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Research Article

EFFICIENCY OF PHYTOCHEMICAL COMPOUND FROM THE PEEL EXTRACT OF *Citrus sinunsis* AND *Citrus medica* AGAINST CLINICAL ISOLATES FROM WOUND INFECTION

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Abstract

An antimicrobial is a substance which kills or inhibits the growth of much type of microorganisms. The citrus product has an important and physiological role because of its commercial value in food and pharmaceutical industries of the entire world. Many pharmacological studies have been conducted to investigate the properties of Citrus in an attempt to authenticate its use as a multi-purpose medicinal agent. Finding healing powers in plants is an ancient idea. According to the World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. Citrus sinensis and Citrus medica peel extracts were collected for their phytochemical compounds against ethanol, methanol and aqueous extract. The antimicrobial activity of these extracts was tested against bacterial wound pathogens *viz.*, *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*. Among the peel extracts examined *Citrus medica* showed highest antibacterial activity against wound pathogens.

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1. Introduction

Medicinal plants are used for several microbial and non-microbial originated diseases due to their valuable effects in health care (Abalaka and Bello, 2006). The affordability, reliability, availability & low toxicity of medicinal plants in therapeutic use have made them popular and acceptable for implementation in medicinal health care all over the world (Siva Sakthi *et al.*, 2011; Prabhahar *et al.*, 2012). Plants are indeed the first material used in alternative medicine type of remedy against many diseases (Adewusi and Afolayan, 2010; Siva Sakthi *et al.*, 2011). Bacterial infections are one of the prominent

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causes of health problems around the world. Medicinal plants are a rich source of antimicrobial and provide a safer and cost-effective way of treating bacterial infections. The antibacterial activity of natural products from medicinal plants is applicable for the treatment of bacterial, fungal and viral diseases. Medicinal plants have been recognized as potential drug for their properties. Secondary metabolites or phytochemicals such as phenols, flavonoids, alkaloids, terpenoids and essential oil have provided to be responsible for the antimicrobial activity of plants (Hwang *et al.*, 2002; Saranraj *et al.*, 2010; Saranraj and Murugan, 2011; Saranraj *et al.*, 2012).

*Citrus medic*a is widely used in traditional system of medicine. Many pharmacological studies have been conducted to investigate the properties of *citrus medica*. The *citrus medica* possesses analgesic, hypoglycaemic, anticholinesterase, anticancer, antidiabetic, hypocholesterolemic, hypolipidemic, insulin secretagogue, anthelmintic and antimicrobial properties. (Singh *et al.*, 2012).

2. Materials and Methods

Collection of Sample

Citrus sinensis peel and *Citrus medica* peel were collected for local market at Tiruvannamalai and their peel were separated from the edible portion. These peels were washed with sterile distilled water and air dried at room temperature. Dried peels were coarsely powdered using mortar and pestle and were further reduced to powder using and electric blender. The powder was transferred into closed container for further use.

Preparation of Extract

The air dried powdered cumin seeds were extracted in a continuous extraction apparatus (Soxhlet) until exhaustion with ethanol for preparation of crude ethanol extract. The solvent was completely removed by evaporation under reduced pressure at a temperature not exceeding 40 ˚C. Crude extract of cumin was kept in deep freeze till used.

Qualitative assessment of phytochemical compounds from peel extracts of *Citrus sinensis* and *Citrus medica*

Screening of major phytochemical constituent *viz.*, Alkaloids, flavonoids, tannins, glycosides, saponins, steroids and phenols (Sofowora, 1994).

Alkaloids

About 5.0 g of sample is prepared in a beaker and 200 ml of 10 % acetic acid in ethanol is added. Mayer's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Flavonoids

A few drops of 1 % Ammonium solution are added to the aqueous extract sample in a test tube. A yellow coloration is observed if flavonoids compounds are present.

Tannins

About 0.5 g of powdered sample is boiled in 20 ml of distilled water in a test tube and filtered. Then 0.1 % ferric chloride is added to the filtered sample and observed for brownish Green or a Blueish black coloration which shows the presence of tannins.

Glycosides

One ml of concentrated sulphuric acid was prepared in test tube 5 ml of aqueous extract from sample was mixed with 2 ml of glacial acetic acid containing 1 drop of ferric chloride. The above mixture is carefully added to 1 Ml of concentrated sulphuric acid so that the concentrated sulphuric acid is underneath the mixture. If Cardiac glycoside was present in the sample, a browning will appear indicating the presence of the cardiac glycoside constituent.

Steroids

Crude extract was mixed with 2 ml of chloroform and concentrated sulphuric acid was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Phenols

The plant extract (500 mg) was 2 dissolved in 5 ml of distilled water. To this, few drops of neural 5 % ferric chloride solution were added. A dark green colour indicated the presence of phenols.

Collection of Wound Sample

Wound swab samples were collected from wound patients Government Medical College and Hospital, Thiruvannamalai. Samples were collected from the patient before an antiseptic dressing applied by wearing sterile gloves and using sterile gloves and using sterile swabs. The collected samples were immediately placed in sterile plastic container, Sealed and preserved in a thermal cool box containing coolant packs. This sample was immediately brought to the lab for further studies.

Isolation and Identification of Bacterial isolates from Wound sample

All the collected swabs were inoculated on Nutrient agar, 5 % Blood agar, MacConkey agar and Chocolate agar plates and incubated overnight at 37 °C aerobically. The sample was also put into liquid media like nutrient broth and Brain Heart Infusion broth. And it was sub cultured after overnight incubation onto Blood agar and MacConkey agar. Bacterial pathogens were identified by conventional biochemical methods according to Standard Microbiological Techniques (Forbes *et al.*, 2007).

Antibacterial activity test

The agar well diffusion method was used to investigate the antibacterial activity of the crude extracts. Within 15 minutes after adjusting the turbidity of the inoculum's suspension, a sterilized swab was aseptically dipped into the suspension. The dried surface of a Muller Hinton agar plates was inoculated by swabbing over the entire agar surface with bacteria pathogens. A sterile cork borer of an internal diameter of about 5 mm was used to punch holes in the medium *Citrus Sinensis* and *Citrus medica* peel extract and Standard antibiotic (Streptomycin) each 100 µl were dispensed into the respective labeled holes. The plates were kept in the refrigerator for about 1 hour for complete diffusion of the extract and incubated at 37 °C for 24 hours. After the incubation period, the diameter of zone of inhibition was measured in millimeter (mm) with zone measuring scale following the method (Durgesh and Tumane, 2014).

3. Results and Discussion

Citrus sinensis and *Citrus medica* peel contains various bioactive compounds and some essential oils. It also contains higher ascorbic acid (Vitamin C) content. The present study was undertaken to determine the phytochemical and antibacterial activity of *Citrus sinensis* and *Citrus medica* fruit peel.

Identification and characterization of pathogens from the wound sample

The colonies grown on the various selective agar plates were observed after incubation period and were differentiated by using the colony morphology, colour and shape. Further it was identified and characterized by using Gram staining, motility and biochemical reactions (Table - 1). Based on the above biochemical results the isolated wound pathogens are tentatively identified as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli, Proteus vulgaris and Pseudomonas aeruginosa.* A number of reports done previously on wound infection from Ethiopia and different parts of the world indicated that *Staphylococcus aureus* and *Klebsiella pneumoniae* were the most frequent isolates (Bhatt and Lakhey, 2006; Muluer *et al*, 2006). The high prevalence of *Staphylococcus aureus* infection may be because it is an endogenous source of infection.

Analysis of Phytochemical constituents in *Citrus sinensis* peel and *Citrus medica* peel extract

Qualitative assessment of different phytochemical contents *viz.*, Alkaloids, Flavonoids, Tannins, Glycosides, Saponins, Steroids and Phenols in the Orange peel extract are presented in (Table - 2, 3 and 4). The results

clearly revealed that all the biochemical contents *viz*, alkaloids, flavonoids, tannins, Saponins, Steroids and Phenols are significantly present in the methanol, ethanol, aqueous extract and glycosides only absent in the methanol extract of orange peel.

The orange peel and lemon peel is rich in soluble sugars and contains significant amounts of vitamin C, pectin, fibers, different organic acids and potassium salt which give the fruits its characteristics citrus flavor (Ganesh *et al.*, 2014; Kolanjinathan and Saranraj, 2015; Ahmed Abd El-Ghfar, 2016; Saranraj *et al.*, 2016; Silva *et al.*, 2018). It contains volatile essential oils which are said to be effective in inhibiting microbial growth and in disinfecting wounds; among its other medicinal capabilities. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities.

Antibacterial activity of Methanol, Ethanol and Aqueous extract of *Citrus sinensis* and *Citrus medica* peel

The antibacterial activities of methanol, ethanol and aqueous extract of *Citrus sinensis* and *Citrus medica* was studied by well diffusion assay against certain isolated burn pathogens *viz*., *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris,* and *Pseudomonas aeruginosa*. Their antimicrobial activities were compared with standard antibacterial disc (Streptomycin) (Table – 5, 6 and 7). It was observed that, higher zone of inhibition against all bacterial wound infection tested viz., 20 mm in *Citrus sinensis* and 19 mm in *Citrus medica* (*Staphylococcus aureus*), 18mm in *Citrus sinensis* and 21 mm in *Citrus medica* (*Pseudomonas aeruginosa*), 19 mm in *Citrus sinensis* and 18 mm in *Citrus medica* (*Klebsiella pneumoniae*) and 20 mm in *Citrus sinensis* and 21 mm *Citrus medica* (*Escherichia coli*) and the same results were observed in ethanol and aqueous extract respectively.

Whereas, the standard antibiotic (Streptomycin) showed lesser zone of inhibition of 13, 12, 13 and 15 mm against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* respectively. The methanol alone does not show any zone of inhibition against any pathogen.

Kavi Karunya *et al.* (2021) reported the antibacterial activity of cumin seeds with ethanol extract at various concentration 1000 µg/ml, 750 µg/ml and 500 µg/ml against organisms were tested. The maximum zone of inhibition was showed at *Streptococcus* sp. of 29 mm at 1000 µg/ml, *Pseudomonas aeruginosa* of 24 mm at 750 µg/ml, *Pseudomonas aeruginosa* of 21 mm at 500 µg/ml. There are strong evidences showing that the essential oil of *Citrus sinensis* and *Citrus medica* have larvicidal, repellent and fumigant activities against *Aedes aegypti* L. mosquitoes (Roger, 2002; Bharathi *et al.*, 2014; Faria *et al.*, 2019). It has also been used as antimicrobial (Omobuwajo *et al*, 2008; Saranraj and Sivasakthivelan, 2012; Alves *et al.*, 2020), antioxidant (Kumar *et al.*, 2011; Sekar *et al.*, 2012; Souza *et al.*, 2019; Nascimento *et al.*, 2020), carminative, insect repellent, antibacterial, larvicidal, antiviral, uricosuric, antiyeast, antihepatotoxic and antimutagenic agent (Kanaze *et al.*, 2008; Kolanjinathan *et al.*, 2012; Saranraj and Sivasakthi, 2014; Kolanjinathan and Saranraj, 2014; Barreto *et al.*, 2018; Dos Santos *et al.*, 2019; Dias *et al.*, 2020; Souza *et al.*, 2020; Alves *et al.*, 2021; Saranraj *et al.*, 2022).

Table - 1: Identification and characterization of pathogenic Bacteria from the Wound sample

S. No.	Grams Staining	Indole	MR	VP	Citrate	Catalase	Oxidase	TSI	Urease Test	Tentative Identification
1	+	-	+	+	+	+	-	A/A	+	*Staphylococcus aureus*
2	-	-	+	-	+	+	-	A/A	+	*Klebsiella Pneumoniae*
3	-	+	+	-	-	+	-	A/A	-	*Escherichia coli*
4	-	+	+	-	+	+	-	A/A	+	*Proteus vulgaris*
5	-	-	-	-	+	+	+	A/A	-	*Pseudomonas aeruginosa*

Table – 2: Phytochemical analysis of the Ethanol extract of the *Citrus sinensis* and *Citrus medica* peel

S. No.	Phytochemicals	*Citrus sinensis*	*Citrus medica*
1	Alkaloids	-	+
2	Flavonoids	+	+
3	Tannins	+	-
4	Glycosides	-	-
5	Saponins	+	-
6	Steroids	+	+
7	Phenols	+	+

Table – 3: Phytochemical analysis of the Methanol of the *Citrus sinensis* peel and *Citrus medica* peel

S.No.	Phytochemicals	*Citrus sinensis* peel	*Citrus medica* peel
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannins	+	-
4	Glycosides	-	-
5	Saponins	+	+
6	Steroids	+	+
7	Phenols	+	+

Table – 4: Phytochemical analysis of the aqueous of the *Citrus sinensis* peel and *Citrus medica* peel

S. No.	Phytochemicals	*Citrus sinensis* peel	*Citrus medica* peel
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Tannins	+	+
4	Glycosides	-	-
5	Saponins	+	+
6	Steroids	+	+
7	Phenols	+	+

Table – 5: Antimicrobial Activity of the Ethanol extract on *Citrus sinensis* and *Citrus medica* peel Extract against bacterial wound infection.

S. No.	Name of the bacterial wound pathogens	*Citrus sinensis*	*Citrus medica*	Standard Antibiotic (streptomycin)
1	*Staphylococcus aureus*	17	21	12
2	*Klebsiella pneumoniae*	19	21	12
3	*Escherichia coli*	18	18	13
4	*Proteus vulgaris*	20	21	12
5	*Pseudomonas aeruginosa*	16	22	12

Table – 6: Antimicrobial Activity of the Methanol extract on *Citrus sinensis* and *Citrus medica* peel Extract against bacterial wound infection.

S. No.	Name of the bacterial wound pathogens	*Citrus sinensis*	*Citrus medica*	Standard Antibiotic (Streptomycin)
1	*Staphylococcus aureus*	20	22	12
2	*Klebsiella pneumonia*	20	21	12
3	*Escherichia coli*	18	19	13
4	*Proteus vulgaris*	20	21	12
5	*Pseudomonas aeruginosa*	20	23	12

Table – 7: Antimicrobial Activity of the aqueous extract on *Citrus sinensis* and *Citrus medica* peel Extract against bacterial wound infection.

S.No.	Name of the bacterial wound pathogens	*Citrus sinensis*	*Citrus medica*	Standard Antibiotic (streptomycin)
1	*Staphylococcus aureus*	21	20	12
2	*Klebsiella pneumoniae*	18	21	12
3	*Escherichia coli*	19	19	13
4	*Proteus vulgaris*	21	21	12
5	*Pseudomonas aeruginosa*	20	19	12

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