Exploring the effects of

Zanthoxylem and moringa

and phytochemical screening of

agriculture soil derived bacteria isolates

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“Exploring the effects of Zanthoxylem and moringa and phytochemical screening of agriculture soil derived bacteria isolates”

Aayushi Rawat

DR. P.D.B.H. P.G COLLEGE KOTDAWAR

Corresponding e mail: aayushirawat0705@gmail.com

Abstract

The increasing concern over soil contamination and microbial imbalance caused by waste accumulation and excessive agricultural practices has encouraged the search for eco-friendly plant-based antimicrobial agents. This study explores the effects of phytochemicals obtained from Zanthoxylum and Moringa oleifera on soil-derived bacteria collected from garbage disposal sites and agricultural soils. The research is mainly about the comparative analysis of the antibacterial properties and phytochemical components of these two plants against natural soil microorganisms.

The plant extracts were obtained with suitable solvents after which phytochemical analysis was done on these extracts to identify whether alkaloids, tannins, flavonoids, saponins, terpenoids, and phenolics were present. Soil samples from agricultural farms were used from which bacterial isolates were isolated using serial dilution techniques. The antibacterial property of the plant extracts was analyzed using agar diffusion and other relevant microbiology methods.

The results showed that all extracts had bioactive compounds that were capable of preventing bacterial growth. However, the Zanthoxylum extract had significantly more potent antimicrobial properties compared to Moringa extracts against different soil bacterial isolates, while the latter only had moderate yet broad-spectrum properties against these isolates in the studied agricultural soil. The presence of flavonoids, tannins, and alkaloids in these plants could have contributed to their antibacterial activities.

This research is important in understanding how plant-based phytochemical compounds can be used as effective antimicrobial agents against harmful soil bacteria. In addition, comparative analysis of these two plants could assist in the development of bioactive compounds.

Keywords: phytochemical, nanotechnology, antimicrobial agents, contaminated,

,accumulation, anti-inflammatory

1.Introduction

Soil a vibrant habitat for diversified life-forms shelters many animals from invertebrates such as worms and insects to mammals like rabbits, rodents and badgers. It is also an ideal habitat of complex groups of microorganisms including bacteria, archaea, fungi and protozoa [1]. Bacteria, involved in decomposition of organic substances, nutrient cycling, soil aggregation and humus formation can reach up to 10 billion cells/g in soil [2]. Bacteria constitute the principal group of soil microbes mostly because of their capacity to produce diverse extracellular enzymes such as amylase, protease, lipase, pectinase, cellulase and chitinase. Bacterial

population can be manipulated to produce enzymes which are commercially important in organic compound synthesis, clinical analysis, pharmaceuticals, detergents, food production and fermentation [3].Microbial, mainly bacterial production of amylase is more fruitful than that of other sources like plants or animals, because of their short growth period, biochemical diversity and the ease with which enzyme production capability might be increased by environmental and genetic manipulation [4].

1.1Moringa oleifera

Moringa (Moringa oleifera Lam). is a type of local medicinal Indian herb which has turn out to be familiar in the tropical and subtropical countries. Moringa oleifera is one of the vegetables of the Brassica order and belongs to the family Moringaceae. The Moringaceae is a single genus family with 13 known species [5] Moringa oleifera is a small native tree of the sub-Himalayan regions of North West India, which is now indigenous to many regions in Islands and South America. Traditionally, besides being a daily used vegetable among people of these regions, the Moringa is also widely known and used for its health . Among commoners, it has earned its name as 'the miracle tree' due to its amazing healing abilities for various ailments and even some chronic diseases. Several investigations were carried out to isolate bioactive compounds from various parts of the plant due to its various applications Therefore, herbal plants in medicine or known as phytomedicine are still trustworthy and widely applied as one of cost[6]. For centuries and in many cultures around the world, the medicinal usage of the Moringa has been used to asthma, blackheads, blood impurities, bronchitis, catarrh, chest congestion, cholera and many other illnesses[7,8,9]. Moringa anti-pyretic, anti-ulcer, anti-epileptic, diuretic, cholesterol lowering, renal, anti-diabetic, [10,11]. and hepatoprotective activities [12,13]. It has also long been labelled for its great cosmetic value in which in recent years, the Moringa has commonly been found to be used in various health care products including body and hair moisturisers and conditioners. It was also discovered that Moringa oil was used in skin ointments ever since the Egyptian times. The Moringa was claimed to be 'the most nutrient-rich plant yet discovered' by [14].

Taxonomic Rank	Classification
Kingdom	Plantae
Division	Magnoliophyte(Angiosperms)
Class	Magnoliophyte(Dicotyledons)
Subclass	Dilleniid
Order	Brassicas
Family	Moringaceae
Genus	Moringa
Species	Moringa oleifera Lam.

Table 1.0 Moringa classification

Fig 1.0 Moringa plant

1.2 Zanthoxylum

Zanthoxylum are deciduous shrubs and trees of the family Rutaceae which comprise 250 species that are native to warm temperate and subtropical region of the world. The genus has much ethanobotanical importance and is used as sources of pharmaceutical and cosmetics raw material. Traditionally, leaves and fruits are used for mouth fresh and tooth care while bark is used for intoxicating the fishes [15]. Leaves, fruits and barks are used as spice [16]. Alkaloids were isolated from *Zanthoxylum*[17,18,19,20]. Twenty microelements were also determined in the root, stem and leaf of traditional Chinese herbs *Zanthoxylum nitidum* by ICP-AES [21].Due to presence of nitrogen containing compound, *Zanthoxylum* has wide spectrum of biological activities.The purpose of this review to collect all the possible information regarding the chemical constituents and biological effects of the genus *Zanthoxylum*, thus this will help to researchers and pharmaceutical companies to take action in this discipline. The letter Zanthoxylum L. plants (ZLP) pertains to the taxonomic classification of Rutaceae shrubs, encompassing thorn trees and woody vines, with approximately 250 species dispersed globally. According to the 2020 edition of the Chinese Pharmacopoeia, Z. bungeanum possesses attributes encompassing the warming of the gastrointestinal tract, alleviating discomfort, insecticidal properties, and itch relief[22]. Its primary applications involve the treatment of conditions such as vomiting, diarrhea, abdominal cold pain, and abdominal

Taxonomic Rank	Classification
Kingdom	Plantae
Division	Magnoliophyte(Angiosperms)
Class	Magnoliophyte(Dicotyledons)
Subclass	Rosidae
Order	Sapindales
Family	Rosacea
Genus	Zanthoxylum
Species	Zanthoxylum armatum

discomfort arising from insect bites [23].

2.Methodology

2.1.Sample collection

Collection and Preparation of Soil Sample: In systematic screening program for isolation of bacteria 1 soil samples were collected at different location within Dehradun were collected from upper layer where most of the microbial activity take place and thus where most of the bacteria population is concentrated. Soil samples (approximately 5g) were collected using some clean dry and sterile polythene bag along with sterile spatula. 1g of the soil samples were dissolved in 10ml of water to make soil suspension.

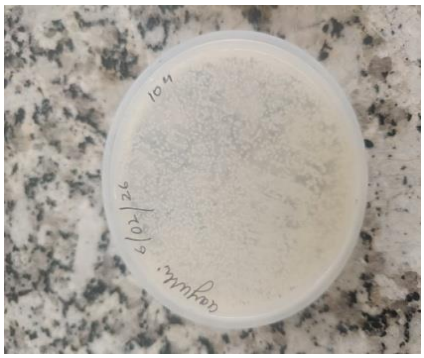
Fig 1.2 sample collection

2.2 Isolation of Bacteria: The media used in this research was nutrient of agar media. 28g of nutrient agar powder was weighed and dissolved in 1000ml and of distilled water. It was stirred vigorously and dissolved using hot plate after which was sterilized in autoclave for 15 minutes at 121°C. It was then allowed to cool after which it was dispensed in Petri dishes.

1.Serial Dilution:A serial dilution is the process of gradually diluting a substance in a series of steps, each using the same dilution factor (like 1:10 or 1:2), to reduce the concentration of microorganisms or chemicals to a countable or workable range.

2.Spreading

Spreading bacteria on a Petri plate is a common microbiological technique used to distribute bacterial cells



evenly over the surface of a solid growth medium, usually nutrient agar.

Fig 1.3 spreading plate

3.Streaking: Streaking is a technique used to isolate individual colonies of bacteria from a mixed culture culture on a solid medium like nutrient agar.

Fig 1.4 streakingplate

3.Chemical used

The chemical and reagent required for the study were provided by rapture biotech pvt.lmt. There will be different test conducted and different chemicals and reagent were used such as nutrient agar, distilled water were used for isolating bacteria. Hydrogen peroxide was used to check catalase activity of the isolate. Gram staining reagent and immersion oil is used to detect which bacteria is gram positive and gram negative. To check nitrogen fixation potassium phosphate, calcium carbonate, magnesium sulphate, dextrose, calcium sulphate and agar is used. For anti-microbial properties by disk diffusion method, agar plates, positive control, negative control.

4.Biochemical Tests

4.1 Gram staining

Gram staining is done to differentiate between gram positive and gram-negative bacteria. The presence of thick peptidoglycan layer and thin lipid layer present in gram positive bacteria retain crystal violet colour whereas thick lipid layer and thin peptidoglycan layer of gram negative bacteria retain pink colour.

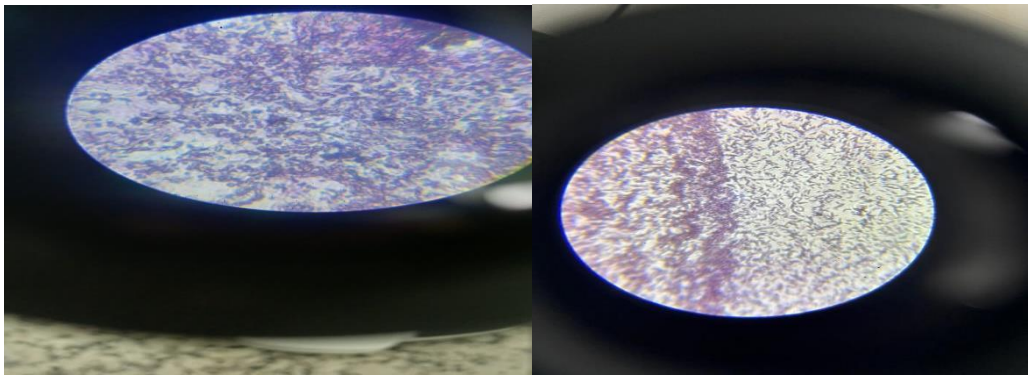


Fig:1.5 Gram staining

4.2 Urease test

The urease test is a way to determine the presence of urease enzyme, which is made by many different types of bacteria. Urease catalyzes the hydrolysis (breaking down) of urea into carbon dioxide and ammonia. When ammonia is released, the pH of the medium goes up and becomes alkaline

4.3Nitrate test

Growing Inoculated representative organisms from specimens in It is an important test for determining type organ and organism primarily uses that same replicable substrate as a type of "biological molecule".

We use the Enzyme activity of nitrate utilizing bacteria to determine if they possess the enzyme activity for reducing a nitrate (by using the Nitrate Reductase).

4.4 Indole Test

The indole test is used for determining the presence of tryptophanase-producing organisms. Indole, pyruvate, and ammonia will be formed if the organism produces tryptophanase and has the enzyme present

4.5 Citrate Test

The citrate test is used to identify if a bacterial organism is able to use sodium citrate as its only carbon source and produce alkaline by-products which create a color change in the medium.

4.6 Amylase Test

The amylase or starch hydrolysis test is a procedure that assesses whether specific types of bacteria create amylase enzymes to break down starch into simple sugars such as maltose and glucoseTo determine if amylase is produced, cultures of bacteria are spread onto starch agar plates (nutrient agar plates with 1% soluble starch). The bacteria will then be incubated at temperatures between 30 - 37 degrees Celsius for 24 - 48 hours.

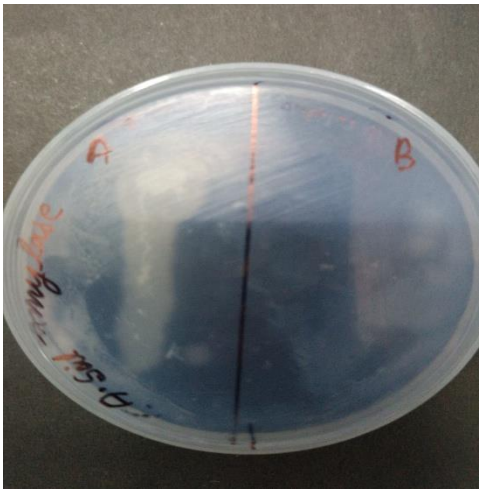


Fig 1.6 amylase test

5.Extraction method

We used ethanol to extract compounds from the herbs Zanthoxylum and Moringa. Ethanol works well for this because it can extract different types of compounds. It is also safe and good for the environment.The powdered material was extracted using methanol or distilled water (1:10).extract was stored at 4 degree Celsius for further phytochemical used.

Steps

Step 1: Preparing the Plants.

We collected Moringa and Zanthoxylum plants. We cleaned them to remove dirt.We dried them in the shade for 6 to 10 days and Then we crushed them into a powder.

Step 2: Choosing a Solvent

We chose ethanol as our solvent .Many helpful compounds are found in ethanol. These compounds include alkaloids, flavonoids, tannins and phenolics .Ethanol is less toxic, than methanol and We used a mix of ethanol and water 10:10.

Step 3: Extraction Process

We took 10 grams of the plants and put them in a special thimble. We placed the thimble in a Soxhlet extraction machine.We filled a 500 mL flask with 200 mL of ethanol.

Step 4: Final Extract

The extract of Zanthoxylum and Moringa is now prepared.The Zanthoxylum and Moringa extract is complete.

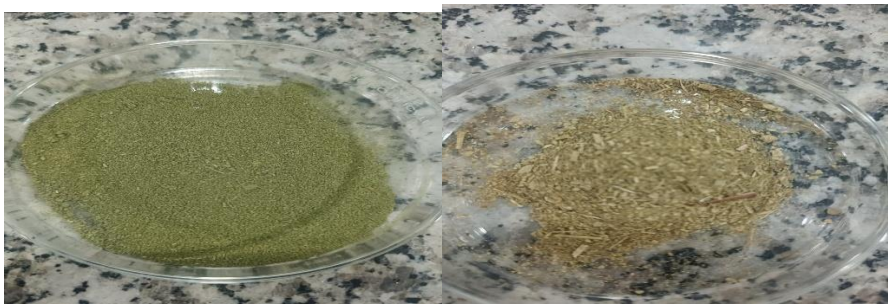


Fig 1.7 Dried powder of Moringa and Zanthoxylum

Fig 1.8 plant Extract of Moringa and Zanthoxylum

6.Phytochemical Screening

Phytochemical screening was carried out to identify major classes of bioactive compounds present in the plant extract using standard qualitative tests. Collectively, these classical assays provided a preliminary phytochemical profile of the extract, enabling differentiation of compound classes for further biochemical and pharmacological analysis.t aqueous extract are made by the help of Soxhlet .

Fig 1.8 Soxhlet appratus

6.1) Alkaloid Test

The alkaline test is a chemical method in which a plant extract is treated with sodium hydroxide (NaOH); the appearance of an intense yellow color indicates the presence of flavonoids, which disappears on adding dilute acid (HCl), confirming the result.

6.1.1Mayer's Test: Mayer's test detects alkaloids by adding Mayer's reagent (potassium mercuric iodide solution) to the sample. The appearance of a cream or white precipitate indicates alkaloids.

6.1.2Wagner's Test: Wagner's test detects alkaloids by adding Wagner's reagent (iodine in potassium iodide). A reddish-brown precipitate confirms alkaloids.

6.2) Tannin Acid Test

This test identifies tannins using reagents such as ferric chloride ($FeCl_3$), lead acetate, or gelatine solution, which give characteristic colour changes or precipitates.

6.2.1Ferric Chloride Test: Adding neutral $FeCl_3$ solution to the extract produces blue, green, purple, or black coloration depending on the type of phenol or tannin present.

6.2.3Gelatine Test: Adding 1% gelatine solution with sodium chloride forms a white precipitate in the presence of tannins.

6.3) Steroid Test

This test detects steroids or sterols, which react with concentrated acids to give colour changes (green, blue, or red).

6.3.1Liebermann–Burchard Test: The extract is treated with acetic anhydride followed by sulfuric acid. A blue-green or emerald-green colour confirms steroids or sterols.

6.4) Flavonoid Test

Flavonoids are detected by qualitative tests producing colour changes or precipitates.

6.4.1Alkaline Reagent Test: Adding NaOH gives an intense yellow colour, which disappears upon adding dilute HCl.

6.4.2Lead Acetate Test: Adding 10% lead acetate forms a yellow or white precipitate confirming flavonoids or tannins.

6.5) Terpenoid Test

Terpenoids are detected by the Salkowski test: mixing the extract with chloroform and carefully adding sulfuric acid produces a reddish-brown interface, indicating terpenoids.

6.5.1 Salkowski Test: Steroids or terpenoids dissolve in chloroform and react with sulfuric acid to give a red, reddishbrown, or golden-yellow colour at the interface.

6.6) Saponin Test

Saponins are confirmed by the **foam (froth) test:** vigorous shaking of the extract with water forms a persistent froth lasting 10–15 minutes.

6.7) Test for Phenols

Phenols are detected by colour or precipitation reactions.

6.7.1 Ferric Chloride Test: Adding neutral $FeCl_3$ produces blue, green, purple, or black coloration depending on phenol type.

6.7.2Lead Acetate Test: Adding 10% lead acetate solution forms a yellow or white precipitate confirming phenols, flavonoids, or tannins.

6.8) Cyanidin Test

This test detects cyanidin-type flavonoids. Treating the extract with concentrated HCl and heating develops a pink, red, or purple colour.

6.9) Benedict's Test

This test detects reducing sugars using Benedict's reagent ($CuSO_4$ + Na_2CO_3 + sodium citrate). On heating, blue Cu^{2+} is reduced to red Cu_2O, causing a color change from blue to green, yellow, orange, or brick-red depending on sugar concentration.

6.10) Drage Dorff's Test

6.10.1Dragendorff's testdetects alkaloids using potassium bismuth iodide reagent. Adding the reagent forms an orange, reddish-brown, or yellow precipitate confirming alkaloids.

6.10.2Kraut's Test: Kraut's test uses potassium mercuric iodide reagent. The formation of a reddish-brown precipitate indicates alkaloids.

Test	A	B
Amylase	Positive	Negative
Indole	Positive	Positive
Catalase	Negative	Negative
Citrate	Negative	Positive
Nitrate	Positive	Negative
Urease	Negative	Negative
MR	Negative	Negative
VP	Negative	Negative
Sucrose	Positive	Positive
Dextrose	Positive	Positive
Maltose	Negative	Negative
Mannitol	Negative	Negative
Motility	Positive	Negative
Gram staining	Negative	Negative

7.Result Table 1.2 biochemical test

Biochemical result A bacteria belong to Enterobacter cloacae (or similar amylase-producing coliform). B bacteria belong to Klebsiella pneumoniae or similar non-motile Enterobacteriaceae

8.PHYTOCHEMICAL TEST RESULTS

Table 1.3 phytochemical result

S.No	**Phytochemical test**	**Zanthoxylum**	**Moringa**
1	Alkaloid test	+	+
2	Steroid test	+	++
3	Tannin acid test	++	+
4	Terpenoids test	++	+++
5	Flavonoid detection test	+	+
6	Test for phenols	+	+++
7	Test for saponins	++	+
8	Dragendroff test	+	++
9	Essentials oils	+	-
10	Coumarins	+	-

9.Antibacterial activity against agriculture bacteria

The disc diffusion method was employed to assess the inhibitory potential of *Zanthoxylum* and *Moringa* extracts against common soil-borne pathogens, measuring zones of inhibition to quantify bacteriostatic efficacy[24]. The observed inhibitory zones were compared against standard antibiotic controls to determine the relative susceptibility of the isolated microbial strains to the phytochemical constituents[25]. Furthermore, the synergistic interaction between alkaloids in *Zanthoxylum* and the elevated phenolic content in *Moringa* suggests a multi-target mechanism that disrupts bacterial cell wall integrity and metabolic pathways [26]. Isolation of bacteria from Agriculture soil checking its antimicrobial property against zanthoxylum and moringa .In performing a Minimum Inhibitory Concentration (MIC) test on various species of bacteria grown in pure culture, bacteria are exposed to a range of concentrations of zanthoxylum and moringa extracts. By inhibiting specific metabolic pathways, the active substances contained within moringa extract inhibit bacterial cell wall integrity, protein synthesis, and metabolism. Zone of inhibition in bacteria 1 is moderate in zanthoxylum and weak in moringa and in bacteria 2 moderate in Zanthoxylum and moringa.

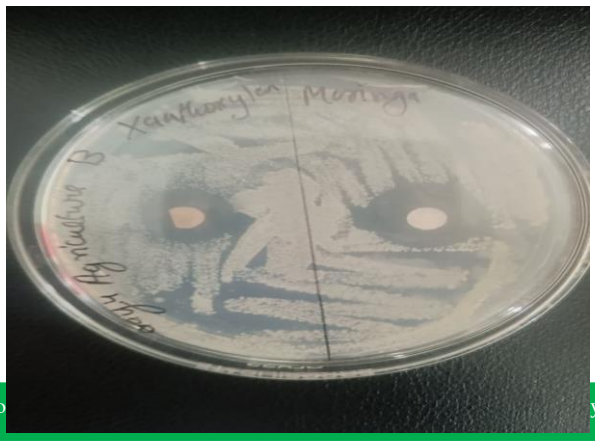


Fig 1.9 Antibacterial activity by agriculture Bacteria

10.Conclusion

Comparing the phytochemical constituents between the two plant extracts, namely Zanthoxylum and Moringa, there seemed to be variation in the distribution of the secondary metabolites, indicating variation in the efficacy of the plants. In addition, running the antioxidant tests in tandem with these screenings will connect the fingerprint of the metabolites to the physiological activity of the studied plants, which are Zanthoxylum and Moringa. Repeating the standard procedure in extracting the phytoconstituents in triplicate will ensure the reproducibility of the results. Measurement of these phytochemicals The unique chemistry of Zanthoxylum zanthoxyloides is attributed to the presence of tannins and alkaloids, while the presence of these chemical compounds in different parts of Moringa oleifera makes its morphology valuable

11.Discussion

The study looked at soil bacteria around Dehradun. We checked these bacteria and two plants, Zanthoxylum and Moringa to see if they have properties.We took soil samples and isolated bacteria from them. Then we tested these bacteria against extracts from Zanthoxylum and Moringa. The results collectively highlight the plants antimicrobial property

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