



ORIGINAL ARTICLE

Screening Of Phytochemical Constituted, Antimicrobial And Antioxidant Activities Of Orange Peel (*Citrus Sinensis*) Extract

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ABSTRACT

Peel of orange (*Citrus sinensis*) subjected to identification of Phytochemical constituted, antimicrobial and antioxidant activities. The antibacterial activity of orange peel extract was highly promising among *Escherichiacoli*, *Salmonella typhi* and *Staphylococcus aureus* with level of significance was from $P < 0.05$. Among the two species of fungus *Rhizopus nigricans* showed very little restrain activity against orange peel. By DPPH free radical scavenging activity the overall antioxidant activity of *Citrus sinensis* was fair compared to ascorbic acid. In phytochemical test orange peel confirmed the presence of alkaloids, phytosterols, phenols, flavanoids, quinines, proteins and amino acid.

Key words: Phytochemical, Antioxidant, Scavenging, restrains

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INTRODUCTION

Disease causing microbes are the main reason of morbidity and mortality in human population. Nowadays, there have been huge generations in antibiotic resistant strains of bacteria, which results for the emerging of new disease, infection and epidemics [1-3]. Therefore, the scientist looking forward for the substance that provide excellent antimicrobial agent. Hence the main of this study was to evaluate the phytochemical, antioxidant and antimicrobial activity of Orange (*Citrus sinensis*) peel, which is considered as a waste causing environmental pollution.

Orange (*Citrus sinensis*) belongs to the family Rutaceae. It has the highest rate of production their peel shows the total 50% to 65% of the total weight of the fruit. Their trees are heavily growing in tropical and subtropical climate region. The rate of production of *Citrus sinensis* in 2008 was 122.90 million tons [4]. A large proportion of this transfer to the industrial process plant for the juice manufacturing, hence this process generates huge amount of waste, causing environmental pollution [5,6].

As with the comparison of seed the peel of orange bear high quantity of flavonoids, the three main types of flavonoids present in citrus fruits are, flavones, flavanones and flavonols [7]. The main leading compound of flavonoids present in citrus species are narirutin, hesperidine, naringin and eriocitrin [8]. With the reference of epidemiological studies, in human citrus flavonoids can lowdown the risk of coronary heart disease [9,10]. In past, many research have been done on antimicrobial potential of orange peel extract in different solvents, according to Khushwaha *et al* [11] cold water, Ethyl acetate, Acetone and ethanolic extract of peel shows significant result against *S. typhimurium*, *P. aeruginosa*, *E. coli*, *S.aureus*, *S. typhi*, *B. subtilis*, *K.pneumonia*. According to another researcher Kumar *et al* [12] Petroleum ether and Aqueous extract of peel shows inhibition against *E. coli*, *S.aureus*, *S. typhi*, *B. subtilis*, *K.pneumonia*.

MATERIALS AND METHOD

Collection and Preparation of Extract

Orange was purchased from the local market of Karachi in Shah Faisal colony. The peel of orange in wash and dried in nature sunlight ($37 \pm 2^\circ\text{C}$) for 6 days and then keep in a hot air oven for 15 mins at 20°C to remove the remaining moisture. After 6 days 6 grams of dried peel were macerated for 2 days in 50ml ethanol at room temperature ($25 \pm 2^\circ\text{C}$). The solvent extracted material was filtered using Whatman filter paper and the extract were preserved in the refrigerator until used.

Phytochemical analysis

For screening the constituent in Orange peel, the freshly prepared ethanol extract were subjected to various phytochemical tests.

Detection of Alkaloids

Wagner's Test: Added 1 ml of hydrochloric acid into a test tube containing a few drops of extract and filter this with filter paper. The filtrate was treated with 2-3 drops of Wagner's reagent (Iodine in potassium Iodide). Brownish/reddish precipitation indicates the presence of alkaloids [13].

Detection of Saponins

Foam Test: 2 ml of extract is added with 6ml of water into a test tube. The mixture was shaken continuously for 10 to 15 minutes and waits for the formation of persistent foam that confirmed the saponins property of test sample [14].

Detection of Phytosterols

Salkowski's Test: 4-5 drops of extracts were treated with chloroform and filtered. Then, filtrate reacts with few drops of Concentrated Sulphuric acid, allowed to stand. Golden yellow color indicates the presence of triterpenes [13].

Detection of Tannins

Gelatin Test: 1% gelatin solution containing sodium chloride was added in test tube containing 4-6 drops of extract. Appearance of whitish precipitate in test tube confirmed the prevalence of tannins [13].

Detection of Flavonoids

Ferric chloride Test: 2-4 drops of ferric chloride added in extract that produce blackish red colour which shows the positive result of flavonoids [15].

Detection of Proteins and Amino acids

Xanthoproteic Test: The extracts were treated with 2-3 drops of conc. Nitric acid. Yellow color precipitation in the test tube indicates the presence of proteins [13].

Detection of Quinones

Few drops of extract were treated with concentrated Hydrochloric acid and observed for the appearance of yellow precipitation or coloration [14].

Detection of Terpenoids

Few drops of chloroform was reacted with 2ml of extract latterly, added a few drops of Concentrated Sulphuric acid. A reddish brown precipitation produced immediately indicated the presence of terpenoids [14].

Detection of Phenol

Ferric Chloride Test: 3-4 drops of ferric chloride solution were treated with extract solution. Appearance of bluish black color indicates the presence of phenols [13].

Antioxidant Activity

In vitro Antioxidant screening was carried by the method of Lee [16]. In this activity, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was prepared in ethanol (300 µM). Then, 10 µL of test sample and 90 µL solution of stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was poured in 96 - well micro titer plates and incubated for 30 minutes at 37°C. Absorbance of sample was calculated through a spectrophotometer at 515 nm. Percent inhibition of radicals by treatment of test sample was concluded by comparison with a DMSO treated control group.

Percentage of Inhibition = (absorbance of the control - absorbance of the test sample / Absorbance of the control) * 100

Ascorbic acid was used as standard. The measuring concentration of EC₅₀ value denotes in (in µg/ml) of sample need to scavenge 50% of DPPH.

Culture media

Nutrient agar and nutrient broth were used for examination of bacterial growth, and for the fungus Sabouraud dextrose agar (SDA) was used. The microbial culture used for the antibacterial test are *Bacillus subtilis*, *Bacillus licheniformis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella paratyphi B*, *Salmonella typhi*, *Salmonella paratyphi A*, *Shigella dysenteriae*, and *Pseudomonas aeruginosa*. For the Antifungal assay *Rhizopus nigricans* and *Ganoderma applanatum* were used. The culture of these microbes was collected from the Institute of Environmental studies and Department of Microbiology, University of Karachi.

Antibacterial assay

For the assessment of antibacterial activity disc diffusion method [17] and agar well diffusion method [18] was used to identify. In Disc diffusion method filter paper disc of 6 mm contained 0.025 ml of extract and in well method 0.1 ml extract was added in each well. Antibiotic disc contained Cefixime (100mg/5ml) and levofloxacin (125 mg/5ml) were used as a control. The antibacterial activity was an analysis by measuring the diameter of the zone of inhibition formed around the discs and wells. These studies were performed in triplicate.

Antifungal assay

The extracts of *Orange peel* were screened for antifungal activity by agar well diffusion method [19] and agar disc diffusion assay. All the isolated fungus checked for the purity subculture on Sabouraud dextrose agar (SDA) at 4 °C in the refrigerator until required for the use. For the preparation of spore suspension autoclaved distilled water used. In agar well diffusion method 0.05ml of the extracts was introduced in the well aseptically. In the Agar disc diffusion method each disc occupies 0.025 ml of extract. For control Fluconazole (75mg/5ml) were used. Triplicate test was done to get accuracy.

Incubation of plates

The plates contain bacterial culture were incubated at 37°C for 24 hours and plates contain fungal culture were incubated for 24 to 48 hours at 37°C.

Statistical Analysis:

Given all the data were statistical analyses and expressed as mean ± standard deviation, a further calculation of T-test and the level of significance was from P < 0.05. As for the data and graphs they were analysis using Microsoft® Office Excel 2007.

RESULT AND DISCUSSION

Antimicrobial activity

The antibacterial activity pattern of orange peel was shown in table 1 and table 2 by both test method against selected bacteria. The results of this study showed that the ethanolic extract of the peel of the plant demonstrated good activity on all the tested bacteria except *Salmonella paratyphi A*. However, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* are the most sensitive bacterial specie to the orange peel and their zone of inhibition higher than the reference antibiotics as seen in figure 1 and 2.

Figure 1: Graph shows the comparison between standard antibiotics and *Orange peel* through the Zone obtained from the Disc diffusion method

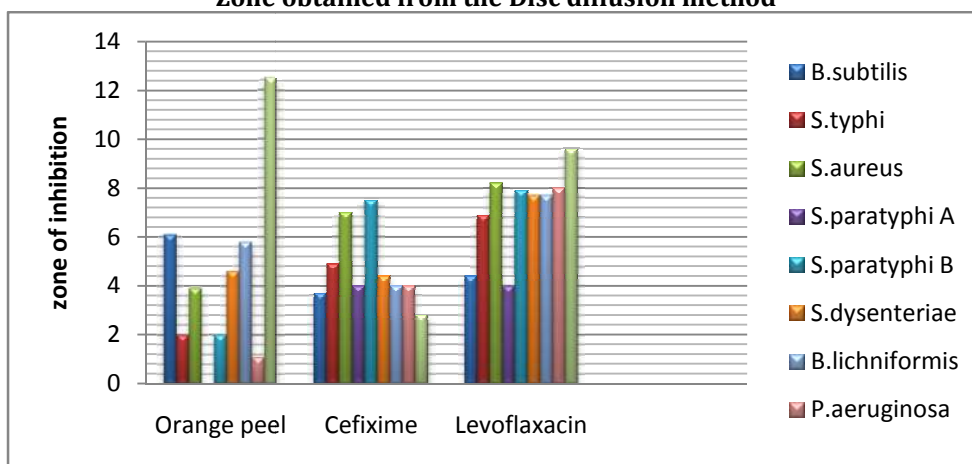


Figure 2: Graphical representation between standard Antibiotics and *Orange peel* extract through the Zone obtained from the Well diffusion method

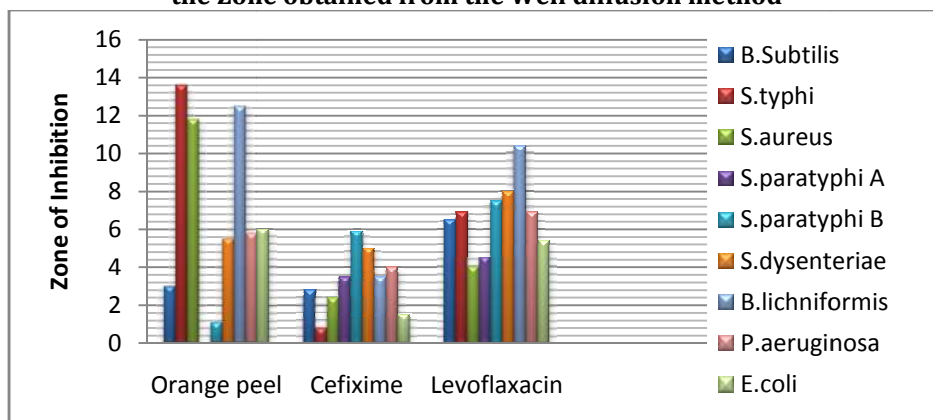


Table 1: Zone of inhibition of Orange peel extracts and reference antibiotics from Disc diffusion method

Bacteria Species.	Gram reaction	Zone of inhibition in mm (0.025 mg)	Zone of inhibition of Reference Antibiotics	
			Cefixime (100 mg)	Levofloxacin (125 mg)
<i>Bacillus subtilis</i>	Gram positive	6.1 ± 0.1	2.8±0.1	6.5±0.7
<i>Salmonella typhi</i>	Gram negative	2 ±0.08	0.8±0.5	6.9±0.5
<i>Staphylococcus aureus</i>	Gram positive	3.9 ± 0.1	2.4±0.3	4±0.5
<i>Salmonella Paratyphi A</i>	Gram positive	-	3.5±0.2	4.5±0.2
<i>Salmonella Paratyphi B</i>	Gram positive	2±0.1	5.9±0.1	7.5±0.2
<i>Shigella dysenteriae</i>	Gram negative	4.6 ± 0.1	5±0.5	8±0.5
<i>Bacillus lichniformis</i>	Gram negative	5.8 ±0.4	3.5±0.2	10.4±0.3
<i>Pseudomonas aeruginosa</i>	Gram negative	1.1±0.1	4±0.5	6.9±0.5
<i>Escherichia coli</i>	Gram negative	12.5 ±0.2	1.5±0.2	5.4±0.3

Table 2: Zone of inhibition of Orange peel extracts and reference antibiotics from agar well Diffusion method

Bacteria Species.	Gram reaction	Zone of inhibition in mm (0.025 mg)	Zone of inhibition of Reference Antibiotics	
			Cefixime (100 mg)	Levofloxacin (75 mg)
<i>Bacillus subtilis</i>	Gram positive	3 ±0.08	3.7±0.3	4.4±0.3
<i>Salmonella typhi</i>	Gram negative	13.6 ±0.8	4.9±0.05	6.9±0.5
<i>Staphylococcus aureus</i>	Gram positive	11.8 ±0.4	7±0.4	8.2±0.1
<i>Salmonella Paratyphi A</i>	Gram positive	-	4±0.5	4±0.1
<i>Salmonella Paratyphi B</i>	Gram positive	1.1 ±0.1	7.5±0.2	7.9±0.1
<i>Shigella dysenteriae</i>	Gram negative	5.5 ±0.2	4.4±0.3	7.7±0.5
<i>Bacillus lichniformis</i>	Gram negative	12.5±0.7	4±0.5	7.7±0.2
<i>Pseudomonas aeruginosa</i>	Gram negative	5.8 ±0.4	4±0.1	8±0.08
<i>Escherichia coli</i>	Gram negative	2.9 ±0.1	2.8±0.08	9.6±0.3

Among the two species of fungus *Rhizopus nigricans* showed very little restrain activity against orange peel extract. However, on *Ganoderma applanatum* no zone of inhibition observed. With the comparison of standard antifungal drug the orange peel extract is not so much promising showed in figure 3.

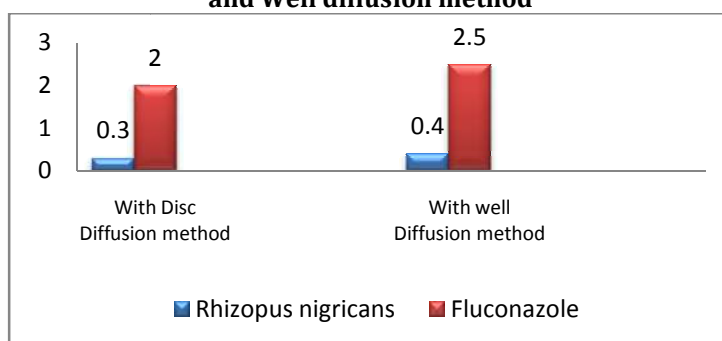
Figure 3: Graphical representation of Zone of fungal strain with reference antifungal drug by Disc and Well diffusion method

Table 3: Zone of inhibition of *Orange peel* on fungal strain and reference antibiotics from Disc diffusion method

Organism	Zone of inhibition in mm (0.025 mg)	Zone of inhibition of Fluconazole (75 mg) in mm
<i>Rhizopus nigricans</i>	0.3 ±0.03	2±0.08
<i>Ganoderma applanatum</i>	-	-

Table 4: Zone of inhibition of *Orange peel* on fungal strain and reference antifungal drug from Well diffusion method

Organism	Zone of inhibition in mm (0.025 mg)	Zone of inhibition of Fluconazole (75 mg) in mm
<i>Rhizopus nigricans</i>	0.4 ± 0.03	2.5±0.02
<i>Ganoderma applanatum</i>	-	-

Phytochemical screening

Phytochemicals are biologically active plant constituents present in the plant naturally. It is believed that phytochemicals may be resisting the formation of disease due to their antioxidant effect [20-21]. The phytochemical screening of *Citrus sinensis* (Orange) peels explore the presence of flavonoids, quinines, alkaloids, protein, amino acid and phytosterols showed in table 5. The presence of these compounds gives an indication of the medicinal importance of the *Citrus sinensis* peels, for example, flavonoids has antioxidant, antibacterial and antimicrobial properties [22] and the phenolic compound has inflammatory and anticarcinogenic activity.

Table 5: Phytochemical screening of extract of *Orange peel*

Phytochemical	Ethanollic Extract
Alkaloids	+
Phytosterols	+
Phenols	+
Tannins	-
Flavanoids	+
Proteins and amino acid	+
Saponins	-
Quinones	+
Terpenoids	-

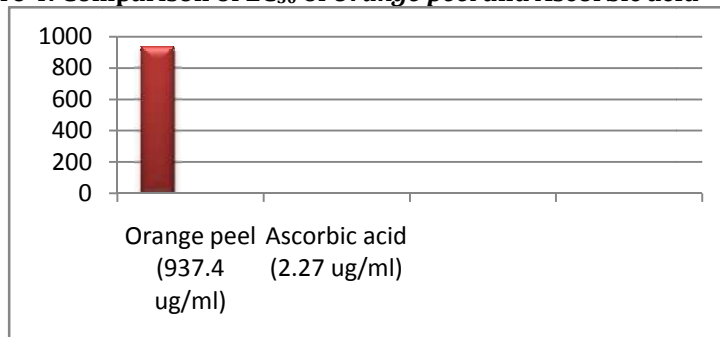
Key: + =Present, - = Absent

Antioxidant Potential

In the present study standard test were perform for the in vitro antioxidant activity using the DPPH method. The crude ethanol extract of orange peel showed antioxidant activity, with EC₅₀ value of 937.4. This value was very low compared to the ascorbic acid Figure 4 with EC₅₀ value 2.27. However, the phytochemical chemical constituents like alkaloids, glycosides, tannins, and flavonoids present in the extract, which are responsible for this antioxidant activity [16]. Hence, it can be viewed that this antioxidant activity may be due to the presence of any of these constituents.

Table 6: Antioxidant of Orange peel

Sample	Percentage Inhibition \pm SD	EC ₅₀ ug / ml
Orange peel	68 \pm 0.02	937.4

Figure 4: Comparison of EC₅₀ of Orange peel and Ascorbic acid

CONCLUSION

Plants can be considered as a promising natural source for pharmaceuticals and herbal medicinal preparations and can be used as natural bactericidal and fungicides or as a synergist agent with antibiotics. Based on the results obtained from our studies, we conclude that with more resources and time, Orange peel would be used in pharmaceutical industries to make natural origin antibiotics and supplements as it contains important phytochemical constituents.

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