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BIOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF *CITRUS* PEEL WASTE

Ali, J.[1]; Abbas, S.[2]; Khan, F.A.[2*]; Rehman, S.U.[1]; Shah, J.[1]; Rahman, Z.U.[1]; Rahman, I.U.[1]; Paracha, G.M.U.[1]; Khan, M.A.[2]; Shahid, M.[2**]

[1]Pakistan Council of Scientific and Industrial Research Laboratories Complex, Peshawar, Pakistan
[2]Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, Pakistan

[*]farhat.fls@suit.edu.pk
[**]shahidsalim_2002@hotmail.com

Abstract

The current study investigated the physicochemical composition, mineral analysis, antibacterial and antifungal activity of *Citrus aurantium* (sour orange), *Citrus sinensis* (sweet orange), *Citrus paradisi* (grapefruit) and *Citrus limon* (lemon) peel waste. The proximate composition was determined according to the described standardized methods. The fiber content was 18.00 ± 2% in sweet orange, protein content was 7.20 ± 0.5% in grapefruit and total sugar was 3.1 ± 0.10% in *C. aurantium* (sour orange). The mineral analysis was carried out by atomic absorption spectroscopy and flame photometry. Increased levels of Na (21750 ± 20 ppm) and Fe (38.0 ± 2 ppm) were found in *C. aurantium* (sour orange). Similarly, higher levels of K (218000 ± 60 ppm), Pb (3.0 ± 01 ppm), Ag (5 ± 01 ppm), Zn (10.0 ± 01 ppm) and Cr (20 ± 01 ppm) were reported in *C. limon* (lemon). Likewise, increased levels of Ca (11623 ± 10 ppm) and Mg (1508 ± 5 ppm) were observed in *C. paradisi* (grapefruit), while levels of Al (39.5 ± 4 ppm) and Mn (5.5 ± 1 ppm) were found higher in *C. sinensis* (sweet orange). The aqueous extract of *Citrus* peel waste showed strong antimicrobial activity against the tested microorganisms. *C. sinensis* (sweet orange) peel extract showed the highest zone of inhibition (21 ± 0 mm) against *Escherichia coli. C. aurantium* (sour orange) possessed good antifungal activity against *Aspergillus niger* (17 ± 1 mm).

Key words: *Citrus* peel, waste, chemical analysis, minerals, antibacterial, antifungal.

Introduction

Citrus cultivation is one of the most important commercial and industrial agricultural activities in the world. The peel of *Citrus* fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants (1). *Citrus* belongs to family Rutaceae, which include approximately 160 genera and 1700 species, and has been widely used in herbal medicine (2). In addition to various food products from pulp, *Citrus* peel are candied, fed to livestock, and used to scent perfumes and soap products. *Citrus* seeds are used to isolate cooking oil, and other oils for plastic and soaps. Their flowers and foliage are used in perfume manufacturing. *Citrus* species essential oil contains terpenes, aliphatic sesquiterpene, oxygenated derivatives and aromatic hydrocarbons. A number of these monoterpenes possess antitumor proclivity (3).

Preparations from peel, flowers and leaves of bitter orange (*Citrus aurantium* L.) are popularly used in the treatment of central nervous system disorders (4). Some essential oils were used in skincare products and for acne control (5). Pakistan is one of the most important countries amongst *Citrus* fruits producer. This study was designed to investigate the biochemical and antimicrobial activities of *Citrus* peel which is being wasted.

Methods

Procurement of raw materials

The sour orange peel wastes were obtained from the Food Pilot Plant of Pakistan Council of Scientific and Industrial Research Laboratories Complex (PCSIR), Peshawar, Pakistan. The peel wastes were dried in a dehydrator at 50°C for 24 h. A grind mill and sieves were used to obtain a powder, which has a particle size of less than 0.2 mm.

Physicochemical composition

The following variables were evaluated in triplicate according to previously established methods (6-8): moisture, ash, fat, fiber, protein, carbohydrates, pectin, acidity, total sugar, reducing sugar, non-reducing sugar, pH and TSS.

Mineral analysis

The powdered material (5 g) was taken in a silica crucible and heated in a muffle furnace at 400°C till no evolution of smoke. The crucible was cooled at room temperature in a desiccator and the ash was moistened with concentrated H_2SO_4 (0.5 mL). Crucible was placed on a hot plate and heated until fumes of H_2SO_4 ceased to evolve. The crucible with sulphated ash was then heated in a muffle furnace at 600°C till the weight of contents became constant. The ash obtained was cooled, dissolved in 5 mL of 6 N HCl and allowed to stand for 30 min. It was later filtered and the volume was made up to 50 mL with deionized water. The resulting extract was used for the determination of Fe, Ag, Mn, Zn, Cd, Cr, Cu, Pb and Ni by using a Hitachi Zeeman Japan Z-8000, atomic absorption spectrophotometer, which was equipped with standard hallow cathode lamp as radiation source and air acetylene flames, while Na and K concentration was determined on Jenway PFP7 flame photometer (6, 8).

Antimicrobial activities

Procurement of microorganisms

For the antimicrobial activities, bacterial strains of *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa* and *Bacillus cereus;* along with fungal strains of *Aspergillus niger, Aspergillus parasiticus, Aspergillus flavus, Fusarium oxysporum, Rhizopus arrhizus* and *Alternaria alternata* were obtained from the Food Microbiology Laboratory of PCSIR Laboratories Complex, Peshawar, Pakistan. Their cultures were maintained on slants of nutrient agar and potato dextrose agar, respectively in a refrigerator.

Citrus peel extraction

The powder of *Citrus* peel wastes (50 g) were taken and extracted in 250 mL of distilled water for 48 h. These extracts were then filtered under vacuum through a Whatman filter paper (No. 1) in a Buchner flask. The extracts were concentrated in a rotary evaporator and were then transferred to a sterile beaker kept on a water bath at 50°C to obtained a dry residue.

Antibacterial activity

The tested bacterial strains were adjusted to standard 0.5 Mac Farland (1.0×10^8 cells/mL) and were inoculated into nutrient agar. Fifty microliter (50 µL) of 100 mg/mL of each *Citrus* aqueous peel extract was pippetted into holes, bored on the agar. 50 µL of sterile distilled water was used as negative control, while 50 µL of streptomycin (500 µg/mL) was employed as positive control. The plates were incubated at 37°C for 18 h. The antibacterial activity was determined by measuring the diameter of zone of growth inhibition (9).

Antifungal activity

Sabouraud dextrose agar (SDA), prepared by autoclaving (121°C for 15 min) was poured into

sterilized petri dishes. On the SDA plate surface, 1 mL of each spore suspension (10^5 spores/mL) was spread. Wells were made at the center of each inoculated/cultured plate with the help of a sterilized cork borer (6 mm in diameter). Fifty microliter (50 µL) of 300 mg/mL of each *Citrus* aqueous peel extract was pippetted into holes bored on the agar. Fifty microliter (50 µL) of standard drug, bifonazole (300 mg/mL) and sterile distilled water were used as positive and negative control, respectively. The plates were kept in an incubator at 27°C for 48 h and the diameter (mm) of inhibition zone was then measured by using a zone reader (9).

Results

The physiochemical analysis is shown in Table 1. The highest moisture content of 8.0 ± 0.3% was observed in sour orange, while lowest content was reported in lemon (6.20 ± 01%). Highest ash (8.30 ± 0.44%) was measured in sour orange and minimum level was measured in lemon peel i.e. 6.30 ± 01%. Low fat (2.10 ± 0.50%) was found in lemon and maximum was noted in sour orange (3.5 ± 0.45%). The sweet orange peel has high content of fiber (18.00 ± 02%), while maximum protein was observed in grapefruit i.e. 7.20 ± 0.5%. The minimum and maximum pectin contents were found in lemon (0.62 ± 0.10%) and grapefruit (0.90 ± 0.01%), respectively. The maximum acidity, pH and TSS were found in grapefruit (1.20 ± 0.02%), sweet orange (3.25 ± 0.10) and sweet orange (0.86 ± 0.10), respectively. The highest total sugar was observed in sour orange (3.1 ± 0.10%). The minerals composition of *Citrus* peel is shown in Table 2. Higher Na content was observed in sour orange peel (21750 ± 20 ppm). Similarly maximum contents of K and Ca were calculated in lemon and grapefruit as 218000 ± 60 ppm and 11623 ± 10 ppm, respectively. Higher Mg and lower Fe contents were found in grapefruit as 1508 ± 5 ppm and 5 ± 01 ppm, respectively. Maximum Al and Mn contents were observed in sweet orange peel i.e. 39.5 ± 4 ppm and 5.5 ± 1 ppm respectively. Maximum content of Zn was found in lemon as 10.0 ± 01 ppm. Table 3 shows the antibacterial activity of *Citrus* peel waste. Maximum zone of inhibition (21 ± 0 mm) was showed by sweet orange peel extract against *E. coli*, while minimum zone of inhibition was observed against *E. faecalis* (9 ± 0 mm) by lemon peel extract. Sour orange peel extract was ineffective against *P. aeruginosa.* Table 4 shows the antifungal activity of *Citrus* peel waste. Maximum zone of inhibition was observed for sour orange against *A. niger* (17 ± 1 mm), while minimum zone of inhibition was noted for sweet orange peel against *A. flavus* (9 ± 1 mm).

Discussion

Sweet orange, *Citrus sinensis* L. (Rotaceae), is an important medicinal plant that is prescribed in traditional medicine for treating diverse medical conditions (9). Lemon is an important medicinal plant of the family, Rutaceae. It is cultivated mainly for its alkaloids, which possess anticancer and antimicrobial activities (10). *Citrus* is a genus of flowering plants in the family Rutaceae, native to tropical and subtropical areas in Southeast Asia. *Citrus* fruits have peculiar fragrance partly due to flavonoids and limonoids, present in the peel and these fruits are good sources of vitamin C and flavonoids (10). 8-geranyloxypsolaren, 5-geranyloxypsolaren, 5-geranyloxy-7-methoxycoumarin, and phlorin were isolated from lemon peel and show effectiveness against oral bacteria (11).

The *S. typhi* was inhibited by essential oil of sweet orange peel with inhibition zone of 13 mm, while *S. aureus* showed an inhibition zone of 21 mm. No zone of inhibition was reported for the rest of bacteria i.e. *E. coli* and *B. cereus*. Generally, the tested fungal strains showed resistance to these oils (9). The ethanolic extract of lemon peel showed MIC against *S. aureus* as 2.4 mg/mL, while the methanolic extract produced MIC against *P. aeruginosa* as 2.4 mg/mL (12). The phytochemical screening of *Citrus sinensis* peel revealed the presence of reducing sugars, saponins, deoxysugars cardiac glycosides, tannins and flavonoids (13). The difference in the antimicrobial activities may be due to the differences in the phytochemical composition in various parts of the plant or may be due to the extraction method used. The difference can also be attributed to environmental factors or genotypes of the *Citrus* plant used (14). The differences in the antibacterial activities of *Citrus* fruits from the same source when extracted with different solvents have proved that not all phytochemicals that are responsible for antibacterial activity are soluble in a single solvent. Hence solvents of different polarity should be employed (polar: water, acetone, ethanol; non-polar: ethyl acetate, petroleum ether). Sequential or successive solvent extraction is a good option for better solubility of many phytochemicals. However, it is also necessary to know the phytochemicals, extracted by each individual solvent so as to avoid the inclusion of unnecessary solvents during the extraction process. This is also important to understand the role of each solvent in the extraction

of an individual or a class of phytochemicals (15).

Recycling of fruits waste is important for its utilization in various products that are nutritionally essential for human, animal and plant. The evaluation of proximate composition of *Citrus* peel waste showed high contents of valuable nutrients. The elemental analysis established that *Citrus* peel wastes contain some micro-nutrients that are essential for normal functioning of human body system. The prospective antimicrobial activities of sweet orange, sour orange, grapefruit and lemon peel waste aqueous extracts revealed their effectiveness against human and plant pathogenic bacteria and fungi. Further analytical studies are required to isolate the exact bioactive compounds responsible for the antimicrobial properties of *Citrus* peel waste.

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Table 1: Physicochemical analysis of *Citrus* peel waste.

Parameters	Sour Orange	Sweet orange	Grapefruit	Lemon
Moisture %	8.0 ± 0.3	7.00 ± 01	6.80 ± 01	6.20 ± 01
Ash %	8.30 ± 0.44	7.20 ± 01	6.90 ± 01	6.30 ± 01
Fat %	3.5 ± 0.45	3.00 ± 01	2.50 ± 0.5	2.10 ± 0.50
Fiber %	16.0 ± 01	18.00 ± 02	17.20 ± 02	16.50 ± 02
Protein %	6.58 ± 1.20	5.20 ± 01	7.20 ± 0.5	6.25 ± 01
Pectin	0.66 ± 0.22	0.75 ± 0.50	0.90 ± 0.01	0.62 ± 0.10
Acidity	1.06 ± 0.10	1.10 ± 0.50	1.20 ± 0.02	1.11 ± 0.01
Total sugar %	3.1 ± 0.10	3.00 ± 0.50	2.5 ± 0.50	2.60 ± 0.01
Reducing sugar %	2.5 ± 0.10	2.00 ± 0.50	2.05 ± 0.50	2.40 ± 0.01
Non-reducing sugar %	0.6 ± 0.10	01 ± 0.50	0.45 ± 0.50	0.2 ± 0.01
pH	3.10 ± 0.50	3.25 ± 0.10	3.08 ± 0.10	2.98 ± 0.10
TSS (^{o}Brix)	0.80 ± 0.05	0.86 ± 0.10	0.85 ± 0.01	0.78 ± 0.10

Values represent mean **±** standard deviation of three separate experiments.

Table 2: Analysis of minerals in *Citrus* peel waste.

Minerals	***Citrus* peel waste (ppm)**			
	Sour orange	**Sweet orange**	**Grapefruit**	**Lemon**
Na	21750 ± 20	6000 ± 30	1000 ±20	8000 ± 20
K	165750 ± 15	71000 ± 25	200 ± 15	218000 ± 60
Ca	3700 ± 20	5970 ± 20	11623 ± 10	6919 ± 15
Mg	530 ± 10	462.0 ± 20	1508 ± 5	787 ± 20
Fe	38.0 ± 2	33 ± 3	5 ± 01	12 ± 03
Al	6.5 ± 1	39.5 ± 4	ND	7 ± 01
Mn	4.5 ± 1	5.5± 1	3.5 ± 01	3.0 ± 01
Zn	3.5 ± 1	3.5 ± 1	5.0 ± 01	10.0 ± 01
Pb	ND	2 ± 1	1 ± 0	3.0 ± 01
Ag	ND	4 ± 1	3 ± 01	5 ± 01
Cr	ND	15 ± 1	14 ± 01	20 ± 01
Cd	ND	ND	ND	ND
Si	ND	ND	ND	ND
Ba	ND	ND	ND	ND
Ni	ND	ND	ND	ND
Cu	ND	ND	ND	ND

Values represent mean ± standard deviation of three separate experiments. ND = Not detected.

Table 3: Antibacterial activity of *Citrus* peel waste.

Sample	Zone of inhibition (mm)				
	S. aureus	***E. faecalis***	***B. cereus***	***E. coli***	***P. aeruginosa***
Sour orange	17 ± 1	13 ± 1	11 ± 1	14 ± 0	0
Sweet orange	18 ± 0	17 ± 1	15 ± 0	21 ± 0	14 ± 1
Grapefruit	10 ± 0	17 ± 1	16 ± 1	18 ± 1	14 ± 0
Lemon	14 ± 0	09 ± 0	18 ± 0	19 ± 1	14 ± 0
Streptomycin	19 ± 1	18 ± 1	19 ± 1	23 ± 1	17 ± 1
Distilled water	0	0	0	0	0

Values represent mean ± standard deviation of three separate experiments. 0 = No zone of inhibition.

Table 4: Antifungal activity of *Citrus* peel waste.

Sample	Zone of inhibition (mm)					
	A. niger	***A. parasiticus***	***A. flavus***	***F. oxysporum***	***R. arrhizus***	***A. alternata***
Sour orange	17 ± 1	10 ± 0	16 ± 0	0	14 ± 0	11 ± 0
Sweet orange	14 ± 1	0	09 ± 1	13 ± 1	0	10 ± 1
Grapefruit	14 ± 0	15 ± 1	13 ± 1	0	11 ± 1	0
Lemon	13 ± 0	0	14 ± 0	11 ± 0	0	12 ± 0
Streptomycin	15 ± 1	16 ± 1	18 ± 1	19 ± 1	14 ± 1	13 ± 1
Distilled water	0	0	0	0	0	0

Values represent mean ± standard deviation of three separate experiments. 0 = No zone of inhibition.