



PHYTOCHEMICAL ANALYSIS OF AQUEOUS AND METHANOLIC EXTRACT OF APPLE (*MALUSPUMILA*) PEEL

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ABSTRACT

Objective of the study: The main objective of the study is to evaluate the presence of phytochemicals in the extracts obtained by apple peel. The extracts were made by using apple peel in two different phases (fresh & dried). Two solvents, water and methanol were used to prepare two different extracts. To perform these tests standard methods were used. **Result:** The presence of alkaloids, carbohydrate, proteins, flavonoids, phenol, and glycoside component were revealed in the qualitative analysis of aqueous and methanolic extract of apple peel. Quantitative analysis was performed by Reducing power assay and found that reducing sugar is present in each extract. By performing TPC and TFC assays, it was found that in methanolic extract of dried apple peel and aqueous extract of fresh apple peel, flavonoids and Phenolic compound were found in high amount. **Conclusions:** Due to the presence of many important components, apple is able to make its place in every human being's diet. By these experiments it is also proved that in apple peel many phytochemical components are present. Many apple-based industries treat apple peel as waste product, which can be utilized as raw material to produce important products for the betterment of human and environment. The study suggests that peel extract of apple could be useful for various therapeutic and pharmacological activities.

KEYWORD: Aqueous, Methanolic Extract.

INTRODUCTION

“An Apple a day keeps doctor away” this is a famous line which is generally recommended by people. Apple belongs to the Kingdom: - Plantae, family: - Rosaceae, order: - Rosales, Genus: - Malus and species: - *M. pumila* and its binomial name is *Malus pumila* and found all over the world. In India apple is mostly cultivated in Jammu & Kashmir and Himachal Pradesh. With this experiment we tried to explain the fact that apple peel having many factors which can be utilized for the betterment of human being. Therefore, the objective of this study was to evaluate the nutritional value of apple peels. Apple peels are waste of apple sauce and canned apple products. Which is treated as waste product of these manufacturing companies. Although it is biodegradable but may cause many kinds of environmental pollution and is able to cause health issues because it is an excellent example of good media for growth of microorganisms like fungus because of its high carbohydrate and many other nutritional contents. There are many varieties of apple and every apple variety has different concentrations of phytochemical components. In this study a common variety of apple was utilized, collected from the local juice shop. An average 150 gms apple fruit with its peel has carbohydrates, proteins, vitamins, minerals and gives total 52 kcal energy after consumption. Apple peel

is a good source of fiber, iron, vitamins like A, C, phenols, Flavonoids and antioxidants which help us in maintaining good immune system, skin health, eyesight, anaemia, improves digestion and many more.

MATERIAL AND METHOD

Collection of material

Fresh apple peels were obtained by peeling fresh apples and some were collected from local juice shop where they use apple pulp for making fruit custard and peels were no more useful for them. The apple peels were washed twice and thrice well by using distilled water, extra apple pulp was removed by the help of knife before washing. Some apple peel were dried in shade for a period of one week; the dried samples were grinded properly by electric grinder. Remaining apple peels were used in fresh condition for aqueous extract preparation.

EXTRACTION METHOD

Methanol extract of dried apple peel

By Maceration

To obtain extract by this method dried ground apple peels were mixed with methanol and leave this mixture for 5 to 8 days at room temperature in a close container with occasional shaking and stirring. After 7th or 8th day the mixture was filtered with the help of filter paper and the

last residue of extract was collected as thick paste by allowing it to evaporate excess solvent by keeping it in the water bath at 60°C. Extract was transferred to glass vials and kept at low temperature in refrigerator.

Aqueous Extraction of fresh apple peel By Boiling Method

To obtain this type of extract washed fresh apple peel has used. The 20 gm. of apple peel were soaked in 200 ml of distilled water and boil it for 30-40 minute. The extract was then filtered using firstly by muslin cloth then whatman filter paper no. 1. Then excesses water was evaporated to obtain thick paste by using water bath at 60°C. Extract was transferred to glass vials and kept at low temperature.

PHYTOCHEMICAL SCREENING

Qualitative analysis of peel extract

1. Test for carbohydrates

a. **Molish's test**:- 2ml of aqueous solution of each extract was treated with 2 drop of alcoholic α -naphthol solution in a test tube and then carefully along the side of test tube 1 ml of concentrated sulphuric acid was added. Formation of violet ring at the junction indicates the presence of carbohydrates.

b. **Fehling's test**:- 1 ml of Fehling's A and 1 ml of Fehling's B solution were added to the 1ml of aqueous extract in test tube and heated in the water bath for 10 minutes. The presence of reducing sugar indicates by the formation of red precipitate.

c. **Benedict's test**:- Extract and Benedict's reagent were mixed in a test tube in equal amount and heated in water bath for 2 minutes. Red color due to formation of cupric oxide indicates the presence of monosaccharide.

2. Test for Protein and Amino Acids

a. **Ninhydrin Test** :- 3 ml of extract was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue color indicates the presence of amino acids.

3. Test of Glycosides

a. **Legal's Test**:- 1 ml of extract was dissolved in pyridine. 1ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

b. **Bornrager's test**: To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shaken it well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.

c. **Keller-killiani test**:- 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added to 2 ml of extract solution in a test tube then 0.5 ml of concentrated sulphuric acid was added carefully by the side of the test tube. Formation of blue colour in the acetic acid by the presence of cardiac glycosides.

4. Test of alkaloids

a. **Dragendroff's test**:- To 1-2 ml of filtrate, few drops of Dragendroff's reagent were added in a test tube. Formation of red precipitate indicates the presence of alkaloids

b. **Mayer's**:- To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.

c. **Hager's test**:- To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow colour precipitate indicates the presence of alkaloids.

5. Test of Saponins

a. **Froth test**: The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

6. Test of Flavonoids

a. **Lead acetate test**: The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

b. **Shinoda test**: To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicates presence of flavonoids.

c. **Alkaline Reagent test**:- The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.

7. Test of Tannin And Phenolic compound

a. **Lead Aetate test**: Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds.

b. **Gelatin test**: Some quantity of extract dissolved in distilled water. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. Development of white precipitate indicates presence of phenolic compounds.

c. **Ferric Chloride Test**: Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

8. Test of Fats and oil:-Solubility Test:- By adding few ml of chloroform in to 2-3 ml of alcoholic solution of extract, solubility was observed.

By adding few ml of 90 % ethanol in to 2-3 ml of alcoholic solution of extract, solubility was observed.

Quantitative phytochemical analysis of peel extracts

Methanolic Extract obtained from dried apple peel and fresh aqueous extract obtained from fresh apple peel were subjected to standard procedure to determine and quantify the presence of flavonoids, phenolic compounds and reducing sugar by following methods:

Determination of total flavonoids: Different concentrations of extract were prepared (10 to 100 µg/ml) in methanol. 0.5-ml of diluted sample solution mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% NaNO₂ solution was added. After 6 min, 0.15 ml of a 10% AlCl₃ solution was added and allowed to stand for 6 min, then 2 ml of 4% NaOH solution was added to the mixture. Water was immediately added to bring the final volume to 5 ml, and then mixture was mixed thoroughly and allowed to stand for another 15 min. After this the absorbance of the mixture is taken with the help of UV- visible spectrophotometer at 510 nm and then total flavonoid content was calculated and found out line of regression by plotting graph standard curve for different concentration of Quercetin. All determination was carried out in triplicates.

Determination of total phenolic compounds

Different concentration of extracts were prepared in methanol. Then 2 ml of Folin-ciocalteu reagent was added to the 0.5ml of prepared concentrations of extracts in methanol followed by 4 ml of sodium carbonate solution. and mixer was incubated at room temperature for 30 min. Then absorbance was taken at 765 nm by using methanol as blank. The line of regression was found in respect of standard curve different concentration of Gallic acid.

Reducing power assay

2ml of various concentrations of extract were mixed with 2 ml of phosphate buffer and 2 ml of potassium ferricyanide then mixture was incubated at 50°C for 20 min. followed by addition of 2 ml of trichloroacetic acid. A volume of 2 ml of each of the mixture was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm.

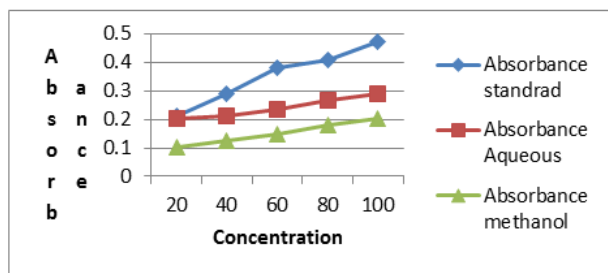
RESULTS**Table: 1.**

S.No.	Phytochemical	Test Performed	Aqueous extract of fresh Apple peel	Methanol Extract of Dried Apple Peel
1.	carbohydrates	Molish's test	-ve	-ve
		Fehling's test	+ve	+ve
		Benedict's test	+ve	+ve
2.	Protein and Amino Acids	Ninhydrin Test	+ve	-ve
3.	Glycosides	Legal's Test	+ve	+ve
		Bornrager's test	+ve	+ve
		Keller-killiani test	-ve	-ve
4.	alkaloids	Dragebdroff's test	-ve	-ve
		Mayer's	-ve	-ve
		Hager's test	+ve	+ve
5.	Flavonoids	Lead acetate test	+ve	+ve
		Shinoda test	+ve	-ve
		Alkline Reagent test	+ve	+ve
6.	Tannin And Phenolic compound	Lead acetate test	+ve	+ve
		Gelatin test	+ve	+ve
		Ferric Chloride Test perform	-ve	-ve
7.	Saponins	Froth test	-ve	+ve
8.	Fats and oil (Solubility Test)	Chloroform	Not Soluble	Soluble
		Ethanol	Little Soluble	Not Soluble

Showing result of quantitative analysis of peel extracts:

Table: 2.

S.No.	Test	Aqueous extract	Methanol Extract
1	Total flavonoid mg/gm equivalent of Rutin	301 mg/g	173 mg/g
2	Total Phenolic compounds mg/gm equivalent of Gallic acid	105 mg/g	158 mg/g



Figuar: 1.

RESULT AND CONCLUSION

Experiment conducted on both extract made by fresh and dried apple peel have shown the presence of carbohydrate, proteins, flavonoids, phenol, glycoside and alkaloids components as show in table no.1.

Table no.2 shows that these two extract are rich in flavonoid and Phenolic compounds. Standard curve of ascorbic acid is shown in Fig.1. shows that the reducing power of aqueous and methanol extracts increased with increase in concentration. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Most of the phytochemicals were found in apple peel extract which means apple peel having many important components more than apple pulp. By these experimental test, it is proved that apple peel which is treated as waste product of apple based food industries like apple sauces, jam, jelly etc. can be used as a raw material for making herbal drug, to utilise its potential of having important phytochemical, for welfare of human being by enriching diet, reducing pollution and to prevent the spreading of disease causing microbes by recycling the fruit waste. The overall experiment represents that peel extracts were rich in phytochemical activity.

BIBLIOGRAPHY

1. Arora Mamta, KaurParminder. Phytochemical Screening of Orange Peel and Pulp. International Journal of Research in Engineering and Technology, 2013; 2. eISSN:2319-1163.pISSN:2321-7308.
2. Baskar Ramakrishnan, Shrisakthi Selvaraj, Sathyapriya Babu, Shyampriya Radhakrishnan, Nithya Radhakrishnan, Poongodi Palanisamy. Antioxidant Potential of Peel Extracts of Banana Varieties (*Musa sapientum*). Food and Nutrition Sciences, 2011; 1128-1133.
3. Bhandary Kumar Satheesh, N. Kumari Suchetha, Bhat S. Vadisha, P.K. Sharmila, Bekal Prasad Mahesh. Preliminary Phytochemical Screening of Various Extracts of Punical Granatum Peel, Whole Fruit and Seeds. Nitte University Journal of Health science, 2. ISSN: 2249-7110.
4. Britto De John A., Gracelin Sheeba Herin D., Kumar Rathna Jeya Benjamin P. Qualitative and Quantitative Analysis of Phytochemicals In Marsilea Minutalinn. International Journal of Pharma and Bio Sciences, 2013. ISSN:0975-6299.
5. Farag S. Radwan, Abdel-Latif S. Mohamed, Sekina, Emam S. and Layla, Tawfeek S. Phytochemical screening and polyphenol constituents of pomegranate peels and leave juices. Landmark Research Journal, 2014; 1(6): 086-093.
6. Ganapaty Seru, Ramajah Maddi, Yasaswini Kanuri, Kumar Vijay, Nuthakki, Dibbanti, Harikrishnareddy. Quantitative Phytochemical Estimation and Evaluation of Hepatoprotective Activity of Methanolic Extract of *Dendrobiumovatum*(L.) Kraenzl. Whole Plant against CCl₄ induced Hepatotoxicity. Journal of Pharmacognosy and Phytochemistry, 2013; 2(3): 113-118. ISSN:2278-4136.
7. G. Ehiowemwenguan, A. Emoghene, O.A. and E.J. Inetianbor. 2014. Antibacterial and phytochemical analysis of Banana fruit peel. IOSR Journal of Pharmacy(e)-ISSN:2230-3013, (p)-ISSN:2319-4219
8. Gracelin Sheeba Herin D., Britto De John A. and Kumar Rathna Jeya Benjamin.2012.Qualitativeand Quantitative Analysis of Phytochemical in Five Pteris Species. International Journal of Pharmacy and Pharmaceutical Science, 2013; 1. ISSN:0975-1491.
9. Justin Showmya Jerlin, Milton Archana, Natesan Geetha. Phytochemical Evaluation of Peel of Citrus Reticulata Blanco Using Various solvent Extracts.International Journal of Pharmaceutical Sciences and Business Management, 2014; 2. ISSN:2310-6913.
10. Kalpana Devi Rajesh, Subramani Vasantha Rajesh, Annamalai Panneerselvam. Qualitative and Quantitative Phytochemical Analysis inFourPteridophytes.International Journal of Pharmaceutical Sciences Review and Research. Article, 2014; 72: 408-412. ISSN 0976-044X.
11. Khaleel Ismael Ayad, Sijam Kamaruzaman, Rashid Sulaiman Tayga, Ahmad Bin Khairulmazmi. Phytochemical Determination and Antibacterial Activity of *Punicagranatum* Peel extracts against Plant Pathogenic Bacteria. American Journal of Plant Science, 2016; 159-166.
12. Kumar Ashok K., Narayani M., Subanthini A., Jayakumar M., Antimicrobial Activity and Phytochemical Analysis of Citrus Fruit peels-Utilization of Fruit Waste. International Journal of Engineering Science and Technology, 2014; 1. ISSN: 0975-5462.
13. KumariSuman R., Venkateshwar C., Samuel G., Samuel, Rao Gangadhar S., Phytochemical Screening of some compounds from plant leaf extracts of *Holopteleaintegrifolia*(Planch.) and *Celestrusemarginata* (Grah.) used by Gondu tribes at Adilabad District, Andhrapradesh, India. International Journal of Engineering Science Invention, 2013. ISSN:2319-6734.
14. K.Velu, D. Elumalatha, M.Babu, A.Janaki, P. K. Kaleena. 2014. Phytochemical screening and larvicidal activity of peel extracts of *Arachishypogaea* against chikungunya and malarial

- vectors. International Journal of Mosquito Research. ISSN:2348-5906.
15. Mathew B Blessy, Jatawa K Suresh, Tiwari Archana. Phytochemical Analysis of Citrus Limonum Pulp and Peel. International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 4(2). ISSN-0975-1491.
 16. Nandani Parvathi M., Vangalapati Meena. Phytochemical Analysis and Effect of Various Parameters on the Extraction Yield of Flavonoids from the Citrus Medica L Peel Extract. International Advanced Research Journal in Science, Engineering and Technology, 2015; 2. ISSN 2393-8021.
 17. Praveeba Jasmine and Estherlydia. 2014. Comparative Study of Phytochemical Screening and Antioxidant Capacities of Vinegar Made from Peel and fruit of Pineapple (*Ananas comosus* L.). International Journal of Pharma and Bio Sciences. ISSN:0975-6299.
 18. Pranoothi Kamala E., Narendra, D.S.D, Joshi Suman, Swathi J., Sowjanya M.K., N.V.K, Rathnakarreddi, J.S. Emmanuel S, Fr. Rev, Ch. Padmavathi, Satya Krishna A. Studies on qualitative, quantitative, phytochemical analysis and screening of in vitro biology activities of *Leucasindica*(L) VAR. *Nagalapuramiana* International Journal of Herbal Medicine, 2014; 2(3): 30-36. ISSN:2321-2187.
 19. Rather Ahmad Riyaz, C. Swetha, and K. Rajagopal. Screening of Peel Extract as Antioxidants, Anticancer Agents and Antimicrobials. *Advances in Bioresearch*, 2010; 1: 29-33. ISSN: 0976-4585.
 20. R. Sangeetha and A. Jayaprakash. Phytochemical Screening of *Punicagranatum* Linn. Peel Extracts. *Journal of Academia and Industrial Research (JAIR)*, 2015; 4. ISSN:2278-5213.
 21. Santhi K., Sengottuvel R. Qualitative and Quantitative Phytochemical analysis of *Moringa concanensis* Nimmo. *International Journal of current microbiology and applied science*, 2016; 5: 1. ISSN:2319-7706.
 22. Sharma Ashok, Sharma K. Ashish, Chand Tara, Khardiya Manoj, Agarwal Suresh. Preliminary Phytochemical Screening of Fruit Peel Extract of Fruit Peel Extracts of *Annona squamosa* Linn. *Journal of current pharma research*, 2013; 4(1): 1038-1043.
 23. Sharma Pradeep Kumar, Ahirwar Pushpak, Mangalshilpi, Kirar Anup, Vishwakarma Shraddha. Phytochemical analysis of Citrus Limonum and citrus Sinensis peels and identification of Beta Carotenoid Pigment Using ethanolic Extract. *International Research Journal Pharmacy*, 2014; ISSN:2230-8407.
 24. Sihombing R. Jenny, Dharma Abdi, Chaidir Zulkarnain, Almahdy, Fachrial Edy, and Munaf Edison. Phytochemical screening and antioxidant activities of 31 fruit peel extract from Sumatera, Indonesia. *Journal of Chemical and Pharmaceutical Research*, 2015. ISSN:0975-7384.
 25. Suja, Bupesh, Nivya Rajendiran, Mohan, Ramasamy, Muthiah, Arul Amutha Elizabeth, Meenakumari and Prabu. Phytochemical Screening, Antioxidant, Antibacterial Activities of Citrus Limon and Citrus Sinensis Peel Extracts. *International Journal of Pharmacognosy and Chinese Medicine*, 2017; 1(2).
 26. V. Sathya, R. Bharathidasan, S. Selvi Tamil, N. Rebecal Sophia, Rilakkiya and M. Prabakaran. Quantitative, qualitative phytochemical analysis and in vitro antibacterial activity of *Bauhinia tomentosa* L. *Scholars Research Library*, 2013; 3(2): 31-36. ISSN:2231-3184.
 27. Velumani S. Phytochemical Screening and Antioxidant Activity of Banana Peel. *IJARIE*, 2016. ISSN(O)-2395-4396.
 28. Yusuf, Z. A., Akir, A, Shemau, Z, Abdullahi, M and Halima, A.S., Phytochemical Analysis of the methanol leaves extracts of *Paullinipinnatalinn*. *Journal of Pharmacognosy and Phytotherapy*, 2014; 6(2): 10-16. ISSA: 2141-25024.
 29. Zawawy EI Ahmed Nessma. Antioxidant, Antitumor, Antimicrobial Studies and Quantitative Phytochemical Estimation of Ethanolic Extracts of Selected Fruit Peels. *International Journal of current Microbiology and Applied Sciences*. ISSN: 2319-7706, 2015; 4(5): 298-309.