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Antimicrobial & Antioxidant Activity of Orange Pulp and Peel

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Abstract: ***The oranges were purchased from the local market of Bela. The orange peel and pulp were subjected to successive extraction with solvents in increasing order of their polarity viz. Acetone, hexane, methanol and distilled water. Orange peel and pulp powder was extracted separately by aqueous extraction. The phenolic content of these samples were studied according to the method described by Folin Ciocalteu. In vitro antioxidant activity of orange peel and pulp were assessed using three different methods (Reducing power, Gallic acid method and DPPH scavenging activity). The in-vitro antioxidant activities on these two samples were evaluated. The antioxidant activity by Gallic acid in the orange peel higher in the distilled water (210mg/g) and low in the hexane (79mg/g) and in orange pulp higher in the acetone (522mg/g) and lower in the hexane (210mg/g) and by the DPPH method the higher in distilled water and hexane of orange peel and pulp.***

Keywords: Antioxidant, antimicrobial, DPPH, in vitro, solvent extraction, polyphenols, nutraceutical

1. Introduction

In Asia oranges originated thousands of years ago, in the region from southern China to Indonesia from which they were spread to the India. It is one of the commercial fruit crops grown in the entire world. 3.23 million tons of citrus fruit was produced in the Egypt which contained 2.14 million tons of the orange in 2008 [1]. Peels are generally wasted while the citrus fruits are mainly used in juice processing industries. Very large amounts of by-product are formed as wastes during the production of citrus juices [2]. The pollution of environment can also be reduced by this. The oranges peels are rich in nutrients, which can used as drugs or as food supplements too [3]. Oranges are cultivated in the many country like in India, UK, France, Germany, Holland, Brazil, China, USA and Spain. Oranges are the ever green tree and the height of tree is generally 9-10 cm. The leaves are arranged alternatively which are 4-8 cm long. With seasonal variation the Oranges are generally available from winter through summer which Depend upon the varity[4].

An antimicrobial is a substance which kills or inhibits the growth of much type of microorganisms such as bacteria, fungi or protozoan's. Antimicrobial drugs either kill or prevent the growth of microbes. The antimicrobial is substances which are used on nonliving objects are disinfectants. Citrus fruit products act as antimicrobial agents against the bacteria and the fungus. The citrus product has an important and physiological role because of its commercial value in food and pharmaceutical industries of the entire world [5].

The antioxidant property is presence the plant materials due to many active photochemical which include the vitamins, flavonoids, terpenoids, carotenoids, cumarins, lignin, saponin, plant sterols etc. The Citrus fruits and their juices are an important source of the bioactive methanol, the compound are an important to human nutrition which including the antioxidants such as ascorbic acid, phenolic compounds, flavonoids and pectins [6].

2. Materials and Methods

2.1 Collection of Material

Fresh orange were collected from in the local market in the month of May 2013. The orange were washed well using tap water. The peel is separated, then the pulp of Orange was separated by cutting them into small pieces and peel is also cut into small pieces then it was dried in the oven for a period of 6-7 days, at an ambient temperature of 30˚C. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form. The powder of the peels and the pulps were stored separately in air tight bottles.

2.2 Preparation of Extracts

2.2.1 Soxhlet extraction

Orange fruits were washed by distilled water then peeled and their edible portions were carefully separated. The peels were air dried in a ventilated oven at 40°C for 48 h and ground to a fine powder and passed through a 24-mesh sieve according to the method described by Van-Acker *et al.* 100g powdered sample was extracted with either 800ml ethanol or methanol or dichloromethane or acetone or hexane or ethyl acetate at room temperature by Soxhelt extraction method for 6 h. The mixture filtered through a Whatman No. 2 filter paper for removal of peel particles. The residue was re-extracted twice under the same condition to ensure complete extraction. The extracts were filtered and evaporated to dryness under reduced pressure at 60°C by a rotary evaporator. The extracts were placed in dark bottles and stored in refrigerator at 4°C until use [6].

2.2.2 Aqueous Extraction

The method of was adopted [6] for extraction with little modification. Briefly, 15g of the powdered plant were soaked separately in 200 ml of distilled water at room temperature for 24 hour under shaking condition. The extract was then filtered using Whatman filter paper No.1 then concentrated to dryness by using the water bath at 70ºc. Yield of the extract is weighed on the weighing balance

(shimadzu). Each extract were transferred to glass vials and kept at 4º C before use.

2.3 In Vitro Testing of Extracts for Antimicrobial Activity

2.3.1. Measurement of Antimicrobial activity of citrus peel and pulp

Nutrient agar medium (NAM)/broth was used for the growth of bacterial culture. Well diffusion method5 was adopted for measurement of antimicrobial activity of extracts. Nutrient Broth was taken separately in different sterilized test-tubes and different bacterium was inoculated separately and the test-tubes were kept in incubator for 48 h at 37ºC. Amphicillin (1mg/ml) for bacterial cultures was used as positive controls. In different sterilized plates the molten medium was introduced along with 1ml of inoculum of different bacterial cultures separately. The plates were kept for some time for hardening and then after they were punctured with a sterilized borer/needle. Different solvent extracts (of both peel and pulp) were introduced separately in the wells. The bacterial culture plates were kept for 24 h and fungal culture plates were kept for 48 h in order to determine the zonal inhibition [5].

2.3.2 Microorganism used

E-coli (MTCC No.118), *Staphylococcus aureus* (MTCC No.1349), *Pseudomonas flourences* (MTCC No.103) is purchased from IMTECH, Sector 39-B Chandigarh were used as the test microorganisms.

2.4 Estimation of Total Phenolic Content (TPC) of peel and pulp extract

Folin-Ciocaltean procedure [5] was used to determine the phenolic activity in Gallic acid by taking the 1.5ml of Folin-ciocaltean reagent and 4ml from stock of sodium carbonate. Tubes were vortexed well and were incubated in dark for 30 minutes at 765 nm.

2.5 Determination of antioxidant activity of samples by DPPH

It was determined by using the procedure [6]. A freshly prepared DPPH solution in 0.5 ml ethanol were added to 3 ml of diluted each orange peel and pulp extract to start the antioxidant reaction. The decrease in absorbance was measured at 517 nm. The absorbance is correlated with the scavenging action of the test compound. The radical scavenging activities were expressed as percentage of inhibition and calculated according to the following formula equation:

DPPH radical scavenging activity (%) = [Abscontrol − Abssample/Abscontrol} x100

Where, Abscontrol is the absorbance of sample at t = 0 min

Abssample is the absorbance of sample at t = 30 min

2.6 Determination of Antioxidant activity by Reducing power assay of extract sample

It was determined by using the procedure [6] with slightly modifications. 1 ml of extract samples was taken in test tubes and added 2.5 ml phosphate buffer and 1 % potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20min. 2.5mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000rpm for 10min. The upper layer of the solution was separated and mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$.The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power.

3. Results

The soxhlet extract of the citrus peel using different solvents yielded different results in each of the experiment conducted in the this study. There existed, a difference in the percentage yield of the extract obtained between various solvents.

Yield of extract obtained after dried the extract of various sample like Acetone, Methanol, Hexane and Distilled water by the Soxhlet apparatus of peel and pulp and the aqueous extraction.

Table 1: Yield peel and pulp extract by soxhlet apparatus

Solvent used according to their polarity	*Yield of peel sample (100gm.)*	*Yield of pulp sample (100gm.)*
Acetone	1.5g	4.8g
Methanol	60.6g	19g
Hexane	1.2g	2.7g
Distilled water	12.7g	2.3g

Table 2: Yield of peel and pulp by aqueous extraction

Peel /100g	2.6g
Pulp/100g	2.1g

3.1 DPPH Radical Scavenging Activity of Orange Peel and Pulp

DPPH assay is used to determine the scavenging potential of the antioxidant extract based on its capability as hydrogen donator. DPPH gives a strong absorption band at 517nm in visible region. When the phenolic compounds in the extract react with the stable DPPH radical, the absorption reduced and DPPH is decolourised from blue complex into light yellow. This discolouration is depend on the intrinsic concentration of present antioxidant and its reactions speed towards DPPH. The degree of reduction in absorbance measurement is indicative of the radical scavenging power of the extract.

3.1.1 Scavenging Activity of Orange Peel

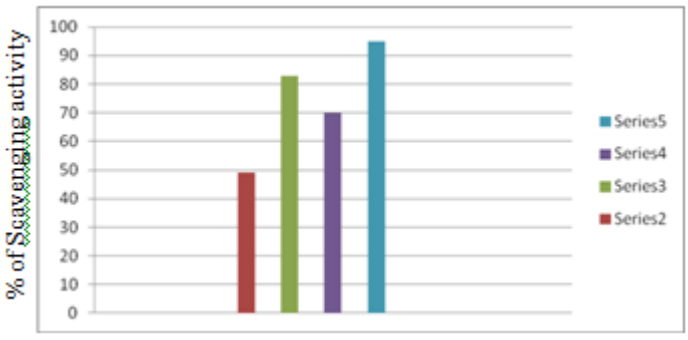

Figure 1: Series2 –**Acetone**, Series3-**Methanol**, Series4-**Hexane**, Series5-**Distilled water**

3.1.2 Scavenging Activity of Orange Pulp

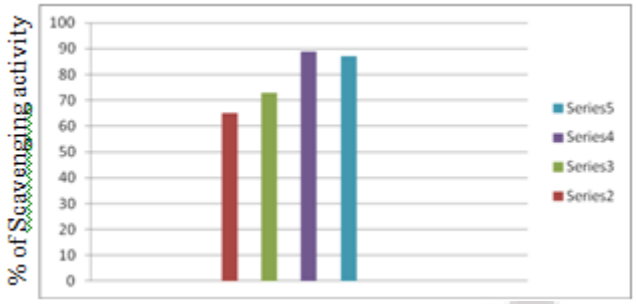

Figure 2: Series2 –Acetone, Series3-Methanol, Series-4-Hexane, Series5-Distilled water

3.1.3 Effect of different solvent on Reducing Power ability

The reducing power of orange peel with solvents like acetone, methanol, hexane and distilled water. The reducing power of methanol shows the highest reducing power shown the different activities which are shown in the graph.

3.2.1 Reducing Power Ability of Orange Peel

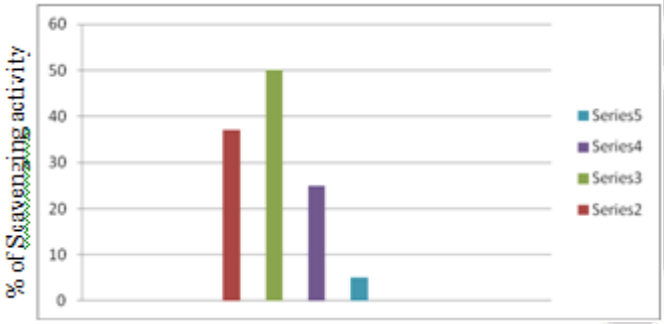

Figure 1.3: Series2 –Acetone, Series3-Methanol, Series4-Hexane, Series5-Distilled water

3.2.2 Reducing Power ability of orange pulp

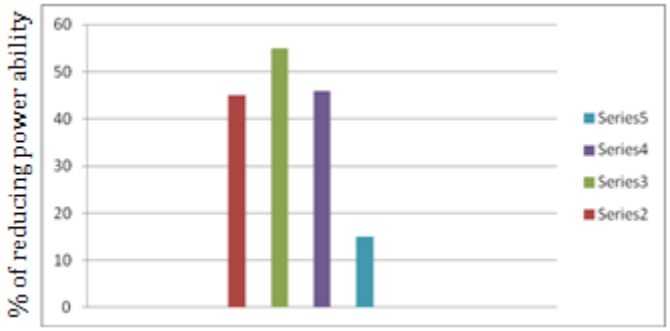

Figure No.4- Series2 –**Acetone**, Series3-**Methanol**, Series4-**Hexane**, Series5-**Distilled water**.

3.3 Antimicrobial activity:-It was found that extract of pulp and peel of oranges possessed maximum antimicrobial activity, which are shown below-

Table 1: Antimicrobial Activity of Orange Peel

Extract of peel	*Microorganism*	*Solvent(cm)*	*Sample (cm)*	*Antibiotic*
Hexane	*E.coli*	0.1	0.4	1.7
Hexane	*S.aureus*	0	0	0
Hexane	*P. fluorescens*	0	0.1	1.7
Methanol	*E.coli*	0	1.1	1.5
Methanol	*S.aureus*	0	1	0.3
Methanol	*P. fluorescens*	0	0.3	0.7
Acetone	*E.coli*	0.2	0.7	1.8

Table 2: Antimicrobial activity of orange pulp

Extract of pulp	*Microorganism*	*Solvent (cm)*	*Sample (cm)*	*Antibiotic*
Hexane	*E.coli*	0.1	0.5	1.7
Hexane	*S.aureus*	0	0	0
Hexane	*P. fluorescens*	0	0.1	1.7
Methanol	*E.coli*	0	1.4	1.7
Methanol	*S.aureus*	0	1.3	0.3
Methanol	*P. fluorescens*	0	0.3	0.7
Acetone	*E.coli*	0.2	0.7	1.8
Acetone	*S.aureus*	0	1.3	0
Acetone	*P. fluorescens*	0.1	1.2	2
Distilled water	*E.coli*	0	0.6	1.8
Distilled water	*S.aureus*	0.2	1.4	1
Distilled water	*P. fluorescens*	0.3	0.3	2

Table 3: Total phenolic content of orange peel and pulp

Acetone	*S.aureus*	0	1.3	0
Acetone	*P. fluorescens*	0.1	1	2
Distilled water	*E.coli*	0	0.2	1.8
Distilled water	*S.aureus*	0.1	0.3	1
Distilled water	*P. fluorescens*	0.2	0.2	2

Table 4: Total phenolic content of orange peel and pulp

Sample	Gallic acid equivalents mg/g of peel	Gallic acid equivalents mg/g of pulp
Acetone	114	522
Methanol	158	465
Distilled water	210	330
Hexane	79	201

Table 5: Total phenolic content of orange peel and pulp of aqueous extract

Sample	Gallic acid equivalents per mg/g peel	Gallic acid equivalents mg/g of pulp
Aqueous extract	215	326

4. Conclusion

The pulp has the more antioxidant activity and antimicrobial activity as compare with the orange peel. Orange peels and pulp can be an alternative use in food, Pharmaceutical and Cosmetic industries. This finding can form the basis for the studies to prepare an optimize preparation of the herbal extract. Recycling of fruit waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential

products required in human, animal and plant nutrition as well as in the pharmaceutical industry

5. Future Scope of the Study

The large amount of waste of orange peel and pulp can be used as nutraceutical at the industrial level. The present study was done to evaluate the antimicrobial and antioxidant potency. Antioxidants are the products that protect from various diseases, aging and UV damage. By combining the antioxidant with another substance like with some vitamins etc. it can be used as a food supplement. It can also be used in the cosmetics like in sunscreen lotion and anti aging substance. That can be used in the antimicrobial drugs for the animal growth promotion.

Reference

[1] H.A. Abd El-aal, F.T. Halaweish, "Food preservative activity of phenolic compounds in orange peel extracts (*citrus sinensis l.),*" *Lucrări Ştiinţifice*, 53, pp.233-240.
[2] Manthey .A and K. Grohmann, "Phenols in citrus peel byproducts: concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses," *J. Agric. Food Chem.* 49,pp. 3268,2001.
[3] K. Ashok kumar, Narayani, Subanthini and Jayakumar, "Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit Peels -Utilization of Fruit Waste,"*International Journal of Engineering Science and Technologyc*,3(6), pp.5414-5421,2011.
[4] Milind P. and Dev C., "Orange: range of benefit," *International research journal of pharmacy*, 3 (7), pp.59-63, 2012.
[5] Mathur A., Satish K. Verma, Purohit R., Gupta V., VK Dua, GBKS Prasad, Mathu D., Santosh K. Singh and Singh S., "Evaluation of *in vitro* antimicrobial and antioxidant activities of peel and pulp of some citrus fruits," *IJPI's Journal of Biotechnolgoy and Biotherapeutics*,1 (2), pp.1-17,2011.
[6] Hegazy A.E. and Ibrahium M.I., "Antioxidant Activities of Orange Peel Extracts," *World Applied Sciences Journal*, 18 (5),pp. 684-688, 2012.

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