

RRST-Pharmacology

Evaluation of Antibacterial and Antioxidant Activity of Methanolic and Hydromethanolic Extract of Sweet Orange Peels

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Article Info	Abstract
Article History Received : 20-04-2011 Revised : 27-04-2011 Accepted : 03-05-2011	The <i>in vitro</i> antibacterial and antioxidant properties of hydromethanolic extract of peel from <i>Citrus sinensis</i> (Sweet orange) was investigated. In this study the antibacterial activity of <i>Citrus sinensis</i> peel extract against different gram positive and gram negative bacteria by disc diffusion method and antioxidant activity by Fenton reaction was undertaken. <i>Citrus sinensis</i> peels had shown mild activity against gram positive and gram negative bacteria and the MIC value was measured. In another experiment antioxidant activity was observed and IC ₅₀ was shown at 600 µg/ml.
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Email: dayananddubey04@gmail.com ©ScholarJournals, SSR	Key Words: Orange peel, Antioxidant activity, Antibacterial activity

Introduction

Citrus is one of the most important commercial fruit crops grown in all countries of the world [1]. Antioxidants are the substances, compounds or nutrients in our foods which can prevent or slow oxidative damage to our bodies. These agents are able to remove the deleterious effects of free radicals within our body. Nowadays, considerable interest is focused on the development and evaluation of natural antioxidants and radical scavengers from plant materials which are rich in polyphenolic compounds. It is well known that plant derived polyphenols have remarkable antioxidant and free radical scavenging activity resulting in multiple beneficial nutritional physiological effects in the humans. The essential oil obtained from citrus fruits is having excellent antimicrobial properties and is used in cosmetic industry [2]. An antimicrobial is a substance that kills or inhibits the growth of microorganism such as bacteria, fungi or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on nonliving objects. Citrus fruit products are known to potent antimicrobial agents against bacteria and fungus. These citrus fruits are rich source of flavonones and many polymethoxylated flavones which are very rare in other plants [3]. Antimicrobial and antioxidant properties of peel and pulps of some citrus fruits were investigated [4]. These compounds, not only play an important and physiological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries activities of the world.

Materials and Methods

Plant material

The fresh sweet orange fruits were collected from local shops of Bhopal (M.P), India.

Preparation of Extract

The whole peels of sweet orange were fresh dried and were mechanically crushed into powder and extracted with distilled water at room temperature up to 48 h. The extract was filtered and concentrated in water bath under reduced pressure to obtain semisolid material which was then powdered. The powdered material was soaked separately in methanol solvent and hydro-methanol (50 % v/v) separately to obtain the extracts [5].

Test organism

The following gram negative and gram positive bacteria viz. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shigella flexneri*, *Bacillus subtilis* and *Escherichia coli* were used for antibacterial activities which were from stock culture of our laboratory.

Antibacterial activity

Antibacterial activities of 50 % methanolic, extract from fruit of Orange peels were investigated using Disk diffusion method [6]. 20ml of sterile Nutient agar was added in the test tube after that petri plates were prepared and cultures were swabbed on the top of the solidified media and allowed to dry. The test was conducted at four different concentrations of the crude extract 25 %, 50%, 75% and 100% (100µl per disk), the disk which was soaked with extract were placed on the surface of the medium the placed on the surface of the medium the

plate and were incubated for 12-18 h at 37°C and the zone of inhibition was recorded.

Antioxidant activity

The hydroxyl radical attached deoxyribose and initiated a series of reaction that eventually resulted in the formation of Thiobarbituric acid reaction substance (TBARS). The measurement of TBARS gives an index of free radical scavenging activity. The reaction mixture consisted of a deoxyribose (3mM, 100µl), ferric chloride (Fe³⁺, 0.2 mM 50µl), EDTA (0.1 mM 50 µl), ascorbic acid (0.1 mM 100 µl), stock solution of orange peel extract 20 mg/ml were prepared from which 200-2000 µl were added in reaction mixture, the final volume was made up to 1ml by adding adequate quantity of phosphate buffer saline (pH 7.4) and incubated for 1 h at 37°C. The reaction was stopped by adding 0.5 ml of 5 % TCA and 0.5 ml of 1 % TBA the mixture was then incubated for 20 min in a boiling water bath. The absorbance was measured at 532 nm. Ascorbic acid was used as positive control. The results are

expressed as the percentage inhibition of TBARS as reported [7].

Results and Discussion

Antibacterial activity

Hydromethanolic extract of orange peel at the different concentration i.e. 25%, 50%, 75%, 100% exhibits antibacterial against *Staphylococcus aureus* (6-14mm), *Bacillus subtilis* (6-9mm), *Staphylococcus epidermis* (5-10mm), *E. coli* (7-12 mm), *Shigella flexineri* (9-12mm), *Pseudomonas aeruginosa* (6-9 mm). The results are recorded in Table 1 and Figure 1-6.

Antioxidant activity

The extract of orange peel showed good antioxidant property in Fenton reaction model, the test drug was compared with a low concentration of ascorbic acid. Table 2 showed the percent TBARS inhibition of Hydromethanolic extract of orange peel and Ascorbic acid. It is clear from the above data that the Orange peels which are considered as waste material of the fruit consist of good Antibacterial and Antioxidant activity.

Table 1: Antibacterial activity of orange peel extract against different bacterial strains

S.No	Microorganism	Concentration of Hydromethanolic extract				Concentration of Methanolic extract			
		Zone of inhibition				Zone of inhibition			
		25%	50%	75%	100%	25%	50%	75%	100%
1.	<i>Staphylococcus aureus</i>	8mm	12mm	14mm	14mm	9mm	13mm	16mm	16mm
2.	<i>Bacillus subtilis</i>	NA	8mm	9mm	9mm	6mm	8mm	9mm	10mm
3.	<i>Staphylococcus epidermis</i>	NA	7mm	8mm	10mm	NA	10mm	10mm	13mm
4.	<i>E. coli</i>	7mm	9mm	10mm	12mm	8mm	12mm	14mm	16mm
5.	<i>Shigella flexineri</i>	6mm	9mm	10mm	12mm	8mm	9mm	10mm	14mm
6.	<i>Pseudomonas aeruginosa</i>	NA	6mm	8mm	9mm	7mm	8mm	9mm	13mm

Table 2: *In vitro* Antioxidant activity of Hydromethanolic Extract of orange peel

S. No.	Concentration (20mg in1ml) Ascorbic acid	% of TBARS	Concentration (20mg In 1ml) Of Orange Peels Extract			
			Hydromethanolic	% of TBARS	Methanolic	% of TBARS
1.	200	50.3	200	45.30	200	45.85
2.	400	51.6	400	45.85	400	46.96
3.	600	81.8	600	49.72	600	63.53
4.	800	85.2	800	55.24	800	113.25
5.	1000	95.3	1000	59.11	1000	74.58
6.	1200	69.7	1200	66.85	1200	82.87
7.	1400	122	1400	63.54	1400	85.63
8.	1600	110.7	1600	67.95	1600	109.39
9.	1800	139.5	1800	72.38	1800	110.01
10.	2000	142.2	2000	74.59	2000	151.9

Methanolic extract of Orange peels consist of good antioxidant and antibacterial activity as compared to Hydromethanolic extract. Among many other bacterial species, *S. aureus* showed good antibacterial activity at 100 % methanolic extract concentration. Both (Hydromethanolic and

Methanolic extracts) showed good antioxidant activity when done by Fenton reaction. Orange peel showed IC₅₀ at 600µg/ml and standard Ascorbic Acid showed IC₅₀ at 200 µg/ml. The results of antimicrobial and antioxidant activity are found to be correlated with some previous work [4].

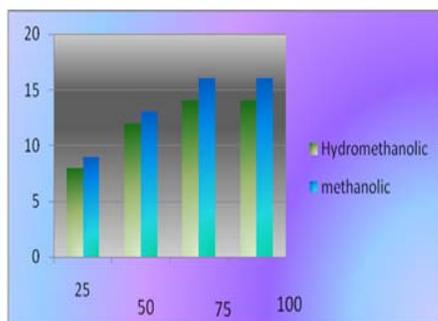


Figure 1: Inhibition of *Staphylococcus aureus* in a different concentration of Hydromethanolic and Methanolic extract of *Orange peel* Extract

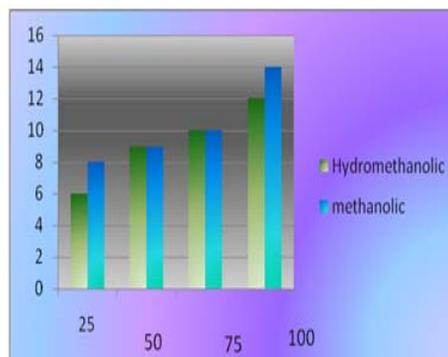


Figure 5: Inhibition of *Shigella flexineri* in a different concentration of Hydromethanolic or Methanolic extract of *Orange peel* Extract

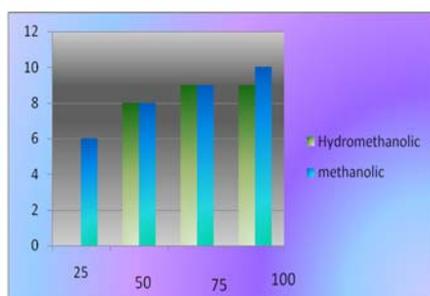


Figure 2: Inhibition of *Bacillus subtilis* in a different concentration of Hydromethanolic and Methanolic extract of *Orange peel* Extract

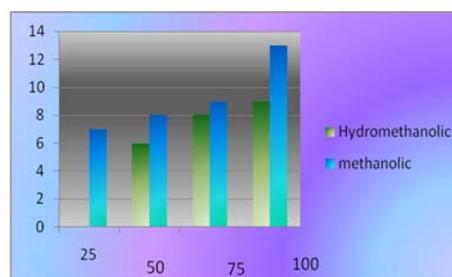


Figure 6: Inhibition of *Pseudomonas aeruginosa* in a different concentration of Hydromethanolic and Methanolic extract of *Orange peel* Extract

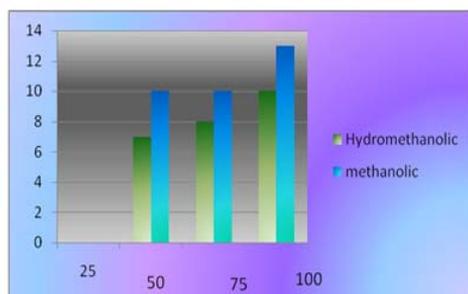


Figure 3: Inhibition of *Staphylococcus epidermis* in a different concentration of Hydromethanolic and Methanolic extract of *Orange peel* Extract

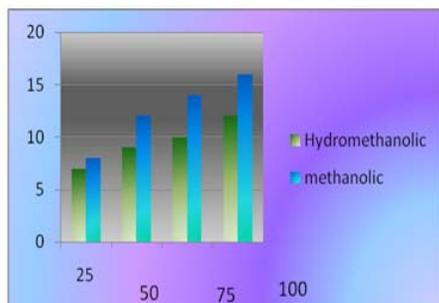


Figure 4: Inhibition of *E coli* in a different concentration of Hydromethanolic and Methanolic extract of *Orange peel* Extract.

Conclusion

From the present investigation it may be concluded that hydromethanolic extract of sweet orange peel has potent antibacterial activity against gram positive and gram negative bacteria. Hydromethanolic extract of *Citrus sinensis* showed good antioxidant activity and IC₅₀ was found at 600 µg/ml concentration. Further studies are however needed to isolate the active molecule responsible for both antimicrobial and antioxidant activity. The studies thus may lead to the formulation of a potent antimicrobial and antioxidant agent.

References

- [1] Tao K. 2007. Chemical composition of essential oil from the peel of Satsuma mandarin. African Journal of Biotechnology. 7(a):1261-1264.
- [2] Caccioni DRL. 1998. Relationship between volatile components of citrus essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. Int. J. Food Microbiology. 43:73-79.
- [3] Ahmed A. 2006. Genetic variability to essential oil composition in four Citrus fruit species. Pak. J. Bot. 38(2): 319-324
- [4] Mathur A., S.K. Verma, R. Purohit, GBKS. Prasad, D. Mathur, V. Gupta, V.K. Dua and S. Singh. 2011. Evaluation of in vitro antimicrobial and antioxidant properties of some citrus fruits. IJPI's Journal of Biotechnology & Biotherapeutics. 1(2):1-17.

- [5] Alade P.I. and O.N. Irobi. 1993. Antimicrobial activity of crude leaf extract of *Acalypha wilkesiana*. J. Ethnopharmacol.39: 170-174.
- [6] Nehru S.S., Z. Zuraini, S. Sasidharan and S. Suryani. 2008. Antimicrobial activity of *Cassia surattensis* and *Cassia fistula*. J. Mol. Bio. & Biotechnology. 1:1-4.
- [7] Kamran G., G. Yousuf and M.A. Ebrahimzadehb. 2009. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Dept. of horticulture, Faculty of Agriculture. 22 (3): 277-281.