1

Boiled Leaf Extract

Procedure: Second procedure is the making of leaf extract by boiling the cleaned leaf at 90°C. After collecting the leaf we washed it. Then we boiled it at 90°C for 60 minute. Leaf extracts (1ml) were collected (in screw cap tube) at several time serially from 15 minute, 20 minute, 25 minute, 30 minute, 35 minute, 40 minute, 45 minute, 50 minute, 55 minute and 60 minute.

A. In Broth Culture:

Procedure: After preparing the MH media we distributed it in 21 test tubes (20 for culture & 1 for control) each with 9 ml of media and autoclaved it. After autoclaving we added 0.5ml of boiled leaf extract with increasing time and add 0.5 ml of inoculum of *Staphylococcus aureus* & *Escherichia coli* in 20 test tubes (10 for *Staphylococcus aureus*, 10 for *Escherichia coli* and one for control) followed by incubating it for 24 hours.

Result: After 24 hour incubation we measured the O. D. value of all the 20 test tubes. In the E. coli culture, the growth is unaffected but in the S. aureus culture we got gradually decrease O. D. value as per the increasing time.

Observation: When we got the result, in case of *S. aureus* O. D. decreases gradually, it means that the bacterial growth decreases. And the boiled leaf extract shows antibacterial activity. So for confirmation we increase the time interval. We done this procedure in solid media (MH media- Muller Hinton Media).

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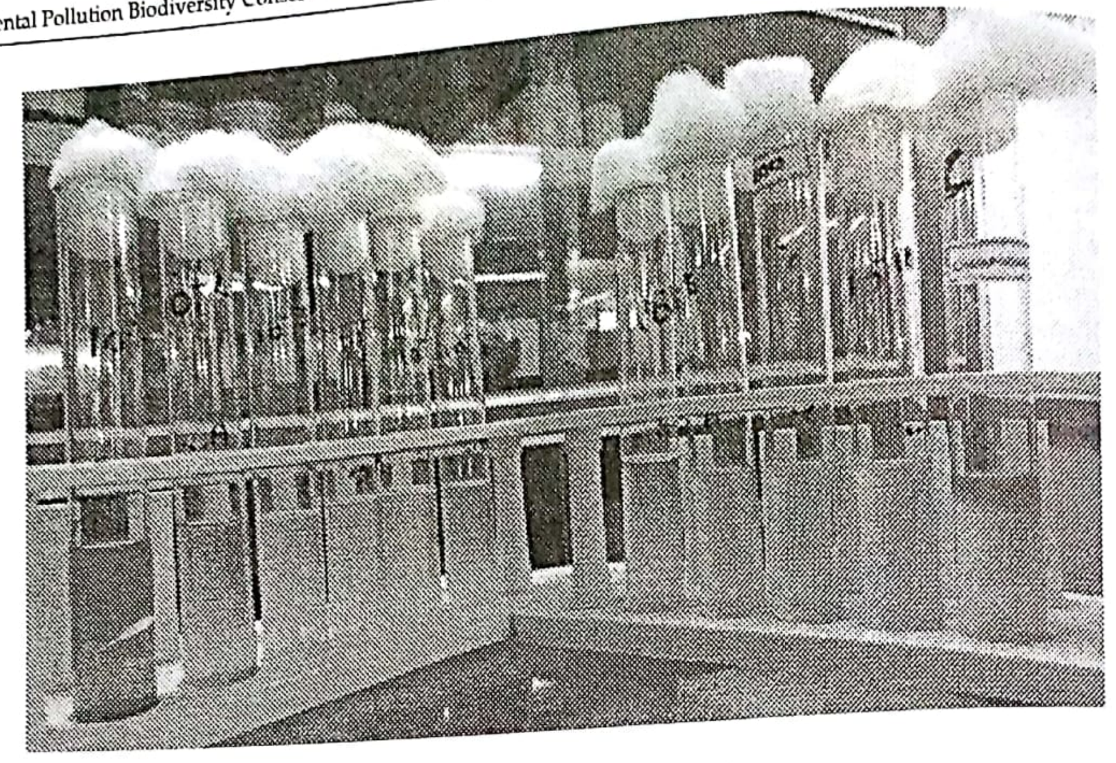
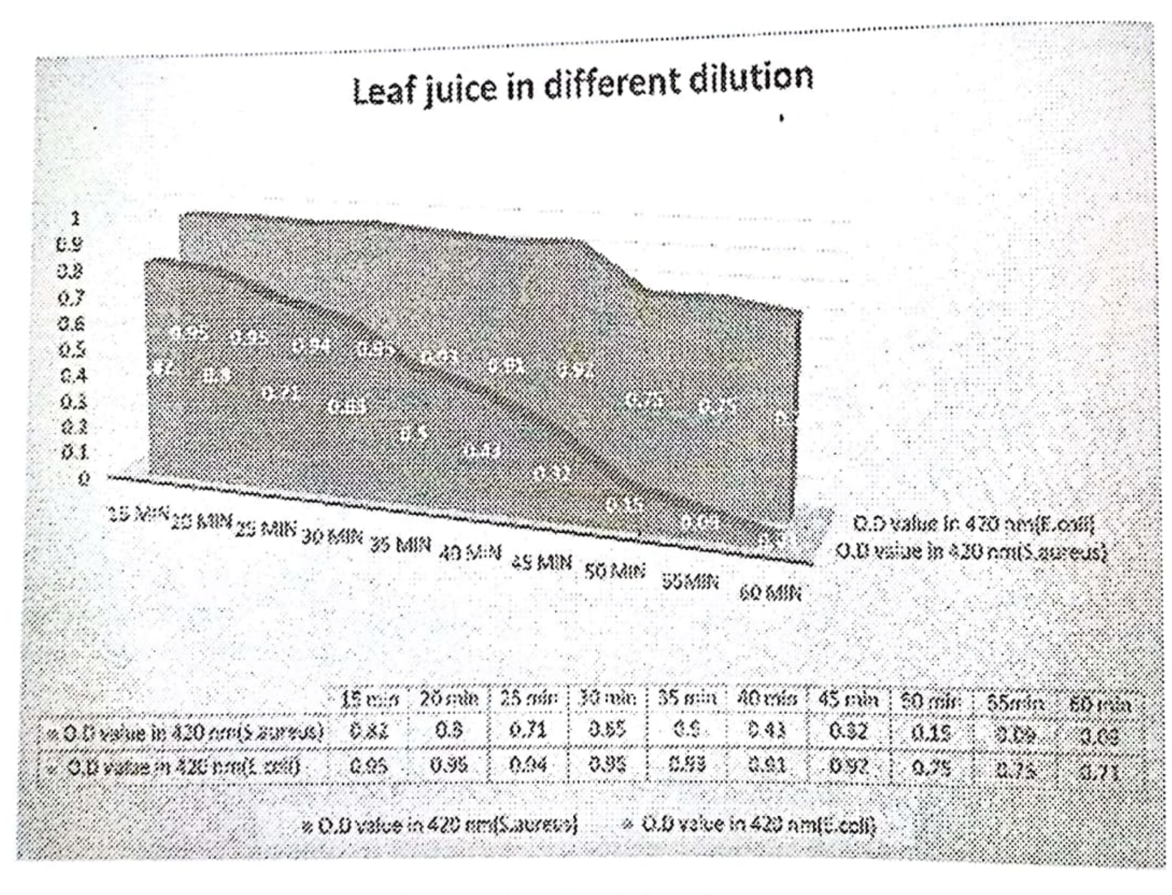


Fig. 3. Different dilution of leaf juice



	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min	55min	60 min
O.D value in 420 nm(S.aureus)	0.82	0.8	0.71	0.65	0.5	0.43	0.32	0.15	0.09	0.03
O.D value in 420 nm(E.coli)	0.95	0.95	0.94	0.93	0.93	0.91	0.92	0.75	0.75	0.71

Fig. 4. Graphical representation of procedure 2

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B. In Agar Plate:

Procedure: In case of agar plate at first we prepared 8 MH agar plate. And also prepared 4 different concentrations of boiled leaf. Now we increase the time interval and take the extract at 30th minute, 60th minute, 90th minute and 120th minute. To ensure the antibiotic effect we applied the "agar cup method" and incubating it for 24 hours.

Agar Cup Method: At first we prepared a bacterial lawn by using two types of bacterial culture, one is *Staphylococcus aureus* and another is *Escherichia coli* in 8 petri dishes and cut the agar cup. We applied 4 different concentrations according to their increasing time.

Result: After 24 hours of incubation period we got the clear zones. The results are as follows

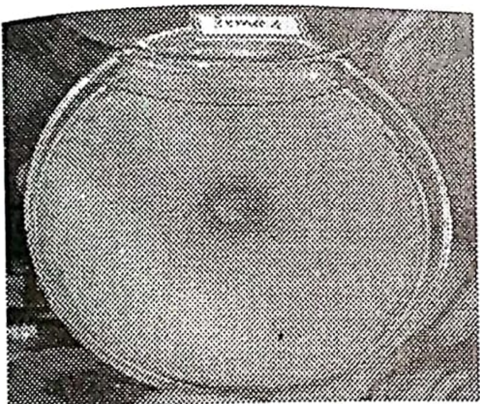

Fig. 5.1. 30 Minute

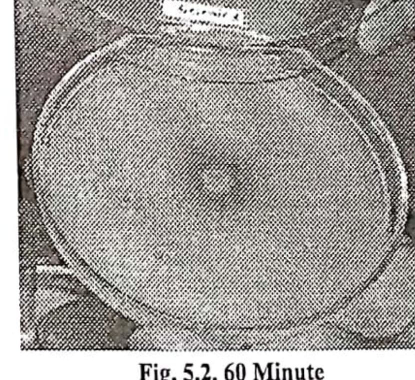

Fig. 5.2. 60 Minute

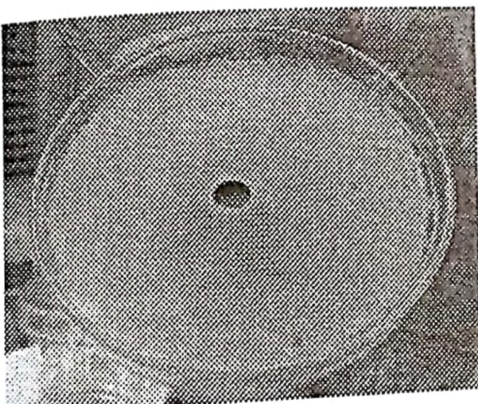

Fig. 5.3. Control

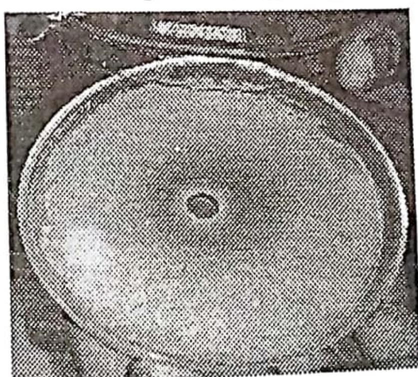

Fig. 5.4. 120 Minute

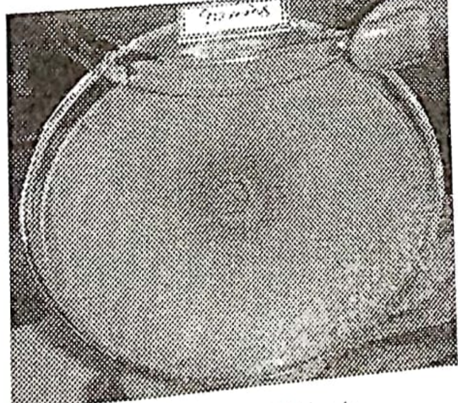

Fig. 5.5. 90 Minute

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Observation: After 24 hours of incubation we got clear zone on the plates. That's clearly indicate the antimicrobial activity of guava leaf. The zone of inhibition become larger as per the increase in time. We got better zone of inhibition in case of boiling the leaf for 120 minute but wasn't got anything in case of 30 minute of boiling.

DISCUSSION

Our results support the findings of antimicrobial activity which was seen with a methanol extract than with other extracts. In table 1 we prepare 20% solution of Methanol, Ethanol & Boiled distilled water and by agar cup method we get clear zone. The higher clear zone we obtain from the centrifuged extract of Methanol that means it shows the highest antimicrobial activity and other clear zone obtained which are 16mm for non-centrifuged methanol extract, 15.6 mm for centrifuged Ethanol extract then 14 mm for non-centrifuged ethanol extract. The water based solution did not show any antimicrobial activity.

The results in table 2 (O. D.) indicates that the leaf extract shows antimicrobial activity because the growth of bacteria decreases with increasing time interval of boiling. When we repeated this process with more time interval on agar plate we got more satisfactory result which is mentioned in table 3.

As we know *S. aureus* is responsible for food poisoning so if a person suffering from this type of problem we can suggest him to consume juice of guava leaf. It also effective against causative agent of diarrhoea and dysentery. Due to their anti-inflammatory qualities, fresh guava leaves can relieve tooth ache, heal gum and mouth sores and treat sore throats when used for gargling. Guava leaves are also considered as a natural remedy for dengue fever because guava leaf extract can increase the number of platelets. It also useful for lowering the blood cholesterol, losing of weight, treatment of prostate cancer and natural remedy for hair loss. From ancient time guava leaves are useful for treating wounds and infections.

CONCLUSIONS

The present work demonstrates the antimicrobial potential of *Psidium guajava* leaves extract by using various solvents. The results indicate that ethanol and methanol are better than boiled distilled water for the extraction of the antibacterial properties of guava. The centrifuged ethanol and methanol extracts are better than non-centrifuged ones. The results also indicate that the plant extracts have no antibacterial effect on the Gram-negative bacteria, showing that they do not contain active ingredients against the organisms. The observed inhibition of Gram-positive bacteria *Staphylococcus aureus*, suggests that guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using methanol or ethanol as the solvent. Comparisons with related data indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of guava. On the basis of the present finding, *P. guajava* leaves possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections and/or diseases caused by *Staphylococcus aureus*.

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