A comparative phytochemical screening for the methanolic peels extract and juices of Apple species via series of qualitative tests

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Abstract

The consumption of apple juice is highly recommended because of various biologically active compounds that have remarkable pharmacological properties. Herein, we reported the comparative screening for the peel extract and juice of Apple via a series of qualitative tests which includes Molish's test, Bendicts's test, Hager's test, Biuret Tests, Brotanger's test, etc. The yield for a methanolic extract of peels of three different color varieties Red Delicious, Golden Delicious, and Granny Smith was recorded to be 20%, 18.2%, and 18% respectively. Apple fruits juices and peels extract were evaluated for bioactive phytochemical which revealed the existence of primary as well as secondary metabolites including terpenoids, phytosterols, and saponins in the peel extract of all varieties, while they were absent in the juices of the same samples. These results indicated that the anthocyanin and anthraquinone glycoside were only present in the peels of Red and Golden Delicious and were absent in Granny Smith peels. Fundamental qualitative analysis will pave the way for the utilization of apple (*Malus Domestica*) in the food and agriculture industries.

Key Words: *Malus Domestica*, Pharmacological, Bioactive, Metabolites, Methanolic extract

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1. Introduction

The plant kingdom is known as the treasury for potential drugs, and within the past few years, awareness about the importance of medicinal plants has been increased. The drugs gained from plants are effortlessly available, cheap, effective, and safe to use with almost no side effects [1]. Plants are the natural prototype of useful prospective medicines. It is believed that about 250,000 to 500,000 different plant species are present on the earth's surface. These plants have been uncovered in a very minor number so far for their antimicrobial and therapeutic properties [2]. Due to the presence of various phytochemicals these Medicinal plants are getting more and more important, that are present in the roots, leaves, stem, vegetables, grains, legumes, nuts, seeds, and fruits that carry potential activity against numerous human and plant diseases [3]. For sustaining life these phytochemicals especially secondary metabolites of plants are not required by the human body as essential nutrients yet they have shown outstanding properties to prevent the human body from diseases [4]. These natural bioactive compounds of plants combine with fibers and nutrients, forming a unified part of human defense mechanisms contrary to diseases and in pressure circumstances [5]. These naturally occurring phytochemicals in plants include proteins, chlorophyll, and common sugar as primary metabolites while Alkaloids, Flavonoids, Phenolic compounds, and Terpenoids, etc. as secondary metabolites [6]. These secondary metabolites or phytochemicals are responsible for the majority of medicinal effects that are present in various parts of the plants [7]. The phytochemicals present in this group are non-nutrients but they have biological activity against chronic diseases [8]. In the field of phytomedicines, for the treatment of innumerable human disorders such as bowels, cholera, wounds & ulcers, etc. different parts of plants including fruits are being used [9]. Numerous epidemiological studies associated with diet and cancer were performed in the previous century by various scientists which concluded that out of 156 almost 128 dietary studies of fruits and vegetables have shown protective effects against many types of cancer [10]. Apple is the common name of *Malus Domestica* which belongs to the family Rosacea that is used in traditional and folklore medicine of many countries and also in modern medicine [11]. Apples are a popular fruit that is high in phytochemicals, and epidemiological studies have linked apple consumption to a lower risk of some malignancies, cardiovascular disease, asthma, and diabetes [12]. The presence of polyphenol contents, especially dihydrochalcones, phenolic acids, flavan-3-ols, anthocyanins, and flavonols, make them interesting for researchers and thus are generally considered as “healthy food” [13]. These compounds are studied intensively in epidemiological research because of their positive impact on human health through the prevention of cancer and cardiovascular diseases, antiviral properties, the inhibition of Helicobacter pylori growth, controlling of plasma cholesterol metabolism, and staphylococcal enterotoxin a toxicity [14]. Due to these reasons, we have selected species of Genus *Malus* of the *rosacea* family *(Malus Domestica)* commonly known as Apple, and worked on the qualitative study of phytochemicals of their fruit parts (Methanolic Peel Extract and pulp juice). (Figure 1

and 2) Such fundamental research on qualitative analysis will pave the way for utilization of apple (*Malus Domestica*) in the food and agriculture industries.



Figure 1: Systematic diagram showing the process adopted for the phytochemical analysis of the selected samples.

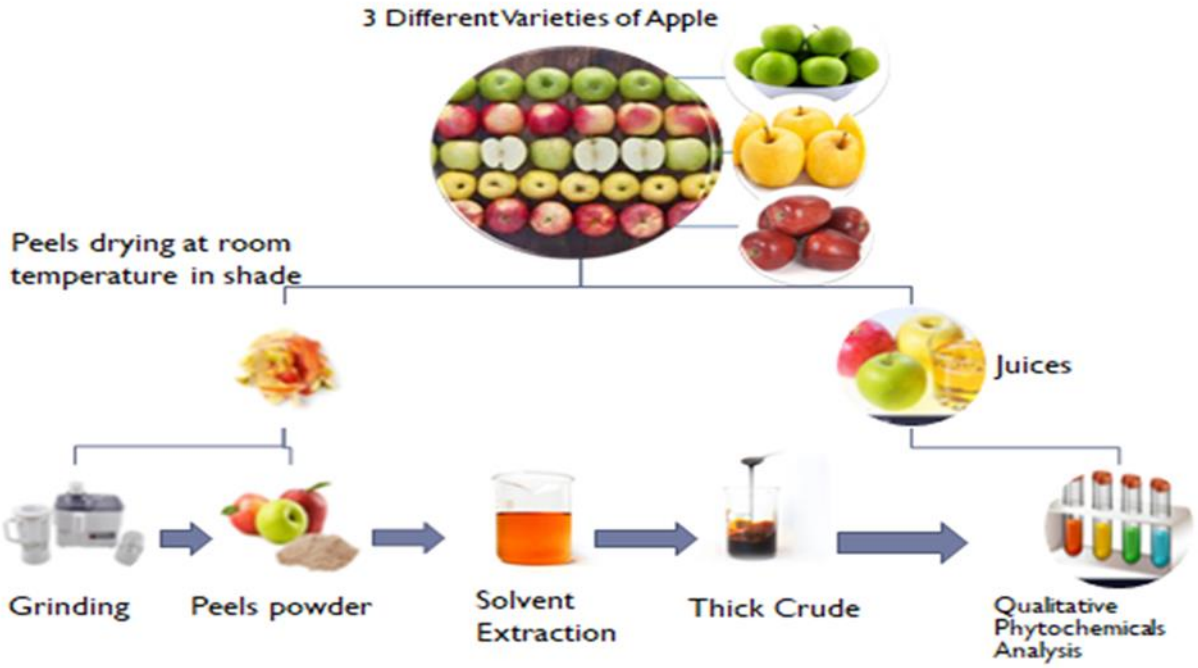


Figure 2: Systematic diagram showing the process adopted for the phytochemical analysis of the selected samples.

2. MATERIALS AND METHODS

2.1 Plant Materials

In the present work we studied three different color varieties of the Rosacea family; Malus mill genus "Malus Pumila or Malus Domestica" species commonly known as apple fruit.

2.2. Sample Collection

We collected 3 kg of three different color varieties of healthy and mature apples, namely (Granny Smith), (Red Delicious), (Golden delicious) Green-colored, Red-colored, and Yellow-colored respectively were purchased from the local market of Dargai Malakand Khyber Pakhtunkhwa Pakistan, during April. After getting information from the sellers it was found that the fruits were about 8 to 10 days old after being picked from the tree. The collected fruit varieties were identified botanically in the department of Botany by Professor Rab Nawaz Khan Govt. Postgraduate College Dargai. During the present investigation varieties of apples that were used for phytochemical analysis are given in (Table 1).

Table 1 – Common names of various varieties of apples

S. NO.	English name	Urdu name	Pashto local name	Pashto countryside name
1.	Granny smith	Sabz Seb	Shna Manrha	Shen Seew
2.	Golden delicious	Peela Seb	Zyarha Manrha	Zyarh Seew
3.	Red delicious	Surkh Seb	Sra Manrha	Sor Seew

2.2 Chemicals

The following chemicals were used for phytochemicals analysis (Table 2).

Table 2– List of chemicals and reagents used for phytochemicals screening test

S. no	Chemicals	Company name
1.	Methanol	Merck, Sigma Aldrich
2.	Barfoed's Reagents	Pancreac
3.	Benedict's reagent	Fluka
4.	Million's Reagents	Aldrich
5.	Wagner's Reagent	Merck, Sigma Aldrich
6.	Sulphuric Acid	Merck, Sigma Aldrich
7.	Hager's reagent	Commercial, BDH, RDH
8.	Selwinoff's Reagents	RDH
9.	Fehling A & B solution	Merck, Sigma Aldrich
10.	Methyl Alcohol	Merck, Sigma Aldrich
11.	HCl	Merck, Sigma Aldrich
12.	Alpha Nepthol	Merck, Sigma Aldrich
13.	Ethyl Acetate	RDH
14.	Ferric Chloride	Merck, Sigma Aldrich
15.	Ammonia	Merck, Sigma Aldrich
16.	Acetic Acid	Merck, Sigma Aldrich

2.3 Preparation of Juice and Peel Extract

The examples (Apples) were first washed with faucet water completely and afterward doused with Distilled water. Strips were taken out from the mash utilizing blades, and the pulps of the apples were cut into little pieces for seeds evacuation. The mash was then ground with the assistance of a juicer at 3200 (RPM) independently for each example until we get the fluid juice structure. The fluid juice was then sifted utilizing cotton texture insect at that point channel paper for capacity at 4°C in fixed jugs till additional investigation. Strips were written down independently and were dried out in conceal for 23 days. In the wake of drying, strips were squashed into adequate powder type of 1 mm size and were pressed in water/air proof polyethylene packs till additional utilization.

Each example was then absorbed utilizing 200ml methanol a different measuring glasses for 3 to 4 days at room temperature and the concentrates acquired were then sifted. To get the second methanolic remove the left buildup, was again exposed to second back to back extraction with methanol, following the recently referenced method. For their TLC profiles, the two concentrates were contemplated and they were blended after getting comparable TLC designs. The methanolic concentrate of past advance was focused by presenting it to air and room temperature to get dry until a thick, gooey mass was gotten. A portion of the thick gooey unrefined was then broken down in refined water in a receptacle and different phytochemical tests were performed.

2.4 Determination of yield value

The values of yield for each extract were found out as:

$$\text{Crude Percentage Yield} = \frac{\text{Crude Extract Obtained}}{\text{Amount of used powdered Sample}} \times 100$$

For methanolic macerated extract

1st Sample (Red Delicious) Yield Value = 20%

2nd Sample (Golden Delicious) Yield Value = 18.2%

3rd Sample (Granny Smith) Yield Value = 18%

2.5 Phytochemicals Screening

The present study was done on various color varieties of Apples (Malus Domestica) Juices and Peels extract which reviled the existence of plentiful medicinal active ingredients. The phytochemical active compounds present in these plants were analyzed quantitatively for peel extract and pulp juice separately using the procedure as described by [15, 16, 17]. and their results are presented in the tables .

The positive symbol shows presence while the Negative symbol shows the absence of the corresponding phytochemicals in corresponding juices and peels extract of *Malus pumila* Mill specie (*Malus Domestica).*

3. Results and Discussion

Yield extraction, qualitative analysis of Juices and peels extract of Malus Domestica

The yield extraction obtained from Red Delicious, Golden Delicious, and Granny Smith by using Methanol was recorded to be 20%, 18.2% and 18% respectively. The extraction method was similar to [18]. The variation in extraction yield is due to difference in solvent polarities [19]. among three species of Malus Domestica.

3.1. *Malus Domestica's* Juices

Juices of Malus Domestica's were taken for the screening through various reagents Carbohydrates, Reducing Sugar, Hexose sugar tests, Amino acid, and hexose sugars were present as primary phytochemicals while protein test was positive for only Red Delicious and Golden Delicious which was negative for Granny Smith.

Furthermore, secondary metabolites such as Tannins, Flavonoids, Polyphenols, and Coumarin were present in all three Varieties. The similar results were also reported by [20]. While Anthraquinie Glycoside and Anthocyanin were only present in Granny Smith and test for Emodins was negative only of Red Delicious. Besides Alkaloids, Cardiac Glycosides, Cyanogenic Glycosides, Phlobotannins, Saponins, and Steroids were absent in all Juices of the selected three varieties.

Table 3: Qualitative screening for primary phytochemicals in juice samples

Phytochemicals	**Reagents used**	**Green Apples**	**Red Apples**	**Yellow Apples**
Carbohydrates	Molish's test	+	+	+
Reducing sugars	Benedict's test	+	+	+
	Fehling's test	+	+	+
Monosaccharaides	Barfoed's test	+	+	+
Hexose sugars	Cobalt Chloride test	+	+	+
	Selwinoff's test	+	+	+
Non-reducing Sugars	Benedict's test	-	-	-
	Fehling's test	-	-	-
Non-reducing Polysaccharides	Iodine test	+	+	+
Protein	Biuret's test	-	+	+
	Million's test	-	+	+
Amino acids	Ninhydrin test	+	+	+
Fixed oils and lipids	Filter paper test	-	+	+

Table 4: Qualitative screening for secondary phytochemicals in juice samples

Phytochemicals	**Regents**	**Green Apples**	**Red Apples**	**Yellow Apples**
Alkaloids	Hager's test	-	-	-
	Wagner's reagent	-	-	-
	Mayer's Reagent	-	-	-
Anthocyanins	Sodium Hydroxide Test	+	-	-
Betacyanins	Sodium Hydroxide Test	-	+	+
Cardiac Glycoside	Acetic acid test	-	-	-
Cyanogenic Glycosides	Sodium Picrate test	-	-	-
Flavonoids	Sulphuric acid test	+	+	+
Phenols	Ferric Chloride Test	+	+	+
Phlobotannins	HCl Test	-	-	-
Saponins	Foam test	-	-	-
Saponins glycosides	Foam Test	-	-	-
Steroids	Salkowiski reaction	-	-	-
Tannins	Ferric Chloride Test	+	+	+
Terpenoids	Chloroform Test	-	+	+
Anthraquinone Glycosides	Borntrager's Test	+	-	-
	Modified Borntrager's test	+	-	-
Chalcones	NH4OH Test	-	+	+
Caumarin	NaOH Test	+	+	+
Emodins	NH4OH Test	+	-	+

3.2 *Malus Domestica's* peels extract.

Methanolic extract from the skin/peels of three different varieties of Malus Domestica was screened for phytochemical analysis and the following results were obtained. Carbohydrates, Reducing Sugar, Hexose sugar tests, Amino acid, Fats/Oils, and hexose sugars were present as primary phytochemicals as same as juices.

Table 5: Qualitative screening for primary phytochemicals in Peel Extract

Phytochemicals	Reagents used	Green Apples	Red Apples	Yellow Apples
Carbohydrates	Molish's test	+	+	+
Reducing sugars	Benedict's test	+	+	+
	Fehling's test	+	+	+
Monosaccharaides	Barfoed's test	+	+	+
Hexose sugars	Cobalt Chloride test	+	+	+
	Selwinoff's test	+	+	+
Non-reducing sugars	Benedict's test	-	-	-
	Fehling's test	-	-	-
Non-reducing polysaccharides	Iodine test	-	+	+
Protein	Biuret's test	-	+	+
	Million's test	-	+	+
Amino acids	Ninhydrin test	+	+	+
Fixed oils and lipids	Filter paper test	-	+	+

There was a slight difference in the secondary metabolites between juices and peels extract. Secondary metabolites such as Tannins, Flavonoids, Saponins, terpenoids, Polyphenols, Phytosterols, and Caumarin were present in peels of all three samples. These results were also supported by [21]. Whereas anthocyanin, Cyanogenic Glycoside, and Anthraquinone Glycosides were present only in Golden and Red Delicious and were absent in Granny smith. Cardiac glycoside was only present in Red Delicious Peel. Furthermore, phytochemicals that were absent in the peels of all three varieties are Alkaloids, Saponins glycoside, and Phlobotannins.

Table 6: Qualitative screening for Secondary phytochemicals in Peel extract

Phytochemicals	**Regents used**	**Green Apples**	**Red Apples**	**Yellow Apples**
Alkaloids	Hager's test	-	-	-
	Wagner's reagent	-	-	-
	Mayer's Reagent	-	-	-
Anthocyanin	Sodium Hydroxide Test	-	+	+
Betacyanin	Sodium Hydroxide Test	+	-	-
Cardiac Glycoside	Acetic acid test	-	+	-
Cyanogenic Glycosides	Sodium Picrate test	-	+	+
Flavonoids	Sulphuric acid test	+	+	+
Phenols	Ferric Chloride Test	+	+	+
Phlobotannins	HCl Test	-	-	-
Saponins	Foam test	+	+	+
Saponins glycoside	Foam Test	-	-	-
Steroids	Salkowiski reaction	-	-	-
Tannins	Ferric Chloride Test	+	+	+
Phytosterols	Sulphuric acid Test	+	+	+
Terpenoids	Chloroform Test	+	+	+
Anthraquinone Glycoside	Borntrager's Test	-	+	+
	Modified Borntrager's test	-	+	+
Chalcones	NH_4OH Test	-	+	+
Caumarin	NaOH Test	+	+	+
Emodins	NH_4OH Test	+	-	+

4. Conclusions

This study has shown that the yield for methanolic extract of Red Delicious was higher among studied species of apple. However, various phenolic compounds and flavonoids were present in both extract. This is the first report of comparative screening of phytochemical of three different kinds of *Malus Domestica*. The information collected in this investigation indicates that Apple tree (*Malus Domestica*) especially its fruit is widely known for its pharmacological properties, which has also been supported by several scientific

studies related to its composition (Mainly flavonoids phenolic compounds, carotenoids quercetin, catechin, phloridzin) and its pharmacological properties.

From the present we have recommended the selected fruits as a medicinal plant for the isolation of active compounds. We are suggesting performing quantitative analysis of these varieties as well as other varieties as well. Furthermore we would like to extend this study to various activities such as antibacterial, antifungal, anticancer and anti-inflammatory.

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Authors Contribution

Kashif Ali performed the measurements, Hazrat Bilal was involved in planning and supervised the work, Iram Islam and Kashif Ali processed the experimental data, performed the analysis, drafted the manuscript, designed the figures, performed the calculations, aided in interpreting the results and worked on the manuscript. Dr. Muhammad Usman provided critical feedback and helped shape the research, analysis and manuscript.

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