Qualitative and Quantitative Analysis of Phytocompounds Present in the Different Varieties of Apple Peels

Project proposal submitted to Midnapore City College for the Partial Fulfilment of the Degree of Master of Science (Nutrition & Dietetics)

Submitted By

Sanjana Mukhopadhyay, Sikta Banerjee, Swikriti Mukherjee, Tanushree Ganguly, Swagata Ghorai

Guided By

Dr. Suchismita Roy

Assistant Professor of Nutrition

Department of Paramedical and Allied Health Sciences

MIDNAPORE CITY COLLEGE

Kuturiya, P.O. Bhadutola,

Paschim Medinipur, Pin – 721129

West Bengal, India

2023

Declaration

We do hereby declare that the present Master project entitled 'Qualitative and Quantitative analysis of Phytocompounds present in the different varieties of apple peels' embodies the original research work carried out by us in the Department of Biological Sciences, Midnapore City College, Paschim Medinipur, West Bengal, India under the supervision of Dr. Suchismita Roy, Designation, Department, College/ Institution name and address. No part thereof has been submitted for any degree or diploma in any University.

Swikriti Mukherjee

Sanjana Mukherjee

Swagata Ghorai

Sikta Banerjee

Tanushree Ganguly

Date:

Place: Midnapore City College,

Paschim Medinipur

Approval Sheet

This project report entitled 'Qualitative and Quantitative analysis of Phytocompounds present in the different varieties of apple peels' by Sanjana Mukhopadhyay, Sikta Banerjee, Swikriti Mukherjee, Tanushree Ganguly, Swagata Ghorai is approved for the degree of M.Sc. in Nutrition and Dietetics.

(Signature of Examiners)

(Name: ……..)

(Signature of Guide)

(Name: Dr. Suchismita Roy)

(Signature of Teacher-in-Charge)

(Name: Dr. Kuntal Ghosh)

(Signature of Director)

(Name: Dr. Pradip Ghosh)

Date:

Place:

Acknowledgment

We would first like to acknowledge Dr. Pradip Ghosh, Honorable Founder, and Director, Midnapore City College, Paschim Medinipur for providing us the opportunity to study and complete our project work in this college. We are gratefully indebted to him for his very valuable comments on this project work.

We like to thank Dr Kuntal Ghosh Principal, Teacher in Charge, Midnapore City College, Paschim Medinipur, for his guidance and providing opportunity for completion of our project work.

We would like to thank our project adviser Dr Suchismita Roy, Assistant Professor of Nutrition, department of biological Sciences, Midnapore City College. The door to Dr Roy's office was always open whenever we ran into a trouble spot or had a question about our project or writing. She consistently allowed this paper to be our own work, but steered us in the right the direction whenever she thought we needed it.

Dr Kuntal Ghosh, Teacher in Charge, Dr Shrabani Pradhan, Assistant Professor of Nutrition and Miss Titli Panchali, Technical Assistant of Nutrition, Midnapore City College, for their support and co-operation to carry out this research project. Without their passionate participation and input, this project work could not have been successfully conducted.

Finally, we must express our very profound gratitude to our parents and friends for providing us with unfailing support and continuous encouragement throughout our years of study and through the process of researching and writing this project work. This accomplishment would not have been possible without them. Thank you.

Swikriti Mukherjee

Sanjana Mukherjee

Swagata Ghorai

Sikta Banerjee

Tanushree Ganguly

Abstract

Fruits have an antioxidant and phenolic compound which have potential for reducing different types of diseases like cancer, heart-related diseases, asthma, and Alzheimer's. Apples are crisp, white-fleshed delicious fruits, which comes in all sizes and varieties of colour such as red, yellow, pink or green. The variation in the colour indicate the presence of several phytocompounds present in the apple skin. Apples contains essentials nutrients like vitamins, and minerals, including fiber, and are most commonly eaten fresh , though they are used for cooking and baking purposes as well. The aims of this project is to focus to measure changes in the amount of phenol & antioxidant compound of the varieties of different Apple by qualitative & quantitative analysis in different types of apples. To find out the qualitative and quantitative analysis of phytocompounds present in the different apple peel food extract were prepared and divided the apple peel sample into six groups: green apple peel (chloroform), red apple peel (chloroform), light red apple peel (chloroform), green apple peel (methanol), red apple peel (methanol), light red apple peel (methanol).We had done several test such as preliminary phytochemical screening, TLC for antioxidant activity and flavonoid constituents, DPPH, analysis of total phenolic compound by HPLC. Result showed that different apple peels contains different phytocompounds such as tannins, phenol, saponin, steroids, terpenoids etc. and methanol fraction of different apple peel samples showed better result compared to the chloroform fraction.

Keywords: Antioxidant, Flavonoid, DPPH, Phenol, Phytochemicals

CONTENTS

Introduction

1. INTRODUCTION

Apple is a very well-known edible fruit which is consumed throughout the world. Apples grown from *Malus sieversii* are descendants of Central Asia, with most of the genome of *Malus sylvestris*. Apples are usually red when ripe and ready to eat, but also can be green or yellow skin, fruit skin is a bit soft, and the flesh was hard. The first began to grow apples in Central Asia. Apple today is grown in many regions of the world where the air and temperature are cooled. Most apples are eaten raw or cooked and are also used in many Food pastas. Apples are cooked until they become mushy for applesauce. Apples are also made into apple cider drinks **(Mariadoss et al., 2019)**. In our diet, the consumption of apple fruit or its products is a rich source of natural antioxidants. Apples can be processed into sauce, slices, or juice and are favored for pastries, cakes, tarts, and pies. The apple fruit matures in late summer or autumn and cultivars exist in a wide range of sizes. The skin of ripe apples is generally red, yellow, green, pink, or russeted although many bi- or tri-colored cultivars may be found. The skin may also be wholly or partly russeted i.e., rough and brown. The skin is covered in a protective layer of epicuticular wax. The exocarp (flesh) is generally pale yellowish-white, though pink or yellow exocarps also occurs. Commercially, apples can be stored for some months in controlled atmosphere chambers to delay ethylene-induced ripening. Apples are commonly stored in chambers with higher concentrations of carbon dioxide and high air filtration. This prevents ethylene concentrations from rising to higher amounts and preventing ripening from occurring too quickly. Ripening continues when the fruit is removed from storage. For home storage, most cultivars of apples can be held for approximately two weeks when kept at the coolest part of the refrigerator (i.e., below 5 °C). Some, including 'Granny Smithland 'Fuji', can be stored up to a year without significant degradation. Non-organic apples may be sprayed with 1-mmethyl cyclopropane blocking the apples' ethylene receptors, temporarily preventing them from ripening **(Eberhardt et al., 2000).**

It gives up to 22% of total dietary phenolics. Apple peel has approximately 3- to 6-times higher flavonoids as compared to the flesh of an apple and apple peel also has unique flavonoids like quercetin glycosides, which are absent in the apple flesh. The extract obtained from apple skin possesses strong antioxidant ability (**Rupasinghe et al., 2010**). Different phenolic compounds present in apple peel and apple flesh are catechins, procyanidins, phlorizin, phloretin glycosides, caffeic acid, and chlorogenic acid. Besides these polyphenols, the apple peel also has some additional flavonoids (**Gornas et al., 2015**).

A high number of polyphenols has protective effects against oxidation, tumoral formation, and cell signaling. The more the use of apple and its various products in the diet, the lower the chance of chronic diseases like cancer, heart-related diseases, asthma, and Alzheimer's (**Hyson, 2011**). It is assumed that the addition of apple peel extract in mayonnaise may enhance its shelf life by suppressing lipid oxidation. Due to consumer health safety issues, there is increasing demand to use natural antioxidants of plant origin which are considered to be safe (**Hameed et al., 2017**). Among the natural antioxidants, phenolic compounds exhibit the highest antioxidant activity and protect oxidizable food products from oxidation. The polyphenol extracted from different plant parts such as green tea, potatoes peel, apple pomace, grape seed, raspberry leaves, ginger extract, rosemary and different oriental herbs has been successfully utilized to inhibit the oxidation in a variety of food products. In addition to antioxidant properties, the polyphenols extracted from different plant parts also show antimicrobial, antimutagenic, anti-allergic, and anti-inflammatory properties (**Hussain et al., 2018).**

I.1 Types of apples

There are many varieties of apples are grown all over the world but we have discussed only on those apples available here in India. (**Gorgi et al., 2016**)

- **Ambri apple:** Ambri apples popularly known as Kashmiri apples come from the lush green Kashmir valley. The texture, taste and classic sweet aroma of these apples makes the most widely consumed variant in Northern India. The flesh of these apples has a nice sweet and crisp texture and on the exterior this apple variant has a greenish and red stripe. This is often addressed as the pride of Kashmir.
- **McIntosh apple:** These are the real McIntosh apples, which hail from the hilly areas of Uttarakhand, Uttar Pradesh and Himachal Pradesh. The slightly sweet and tarty taste and crisp texture of these apples are widely used for making desserts, jellies, jams, pies and cakes. However, the skin of these apples has a slight mix of red and green, whereas the flesh of these apples has an off-white colour. In hilly areas this apple is mostly consumed raw.
- **Granny Smith:** The name Granny Smith sounds exotic and much-like the name, its eye-grabbing colour, taste, aroma and texture makes it one of the most classic apple variants.

Used in making beverages, jams, desserts, cakes and candies, this crunchy, tarty and juicy apple variant is packed with the goodness of nutrients, antioxidants like polyphenols. This apple variant is usually grown in Himachal Pradesh.

- **Honeycrisp:** This classic sweet yet tarty apple variant is known for its sweetness and crispy texture. This apple has a long shelf life and the freshness and crunch of this apple lasts for a very long time, if stored properly. These apples make for delightful sauces, beverages and are great for eating raw or as a salad.
- **Chaubattia Anupam:** Chaubattia Anupam apples are hybrid apples developed in India by crossing Early Shanburry and Red Delicious. This hybrid variety is cultivated extensively in Uttar Pradesh and Uttarakhand. These apples have red vertical stripes on the exterior with a glossy peel. However, the flesh of this apple is whitish in colour and has a sweet and tangy taste.
- **Golden Delicious:** Much like the name of this variant, these apples have a beautiful greenish-yellow skin. These apples have a delightful sweet flavour and smooth texture and a subtle aroma, which can instantly accentuate the taste of any delicacy. It is used to make sauces, apple butter, jams and is also added to salads to give it a nice flavorsome twist. This variant is also found around the hilly areas of Jammu and Kashmir and Himachal Pradesh.
- **Sunehari:** This is another popular variant of apples made by crossing the genes of Ambri apples and Golden delicious and the results of this hybrid apple is worth the effort. Sunehari apples have nice yellow peels with crimson streaks. The flesh of this apple is crunchy and juicy with a sweet acetous taste.
- **Fuji apple:** These apple variants have gained popularity for their delicious taste, crisp texture, sweet flesh and subtle flavour. The size of these apples is a bit large as compared to other apples. Moreover, these apples have a pale red-green skin and are mostly consumed raw.
- **Tydeman's Early:** Tydeman's Early is a popular apple variant which hails from the hilly areas of Himachal Pradesh and J & K. The classic red coloured exterior of these apples with a yellowish green base, makes Tydeman's Early a classic apple variant, which is majorly relished in the form of desserts and fresh salads.
- **Red Delicious:** This variant is dark red in colour and has a sweet and subtle taste and flavour. This popular variant of apple hails from the mountains of Himachal Pradesh.

This apple is usually relished raw or by adding to salads, chutneys or locally produced jams. What makes this variant different is its oblong-conical shape, which is created by using tricks by cultivators.

- **Gala apple:** This apple variant is consumed all around the country and is usually large in size. Majorly used to make sweets, cakes, salads and juices. This apple variant is known for its sweetness, which is why it is used in making apple juices.
- **Irish Peach:** The name might confuse you, but the taste of this apple variant will not disappoint your palate. This apple variant hails from the Jammu Kashmir region and is widely consumed raw or used in local cuisines for its sweet taste and a peachy aroma and flavour, which gives a hint of fresh and fruits notes to your delicacies.
- **Parlin's Beauty:** This apple variety comes from Tamil Nadu, South India. Usually a late season apple, which has a sweet, mild and crunchy texture. Used in making traditional chutneys and jams.
- **Winesap Apple:** Winesap Apple has a unique spiced flavour with a sweet taste. This apple variant is used to make Apple Cider vinegar. The classic reddish and violet colour of this apple makes it different from other variants of apple. The extracts of these apples are fermented to create cider, which is used in several ways in day-to-day life.

I.2 Scientific Classification

- Kingdom: Planta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Rosales
- Family: Rosaceae
- Subfamily: Maloideae or Spiraeoideae
- Nation: Maleae
- Genus: Malus
- Species: M. Domestica
- Binomial name: *Malus domestica*

1.3 Content of apple peel:

The compounds most commonly found in apple peels consist of the procyanidins, catechin, epicatechin, chlorogenic acid, phlorizin, and the quercetin conjugates. In the apple flesh,

there is some catechin, procyanidin, epicatechin, and phlorizin, but these compounds are found in much lower concentrations than in the peels. Quercetin conjugates are found exclusively in the peel of the apples. Chlorogenic acid tends to be higher in the flesh than in the peel. Because the apple peels contain more antioxidant compounds, especially quercetin, apple peels may have higher antioxidant activity and higher bioactivity than the apple flesh. Research showed that apples without the peels had less antioxidant activity than apples with the peels. Apples with the peels were also better able to inhibit cancer cell proliferation when compared to apples without the peels (**Eberhardt et al., 2000**). More recent work has shown that apple peels contain anywhere from two to six times (depending on the variety) more phenolic compounds than in the flesh, and two to three times more flavonoids in the peels when compared to the flesh. The antioxidant activity of these peels was also much greater, ranging from two to six times greater in the peels when compared to the flesh, depending on the variety of the apple (**Wolfe et al., 2003**). This work is supported by who found that rats consuming apple peels showed greater inhibition of lipid peroxidation and greater plasma antioxidant capacity when compared to rats fed apple flesh (**Leontowicz et al., 2003**).

1.4 Benefits of apple peel:

- **Apple peels are high in fiber:** The skin of an apple contains the majority of the fiber. Fiber keeps you fuller for longer, helping you avoid hunger pangs and cravings. Fiber also aids in the maintenance of the liver's health, which in turn aids in the maintenance of bone health. Fiber is also advantageous to diabetic individuals since it aids digestion.
- **Contributes to the protection of lung health:** Apple peel contains quercetin, a powerful anti-inflammatory compound that protects your lungs and heart from a number of diseases.
- **Helps you maintain a healthy heart:** Polyphenols in apple skin have been shown in tests to help lower blood pressure and cholesterol levels, as well as increase vascular flexibility for a healthy heart.
- **Apple peel helps you lose weight:** Apple skin's high fiber content keeps you satisfied for longer, preventing overeating. As a result, calorie consumption will be reduced, and weight reduction will occur. Furthermore, polyphenols present in the skin have been shown to inhibit glucose and fat absorption, resulting in weight reduction.

- **Apple skin is full of vitamins:** Vitamins A, C, and K are found in apple skin. It also contains essential minerals such as potassium, phosphorus, and calcium, all of which are beneficial to overall health. All of these nutrients contribute to the health of your heart, nerves, brain, skin, and bones **(Wolfe et al., 2003).**

1.5 Application of apple peel

The peels of processed apples can be recovered for further food applications. Limited information on the valorizations of this type of waste is available for cooking varieties, e.g., Bramley's Seedling. Extracts from fresh or dried (oven-dried or freeze-dried) peels were obtained with solvents of different polarity (aqueous acetone or ethanol) and assayed for their total phenolic content and antioxidant capacity; their antiradical power was compared into herb extracts. The dried peels were also characterized as bulk powders by assessing their nutritional value and total phenolic content. High amounts of ascorbic acid (up to 4 mg/g, dry weight) and polyphenols (up to 27 mg gallic acid equivalents/g, dry weight) were found in the peels, with the latter contributing significantly to the antioxidant capacity; the nutrient profile was low in protein (less than 10%, w/w) and total dietary fiber content (less than 40%, w/w).

Higher yields of phenolic antioxidants were recovered with acetone from freeze-dried peels; the resulting extracts had equivalent antioxidant power to oregano leaves **(Leontowicz et al., 2003).** The combination of oven-drying/ethanol led to lower recovery yields of phenolic antioxidants; however, these conditions could increase the feasibility of the extraction process, leading to antioxidant extracts with lower energy or cost input, and higher suitability for further food use. The recovery of phenolic antioxidants from the peels of processed apples could be a valuable alternative to traditional disposal routes (including landfill), in particular for cooking varieties. The recycling process could enhance the growth of traditional culinary apple markets in the UK and Ireland thanks to the new business opportunities for the peel-derived materials.

1.6 Research progress on different coloured apples:

Apple is one of the most widely produced and economically important fruits in temperate regions. Fruit colour development in apple is a major focus for both breeders and researchers as consumers associate brightly coloured red apples with ripeness and a good flavour. In recent years great progress has been made in recent years, great progress has been made in the research of apple fruit colour development, but its development mechanism but not been

systemic dissected from the aspect of genetics, transcription or environmental factors. It is popular with growers and consumers due to its strong ecological adaptability and high nutritional value. According to the statistical data in the World Apple Review (2018), apples accounted for 12.26% of the world's fruit production from 2012 to 2014, second only to bananas and citrus (World Apple Review, 2018). According to the Food and Agriculture Organization of the United Nation (FAO), global apple production in 2015 exceeded 80 million tons. The main apple cultivars can be roughly divided into red varieties such as 'red delicious', 'Fuji' and 'Royal Gala' and non-coloured varieties such as 'Golden delicious', 'Granny Smith' and 'Orin'. Consumers associate red apples with ripeness and a good flavour. Consequently, red apple varieties often have better marketability and a higher economic value. Fruit colour is mainly determined by anthocyanins, an important class of secondary metabolites synthesized in higher plants (**Chen et al., 2021**).

Review of Literature

2. REVIEW OF LITERATURE

2.1 Phenolic content, chemical composition and anti-/pro-oxidant activity of Gold Milenium and Papierowka apple peel extracts

In this study the peels of ecologically grown apple (*Malus domestica*) cultivars: Gold Milenium (a new scab-resistant variety) and Papierowka (Papirovka; an old, sensitive to apple scab variety) were examined for their composition (phenolic compounds, triterpenoids, simple organic acids, macro-, microelements, reducing sugars, L-ascorbic acid), pro- and antioxidant properties as well as their application in reduction of the oxidative stress in cultured human skin fibroblast. The higher content of phenolic compounds correlated with the greater pro- and antioxidant activity of the peels of Papierowka compared to Gold Milenium in DPPH·, ABTS+, FRAP and CUPRAC assays as well as an ability to inhibition of lipid peroxidation. The quantity of the compounds strongly depended on the type of extraction. The extract of Papierowka peels possessed much higher number of phenolic compounds compared to Gold Milenium (Papierowka: 3.68 ± 0.20 mg/g peel ultrasound assisted extraction (u.a.e); 2.02 ± 0.13 mg/g peel conventional extraction (c .e.); Gold Milenium: 1.46 ± 0.19 mg/g peel u.a.e; 1.15 ± 0.04 mg/g peel c .e. according the HPLC measurement). The pro-oxidant activity of the extract from Papierowka peels can be correlated with the content of phenolic compounds and metal ions as well. The apple peel extract is promising agent reducing the oxidative stress in skin fibroblast **(Kalinowska et al., 2020).**

2.2 Phytochemical Content and Antioxidant Activity of *Malus domestica* Borkh Peel Extracts

Apple is an important dietary source of carotenoids and phenolic compounds, and its regular consumption is associated with several health benefits. The study was done to evaluate the phytochemical composition of fresh peels of four red-skinned (“Champion”, “Generos”, “Idared”, “Florina”) and two yellow-skinned (“Golden Delicious”, “Reinette Simirenko”) apple varieties. Antioxidant activity of apple peel extracts was determined by ferric reducing antioxidant power (FRAP) and ABTS radical scavenging capacity assays. Total carotenoid and polyphenolic contents were determined spectrophotometrically, while the profile of individual carotenoids and anthocyanins (in red-skinned varieties) was analysed using high-performance liquid chromatography coupled to a photodiode array detector (HPLC-PDA). Carotenoid composition was specific for each variety, and total carotenoid content was

slightly higher in yellow-skinned apple peels compared to red-skinned varieties. In contrast, total phenolic content was higher **(Vasile et al., 2021).**

2.3 In vitro inhibitory effect of apple peel extract on the growth of Helicobacter pylori and respiratory burst induced on human neutrophils

In this study, the in vitro effect of a standardized extract of apple peel APPE (Apple peel polyphenol rich extract) was evaluated with regard to the viability of Helicobacter pylori. The cytotoxic effect of APPE on H. pylori was also evaluated through the resazurin assay and ATP level determination. In both assays, APPE showed an early cytotoxic effect, which was both concentration and time-dependent. Additionally, the effect of APPE on the intra and extracellular production of reactive oxygen species (ROS) was evaluated in human neutrophils stimulated by H. pylori, phorbol myristate acetate (PMA), and formyl-methionyl-leucyl-phenylalanine (fMLP). The extracellular and intracellular production of ROS was evaluated through chemiluminescence with the isoluminol-horseradish peroxidase (HRP) and luminol-superoxide dismutase (SOD)-catalase systems, respectively. APPE showed an inhibiting effect on the multiplication of two H. pylori strains (ATCC 43504 and TX136) with a minimum inhibitory concentration (MIC) value of 112.5 microg gallic acid equivalent (GAE)/mL. APPE inhibited the respiratory burst of neutrophils induced by H. pylori, PMA, and fMLP in concentration-dependent form. Interestingly, this effect was observed on both the interior and exterior of the neutrophil. This result suggests that apple peel polyphenols have an attenuating effect on the damage to gastric mucosa caused by neutrophil generated ROS and, particularly, when H. pylori displays its evasion mechanisms **(Pastene et al., 2009).**

2.4 Development of an ingredient containing apple peel, as a source of polyphenols and dietary fibre

Apple peel is a waste product from dried apple manufacture. The content of phenolic compounds, dietary fibre, and mineral are higher in apple peel, compared to other edible parts of this fruits. The objective of this study was to develop an ingredient from Granny Smith apple peel, using a pilot scale double drum-dryer, as drying technology. The control of all steps to maximize the retention of phenolic compounds and dietary fibre was considered. Operational conditions, such as drying temperature and time were determined, as well as important pre-processing steps like grinding and PPO inhibition. In addition, the physical-chemical characteristics, mineral and sugar content, and technological functional properties

such as water retention capacity, solubility index, and dispersibility among others, were analysed. A simple, economical, and suitable pilot scale process, to produce a powder ingredient from apple peel by-product, was obtained. The drying process includes the application of ascorbic acid at 0.5% in the fresh apple peel slurry, drum-dryer operational conditions were 110 degrees C, 0.15 rpm and 0.2 mm drum clearance. The ingredient developed could be considered as a source of phenolic compounds (38.6 mg gallic acid equivalent/g dry base) and dietary fibre (39.7% dry base) in the formulation of foods. Practical Application: A method to develop an ingredient from Granny Smith apple peel using a pilot scale double drum-dryer as drying technology was developed. The method is simple, economical, feasible, and suitable and maximizes the retention of phenolic compounds and dietary fibre present in the raw matter. The ingredient could be used in the formulation of foods **(Henriquez et al., 2010)**.

2.5 Antioxidant activity of apple peels

Apples are commonly eaten and are large contributors of phenolic compounds in European and North American diets. The peels of apples, in particular, are high in phenolics. During applesauce and canned apple manufacture, the antioxidant-rich peels of apples are discarded. To determine if a useful source of antioxidants is being wasted, the phytochemical content, antioxidant activity, and anti-proliferative activity of the peels of four varieties of apples (Rome Beauty, Idared, Cortland, and Golden Delicious) commonly used in applesauce production in New York state were investigated. The values of the peels were compared to those of the flesh and flesh + peel components of the apples. Within each variety, the total phenolic and flavonoid contents were highest in the peels, followed by the flesh + peel and the flesh. Idared and Rome Beauty apple peels had the highest total phenolic contents. Rome Beauty and Idared peels were also highest in flavonoids. Of the four varieties, Idared apple peels had the most anthocyanins. The peels all had significantly higher total antioxidant activities than the flesh + peel and flesh of the apple varieties examined. Idared peels had the greatest antioxidant activity. Apple peels were also shown to more effectively inhibit the growth of HepG (2) human liver cancer cells than the other apple components. The high content of phenolic compounds, antioxidant activity, and anti-proliferative activity of apple peels indicate that they may impart health benefits when consumed and should be regarded as a valuable source of antioxidants **(Wiersma et al., 2007).**

2.6 Polyphenolic compounds and antioxidant activity of new and old apple varieties

Apple fruit is a major source of phenol compounds, because its consumption is widespread in many countries and it is available on the market for the whole year. The phenolic composition of 67 varieties of apple cultivars was examined for the concentration of some important phytochemicals and antioxidant activity. For the first time, we have looked at the correlation and compared polyphenolic compounds in Golden Delicious variety and new varieties grown from it. Up to 18 compounds, including catechin, procyanidin, hydroxycinnamates, flavanols, anthocyanins, and dihydrochalcones, were analysed by high-performance liquid chromatography with diode array detection analysis of crude extracts and after thiolysis and LC-MS (Liquid chromatography–mass spectrometry). Flavanols (catechin and oligomeric procyanidins) are the major class of apple polyphenols, representing more than 80%, followed by hydroxycinnamic acids (1-31%), flavonols (2-10%), dihydrochalcones (0.5-5%), and in red apples, anthocyanins (1%). The presented data clearly demonstrated that new varieties, i.e., Ozark Gold, Julyred, and Jester, of apple had the same or higher value of bioactive compounds in comparison to the old varieties, i.e., Golden Delicious, Idared, and Jonagold **(Wojdylo et al., 2008).**

2.7 Apple peels as a value-added food ingredient

Apple peels have high concentrations of phenolic compounds and may assist in the prevention of chronic diseases. A study proposed that millions of pounds of waste apple peels are generated in the production of applesauce and canned apples in New York State each year. We proposed that a valuable food ingredient could be made using the peels of these apples if they could be dried and ground to a powder without large losses of phytochemicals. Rome Beauty apple peels were treated with citric acid dips, ascorbic acid dips, and blanches before being oven-dried at 60 degrees C. Only blanching treatments greatly preserved the phenolic compounds, and peels blanched for 10 s had the highest total phenolic content. Rome Beauty apple peels were then blanched for 10 s and dried under various conditions. The air-dried and freeze-dried apple peels had the highest total phenolic, flavonoid, and anthocyanin contents. On a fresh weight basis, the total phenolic and flavonoid contents of these samples were similar to those of the fresh apple peels. Freeze-dried peels had a lower water activity than air-dried peels on a fresh weight basis. The optimal processing conditions for the ingredient were blanching for 10s and freeze-drying. The process was scaled up, and the apple peel powder ingredient was characterized **(Wolfe et al., 2003).**

Aims and Objectives

3. AIMS AND OBJECTIVES

There are many various types of apples available in West Bengal. According to nutritive value of Indian foods by C. Gopalan, ICMR, NIN there are a fixed amount given for nutrients of all the foods but there was no such information related to their peel varieties. But we do not know if there are any alteration of apple/apple peel. The study was done to investigate the variation on the phenolic content & phytochemical content in different varieties of apple peel. The objectives of this study were –

- To study the preliminary phytochemical screening for presence of different phytochemicals in different varieties of apple peel.
- To study the quantitative and qualitative analysis of phenolic compounds in different varieties of apple peel.
- To study about different phytocompounds present in different varieties of apple peel by HPLC analysis using standards.

Methods and Materials

4. METHODS AND MATERIALS

4.1 Collection of samples: Different Samples were collected from different areas of Pashchim Medinipur, West Bengal. The different samples were stored in the nutrition laboratory of Midnapore City College.

4.2 Preparation of food extract: Fruits were washed and cut carefully and dried in a hot air oven up to minimum amount of moisture content. After full dried food products were grinded in a mixture grinder then stored in a cool dried place in an air tight container. 10g of each sample of apple peel was dissolved in 50ml of methanol and chloroform and marked as MEGA (methanol extract of green apple), CEGA (Chloroform extract of green apple), CERA (Chloroform extract of red apple), MERA (Methanol extract of red apple), CELRA (Chloroform extract of light red apple), MELRA (Methanol extract of light red apple)

4.3 Preliminary phytochemical screening: Different extracts were prepared and qualitative phytochemical analysis were done for presence of tannins, phenols, saponins, flavonoids, glucosides, terpenoids, and steroids.

4.4 Thin layer chromatographic (TLC) analysis for qualitative antioxidant assay: TLC plates were prepared by using Silica gel G 8g with 24 ml distilled water. Plates were developed in Methanol: Chloroform: Hexane =7:2:1 (v/v/v). Ethanol extract of different varieties of apple skin and ascorbic acid as Standard were spotted on the plate and run just below the upper end of the plate. The plate was dried and 0.05% DPPH in methanol was sprayed on the plate. The plate was again dried and the yellow spot on the plate was the indication of positive antioxidant activity.

4.5 Total antioxidant activities: Determination of DPPH radicals scavenging activity was estimated with the method used by Kato. 1mM solution of DPPH in ethanol and also 1mg/1ml extract solution in ethanol was prepared and 1.5ml of this solution was added to 1.5ml of DPPH. The absorbance was measured at 517m against the corresponding blank solution which is prepared by taking 3ml ethanol and control O.D. was prepared by taking 3ml of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH. The assay was performed in triplicates.

4.6 TLC analysis for flavonoid constituents: TLC was performed on the 20*20cm plates precoated with precoats with microcrystalline cellulose. A volume of 10 micro litre of 1% methanol, chloroform solution of apple peel (green, red, light red) fraction was spotted on the

plate. One dimensional TLC analysis was performed with chloroform: ethyl acetate: formic acid in volume ratio 20:16:4 as mobile phase. Spots were observed under UV transilluminator at 215nm.

$$\text{RF Value} = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent}}$$

4.7 Total phenolic content: The amount of phenol in the aqueous food extracts was determined by Folin-Ciocalteu phenol Reagent method with some modifications.2.5 ml of 10 %Folin-Ciocalteu phenol reagent (V/V) And 2 ml of 7.5 % of Na2CO3 were added to the 1 ml of food extract. The resulting mixture was Incubated for15minutes at room temperature. The absorbance of the sample was measured at 765 nm spectrophotometrically. Gallic acid was used as standard (1mg/ml) and was prepared by 0.01, 0.02.0.03, 0.04 and 0.05 mg/ml of gallic acid in methanol. All the readings were recorded in triplicates. The results were determined from the standard calliberation Curve of gallic acid and total phenolic contents were expressed as gallic acid equivalents (mg/g Of GAE of extracted compound) **(Roy et al., 2014).**

4.8 HPLC analysis: High Performance Liquid Chromatography was carried out on the extracts from experimental plan Using a HPLC Agilent 1100 Series (Palo Alto, CA), equipped with a C18 reverse-phase column (Model 201TP54, Vydac, Hesperia, CA) and coupled with a Diode Array Detector. Samples and standard were run at 273nm. Before the Analysis, extracts were properly diluted, filtered by 0.2 µm cellulose acetate membrane filter and 100 µL of sample were injected for each run. Water/acetic acid (99:1 % (v/v); solvent A) and methanol/acetonitrile (50:50 % (v/v); solvent B) were used as mobile phase, and the solvent gradient was set to change according to the following conditions: 100 % A for 5 min, from 5 % to 30 % B in 25 min, from 30 % to 40 % B in 10 min, from 40 % to 48 % B in 5 min, from 48 % to 70 % B in 10 Min, from 70 % to 100 % B in 5 min, isocratic at 100 % B for 5 min, followed by returning to the Initial conditions (10 min) and column equilibration (12 min). A flow rate of 1.0 mL/min was used at 30 °C **(Yang et al., 2016).**

Results

5. RESULTS:

Table 1: Preliminary phytochemical screening of methanol & chloroform extract of different varieties of apple peel

SL NO.	Name of the phytochemicals	Name of the biochemical test	Green apple (c)	Red apple (c)	Light red apple (c)	Green apple (m)	Red apple (m)	Light red apple (m)
1.	Phenols	Ferric chloride test	-	-	+	+++	+++	-
2.	Flavonoid	Flavonoid test	+	-	+	+++	+++	+++
3.	Terpenoid	Terpenoid test	+	+	+	-	+++	+++
4.	Steroids	Steroid test	+	+	+	-	+++	+++
5.	Lignin	Labatt test	+	+	+	-	+++	-

Fig 1: TLC analysis for qualitative antioxidant activity in methanol & chloroform extract of different varieties of apple peel

G(M)- Methanol extract of green apple peel, R(C)- Chloroform extract of red apple peel, G(C)- Chloroform extract of green apple peel, LR(M)- Methanol extract of light red apple

peel, LR(C)- Chloroform extract of light red apple peel, R(M)- Methanol extract of red apple peel

Table 2: RF value for antioxidant constituent:

SAMPLE	RF VALUE
Green apple peel (c)	0.911
Red apple peel (c)	0.855
Light red apple peel (c)	0.881
Green apple peel (m)	0.930
Red apple peel (m)	0.885
Light red peel (m)	0.852

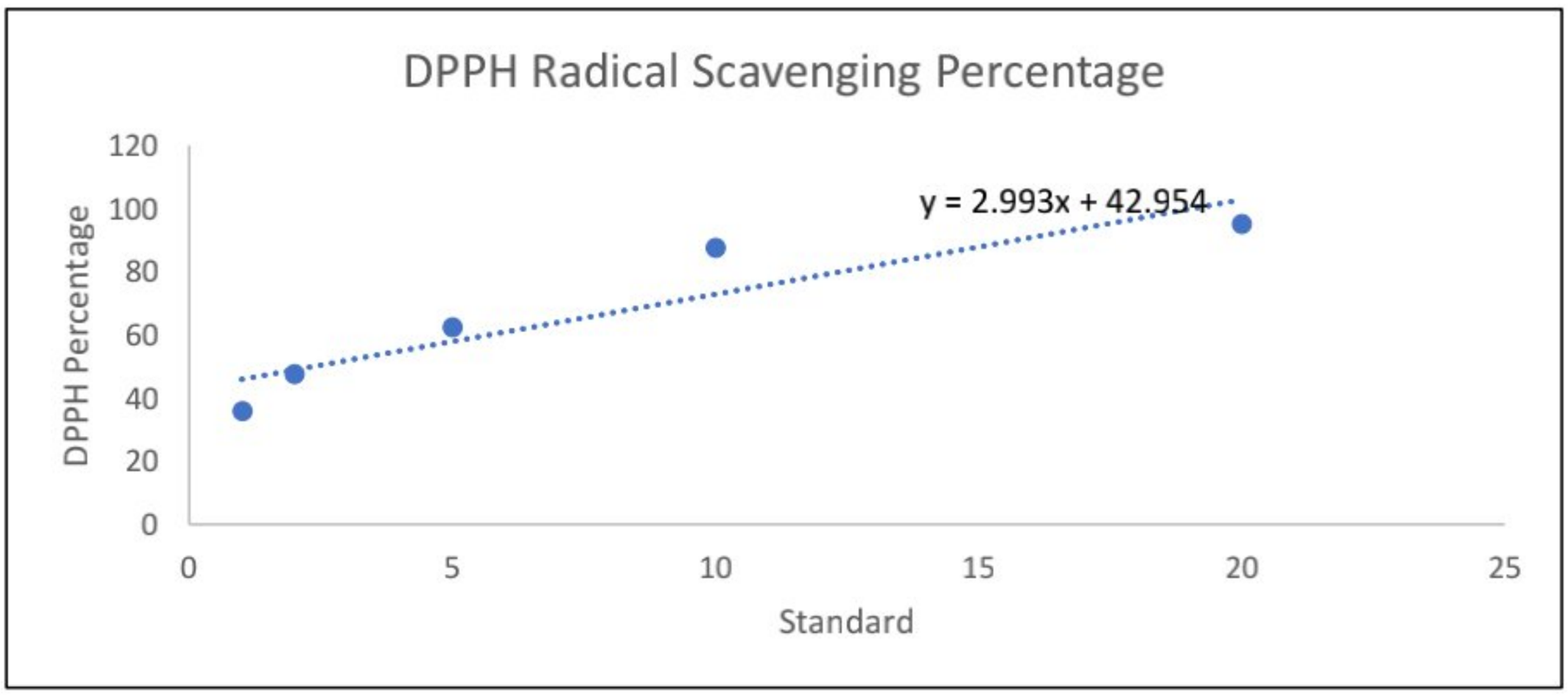

Fig 2: DPPH radical scavenging percentage of standard ascorbic acid at different concentrations

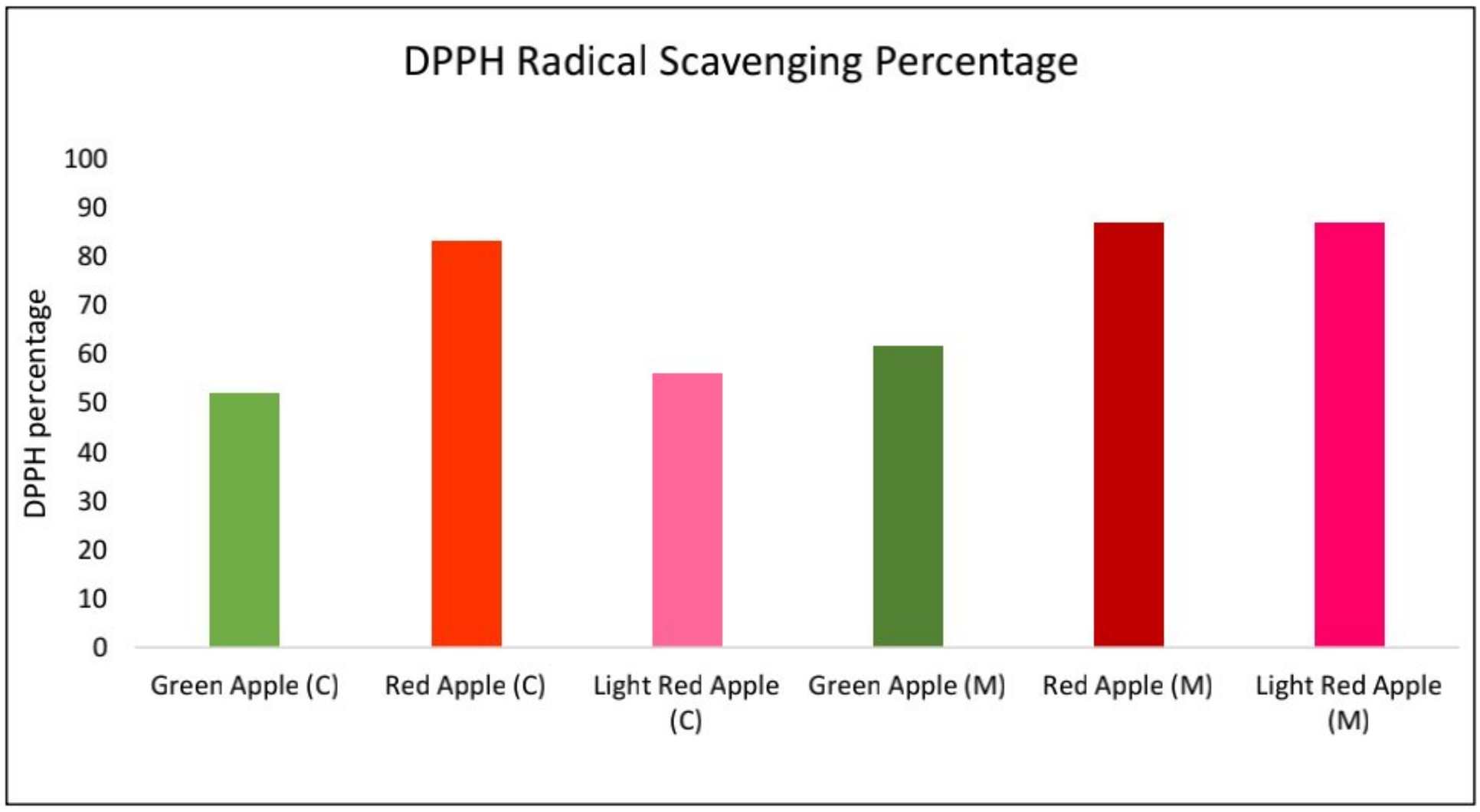

Fig 3: DPPH radical scavenging activity of Chloroform and Methanol extract of different varieties of apple peels

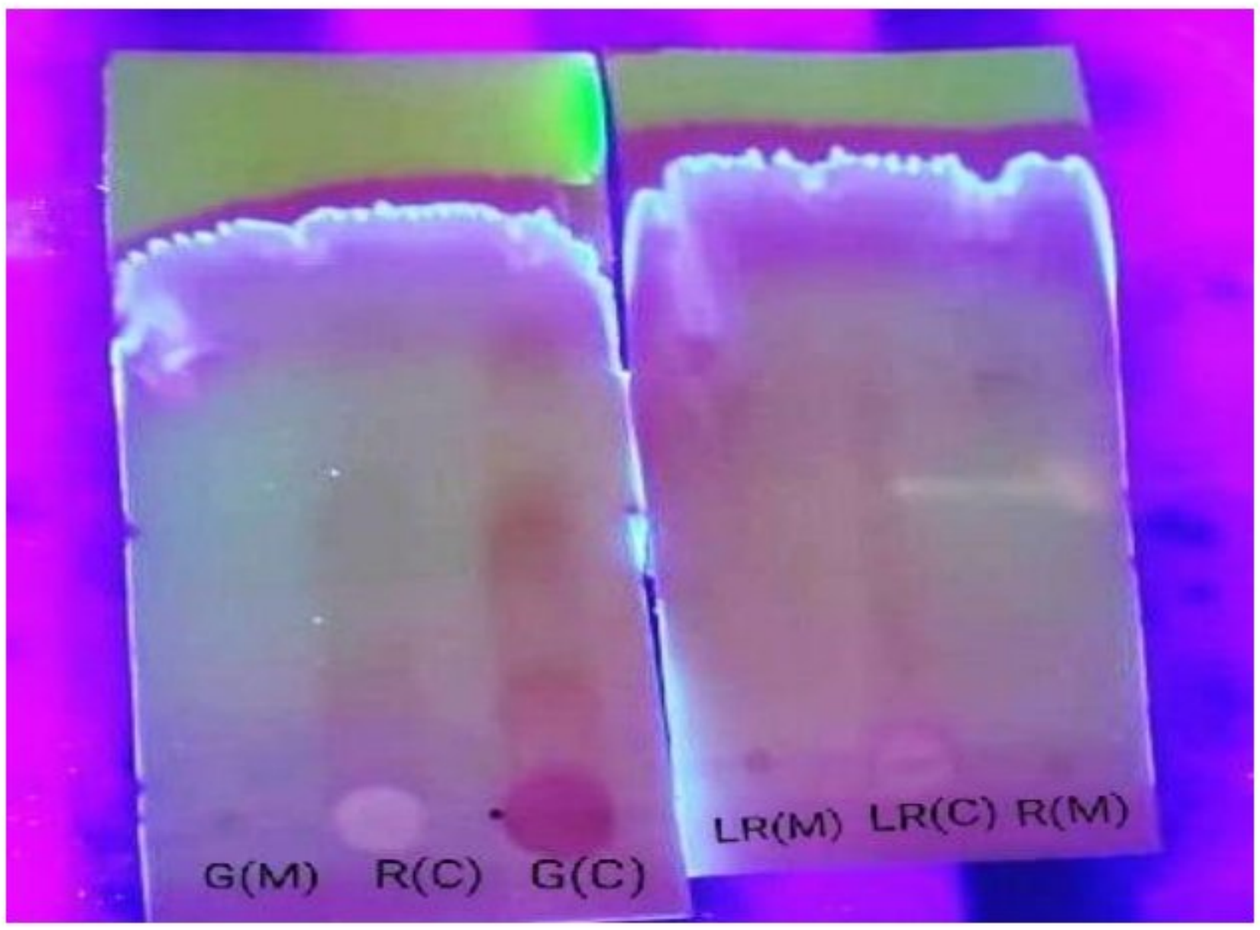

Fig 4: TLC analysis for flavonoid properties in methanol & chloroform fractions of different varieties of apple peel

G(M)- Methanol extract of green apple peel, R(C)- Chloroform extract of red apple peel, G(C)- Chloroform extract of green apple peel, LR(M)- Methanol extract of light red apple peel, LR(C)-Chloroform extract of light red apple peel, R(M)- Methanol extract of red apple peel

Table 3: Rf value for flavonoids constituents obtained from TLC analysis:

SAMPLE	Rf VALUE
Green apple peel (c)	0.861
Red apple peel (c)	0.897
Light red apple peel (c)	0.888
Green apple peel (m)	0.812
Red apple peel (m)	0.896
Light red apple peel (m)	0.695

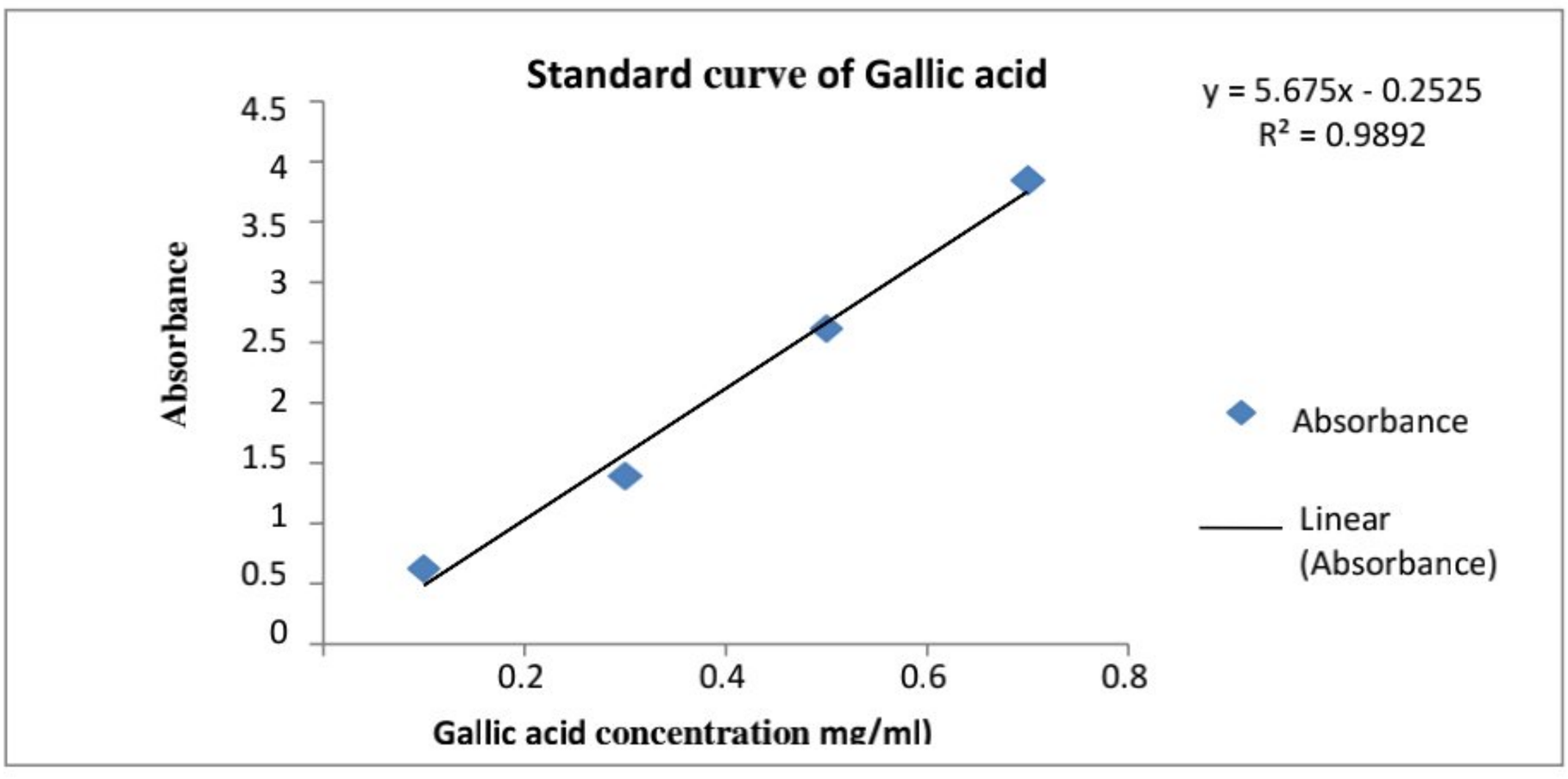

Fig 5: Standard curve of Gallic Acid.

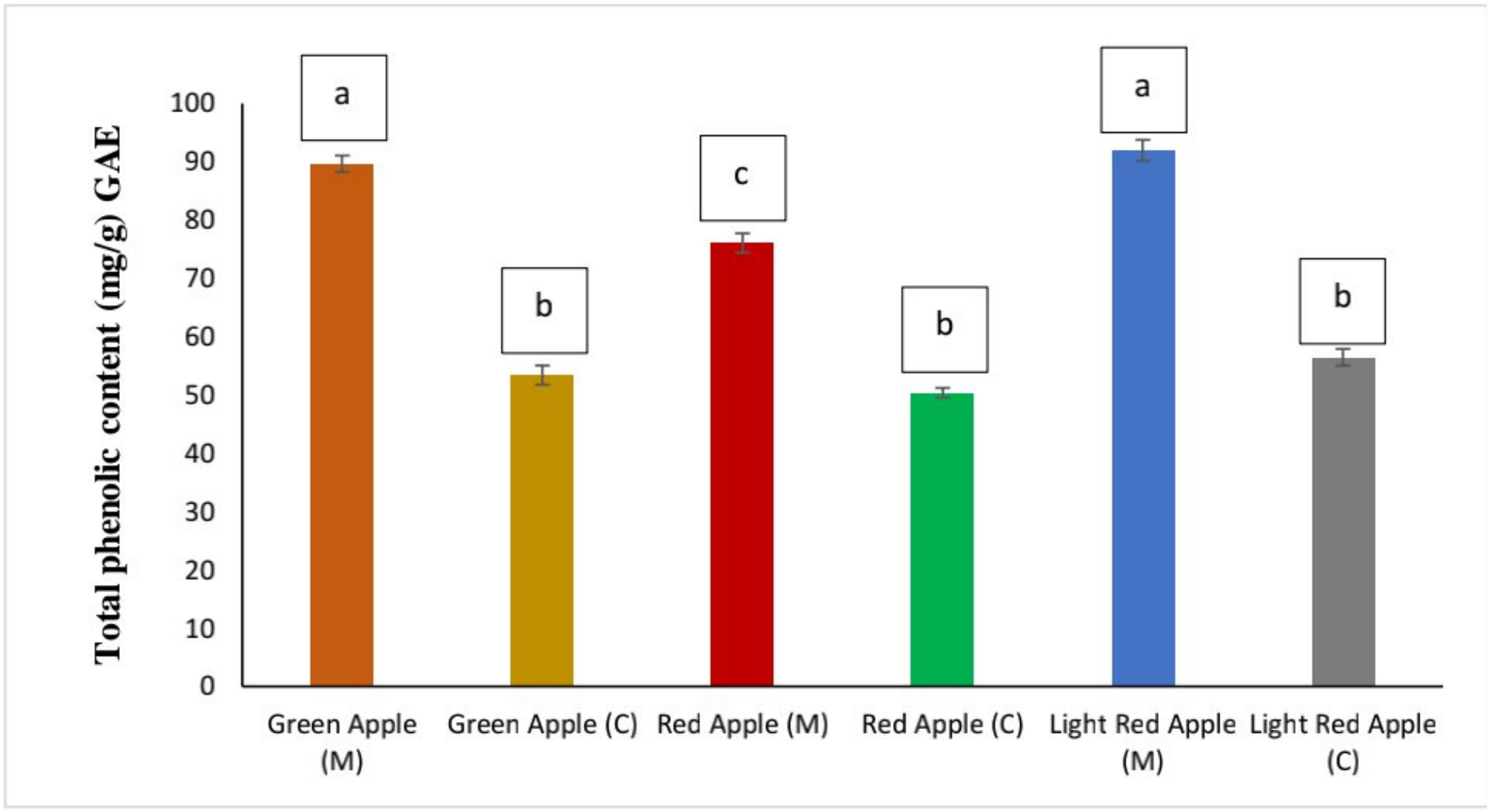

Fig 6: Total phenolic content (mg/g) GAE of different varieties of apple peel

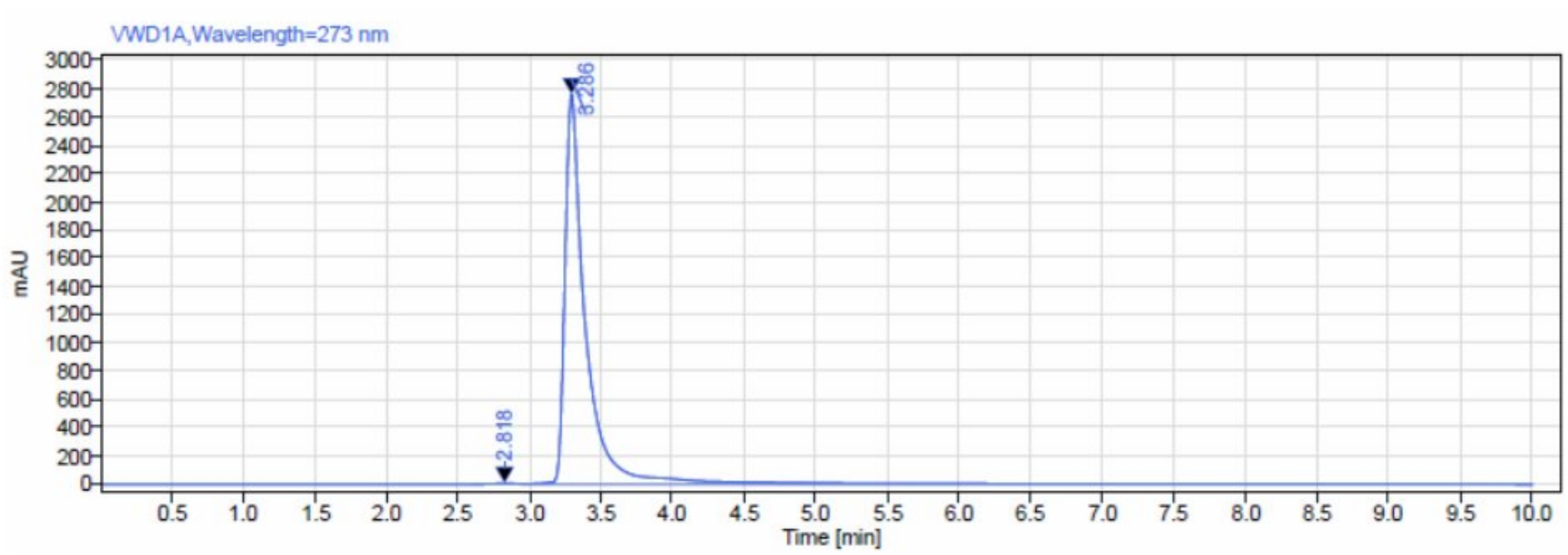

Fig 7: HPLC analysis of Standard Quercetin

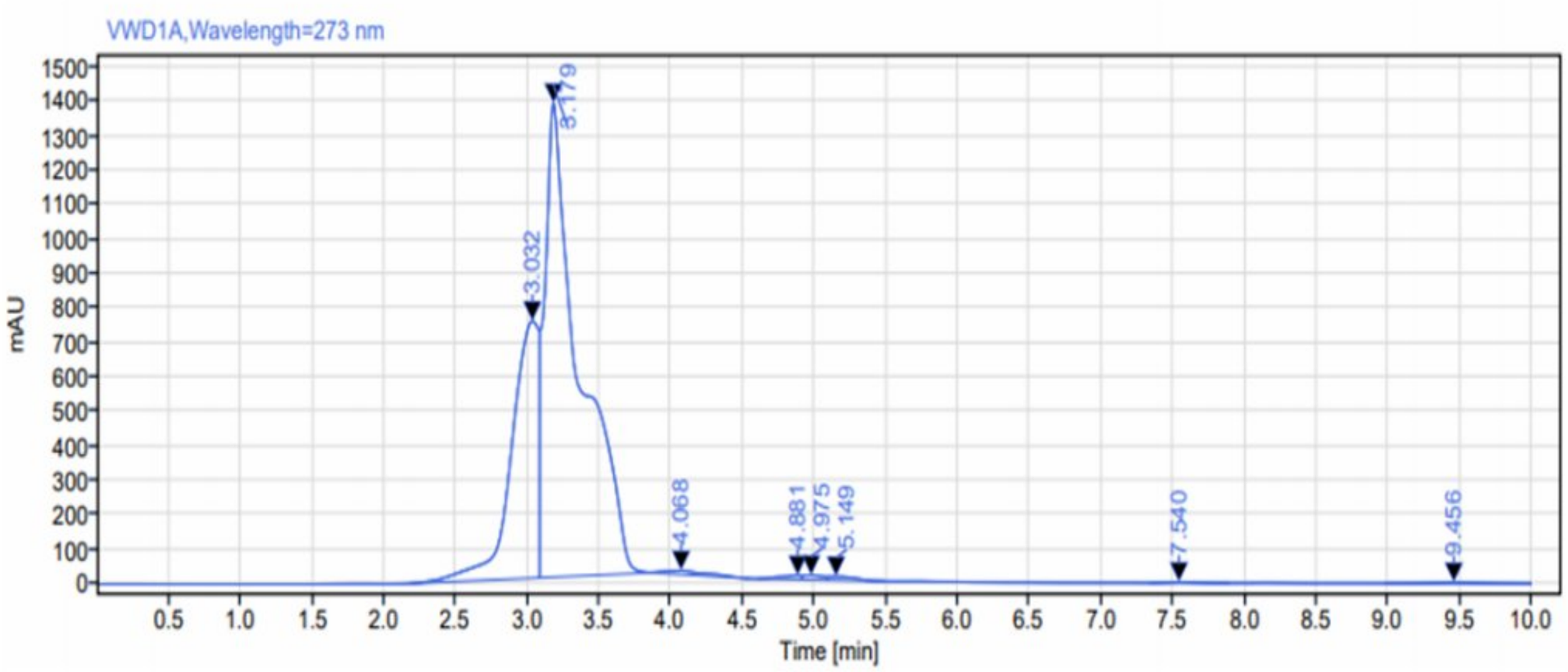

Fig 8: HPLC analysis for methanol extract of red apple peel

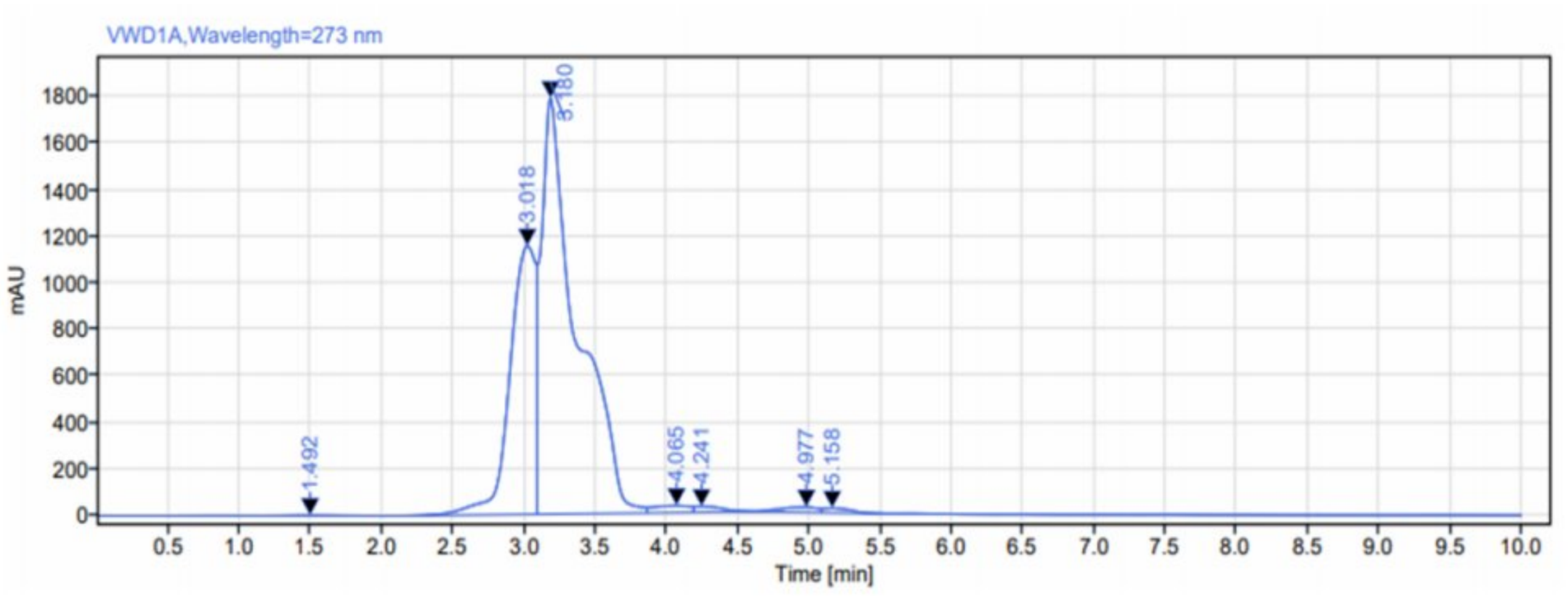

Fig 9: HPLC analysis for methanol extract of light red apple peel

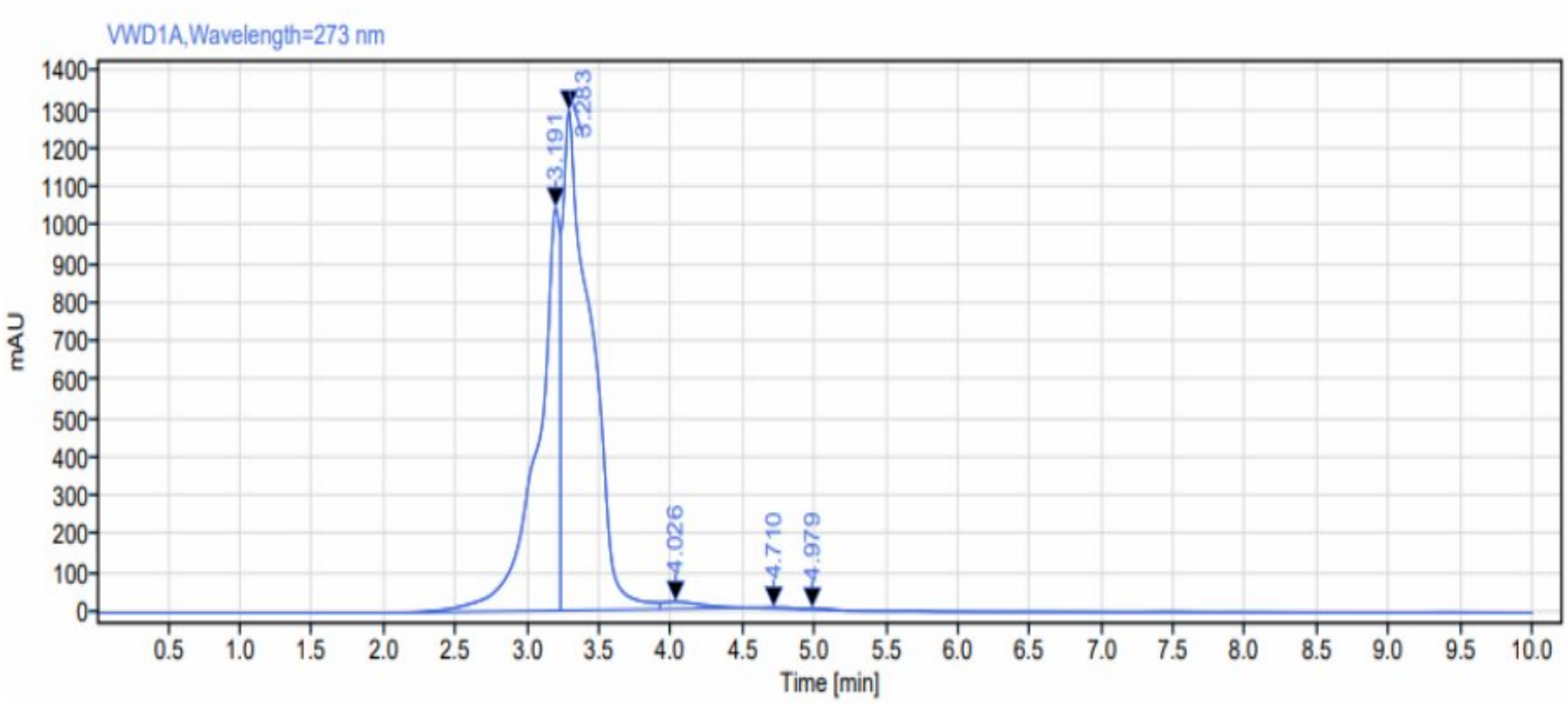

Fig 10: HPLC analysis for methanol extract of green apple peel

Discussion

5. DISCUSSION

Apples are commonly eaten and are large contributors to many bioactive phytochemicals including, antioxidants, flavonoids, polyphenols etc. The peels of apples, in particular are high in phenolic compounds. During apple sauce and canned apple manufacture, the antioxidant-rich peels of apples are discarded. To determine it a useful source of antioxidants is being wasted, the phytochemical content, antioxidant activity, anti-proliferative activity of peels. The high content of phenolic compounds, antioxidant activity and anti-proliferative activity of apple peels indicate that they may impart health benefits when consumed and should be regard as a valuable source of antioxidant. Apple contributes to improve health by reducing the risk of diseases, such as cardiovascular disease and some forms of cancer. Apple fruit is a major source of phenol compounds, because its consumption is widespread in many countries and it is available in the market for the whole year.

Preliminary phytochemical screening was done to assesses the presence of different phyto-compounds present in different varieties of apple peel, It has been shown that different types of apple peel like chloroform extract of green apple peel contain flavonoid, terpinoids, steroid lignin. Methanol extract of green apple peel contain phenol, flavonoid. Chloroform extract of red apple peel contain terpinoid, steroid, lignin. Methanol extract of red apple peel contain flavonoid, terpinoid, steroid, lignin. Chloroform extract of light red apple peel contain phenol, flavonoid, terpinoid, steroid, lignin. Methanol extract of light red apple peel contain flavonoids, terpinoids, and steroids (Table 1).

Antioxidants are major compounds which believed to have protection of against certain disease by preventing the destructive effect of the free radical mediated processes in cell membrane and by reducing the susceptibility of tissue to oxidative stress. For qualitative analysis of antioxidants activity TLC analysis was performed using standard ascorbic acid. A yellow spot on TLC plates indicate the presence of antioxidant compound (Figure-1). All the samples of apple peel contains antioxidative properties as shown in the TLC plate with Rf value 0.911, 0.855, 0.881, 0.930, 0.885, 0.852 for green apple peel (chloroform), red apple peel (chloroform), light red apple peel (chloroform), green apple peel (methanol), red apple peel (methanol) and light red apple peel(methanol) respectively (Table 2).

In DPPH scavenging activity, it was observed that all the samples have good DPPH scavenging properties when compared to standard ascorbic acid(Figure-2). The DPPH radical scavenging percentages are 52, 83, 56, 61.5,87 and 87 for green apple peel (chloroform), red

apple peel (chloroform), light red apple peel (chloroform), green apple peel (methanol), red apple peel (methanol) and light red apple peel (methanol) respectively (Figure-3).

Flavonoids are good free radical scavengers, donate hydrogen atoms. Free radicals scavenging activity of flavonoids are also distinguished. The anti-oxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as ions and coppers and inhibitions of enzymes responsible for free radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS. Phenols are well established to exhibit antioxidant activity, contribute to human health. Figure-2 suggested that the presence of flavonoids in all the six samples analyzed through TLC plate with the RF value 0.861, 0.897, 0.888, 0.812, 0.896, 0.695 for green apple peel (chloroform), red apple peel (chloroform), light red apple peel (chloroform), green apple peel (methanol), red apple peel (methanol) and light red apple peel (methanol) respectively(Table-3).

Phenolic contents have gained considerable interest because of their potential beneficial effects on human health. Phenolic contents have been reported to show antiviral, anti-allergic, antiplatelet, anti-inflammatory, anticancer, and antioxidant activities (Sultana et al., 2012). Quantification of total phenolic content in extracts of different apple fruits was carried out by using the Folin-Ciocalteau reagent. The total phenolic content ranged from 30 to 90 mg gallic acid equivalents (GAE)/g of apple peel samples(Figure-5). All the three varieties of apple peel and their chloroform and methanol fraction both content phenolic constituents. Green apple of chloroform fraction content 53.4 mg/g, green apple of methanol fraction content 89.66 mg/g, light red apple of chloroform fraction content 56.46 mg/g, light red apple of methanol fraction content 91.14 mg/g, red apple of chloroform fraction content 50.39 mg/g, red apple of methanol fraction content 76.06 mg/g. According to Fig -6 study it was studied that green apple & light red apple have significantly ($p<0.05$) high level of total phenolic content compared to all other type of apple samples. This indicates that peels of the apple fruits considered here may be used to produce natural antioxidant supplements in food and pharmaceutical industry.

After studying all the sample, it was observed that all test (preliminary phytochemical screening, TLC for antioxidant activity, DPPH/total antioxidant activity, total phenolic compound and TLC for flavonoid constituents) are good for methanol extract of all the apple peel. So we continue the further study by HPLC analysis. The HPLC chromatogram of the

methanol fraction of red apple peels showed two major and six other minor peaks at 273 nm (Fig 8), methanol fraction of light red apple peel showed two major peaks as well as five minor peaks at the wavelength of 273nm (Fig 9); and the methanol fraction of green apple peel showed two major and three minor peaks at 273nm (Fig 10). Methanol fraction of different apple peels at the retention time around 3.2 compares with standard quercetin of the major peak at the retention time 3.2 (Fig 7).

Conclusion

6. CONCLUSION:

Depending on the varieties of apple peel there are some alterations in their phytochemical profile in both case of qualitative and quantitative analysis. Through phytochemical screening we found that there are presence of many phytochemical compound like terpinoids, steroids, tannins, saponins etc. Through different analysis the study revealed that different varieties of apple peel contain good amount of flavonoids, phenol, antioxidant. Apples and it peels contain a wide variety of phytochemicals, many of which have been found to have strong antioxidant activity. So we encourage the people to consume apple with their peel because it has many health beneficial effect. Regular consumption of apples as a part of a healthy diet may aid in the prevention of chronic disease and maintenance of good health.

.

Future Scope

7. FUTURE SCOPE

There are many compounds and substances present in the apple skin but all of the compounds may be still not identified. From this study the nature of the compounds can be identified and in future many separation methods and isolation of compounds can be used to find out the presence of bioactive substances as well as if any type of anti-nutritional substances presents or not can be studied.

Depending on the presence of effective bioactive compounds further studies can be done by using the compounds for prevention and protection of many degenerative diseases.

References

8. REFERENCES

Barickman, T. C., Kopsell, D. A., & Sams, C. E. (2013). Selenium influences glucosinolate and isothiocyanates and increases sulfur uptake in Arabidopsis thaliana and rapid-cycling Brassica oleracea. Journal of agricultural and food chemistry, 61(1), 202-209.

Chen, Z., Yu, L., Liu, W., Zhang, J., Wang, N., & Chen, X. (2021). Research progress of fruit color development in apple (Malus domestica Borkh.). *Plant physiology and biochemistry : PPB*, *162*, 267–279.

Eberhardt, M. V., Lee, C. Y., & Liu, R. H. (2000). Antioxidant activity of fresh apples. Nature, 405(6789), 903-904.

Gorji, S. G., Smyth, H. E., Sharma, M., & Fitzgerald, M. (2016). Lipid oxidation in mayonnaise and the role of natural antioxidants: A review. Trends in Food Science & Technology, 56, 88-102

Górnaś, P., Mišina, I., Olšteine, A., Krasnova, I., Pugajeva, I., Lācis, G., ... & Segliņa, D. (2015). Phenolic compounds in different fruit parts of crab apple: Dihydrochalcones as promising quality markers of industrial apple pomace by-products. Industrial Crops and Products, 74, 607-612.

Hameed, A., Hussain, S. A., Yang, J., Ijaz, M. U., Liu, Q., Suleria, H. A. R., & Song, Y. (2017). Antioxidants potential of the filamentous fungi (Mucor circinelloides). Nutrients, 9(10), 1101.

Henríquez, C., Speisky, H., Chiffelle, I., Valenzuela, T., Araya, M., Simpson, R., & Almonacid, S. (2010). Development of an ingredient containing apple peel, as a source of polyphenols and dietary fiber. Journal of Food Science, 75(6), H172-H181.

Hyson, D. A. (2011). A comprehensive review of apples and apple components and their relationship to human health. Advances in nutrition, 2(5), 408.6

Leontowicz et al ., Leontowicz M, Gorinstein S, Leontowicz H, Krezeminski R, Lojek A, Katrich E, Ciz M, Martin-Belloso O, Soliva-Fortuny R, Haruenkit R, Trakhtenberg S. Apple and pear peel and pulp and their influences on plasma lipids and antioxidant potential in rats fed cholesterol-containing diets. J Agric Food Chem. 2003; 51:5780–5785.

Ma, X. M., Li, Y. X., Zhang, H. X., Liu, Q., Su, X. H., & Xing, L. X. (2020). Transcriptomic evidence that insulin signalling pathway regulates the ageing of subterranean termite castes. Scientific reports, 10(1), 1-13.

Mariadoss, A. V. A., Ramachandran, V., Shalini, V., Agilan, B., Franklin, J. H., Sanjay, K., ... & Ernest, D. (2019). Green synthesis, characterization and antibacterial activity of silver nanoparticles by Malus domestica and its cytotoxic effect on (MCF-7) cell line. Microbial pathogenesis, 135, 103609.

Roy S, Das K, Mandal S et al. Phytochemical analysis, antimicrobial activity and assessment of potential compounds by thin layer chromatography of ethanol fraction of Asparagus racemosus roots. Int J Pharm PharmSci2014; 6: 367-370.

Rupasinghe, H. V., Erkan, N., & Yasmin, A. (2010). Antioxidant protection of eicosapentaenoic acid and fish oil oxidation by polyphenolic-enriched apple skin extract. Journal of Agricultural and Food Chemistry, 58(2), 1233-1239.

Sultana, B., Hussain, Z., Asif, M., & Munir, A. (2012). Investigation on the antioxidant activity of leaves, peels, stems bark, and kernel of mango (Mangifera indica L.). *Journal of food science*, *77*(8), C849–C852.

Vasile, M., Bunea, A., Ioan, C. R., Ioan, B. C., Socaci, S., & Viorel, M. (2021). Phytochemical Content and Antioxidant Activity of Malus domestica Borkh Peel Extracts. Molecules, 26(24), 7636.

Wiersma, P. A., Zhang, H., Lu, C., Quail, A., & Toivonen, P. M. (2007). Survey of the expression of genes for ethylene synthesis and perception during maturation and ripening of 'Sunrise' and 'Golden Delicious' apple fruit. Postharvest Biology and Technology, 44(3), 204-211.

Wojdyło, A., Oszmiański, J., & Laskowski, P. (2008). Polyphenolic compounds and antioxidant activity of new and old apple varieties. Journal of Agricultural and Food Chemistry, 56(15), 6520-6530.

Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. Journal of agricultural and food chemistry, 51(3), 609-614.

Yang, L. L., Xiao, N., Li, X. W., Fan, Y., Alolga, R. N., Sun, X. Y., & Qi, L. W. (2016). Pharmacokinetic comparison between quercetin and quercetin 3-O-β-glucuronide in rats by UHPLC-MS/MS. Scientific Reports, 6(1), 1-9.