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ANTIOXIDANT POTENTIAL OF THE SELECTED BRIGHT COLOURED FRUIT PEELS: AN *IN VITRO* STUDY

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ABSTRACT

Fruits are considered as a heterogeneous food group with different contents of vitamins, minerals and other bioactive phytochemicals. Since not many studies have been carried out to evaluate the association between peels of the specific fruits and it is biological importance. The major goal of our work is to analyze both qualitatively and quantitatively the presence of phytochemicals and their antioxidant potential present in the peel of banana, green apple, orange and pomegranate samples. The phytochemicals present in the methanolic extract of banana, green apple, orange and pomegranate peel contains Alkaloids, Flavonoids, Tannins / Phenols, Saponins, Steroids, Terpenoids, Carbohydrates, Amino Acids and Proteins. Overall these phytochemicals possess antioxidant activity as well as Anti-molluscicidal activity, Anti-implantation activity, Anti-fungal activity, Anti-inflammatory activity, Insecticidal activity and Hypotensive.

KEYWORDS: Antioxidant, phenols, spectrophotometer, vitamins**,** phytochemicals.

1. INTRODUCTION

Many prospective studies have suggested that people with high blood antioxidant concentration have shown low risks for epithelial cancer, coronary heart diseases and strokes. This is mainly due to their large intake of fruits and vegetables in their diet. The anti-carcinogenic properties of the antioxidants and their ability to reduce the oxidation of low density lipoproteins has led to the suggestion that people with potential risks for either cardiovascular diseases or epithelial cancer must increase their intake of fruits and vegetables which in turn increases the dietary anti-oxidant concentration.[1]

Recent trends have been focusing on promoting the consumption of health enhancing photo-chemicals found in fruits. These naturally occurring compounds impart bright colour to fruits and vegetables and act as antioxidants in the body by scavenging harmful free radicals, which are implicated in most degenerative diseases. Hence suggesting that by consuming fruits of different colours we tend to benefit from the unique array of photo-chemicals as well as essential vitamins, minerals, and fiber that each colour has to offer. These fruits are considered to have an abundance of flavonoids and carotenoids which are powerful compounds that have the ability to bind the damaging free radicals in the body.[2]

According to the authors of a study published in the Journal 'Food Chemistry' in 2012, compounds such as phenolic flavonoids and ellagic acids which are anti-inflammatory and may reduce the risk of certain types of cancer and benefits cognitive function. These compounds also have antioxidant properties and are mostly found in blue, red, and purple fruits such as apples, pomegranates, black berries, plums, raisins. Another benefit of consuming fruits is their protein content since proteins are the major structural components of muscles and other body tissues. They are mainly used to produce hormones, enzymes and haemoglobin. Adequate dietary intake of proteins is essential for the growth and repair of the body's cells as well as the normal functioning of the muscles, transmission of nerve impulses and immunity. Proteins have also been proved to be a good source of energy and are mainly used when the large amounts of carbohydrates and fats consumed are insufficient by the body. Certain fruits contain proteins with large amounts of amino acids which are often referred to as high-quality proteins.[3]

Studies have suggested that the quality of proteins present in fruits can be determined by the PDCAAS

(Protein Digestibility-Corrected Amino Acid Score) which evaluates the protein quality and their ability to digest. It is seen that most animal protein (including meat, eggs and milk) have a PDCAAS close to or equal to 1.0 (maximum score) this is also the same for soy protein. However, the scores of plant proteins (fruits) are generally lower. Hence a combination of different fruits is required to maintain the adequate energy intake required to provide enough amino acids of good quality which can meet the body's physiological needs.[3]

Fruits are considered as a heterogeneous food group with different contents of vitamins, minerals and other bioactive phytochemicals. Since not many studies have been carried out to evaluate the association between specific fruits and it is unclear as to which fruits subgroup may be considered as the most protective against CVD. Also, fruits consumed in different regions differ and this was able to explain the difference observed in the studies.[4]

Another main use of fruits is seen in obesity wherein certain epidemiological research has consistently shown that most types of fruit have anti-obesity effects. Thus, due to their anti-obesity effects as well as their vitamin and mineral contents, health organizations are suggesting the consumption of fruit for weight reduction purposes even though they have high sugar content in them. These contradictory characteristics of fruit with respect to human body weight management motivated us to study previous research to understand the contribution of different types of fruit to weight management.[5]

The major goal of this work is to analyze both qualitatively as well as quantitatively the presence of proteins, antioxidants, vitamin C, phenols, flavonoids and fiber present in the peel of banana, green apple, orange and pomegranate methanolic extracts.

2. MATERIALS AND METHODOLOGY

2.1. Preparation of selected fruit peels samples

The dried fruit peels were ground into fine powder and extracted by 85% methanol for two days, twice, at room temperature. The filtrates were pooled and concentrated by rotary evaporator at 40°C. The obtained extracts were kept in a desiccator at 4°C until further use.

2.2. Phytochemical analysis

A small amount of the methanol extract was used for the phytochemical analysis. The phytochemical tests include the test for alkaloids, flavonoids, tannins and phenols, saponins, steroids, terpenoids, carbohydrates, amino acids and proteins.

2.3. Determination of moisture content

Moisture is an inevitable component of crude sample which must be eliminated as far as Possible. Drying the sample has very critical role in determining the quality as well as impurity of the sample .Moisture leads to the activation of certain enzymes and gives the suitable condition for their proliferation. About 3g of the sample was taken in the watch glass, it was kept in the hot air oven at 105 degree centigrade and dried for the period until constant weight was obtained weight loss on drying was noted and difference in weight gives the moisture content of powdered sample. Total moisture content of crude sample was expressed as % w/w.

2.4. Determination of ash content

About 3g of powdered drug was weighed and placed in the silica crucible, which was previously ignited and weighed. Powdered sample was spared uniformly in a layer at the bottom of tarred silica crucible. Crucible was kept inside the muffle furnace ad the temperature increased to make crucible dull red hot until free form carbon. Crucible was cooled, kept in desiccators and weighed same procedure was repeated to arrive at the constant weight. The percentage of the total ash obtained was calculated with reference to the air dried drug .Total ash values of powdered crude drugs were recorded and expressed as % w/w.

2.5. Estimation of Carbohydrates by Ortho-Toluidine Method

Glucose condenses with ortho-toludine in glacial acetic acid when heated to 100°C. The product formed is N-Glycosylamine which was blue green in colour, the absorbance which is measured at 630 nm.

2.6. Estimation of Protein by Lowry's Method

Protein content was determined by the method of Lowry et al., (1951). Protein reacts with Folin-Ciocalteau reagent to give a coloured complex. The colour so formed was due to the reaction of alkaline copper protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic amino acids and read at 660 nm using colorimeter.

2.7. Estimation of Total Phenols by FC Method

The colorimetric method is the most widely used method for the estimation of total phenolic content. The reagent used for this estimation is the Folin-Ciocalteu reagent, which is a mixture of phosphomolybdate and phosphotungstate. This method consists of calibrating using the standard phenolic compounds. FC reagent reacts with the nitrogen-containing compounds to form a blue coloured complex. The intensity of the color was read at 650 nm.

2.8. Estimation of Vitamin C using DNPH

The dinitrophenylhydrazine method for the determination of ascorbic acid is based upon treatment with 85% H_2SO_4 of the chromogen formed by the coupling of 2, 4 –dinitrophenylhydrazine with oxidized ascorbic acid.

2.9. Estimation of antioxidant property by FRAP assay

Ferric reducing antioxidant power (FRAP) is a widely used method to determine the antioxidant capacity of the samples. This method uses antioxidants as reductants in a redox-linked colorimetric reaction in which ferric (Fe^{3+}) is reduced to ferrous (Fe^{2+}). The reduction of ferric to ferrous at low pH leads to the formation of a coloured ferrous-probe complex from a colourless ferric-probe complex. The intensity of the colour was read at 590 nm using colorimeter.

3. RESULTS

3.1. Phytochemical analysis

The observation for qualitative phytochemical analysis on methanolic extract of peels of Pomegranate, Banana, Orange and Green apple leaves are shown in the Table – 1.

Table – 1: Qualitative analysis for phytochemicals present in methanolic extracts of peels of Banana, Green apple, Orange and pomegranate.

Test for Phytochemicals	General Observations	Banana	Green apple	Orange	Pomegranate
Alkaloids Mayer's Test Wagner's Test	Orange coloured precipitate Reddish-brown coloured precipitate	+	+	+	+
Flavonoids: 2 M HCl + Aq.NaOH	Yellow colour is observed	+	+	+	+
Tannins and Phenols: 10% Lead Acetate + $FeCl_3$	Brick red colour is observed at top layer of the test tube White colour is observed at the bottom of the test tube	+	+	+	+
Terpenoids: Salkowski's Test	Presence of Reddish Brown colour	+	-	+	+
Carbohydrate: Benedict's test	Red colour precipitate is observed	+	+	+	+
Amino Acids: Ninhydrin Test	Purple Colour	+	+	-	+
Proteins: FC Reagent	Green Colour	+	+	+	+

3.2. Estimation of the moisture content

Table – 2: Moisture content of the samples.

Samples	Time of Incineration (min.)							Moisture Content (%)
	0	10	20	30	40	50	60	
Banana (g)	3.0	2.73	2.20	1.80	1.47	1.17	0.98	**64**
Green apple (g)	3.0	2.42	1.61	0.95	0.58	0.41	0.27	**33**
Orange (g)	3.0	2.43	1.85	1.58	1.49	1.48	1.46	**51**
Pomegranate (g)	3.0	2.32	1.55	1.09	0.87	0.78	0.76	**73**

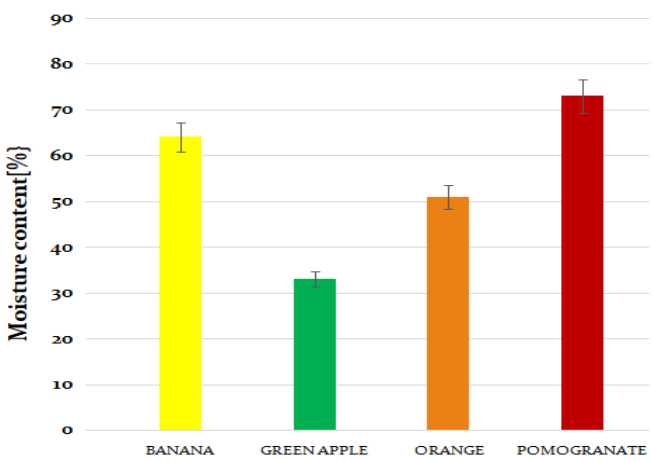

Figure 1: Moisture content of the samples.

3.3. Estimation of ash contents

Table – 3: Ash contents of the samples.

Sample	Trial	Weight (g)		Total Ash % (W/V)	Average (%)
		Original	Ash		
Banana	1.	3.0	2.80	93.3	**94**
	2.	2.0	1.89	94.5	
	3.	1.0	0.95	95.0	
Green apple	1	3.0	0.20	6.6	**7**
	2	2.0	0.14	7.0	
	3	1.0	0.07	7.0	
Orange	1.	3.0	1.70	56.7	**60**
	2.	2.0	1.26	63.0	
	3.	1.0	0.61	61.0	
Pomegranate	1.	3.0	2.65	88.3	**86**
	2.	2.0	1.69	84.5	
	3.	1.0	0.85	85.0	

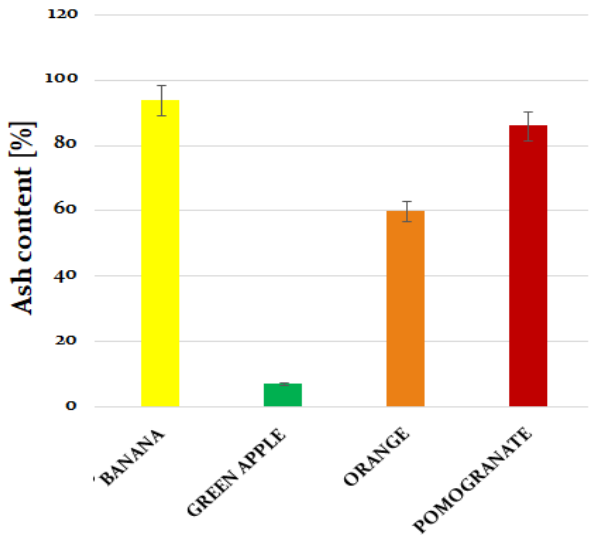

Figure – 2: Ash contents of the samples.

3.4. Estimation of Carbohydrates by Ortho-Toluidine Method

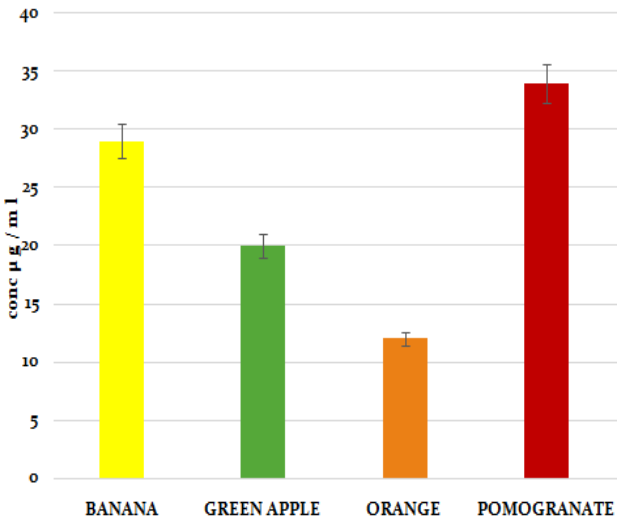

Figure – 3: Carbohydrates concentration of the samples.

The total amount of carbohydrates present in the methanolic extracts of Banana, green apple, orange and pomegranate peels are found to be 29, 20, 12 and 34µg/ml respectively.

3.5. Estimation of Protein by Lowry's Method:

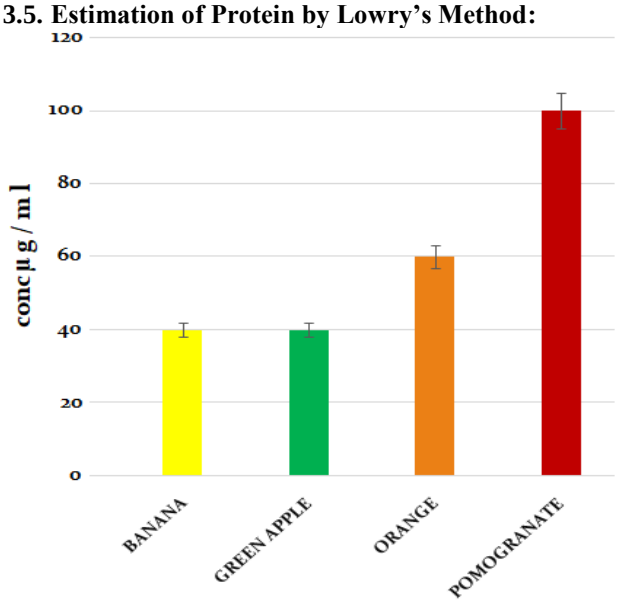

Figure – 4: Protein concentration of the samples.

The total amount of proteins present in the methanolic extracts of Banana, green apple, orange and pomegranate peels are found to be 40, 40, 60 and 100µg/ml respectively.

3.6. Estimation of total phenols by F C Method

The total amount of Phenols present in the methanolic extracts of Banana, green apple, orange and pomegranate peels are found to be 13, 13, 24 and 136µg/ml respectively.

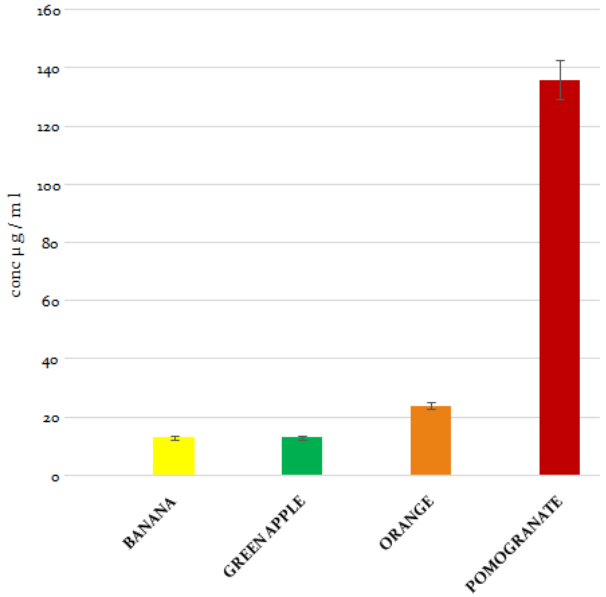

Figure – 5: Total Phenol concentration of the samples.

3.7. Estimation of ascorbic acid by DNPH

The total amount of ascorbic acid present in the methanolic extracts of Banana, green apple, orange and pomegranate peels are found to be 80, 20, 15 and 100µg/ml respectively.

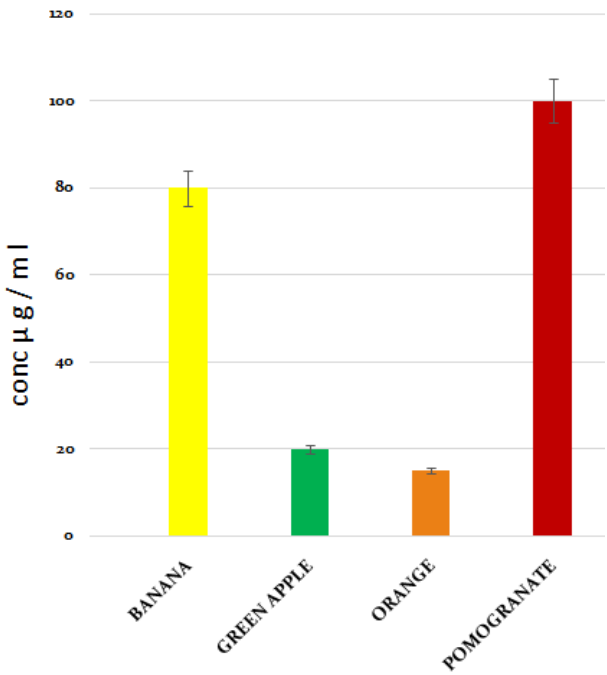

Figure – 6: Ascorbic acid concentration of the samples.

3.8. Estimation of antioxidant property by FRAP assay

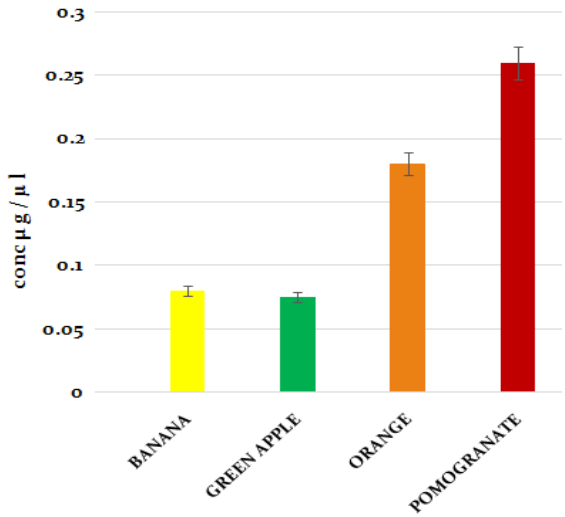

Figure – 7: Reducing Potential of the samples

The total amount of antioxidants present in the methanolic extract of Banana, green apple, orange and pomegranate peels are found to be 0.080, 0.075, 0.180 and 0.260µg/ml respectively.

4. DISCUSSION

In our study, it was found that the difference in colour among the selected fruit samples does in fact play a major role in their phytonutrient content as well as antioxidant ability. Our observation is in consistence with earlier foundlings confirming that a regular diet of fruit in our day to day life does in fact increase the body's antioxidant concentration as well as enhance our health.

4.1. Phytochemical analysis in the methanolic extracts of fruits peel

From the Table – 1, phytochemicals such as Alkaloids, Flavonoids, Tannins and Phenols, Terpenoids, Carbohydrates, Amino Acids and proteins are present widely in all the peels of fruit sample. Except amino acid in orange all the constituents are common in all samples. Phytonutrients that are found in the skin of fruits give flavour, colour, and scent. The pulp and seeds carry these nutrients. Fruits such as orange, banana, pomegranate and green apples are examples that contain these nutrients. Recent researches have shown that Phytonutrients are in fact surprisingly good for our body. They tend to strengthen the immune system and may in fact act as a preventive measure against certain diseases.[39] Phytonutrients are also very powerful antitoxins. These substances provide neutralize free radicals. The liver releases free radicals when metabolism occurs and can be detrimental to the body. When they build up, they can invite sickness and give rise to premature aging. The fruit peels were proved to have therapeutic activity and antimicrobial activity.[6]

4.2. Moisture content of the peel extracts

From the Table – 2 and Figure – 1, the moisture content was increased in pomegranate (73%) followed by banana (64%), orange (51%) and green apple (33%). Moisture content is the quantity of water contained in a material. The moisture content influences the physical properties of a substance such as the weight, density, viscosity, refractive index, and other physio-chemical properties of fruits. The technique of water loss on drying is used to determine the moisture content of the fruits. Various studies proved that variations in moisture contents in fruits occur mainly during ripening and storage periods. Increase in moisture content reduces the proximate principles such as fat, protein and carbohydrate thereby decreasing the energy value. During storage ripening of the pomegranate fruits a decrease in moisture content was observed. This decrease was due to the evaporation of moisture from the surface of the fruit during the storage ripening period.[7]

4.3. Ash content of the peel extracts

From the Table – 3 and Figure – 2, the ash content was increased in banana (94%) followed by pomegranate (85%), orange (60%) and green apple (7%). Ash is an inorganic material which results after removing the water and organic matter from the sample by heating in the presence of oxidising agent. This process provides the total amount of the minerals such as sodium, potassium and iron present within in the samples taken. Hence, we analysed the difference in the mineral content among the selected fruits. The ash determination will be used to assess the quality of the fruit peels. Although such minerals constitute only a small portion of the body tissue but they are the structural components for carrying out many important processes in the body. High ash content signifies the presence of adulterants.[8]

4.4. Carbohydrates concentration of the peel extracts

From the Figure – 3, the extracts of banana, green apple, orange and pomegranate contained a considerable amount of glucose content. The difference in the carbohydrates content is observed to be increased in pomegranate (34µg), banana (29µg), green apple (20µg) and orange (12µg) for per ml of the sample taken. The presence of carbohydrates in many fruits may differ between individual species depending on their ripeness and source. Since the fruits lowest in sugar usually have some of the highest nutritional values, including antioxidants and other phytonutrients.[9]

4.5. Protein concentration of the peel extracts

From the Figure – 4, the extracts of banana, green apple, orange and pomegranate contained a considerable amount of protein content. The difference in protein content is observed to be increased in all samples, pomegranate (102µg), orange (54µg), banana (40µg) and green apple (36µg). The concentration of protein present in the fruit peels determines the quality of fruit. Various studies proved that as the quality of the protein present in the peel increases, the nutritional quality also increases. Proteins are the major sources of nitrogen and sulphur. They are essential dietary constituents.[10]

4.6. Total phenol concentration of the peel extracts

From the Figure – 5, the extracts of banana, green apple, orange and pomegranate contained a considerable amount of phenol content. The difference in total phenolic content is observed to be increased in pomegranate (136µg), orange (24µg), banana (13µg), and green apple (13µg) for per ml of the sample taken. Phenols are protective chemical compounds that offer resistance to illness raging from cancer to arthritis. Polyphenolic compounds from plant-based foods are responsible for the color, flavor, taste, and metabolic activity to humans. The concentration of polyphenols is influenced by their variety and other environmental factors.[11] The wide ranges of polyphenol content present in the fruits peel are Hydroxycinnamic acids, Flavan-3-ols/procyanidins, Flavonols, Dihydrochalcones and Anthocyanins.[12] The antioxidant mechanism of phenolic compounds is based on hydrogen donation abilities and chelation tendencies of antioxidants. After donating a hydrogen atom phenolic compounds become resonance stabilized radical, which does not easily participate in other radical reactions. However, phenolic compounds act as pre-oxidants under certain conditions, such as high concentration of phenolic compounds or metal ion, and high pH, chemical structure also affects the antioxidant activities.[13]

4.7. Ascorbic acid concentration of the peel extracts

From the Figure – 6, the extracts of banana, green apple, orange and pomegranate contained a considerable amount of ascorbic acids content. It is observed to be increased in pomegranate (100µg), banana (75µg), green apple (50µg) and orange (37.5µg) for per ml of the sample taken. The high values of ascorbic acid in the fruits peel sample signifies the potential use of the fruit as a good antioxidant and can be used for lowering the risk of heart disease, blood pressure, and carcinogens. It can be also used for collagen synthesis.[14]

4.8. Reducing potential of the peel extracts

From the Figure – 7, the extracts of banana, green apple, orange and pomegranate contained a considerable amount of reducing potential. It is observed to be increased in pomegranate (0. 26µg) followed by orange (0.18 µg) banana (0.08µg) and green apple (0.075µg) for per ml of the sample taken. The differences in the reducing potential among the fruits peel could be due to their phenolic contents and other non-phenolic antioxidants present in the samples.[15]

5. CONCLUSION

The phytochemicals present in the methanolic extract of banana, green apple, orange and pomegranate peel contains Alkaloids, Flavonoids, Tannins / Phenols, Saponins, Steroids, Terpenoids, Carbohydrates, Amino Acids and Proteins. Overall these phytochemicals possess Anti-molluscicidal activity, Anti-implantation

activity, Anti-fungal activity, Anti-inflammatory activity, Insecticidal activity and Hypotensive.

The moisture content was found increased in pomegranate (73%) followed by banana (64%), orange (51%) and green apple (33%) shows the pomegranate has the high influence in physiochemical property compared to other peel extracts.
Ash is an inorganic material which results after removing the water and organic matter from the sample by heating in the presence of oxidising agent and was found increased in banana (94%) followed by pomegranate (85%), orange (60%) and green apple (7%).

The carbohydrates content is observed to be increased in pomegranate (34µg), banana (29µg), green apple (20µg) and orange (12µg) for per ml of the sample taken. In the protein content pomegranate (102µg) was found to have increased followed by orange (54µg), banana (40µg) and green apple (36µg). These differences may be depending on their ripeness and source.

The total phenols, ascorbic acid and antioxidant activity was observed to be increased in pomegranate when compared to other peel extracts and hence pomegranate peel extracts can act effectively as antioxidants.

5.1. Suggestion for further research:
The present methanobotanical study on banana, green apple, orange and pomegranate peel extracts proved the existence of various biologically active molecules. Moreover, these peel extracts are already in traditional medicinal practice for a wide range of treatments such as skin rashes etc. The present study may be an initiative for further phytochemical and pharmacological investigations required to identify as well to separate the novel active compounds from the peel to formulate new drug to treat incurable diseases.

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