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Antimicrobial Activity and Phytochemical Analysis of Citrus Sinensis Leaves Extracts

Pramila Kori[1], Meenakshi Nagar[2]

Department of Chemistry, Govt. Holkar Science College, Indore (M.P.), India

Abstract: ***The leaf extract of Citrus sinesis were screened for its antimicrobial and phytochemical activities. The solvents used for the leaves and root extraction were benzene, acetone, aqueous. The extract was tested against infectious disease causing bacterial such as Escherichia coli, Pseudomonas aerginosa, staphylococcus aureus using the well diffusion method. The benzene, acetone, aqueous extract of leaf of Citrus sinensis inhibition against the entire test microbe ranging from 8 to 16 mm diameter inhibitory zone. In present study, bacterial extract showed a varying zone of inhibition of growth of tested organism than benzene, acetone, aqueous. Phytochemical properties of leaf of Citrus sinesis obtain from benzene, acetone, aqueous extract were investigated. The result confirmed that presence of antibacterial activity and phytochemical in shade dried extract of Citrus sinensis against the human pathogenic bacteria.***

Keywords: Antimicrobial and phytochemical activity, *Citrus sinensis*, S. aureus, P. aerogenosa, E. coli.

1. Introduction

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmocological screening is even smaller. Thus any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Random screening as tool in discovering new biologicaliy active molecules has been most productive in the area of antibiotics.[1] medicinal plants represent a rich source of antimicrobial agents.[2] although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated. Considering the vast potentiality of plants as source for antimicrobial drugs with refrence to antibacterial activity. In current work the antimicrobial activity of various extracts of a valuable medicinal plant *Citrus sinensis* is systematically studied on some common pathogenic microorganisms, which may result into the development of potent natural remedy for many infections after advance studies in future.

Citrus sinensis belongs to *Rutaceae* family and it is commonly known as sweet orange [3] It is the most commonly grown tree fruit in the world [4] The sweet orange is an evergreen flowering tree generally growing to 9– 10m in height. Its fruit is strengthening, cardiotonic, Laxative, anthelmintic and removes fatigue [5.] It possesses anti-inflammatory, antibacterial and antioxidant properties. [6] Its leaves are shiny and leathery, arranged alternatively. Oranges are said to lower cholesterol and aid in the digestion of fatty foods [7]. The vitamin C in Oranges is concentrated mainly in the peel and the white layer just under the peel. The peel contains citral, an aldehyde that antagonizes the action of vitamin A. Therefore, anyone eating quantities of orange peel should make certain that their dietary intake of vitamin A is sufficient [8.]

2. Materials and Methods Collection of Plant Material

Collection of plant material the leaves of *Citrus sinensis* were done from the area around pachore, Madhya Pradesh. The whole plant and parts were done by Phytochemical extraction and screening.

a) Extraction of Plant

The leaves of *Citrus sinensis* were allowed to dry in shade for a week and then grounded into fine powder in mixer grinder. 10 gm of dried powder was subjected to soxhlet extraction with 200 ml of solvents starting from Benzene followed by extraction with other solvents acetone and pure distilled water in separate ways. Soxhlet process was allowed to carry out till the complete exhaustion of sample material use for extraction with the maintenance of temperature below the boiling points of the solvents used. The extract so obtained is subjected to evaporation of solvent to get the extract in crystalline/slurry form which were suitably diluted and used for preliminary phytochemical analysis and studies of their antimicrobial activity.

b) Phytochemical Analysis of the Extract

A small portion of the extracts were subjected to the phytochemical test using Harbourne's (1983) methods to test for alkaloids, tannis, saponins, flavonoids, glycosides, steroids, phenolic compound, amino acids.[9]

Test for alkaloids: About 0.2 g extract warmed with 2% H2SO4 for two minutes, filtered and few drops of Dragendorff's reagent added orange red precipitate indicates the presence of alkaloids. And or filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for glycosides: The extracts hydrolyzed with HCl solutions and neutralized with NaOH solutions. A few drops of Fehling solution A and B were added. Red precipitate indicates the presence of glycoside. Another test use was Benedict's test, in which the filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for tannins: Small quantity of extracts mixed with water, heated, filtered and ferric chloride added. A dark green solution indicates the presence of tannins.

Test for saponins: About 0.2g of the extracts shaken with 5ml of distilled water and then heated to boil frothing (appearance of creamy mix of small bubbles) shows the presence of saponins.

Test for flavonoids: Extract of about 0.2 g of the extracts shaken with 5ml of distilled water and then a few drops of 10% lead acetate solution is added. A yellow or dirty white precipitate shows the presence of flavonoids.

C.Culture Media And Inoculum Preparation

Nutrient agar broth cultures of the pure culture isolates of *Staphylococcus aureus, E. coli and Pseudomonas aeruginosa* were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37°C for 48hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture.

D.Antimicrobial Activity

The well diffusion method was used to determine the antibacterial activity of the extracts prepared from the *Carica papaya* leaves and roots using standard procedure. In this method, first the test bacteria broth of bacteria is used to inoculate on the nutrient agar plates with the help of sterile cotton swabs to develop the lawn culture. Then to these plates 6 mm diameter well are punched in agar plates pre-inoculated with test microorganisms Undiluted over night broth cultures should never be used as an inoculum. Routine direct application of suitably diluted extracts is poured into the well. The plates were incubated at 37oC for 24 hr. and then examined for clear zones of inhibition. Sterile water was used as control.[10]

3. Result and Discussion

Phytochemical Analysis of Bioactive Compound in Different Solvents Extracts of Citrus Sinensis

The plant leaf extracts in different solvent were screened for the presence of various bioactive phytochemical compounds. The analysis of alkaloids, glycosides, tannins, saponins, flavonoids, steroids, phlobatannis. The benzene extract tannins are absent and alkaloids, glycosides, saponins, flavonoids, steroids, phlobatannis are presnt. In Acetone extract alkaloids, steroids are absent and phlobalycosides, tannins, saponins, flavonoids, phlobatannis are presnt. In aqueous extract alkaloids, phlobatannins, steroids are absent and glycosides, tannins, saponins, flavonoids are presnt. This documented in table 1

Table 1: Phytochemical analysis of *Citrus sinensis* extracts from leaves

S.N.	Constituents	Benzene extract	Acetone extract	Aqueous extract
1	Alkaloids	+	-	-
2	Tannins	-	+	+
3	Glycosides	+	+	-
4	Saponins	+	+	+
5	Flavonoids	+	+	+
6	Phlobatannis	+	+	-
7	Steroids	+	-	-

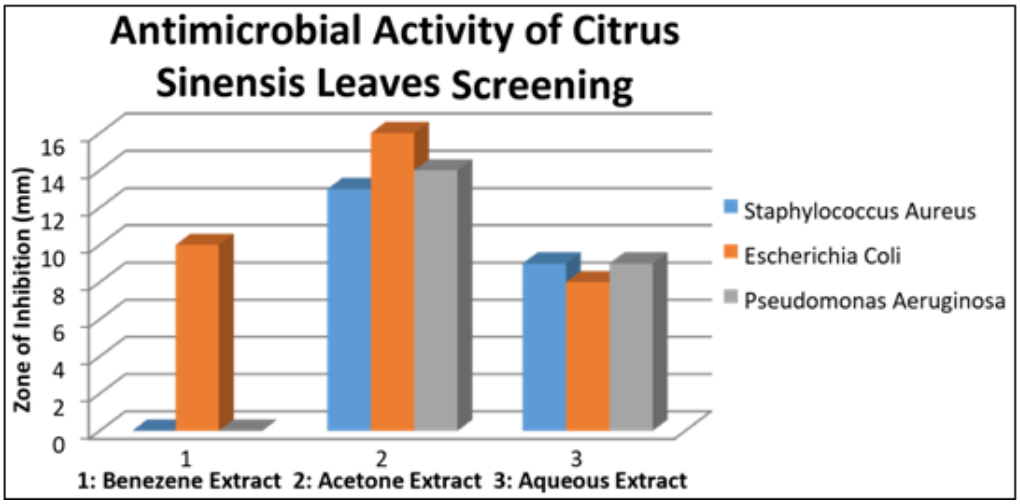
[(+) means present (-) means absent]

Antimicrobial Activity of Different Organic Solvent Extracts of *Citrus Sinensis*

Antimicrobial efficacy of different solvent extract of *Citrus sinensis* is shown. The result from leaf extracts of Citrus *sinensis*, acetone extract shows maximum antimicrobial activity against E. coli out of the all test microbes with zone of inhibition lying in the range of 16mm approx. on the basis of result depicted in the table 1. The least inhibitory range was 8mm for aqueous extract against E. coli. but there was no inhibition observed against S, aureus, P. aeruginosa in benzene leaf extract.

Table 1: Result of antimicrobial activity of leaves of Citrus sinensis

S.N.	Test microbes	Zone of Inhibition due to *Citrus sinensis* leaves extract 1mg/ml (in mm)		
		Benzene extract	Acetone extract	Aqueous extract
1	Staphylococcus aureus	Nil	13	9
2	Escherichia coli	10	16	8
3	Pseudomonas aeruginosa	Nil	14	9



Graph 4.3: Screening for the antimicrobial activity of Leaves extracts of *Citrus sinensis* for three test species

4. Conclusion

The phytochemical analysis revealed the bioactive compounds which are responsible for the in vitro antimicrobial of *Citrus sinensis* our all bacterial strains in all extracts could be benzene, acetone, aqueous extract of various parts of *Citrus sinensis* might be exploited as a natural drug for the treatment of several infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications.

Our results showed that in present work that extracts obtained leaves of the plant *Citrus sinensis* using various solvent are rich sources of potent phytochemicals especially the leaves extract and has inhibitory effects on the experimental microbes. From previous studies and the current work, it is clear that the plants are rich source of alkaloids, glycosides, tannins, saponins, flavonoids, steroids, phlobatannis. These bioactive complex phytochemicals can be used for the development of potent drugs, medicines or antimicrobial agents that can be used for various purpose for human welfare upon further extensive and systematic studies.

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