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In-Vitro Anti-Bacterial and Anti Fungal Activities of *Citrus Maxima* and *Citrus Sinensis* Linn Leaf Extracts.

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ABSTRACT

Antimicrobial and anti-fungal activity of ethanolic and aqueous leaf extracts of *Citrusmaxima* and *Citrus sinensis* (Family**:** Rutaceae) was examined against 4 bacteria which includes two Gram +vebacterias(*Bacillus subtilis, Staphylococcus aureus),* two Gram -ve bacteria's (*Escherichia coli*, *Pseudomonas aeruginosa*). It was also examined against 4 fungi (*Aspergillusniger*, *Fusariummoniliforme*, *Candida albicans*and *Mucorplumbeus*). All these were tested for antibacterial and antifungal activities by agar well diffusion method and Disc diffusion method respectively. At different concentrations (40µl, 70µl, 100µl) of leaf extracts were studied, 40µl was found to be no activity against both bacteria and fungi. The concentration at 100µl all the four bacterial species were found to be significant anti-bacterial and anti-fungal activity, where as the *Candida albicans* shown the less significant anti-fungal activity compared to standard

KEY WORDS:*Citrus maxima, Citrus sinensis,* antibacterial, antifungal, Physiochemical, Pharmacological.

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INTRODUCTION:

A genus of *Citrus* (Linn) of family Rutaceae is an evergreen aromatic shrub or small trees, its leaflets are 3-6 inch long,elliptic-ovate or ovate-lanceolate with sort, wingless or nearly wingless petioles flowers are 5-10 in a raceme, small or middle-sized; petals are generally more or less pink; fruit is globes ovoid or oblong often mamillate at the apex [1]. This fruit occupies an important place in the medicine and also in the fruit cost-cutting. Scientifically it is also known as *Aurantium maximum* Burm, *Citrus aurantium* L, Var*grandis* L, *Citrus Decumana* L, *Citrus grandis* & *Citrus pamplemos*. *Citrus grandis* (Linn) is a crop plant of India, China, Indonesia, America, Thailand etc. The citrus fruits are well competent with a variety of phyto-fungicides that are necessary to inhibit fungal growth and development[2]. Citrus oil has a toxic effect on fleas, fire ants and houseflies due to 90–95% limonene. In southern parts of Nigeria, *Citrus sinensis*is used to control malaria and skin diseases[3]. In traditional medicine, the fruit peel has been used for cough, swelling, and epilepsy, because of the effectiveness of the volatile [4]. The root bark contains β- sitosterol and several acridone alkaloids, and coumarins which shows antimicrobial activity.

Citrus maxima are a perennial shrub commonly known as Papanus, distributed throughout India. Bark and root of *Citrus maxima* contain β-sitosterol, acridone alkaloids. Essential oil from the leaves and unripe fruits contain limonin, nerolol, nerolyl acetate and geraniol. It is said to possess appetizing, cardiac stimulant and antibacterial activity[5]. The root bark contains β- sit sterol and several acridone alkaloids and coumarins which shows antimicrobial activity. Petiole narrowly winged, wings oblanceolate. Rind deep yellow to orange-red; thick or thin; pulp orange-yellow or reddish, sweet or slightly acidic.

Citrus sinensis (L.)orange or sweet orange, Spinous tree, up to 10 m tall; spines few slender and flexible. Leaves are aromatic 7.5-11 cm, ovate-oblong to ovate, entire or serrulate, acute to obtuse. Flowers are axillary, in 1-6-flowered cymes. Petals reflexed. The fragrant white flowers 20 to 25 yellow stamens[6]. Fruits are oblate to slightly oval, 6-9 cm in diameter. Fruit somewhat longer than broad, 8-9 cm in diameter; rind deep orange, apex marked with a distinct open or closed navel, containing a rudimentary secondary fruit. Pulp juicy, sweet, highly flavoured, excellent source of vitamin C. *Citrus sinensis*is used for skin disorders. Citrus flavonoids reported for large spectrum of activities like antibacterial, antifungal, antidiabetic, anticancer and antiviral activities[7]. It is excellent source of vitamins, minerals and other nutrients. Many phytoconstituentsincludes, Monoterpenes, sesquiterpenes & bioflavonoid like limonoids, synephrine, hesperidin, pectin and also contain calcium, potassium, thiamine, niacin etc. These biologically active compounds prevent pulmonary disorders, stomach troubles, febrifuge, cancer,, stomach ulcer and cause a reduction in

cholesterol levels and high blood pressure, promoting human health, as well as significant anti-inflammatory, antibacterial, anti-malarial and antifungal activity[8], [9].

The present study is aimed to determine the screening of antibacterial and antifungal activities against two Gram positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), two Gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa) and four different fungi (*Aspergillusniger, Candida albicans, Fusariummoniliforme* and *Mucorplumbeus*)of ethanolicand aqueous leaf extracts of ***Citrus maxima*** and ***Citrus sinensis***

MATERIALS AND METHODS

Plant material

Citrus maxima and *Citrus sinensis* Linn(Family: Rubiaceae) plants were collected in local areas of Bangalore, Karnataka, India. And authenticated by Dr. T. Sridhar Bairy by comparison with the standard specimens deposited at the Department of Drava Guna, SDM College of Ayurveda, Udupi. Voucher specimen is kept at the Acharya and BM Reddy College of pharmacy Bangalore, Karnataka. India

Preparation of extracts

Leaves of *Citrus maxima* and *Citrus sinensis* were collected from local areas of Bangalore, Karnataka, India and then dried in shade. After complete dry, fine powder was made by electric grinder.

Aqueous extract: The aqueous extract of dried plant leaves was made in the distilled water. About 5 grams of each plant leaves powders (*Citrus maxima* and *Citrus sinensis*) were taken and mixed in 50 ml of distilled water. The mixtures were taken into 250 ml sterile conical flasks, plugged with sterile cotton and kept in shaking Incubator with the 200 rpm for 24 h. The solutions were filtered through muslin cloth followed by what man filter paper[10]. This process was repeated three times after which a clear aqueous extracts of the both plant leaves.

Ethanolic extract: The ethanolic extracts of dried both plant leaves were prepared. The ethanolic extracts were prepared through the same protocol followed for that of cold water extraction. . The extracts were added into clean Petri plate then allowed for evaporation. After evaporation, the plates were weighted. Residual concentrates were dissolved in 5 ml of DMSO. The extracts were collected in screw capped bottles. The extracts were used for antibacterial and antifungal activity, MIC test and phytochemical test. All these extracts were stored at 20°C for experimental use. Bio efficacy of the extracts was checked *in-vitro* by well in agar diffusion method and Disc diffusion method[12].

Organisms

Four bacterial cultures were utilized in the present study which includes two Gram positive (*Staphylococcus aureus, Bacillus subtilis*) andtwo Gram negative (*Escherichia coli, Pseudomonas aeruginosa*), the fungal cultures (*Aspergillusniger, Fusariummoniliforme, Candida*albicansand *Mucorplumbeus*).All these strains were obtained from culture bank of our laboratory. The bacteria were grown in nutrient broth at 37°C and maintained on nutrient agar slants at 4°C and fungal cultures were grown and maintained on Sabourauds dextrose agar (SDA) at 4°C.

Agar-well diffusion method:

The assay was conducted by agar well diffusion method. The bacterial strains were grown on nutrient agar at 37°Cfor 18 h and were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). The suspension was used to inoculate 90 mm diameter Petri. Wells (6 mm diameter) were punched in the agar plates filled with the aqueous and ethanolic leaf extracts, which was dissolved in DMSO. Preparethe different concentrations viz. 40µl, 70µl, 100µl of both plant extracts. Controls were maintained by inoculating pure DMSO, which did not affect the growth of microorganisms. Plates were incubated at 37°Cfor 24 h. Antibacterial activity was evaluated by measuring inhibition zone diameters. The experiments were conducted in triplicates. The same method was followed for testing antifungal activity using potato dextrose agar medium[12].

Disc-diffusion method:

Disc diffusion method was followed by taking the Flucanazole as standard. The agar plates were inoculated by dipping a sterile cotton swab into the inoculums suspensions and streaking the swab in three directions over the entire agar surface. The plates were allowed to dry for 10–15 min before the disks loaded with different concentration viz. 40µl, 70µl, 100µl of both leaf extracts were applied. The inhibition zone diameter was measured in mm using a dial caliper after 4–7 days of incubation at 30°C for *Aspergillusniger, Fusariummoniliforme, Candida albicans*and *Mucorplumbeus*, the plates were examined and the diameters of the inhibition zones were measured to the nearest millimetre[13].

RESULTS AND DISCUSSION

In the present investigation, four bacterial species viz. Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and fungal species viz. *Aspergillusniger, Fusariummoniliforme, Candida albicans*and *Mucorplumbeus* were tested to determine antibacterial

and antifungal activitiesfor both aqueous and ethanolic extracts (*Citrus maxima* and *Citrus sinensis)* of leaves. The values were given in the table 1 & 2 are the mean of the three sets of observations.

Anti- microbial activity:

Alcoholic and aqueous leaf extracts of *Citrus maxima* and *Citrus sinensis* at concentrations of 40µg, 70µl and 100µl were used to study anti bacterial and antifungal activities. The concentration at 40µl did not shown anti-bacterial activity, whereas, at 70µl concentration of both the leaf extracts shown moderate activity on *Bacillus subtilis* and *Staphylococcus aureus* and more significant activity (Table No 1) on *Escherichia coli* and *Pseudomonas aeruginosa*, mean while**,** concentration at 100µl all the four bacterial species were found to be significant**.**

Table No 1. Anti-bacterial activity of alcoholic and aqueous extracts of *C maxima* and *C sinensis*

Bacteria	Alcoholic extract of *Citrus maxima*			Aqueous extracts of *Citrus maxima*			Alcoholic extract of *Citrus sinensis*			Aqueous extract of *Citrus sinensis*			Kanamycin
	40 µl	70 µl	100 µl	40 µl	70 µl	100 µl	40 µl	70 µl	100 µl	40 µl	70 µl	100 µl	30µg/ml
Bacillus subtilis	-	7.3	11.3	-	9.2	15.6	-	8.7	14.2	-	11.7	14.5	17.2
Escherichia coli	-	8.9	11.9	-	11.1	12.3	-	8.5	9.7	-	10.2	11.8	16.4
Pseudomonas aeruginosa	-	6.9	12.5	-	7.2	15.1	-	7.4	15.3	-	7.1	16	18.3
Staphylococcus aureus	-	9.7	11.7	-	10.4	13.3	-	9.6	10.3	-	10.8	12.9	16.7

Anti-fungal activity:

Antifungal activity of alcoholic and aqueous leaf extracts of *Citrus maxima* and *Citrus sinensis* were performed against four fungi. All the concentrations 40µl, 70µl and 100µl inhibited the growth of four fungi under study.All the different concentrations of extracts of *Citrus maxima* and *Citrus sinensis*were found to show maximum inhibition of all the three fungi species ie, *Aspergillu sniger*, *Fusariummoniliforme* and *Mucorplumbeus* under study, where as the *Candida albicans*shown the less significant activity (Table No. 2) compared to standard. Based on previous reports and present investigation can be said that the plant possess wide range of anti bacterial and antifungal phyto-constituents. Further investigations should be carried out in finding other activities of the extracts of other parts of plants.

Table No 2. Antifungal activity of alcoholic and aqueous extracts of *C maxima* and *C sinensis*

Extracts	Alcoholic extract of *Citrus maxima*			Aqueous extracts of *Citrus maxima*			Alcoholic extract of *Citrus sinensis*			Aqueous extractof *Citrus*			Flucan azole
	40 µl	70 µl	100 µl	40 µl	70µl	100µl	40µl	70µl	100 µl	40µl	70µl	100 µl	10µg/ml
Aspergillus niger	11	14	19	9	10	14	10	12	17	9	10	15	18
Fusariummonili forme	35	42	46	31	36	39	36	41	44	28	32	37	42
Mucorplumbeus.	13	14	21	15	16	19	16	17	20	15	16	19	19
Candida albicans	5	6	6	4	5	7	5	5	6	6	7	7	13

DISCUSSION

Various publications have documented the antimicrobial activity of plant extracts and essential oils including lemon juice against HIV, syphilis, gonorrhoea[14] and topical microbicide[15]. In the present study leaf extracts of *Citrus maxima* and *Citrus sinensis* were found to be the most effective agent against all tested bacteria and fungi. The basis of varying degree of sensitivity of test organisms may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the crude extracts. One or more of the common phytoconstituents like alkaloids, tannins, phenols, glycosides, flavonoids and acids were already reported in some of these active extracts[16, 17]. These major compounds are known to have antimicrobial activity[18]. On the basis of the present investigation it can be highlighted that some of these extracts of ***Citrus maxima*** and *Citrus sinensis*Linnshowed promising antibacterial and antifungal properties and could be exploited in herbal preparations for both external and internal uses. Since the fruit is edible and used for preparation of pickles and juices by Kumaonpeople, various herbal preparations for internal use. Root is also reported in ancient Indian literature to be used as anthelmintic and in urinary calculus treatment[19].

CONCLUSION:

The active extracts can also be screened against more human pathogens including human immunodeficiency virus (HIV) and antibiotic resistant strains. The active principles of these extracts are required to be isolated, characterized and tested for their safety and efficacy to uncover their therapeutic potential in modern medicine against infectious diseases. Furthermore of these compounds can be subjected to animal and human studies to determine their effectiveness in whole organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal micro biota.

Further, pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of this herbal extract in treating various infections and skin diseases.

Conflict of interest: NIL

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