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Phytochemical screening and antimicrobial activity of select medicinal plants in Thellanthi village, Kanyakumari district, Tamilnadu, India

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Abstract
Phytochemicals are bioactive compounds present in plants, provide nutritional benefits and reduce the risk of major chronic diseases. Screening of the phytochemicals is the pioneer step in drug discovery. This study aims to evaluate the antimicrobial activity of aqueous, chloroform, and ethanol extracts of *Moringa oleifera* and *Citrus maxima* medicinal plants against two Gram-positive bacteria and two Gram-negative bacteria using phytochemical analysis. In the study, it was revealed that plants with phytochemicals show significant antibacterial activity.

Keywords: *Citrus*, *Moringa*, phytochemicals

Introduction
Since ancient times, plants have been used to treat many diseases. Phytochemicals have become great interest owing to their springy application [1]. These plants are valued for their medicinal properties because they contain chemical substances that produce a specific physiological effect on the body [2]. Compounds, especially secondary metabolites, were isolated from plants, and studies have shown that these compounds have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral, and many other activities. [3.4]. They are considered secondary metabolites and are synthesized in all parts of the plant body such as bark, leaves, stems, roots, flowers, fruits, and seeds. Plants produce these chemical compounds as a defense against herbivores, and many of these compounds protect humans from harmful diseases as well [5]. Phytochemicals are naturally occurring chemical substances found in plants that are biologically energetic materials that offer health benefits to humans and are rich in macronutrients as well as micronutrients. They protect plants from illness and damage and thus confer odours (terpenoids), pigmentation (tannins and quinines), and flavors (capsaicin) on plants. [6]. These phytochemicals protect plant cells from various environmental threats, including pollution, stress, drought, UV exposure, and pathogenic assault. Phytochemicals include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides, tannins, nitrogen compounds (alkaloids, amines, betalains), terpenoids, and endogenous metabolic compounds [7].
Citrus maxima (Burm.) Merr. generally called as Papanus. Bark and roots of *Citrus maxima* incorporate β-sitosterol, acridone alkaloid. It also contains limonine, nerolidol, nerolyl acetate, nerolidol, nerolol, and geraniol in its unripe form. Pommelos are wealthy in Vitamin C [8]. *Moringa oleifera* Lam is used to treat anemia, arthritis and different joint pain (rheumatism), asthma, constipation, diabetes, diarrhea, seizures, belly pain, stomach and intestinal ulcers, intestinal spasms, headache, high blood pressure, kidney stones, menstrual disorders, and thyroid disorders. The plant poses antispasmodic, stimulant and expectorant properties [9, 10].
This study aimed to identify the presence or absence of different phytochemicals in various extracts of *Moringa oleifera* and *Citrus maxima* medicinal plants and to determine their antimicrobial activity against two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) pathogenic bacteria.

Materials and Methods
Collection of sample
These leaves of *Citrus maxima* and *Moringa oleifera* were collected from Thellanthi, a village located in the Kanyakumari district of Tamilnadu. Cleaned leaves were shade dried.

Preparation of sample
Leaves of *Citrus maxima* and *Moringa oleifera* were shade dried at room temperature for 15 days. They were ground into fine powder using a mechanical blender.

Phytochemical Studies
The crude extracts of *Ciitrus maxima Moringa oleifera* were pull out and subjected to various phytochemical tests for the detection of phytoconsitituents such as carbohydrates, amino acids, proteins, vitamins, vitamin-C, chloride test, flavonoids, phytosterols, phlobatannins, saponins, steroids, glycosides and saponin glycosides.

Tests for Carbohydrates
Benedict' test
An equal volume of Benedict's reagent and test solution is placed in a test tube and heated for five minutes in a boiling water bath. The color of the solution become green, yellow or red depends on the amount of reducing sugar present in the test solution.

Tests for Amino Acids
Ninhydrin test
A purple or bluish colour appears in 4ml of test sample and 3 drops of Ninhydrin solution heated in a water bath for 10 minutes.
Test for Proteins

Biuret test
Add 4% NaOH and a couple of drops of 1% CuSO4 solution to 3ml of sample solution and observe the violet or pink color.

Test for Vitamins
Test for B2 (Riboflavin)
Dissolve 1mg of sample in 100 ml of water. By transmitted light, the solution is pale greenish yellow, and by reflected light, it is intense yellowish green, which disappears with the addition of mineral acids

Test for Nicotinic Acid
Heat a small quantity of sample with twice its weight of soda lime, pyridine is evolved.

Test for Vitamin C
Add 2ml of a 2% w/v solution, 2ml of water, 0.1 gm of sodium bicarbonate, and about 20 mg of ferrous sulphate, shake and allow to stand; a deep violet colour develops. To this add 5 ml of 1 M sulphuric acid, the violet colour disappears.

Tests for inorganic elements
Test for Iron
Add a few drops of 2% potassium ferrocyanide to 5 ml of sample solution. Dark blue colour is formed.

Test for Tannins
2 ml of acetic acid is added to 2-3 ml of the test solution, resulting in a red color solution.

Test for phenolic compounds
Ferric chloride test
Dilute the extract with 5 ml distilled water. A few drops of neutral 5% ferric chloride added. The presence of phenolic compounds indicated by dark green colour.

Detection of flavonoids
Lead acetate Test
In addition to Libermann-Buchard solution, sample extracts were treated with a few drops of 10% lead acetate solution. The formation of a yellow precipitate indicated the presence of flavonoids.

Test for phlobatannins
A red precipitate forms when aqueous extract of plant samples is boiled with 1 % aqueous hydrochloric acid, indicating that phlobatannins are present. Test for steroids

Salkowski Reaction
The sample extract was added to 2 ml of chloroform and 2 ml of concentrated H2SO4 and the chloroform layer turned red..

Test for saponin Glycosides
Foam test
A persistent foam was observed after shaking the sample powder with water.

Determination of antibacterial activity
Bacterial strains
Several human pathogens, including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis, have been obtained from the Centre for Marine Science and Technology (CMST), Rajakamangalam, Manonmaniam Sundaranar University, Tamilnadu, India. Bacterial stock cultures were grown on nutrient agar at 4°C.

Disc diffusion method

Diffusion discs were used for preliminary screening of the extracts. [11]. Freshly grown liquid cultures of the pathogens were seeded on nutrient agar plates with sterile swab. Sterile filter paper discs (8mm) had been soaked in 40 ml of extracts and air dried to evaporate the solvents. The discs were then placed over Muller Hinton Agar plates at equidistant intervals. After incubation at 37°C for 18 - 24 h, the plates were measured for a clearance zone around the discs. The clearance zone formed around the disc is measured. Each test was done in triplicates.

Result and Discussion

The phytochemicals such as carbohydrate, vitamin, Nicotinic Acid, flavonoids and saponin were present in all the plants.

***Citrus maxima* (Burm,) Merr.**

The phytochemical screening of an aqueous extract of Citrus maxima revealed the presence of carbohydrates, amino acids, vitamins, proteins, nicotinic acid, vitamin C, phenolic compounds, flavonoids, and steroids. The phytochemical screening of ethanol extracts of Citrus maxima revealed various phytoconstituents including carbohydrates, amino acids, vitamins, proteins, nicotinic acid, vitamin C, phenolic compounds, flavonoids, steroids, and saponin glycosides.. The phytochemical screening of chloroform extract of *citrus Maxima* showed the presence of different type of phytoconsitituents namely carbohydrate, vitamin, protein, vitamin C, flavonoids, Steroids, Saponin glycosides, nicotinic acid. Phytochemical screening of chloroform, ethanol and aqueous extract plant showed the presence of flavonoids, Tannins, Amino Acids, Carbohydrate, Saponin. This study is similar to the study of [12, 13,14,15,16,]. These bio components with many biological effect and helps in the treatment of variety of diseases. Tannins are considered to be superior antioxidants since they protect the protein from oxidation and glycation, as well as scavenging copper from the cells. [17]. Furthermore, the ability of plant extracts to fight pathological diseases is due to the presence of total phenolics and flavonoids in the extracts, as they are used to exhibit many potent biological effects [18, 19]. Secondary metabolites, such as saponin, flavonoids, phlobatannins, and glycosides play a crucial role in preventing free radicals that undergo oxidation

***Moringa oleifera* Lam.**

The phytochemical screening of aqueous extract of *Moringa oleifera* showed the presence of different types of phytoconstituents namely Carbohydrate, Amino Acids, vitamin, protein, Nicotinic Acid, Vitamin C, flavonoids, Phylopatanic and Saponin glycosides. The phytochemical screening of ethanol extract of *Moringa oleifera* showed the presence of different types of phytoconstituents namely Carbohydrate, Amino Acids, vitamin, Nicotinic Acid, inorganic elements, phenolic compounds, Saponin glycosides. The phytochemical screening of chloroform extract of *Moringa oleifera* showed the presence of different types of phytoconstituents namely Carbohydrate, protein, vitamin, vitamin C, flavonoids, Steroids, Saponin glycosides, phlobatannin.

The wound healing property and Hemostasis (prevent bleeding) of *Moringa oleifera* is due to the presence of Saponin. *Moringa oleifera* leaf extract processes property to precipitate and regulate red blood cells. Presence of flavonoids in the plants is responsible for its antioxidant and anti-inflammatory activity because flavonoids are water-soluble antioxidants and they are free radical scavengers and prevents oxidative cell damage. Glycosides and terpenoids contain anti-diarhoeal property, they inhibits release of autocoids and prostaglandins, and thus anti-diarhoeal property is due to glycosides and terpenoids. Tannins contain antihelmintic property which increases supply of digestible protein forming complex in humans interference with energy generation and by coupling oxidative phosphorylation [21]. Tannins poses antiviral, antibacterial and antiparasitic properties [22].

The results of the present study are substantiated [23] by finding out that Moringa oleifera leaves collected from Puducherry have high levels of phenolic compounds, flavonoids, and Vitamin C and show strong antioxidant activity. There were stronger concentrations of some compounds in this study, such as protein, amino acids, flavonoids, steroids, and saponin, in agreement with [24]. Extracts of leaves of Moringa oleifera from India revealed that leaves contained the most total phenolics [25]. Crude ethanolic extract of *Moringa oleifera* with compounds like steroids, flavonoids, Saponin, tannins [26].

Table 1: Phytochemical screening of Aqueous, Ethanol, Chloroform leaf extract of *Citrus maxima* (Burn.) Merr.

S. No	Extract	Carbohydrates	Amino acid	Protein	Vitamin	Nicotinic Acid	Vitamin C	Inorganic element	Tannin	Phenolic compound	Flavonoids	Phlobatannin	Steroids	Saponin Glycosides
1	Water	+	+	+	+	+	+	-	-	+	+	-	+	+
2	Ethanol	+	+	+	+	+	+	+	-	+	+	-	+	+
3	Chloroform	+	-	+	+	-	-	-	-	+	+	+	+	+

(+) Presence (-) Absence

Table 2: Phytochemical screening of Aqueous, Ethanol, Chloroform leaf extract of *Moringa oleifera Lam.*

S. No	Extract	Carbohydrates	Amino acid	Protein	Vitamin	Nicotinic Acid	Vitamin C	Inorganic element	Tannin	Phenolic compound	Flavonoids	Phlobatannin	Steroids	Saponin Glycosides
1	Water	+	+	+	+	+	+	-	-	-	+	-	-	+
2	Ethanol	+	+	-	+	+	-	+	-	+	-	-	-	+
3	Chloroform	+	-	+	+	-	-	-	-	+	+	+	+	+

(+) Presence (-) Absence

Antibacterial activity

The results reveals, that the ethanolic extract of select plants with efficiency suppressing the activity of harmful microorganisms. In this study, the ethanolic leaf extract of all the plants showed potential activity against all the microbial cultures of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilus.*

Moringa oleifera highest zone of inhibition, (9.5mm) was recorded against *Escherichia coli* and also the smallest zone of inhibition (3mm) was recorded against genus *Pseudomonas aeruginosa*. The result was in in line with the findings of [27].

In *Citrus maxima* zone of inhibition, (5mm) was recorded against *Escherichia coli* and the minimal zone of inhibition (3mm) was recorded against *Staphylococcus aureus.* Among all the plant assigned comparatively showed better results of antibiotic density reactions than the other plant extracts all the selected plants showed plant inhibitory activity against gram negative bacteria than gram positive bacteria. Among the selected bacterial organisms, the highest antibacterial activity was noted against gram negative bacterium *Pseudomonas aeruginosa* and *Escherichia coli*.. The result was in line with the findings of [28].

Table 3: Antibacterial activity of Ethanolic extract of *Citrus maxima* and *Moringa oleifera*

Sl. No	Microorganism	Diameter of zone of inhibition (mm)		
		Control	*Moringa oleifera*	*Citrus maxima*
1	*Staphylococcus aureus*	5	6	3
2	*Pseudomonas aeruginosa*	5	3	5
3	*Escherichia coli*	5	9.5	5
4	*Bacillus subtiilis*	5	5	4

Antimircobial substances of the plant extracts (terpenoids, alkaloid and pheb=nolic compounds and synthetic resin compounds) interact with enzymes and proteins of the microorganism cellular membrane its disruption to disperse a flux of protons in exterior of cell, that causes death inhibit enzymes for aminoacids synthesis [29].

Conclusion

From the current study, it's evident that the leaves of *Citrus maxima* and *Moringa oleifera*, contains phytochemical elements, probably, these compounds have the foremost vital applications against human pathogens.

They have important antibacterial drug action and might use as antibacterial drug agent. The results of assorted screening tests indicate that the leaves have some measurable restrictive action against gram-negative bacterium Pseudomonas aeruginosa. The antibacterial activity may be attributed to the presence of various secondary metabolites like amino acids, tannin, vitamin, saponin, glycosides and also the mechanism of action may well be because of their individual or collective participation. The study concludes that, ethanolic extract of Citrus maxima and Moringa oleifera with potent antibacterial potency.

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