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In Vitro Microbicidal Activity of Essential Extracts Against Various Pathogens

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Abstract:

This study checked out how well methanol extracts from orange peels, neem leaves, and lemongrass leaves killed certain germs in test tubes. The germs tested were Staphylococcus, E. coli, Streptococcus, and Aspergillus niger. The extracts were made by soaking the plant parts in methanol, and then diluted with DMSO. How well the extracts worked was tested by seeing how big the clear areas were around the wells where the extracts were placed on agar plates.

Orange peel extract worked well against Staphylococcus but not so well against A. niger. Neem extract stopped A. niger pretty well, had a small effect on Staphylococcus, and did nothing to E. coli. Lemongrass extract did a good job on the A. niger fungus, but not so much on Streptococcus. All in all, most of the extracts stopped at least one of the germs from growing.

The results suggest that these plant extracts could be good sources of germ-killing stuff that could be used to create medicine. More testing is needed for clinical and molecular studies before they can be used as medicine, because germs are becoming more resistant to antibiotics due to overuse and misuse.

Keywords**: **Methanol Extracts, Soaking, Agar Well Tests, Germ-Killing Power, Clear Zone, Medicinal Plants, Antibiotic Resistance.

Introduction:

People have been using plants as natural cures for ages to treat sickness and stay healthy. Think of medicinal plants as plants that have stuff in them that can help cure diseases or ease pain. Some old books mention how useful herbs and plant stuff are, which shows that plant-based medicine has been around for a long time. Lots of people still use herbal cures today because they don't cost a lot and are easy to get.

Plants create chemicals that help them fight off different conditions. These ingredients can also fight germs that cause diseases. Germ-caused diseases are still a big problem, especially since germs are resisting drugs. That's why we should check out what nature offers us as new germ-fighters.

Plant extracts might be good backups for synthetic drugs. A bunch of modern meds come straight from plants. Natural things can kill germs well. So, looking at medicinal plants for germ-killing stuff is a way to find new ways to treat diseases.

Citrus, Neem, and Lemongrass are plants that are said to have antibacterial, antifungal, anti-inflammatory, and antioxidant stuff. Neem is known for fighting germs, while lemongrass has oils that clean wounds. Citrus peel extracts can stop germs from growing.

Since drug resistance is becoming a bigger deal, checking how well plant extracts kill germs is important. This helps us spot useful ingredients that could lead to new drugs.

Literature Review:

Medicinal plants are traditionally used worldwide as remedies for the treatment of various diseases, including asthma, gastrointestinal symptoms, skin disorders, respiratory and urinary problems, and hepatic and cardiovascular disease.(Patel, R.P. and B.M. Trivedi. 1962)

These plants synthesize a diverse array of biologically active compounds that are important for them to survive and flourish in the natural environment, including protective functions with respect to abiotic stresses derived from temperature, water status, mineral nutrient supply and to insect pests

The composition of biologically active compounds of medicinal plants varies widely depending on the plant species, soil type and on their association with microbes. These bioactive secondary metabolites synthesized by medicinal plants can also strongly affect plant-associated microbial communities and their physiological functions.(Jieyu Zhu, Qingrong Huang)

Moreover, plants rely on their microbiome for specific traits and activities, including growth promotion, nutrient acquisition, induced systemic resistance and tolerance to abiotic stress factors. Although a vast number of medicinal plants have been well-studied with respect to their phytochemical constituents and pharmacological properties, their microbiome and the physiological interactions between host and microbes remain poorly understood. The plant-associated microbiome consists of distinct microbial communities living in the roots, shoots and endosphere.Köberl et al. (2013)

The rhizosphere of many plants is well-studied and known to be a potential source for selecting beneficial microbes that can positively affect plant health. Hence, understanding the response of microbial communities to alterations in the physicochemical environment of the rhizosphere may provide valuable insights into the microbial ecology of plant-associated bacteria.Köberl et al. (2013) observed a high abundance of antagonistic bacteria in the rhizosphere of the medicinal plants Matricaria chamomilla, Calendula officinalis, and Solanum distichum.

The root-associated bacteria of Ajuga bracteosa exhibited a wide range of plant growth promoting activities by producing siderophores and indole acetic acid and exhibiting antioxidant activity. Recently, endophytic microorganisms have been under increased investigation due to their intimate interaction with the host (Hardoim et al., 2015); it is believed that the phytochemical constitutes of plants are related either directly or indirectly to endophytic microbes and their interactions with host plants.Despite first studies of endophytes in medicinal plants, the potential of medicinal plants is far from exhausted.

Orange is consumed fresh or in the form of juice, jam, squash and syrup. It is the main source of peel oil, citric acid and cosmetics which have international market value. Citrus industry in India is the third largest fruit industry of the country after mango and banana. India ranks ninth among top orange producing countries contributing 3% to the worlds total orange production. Only 1.72% of the country's production is exported. Nagpur mandarin is one of the best Oranges in the world. Production of this fruit crop in central and western part of India is increasing every year.

Mrig crop (monsoon blossom) which matures in February-March has great potential for export since arrivals of mandarin fruit in international market are very less during this period. Selection of desired quality fruit as per specific market demand and careful post-harvest handling to retain most of natural qualities and freshness plays a key role in expanding exports of Nagpur mandarin. At present fruit consignments

are being exported to neighbouring countries by road without cooling and any other treatments.

Neem trees are attractive broad-leaved evergreens that can grow up to 30 m tall and 2.5 m in girth. Their spreading branches form rounded crowns as much as 20 m across. They remain in leaf except during extreme drought, when the leaves may fall off. The short, usually straight trunk has a moderately thick, strongly furrowed bark. The roots penetrate the soil deeply, at least where the site permits, and, particularly when injured, they produce suckers. This suckering tends to be especially prolific in dry localities.

Lemon grass belongs to *Cymbopogon*, a genus of about 55 species of grasses, native to temperate and tropical regions. Lemon grass is, also called fever grass, a perennial plant with thin, long leaves that is indigenous to many Asian countries. lemon grass is a good source of vitamins A and C, folic acid, magnesium, zinc, copper, iron, potassium, calcium and manganese. Lemon grass oil (citral) is hydro distilled and IR and GC are conducted to verify its constituents

Microbicidal agent: agent is a chemical substance that either kills microorganism or prevent their growth. The action of microbicidal agents upon microorganism may be either Antimicrobiotic i.e. inhibiting growth and reproduction or microbicidal i.e. actually killing the microorganism

Staphylococcus species. (Bacteria): Staph is the common name for a group of bacteria in the genus *Staphylococcus*. *Staphylococcus aureus* is Gram-positive bacteria (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters that are described as "grape-like." On media, these organisms can grow in up to 10% salt, and colonies are often golden or yellow (aureus means golden or yellow).

Escherichia coli (Bacteria): *Escherichia coli* (E. coli) is a bacteria that is commonly found in the lower intestine of warm-blooded organisms. Most E. coli strains are harmless, but some can cause serious food poisoning. Shiga toxin-producing E. coli is a type of bacteria that normally lives inside our intestines, where it helps the body break down and digest food.

Streptococcus species. (Bacteria): Streptococci are Gram-positive, no motile, nonsporeforming, catalase-negative cocci that occur in pairs or chains. Older cultures may lose their Gram-positive character. Most streptococci are facultative anaerobes, and some are obligate (strict) anaerobes. *Streptococci* are coccoid bacterial cells microscopically, and stain purple (Gram-positive) when Gram staining technique is applied.

Aspergillus Niger (fungus): *Aspergillus* species are filamentous fungi that are commonly found in soil, decaying vegetation, and seeds and grains, where they thrive as saprophytes. *Aspergillus Niger* is a fungal microbe of great industrial importance. It is a fungus and one of the very common species of the genus *Aspergillus*. Furthermore, it can cause disease we call it black mold. This mold is used extensively in the production of citric acid and in the production of several enzymes such as amylases, pectinases, and proteases.

Agar well diffusion plate technique:

Agar well diffusion method is widely used to evaluate the microbicidal activity of plants or microbial extracts.

The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface.

Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 μL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well.

Then, agar plates are incubated under suitable conditions depending upon the test microorganism.

The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested (Mounyr Balouiri)

Materials And Methodology:

We tested the activity of extracts from one citrus fruit, which is Citrus reticulata, one medicinal plant, which is Azadirachta indica and one herb, which is Cymbopogon citratus against three bacterial and one fungal pathogen.

1. Materials: We used culture media like nutrient agar and broth reagents like methanol, DMSO and staining chemicals, glassware and laboratory instruments like autoclave, incubator, micropipettes and microscope.

2. Sample Collection And Processing: We collected plant materials from Pune. We got orange from the market neem leaves from an area and lemongrass from a home garden. We washed the samples with water then we dried them in the shade at 35–40°C and then we ground them into fine powder.

3. Extraction Procedure: We took the dried powders, which were 10 g. We did cold maceration using 100 ml of 80% methanol for three days. We filtered the extracts through cloth and Whatman filter paper then we evaporated the solvent using the open-dish method. We made stock solutions by dissolving the extracts in DMSO. We stored them at 2–8°C.

4. Test Microorganisms: We got cultures of bacteria and fungi from the microbiology department. We confirmed that the cultures were pure by looking at the colony morphology and doing Gram staining before we used them.

5. Preparation Of Inoculum And Media: We put cultures into nutrient broth and we incubated them. We prepared the media according to the manufacturer instructions under conditions.

6. Screening Of Microbicidal Activity: We tested the activity using the agar well diffusion method. We made inoculated agar plates we made wells. We added 100 µl of each extract. We used DMSO as a control. We incubated the plates at 37°C for 24–48 hours. We measured the zones of inhibition to see the activity of the antimicrobial activity of the extracts, from Citrus reticulata, Azadirachta indica and Cymbopogon citratus.

Observation And Result:

1. Percentage yield of crude extracts of medicinal plants:

Dried powder of 3 types of essential products were subjected to continuous extraction with the solvent: methanol in the order to increasing the polarity for 24 to 48 hours by using Maceration to obtain crude methanol extracts of the respective essential products.

Percentage yield = Weight of extract / Weight of samples × 100

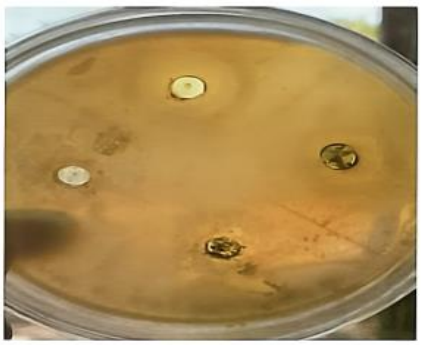
Sr. No.	Name of Samples Used	Solvent used for extraction	% yield
1	*Citrus reticulata*	Methanol	3.80
2	*Azadirachta Indica*	Methanol	2.56
3	*Cymbopogon citratus*	Methanol	1.92

Table 1: The Percentage yield of the crude extracts

Table 1 shows that the highest yield was observed from the methanol extract of *Citrus reticulata* (3.80%), followed by *Azadirachta indica* (2.56%). The lowest yield was obtained from *Cymbopogon citratus* (1.92%).

2. Evaluation of Microbicidal Activity:

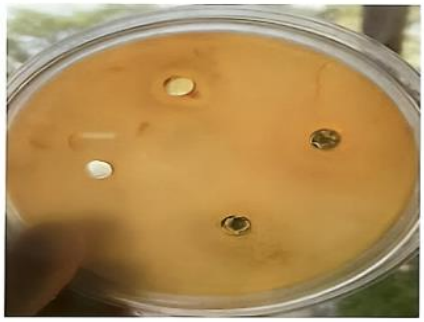
The observation and mean diameter of zones of inhibition of methanol fractions of different essential extracts are shown in the tables and photographs.



Photograph 1: Zone of inhibition produced by essential extracts against *Staphylococcus* species.

Sr. No.	Test Organisms	Negative Control (Solvent)	Zone of Inhibition Diameter (mm)
1	*Citrus reticulata*	DMSO	12 mm
2	*Azadirachta indica*	DMSO	7 mm
3	*Cymbopogon citratus*	DMSO	4 mm

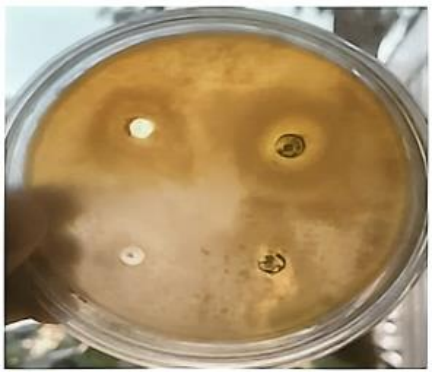
Table 2: Zone of inhibition given by essential extracts against *Staphylococcus* species



Photograph 2: Zone of inhibition produced by essential extracts against *Escherichia coli*.

Sr. No.	Essential Extracts	Negative Control (Solvent)	Zone of Inhibition Diameter (mm)
1	*Citrus reticulata*	DMSO	4 mm
2	*Azadirachta indica*	DMSO	2 mm
3	*Cymbopogon citratus*	DMSO	—

Table 3: Zone of inhibition given by essential extracts against *Escherichia coli*



Photograph 3: Zone of inhibition produced by essential extracts against *Streptococcus* species.

Sr. No.	Essential Extracts	Negative Control (Solvent)	Zone of Inhibition Diameter (mm)
1	*Citrus reticulata*	DMSO	6 mm
2	*Azadirachta indica*	DMSO	8 mm
3	*Cymbopogon citratus*	DMSO	2 mm

Table 4: Zone of inhibition given by essential extracts against *Streptococcus* species

Photograph 4: Zone of inhibition produced by essential extracts against *Aspergillus niger*.

Sr. No.	Essential Extracts	Negative Control (Solvent)	Zone of Inhibition Diameter (mm)
1	*Citrus reticulata*	DMSO	4 mm
2	*Azadirachta indica*	DMSO	12 mm
3	*Cymbopogon citratus*	DMSO	—

Table 5: Zone of inhibition given by essential extracts against *Aspergillus niger*

Discussion And Conclusion:

1. Discussion:

Medicinally utilised plants (Citrus reticulata, Azadirachta indica, Cymbopogon citratus) were obtained from the state of Pune and processed using traditional knowledge of their medicinal usage. The plant samples were dried to powder, extracted with a cold methanol maceration, filtered, evaporated dry, and diluted in DMSO to prepare stock solutions. All standard strains for bacterial and fungal cultures were obtained through the Microbiology Division (UMC) University of Michigan for the purpose of producing inocula. The antimicrobial properties of each plant extract were evaluated by the "Agar Well Diffusion Method." The antimicrobial properties of the plant extracts were determined by measuring the diameters of inhibition zones.

The results showed microbicidal properties of all of the tested plant species against Staphylococcus sp. and Escherichia coli, and Streptococcus, and Aspergillus niger. However, bacterial and fungal species exhibited different levels of activity against the respective plant extracts. In comparison, Cymbopogon citratus showed a limited level of activity, and no inhibitory activity against either E. coli or A. niger. The present study confirms previous studies that methanol is an adequate extractor of bioactive compounds from each of, the three traditional plant sources.

2. Conclusion:

1. The methanolic extract of Citrus reticulata exhibited the most significant antimicrobial activity against all tested organisms, indicating its potential as a natural antimicrobial agent.
2. Azadirachta indica leaf extract showed strong activity against Staphylococcus, Streptococcus, and Aspergillus niger, which proves that it has medicinal value.
3. The extract from Cymbopogon citratus only worked a little against some bacteria and not at all against E. coli and A. niger, which means it wasn't very good at killing germs.

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