

Evaluation of antimicrobial activity of different powders using natural micro flora

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Abstract

Plants integrate many synthetic mixtures for capabilities including protection against bugs, organisms, sicknesses, and herbivorous warm-blooded animals. Plants are created to supply man with food, medical treatment, and other effects. A spice is a plant or plant part utilized for its fragrance, flavour, or remedial properties. Natural drugs are one kind of dietary enhancement. Individuals utilize natural drugs to attempt to keep up with or work on their wellbeing. The herbal powder extract can be used as effective Anti-microbial agent. Antimicrobial potential in the above-mentioned herbal powder attracted the attention of the agricultural industry with herbal powder being considered for the control of postharvest pathogens. Application of powdered extract on fruits and vegetables may prevent spoilage.

Keywords: Synthetic Mixture; Medical Treatment; Anti-Microbial Agent; Pathogens

1. Introduction

Plants integrate many synthetic mixtures for capabilities including protection against bugs, organisms, sicknesses, and herbivorous warm blooded animals. Plants are created to supply man with food, medical treatment, and other effects⁽¹⁾. Medicinal plants contain active compounds which are responsible for the curing of acute to chronic diseases hence they are used in our traditional medicine system.

Ever since ancient times, in search for rescue for their disease, the people looked for drugs in nature⁽³⁾. Considering the way that at the time there was not adequate data either concerning the purposes behind the sicknesses or concerning which plant and how it very well may be used as a fix, everything depended on experience. In time, the purposes behind the utilization of explicit restorative plants for treatment of specific sicknesses were being found; consequently, the therapeutic plants use bit by bit deserted the empiric system and became established on explicatory realities. Regardless, the diminishing adequacy of engineered drugs and the rising contraindications of their use make the utilization of normal medications skin once more. Restorative plants are broadly utilized in non-industrialized social orders, primarily on the grounds that they are promptly accessible and less expensive than present day prescriptions.

1.1. Herbal medicine

A spice is a plant or plant part utilized for its fragrance, flavor, or remedial properties. Natural drugs are one kind of dietary enhancement. Individuals utilize natural drugs to attempt to keep up with or work on their wellbeing. A couple of flavors can Microorganisms - by and by on occasion called "eubacteria" or "real tiny creatures" to isolate them from archaeobacterial - are the kind of microorganism you probably find out about the most. This is on the grounds that they're the sort probably going to make you debilitated. Microbes are the reason for most skin diseases, and can likewise cause food contamination, pneumonia, strep throat, and numerous other illnesses.(4-7)

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1.1.1. Archaea

Archaea, or archaeobacteria, were once remembered to be essential for the microorganism's family. Regardless, progressing assessment has shown that they are immeasurably unique in relation to eubacteria, and may attempt to be more immovably associated with us than they are to introduce day microorganisms.

1.1.2. Protozoa

Protozoa are a different gathering of unicellular eukaryotic life forms. Like microorganisms and archaea, they are single-celled; yet their cells seem to be those of animals and plants more than those of organisms or archaea.

1.1.3. Fungi

A fungus is a eukaryote that digests food externally and absorbs nutrients directly through its cell walls. Most parasites recreate by spores and have a body (thallus) made out of minute cylindrical cells called hyphae.

1.1.4. Molds

Molds are microorganisms that share a few properties of parasites, yet are false growths. These incorporate pathogenic molds that taint plants and have caused wrecking crop disappointments, for example, the Incomparable Irish Starvation of the 1840.

1.1.5. Algae

Minute green growth were once remembered to be plants, yet late examinations have shown that green growth don't squeeze into the plant family. All things being equal, these single-celled photosynthetic creatures are believed to be family members of the genealogy that prompted land plants.

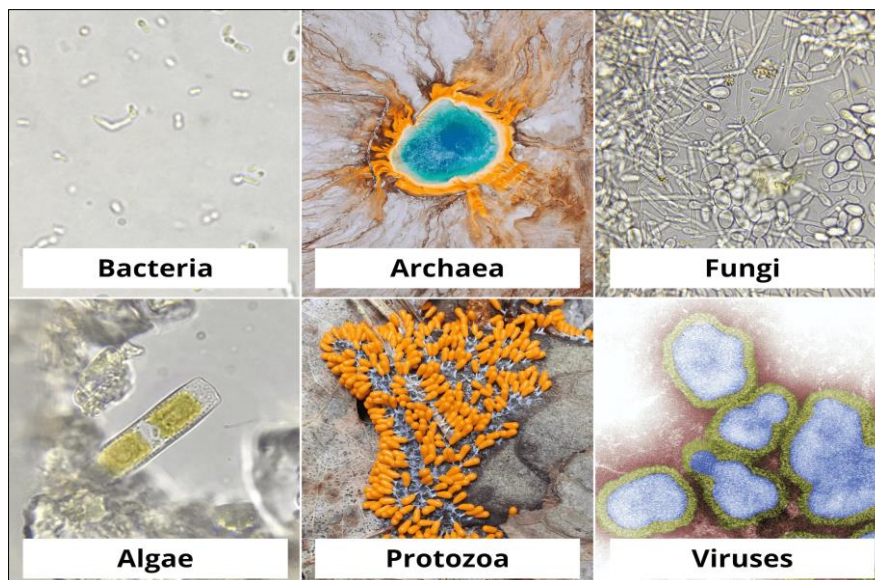


Figure 1 Different types of bacteria

2. Diseases caused by microorganisms

2.1. In fungi

2.1.1. *Pencilium notatum*

Sporadically in people they produce contaminations of the outside ear, skin, or respiratory section ways. They are normal allergens which cause fundamental mycosis with fever and neutropenia. Albeit human diseases with *P. notatum* are very uncommon, clinicians ought to realize that detachment of this microorganism in resistant compromised host could show the presence of serious parasitic contamination.

2.1.2. *Aspergillus niger*

Aspergillosis is an illness brought about by *Aspergillus*, a typical form (a sort of organism) that lives inside and outside. The vast majority take in *Aspergillus* spores consistently without becoming ill.

2.1.3. *Verticillium alboatrum*

Inside, staining or streaking of the sapwood (xylem vascular tissue) happens in many plants. The sapwood staining might show up as a striping of the wood when seen on a branch with the bark stripped away.

2.1.4. *Cladosporium*

Cladosporium shape is a typical parasites animal varieties you can see as both indoor and open air. Its ubiquitous presence is generally harmless, but unfortunately, there are still several side effects that can happen when people are in contact with the spores.

2.2. Neem

Neem (*Azadirachta indica*) is seen as a helpful plant eminent for its antibacterial, antimalarial, antiviral, and antifungal properties. Neem nanoemulsion (NE) (O/W) is figured out utilizing neem oil, Tween 20, and water by high-energy ultrasonication. The figured out neem NE showed antibacterial action against the bacterial microorganism *Vibrio vulnificus* by upsetting the uprightness of the bacterial cell layer. Notwithstanding the utilization of neem NE in different biomedical applications, the poisonousness concentrates on human cells are as yet deficient.

2.2.1. Antibacterial movement

+Bacterial culture The bacterial culture of *V. vulnificus* was bought from Microbial sort cell culture. Toxicity studies: Isolation of human lymphocytes The human lymphocyte cells were isolated from blood using lymphocyte separation media.⁽⁹⁾

2.2.2. Membrane integrity and cell damage

The antibacterial activity of NE was further confirmed by the film uprightness and cell harm appraisals of the microorganisms. The film uprightness of the NE was concentrated on in *V. vulnificus* after exposing it to 150 µg/mL NE at 0, 3, 6, 9, membrane integrity assessment on bacteria.^(10,11)

2.2.3. Cytotoxicity of neem NE

The human lymphocytes assume a significant part in the protection component of disease, and the refined human lymphocytes were utilized to concentrate on the in vitro harmfulness evaluation of the nanoparticles⁽¹²⁾. The cytotoxicity of NE was evaluated in human lymphocytes by measuring the percentage of viable cells after 24 hours of interaction.

2.3. Pomegranate

Punica granatum (Pg), commonly known as pomegranate (Pg), is a member of the monogeneric family Punicaceae. *Punica granatum* (Pomegranate) is a small tree which measures between five and eight meters tall and mainly found in Iran, the Himalayas in northern India, China, USA and throughout the Mediterranean region⁽¹³⁾

2.3.1. Chemical properties

Synthetic parts have different pharmacological and toxicological properties including cell reinforcement, mitigating (by hindering favorable to fiery cytokines), and hostile to disease and against angiogenesis exercises.

2.3.2. Antioxidant properties

Oxidative stress (OS) produces toxic metabolites which can initiate and promote cancers. Consumption of polyphenoles and flavonoids are beneficial for the prevention of cardiovascular, inflammatory, and other diseases by preventing OS that ages and in lipoproteins^(14,15)

2.3.3. Carcinogenesis

Pomegranate possesses inhibitory effects on different type of cancers such as prostate^(17,18) breast⁽¹⁹⁾, colon^(20,21) and lung cancers⁽²²⁾. Various components have been framed for pomegranates hostile to disease exercises in these examinations-

2.3.4. Anti-mutagenicity

A mutagen is a physical or chemical agent that alters the genetic material of an organism, usually DNA, permanently and thus increases the frequency of mutations above the natural background level. Mutagenicity is the capacity of a chemical or physical agent to cause such permanent change⁽²³⁾.

2.4. Onion

Onion or *Allium cepa* (*A. cepa*) is one of the main topping plants developed and consumed everywhere. Various impacts of onion might be because of many constituents, including quercetin, thiosulphinates and phenolic acids which have frequently been accounted for from this plant. The primer phytochemical examinations have showed that onion contains water, starches (for example inulin, fructooligosaccharides, isorhamnetin-4-glucoside, galactose, glucose and mannose), proteins, vegetal hormone. There are different flavonoids in onion skin, including quercetin aglycone, quercetin diglucoside, quercetin 4-glucoside and isorhamnetin monoglycoside or kaempferol monoglycoside⁽²⁴⁾.

2.5. Orange peel

Citrus vulgaris, citrus bigaradia, citrus aurantium amla, Biga-rode orange, bitter orange, Seville orange, (sweet) Portugal orange, china orange citrus dulcis, cortexaurantiamar L, Seville orange peel.

2.5.1. Chemical constituents

Bitter orange peel contains of 1-2.5% volatile oil. The principle component of volatile oil is 90% limonene and small quantities of aldehydes citral, citronellal, bitter, amorphous, glycoside like aurantiamarin and its acids, hesperidin, isohesperidin, vit c and pectin. Although citrus peels are not edible, they possess significant biological activities including antimicrobial, antioxidant and anti-cancer activities. This antioxidant activity is due to the presence of a number of bioactive components in citrus such as phenolic compounds, limonoids, flavonoids and polysaccharides scavenging single oxygen, hydroxyl radicals, and lipid peroxyl radicals⁽²⁵⁾.

2.6. Guava

The guava (*Psidium guajava*) is a phytotherapeutic plant utilized in people medication that is accepted to have dynamic parts that assistance to treat and oversee different illnesses. The many pieces of the plant have been utilized in customary medication to oversee conditions like jungle fever, gastroenteritis, regurgitating, looseness of the bowels, diarrhea, wounds, ulcers, toothache, hacks, sore throat, excited gums, and various other conditions (27).

It is powerful against killing or repressing the development of foodborne bacterium *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, and *Bacillus cereus* which can cause food borne disease and waste.

The component by which they can hinder the microorganisms can include various methods of activity. Sanches et al. Vieira et al. have likewise detailed the antibacterial impact of guava leaves concentrates and tracked down that they hindered the development of the *S. aureus*. Gnan and Demello testing guava leaf extricate found great antimicrobial movement against nine unique types of *Staphylococcus aureus*.

2.7. Castor seeds

Castor plant, (*Ricinus Communis* L.), is an animal group's offlowering plant in the spurge family, Euphorbiaceae. Albeit, the plant is local to the Ethiopian area of tropical east Africa it has become naturalized in both tropical and warm mild districts all through the world.

The oil and its derivatives are used in the production of germicides, insecticides and as raw material in the manufacturing of various chemicals such as sebacic and undecylenic acids used in the production of plasticizer and nylon. It is, also, used in production of bio-diesel, chocolate bar (in processed form) and its toxin provides the castor oil plant with some degree of natural protection from insect pests. Global castor seed production is around 1.2 million- tonnes per year (FAO, 2008).

Uses

Castor seeds without the body are utilized for anti-conception medication, clogging, disease, and syphilis.

2.8. Cinnamon

Cinnamon basically contains crucial oils and different subordinates, for example, cinnamaldehyde, cinnamic corrosive, and cinnamate. As well as being a cancer prevention agent, calming, antidiabetic, antimicrobial, anticancer, lipid-bringing down, and cardiovascular-infection bringing down compound.

One of the major constituents of essential oil extracted from while cinnamaldehyde is the principal compound responsible for this activity. Cinnamon bark contains procyanidins and catechins ⁽²⁹⁾. The components of procyanidins include both procyanidin A-type and B-type linkages. These procyanidins separated from cinnamon and berries additionally have cell reinforcement exercises

2.8.1. Chemical Constituents

Cinnamon comprises of different resinous mixtures, including cinnamaldehyde, cinnamate, cinnamic corrosive, and various natural ointments . Singh et al. reported that the fiery taste and aroma are because of the presence of cinnamaldehyde and happen because of the assimilation of oxygen.

2.8.2. Antidiabetic Activity

A substance from cinnamon has been segregated and instituted as "insulin-potentiating factor" (IPF). A few examinations have likewise uncovered that cinnamon separates lower blood glucose as well as cholesterol levels.

2.8.3. Antimicrobial Activity

Until this point in time, a few antimicrobial exercises of cinnamon and its oils have been accounted for in different examinations. The impacts of cinnamon oils on various bacterial (*Pediococcus halophilus* and *Staphylococcus aureus*), contagious (*Aspergillus flavus*, *Mucor plumbeus*, *Penicillium roqueforti*, and *Eurotium* sp.), and yeast species (*Candida lipolytica*, shows that cinnamon is a characteristic antimicrobial agent⁽³⁰⁾.

2.9. Tomato

The spoiled tomato samples collected from three different samples were found to be severely infected with fungi with *Aspergillus niger*. and *Fusarium* sp. to be predominantly present in all the spoiled samples with some plates containing *Penicillium* as well .The Nutrient Agar plates were having scanty growth of bacteria and predominately occupied by the above two major fungi⁽³¹⁾.

The Nutrient agar plates were predominantly occupied by fungi and very scanty growth of bacterial in 3-4 plates. Hence we can conclude that fungi with *Aspergillus niger*, *Fusarium* and *Penicillium* to be the major cause of damage to the tomato fruit. Further, these fungi are a source of potent mycotoxins which exhibits a wide spectrum of diseases which can even be fatal. *niger* is a wellspring of ochratoxin while *Fusarium* produces Trichothecenes. Both these toxins have detrimental effect in humans which can even be fatal.

2.10. Bread

Microbes are organisms that are too small to see without the use of a microscope.

The microbe can be found in its single-celled form or as a colony of cells. There are five major kinds of microbes namely Viruses, Bacteria, Archaea, Fungi, and Protists.

2.11. Banana

Numerous microorganisms have been segregated from banana, really spoilt banana.. These micro-organisms have been associated with many diseases caused by the bacterium, *pseudomonas solanacearum*, resulting in internal decay. It is transmitted by insects, machetes and with the roots of sick plants. There are said to be 4 different types transmitted by different means. It also contains *latctobacillus*, *leuconostoc*.

2.12. Cucumber

The adhesion of bacteria to the fruit in wash water was less extensive at lower temperatures and shorter exposure times. Various species of bacteria were adsorbed to cucumber surfaces in the following relative order: Salmonella Typhimurium > Staphylococcus aureus > Lactobacillus plantarum > Listeria monocytogenes

	Spoilage Agents	Properties of Colony
Moulds	<i>Penicillium</i> spp.	Blue/green, flat, spread rather slowly
	<i>Aspergillus niger</i>	Black, fluffy, spreading with sporeheads often clearly visible
	<i>Aspergillus flavus</i>	Olive green
	<i>Aspergillus candidus</i>	Cream
	<i>Aspergillus glaucus</i>	Pale green
	<i>Cladosporium</i> spp.	Dark olive green, flat, spread slowly
	<i>Neurospora stophila</i>	Salmon pink, fluffy and fast spreading
	<i>Rhizopus nigricans</i>	Grey/black, very fluffy and fast spreading
	<i>Mucor</i> spp.	Grey
Bacteria	<i>Bacillus subtilis</i> or <i>Bacillus licheniformis</i>	Irregular shape, white and dull colour
Yeasts	<i>Hyphopichia burtonii</i>	Slow growth on bread surface, low, white, spreading colonies
	<i>Pichia anomala</i>	
	<i>Scopsisfi buligera</i>	
	<i>Pichia burtonii</i>	Very fast growth on bread
	<i>Zygosaccharomyces bailii</i>	Smooth, round, convex and white to cream coloured
	<i>Torulaspota delbrueckii</i>	
	<i>Pichia membranifaciens</i>	
<i>Candida parapsilosis</i>		

Figure 2 Organisms grown on bread

Two bacteria cause the disease:

Erwinia tracheiphila and *Curtobacteriumflaccumfaciens*sp

2.13. Bitter guard

Sphaerotheca fuliginea,

*Fusarium oxysporum*f.sp.niveum

Pseudoperonosporacubensi.

Aim and objectives

The main objective of the present study was to “Evaluate the antimicrobial activity of different powders using natural micro flora.”

Depending on the extent of literature available on these powders, the present work is planned with the following objectives:

- Selection of powders based on their antimicrobial action.
- Preliminary phytochemical investigational of different powders.
- Estimation of phytochemical constituents.
- Antimicrobial activity using cup plate method.

2.14. Phytochemical constituents

2.14.1. Onion

Table 1 list of onion activities

Constituent	Activity
Organosulfur compound	Anti –carcinogenic
Poly phenols	Anti –oxidant activity
Quercetin	Anti –oxidant
Anthocyanins	Anti - microbial

2.14.2. Neem

Table 2 list of neem activities

Constituent	Activity
Nimbin	Anti – feedent activity
Nimbidin	Anti – bacterial activity
Nimbidol	Anti- pyretic activity
	Anti- fungal activity
	Anti-ulcer activity
	Anti bacterial activity

2.14.3. Guava

Table 3 list of guava activities

Constituent	Activity
Quercetin	Anti –viral
	Anti – inflammatory
	Anti – plaque
	Anti - mutagenic
Apigenin	Anti – proliferative activity against carcinoma cells
Terpenoids	Potent Anti- microbial activity

2.14.4. Orange peel

Table 4 list of orange peel activities

Constituent	Activity
Citral	Anti – microbial activity
Vit C	Anti – microbial activity
Citronella	Flavouring agent
Limonene	Anti – bacterial activity

2.14.5. *Cinnamon***Table 5** list of cinnamon activities

Constituent	Activity
Sulfides	Anti – bacterial activity
Eugenol	Anti – microbial activity
cinnamaldehyde	Bactericidal action

2.14.6. *Pomegranate***Table 6** list of pomegranate activities

Constituent	Activity
Luteolin	Antimicrobial activity
Steroids	Anti – microbial activity
terpenoids	Potent Anti- microbial activity
gallic acid	Anti –oxidant activity
punicalins	Anti –oxidant activity
punicalagin	Anti –oxidant activity
ellagitannin	Anti –oxidant activity

2.14.7. *Castor seed***Table 7** List of castor oil activitie

Constituent	Activity
Linoleic acid	Anti – bacterial activity
Leucine	Anti – bacterial activity
steroids	Anti – microbial activity
terpenoids	Potent Anti- microbial activity

3. Materials and methods
3.1. Materials**Table 8** List of materials and chemicals used

S.no	Name of material and chemical used
1	Dragendroff's reagent
2	Mayer's reagent
3	Wager's reagent
4	Hager's reagent
5	ammonia solution
6	chloroform

7	5 % FeCl ₃ solution
8	dilute hydrochloric acid
9	pyridine
10	Sodium Nitroprusside solution.
11	70% ethanol
12	lead acetate
13	glacial acetic acid
14	Concentrated sulphuric acid.
15	gelatin solution,
16	Acetic anhydride.
17	sodium hydroxide
18	KmNo ₄
19	Distilled water

3.2. Methods

3.2.1. Agar preparation

Nutrient broth

- Put the weighed amount of ingredients in 500ml distilled water
- Heat with agitation to dissolve the constituents
- Add more distilled water to 1 liter
- Adjust the P^H of medium to 7 by using PH meter on by adding alkaline.
- Pour 10 ml per tube
- Apply cotton plug
- Autoclave at 121°C, 15Lb pressure for 15-20 min.
- Remove the nutrient broth tubes and store at room temperature for further use, cover the tubes with butter paper.

Table 9 Ingredients used in preparation of agar ⁽⁴¹⁾

Ingredients	Quantity
Peptone	5 gm
Sodium chloride	5 gm
Beef extract	3 gm
Agar	20 gm
Distilled water	1000 mL

PH adjusted to 7.2

3.3. Streak plate method

Clean every one of the instruments, jars and media that are expected for the streaking procedure. Clean your workspace utilizing a sanitizer to painstakingly limit any contamination. Set up the bunsen burner in your workspace. Clean up with a germ-free arrangement prior to taking care of any microbial arrangement. Mark the petri dish with terrifically significant data, for example, your name, date, media utilized and the way of life being inoculated. To get the example, you can utilize either a metal circle or expendable plastic loops. A loopful of test is streaked on the main quadrant in an ever changing movement on the agar plate. Sterilise the circle by warming it in the bunsen burner if utilizing a metal

loop. Streak the other three quadrants by a comparable method. Close the cover of the plate subsequent to streaking, and store the dish upside down in an incubator with ideal temperature.

3.4. Test for alkaloids

3.4.1. Dragendroff's test

Add Dragendroff's reagent (potassium bismuth iodide solution) to the extract. Orange red precipitate is formed.

3.4.2. Mayer's test

To the 1 ml of extract add 1ml of Mayer's reagent (potassium mercuric iodide). Cream coloured precipitate is formed.

3.4.3. Wager's test

To the 1 ml of the extract add 2 ml of Wager's reagent (iodide in potassium iodide) formation of reddish brown precipitate indicates the presence of alkaloids.

3.5. Test for glycosides

3.5.1. Brontrager's test

A few ml of dilute were added to the extract solution, boiled, filtered and treated the filtrate with chloroform and shaken well. Then separated the chloroform layer and treated with a few ml of ammonia solution. The ammonical layer gets pink or red colour.

3.5.2. Modified brontrager's test

Add a few ml of 5 % FeCl_3 solution and dilute hydrochloric acid to the extract. Then boiled for 5 min, cooled and shaken well with organic solvent. Then add equal quantity of dilute ammonia. The ammonical layer acquires pinkish red colour.

3.5.3. Legal's test

Add extract and dissolved with pyridine and made alkaline with sodium

Nitroprusside solution. The solution becomes pink or red.

3.6. Test for tannins

Diluted small quantities of extracts with distilled water and subjected to.

3.6.1. Ferric chloride test

Extracts treated with ferric chloride solution, blue colour.

3.6.2. Gelatin test

Extracts treated with gelatin solution, white precipitate.

3.6.3. Lead acetate test

Extracts treated with lead acetate solution, yellow precipitate.

3.7. Test for saponins

3.7.1. Foam test

Add small quantity of extract add 20 ml of distilled water, shaken in a graduated cylinder for 15 minutes, 1 cm layer of foam develops.

3.7.2. Hemolytic test

Add drug extract or dry powder to 1 drop of blood, placed on the glass slide. Hemolytic zone appears.

Pomogranate and onions does not pass the above tests they do not contain saponins.

3.8. Test for terpenoids

3.8.1. Liberman-burchard test

Drug is blended in with acidic anhydride. To this blend conc. Sulfuric corrosive is added. There structures two layers with sautéing at intersection. Upper Layer with green tone address steroids though lower layer address Terpenoid red tone.

3.8.2. Salwoski test

Drug is blended in with con. Sulfuric corrosive. Upper layer is of steroids which are red in variety and lower layer yellow variety layer address tri terpenoid. Pomegranate don't shows precise outcomes with above tests so it needs terpenoids.

3.9. Test for flavanoids

3.9.1. Shinoda test

Shinoda test to dry powder or concentrate, add 5 ml of 95% ethanol, scarcely any drops of conc. HCl and 0.5 g magnesium turnings Pink colour observed.

3.9.2. General test

To small quantity of residue, add lead acetate solution. Yellow coloured precipitate is formed. Expansion of expanding measure of sodium hydroxide to the buildup show yellow hue, which decolouration after expansion of corrosive.

3.10. Test for phenols

3.10.1. Ferric chloride test

Test extract were treated with 4 drops of alcoholic FeCl_3 solution. Formation of bluish black colour indicate the presence of phenol.

3.11. Test for oxalic acid

Take the sample and dissolve. Test with litmus paper to identify its acidic nature. To the above sample add KMnO_4 . This results decolouration of above KMnO_4 sample solution, this indicates presence of oxalic acid.

4. Results

4.1. Estimation of Phytochemical Constituents

Table 10 Estimation of phytochemical constituents

Chemical constituents	Pomegranate	Guava	Neem	Castor oil	Onion	Cinnamon	Orange peel
Alkaloids	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+
Saponins	-	+	+	+	-	+	+
Terpenoids	-	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+
Oxalic acid	+	+	+	+	+	+	+

PRESENT + ABSENT -

4.2. Control



Figure 3 Penicillin standard

4.3. Bitter guard

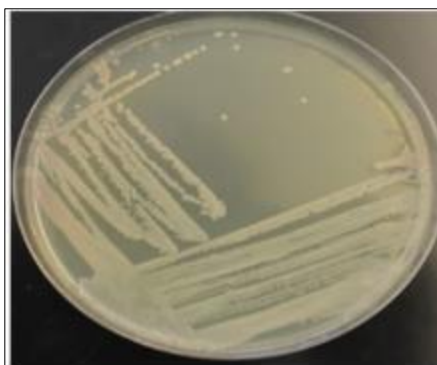


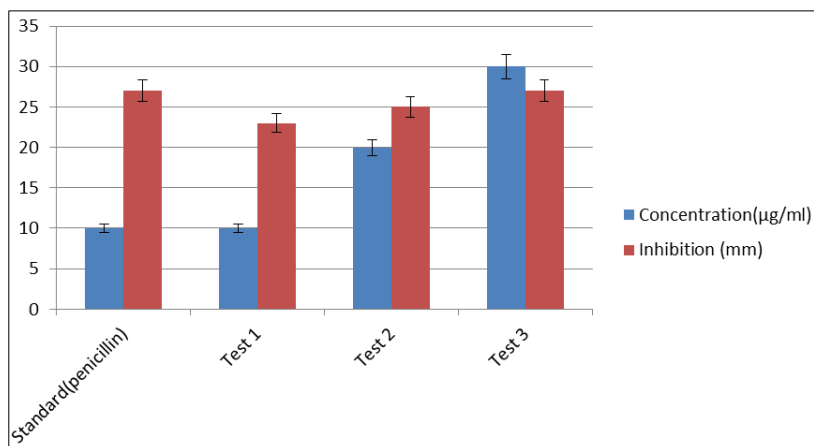
Figure 4 Bitterguard control



Figure 5 Bitterguard zone of inhibition

Table 11 Results showing zone of inhibition in Bitter Guard by cup plate method

Sample	Concentration($\mu\text{g/ml}$)	Inhibition (mm)
Standard(penicillin)	10	27
Test 1	10	23
Test 2	20	25
Test 3	30	27



Graph 1 Graphical representation of zone of inhibition of Bitter Guard

4.4. Tomato



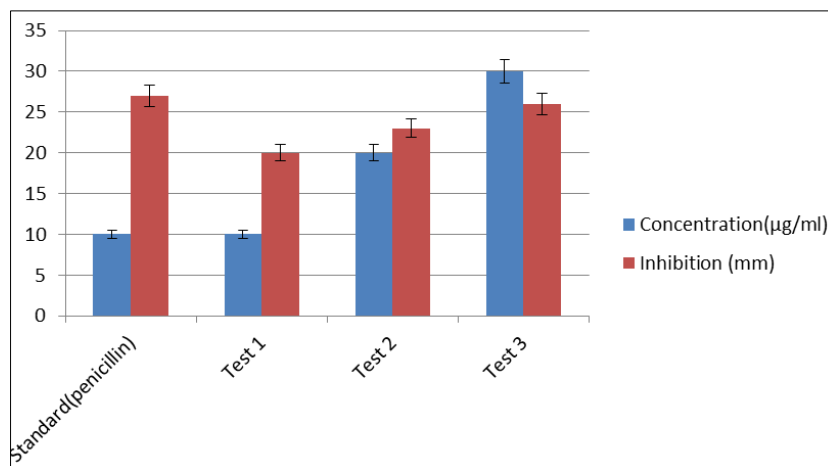
Figure 7 Tomato control



Figure 8 Tomato zone of inhibition

Table 12 Results showing zone of inhibition in Tomato by cup plate method

Sample	Concentration(µg/ml)	Inhibition (mm)
Standard(penicillin)	10	27
Test 1	10	20
Test 2	20	23
Test 3	30	26



Graph 2 Graphical representation of zone of inhibition of Tomato

4.5. Cucumber

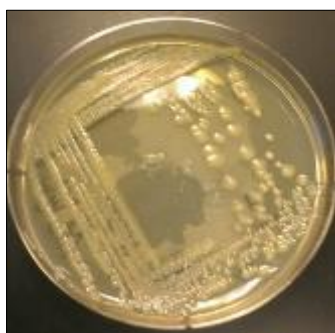


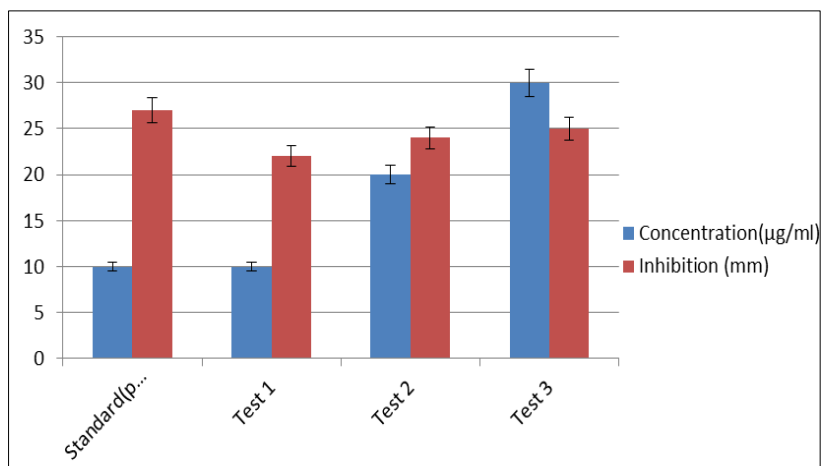
Figure 10 Cucumber control



Figure 11 Cucumber zone of inhibition

Table 13 Results showing zone of inhibition in cucumber by cup plate method

Sample	Concentration(µg/ml)	Inhibition (mm)
Standard(penicillin)	10	27
Test 1	10	22
Test 2	20	24
Test 3	30	25



Graph 3 Graphical representation of zone of inhibition of cucumber

4.6. Bread

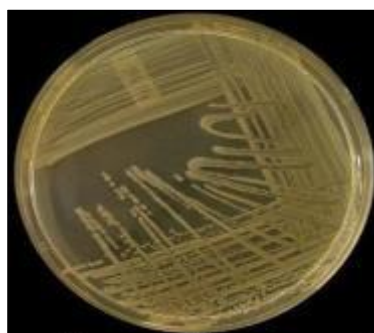


Figure 13 bread control

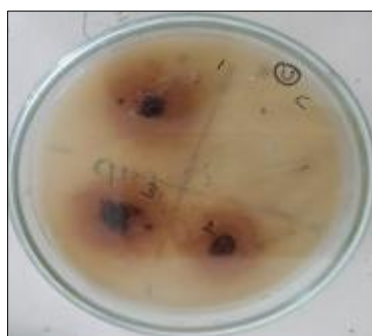
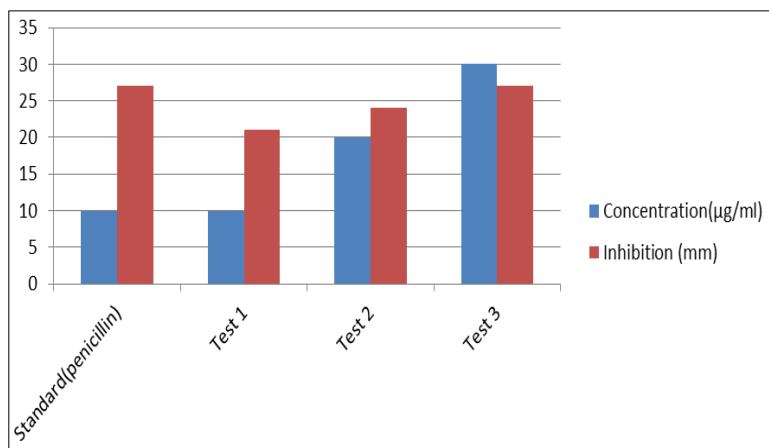


Figure 14 Bread zone of inhibition

Table 14 Results showing zone of inhibition in Bread by cup plate method

Sample	Concentration(µg/ml)	Inhibition (mm)
Standard(penicillin)	10	27
Test 1	10	21
Test 2	20	24
Test 3	30	27



Graph 4 Graphical representation of zone of inhibition of Bread

4.7. Banana



Figure 16 Banana control

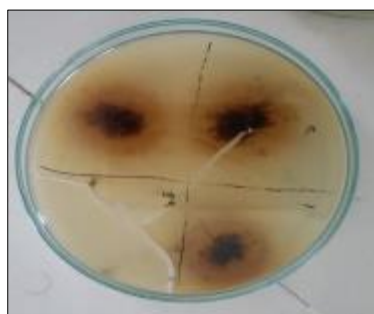
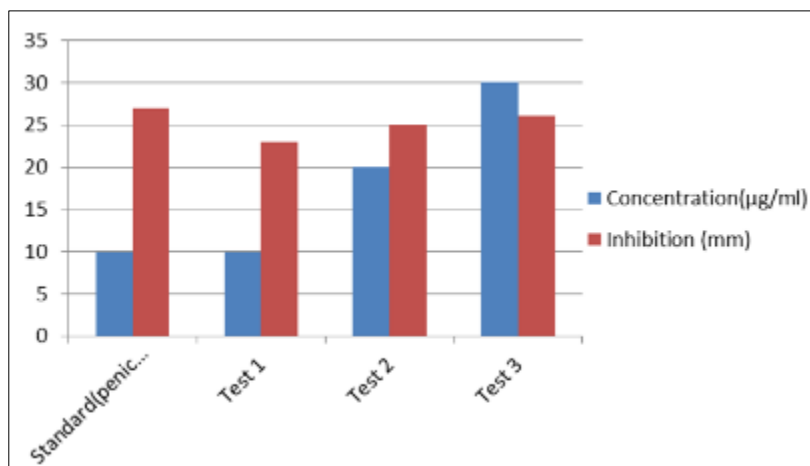


Figure 17 Banana zone of inhibition

Table 15 Results showing zone of inhibition in Banana by cup plate method

Sample	Concentration(µg/ml)	Inhibition (mm)
Standard(penicillin)	10	27
Test 1	10	23
Test 2	20	25
Test 3	30	26



Graph 5 Graphical representation of zone of inhibition of Banana

5. Discussion

The percentage yield of zone of inhibition of different powders extracted from natural microflora in:

Table 16 yield of zone of inhibition of different powders

Sample	Zone of inhibition
Cucumber	25 mm
Bitterguard	27 mm
Tomato	26 mm
Bread	27 mm
Banana	26 mm

The powder shows definite anti-microbial activity. The major constituents of the above powder has been associated with anti-microbial, anti-fungal, anti-bacterial, anti-viral activities. The result of agar cup plate method regarding the growth inhibition zones of the standard isolated against various concentrations of mixture of different powders extracted from natural micro flora are summarized in table11, 12,13,14,15 against natural micro flora growing on spoiled vegetables, fruits and bread. Two test concentrations T1 and T2 are taken with 10µg/ml, 20µg/ml, 30µg/ml concentrations respectively. Here the standard is taken as penicillin at a concentration 10µg/ml.

The anti-microbial action of Pencillin on all microorganisms tested was superior to that of powder extract. Pencillin alone showing inhibition zones ranging from 27mm in diameter. Test 3 concentrations of tomato (96%), bitter guard (100%), cucumber(92%), bread(100%), banana(96%) are showing more inhibition zone diameter than Test 2,1 concentrations of tomato(85%,74%), bitterguard(92%,85%), cucumber(88%,81%), bread(88%,77%), banana(92%,85%) respectively. Inhibition zones produced by pencillin in all vegetables, fruits, bread on all microorganisms shows 27mm.

The zones formed by Pencillin (std) are nearer to 92% concentration of powder extract. So 92% concentration powder extract can be used as effective Antimicrobial agent.

Vegetables, fruits, bread are intentionally rotten and various bacteria, fungi, molds are grown on them. The inoculum was prepared by isolating microbes from rotten fruits and Streaked on potato dextrose agar media and incubated for 24 hrs. Now a loop of microbial colonies are taken and dissolved in distilled water. The powder extract is extracted by maceration process and three different concentrations 10 µg/ml, 20µg/ml and 30µg/ml are taken and tested against microbial culture plates of Vegetables, fruits and bread with the help of small discs. Standard Pencillin is taken at a concentration of 10µg/ml and zones of inhibitions are observed. The zones formed by Pencillin (std) are nearer to

92% concentration of herbal powder extract. So 92% concentration herbal powder extract can be used as effective Anti-microbial agent. Antimicrobial potential in the above mentioned herbal powder attracted the attention of the agricultural industry with herbal powder being considered for the control of postharvest pathogens. Application of powdered extract on fruits and vegetables may prevent spoilage.

6. Conclusion

Ayurvedic medications are fundamentally regular medications which are generally innocuous to our body. Allopathy treats the illnesses by offering an answer that might create side results. A significant issue with allopathy is the maltreatment of anti-toxins. Doctors indiscriminately prescribe antibiotics which give temporary relief but causes long term damage to the patient. It causes bloating, belching, gas, constipation, diarrhoea, hyperacidity and allergic reactions in around 10% of the population like skin rashes and itching. So, allopathy is producing more side effects Herbal medicine is used. In our Present study we collected different herbal substances and evaluated for Anti-microbial properties by maceration method. Vegetables, fruits, bread are intentionally rotten and various bacteria, fungi, moulds are grown on them. The inoculum was prepared by isolating microbes from rotten fruits and streaked on potato dextrose agar media and incubated for 24 hrs. Now a loop of microbial colonies is taken and dissolved in distilled water. The powder extract is extracted by maceration process and three different concentrations 10 µg/ml and 20µg/ml 30 µg/ml are taken and tested against microbial culture plates of Vegetables, fruits and bread with the help of small discs. Standard Pencilling is taken at a concentration of 10µg/ml and zones of inhibitions are observed. The zones formed by Pencillin(std) are nearer to 92% concentration of herbal powder extract. So 92% concentration herbal powder extract can be used as effective Anti microbial agent.

Antimicrobial potential in the above mentioned herbal powder attracted the attention of the agricultural industry with herbal powder being considered for the control of postharvest pathogens. Application of powdered extract on fruits and vegetables may prevent spoilage.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest in publishing this paper.

References

- [1] Farhat Ali Khan, Muhammad Zahoor, Naseem Ullah, Shazeb Khan, Muhammad Khurram, Sartaj Khan, Javid Ali.
- [2] Ahn, K. (2017). "The worldwide trend of using botanical drugs and strategies for developing global drugs". *BMB Reports*. 50 (3): 111–116.
- [3] Stojanoski N. Development of health culture in Veles and its region from the past to the end of the 20th century. *Veles: Society of science and art*. 1999:13–34
- [4] Barrett, Stephen (23 November 2013). "The herbal minefield". *Quackwatch*.
- [5] Zhang, J.; Wider, B.; Shang, H.; Li, X.; Ernst, E. (2012). "Quality of herbal medicines: Challenges and solutions". *Complementary Therapies in Medicine*. 20 (1– 2): 100–106.
- [6] Morris, C. A.; Avorn, J. (2003). "Internet marketing of herbal products". *JAMA*. 290 (11): 1505–9 7. Coghlan, M. L.; Haile, J.; Houston, J.; Murray, D. C.; White, N. E.; Moolhuijzen, P; Bellgard, M. I.; Bunce, M (2012). "Deep Sequencing of Plant and Animal DNA Contained within Traditional Chinese Medicines Reveals Legality Issues and Health Safety Concerns" ..
- [7] Finegold SM, Martin WJ. *Diagnostic Microbiology*. 6th edit. St Louis: C.V. Mosby Co; 1982.
- [8] Ghiraldini F, Mello ML. Micronucleus formation, proliferative status, cell death and DNA damage in ethosuximide-treated human lymphocytes. *Cell Biol Int Rep*. 2010;17(1):27–31.
- [9] Daima HK, Selvakannan PR, Kandjani AE, Shukla R, Bhargava SK, Bansal V. Synergistic influence of polyoxometalate surface corona towards enhancing the antibacterial performance of tyrosine-capped Ag nanoparticles. *Nanoscale*. 2014;6:758–765.

- [10] Connell S, Li J, Shi R. Synergistic bactericidal activity between hyperosmotic stress and membrane-disrupting nanoemulsions. *J Med Microbiol.* 2013;62:69–77.
- [11] Lankoff A, Arabski M, Wegierek-Ciuk A, et al. Effect of surface modification of silica nanoparticles on toxicity and cellular uptake by human peripheral blood lymphocytes in vitro. *Nanotoxicology.* 2013;7:235–250.
- [12] Facciola S. *Cornucopia: a Source Book of Edible Plants.* Vista, California: Kampong Publications; 1990.
- [13] Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, and pelargonidin. *J. Agric. Food Chem.* 2002;50:166–171.
- [14] Miguel G, Dandlen S, Antunes D, Neves A, Martins D. The effect of two methods of pomegranate (*Punica granatum L*) juice extraction on quality during storage at 4°C. *J. Biomed. Biotech.* 2009.
- [15] 16. Okamoto JM, Hamamoto YO, Yamato H, Yoshimura H. Pomegranate extract improves a depressive state and bone properties in menopausal syndrome model ovariectomized mice. *J. Ethnopharmacol.* 2004
- [16] Koyama S, Cobb LJ, Mehta HH, Seeram NP, Heber D, Pantuck AJ, Cohen P. Pomegranate extract induces apoptosis in human prostate cancer cells by modulation of the IGF-IGFBP axis. *Growth Horm. IGF Res.* 2010;20:55–62.
- [17] Rettig MB, Heber D, An J, Seeram NP, Rao JY, Liu H, Klatte T, Beldegrun A, Moro A, Henning SM, Mo D, Aronson WJ, Pantuck A. Pomegranate extract inhibits androgen-independent prostate cancer growth through a nuclear factor-kappaB-dependent mechanism. *Mol. Cancer Ther.* 2008.
- [18] Sturgeon SR, Ronnenberg AG. Pomegranate and breast cancer: possible mechanisms of prevention. *Nutr. Rev.* 2010.
- [19] Kasimsetty SG, Bialonska D, Reddy MK, Ma G, Khan SI, Ferreira D. Colon cancer chemopreventive activities of pomegranate ellagitannins and urolithins. *J. Agric. Food Chem.* 2010;58:2180–2187.
- [20] Khan SA. The role of pomegranate (*Punica granatum L.*) in colon cancer. *Pak. J. Pharm. Sci.* 2009;22:346–348.
- [21] Khan N, Afaq F, Kweon MH, Kim K, Mukhtar H. Oral consumption of pomegranate fruit extract inhibits growth and progression of primary lung tumors in mice. *Cancer Res.* 2007;67:3475–3482.
- [22] Hong MY, Seeram NP, Heber D. Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor. *J. Nutr. Biochem.* 2008;19:848–855.
- [23] Stajner D, Varga IS. An evaluation of the antioxidant abilities of *Allium* species. *Acta Biologica.*
- [24] Benavente-García O., Castillo J., Marin F.R., Ortuño A., Del Río J.A. Uses and properties of citrus flavonoids
- [25] Brand-Williams W., Cuvelier M.-E., Berset C. Use of a free radical method to evaluate antioxidant activity.
- [26] G. D. Lutterodt, "Inhibition of Microlax-induced, vol. 37, no. 2, pp. 151–157, 1992.
- [27] S. Begum, S. I. Hassan, S. N. Ali, and B. S. Siddiqui, "Chemical constituents from the leaves of *Psidium guajava*," *Natural Product Research*, vol. 18, no. 2, pp. 135–140, 2004.
- [28] C.-W. Chang, W.-L. Chang, S.-T. Chang, and S.-S. Cheng, "Antibacterial activities of plant essential oils against *Legionella pneumophila*," *Water Research*, vol. 42, no. 1-2, pp. 278–286, 2008.
- [29] B. Marongiu, A. Piras, S. Porcedda et al., "Supercritical CO₂ extract of *Cinnamomum zeylanicum*: chemical characterization and antityrosinase activity," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 24, pp. 10022–10027, 2007
- [30] Filtenborg, O., Frisvad, J.C., Thrane, U. 1996. Moulds in food spoilage. *Int. J. Food Microbiol.*
- [31] D.Y.C. 2009. Food Spoilage, Preservation and Quality Control. *Encyclopedia of Microbiology*, 54-79.
- [32] Singh V, Shri R, Krishan P, Singh IP, Shah P. Isolation and characterization of components responsible for neuroprotective effects of *Allium cepa* outer scale extract against ischemia reperfusion induced cerebral injury in mice. *J Food Sci.* (2020) 85:4009–17. doi: 10.1111/1750-3841.15474
- [33] Gupta S, Kataria M, Gupta PK, Murganandan S, Yashroy RC. Protective role of extracts of neem seeds in diabetes caused by streptozotocin in rats. *J Ethnopharmacol.* 2004;90(2–3):185–189.
- [34] Kim H.-S. Do not put too much value on conventional medicines. *J. Ethnopharmacol.* 2005;100:37–39. doi: 10.1016/j.jep.2005.05.030.
- [35] Bruneton, J., 1999. *Pharmacognosy, phytochemistry, Medicinal plants.* 2nd Edn., intercept Ltd., Hampshire, UK., pp: 385-386