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Antimicrobial & Antioxidant activity Pulp and Peel of Orange (*Citrus sinensis* L.)

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SUMMARY

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. Therefore, the scientist looking forward for the substance that provide excellent antimicrobial agent. Hence the main of this study was to evaluate the photochemical, antioxidant and antimicrobial activity of Orange (Citrussinensis) peel, which is considered as a waste causing environmental pollution.

INTRODUCTION

Orange is one of the most important commercial fruit crops grown in all continents of the world. In Asia oranges originated thousands of years ago in the region from southern China to Indonesia from which they were spread to the India. *Citrus sinensis* L. is the scientific name of Orange and *Rutaceae* family. The chromosome number is 2n=18 and genome size is 380 Mb. In world the orange production is about 54.23 million metric tons (2017-2018). India produces 2,546 metric tons (2017-2018) of orange. The top orange producing states of India are Panjab, Madhya Pradesh, Andhra Pradesh, Maharashtra, Rajasthan, Assam, Karnataka. In the Maharashtra production of orange is 33.86 metric tons (2017-2018) and area production is about 80,000 hectors. Orange is cultivated in Amravati, Nagpur, Akola, Wardha and Yavatmal. The major varieties of orange produced in India include Khasi mandarin, Nagpur mandarin and Kinnow. Peel is generally wasted while the citrus fruit are mainly used in juice processing industries. The very large amount of by product is form by wastes during the production of citrus juice. The pollution of the environment can also be reduced by this. The oranges peel is rich in nutrients which can use as drugs or as food supplement too. An antimicrobial is a substance which kills or inhibits the growth of much types of organisms such as bacteria, fungi or protozoan. Antimicrobial drugs either kill or prevent the growth of microbes. The antimicrobial is substance which is used on non-living objects are disinfectants. Citrus fruit products act as antimicrobial agent against the bacteria and fungus.The antioxidant property is presence of the plant materials due to many active photochemical which include the vitamins, flavonoids, terpenoids, carotenoids, coumarins, lignin, saponins plant etc. The orange is cross-pollinated crop. Deep well drained loamy soils are the best for cultivation of orange. The pH of soil should be 6.5 to 7.5. The orange is grown successfully in all frost-free tropical and sub-tropical regions. Annual rainfall of 100-120 cm. The temperature is 10º C to 15º C. Orange peel extract has a lot of medicinal properties which have been reported just like against cancer, diuretic, stomachic, immune enhancing, colic, communitive, upset stomach, tonic to digestive system, immune system and skin. It is also used to treat and prevent vitamin deficiencies, colds, flu, and scurvy and helping to fight viral and bacterial infections. The major phenolic compounds present in the orange include hydroxyl cinnamic acids (HCA) and flavonoids, among which flavanones are the most prevalent. Citrus flavonoids, especially hesperidin, have a wide range of therapeutic properties, including anti-inflammatory, antihypertensive, diuretic, analgesic, and hypolipidemic activities.

Table 1. Taxonomy of *Citrus Sinensis* L.

Common name	**Sweet orange**
Botanical name	*Citrus sinensis* L.
Kingdom	Plantae
Subkingdom	Tracheobionta
Division	Magnoliophyta
Class	Magnoliosida
Subclass	Rosidea
Order	Sapindales
Family	*Rutaceae*
Genus	*citrus*

The present study or research work "Antimicrobial & Antioxidant activity of Orange (*Citrus sinensis* L.) Pulp and peel" done according to following objective: 1.To prepare the extract from orange peel and pulp.2.To test in vitro antimicrobial and antioxidant activity on the microorganism.

Materials and Methods

The project has been performed at Department of Plant Biotechnology and Molecular Biology of College of B.Sc. Agriculture Biotechnology, Saralgaon, Tal-Murbad, Dist Thane, MS. The present study was under take aqueous extraction of orange pulp and peel and check the antimicrobial and antioxidant activity of orange pulp and peel extract against using different organisms.

A. Material:

1. Collection of plant sample

The fresh orange was collected from the local market Saralgaon. The orange was 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. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form.

Fig 1. Orange Pulp

Fig 2. Orange Peel

Fig.3. Peel powder

Fig 4. Pulp powder

2.Chemicals: Ethanol, Methanol, Dichloromethane, Acetone, Hexane, Ethyl acetate, Ampicillin, Penicillin, streptomycin, Sodium carbonate, DPPH solution, Gallic acid, Folinciocaltean reagent, Phosphate buffer, potassium tricyanide, Ferric chloride, Trichloroacetic acid, Peptone, Yeast extract, Beef extract, NaCl, Agar.

3.Instruments and Equipments : Hot air oven, Incubator, Mortar and pestle, Grinder, Rotary evaporator, Refrigerator, Orbital shaker, Water bath, Weighing balance, Spectrophotometer, Centrifuge machine, Distilled water unit. Knife, 24 mess sieves, Whatman No.1 filter paper, Whatman No.2 filter paper, Needle, Nichrome wire loop, Spreader, Borer, Tips, Vials, Micropipette, Ambient glass bottles, Glass vials, Test tube, Petri plates, Beaker, Conical flaks.

2 Methods 1. Aqueous extraction: The orange powered of pulp and peel each 15g of powdered were soaked separately in 200 ml ofdistilled water at room temperature for 24 hours under shaking condition. The extract was then filtered using Whatman filter paper No.1then concentrated to dryness by using the water bath at 70ºc.Yield of the extract is weighed on the weighing balance each extract was transferred to Glass vials and kept at 4º C before use.

2. In Vitro Testing of extracts for Antimicrobial activity: -

2.1. Measurement of Antimicrobial activity of Citrus peel and pulp: -Nutrient agar medium (NAM) was use for the growth of bacterial culture. Agar well diffusion method5 was adopted for measurement of antimicrobial activity of extracts. Nutrient Broth was taked separately in different sterilized test-tube sand different bacterium was inoculated separately and the test-tubes was kept in incubator for 48 h at 37ºC.*Ampicillin* (1mg/ml) for bacterial cultures was use as positive controls. In different sterilize plates the molten medium was introduce along with1mlofinoculumofdifferent bacterial cultures separately. The plates were kept for some time for hardening and then after they were puncture with a sterilize borer/needle. Different solvent extracts (of both peel and pulp) was introduce separately in the wells. The bacterial culture plates will be kept for 24 hand fungal culture plates was kept for 48 h in order to determine the zonal inhibition.

2.2Microorganisms used: -*E-coli* (MTCC No.118), *Staphylococcus aureus* (MTCCNo.1349), *Pseudomonas fluorenes* (MTCC No.103), *Bacillus subtilis* (MTCC No.441) was purch from Mitcon institute Shivajinagar, Pune was use as the test microorganisms.

2.3 Estimation of Total Phenolic Content (TPC) of peel and pulp extract: - Take 1 ml aliquots and standard gallic acid (200, 400, 600, 800, 1000, 1200 µg/ml) was position into test tubes and 5 ml of distilled water and 0.5 ml of Folin ciocalteu (FC) reagent was mix and shake after 5 min of incubation 1.5 ml 20% NO_2CO_3 was add and volume made up 10 ml with distilled water. It was allowed to incubate for 2 hours at dark condition. Intense blue colour was developed. After incubation absorbance measure of 765nm UV-visible spectrophotometer. The extract was performed in triplicate. The blank was performed using reagent blank with solvent. Gallic acid is used as standard. The calibration curve was be plot using standard gallic acid. The data for total phenolic compound content of polyherbal formulation where express as mg of gallic acid equivalent weight (GAE/100gm) dry mass.

$$\mathrm{TPC} = \mathrm{C} \times \frac{\mathrm{V}}{\mathrm{M}}$$

Where,

C=concentration of gallic acid (mg/ml)
V=volume of plant extract (ml)
M=Weight of pure extract(ml)

2.4 Determination of antioxidant activity of samples by DPPH: -

A freshly prepare DPPH (2-dipheny-1-picrylhydrazyl) solution in 0.5 ml ethanol was added to 3ml of dilute sample orange peel and pulp extract to start the antioxidant reaction. The decrease in absorbance will be measure at 517 nm by using spectrophotometer. The absorbance is correlated with the scavenging action of the test compound. The radical scavenging activities was expressed as percentage of inhibition and calculate according to the following formula equation:

$$\text{DPPH radical scavenging activity}(\%) = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

2.5 Determination of Antioxidant activity by Reducing power assay of extract sample:

Take 1 ml of extract samples was taken in test tubes and added 2.5 ml phosphate buffer and 1 % potassium ferricyanide [K3Fe (CN)6]. The mixture was incubated at 50°C for 20 min. 2.5 ml of trichloroacetic acid (10%) was to the mixture, which was then centrifuge at 3000 rpm for 10 min. The absorbance was measure at 700 nm by using UV-Vis spectrophotometer. Increase absorbance of the reaction mixture indicates the increase reducing power.

Results:

1. Aqueous extraction

The aqueous extraction of the citrus peel and pulp using different solvents yield different results in each of the experiment conducted in 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 aqueous extraction of peel and pulp.

Solvent used according to their polarity(ml)	Yield of peel sample (100gm)	Yield of pulp sample (100gm)
Distilled water	3.9 gm	3.1 gm

Fig 5. peel yield

Fig 6 pulp yield

2. In Vitro Testing of Extracts for Antimicrobial activity: -

2.1DPPH 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 decolorized from blue complex into light yellow. This discolouration is depending 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.

Table 2. Scavenging Activity of Orange Peel

Sr. No	Series	Percentage of scavenging activity
1	Acetone	50
2	Methanol	85
3	Hexane	70
4	Distilled water	95

Table 3. Scavenging Activity of Orange Pulp

Sr. N	Series	Percentage of scavenging activity
1	Acetone	65
2	Methanol	75
3	Hexane	90
4	Distilled water	85

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.

Table 2. Scavenging Activity of Orange Peel

Sr. No	Series	Percentage of scavenging activity
1	Acetone	38
2	Methanol	50
3	Hexane	23
4	Distilled water	8

Table 4. Reducing Power Ability of Orange Peel

Sr. No	Series	Percentage of scavenging activity
1	Acetone	45
2	Methanol	55
3	Hexane	44
4	Distilled water	15

3.1 Antimicrobial activity:-It was found that extract of pulpand peel of oranges possessed maximum antimicrobialactivity, which are shown below.

Aqueous Extract (µL)	Zone of inhibition of inhibition in mm			
	P.aeruginosa	*B.subtilis*	*S.aureus*	*E. coli*
10	2	2	3	2
20	4	4	2	3
30	6	6	4	8

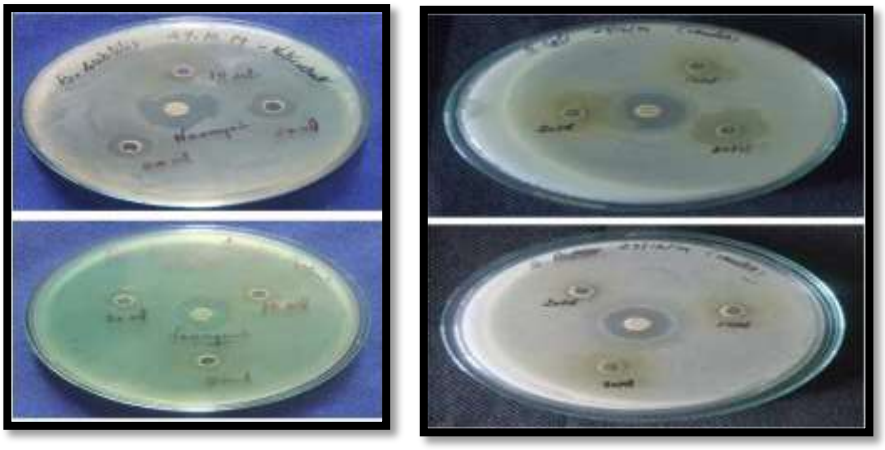

Fig 7. Result of Antimicrobial activity of aqueous extract

CONCLUSION:

The pulps have 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.

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