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Relative Phenolic Profile, ROS Scavenging, and Anti-haemolytic Potential of Polarity-Driven Peel Bioactive Compounds of Distinct *Malus* species Indigenous to Kashmir

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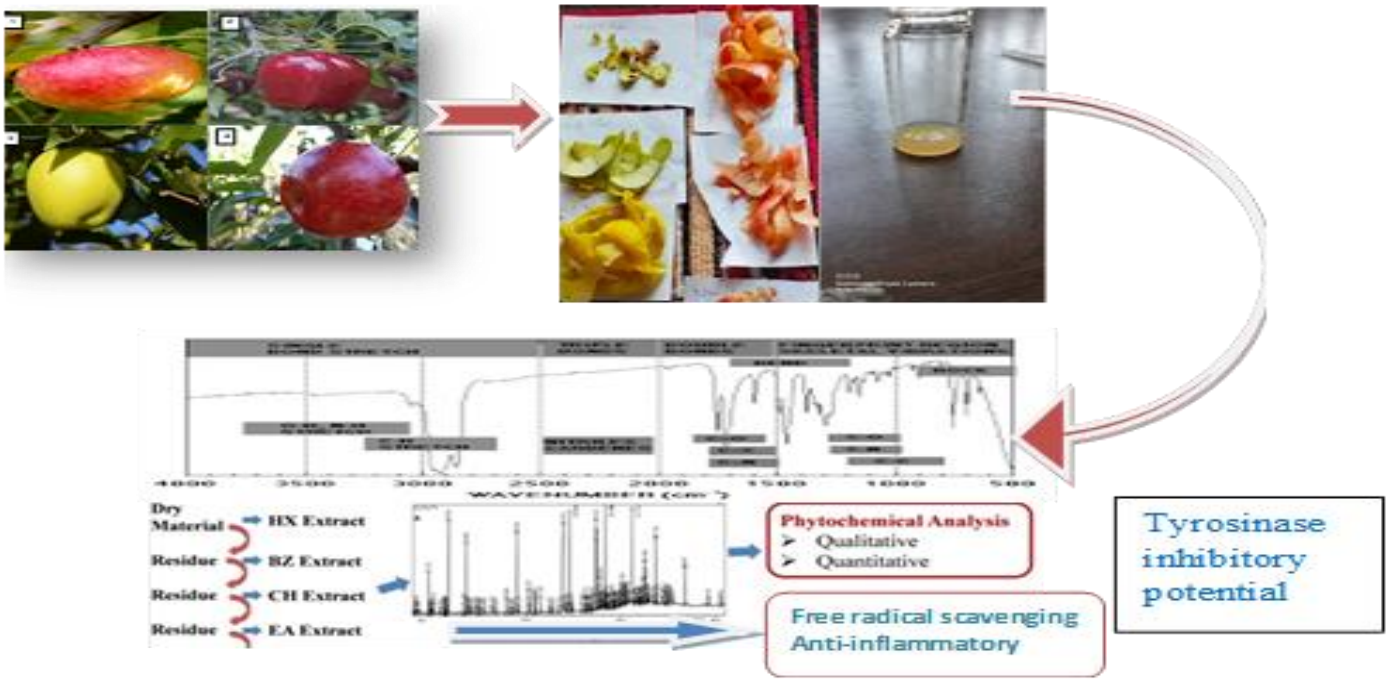
ABSTRACT

Malus domestica, Brokh, is a vital source of phenolic and polyphenolic compounds whose accumulation in the different parts of the fruit depends upon various intrinsic and extrinsic factors. This study aimed to perform a comparative evaluation of secondary bioactive compounds of peels of three red-skinned ("Delicious", "Maharaji", "Royal delicious"), and one yellow-skinned ("Golden Delicious") Malus varieties followed by estimation of Total phenol and total flavonoid contents. Secondary phytocompounds being usually polar in nature were extracted from peels via the Soxhlet and hot maceration processes using different menstruum systems of varied polarity. Comparative assessment of free radical scavenging and anti-haemolytic potential of all the polarity driven compounds was analyzed thereafter. TPC/TFC estimation was analyzed via spectrophotometry as the equivalents of standard phenol and a flavonoid. Free radical scavenging capacity was analyzed via 2, 2-diphenyl-1-picrylhydrazyl assay. The anti-hemolytic flair was gauged by the degree of suppression of HRBC membrane lysis triggered via H_2O_2 exposure. Methanol crude peel extracts of all malus species revealed rich phytochemical content. In a dose-dependent manner, all the crude peel extracts demonstrated considerable free radical scavenging capacity. Maharaji methanolic fraction revealed comparatively higher free radical scavenging activity by showing the lowest IC50 value while Royal delicious acetone extract showed the highest IC50 value when collated with the potent reference antioxidant ascorbic acid with an IC50 value of 114.62µg/ml. The significant level of HBRC membrane stabilization was shown by the methanolic fractions followed by ethyl acetate retrieved exudates when juxtaposed with NSAID standard diclofenac sodium showing 94.58 % inhibition at a dose of 1600 mcg/ml. Maharaji methanolic extract again outperformed in stabilizing HRBC membrane, showing 69.16 % inhibition at a dose of 1600 mcg/ml. Methanol extracts of *Malus domestica* var Maharaji outperformed the other Malus peel extracts in terms of all the parameters analyzed, according to the current study. So, this study is giving the highest pharmaceutical priority to the *Malus domestica* var Maharaji peel bioactive compounds, when it comes to human-health benign bioactive compounds in oxidative stress-related diseases, inflammatory disorders, erythrocyte cell membrane disorders like hereditary pyropoikilocytosis (HPP), and other hemolytic ailments.

Keywords: Malus Bioactive compounds; HRBC membrane stabilization, *Malus domestica* var Maharaji; Anti-inflammatory, Oxidative stress.

Figure 1: Vital source of phenolic and polyphenolic compounds



INTRODUCTION

Apple, (*Malus domestica*, Brokh), the fruit of the domesticated tree *Malus domestica* (family Rosaceae) is one of the most popular and widely cultivated fruit crops in temperate regions. Most of the particular properties of apple fruits, such as flavor, aroma, and color, are intimately correlated with their metabolic profile. At the same instant, many bioactive compounds might be found as indicators of a certain apple type. In general, apples have a diverse and well-balanced constitution of nutrients; water makes up more than 84 % of their weight, in which minerals like Na, Mg, k, and Ca as well as trace minerals like Se, F, Fe, Mo Mn Cu B, and Zn, are dispersed [1, 2].

They are high in practically all vitamins, with the B complex and vitamin C being the most plentiful. Proteins and lipids contribute just a modest amount of nutrients to *Malus domestica* fruits due to their low quantities [3]. When contrasted to other fresh fruits, apples have a high fiber content, which includes complex polysaccharides including celluloses, pectin, lignin, and hemicelluloses. Furthermore, apples are a rich source of phenolic and polyphenolic secondary phytochemicals, which provide them with a lot of antioxidant potential [4]. As a result, frequent apple eating has been related to better health advantages and a lower risk of degenerative illnesses. To stay fit and healthy, the popular phrase "An apple a day keeps the doctor away!" is widely encouraged and actively pushed nowadays by the general population. The existence of a wide spectrum of phenolic and polyphenolic bioactive molecules in apples, as in many other fruits, may be substantially responsible for their favorable biological influence. Apple peels had the highest concentration of these compounds [5], suggesting that apple peels may have more antioxidant potential and bioactivity than apple flesh. As a result, eating an apple with the skin on is strongly recommended [6].

Plant secondary metabolites (PSM) are physiologically active molecules that have a unique defence and ecological role in plants, and their discovery has sparked a lot of interest in pharmacognosy research in the last two decades because they revealed anti-inflammatory, anti-oxidative, anti-carcinogenic, anti-tumour, anti-hypertensive, anti-viral, anti-allergic, anti-mutagenic, anti-ageing, cardio protective, and immune modulatory properties, as well as the ability to modulate enzymatic functions, inhibit cell proliferation, induce apoptosis, and inhibit bacterial and fungal growth among others [7, 8] **Figure 1**. Current research, by applying the principles of bioinformatics and molecular docking, is now forecasting their possible use as substrates for biochemical reactions, cofactors for enzymatic reactions, ligands that antagonize or agonize cellular receptors, etc. The primary goal of incorporating plant-based bioactive chemicals into healthcare is to produce a beneficial interaction with the body's physiology while eschewing the unintended and off-target detrimental effects that pharmaceuticals are notorious for [9]. Among the different varieties of apples, flavonoids, hydroxycinnamic acids, anthocyanins, chlorogenic acids, quercetins conjugates, procyanidins, polyphenols, carotenoids, terpenes, and organic acids are the main categories of organic compounds that are significantly present [10]. Considering the beneficial effects of plant bioactive compounds on human health, this study was undertaken to investigate the therapeutic potential of polar peel decoctures of distinct Malus species. Oxidative stress in the current era where pollution is at its peak has wreaked havoc on human health. An ample number of diseases and disorders have been identified which are induced primarily by the load of reactive oxygen species. Cancers, cardiovascular diseases like ischemia, neurological disorders like

paralysis agitans, inflammatory diseases like Chron's disease, ulcerative colitis and atopic disorders which are causes of significant morbidity and mortality, are all linked to ROS damage.

Figure 2: Human health related benefits of plant secondary metabolites

As a result, bioactive substances from organic sources are required that have free radical scavenging, immunomodulatory, and inflammation-reducing capabilities. Although apples contain a variety of bioactive chemicals, these phenolics are principally responsible for their potent free radical scavenging, anti-inflammatory, anti-aging, cardioprotective, and immunomodulatory properties. The antioxidant potential of most of these molecules is due to the occurrence of many double bonds and hydroxyl groups in their structures [11]. Hydroxycinnamic acids mainly chlorogenic acid, flavan-3-ols mainly (+)-catechin, (-)-epicatechin, flavonols mainly different quercetin glycosides, dihydrochalcones such as phloridzin, anthocyanidins, and anthocyanins are the fundamental families of polyphenolic chemicals found abundantly almost in all apple cultivars [12,13,14]. As per research, monomeric and polymeric flavan-3-ols account for 60% of the total phenolic content in apple peel, while flavonols account for 5%, hydroxycinnamic acids 18%, dihydrochalcones 9% and anthocyanins 5% of the total phenol concentration [15]. Procyanidin, (+)-catechin, (–)-epicatechin, chlorogenic acid, cyanidin-3-galactoside, Quercetin-3-galactoside, Quercetin-3-glucoside, quercetin-3-rhamnoside, gallic acid, coumaric acid, and phloridzin are some of the most well-studied flavonoids/phenolics in different apple varieties (Figure 2). Because of their good degree of Cyanidin glycosides (anthocyanins), which are mostly concentrated in the peel, red-skinned apples are gaining popularity. Even though the pigments associated with colour may vary, the amounts of anthocyanins within the phenolic categorization are considered the major contributors to apple skin reddening. Light, temperature, nutrition, and genetic factors significantly influence apple anthocyanin levels and colour development as the fruit matures [16]. Individual phenolic and polyphenolic phytochemical concentrations in apples and other fruits differ according to the type of soil and climatic conditions, cultivation method, type of cultivar, stage of growth and ripeness, environmental stress circumstances, and other aspects like storage spell, postharvest conditions, etc. [17] So, the results from different researches can't be readily compared to arrive at final conclusions. The aim of this study was to comparatively evaluate the phytochemical makeup, total phenolic and flavonoid content, free radical scavenging capacity, and anti-hemolytic potential of four distinct apple cultivars peels that are grown on a large scale in Kashmir viz *Malus domestica* var Maharaji, *Malus domestica* var Delicious,−*Malus domestica* var Golden,−*Malus domestica* var Royal Delicious to reveal that which species is phytochemically and pharmaceutically striking.

MATERIALS AND METHODS

Sampling and preparation of apple peel polar decoctures

All of the apple varieties utilized in this investigation (Delicious, Maharaji, Royal delicious, and Golden) were obtained in September 2022 from various apple gardens in Dodarkoot, a district in Kashmir's Kulgam region, which is situated at 33°38′24″N 75°01′12″E and has an average altitude of 1739 metres. Only healthy-looking fruits were selected via simple random sampling. Fruits were collected and cleaned thoroughly with tap water before being scoured with distilled water. Subsequently, all the fruits were peeled aseptically, dried in shade at room temperature for 20-25 days, then pulverized and finally stored in airtight containers until proceeded for further investigations. Polar menstruum systems of variable polarity (Methanol, Acetone, and Ethyl acetate) were employed to extract the pulverized samples involving the aid of hot maceration and Soxhlet extraction [20].The cotton-plugged conical flasks containing the 20g of pulverized samples and 100 ml of respective menstruum systems were constantly swirled at 120 rpm in a shaker incubator for 72 hours at 37°C. The retrieved extracts were filtered thru the Whatman (No. 1) filter paper and muslin cloth before being concentrated using a rotary evaporator at a temp that didn't go above the boiling point of the solvents. The derived gummy exudates were then placed in plastic screw-cap tubes and kept below room temperature until they could be weighed in order to determine the extractive yield. Before being put through analysis of phenolics, free radical scavenging, tyrosinase inhibitory and anti-inflammatory assays, the crude extracts were kept at 4°C [18,19,20].

Preliminary screening of Secondary Phyto active compounds extracts

All of the crude extracts underwent traditional procedures

developed by Trease and Evans in 2002 [21] to screen for the presence or absence of particular secondary Phytoactive compounds. Like flavonoids, quinones, alkaloids, phytosterols, phenols, polyphenols, terpenoids, glycosides, chalcones, phlobatannins, coumarins, anthraquinones, emodins, saponins, tannins**,** andanthocyanins

Assessment of total phenolic content

Total phenolic content was determined via Folin-Ciocalteau's reagent assay as reported by Singleton *et al*., [22] with minor adjustments and the findings were represented as gallic acid equivalent (GAE) per gram dry weight.

Preparation of standard

Known Gallic acid concentrations of 50, 100, 150, 200, and 250 µg/ml were used to create a standard curve. To begin, 100 mg of gallic acid was dissolved in 100 mL of 80 % methanol to make a stock gallic acid solution. The standard working solutions were then made by pipetting 0.5, 1, 1.5, 2-, and 2.5-mL aliquots of a stock gallic acid solution into volumetric flasks and diluting to volume with 80 % methanol (final volume 10ml). After that, 1 mL of each of the standard solutions was pipetted into a separate test tube, followed by the addition of 5 mL of a 10% aqueous dilution of Folin-Ciocalteu's reagent, which was mixed well for about 1 minute using a vortex mixer. 8 mL of a 7.5 % Na_2CO_3 solution was added after 3- 8 minutes. The mixture was well stirred for another minute before being incubated in the dark for half an hour. Finally, the absorbance at 760 nm was measured, and a standard curve was created beforehand.

Preparation of sample

1 mL of each sample extract at a concentration of 1 mg/mL was combined with 1 mL Folin-Ciocalteu's reagent, and then 10 mL of 7% sodium carbonate solution was added. A UV Vis Spectrophotometer was used to measure absorbance at 725 nm after the mixture was allowed to stand for 1 hour at 25 ± 2^0C in the dark. TPC was measured in milligrams of gallic acid equivalent (GAE) per gram of dry weight of the sample.

Assessment of total flavonoid content

Colourimetric analysis with aluminium chloride was used to determine the flavonoid content [23].

Preparation of standard

To get a concentration of 1 mg/ml, a standard stock solution (primary) was generated by dissolving 100 mg of quercetin in 10 ml of 80 % methanol and then diluting it to 100 ml in a 100 ml volumetric flask. 0.5, 1, 1.5, 2-, and 2.5-mL aliquots of this stock solution in a final volume of 10mL were used to create a standard curve of known quercetin concentrations (50, 100, 150, 200, and 250 µg/ml). 1 mL of each standard solution was mixed with 3 mL of 95% ethanol, 0.2 mL of a 10% aqueous dilution of $AlCl_3$ reagent, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water in test tubes. The mixture was vigorously stirred with a vortex mixer for about 30 seconds before being set aside at room temperature for 30 minutes. A UV/Visible Spectrophotometer was used to collect absorbance data at 415 nm, and a standard curve was created beforehand.

Preparation of sample

To get a concentration of 1 mg/ml, 100 mg of dry extract was dissolved in 10 ml of 80 percent methanol and diluted to 100 ml with the solvent. A 10ml volumetric flask holding 3ml of 95% methanol (v/v) was filled with 1 ml of this solution. A 0.2 ml 10% aqueous dilution of aluminium chloride was added to the flask. 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water were added after 5 minutes. The solution was well mixed and incubated for 30 minutes at room temperature. At 415 nm, the absorbance of the reaction mixture was measured. The calibration curve was used to determine the conc. of test samples, which were examined in triplicate. The mean value is expressed in mg/g of extract as quercetin equivalent.

DPPH free radical scavenging activity

DPPH test is the classic and simple colorimetric approach for evaluating the antioxidant capabilities of pure substances as well as the scavenging activity of plant extracts [24]. The antioxidant compounds cause the reduction of 2, 2-Diphenyl-1-Picrylhydrazyl radical to the yellow product 1, 1--Diphenyl-2-Picrylhydrazine in this test, and the amount of the reaction is determined by the hydrogen donating ability of the compounds under examination. DPPH solution 0.2mM (0.008% w/v) was prepared in 95% methanol. All apple peel extracts were mixed with 95 % methanol to prepare their stock solutions (5 mg/mL). Five working concentrations of each extract (100-500 µg/ml) were added individually from their stock to the newly made DPPH solution in a final volume of 3 mL, and the absorbance was measured at 517 nm using a spectrophotometer after 30 minutes. Ascorbic acid was used as a reference standard for which a standard curve was developed beforehand. The calibration curve was created by making ascorbic acid solutions in distilled water at concentrations ranging from 40 to 200 µg/ml from a stock solution of the same concentration (5 mg/mL). A control sample with the same volume of DPPH but no extract or reference ascorbic acid was created. 95 % methanol was served as blank. % Inhibition of the DPPH free radical was derived by using the following equation:

$$\% \text{ RSA} = \frac{(\text{Abs}_{517} \text{ control} - \text{Abs}_{517} \text{ sample})}{\text{Abs}_{517} \text{ control}} \times 100$$

For repeated trials, the inhibition curve was plotted and expressed as a percentage of mean inhibition ± standard deviation. Interpolation from linear regression analysis was used to establish the inhibitory concentration IC_{50}.

Anti-hemolytic potential via cell membrane stabilization

Membrane stabilization is the method by which anti-inflammatory medications preserve the structural integrity of the cell membranes by stabilizing the membrane proteins and prevent hemolysis. This is especially important for lysosomal membrane.

Lysosomes comprise numerous enzymes that may be connected to the process of inflammation [23]. Lysosomal enzymes get discharged into the cytosol following inflammatory response, triggering wreckage to the tissues adjacent to it and consequently initiating inflammation. It any bioactive compound stabilizes HRBC membrane (anti-Hemolytic), which is akin to lysosomal membrane, we anticipate that it will also stabilize the lysosomal membrane. By limiting the ejection of lysosomal enzymes, most anti-inflammatory like NSAIDs stabilize the membrane of lysosomes and reduce inflammation. The in vitro membrane efficacy of the polar fractions of the Malus peels was estimated using the human red blood cell stabilization (HRBC) approach. The primary concept at work here is to stabilize the human red blood cell membrane to prevent lysis triggered by ROS exposure [24].

Healthy person's blood was obtained after taking consent and mixed with an equal volume of sterilized Alsever's solution, which is made up of 0.8% sodium citrate, 0.42% sodium chloride, 2% dextrose, and 0.05% citric acid in water. This solution acts as an anti-coagulant and blood preserver. The compacted cells in this blood mixture emerged after 12 minutes of centrifugation at 3000 rpm. A 10% v/v suspension of RBC's with isosaline solution (0.87%, pH 7.1) was made after isosaline washing of packed cells. The assay mixture was prepared by mixing 1mL phosphate buffer (pH 7.4, 0.15M), 2mL hyposaline solution (0.36%) and 0.5mL HRBC suspension (10% v/v) with 1 mL of each decoctures of various concentrations (100, 200, 400, 800 and 1600 µg/mL) and standard NSAID indomethacin (100, 200, 400, 800 and 1600 µg/mL), respectively.

A reaction mixture of erythrocytes with hydrogen peroxide (20%) served lysis triggering control to induce complete hemolysis (anticipated 100%). A reaction mixture of erythrocytes in the isotonic solution (composed of 154 mM NaCl in 10 mM sodium phosphate solution, Ph 7.4) served as another control that would induce 0% hemolysis. The mixtures were incubated at 37°C for 30 minutes and then centrifuged at 3000rpm. The hemoglobin content in the supernatants of all reaction mixtures was estimated spectrophotometrically at 560nm, the release of which from erythrocytes is dependent on % hemolysis. Anti-hemolytic, membrane stabilizing, or Anti-inflammatory activity was expressed as the % hemolysis and % Protection as follows when it is anticipated that the H_2O_2 triggered 100% hemolysis.

% Hemolysis = [(OD540 of Test sample / OD540 of lysis triggering control) × 100]

% Protection = 100 – [(OD540 of Test sample / OD540 of lysis triggering control × 100]

Statistical analysis

At the very least, all measurements were recorded in triplicate and approximated. The 95% confidence interval (P 0.05) was used in this investigation. To reveal all data, the mean standard deviation of three replicates has been used. The analysis of variance (ANOVA) method was utilized to evaluate the findings using SPSS software.

RESULTS AND DISCUSSION

Secondary phytochemical analysis

The extraction yield differs depending on the solvent systems utilized and the properties of dried samples. Methanol extract gave the maximum extractive yield compared to the other extraAcetone and Ethyl acetate produced the least amount of extract. The phytochemical screening for the existence or lack of a particular class of secondary compounds produced the qualitative results listed below **Table 1.**

Total phenolic and total flavonoid content

The total phenolic content was calculated using a calibration curve that was created beforehand ($y = 0.0079x + 0.2866$, $R^2 = 0.9861$) of gallic acid (50–250 µg/mL) and was demonstrated in gallic acid equivalents (GAE) per gram dry extract weight (DW). All the extracts in different solvents contained different amounts of phenolics. The total phenolic content varied from 105.78 to 198.99 mg GAE/G dry weight of the sample **Table 2**.

Methanolic extract of Maharaji peel, according to the present study, contained the highest phenolics and Royal delicious acetone extract contained the least amount of total phenolics comparatively. The results for total flavonoid content were derived from a calibration curve, developed beforehand ($y = 0.002x – 0.0076$, $R^2 = 0.9988$) of quercetin (50–250 µg/mL) and were demonstrated as quercetin equivalents (QE) per gram dry extract weight (DW). The total flavonoid content also varied in the range from 19.1 mg to 54.26 mg QE/G dry weight of the sample **Table 2.** The Methanolic extract of Royal delicious peel, according to the present study, contained the highest flavonoid content and the peels of the Golden variety extracted in acetone showed the least amount of total flavonoid content comparatively **Figure 2**.

Figure 2: IC50 values of peel extracts in different solvents

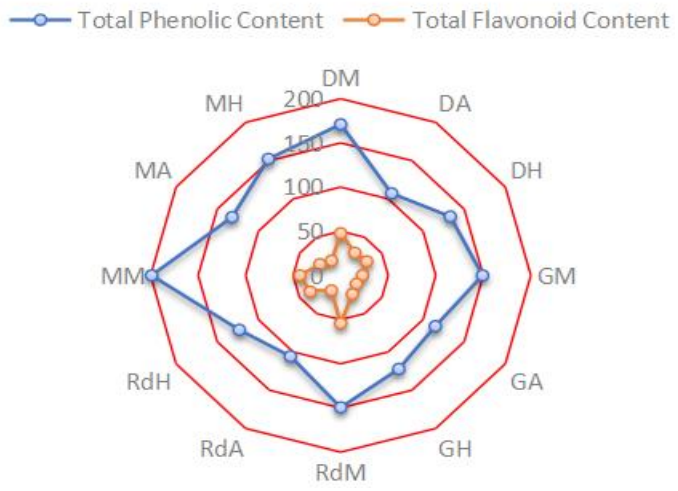


Table 1: A preliminary examination of the Phenolics in four different apple varieties' peel extract

	DM	DA	DE	GM	GA	GE	RdM	RdA	RdE	MM	MA	ME
FLAVONOIDS	+ +	+	+ +	+	+	+ +	+	+	+	+ +	+	+ +
ANTHRAQUINONES	-	-	-	-	-	-	-	-	-	-	-	-
ALKALOIDS	+	-	+	-	-	+ +	+ +	+	+	+	-	+
QUINONES	+	-	-	-	-	-	-	+	-	+	-	-
GLYCOSIDES	+ +	+	+ +	+	+	+	-	+	-	+ +	+	+
PHENOLS	+	+	-	+	+	+	+	+	+	+	+	-
POLYPHENOLS	+	+	+	+ +	+	+ +	+	+	+ +	+ +	+	+
TANNINS	+	-	+	+ +	-	+	+ +	+	-	+ +	-	+
PHYTOSTEROLS	+	-	-	-	-	+	-	+	-	-	-	-
PHYTOBATANNIN	-	-	-	+	+	-	+	+	+	-	-	-
SAPONINS	-	-	-	-	-	-	+ +	+	+	+	+	-
CHALCONES	+	-	+	-	-	+	+ +	-	+	+	+	-
TERPENOIDS	+ +	+	-	+	+	-	-	+	-	+ +	+	+
ANTHOCYANINS	+ +	+	+	-	-	-	++	+	+	+	+	+
COUMARINS	-	-	-	-	-	-	-	-	+	+	-	+
EMODINS	-	-	-	-	-	-	-	-	-	-	-	-

+ = Present, + + = Relatively abundant, - = not detected, DM- Delicious Methanol, DA-Delicious Acetone, DE-Delicious Ethyl acetate, GM-Golden Methanol, GA- Golden Acetone, GE- GoldenEthyl acetate, RdM-Royal delicious Methanol, RdA- Royal delicious Acetone, RdE- Royal delicious Ethyl acetate, MM-Maharaji Methanol, MA-Maharaji Acetone, ME-Maharaji Ethyl acetate.

Table 2: Total phenolic and total flavonoid content of different apple peels in different extracts

PEEL EXTRACT	Total Phenolic Content (mg GAE/G DW)	Total Flavonoid content (mg QE/G DW)
DM	170.96 ± 16.512	47.62 ± 9.354
DA	107.19 ± 5.011	29.57 ± 4.882
DH	133.17 ± 8.376	31.65 ± 5.277
GM	149.23 ± 5.891	22.8 ± 6.878
GA	114.94 ± 3.554	19.1 ± 4.835
GH	122.25 ± 27.460	24.52 ± 5.278
RdM	149.49 ± 8.718	54.2611 ± 2.547
RdA	105.78± 11.377	19.74 ± 4.623
RdH	123.20 ± 20.369	36.98 ± 3.563
MM	198.99 ± 16.416	42.96 ± 2.282
MA	131.95 ± 7.645	25.33 ± 5.232
MH	152.23 ± 12.652	19.267 ± 2.100

GAE- gallic acid equivalent; FW- fresh weight; QE- quercetin equivalent; The values are presented as the mean ± standard deviation (n=3). Different letters within a column denote significant differences ($p < 0.05$) by ANNOVA test

Antioxidant potential of peels

In a dose-dependent manner, all the crude peel extracts demonstrated considerable antioxidant capacity when collated with the reference antioxidant ascorbic acid. Table 3 shows the % inhibition values of all peel extracts in various solvents. The IC50 value was calculated using the percent scavenging versus concentration curve, which reflects the amount of extract that scavenged/reduced 50% of the DPPH radical. Antioxidant activity is said to be low when the higher concentration reduces 50% of the DPPH solution. The reference standard ascorbic acid had an IC50 value of 114.62µg/ml in this experiment, while the IC50 values of the various extracts varied but were substantial **Figure 3**. The total antioxidant activity of the samples significantly elevated the pattern. Maharaji methanolic extract showed the lowest IC50 value, thus the highest free radical scavenging activity while Royal delicious acetone extract showed the highest IC50 value and thus comparatively lower antioxidant activity.

Figure 3: IC50 values of peel extracts in different solvents

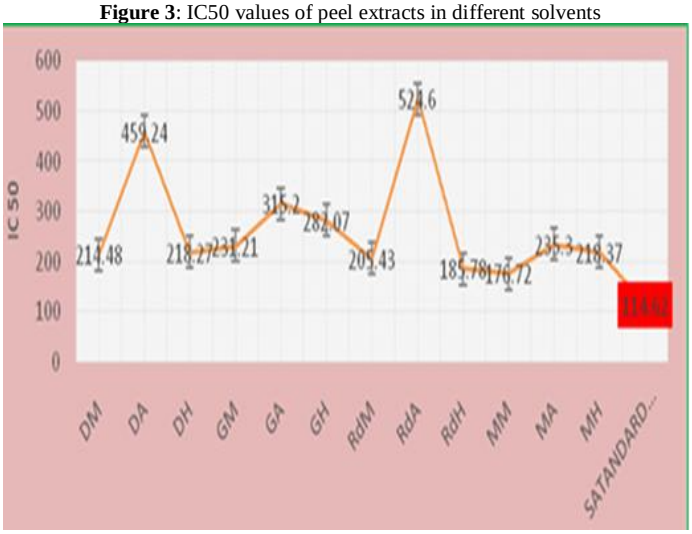


Table 3: % DPPH radical scavenging activity of samples at different concentrations

Peel extract	% DPPH radical scavenging activity of sample at different concentrations				
	0.05mg/ml	0.1mg/ml	0.15mg/ml	0.2mg/ml	.25mg/ml
DM	14.93	24.21	36.03	46.49	58.20
DA	7.47	12.83	18.84	23.58	27.88
DH	12.20	26.12	35.63	45.75	56.46
GM	12.26	23.09	32.90	41.88	55.06
GA	5.60	12.01	18.64	27.18	34.86
GH	7.95	16.11	24.45	33.85	42.46
RdM	12.84	26.90	37.61	48.39	60.01
RdA	5.05	10.60	14.65	19.10	24.33
RdH	13.60	25.82	41.56	56.07	65.32
MM	14.52	28.09	42.90	57.26	69.86
MA	10.73	20.95	32.50	42.80	52.72
MH	11.91	22.17	34.25	45.73	57.85
All values are means of triplicate measurements of absorbances included in the % inhibition formula (n=3) P<0.05 by ANOVA test.					

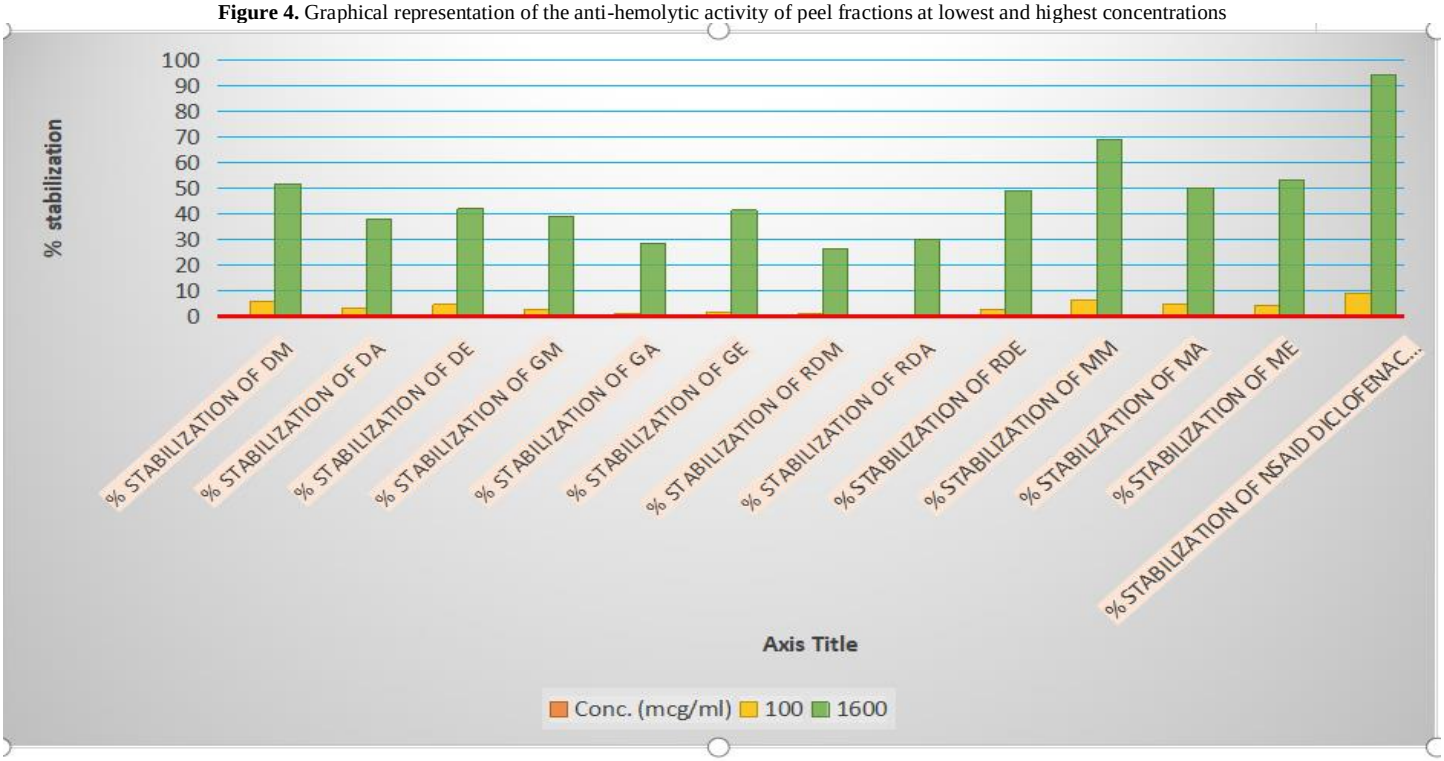
Anti-hemolytic capacity of the fractions via HRBC membrane stabilization

The ability to prevent HRBC membrane lysis brought on by H_2O_2 exposure served as a measure of the anti-hemolytic potential, which in other words is the degree of stabilization of the HRBC membrane. All fractions and standard NSAID Diclofenac sodium were tested for membrane stabilization at doses of 100, 200, 400, 800 and 1600 µg/mL. The significant level of HBRC membrane stabilization was shown by the methanolic fractions followed by ethyl acetate retrieved exudates when juxtaposed with NSAID standard diclofenac sodium. Methanol derived exudate of the Maharaji peels turned out to be potent in inhibiting the H_2O_2-triggered hemolysis of HRBC (69.16 % at a dose of 1600 mcg/ml) when compared with standard NSAID showing 94.58 % inhibition at a dose of 1600 mcg/ml. At 1600 mg/ml, the other extracts also exhibited low to moderate activity **Table 4**. The membrane hemolysis is lowered, and the membrane stabilization is enhanced with increasing concentration of the samples **Figure 4**. Consequently, the anti-hemolytic or membrane stabilizing action of the polarity derived bioactive compounds will be said to be dose-dependent.

Table 4: % HRBC membrane stabilization activity of different extracts and standard NSAID at different concentrations.

Peel extract	% HRBC membrane stabilization activity of different extracts and standard NSAID at different concentrations.				
	100mg/ml	200mg/ml	400mg/ml	800mg/ml	1600mg/ml
DM	5.84	7.31	13.83	28.01	51.92
DA	3.41	4.62	8.92	18.34	38.31
DE	4.72	9.41	16.90	28.41	42.03
GM	2.82	5.62	11.06	20.96	39.10
GA	1.42	3.61	8.01	14.41	28.50
GE	1.78	4.42	10.21	24.57	41.56
RdM	1.08	2.63	5.22	12.63	26.41
RdA	0.99	3.21	7.11	15.24	30.21
RdE	2.70	6.21	13.32	26.56	49.21
MM	6.68	11.27	19.31	36.52	69.16
MA	4.82	9.42	15.99	27.81	50.31
ME	4.32	8.72	17.06	31.31	53.31
All values are means of triplicate measurements of absorbances included in the % inhibition formula (n=3) P<0.05 by ANOVA test.					

Figure 4. Graphical representation of the anti-hemolytic activity of peel fractions at lowest and highest concentrations



The current study aimed to perform a comparative evaluation of secondary metabolites of peels of three red-skinned ("Delicious", "Maharaji", "Royal delicious"), and one yellow-skinned ("Golden Delicious") Malus varieties incorporating preliminary secondary metabolite perusal followed by TPC/TFC scrutiny. Comparative assessment of Free radical scavenging potential and HRBC membrane stabilization potential was also analyzed. Secondary metabolites being usually polar in nature were

extracted from peels via the Soxhlet and hot maceration processes using different menstruum systems of varied polarity. Apple is an important source of phenolic and polyphenolic compounds whose accumulation in the different parts of the fruit depends upon various intrinsic and extrinsic factors. Fruit cultivar, fruit growth stage, the pattern of cultivation, soil type, environmental stress circumstances, weather variables, and other factors like storage time, postharvest scenarios, and so on all affect individual phenolic and polyphenolic phytochemical concentrations in apples [7, 17].

All of these factors impact the overall phenolic content of fruits, as well as their antioxidant and pathogen-defending abilities. As a result, data from the original study done on a variety of cultivars under a variety of conditions cannot be easily compared to arrive at a conclusion. The menstruum included and the chemical properties of the samples are the two most significant factors affecting the retrieval of various classes of phytochemicals under the same time and temperature situations. The kind of menstruum utilized, and the process employed to create the extract also have a major impact on the extraction of phytochemical content [25]. Methanolic extract yielded the maximum extraction yield and had a more complex phenolic content in our investigation. This conclusion is consistent with sultana et al., 2009 [26], who discovered that methanolic extract had a greater extractive yield than other solvents. Flavonoids and polyphenols were found in all four apple species peels, but anthraquinones and emodins were not found in any of them, according to the present study. Except for Golden, all types have anthocyanins. This conclusion is consistent with vasile et al., 2021 [27], who discovered that anthocyanins are exclusively detected in red-skinned apples and are strongly linked to apple reddening. Other phytochemicals that were tested for were present in some extracts while absent in others. Some extracts showed their relatively abundant quantity while some extracts contain them moderately. In this investigation, the total phenolic content of apple peels varied from 105.78 ± 11.377 to 198.99 ±16.416 mg GAE/g, which is comparable to the concentration in grape extract, a phenolic beverage. The methanolic extract of the Maharaji peel had the highest phenolic content of 198.99 ± 16.416 mg GAE/G DW, followed by 170.96 ± 16.512 mg GAE/G in the methanolic extract of the delicious peel, and the lowest phenolic content of 105.78 ± 11.377 mg GAE/G DW in Royal delicious acetone extract. The rest of the kinds' ranges were modest. TPC value of peel of delicious variety as reported from the present study is slightly higher than the findings by Shafi et al., 2019 [28] that reported (75.2 ± 8.98 mg GAE/G) in Red delicious but the same study reported higher TPC values 653.8 ± 20.78 for Maharaji, 342.5 ± 6.89 for royal delicious than our study revealed. In different solvent systems, the total flavonoid content of different apple peels differed from 19.1 ± 4.835 to 54.2611 ±2.547 mg QE/ G DW.

The methanolic extract of royal delicious peel had the highest flavonoid content of 54.2611 2.547 QE (mg/g), preceded by the methanolic extract of delicious peel (47.6 ± 2 9.354 QE (mg/g), and the acetone extract of golden variety peel had the lowest flavonoid content of QE (mg/g), preceded by 19.267 ± 2.100 QE (mg/g) in Ethyl acetate extract of Maharaji peel. The remaining peel extracts exhibited modest concentrations in various solvents. Wolfe K et al., 2003 [29] discovered that the peels had the greatest total flavonoid concentration within each variety, followed by the flesh plus peel and the flesh. With reference to the antioxidant potential of apple samples, at 0.25 mg/ml concentration of crude extract, the DPPH assay exhibited a maximum of 69.86 % scavenging activity in Maharaji methanolic extract followed by 65.32 % in royal delicious Ethyl acetate extract and a minimum of 24.33 % was found in royal delicious acetone extract. The IC50 value was calculated using the percent scavenging versus concentration curve, which reflects the amount of extract that scavenged 50% of the DPPH radical. Maharaji methanolic extract showed the lowest IC50 value (176.72 µg/mL), thus the highest antioxidant activity while royal delicious acetone extract showed the highest IC50 value (524.6 µg/mL) and thus comparatively lower free radical scavenging activity. According to Yishak et al., 2021 [30] fuji rose variety showed the strongest DPPH radical scavenging activity (EC50 = 86.20 ± 2.28 µg/mL). As this species was not incorporated in the current study, no active relation can be made. The significant link between apple phenolic concentration and antioxidant activity has been well documented [31], and the current research backs it up as there is a virtually linear association between total phenols and antioxidant capacity in all extracts of four apple varieties analyzed in this study. Total phenolic content and % scavenging activity had a positive coefficient of determination (R2 = 0.9542), suggesting that phenolic content exerts a substantial influence on the antioxidant ability of the extracts. The current study found that Malus peels are a fantastic source of bioactive compounds especially dietary fiber and polyphenols, which have been revealed to reduce inflammation. The stability of the HRBC membrane, or suppression of hydrogen peroxide-induced HRBC membrane lysis, was used in the current investigation as a proxy for anti-hemolytic and anti-inflammatory action. According to the current investigation, methanolic fractions were shown to have a substantial amount of HBRC membrane stabilization, followed by ethyl acetate recovered exudates, when compared to the NSAID standard diclofenac sodium, which showed 94.58% inhibition at a dosage of 1600 mcg/ml. At a dosage of 1600 mcg/ml, Maharaji methanolic extract inhibited HRBC membrane stabilization by

69.16%.

According to our findings, the membrane stabilizing capacity of peel chemicals, which can be used to assess anti-hemolytic and anti-inflammatory potential, is consistent with previous research. The instances of such studies include the findings of Jensen et al., 2014 [32] according to which dried apple peel powder has diverse anti-inflammatory capabilities. Dried apple peel powder supplementation was shown to be linked to enhanced joint mobility and serum protective antioxidant capacity. The reported decrease in pain, according to their study, is attributed to enhanced antioxidant activity and the anti-inflammatory actions of apple polyphenols. According to Liddle et al., 2021 [33], eating six whole Gala apples diurnal for six weeks may be a successful nutritional plan to reduce inflammation associated with weight gain. Another investigation by Andre et al., 2012 [34] indicated that triterpene-rich fractions decreased the expression levels of the TNF gene, whereas cultivars with high procyanidin concentration were most effective at suppressing NF-B. To conclude, the peel phenolics of investigated cultivars that indigenous to Kashmir revealed several biological properties with potential pharmacological activity and hence can be exploited for novel cell membrane stabilizing, anti-hemolytic, anti-oxidant, and anti-inflammatory agents. The phytochemical profile of the Malus peels, which may be established according to the current study, have great therapeutic potential.

CONCLUSION

Amidst their function in plant defense, secondary metabolites also benefit human health. Utilizing cutting-edge dimensions of nanotechnology, secondary metabolites are now being used in the management of refractory diseases and perform a crucrole in the prevention of diseases and the promotion of good health. Comparative phenolics perusal of peel extracts of different *Malus domestica* cultivars (both red-and yellow-skinned) indigenous to Kashmir was investigated in this stud using well-established methodologies. The crude decoctures were revealed to be high in phenolic and polyphenolic compounds and have the ability to neutralize reactive oxygen species by acting as antioxidants and have the ability to inhibit the HRBC membrane lysis tigered by ROS (H_2O_2). According to the findings, there is a correlation between the extract's chemical makeup, particularly phenolic chemicals, and its strong antioxidant and anti-inflammatory activities.

Peels of *Malus domestica* var Maharaji followed by the delicious variety reflected the striking activities in all the analyzed parameters kindred with human health and disease. According to the findings of the current investigation, it can be asserted that varietal variations leading to alterations in the biosynthesis of benign Phytoactive metabolites may be responsible for the significant deviations in TFC\TPC, free radical scavenging, and anti-inflammatory action of *Malus domestica* peels. This study offers fresh perspectives on how polar decoctures or dried powders of *Malus domestica* peels could be used to treat illnesses linked to oxidative stress, hemolysis, erythrocyte disorders, and inflammatory disorders.

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Conflict of interest

During the research, there were no connections that could be viewed as having a potential conflict of interest in terms of business or finances.

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