



Comparative Assay of Antioxidant and Antibacterial Properties of Indian Culinary Seasonal Fruit Peel Extracts obtained from Vellore, Tamilnadu

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ABSTRACT

The study was carried out to enhance the utilisation of the waste products from the food processing industries. Fruit peels are the major by product of any food processing industry. These fruit peels, as a waste product, causes tremendous investment of capital to decompose it safely and to prevent any environmental pollution. If these waste products can be converted as a raw material for the production of any bioactive compounds, then it will keep the food processing industries free from investing its capital in decomposing these wastes. Instead, it can be used efficiently for any useful products, giving them an extra profit. The fruit samples studied in our work were *Maclura pomifera* (Orange), *Punica gramatum* (Pomegranate or Anar), *Musa balbisiana* (Banana), *Citrus limetta* (Sweet lime or Mousambi), *Malus domestica* (Apple), *Carica papaya* (Papaya), and *Mangifera indica* (Mango). The fruit peels were collected from Vellore district of Tamilnadu. The fruit peel extracts were prepared using water as a solvent. The Reductive power assay was used to determine the antioxidant property and well diffusion assay for the antibacterial property. The absorbance of the sample, observed in spectrophotometer at 595nm for different concentrations is being compared for fruit peel extracts in order to analyse the antioxidant property and the zone of exclusion is being studied for determination of antibacterial property of the fruit peel extracts. Apple peel extracts were found to have the maximum antioxidant activity and pomegranate peel extract was found to have the maximum antibacterial activity, among the fruit samples studied.

Keywords: Fruit peels, antioxidant property peel extract, antibacterial activity, reductive power assay.

INTRODUCTION

Antioxidants are the substances that prevent oxidation and these are especially used to contravene the deterioration of stored food products. Numerous diseases have been proposed to have a radical or oxidant involvement. Antioxidants can block, prevent or inhibit the radical initiated reactions.

Antibacterial agents are the synthetic or natural compounds which check the growth and division of bacteria. There are many cases in recent years showing the emergence of resistance to drugs, in the pathogenic microorganisms found in human and animal bodies. This drug resistance is due to the random usage of some commercial antimicrobial agents. These antimicrobial agents are very commonly used in the treatment of various infectious diseases¹. This arouses the need for search of a new antibacterial agent. This is the reason why the present researchers are concentrating on the study of herbal products which can provide new routes to formulate a better drug against the pathogenic microorganisms². The antibiotics presently available in the market are continuously losing their biochemical effect at a tremendous percentage rate whereas the development of any new antibiotics is at very slower rates. This creates an emerging scope of any unique antimicrobial agent from various biological sources. There are many plants which produces secondary metabolite having antibacterial properties³. Plants constitutes of antimicrobial compounds in various parts such as bark, stalk, leaves, roots, flowers, pods, seeds, stems, hull, latex and fruit rind⁴⁻⁶.

Fruit peels are the outer covering of the fruits which behaves as a physical as well as a chemical barrier, by virtue of the presence of many antimicrobial constituents in it, for any external pathogens as well as for any other factors intended to spoil the quality of the fruit products. The fruit peels are the major by product of any food and beverages industry, and it becomes a financial burden on the industry to decompose it, without causing any severe environmental pollution. If these fruit peels, being a by product, can be converted to some useful components to be used in food and healthcare industries, then the industry can gain a better profit, and at the same time the product can be affordable to common people. The fruit peels are very rich in bioactive compounds which can be used as an antioxidants and antimicrobial agents. Till now, the by products of food and beverages industries are being thrown away or being fed to animals. But, the current researches on the fruit peels are following a trend to identify an efficient way to extract the bioactive compounds having antioxidant and antimicrobial properties from the fruit peels, to use it for better health development. Thus, these fruit peels are the novel, easily available, efficient, affordable, eco friendly, natural and economic source for antioxidants and antimicrobial agents.

Mango (*Mangifera indica* L. Anacardiaceae) is listed among the most important tropical fruits in the world which ranks among the top 10 fruits in overall production of major fruit crops all over the world⁷. During the processing of mango, waste products like peels, seeds and kernels are generated. Peels contribute around 15-



20% of the whole fruit⁸. Bioactive compounds such as antioxidants, ascorbic acid, phenolic compounds, flavonoids, and pectins are found mostly in citrus fruits like Oranges and Mousambi. These are important for human nutrition⁹. The extracts from the orange peels were found to have a good total radical antioxidative potential¹⁰.

In our current investigation, we are reporting the antioxidant and antimicrobial properties of peels of various common fruits consumed in India, particularly in South Indian region. The fruit samples were collected from Vellore, which lies between 12° 15' to 13° 15' north latitudes and 78° 20' to 79° 50' east latitudes in Tamil Nadu state. The comparison between the antioxidant and antibacterial properties has been done to bring out the most efficient fruit peels for the industrial purpose. Seven fruit peels are being investigated for the antioxidant and four fruit peels for antibacterial properties.

MATERIALS AND METHODS

Chemicals

Ascorbic acid (1%), Ferric chloride (0.1%), Phosphate buffer (0.2M, pH6.6), Potassium ferricyanide (1% w/v), Trichloroacetic acid (10%), Nutrient agar media and Phosphate buffer used in the experiment were obtained from Instrumental & Food analysis lab of the School of Biosciences and Technology, VIT University, Vellore. Phosphate buffer was prepared by mixing dibasic sodium phosphate (37.50ml of 0.2M) along with 62.5ml monobasic sodium phosphate, which was diluted with

water, to 100ml of final solution. All the chemicals were obtained in highly purified state.

Bacterial culture

The bacterial cultures of *Bacillus*, *Pseudomonas*, *Escherichia coli*, and *Klebsiella* were obtained from Instrumental & Food analysis lab of School of Biosciences and Technology, VIT University, Vellore. The bacterial cultures were sub cultured in the nutrient media and pure cultures were obtained by streaking technique. The purity of the bacterial cultures was tested by gram staining and microscopic investigation. The staining and microscopic investigation of *Bacillus* strain is shown in Fig. 1. *Bacillus* is a Gram negative bacteria and the appearance of pink colour proves it to be the pure culture of *Bacillus cereus*.

Preparation of Fruit peel extracts

The fruit peels under our investigation are mentioned in Table 1. The fruit peels, being the by product of juice industries, were obtained from different juice corners of Vellore. The peels were cut into small pieces and dried in open sun for few days on a clean dry paper (Fig. 2, 3). After complete removal of moisture, the fruit peels were churned to powdered form (Fig. 4) in the grinder. 10mg of fruit peels powder was mixed in 10 ml of distilled water (Fig. 5) and kept on a shaker for overnight. The resulting mixture was centrifuged at 3000rpm for 20 minutes in the centrifugation machine. 2ml of the supernatant was taken for the study of radical scavenging property. Three different volumes, such as, 0.1ml, 0.3ml and 0.5ml of the fruit peel extracts were prepared in separate test tubes for the comparative study of the antioxidant activity.

Table 1: Classification of fruit samples

Fruits	Kingdom	Order	Family	Genus	Species
Orange	Plantae	Urticales	Moraceae	Maclura Nutt.	Maclura pomifera
Pomegranate or Anar	Plantae	Myrtales	Punicaceae	Punica L.	Punica gramatum L.
Banana	Plantae	Zingiberales	Musaceae	Musa L.	Musa balbisiana Colla
Sweet lime	Plantae	Sapindales	Rutaceae	Citrus	Citrus limetta
Apple	Plantae	Rosales	Rosaceae	Malus	Malus domestica
Papaya	Plantae	Violales	Caricaceae	Carica L	Carica papaya L.
Mango	Plantae	Sapindales	Anacardiaceae	Mangifera L.	Mangifera indica L.

Antioxidant property assay

The Reducing power assay¹¹ has been used for the determination of the antioxidant property of fruit peels under consideration, with respect to Ascorbic acid as the standard Antioxidant compound. Substances with reduction potential has a reaction with potassium ferricyanide (Fe^{3+}), forming potassium ferrous cyanide (Fe^{2+}), which upon reaction with ferric chloride, forms ferric ferrous complex that has absorption maximum at 700nm. The three different volumes of the fruit peel extracts were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml). This mixture was kept at a temperature of 50°C in hot water bath for the time

duration of 20 mins. After cooling the mixture at room temperature, 2.5ml of 10% Trichloroacetic acid was added and further centrifuged at 3000rpm for 10 mins. The supernatant (2.0ml) was separated in a clean test tube and mixed with distilled water (2.5ml) and freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 595nm in the spectrophotometer (Fig.6). Controls were prepared for each fruit peels by excluding the sample and mixing all other remaining components in the same manner. Antioxidant activity was comparatively studied by measuring the absorbance of the fruit peel extracts with various concentrations at 595nm.



Antibacterial property assay

The antibacterial property was determined by well diffusion method¹². The test organisms were inoculated in a 100ml conical flask containing nutrient broth media and it was incubated overnight at 37°C. Streaking was done to obtain a pure culture line from the conical flask mixture. The pure bacterial colonies were taken and grown into 20ml agar broth. Agar test plates were prepared for each test organisms. After the agar was hardened, the required test organism taken in the volume of 0.2ml was spread over the hardened agar media with the help of sterilised L-glass rod. Holes, in the number of four, were made using sterilised cork borer, making sure that the holes are distributed properly in the periphery. After the agar plugs were removed, the cork borer was changed with another sterilised cork borer to make hole for another test organisms. Ampicillin was used as a standard antibacterial agent for comparison of antibacterial activity of the fruit peel extracts from the test organisms. The concentration as well as the volume of ampicillin and the fruit peels

extracts were kept same to maintain uniformity in the comparison of the antibacterial property of the samples. The concentration of ampicillin and the fruit peels were taken as 1mg/ml and the test volume used was 0.2ml for both of them. In each hole, sample was loaded, except one, in which the ampicillin was loaded. Then the plates containing the fruit peel extracts along with the test organisms were left at ambient room temperature to facilitate the diffusion of test samples and then incubated with face upwards at 37°C for overnight. The diameter of the zone of exclusion (Fig. 7, 8, 9, 10) was measured using a measuring scale (Table 4).

Statistical analysis

All the analysis was performed on triplicate samples. The results were expressed as absorbance value at 595nm for studying antioxidant activity and the diameter of zone of exclusion for the study of antibacterial property.

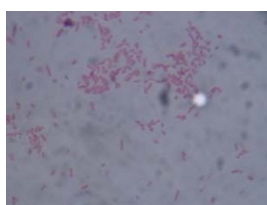


Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5

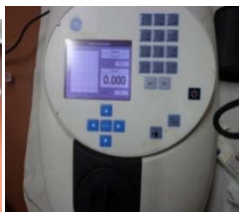


Fig. 6



Fig. 7



Fig. 8



Fig. 9



Fig. 10



Fig. 11

RESULTS

The absorbance of the standard solution at 595nm is shown in (Table 2) whereas (Table 3) shows the absorbance of sample solution at 595nm. The absorbance value in Table 3 shows a directly proportional relation with the concentration of the sample solution. The horizontal axis in the Graph 2 shows the fruit peels in the serial number as in the same order as given in Table 2. It is obvious from the graph 2 and Table 3 that 0.5ml volume of Apple peel extracts at the concentration of

1mg/ml is having comparatively highest antioxidant activity than the rest of the fruit peel samples.

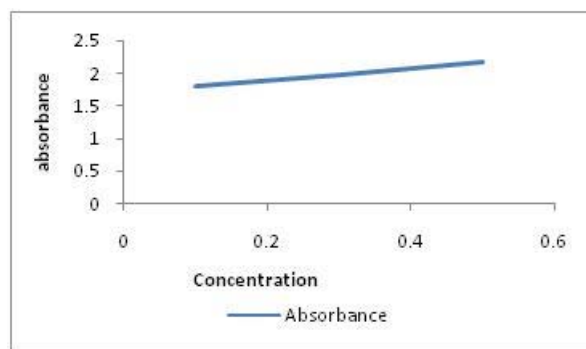
From the Table 4, it is obvious that Pomegranate peel extract has comparatively maximum antibacterial effect as the zone of exclusion is 34.66mm. Orange peel extracts has comparatively minimum level of antibacterial activity. The comparative relation can be interpreted clearly from the Graph 3.

Table 2: Absorbance of standard drug

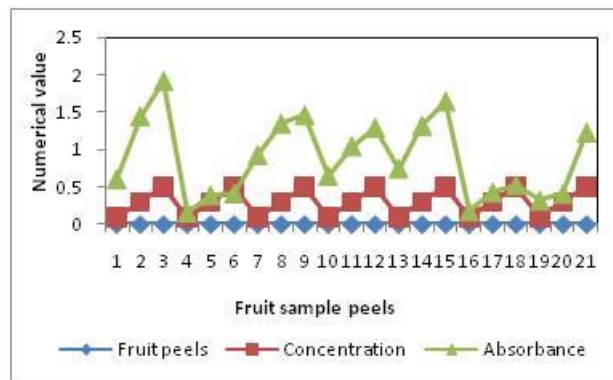
Concentration	Absorbance
0.1	1.809
0.3	1.971
0.5	2.181

Table 3: Absorbance of fruit peel samples

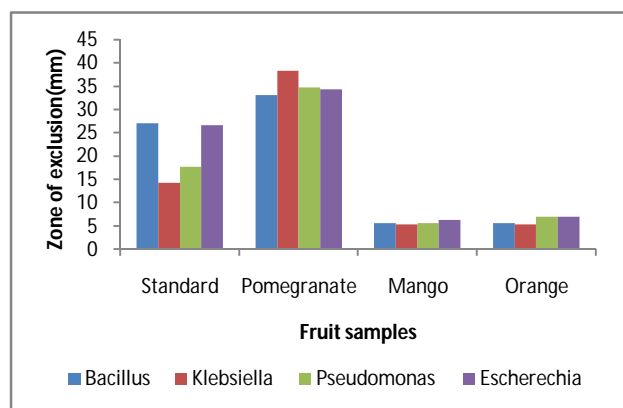
Fruit Peels	Volume (ml)	Absorbance
Apple	0.1	0.607
Apple	0.3	1.449
Apple	0.5	1.919
Orange	0.1	0.160
Orange	0.3	0.394
Orange	0.5	0.421
Mango	0.1	0.934
Mango	0.3	1.351
Mango	0.5	1.463
Pomegranate	0.1	0.654
Pomegranate	0.3	1.050
Pomegranate	0.5	1.295
Banana	0.1	0.749
Banana	0.3	1.324
Banana	0.5	1.647
Sweet lime	0.1	0.178
Sweet lime	0.3	0.432
Sweet lime	0.5	0.531
Papaya	0.1	0.324
Papaya	0.3	0.413
Papaya	0.5	1.231



Graph 1: Absorbance of standard drug



Graph 2: Antioxidant activity of fruit peels



Graph 3: Antibacterial activity of fruit peel

Table 4: Antibacterial activity exhibited by zone of exclusion in mm

S.no.	Sample extracts (10mg/10ml)	Bacillus	Klebsiella	Pseudomonas	E. Coli
1	Ampicillin	27	14.33	17.66	26.66
2	Pomegranate peels	33	38.33	34.66	34.33
3	Mango peels	5.66	5.33	5.66	6.33
4	Orange peels	5.66	5.33	7	7

DISCUSSION

Antioxidant activity is associated with reducing power. It is significantly reflected by the reducing power of any compound. Compounds with reducing power indicate that they are electron donors and can reduce the oxidised intermediates. Various shades of green and blue (Fig. 11) are obtained from the yellow colour of the test solution, in the reductive power assay and this depends on the reducing power of each compound. Presence of

antioxidant compounds causes the Ferricyanide (Fe^{3+}) conversion to Ferrous cyanide (Fe^{2+}). The concentration of the Fe^{3+} ion to Fe^{2+} can be determined by the measurement of the formation of pearl's Prussian blue at 595nm, which is given by the absorbance value.

The antibacterial property of the fruit peel extracts was determined by the well diffusion assay in our study. The zone of exclusion of different fruit peel extracts is shown in Table 4. The readings were taken in mm.

CONCLUSION

The absorbance of the sample solution and the reductive potential is directly proportional to each other. Thus, an increase in the absorbance leads to the fact that the reductive potential of the sample is increasing. The Antioxidant activity of a solution can be significantly indicated by the reductive potential of the compounds present in the sample. Further studies are warranted for the identification of the individual compounds which are responsible for the reducing power and also for the identification of the synergistic effect. The correlation between the antioxidant activity and the reducing potential can also be determined as a mathematical formula by further studies. In the current study, we collected the information of antioxidant activity of seven fruit peels extracts from Vellore by determining and interpreting the absorbance of test samples at 595nm in spectrophotometer.

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A hands on Research oriented student, well knowledgeable with the tools and techniques of Biotechnology along with the conceptual and modern basics of Life Science. The experience of Internships from various Industrial Organisations have given him the exposure to the practical applications of Biotechnology and he has proved it in his work experience in the laboratory since last two years, working under well guidance of Dr. Suneetha V, Associate Professor, VIT University, Vellore, India. His area of interest is Microbial technology and genetic engineering.