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Phytochemical Screening and *In vitro* Antimicrobial Activity of Combined *Citrus paradisi* and *Ficus carica Linn* Aqueous Extracts

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Abstract: To investigate the antimicrobial properties of the combnined essential oil of *Citrus paradisi* var. star ruby and *Ficus carica* extracts. The *Citrus paradisi* and *Ficus carica* were tested against pathogenic microorganisms; *S. aureus, E. coli, K. pneumoniae, B. subtilis, M. luteus* and *Candida albicans*. The extracts tested exhibited good antimicrobial activity against all the clinical isolates when compared with standard. The different extracts showed remarkable inhibitory action against various Gram positive and Gram negative bacteria and two fungal species. The methanolic, petroleum ether, chloroform, ethyl ether, ethanol extract *Citrus paradisi* and *Ficus carica* was screened for its antimicrobial activity. Antimicrobial activity was detected by observing the growth response of different organisms to the methanolic extract. It was generally based on the inhibition of growth of microorganisms which were measured with a desired concentration of the plant extract of *Citrus paradisi* to be examined with the standard concentarion preparation.

Key words: Antibacterial · Antifungal · *Citrus paradisi* · *Ficus carica Linn* · TLC

INTRODUCTION

The worldwide emergence of *E. coli*, *K. pneumoniae, Haemophilus* and many other â-lactamase producers has become a major therapeutic problem. Multi-drug resistant strains of *E. coli*, *K. pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community acquired infections [1]. *Candida albicans*, also a nosocomial pathogen, has been reported to account for 50-70% cases of invasive candidiasis [2]. Alarmingly, the incidence of nosocomial candidemia has risen sharply in the last decade [3]. All this has resulted in severe consequences including increased cost of medicines and mortality of patients. Plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts [4]. Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. *Citrus* fragrances have been particularly attributed with mood enhancing properties by aroma therapists. The essential oils obtained from genus *Citrus* are recommended for the treatment of anxiety also. Volatile oils isolated from grapefruit (*Citrus paradisi*), lemon (*Citrus limon*), bergamot (*Citrus bergamia*), lime (*Citrus aurantifolia*), mandarin (*Citrus nobilis*) and orange. (*Citrus aurantium*) are often used in the treatment of anxiety [5]. *Ficus carica* Linn. (Syn: *Ficus sycomorous*; family: Moraceae) is grows in tropical and subtropical regions of India, used for variety of purpose in traditional medicine. The usefulness of this plant is scientifically evidenced and different biologically active phytoconstituents were isolated form plant. In traditional medicine the roots are used in treatment of leucoderma and ringworms and its fruits which are sweet, have antipyretic, purgative, aphrodisiac properties and have shown to be useful in inflammations and paralysis [6]. *F. carica* has been reported to have numerous bioactive compounds such as arabinose, β-amyrins, β- carotines, glycosides, β-setosterols and xanthotoxol [7-10].

MATERIALS AND METHODS

Plant Material: All other chemicals and reagents were procured from authorized suppliers and were of analytical

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grades. The plant material *Citrus paradisi* and *Ficus carica* whole parts were collected from local area of Indore Madhya Pradesh India and identified at the Department of Pharmacogonsy Dr.Hari Singh Gaur University Sagar [M.P].

Preparation of Extracts: Leaves were taken and air dried in shade for ten days. Then whole dried plants are made coarse powder. Now the extraction was occurred by the soxhlet apparatus using the methanol (50-60°C), petroleum ether, chloroform, ethyl ether, ethanol extract of the leaves in their increasing order of polarity as a solvent. Then the extracts were concentrated by putting the extract on water bath or by distillation. The extracts so obtained were concentrated to dryness by evaporating the solvent under reduced pressure using rotary evaporator. The aqueous extract was prepared by cold maceration of 250 g of the shade-dried leaf powder in 500ml of chloroform water (1:99) for 7 days. The various extracts obtained were filtered, concentrated, dried in vacuum and the residue stored in a refrigerator at 2- 8°C for use in subsequent experiments.

Preliminary Photochemical Screening: The dry extracts were subjected to various chemical tests [11,12] to detect the presence of different phytoconstituents. The coarse powder of leaves of *Citrus paradisi* and *Ficus carica* (100g) was subjected to successive extraction with different solvents in their increasing order of polarity from methanol, petroleum ether (60°-80°C), chloroform, acetone, ethyl ether and ethanol. The extracts were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents.

Formulation of Extract: For anti-microbial activity study on the day of experimentation, the different amount of powder was suspended in distilled water to get different concentration of suspension.

Antibacterial and Antifungal Studies: The various extracts were tested for their effect on Gram +ve bacteria Gram – ve bacteria such as *S. aureus, E.coli, K. pneumoniae, B. subtilis, M. luteus* and *Candida albicans*. Fungi used for the present study were *Candida albicans*. Minimum inhibitory concentration of the extracts was evaluated by cup plate diffusion method for antibacterial and antifungal activity [13,14]. 0.1 ml of overnight grown nutrient broth culture of the bacteria was transferred aseptically to sterile glass Petri dish. Sterile molten nutrient agar (45°C) was then poured, mixed uniformly rotating the plate and allowed to solidify. Cups were made out in the centre of the seeded nutrient agar plate using a sterile cork borer (6mm). The various extracts of the *Citrus paradisi* leaf of different concentrations viz. 5,10,20,50, 100, 200, 400 mg/ml were made using dimethyl sulphoxide (DMSO) as a diluting solvent. The samples were added with a sterile micropipette to each of the cups. The plates were then incubated at 37°C for 24 hrs. Plates with cups containing only DMSO served as a control.

Agar Well Diffusion: The antimicrobial activity of the essential oil was determined by using the agar well diffusion technique. Nutrient agar plates were each seeded with 0.5 ml of an overnight culture of each bacterial, while the sabouraud dextrose agar plates were each similarly seeded with each fungal strain. The 24 hrs broth culture of each bacterium and three days inoculated fungus culture were used to seed sterile molten nutrient agar and sabouraud dextrose agar at 45°C respectively, allowed to set and well made by sterile cork borer and 100 µl (0.1 ml) solution of essential oil added in to in each well.

RESULTS AND DISCUSSION

The antimicrobial affect of plant extract against the different strains are illustrated in Table 1. The five different crude extracts viz. petroleum ether, chloroform, ethyl ether, ethanol and aqueous extract of *Citrus paradisi* and *Ficus carica* were tested against various Gram +ve and Gram –ve bacteria. The results illustrated in Table 1 revealed the extract of *Citrus paradisi* as most active against *S. aureus, E. coli* and *K. pneumoniae* in the dilution of 5 to 400 concentrations. It is clear that all the extract at various concentrations have shown antibacterial activity equivalent to that of standard against the entire tested organism. Petroleum ether, methanol and aqueous extracts have shown better activity than the standard against all the four microorganisms. Chloroform extract was only effective against *Bacillus subtilis, M.luteus* and *C.albicans*. Acetone extract was most effective against *M. luteus* and *C. albicans*. The zone of inhibition varied among suggesting that the varying degree of efficacy and different phytoconstituents of herb on the target organism. Preliminary phytochemical screening of different extracts showed the presence of alkaloids, tannins, saponin, flavonoids, steroids and glycosides.

Table 1: Antimicrobial activity of *Citrus paradisi and Ficus carica* aqueous extract of different micro organisms

	Zone of Inhibition in (mm)					
Sample Conc. in %	*S. Aureus*	*B. Subtillis*	*E. Coli*	*K. pneumoniae*	*M. luteus*	*C. albicans*
400	24	14	22	17	23	19
200	21	17	20	18	22	24
100	22	19	20	22	21	18
50	23	15	19	21	17	21
20	16	11	20	18	22	19
10	14	12	18	14	11	17
5	11	08	11	13	16	15

Table 2: Antimicrobial activity of antibiotics on different micro organisms

	Microorganisms (inhibition zone in mm)					
	Gram-positive			Gram-negative		
Standard Drug	*S. aureus*	*B. subtilus*	*K. pneumoniae*	*E. coli*	*M. luteus*	*C. albicans*
Ampicillin	24	21	20	19	22	16
Cephalexin	21	20	-	-	19	15
Amoxicillin	22	25	21	16	15	18

Table 3: Phytochemical group test for the Petroleum ether, Chloroform, Extract of *Citrus paradisi* and *Ficus carica*

S. No.	Phytoconstituents	Extract of leaves of *Citrus paradisi Linn*
1	Alkaloid	-
2	Amino acid	+
3	Flavonoids	-
4	Glycosides	-
5	Tannins	+
6	Saponins	-

+Present, - Absent

The antibacterial activity of the plants may be due to the presence of various active principles in the leaves. From the antimicrobial screening it was found that the methanolic extract of *Citrus paradisi* and *Ficus carica* possessed significant antibacterial and antifungal activity when compared with the other extracts and standard drugs.Further studies involving the isolation, characterization and purification of the chemical compounds of the plant and screening for antibacterial and antifungal may result in the development of a potent entity which will be of lower toxicity and a high therapeutic value to the mankind. Thus, it can be concluded that while screening of various extracts of *Citrus paradisi* leaf against various Gram +ve and Gram –ve bacteria and fungi, ethanol extracts exhibited very satisfactory inhibitory activity.

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