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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF DIFFERENT CITRUS FRUIT PEELS

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ABSTRACT: *Citrus* fruit is a very rich source of Vitamin C, A, E, and flavonoids, alkaloids, and minerals. *Citrus* fruits are medicinal plants that present *rutaceae* family and include some fruits such as orange (*Citrus sinensis*), kinnow (*Citrus reticulata*), mosambi (*Citrua ltmetta*), grapefruit (*Citrus maxima*) lime, pomelo lemon. Antibacterial antiviral, antifungal activity of different solvent extracts (Acetone, ethanol, methanol, and distilled water) prepared by soxhlet extractor and aqueous solvent from three *citrus* fruit peel orange, kinnow, mosambi (*Citrus sinensis, Citrus reticulata,* and *Citrus limetta* respectively) were screened against four pathogenic bacteria *staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Salmonella typhi*. The highest antibacterial activity was exhibited by the acetone peel and ethanol extract of *Citrus reticulate* peels. MICs were tested at concentrations ranging from 80-5.25 mg/mL as wells as their MBCs. The phytochemical analysis of the *citrus* peel extracts showed the presence of flavonoids, saponins, steroids, terpenoids, tannins and alkaloids.

INTRODUCTION: *Citrus* fruit is the most abundant fruit that grows worldwide and contains very rich amounts of phytochemicals and bioactive compound [1]. *Citrus* fruit is a medicinal plant belonging to the Rutaceae family. They are a rich source of Vitamin C, A, E alkaloids and flavonoids, and other minerals. *Citrus* plants originated from tropical, subtropical and East Asia and it is consumed all over world as an rich source of Vitamin C and other minerals and good source of vitamin A and contains a powerful natural antioxidant antiviral, antifungal and antibacterial activity that builds the strong body immune system.

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The peel of different *citrus* fruit is most abundant source of phenolics content, flavonoids, flavones, flavanones content and many polyethoxylated flavones, which are very rare in other plants [2]. *Citrus* fruit peels are recognized as being a healthful source of bioactive compounds polyphenols, dietary fibre, essential phenolics, and ascorbic acid [3].

The phytochemicals are also known as secondary metabolites, which include many types of useful secondary metabolites such as alkaloids, flavonoids, tannins, saponin, Coumarins, carotenoids *etc.*, which are derived from primary metabolites and are used as drugs [4, 1]. Phytochemical analyses of citrus fruit peels are very useful in the evaluation of some biologically active compounds in some vegetables and medicinal plants. Medically, the presence of phytochemicals explains the use of the

secondary compound in a different area of medical, where used as a beneficial drug.

These secondary components of *citrus* fruit peels are extractable by various solvents that showed varied biological, biochemical and pharmacological actions in human beings. The *citrus* peels are a good and promising source of Vitamin C, minerals, fiber and different nutrients, and contain many rich amounts of phytochemical metabolites. *Citrus* plant can be used as a beneficial drug, food supplement in the food industry and Cosmetic industries and many different areas [5, 6]. *Citrus* peels enhance the antimicrobial activity of harmful bacteria, fungi, and viruses. *Citrus* fruits are rich sources of useful phytochemicals, such as vitamins A, C, and E, mineral elements, flavonoids, coumarins, limonoids, carotenoids, pectins and other compounds [7]. These phytochemicals, consumed through fresh fruits or their derived products, have been suggested to have a wide variety of biological functions, including antioxidant, anti inflammatory, anti-mutagenicity, anti-carcinogenicity, and anti-aging [5, 8, 9, 10].

MATERIALS AND METHODS:

Collection of the Plant Material: Plant material of *citrus* spp. fruit peels used in this study. *Citrus* spp. fruit were collected from the local fruit market of Allahabad from India then after, the peels were dried at room temperature (40 ºC) for 8 days. The Plant was authenticated by the scientist of the Botanical Survey of India (BSI), Prayagraj. After that 20 g of dried citrus peels were crushed by electric blender. The dried *citrus* peels were collected in an air-tight closed container.

Preparation of Solvent Extract:

Extract Prepared by Soxhlet Method: The dried and powdered of *citrus* peels extract (10 g) were dissolved in 100 ml of each different solvent and separated by soxhlet extractor for 6 hours at a room temperature [11]. The solvents acetone, ethanol, methanol and distilled water were used for the study. The extracts were filtered by Whatman filter paper no. 1 and then concentrated to dryness. After that, each different extract was stored in airtight container and kept in a cold place before use.

Aqueous Extraction: 15 g of the dried *citrus* peels powder soaked separately in 100 ml of distilled water at room temperature for 18 hours under shaking conditions at 120 rpm. After that extract was filtered by Whatman filter paper No 1 and extracts were stored to airtight container and kept in cold place for future use.

Phytochemical Analysis: The dried extracts of *citrus* fruit peels of the different extracts are a preliminary estimation of phytochemical [12].

Alkaloids Test: 1 ml acidic and aqueous extract was mixed with few drops of Mayers reagent and add 0.1% HCl. A yellow color showed the presence of alkaloids.

Flavonoids Test: 1 ml of extract was mixed with conc HCl and adds magnesium Ribbon. The pink-red color showed the presence of flavonoids.

Saponins Test: Take 2 ml filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb Froth indicated the presence of saponins.

Tannins Test: 1ml extract was mixed with 0.1% Ferric chloride. The brownish-green indicated the presence of tannins.

Steroids Test: 1 ml of extract was added to 2 ml acetic anhydride with add 1.5 ml sulfuric acid the violet to a blue colour indicating the presence of steroids.

Amino Acid Test: Take 3 ml of extract was mixed with 4 -5 drops of ninhydrin reagent. The purple color showed the presence of amino acids.

Reducing Sugar Test: 2 ml of extract add to 0.5 ml of both A and B Fehling's solution after that mixture was heated in a water bath for 30 min. The brick red colour indicates the presence of reducing sugar.

Terpenoids Test: 1 ml of extract was mixed with 0.5 ml sulfuric acid and adds 0.5 ml of chloroform. A reddish brown colour was formed to show the presence of terpenoids.

Cardiac Glycosides Test: 4 ml of each extract was mixed with glacial acetic acid and 1 ml of conc. sulphuric acid adds few drops of ferric chloride a brown color ring indicates the presence of cardiac glycosides.

Antraquinones Test: 2 ml of the extract was mixed with diluted conc. sulfuric acid and added 1 ml of dilute ammoniarose - pink color showed the presence of anthraquinones.

***In-vitro* Antimicrobial Screening:** Antibacterial activity analysis by agar well diffusion method and disk diffusion method. The four bacteria of Gram-positive and Gram-negative (*S. aureus, B. subtilis and E. coli,* and *S. typhi*) were cultured in 24 hr, and fungi *C. albicans, Aspergillus niger* were prepared on Mueller-Hinton agar plates and Sabouraud dextrose agar (SDA). Where *citrus* extract *C. sinensis C. reticulata C. limetta* tested against two positive *B. subtilis, S. aureus* bacteria and two gram negative *E. coli, S. typhi* bacteria and fungi *C. albicans, Aspergillus niger*. These plates were incubated at 34 °C for 24 hours in an upright position, and the zone of inhibition formed around the wells was measured.

Minimum Inhibitory Concentration: The determination of minimum inhibitory concentration (MIC) by the lowest concentration of different *citrus* fruit extracts. Which essential to inhibit the multiplication of the organism is referred to as [13 14]. Here "Serial dilution technique" was followed using nutrient broth media. The four test bacteria grown at 34 °C in a nutrient broth medium for 24 hr. Here five different concentrations (80, 40, 20, 10, 5 mg/mL) of extracts are used for determination of MIC. 1 ml of nutrient broth medium was transferred to each of the test tubes. Next the test tubes were cotton plugged and sterilized in an autoclave for 20 minutes at 121 °C. After cooling, 1 ml of the sample solution was added to the 1st test tube and mixed well and then 1 ml of this content was shifted to the 2nd test tube. The content of the 2nd test tube was shaken well and then again, 1 ml of this mixture was moved to the third test tube. The entire process of serial dilution was carried up to the 5th test tube. Finally, 1 ml of the sample was added to each of the test tubes and all the test tubes were incubated at 37 °C for 20 h.

Minimum Bactericidal Concentration: The determination of minimum bactericidal concentration (MBC) [15] for the study of each well in the MIC determination after that Inoculated test bacteria in Nutrient agar plates only use for as a control. The nutrient agar plates were incubated at 36 ºC for 18 h. After incubation, agar plates did not show any growth of organisms and were noted as a minimum bactericidal concentration.

Statistical Analysis: All the experiments were carried out in triplicate. One-way analysis of variance was applied to confirm the significance of data (p<0.05). Comparison with control and treatment's means was carried out by using ANOVA by Dunnett's multiple comparison tests and Duncan's Multiple Range Test (DMRT) as per required. The principal component analysis was performed with PAST software (Version 2.17).

RESULTS AND DISCUSSION:

Extract of Citrus Fruit Peels: The extract of the *citrus* peel was carried out using different solvents such as ethanol, methanol, acetone and distilled water. **Fig. 1**, exhibited the comparison of yield of *citrus* peels extracts obtained from different solvents.

For acetone and distilled water extract, C*itrus sinensis* exhibited a good yield and the methanol extract showing the slowest percentage yield. Methanol and ethanol extract of *citrus reticulate* peel showed good yield. Acetone and distilled water extract of *Citrus limetta* showed good yield when compared to the methanol and ethanol extract. The aqueous, methanol, and Acetone extract exhibited a good yield of *citrus* fruit peels extract. So the variation of yield showed the solubility of extract in different solvents.

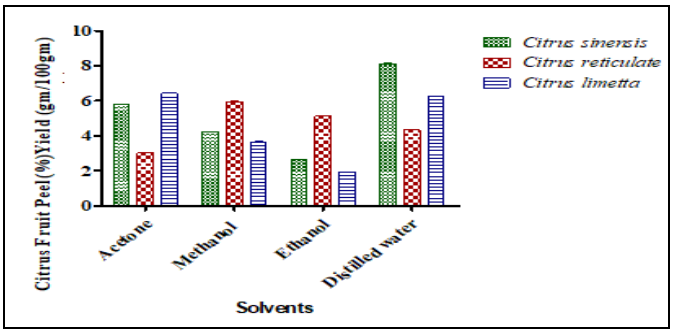

FIG. 1: YIELD (%) OF EXTRACT OF CITRUS FRUIT PEELS BY USING DIFFERENT SOLVENTS

Phytochemical Screening of Citrus Fruit Peels Extract: The phytochemical analysis of the various solvent extract showed the presence of secondary metabolites such as alkaloids, flavonoids, saponin, tannins, starch and terpenoids [16-18].

Phytochemical Analysis of Different Solvent Extract of Citrus Fruit Peel:

TABLE 1: PHYTOCHEMICAL ANALYSIS

Test no.	*Citrus sinensis*				*Citrus reticulate*				*Citrus limetta*			
	acetone	methanol	ethanol	D.W	acetone	methanol	ethanol	D.W	acetone	methanol	ethanol	D.W
Flavonoid	-	+	+	-	-	-	+	+	+	-	-	+
Saponin	-	+	+	+	+	+	+	-	+	+	-	+
Tannin	+	+	-	+	+	+	-	+	+	+	-	+
Alkaloid	+	-	+	+	+	+	+		+	+	-	+
Terpenoid	-	+	+		+		+	+	-	+	+	
Phytosterol	+	+	+	+	+	+	+	+	-	+	-	+
Triterpenoid	+	-	-	+	+	+	-	+	+	+	-	+
Amino acids	+	+	+	+	+	+	+	+	+	+	+	+
Reducing sugar	+	+	+	+	+	+	-	+	+	+	-	+
Steroids	+	-	+	+	+	+	+	-	+	+	-	-
Cardiac glycoside	-	+	+	-	+	-	+	-	+	-	-	-
Anthraquinones	+	+	-	-	+	+	+	+	-	-	+	+

TABLE 2: QUANTITATE ANALYSIS (g/100 g.)

S. no.	*Citrus sinensis* (100 g)	*Citrus reticulate* (100 g)	*Citrus limetta* (100 g)
Carbohydrate	1.04g	0.92g	0.82 g
Protein	0.64g	0.83 g	0.62 g
Fat	0.22g	-	0.8g
Ash content	8.1g	6.8 g	3.1g
Moisture	14.0g	8.1g	12.2g
Iron	0.4mg	0.2mg	0.1mg
Calcium	42mg	38g	36g
Crude fiber	12.48mg	9.6mg	7.2mg
Phosphorus	12mg	8mg	-
Energy	(48 kcal)	(42 kcal)	(38 kcal)

TABLE 3: DETERMINATION OF ANTIMICROBIAL ACTIVITY OF *CITRUS* FRUIT PEEL EXTRACT FROM DIFFERENT SOLVENT BY USING DISC DIFFUSION METHOD

Microbial strains	Diameter of Minimum Zone of Inhibtion (ZOI in mm)					
	Citrus sinsensis		*Citrus reticulata*		*Citrus limetta*	
	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol
B. subtillis	-	18	14	-	14	12
S. aureus	-	13	-	-	-	11
E. coli	13	-	25	12	-	18
S. typhi	15	11	18	11	10	-
C. albicans	14	12	10	-	11	-
A. niger	-	16	12	16	-	12

Antimicrobial Activity of Citrus Fruit Peels: *Citrus* peel extracts showed a good antibacterial, antifungal, and antiviral activity against all different pathogenic bacteria. *C. sinensis* and *C. reticulata* peel extracts exhibited a good antibacterial and antifungal activity. Ethanol extract of *Citrus sinensis* showed a maximum zone of inhibition against bacteria *S. aureus* (13mm), *B. subtilis* (18mm), *S. typhi* (11mm), and showed antifungal activity in *C. albicans* (14mm), *A. niger* (16mm) acetone extract of *Citrus reticulata* exhibited maximum zone of inhibition against bacteria *E. coli* (25mm), *S. typhi* (18mm) followed by fungi *A. niger* (10mm) acetone and ethanol extract of *citrus reticulate* showed good activity against *C. albicans* (10mm), *A. niger* (16mm) and acetone extract of *Citrus limetta* exhibited zone of inhibition against bacteria *E. coli* (18mm) and fungi *C. albicans* (11mm) and ethanol extract showed *A. niger* (12mm) whereas the methanol and aqueous extract of *Citrus sinensis, Citrus reticulate* and *Citrus limetta* did not show such high antibacterial and antifungal activity [19-21]. In the case of *Citrus limetta, Citrus sinensis, Citrus reticulata* methanol and aqueous showed very less the same antibacterial activity and antifungal activity when compared to other solvents. So different extracts may have shown diverse antimicrobial agents that the bacterium was contained special metabolism to maintain its activity. Acetone and ethanol were showed a better solvent for the extraction of antibacterial and antifungal agents, which shown

better higher yield of antibacterial and antifungal activity relating to the high concentration of various phytochemicals, and so citrus peels showed high antibacterial and antifungal activity. This statement can be validated as acetone and ethanol has shown antibacterial and antifungal activity in *Citrus sinensis* and *Citrus reticulate* [22-23]. The determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of different solvent extracts of *C. sinensis*, *C. reticulata*, and *C. limetta* peels are shown in **Table 4** and **Table 5,** respectively. The extracts showed significant activity. MIC activity detects by the nutrient broth dilution method, which showed excellent results. The broth method, carried out in a test tube, has the advantage of lower workloads for a larger number of replicates, and Lack of activity can thus only be proven by using large doses [24-25].

TABLE 4: DETERMINATION OF MIC (MINIMUM INHIBITORY CONCENTRATION) OF DIFFERENT CITRUS PEEL EXTRACT AGAINST PATHOGENIC BACTERIA

Bacteria	*Citrus sinensis*		*Citrus reticulate*		*Citrus limetta*	
	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol
E .coli	14.2	27	12.6	29	50	26
B. subtillis	50	50	-	45	-	50
S. auerus	40	39	65	50	25	-
S. typhi	48	45	40	50	-	-

Concentration- 40 mg/mL and (-); No Inhibition Zone

TABLE 5: DETERMINATION OF MBC (MINIMUM BACTERICIDAL CONCENTRATION) OF DIFFERENT *CITRUS PEEL* EXTRACT AGAINST PATHOGENIC BACTERIA

Bacteria	*Citrus sinensis*		*Citrus reticulate*		*Citrus limetta*	
	Acetone	Ethanol	Acetone	Ethanol	Acetone	Ethanol
E .coli	49	14.6	12.5	50	50	50
B. subtillis	25	50	48	28	25	-
S. auerus	-	50	-	47	-	50
S. typhi	50	27	48	50	-	-

Principle Component Analysis: Protein, Moisture, Fiber, Calcium, Phosphorus, Energy, and carbohydrate contents were further analyzed *via* multivariate analysis by principal component analysis (PCA) to determine principle component in each genus and their strains. PCA clearly resolved the data into two major components *viz.* MIC and MBC for all eight components in each three strains of Citrus. A maximum of 79.17%, 31.8%, 29.78% and 19.9% of the variance was recorded for Energy Calcium, phosphorus content, and carbohydrate content of these Citrus strains **Fig. 2**. PCA scores were determined the highest producing attributes of citrus strains [26] of the *Citrus limetta* for efficient carbohydrate and protein accumulation. PCA was also used in multifactor analysis to easily analyze the probability area of productivity [26].

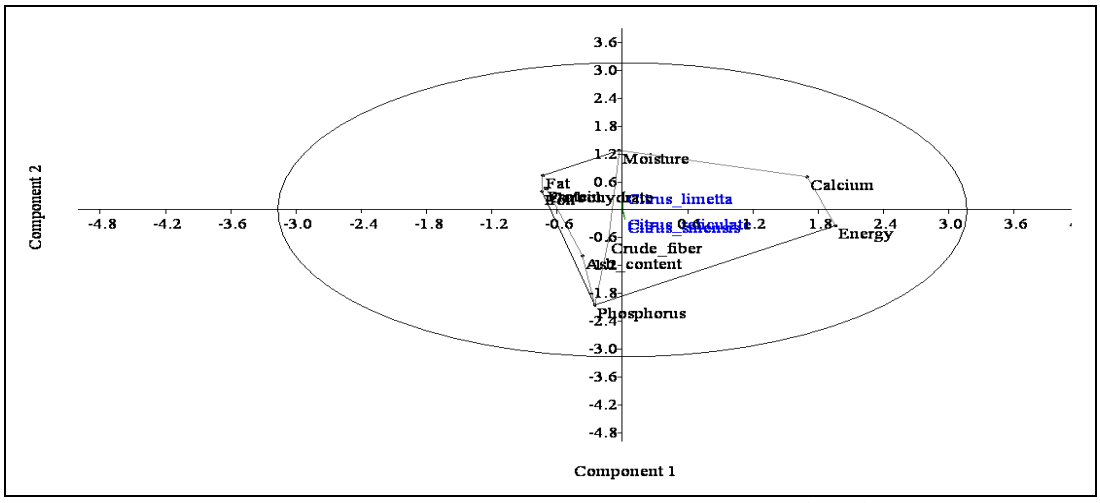

FIG. 2: PRINCIPLE COMPONENT ANALYSES (PCA)

CONCLUSION: Re-use of peels of *citrus* waste which are very important innovative ways where produce the higher amount of beneficial, essential, a healthy new product in a very low cost, utilization of *citrus* fruit peels waste can be used in multiple areas such as food industry where the use of fruit waste peels as a supplement and can be use waste of peels in the cosmetic industry for make a beauty product in a very low price and *citrus* peels also use as drugs which are very effective against the pathogenic bacteria, fungi and virus apart from that *citrus* peels enhance the immunity of human being because it is a natural source of vitamin C. which protect human body to harm full disease. *Citrus* peels rich in nutrients which beneficial and required for human, animal, and plant nutrition. So it is used in the pharmaceutical. This work has identified the antibacterial, antiviral, antifungal, and antioxidant activity of *citrus* peels. Which act against the disease phytochemical and bioactive compounds in *Citrus limetta*, *Citrus reticulate*, and *Citrus sinensis* peels extracts obtained from different solvents. This study showed that dried fruit peels of different *citrus* fruit are rich sources of antioxidant compound and antibacterial activity, and the exploitation of these abundant and low-cost renewable resources could be anticipated for the pharmaceutical and food industry with opportunities of developing new ingredient for the formulation of functional food products and/ or pharmaceutical products.

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Author's Contributions: Shanthy Sundaram and Swati Gupta designed the experiments; Swati Gupta performed all the experiments. Adi Nath and Mahesh Gupta helped Swati Gupta in manuscript drafting. Shanthy Sundaram reviewed the manuscript.

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