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Value addition of fruit waste: Determination of total phenolic content, antioxidant and antibacterial assay of fruit peel.

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ABSTRACT

Fruit waste are one of the main source of municipal waste.In order to explore the potential of fruit waste as natural resources of bioactive compound and total phenolic content, antioxidant and antimicrobial activites of different fruit peels. Fruit peels of punica granatum(pomeogrante), citrus sinensis(orange), musa paradisiaca(Banana) and citrus lemon(lemon) were evalutated.The result reveal the presence of flavonoid, phenol, carbohydrate and tannin in all the samples. DPPH (1,1 diphenyl 2-picrylhydrazyl) radical scavenging activity varied with punica granatum(pomeogrante) and having better scavenging capacities than citrus sinensis(orange), musa paradisiaca(Banana) and citrus lemon(lemon).Antimicrobial studies reveal differential antimicrobial activities against different microbial strains.Zone of inhibition from 15 to 40 mm were for antimicrobial test.The result showed that fruit residues could be inexpensive and readily available resources of bioactive compounds for use in the food and pharmaceutical industries.

Keywords—DPPH(1,1 diphenyl 2-picrylhydrazyl), Fruits peels, vegetable peels, antioxidants, antimicrobial agents, bioactive compounds.

INTRODUCTION

Fruit juice processing industry in India and worldwide generate large quantities of waste. Fruit juice industry while processing the edible part of the fruit, release waste such as fruit peel, seeds and other fruit residue in large quantities and its disposal has become a serious environmental issue (Gui-Fang Deng et al., 2012). Peels represent between 50 to 65% of total fruit weight. If not processed further, it produce odour and soil pollution, harborage for insects and can give rise to serious environmental pollution (A.E. Hegazy et al., 2012).Fruit waste is the major component of municipal solid waste. Disposal of municipal solid waste include two main techniques, landfill and incineration. Improper management of landfill would lead to emission of methane and carbon dioxide and incineration involves releases of pollutants and secondary wastes such as dioxins, furans, acid gases as well as particulate which may cause serious environmental issue (Gui-Fang Deng et al., 2012). For these reasons, there is an urgent need to reduce these waste and value-added use for fruit wastes. In fact, the method apply should be inexpensive and readily available, it should be highly cost-effective and minimizes environmental impact.Fruit wastes are a rich source of Polyphenols. These peels are the source of rough dietary fibres, minerals, carbohydrate, organic acid and phenolics. These molecules show antioxidant, antibacterial, antimutagenic, anti-cancer, anti-inflammatory, reduces heart diseases and antiviral activities (Soma Singh et al., 2014). So the beneficial approaches in order to reduce waste and make full utilization of it is to recover the bioactive constituents, especially the phenolic compounds and use this in the food, pharmaceutical as well as cosmetics industry.

Thus, utilization of the fruit wastes as sources of bioactive compounds may be of considerable economic benefit (Gui-Fang Deng et al., 2012). Extraction of such compound is needed for environmentally utilization of these wastes.Phenolic compound can be used as ingredients in various field like medicine, cosmetics and food industry. For application in food industry, it can be used to prevent oxidation of food containing high amounts of lipid.Use of synthetic antioxidant in food is not preferable due to their toxicity and carcinogenicity. Thus natural antioxidant from fruits and its waste has gain attention due to its safety (TajnubaSharmin, 2016).Natural antioxidants in fruits peels, such as vitamins and polyphenols, are considered to provide health benefits. Phenolic compounds are one of the most important categories of natural antioxidants of interest, and much evidence is derived on the antioxidant potency as well as their prevention of diseases. Yet, in recent studies, the antioxidant potency and the content of phenolic compounds were found to be high in the peel and seed of some fruits, indicating that fruit residues have the potential to

be utilized as a resource of bioactive compounds, such as natural antioxidants. Thus phenolic compound from fruit peel waste can be utilize as natural antioxidant in food industry for prevention of oxidation of food.A number of reactive oxygen species (ROS), including superoxide anion, hydroxyl and hydrogen peroxide radicals, are produced in the human body by numerous enzymatic systems through oxygen consumption. These reactive oxygen species cause cancer, cardiovascular diseases, and aging and neurodegenerative disorders. The ingestion of fruits has been connected with a distinguished health-protecting factor against diseases caused by oxidative stress (Arshad MehmoodAbbasi et al., 2015).

MATERIALS AND METHODS

A) Preparation of plant sample:-Fruit peels were collected and air dried. The air dried sample was grounded into powder using kitchen grinding machine. Thepowder was stored in air tight bottles.

B) Soxhlet Extraction:-The 20 g of fruit peel powder was extracted with 99% methanol as solvent 150 ml in soxhlet apparatus. This method was carried out at boiling temperature of 99% methanol. The extracted solution was collected and stored at 4°C. After extraction, the extracted sample was evaporated using a rotary evaporator at boiling temperature of solvent for 15 minutes.

C) Yield estimation:- Each 0.5 ml extract was measured into a pre-weighed Petri dish. The samples were kept in an oven at 50°C for 30 minutes. The weight differences was used to calculate percentage yield as well as expressed in mg/ml.

D) Total phenolic content:-Total phenolic content was measured by Folin-Ciocalteu method. A sample of different concentration (50,100,150,200,250µg/ml) was mixed with 50% methanol. Then 2 ml of Folin-Ciocalteu reagent was added and allow to withstand for few minutes. Sodium carbonate 1.6 ml was then added to the mixture and incubate for 15 minutes at room temperature in dark. The absorbance was measured at 765 nm using spectrophotometer. Different concentration of gallic acid was used for standard curve(Narkhede et al., 2012).

E) Antioxidant activity by DPPH radical scavenging assay:-Different dilution of extract (20, 40, 60,80and100 µg/ml) was prepared. DPPH solution of concentration 1 millimolar was used then 150µl of DPPH was added to 50µl of extract from each dilution. The absorbance of DPPH diluted in methanol was considered as control. Ascorbic acid was used as standard. The mixture was shaken vigorously and was left to stand in the dark for 30 minute. The absorbance of the resulting solution was measured in ELISA plate reader at 517 nm. The scavenging activity of the extract was calculated using the formula:

Scavenging activity % = (absorbance of control-absorbance of sample/absorbance of control) × 100]
(Naima Chahmi et. al, 2015).

F) Screening for Antimicrobial Activity
Culture used for antimicrobial microbial effect

1. Pseudomonas Aeruginosa, Staphylococcus Aureus And Klebsiellaspp Were Used As A Test Organism.

II. Antimicrobial Effect:- Sterile Molten Nutrient Agar At Around 40°C Was Taken And Seeded With Different Microbial Cultures And Plates Were Prepared. The Methanol Extracts Of Each Fruit Peel Were Used For The Antimicrobial Screening Using The Agar Well Diffusion Method. The Media Was Punched With Wells And Were Filled With Extracts. The Plates Were Then Incubated At 37°C For 24 Hours. After Incubation, Zone Of Growth Inhibition For Each Extract Was Measured In Millimetres.

G) phytochemicalscreening (R.Sangeeta, A. Jayaprakash, 2015)

Table 1: Phytochemical screening.

Sr.No.	Test	Procedure
1.	Flavonoid	extract 1ml(1mg/ml)+ 5% Fecl3 drop wise
2.	Phenol	extract 1ml(1mg/ml)+ 5% Fecl3 drop wise
3.	Tannin	extract 1ml(1mg/ml)+ 5% Fecl3 drop wise

4.	Amino acid	Extract(1ml)(1mg/ml)+Ninhydrin (1mg/ml) drop wise and boil for few minutes
5.	Carbohydrate	1ml extract+ Benedict`s reagent and boil for few minutes
6.	Sterols	Extract(1ml)(1mg/ml)+H2SO4(0.5ml)
7.	Terpenoid	Extract(1ml)(1mg/ml)+chloroform(0.5ml) +H2SO4(0.5ml)
8.	Saponin	Extract(0.5ml)+10 time D/w

RESULTS

Preliminary phytochemical tests:

Phytochemicals in plant may reduce the risk of cancer, possibly due to dietary fibers, polyphenolantioxidants and anti-inflammatory effects. The present study was focused to find out the photochemical analysis of different fruit peel waste.

The result suggest that fruit peel waste is rich in phytochemical such as flavonoid, phenol, tannin etc.

Table 2: Result of phytochemical screening.

Sr.No	Test	orange	Pomegranate	Banana	Lemon
1	**Flavonoid**	+++	+++	+++	+++
2	**Phenol**	+++	+++	+++	+++
3	**Tannin**	+++	+++	+++	+++
4	**Amino acid**	++	--	--	--
5	**Terpenoid**	--	--	--	--
6	**Steroid**	--	--	--	--
7	**Carbohydrate**	+++	+++	+++	+++
8	**Saponin**	+++	--	--	+++

Total phenolic content:

Phenolic compounds are class of antioxidant compounds which act as free radical terminators. The antioxidant activity of phenolic compounds has long been accepted for their ability to scavenge radicals and chain breaking actions, thereby protecting cells against the detrimental effects of reactive oxygen species. Also it has recognized that flavonoids show antioxidant activity and their effect on human nutrition and health is considerable.

Table 3: Total phenolic content.

Concentrationµg/ml	dry mass mg/ml			
	orange	pomegranate	banana	lemon
50	0.020	0.130	0.1005	0.019
100	0.038	0.231	0.0910	0.046
150	0.061	0.372	0.1277	0.054
200	0.072	0.474	0.1535	0.111
250	0.092	0.597	0.1301	0.122

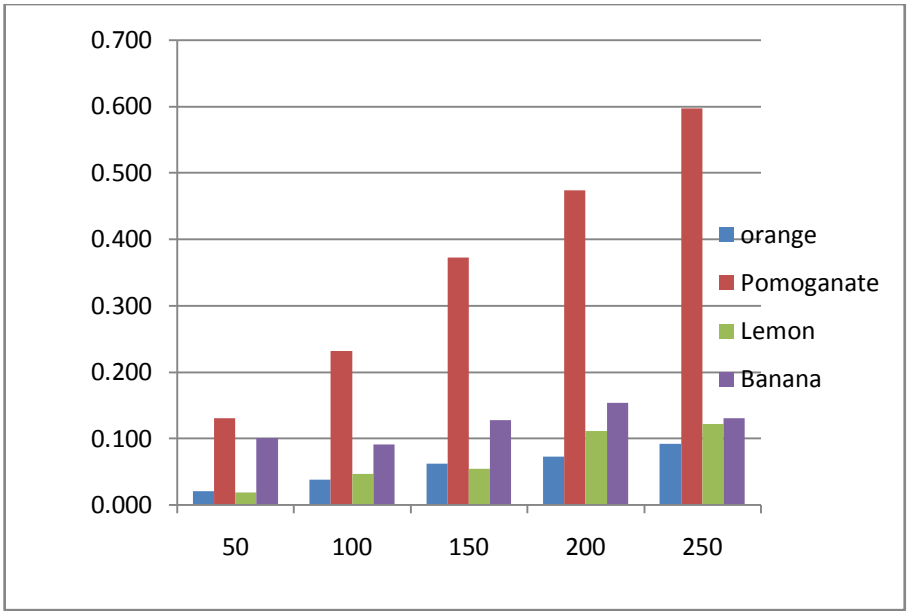


Fig 1: Total phenolic content.

As shown in figure 2, maximum Total phenolic content (0.597 dry mass/conc.250µg/ml fruit peel extract) is found in pomegranate fruit peel extract.

In-vitro antioxidant assay:
DPPH assay:

Being a stable free radical, DPPH is often used to determine radical scavenging activity of natural compounds. The reduction capability of DPPH was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants activity.

Table4: Scavenging activity (%) of the methanol extract of the fruit peel.

		Scavenging activity (%)				
Sr.no.	**Concentration (ug/ml)**	**Orange**	**Lemon**	**Banana**	**pomegranate**	**Ascorbic acid (std.)**
1.	20	16.96	38.39	41.51	70.53	71.42
2.	40	15.63	31.67	33.92	72.32	75.89
3.	60	22.32	27.67	47.32	70.98	76.33
4.	80	27.23	34.37	52.6	71.87	74.55
5.	100	33.48	41.96	67.41	68.64	75

As shown in Table 5, maximum scavenging activity (%) (72.32%.40µg/ml fruit peel extract) is found in pomegranate fruit peel extract**.**

Antibacterial Screening
Well diffusion method

The methanol extracts of each fruit peel were used for the antimicrobial screening using the agar well diffusion method(Nada Khazal Kadhim Hindi et. al, 2013).

Table 5: It shows the zone of inhibition.

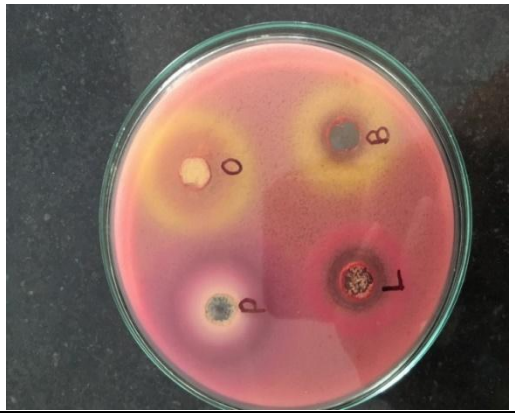
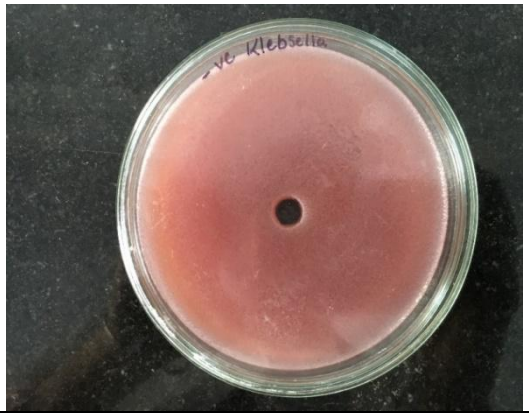
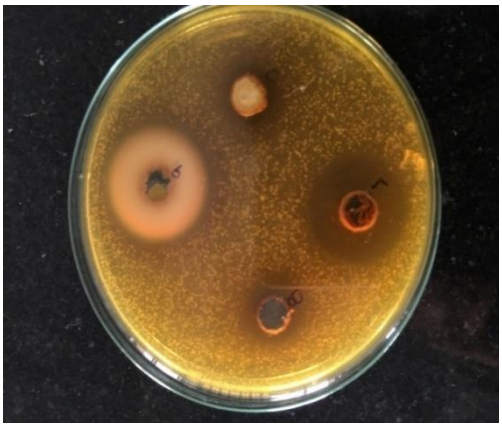
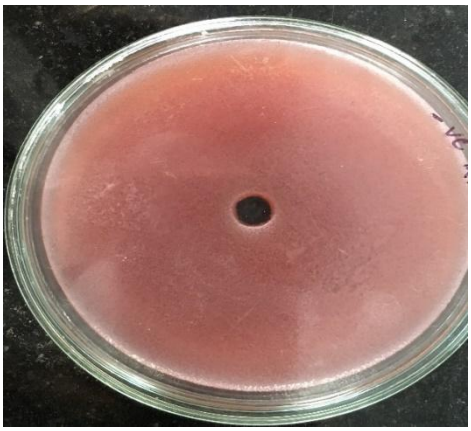
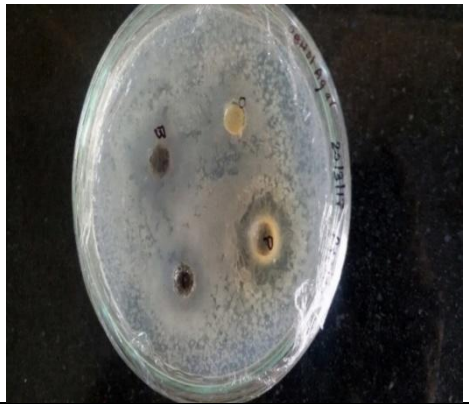
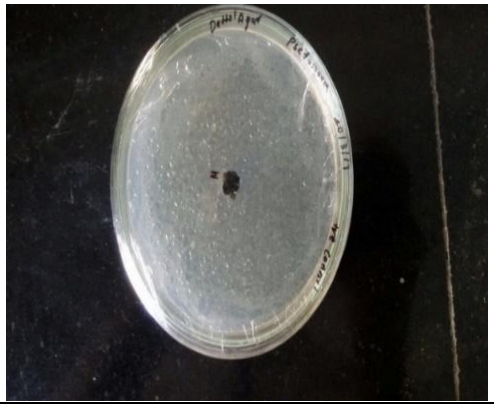
Test organism	Test	Control
Klebsiella Spp		
Staphylococcus aureus		
Pseudomonas aeruginosa		

Table 6: **Measurement of zone of inhibition (mm).**

Source	Test organism Zone of inhibition (mm)		
	Pseudomonas aeruginosa	Staphylococcus aureus	Klebsiellaspp
Orange	-	16mm	-
Pomegranate	24mm	40mm	39mm

Banana	-	15mm	-
Lemon	-	25mm	17mm

As shown in Table 6, pomegranate extract yielded larger zones of inhibition growth for Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella spp using Agar-well diffusion method.

CONCLUSION

It can be concluded that the fruit peel waste contains major phytochemical such as flavanoids, tannin and phenolic compound. comparative in-vitro biochemical assay revealed that punica granatum shows the maximum phenolic content, scavenging activity % and antibacterial activity. Optimization studies colud be undertaken for more bioactive compounds. this compounds can be used in food, pharmaceutical and cosmetic industry.

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