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Review Article

Juicy Gems of Nutrition: Exploring the Nutrient Profile and Antioxidant Activity of Rosaceae Fruits

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ABSTRACT

Fruit consumption provides valuable phenolic antioxidants and nutrients that are essential to subsidize consumers' health. The functional food value of many fruits belonging to the Rosaceae family is considerably high which is derived from their medicinal and nutritional attributes. This value contributes to the overall health and dietary practice of the consumer. Apples, pears, loquats, peaches, plums and apricots are some of the most desirable and economically viable members of this family. The present review aims a detailed evaluation of the nutritional composition, organic acid, sugar contents, and phenolic antioxidants of these beneficial fruits. Sucrose occurs in plum, loquat, peach and apricot fruits predominantly, in contrast, fructose was mainly present in apples and pears. The primary organic acids were citric, tartaric and malic acid in the tested fruits. The FRAP, TEAC and ORAC values were strongly correlated and almost similar to the total phenols (TP) in the extract of the tested samples. The phenolic acid assessment indicated that p-coumaric acid is the major compound in plums, apricot; gallic acid in loquat and caffeic acid in apple, peach and pear fruits. Overall, the results indicate that these Pakistani fruits contain significant levels of phenolic antioxidants and nutritionally valuable compounds, so they can be considered advantageous ingredients for the nutraceutical and food industry.

INTRODUCTION

The natural world offers a diverse range of plant species, including certain fruits and vegetables that can serve as functional foods, providing a means to fulfil both the physiological and medicinal requirements of human beings. Studies have found that phytochemicals, especially phenolics, are the most significant biomolecules for humans that generally exist in fruits and vegetables. Fruits and plants contain biologically active compounds that serve a complementary role in regulating many of the body's functions, for example, immune system excitation, gene expression regulation, and hormonal and antibacterial activity. Research also suggests that there is a strong correlation between the phenolics found in fruits and vegetables and various biological activities within the human body [1]. A sizable quantity of phenolics in fruits and some vegetables triggers the human body to be immune to cardiovascular diseases, cancers, and persistent health problems. These vegetables and fruits also consist of phytochemicals, which hold active antioxidant properties and assorted biological functions such as modification of metabolic activities and detoxification of harmful substances, e.g.; carcinogens and toxins – resulting in the reduction of various health incidents [2]. The presence of bioactive chemicals and antioxidants in vegetables and

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phytochemicals, which hold active antioxidant properties and assorted biological functions such as modification of metabolic activities and detoxification of harmful substances, e.g.; carcinogens and toxins – resulting in the reduction of various health incidents [2]. The presence of bioactive chemicals and antioxidants in vegetables and fruits primarily has therapeutic and medical abilities. Moreover, these chemicals help in the reduction and elimination of free radicals. These are controlling agents of a variety of age-related chronic illnesses. Research has demonstrated a decrease in the incidence of coronary diseases through the consumption of phenolics, including flavonoids and phenolic acids. Additionally, studies have revealed that the antioxidant activity and flavonoid concentration can vary based on the source of fruits and vegetables consumed. Dietary intake of these chemicals is beneficial for treating cancer. Regarding the functional food potential of fruits, there is substantial evidence that fruit intake is linked to a balanced and nutritious diet, providing protection against degenerative illnesses, osteoporosis, and cellular ageing. Fruits' positive benefits are due to a high quantity of natural sugars, fiber, minerals, water, and organic acids, as well as the existence of significant levels of functional bio-actives and antioxidants [3]. Polyphenolics, which have a great potential to scavenge reactive oxygen and free radicals, are among the principal antioxidant chemicals found in fruits. Fruits are well known for their bioactive phenolic components, such as coumarins, phenolic acids, tannins, and flavonoids. These phytochemicals are famous for their physiological and biological advantages, including, anti-inflammatory, antioxidant, anti-carcinogenic, anti-tumour, and so on. In recent literature, a growing body of research has provided evidence for the efficacy of fruits in reducing the risk of various fatal diseases. Citrus fruits and their juices, in particular, are highly effective in reducing the incidence of ischemic stroke. A fruit-rich diet has demonstrated positive effects on blood antioxidant capacity and has been found to lower the risk of lipid peroxidation. Consuming an adequate number of strawberries, approximately 200mg, has been found to increase plasma antioxidant capacity significantly. Apple fruit contains numerous flavonoids, and its intake has been linked to a lower risk of diabetes, lung cancer, cell proliferation, and asthma.

Important Fruits of the *Rosaceae* Family

The *Rosaceae* family, sometimes called the Rose family, is the 19th most prominent flowering plant family with species ranging from 2830-3100 and roughly 95 to more than 100 genera. Sorbus (260), Rubus (250), Alchemilla (270), Cotoneaster (260), and Crataegus (260) are some of the well-known genera. The majority of the species are deciduous; however, there are a few that are evergreen. They are found all across the planet, although their diversity is most extraordinary in the northern hemisphere. Fleshy fruits and other prominent red, purple, and yellow colors make up the majority of *Rosaceae* members, and they are a vital source of human nourishment. Many commercially and medically recommended nutrient-rich fruits, such as raspberry, plum, strawberry, apple, peach, pear and apricot are members of the *Rosaceae* family [4]. For centuries, *Rosaceae* Fruits have been utilized as a source of food, nourishment, and medicine.

Apple – *Malus domestica* Borkh

This interspecific hybrid is the most widely spread domesticated apple. *M.domestica* as a cultivated apple belongs to the subfamily *Pomoideae* of the Rosacea. It is considered an interspecific hybrid as it does not occur in the wild. It is a well-known fruit and is most frequently farmed among the human-eating species of the *Malus* genus. Present-day, the farming of apples is common in temporal regions mainly or in all continent's tropics excluding Antarctica – the southernmost region of the planet [5]. Apples can be dried, fresh, tinned, or made into alcoholic beverages, preserves, juices and cocktails. Its intake reduces the risk of cardiovascular conditions, cancer incidence – essentially for the lungs, and coronary and thrombotic stroke. The delicious apple is one of the most eaten fruits worldwide. It has been studied as a nutrient source of carbohydrates, beneficial phenolics, and dietary fibre. On market shelves, all types of apple fruits and apple-related items are accessible throughout the year. Apples are rich in nutrients, for example, amino acids, sugars, phenolic metabolites, and organic acids – all beneficial to human health [6]. According to reports, Apple is a fountainhead of various substances, including phenolic phytochemical substances, but these fruits' phytochemical types and distribution are significantly different. Peeled apples' proliferative and antioxidant capacity was lower than that of unpeeled apples. Polyphenols in apples have high antioxidant activity, which may be attributed to their radical scavenging characteristics as well as their distinctive structural features, which include double bonds and electron-donor groups (hydroxyl). Consumption of apple juice, fruits and similar products has been shown in recent research to have anti-carcinogenic, antiproliferative, and anti-inflammatory properties. Furthermore, eating apples is linked to a decreased risk of prostate cancer, cardiovascular disease, and viral infections [7].

Loquat – *Eriobotrya japonica* L.

Eriobotrya japonica (Thumb.) Lindl is a fruit tree which blooms evergreen and is found in subtropical regions and has an association with the class Rose family. The loquat and apricots share comparable sizes, having a light yellow

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to orange-tinted skin and a round to oblong shape. The hue of the flesh ranges from orange to pale milky [8]. The fruit consists of one or more seeds, and it is quite juicy. It has a sweet taste with a hint of acidity. Because of its high concentration of organic acids, pectin content, and sugars, it competes nutritionally with apple fruit. Minerals, including potassium, phosphorus, and calcium, as well as vitamins A, B, and C, and natural sugars, are abundant in loquat [9]. It includes a significant quantity of organic acids, primarily malic acid, as well as modest quantities of citric, succinic, and tartaric acids. The majority of consumers prefer loquat with a greater total sugar level (> 10%). Carotenoids, particularly provitamin A, are abundant in loquat. Medicinally, almost every part of the plant is used, including the fruit, kernel, and sensitive leaves [10]. Amygdalin, an anti-cancer vitamin, is found in the leaves and kernels. The extracts of loquats have long been used for folk medicinal and physiological purposes that have antitussive/expectorant and anti-inflammatory properties. These properties are used to treat dermatitis and pain. Important secondary metabolites of plants and antioxidant components such as carotenoids, anthocyanin and flavonoids may be found in loquat fruit.

Apricot – *Prunus armeniaca*

The apricot is another important fruit grown in northern areas of Pakistan. Hunza, Balochistan, Gilgit, and Khyber Pakhtunkhwa are the most common places to find it. The apricot is used to make nectar and jam because of its particular scent [11]. Fresh apricot fruit is accessible to consumers from May to the month of September, but the dried form is available the whole year. The pericarp of an apricot includes organic acids, saccharides, vitamins (polyphenols, provitamin A and vitamins B & C), and minerals (primarily boron, iron and potassium). The fact that this fruit is rich in the aforementioned compounds supports the fact that it is frequently employed in folk and traditional medical systems [12]. This healthy fruit has a lot of medical qualities, including actions against weariness, sleeplessness, stress, anti-anemic and anti-asthmatic potential, and anaemia or cholesterol reduction. Researchers and food scientists have been paying close attention to the fruit as a result of its positive properties. In addition to the nutrients listed above, it has been discovered that apricots contain a significant quantity of phenolic compounds [13].

Plum – *Prunus domestica* L

The plum (*Prunus domestica L.*), also a member of this family, is one of Pakistan's most popular summer fruits. The fruit has an astringent and sour flavor, is almost calories free, and has a high nutritional value. Tannins, fibers (pectin), carbohydrates (fructose, glucose and sucrose), aromatic compounds and organic acids like malic and citric compounds, are all found in it. Plums have a strong nutritional value and flavor because of these compounds. Plums contain vitamin contents including A, B1, B2, and C, necessary for the human body to operate properly. Plums are an effective source of nutrients (both micro and macro), including potassium, phosphorus, calcium, and magnesium, among others [14]. Plums can be advised to people with arterial hypertension because of their balanced sodium-potassium ratio and high potassium concentration [15]. Plums are high in phenolics (flavonoids and phenolic acids) and powerful dietary antioxidants. According to findings in the literature, plum fruit has more potent antioxidant properties than others, such as apples, peaches, pears, persimmon, and apricot. For example, plums are one of the most popular fruits in our diet and contain 4.4 times the total content of antioxidants concerning apples. These are also sources of bioactive chemicals, which can aid in preventing various ailments and is medicinally significant. Plums have been shown to be effective at scavenging oxygen-derived peroxyl and hydroxyl free radicals. Fruits of this category have been shown to improve peristaltic motions, limit carbohydrate absorption, promote lipid breakdown in the human body, and lower overall cholesterol levels. (Especially LDL fraction), and Triglyceride and homocysteine levels in the blood serum have protective and antithrombotic effects in the cardiovascular regions of the body [16].

Pear – *Pyrus communis* L.

Pears (*Pyrus pyrifolia and communis*) are canned or eaten fresh. It contains fabric content and vitamin C. Pears are a favorite summer fruit because of their excellent nutritional value, delicious taste, and low-calorie content. Consumers enjoy the fruit because it is low in protein and fats and high in sweets such as sorbitol, fructose and sucrose [17]. This fruit contains fats (0.3%), protein (0.5%), carbohydrates (12.4%) and fiber (2.8%). They include various nutritional ingredients, such as vitamins, antioxidants, minerals, medically beneficial bioactive and health-promoting chemicals, and their macronutrient content [18]. Pear varieties are produced in Pakistan's summer rainy areas (such as in the districts of Swat, Muzaffarabad and Hazara,) and the interior desert valleys (Chitral and Gilgit-Baltistan), where irrigation is accessible. The Swat Valley and surrounding valleys have the highest diversity of pears, especially on the valley's eastern side. The most widely cultivated Asian pear cultivar 'Shoghori' found in Chitral and Gilgit, is also called *P. pyrifolia*. In Pakistan, the whole area utilized for pear production was calculated to be around 2400 hectares, with 100 hectares in Punjab, 100 hectares in Balochistan and 2400 hectares in the Khyber Pakhtunkhwa (KPK). While taking into account the whole weight of pear production – it stands at 30700 tones, with 29,000 tons

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from KPK, 1200 tones from Punjab and 500 tones from Balochistan[19].

Peach – *Prunus persica* L.

Peach is a tall one that generally grows up to 4m to 10m and usually loses its leaves at the end of fall or early winter – having the deciduous characteristic; this tree also belongs to the Rose family. It is related to the *Prunus* genus, mainly the Amygdalus subgenus, and harbors distinctive properties from the rest of the subgenera due to its complex ridged seed shell. Peaches are among the most admired fruits in summertime. The key ingredients of peaches include minerals, organic acids, dietary fibres, and carbohydrates; almost all contribute to the high quality nutritional fresh and juiced peaches [20]. As discussed above, peaches are the most delicious fruit and currently one of the most abundant eaten fruits, especially in summer. Besides its popularity, it contains phenolic acid, flavonoids, and anthocyanin chemicals that are a massive source of potential antioxidants. Peaches with golden yellow flesh are moderate in acidity and sweet in taste, but those which have only yellow flesh bear a tangy acidic taste along with adequate sugariness. A peach is a potassium and vitamin A & C rich fruit, and a variety of other nutrients are also present, giving it a high nutritional value and benefiting consumer health. Being potassium-rich, peaches help in regulating heart rate and blood pressure. Further studies show that they reduce the free radicals and ROS (Reactive Oxygen Species) generation in human plasma, as well as give protection against chronic illnesses [21]. At the same time, Peach is a conventional food crop in the northern districts of Pakistan, covering 4543 hectares and yielding 48284 tones. The prominent peach-growing locations include Kalat, Peshawar, Quetta, some of the Kohistan highlands and Swat Valley. Shireen, Shahpasand and Golden are the most regularly produced peach fruits in Balochistan and Swat (Khyber Pakhtunkhwa) and have a high market potential[22].

Sugars and Organic Acids in Fruits

Generally, sugars vary in their relative sweetness, the sweetness and flavor of fruits are highly influenced by their sugar content. For example, sucrose has a sweetness value of 1.0, fructose has around 175 calories per gram, glucose has 75 calories per gram, and sorbitol has 5 calories per gram. Organic acids, on the other hand, play an important role in fruits' composition. Since they are less prone to alteration during processing and accumulation than other ingredients of fruits, micro-organic acids can be proved as a favourable implication for standard perusal and authenticity of fruits – and products derived from fruits. These acids can help the body absorb potassium and stimulate the breakdown and digestion of other nutrients, including calcium, copper, and zinc, which help in disease resistance [23]. Many fruits include naturally occurring amino acids, free sugars, and organic acids, which significantly preserve their quality and define their nutritional worth. Because of their close relationship with organoleptic qualities, the type and quantity of these vital nutrients in fruits is also the centre of attention. Free sugars such as fructose in monosaccharides and disaccharides glucose are essential nutritional elements that considerably contribute to the taste and sweetness of the fruit. Later on, Stampar and Hudina investigated the formation of organic acids in four *Pyrus pyrifolia* cultivars and eight *Pyrus communis* cultivars, discovering the Hardy fruits. Malic acid, tartaric acid, fumaric acids and citric acid are known as hardy fruits. Moreover, it is concluded that various pear cultivars can have different flavors and quality qualities due to differences in the amount and composition of acids in the fruits. Research suggests that malic, citric, lactic, tartaric, shikimic, and quinic acids were included in the list of most occur organic acids in the fruits. Researchers have also studied the natural sugars, phenols and acids composition from artificial and organic fruit production – with respect to their impact on body health [24].

Analysis of Sugars and Organic Acids in Fruits

The relative ratio of organic acid and sugars in fruits is an important factor as it affects their sensory and chemical properties (e.g., sweetness, total acidity, microbial stability, pH and acceptability). Food characteristics and quality data is provided by the organic acids and sugar profile of respective foods [25]. For the determination of these contents in fruits, different analytical techniques are executed. These techniques are primarily based on HPLC (high-performance liquid chromatography) and GLC (gas-liquid chromatographic separation). Analysis can also be performed by using a spectrophotometer. It involves the reaction between derivatizing compounds and organic acid that give rise to coloured complex formation. The absorption rate or intensity of these coloured complexes can be calculated by setting a definite wavelength. Ion exchange resins and precipitation techniques are also utilized for the isolation of organic acids to avoid matrix effects and interferences. The major sugars in tested fruits of the *Rosaceae* family were glucose and fructose. While ascorbic acid and citric acids were major organic acids in mulberry and strawberry. Sweet cherries consist of tartaric acid as the predominant organic acid. With ripening, the sugar and organic contents increase in these tested fruits[26].

Antioxidant Activity and Phenolic Contents of *Rosaceae* Fruits

Antioxidant Activity

Epidemiological data indicate that including an adequate

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number of fruits in one's diet can offer various health benefits that can aid in preventing degenerative diseases. These properties are primarily attributed to the presence of phenolic compounds in fruits. Studies suggest that natural health products and foods' protective effects against degenerative and chronic diseases are mainly due to their activity of antioxidants that attributed to phenolics. The naturally occurring phenolic compounds in fruit extracts are largely responsible for their disease-prevention properties through their antioxidant activity. Fruit extracts have been shown to have a positive impact on various conditions such as microbial infections, cancer, immune disorders, cardiovascular disease, viral infections and neurodegenerative diseases [27]. In a study conducted by Proteggente and colleagues, various fruits and vegetables were analyzed to determine their individual phenolic profiles. These were rich in anthocyanins, such as strawberries, raspberries, and red plums, and demonstrated the highest antioxidant activities. Those rich in flavanones, such as oranges and grapefruits, and flavonoids, such as leeks, spinach, onions, and green cabbage, followed closely behind, while fruits high in hydroxycinnamates, including apples, tomatoes, pears, and peaches, had lower antioxidant potential. The antioxidant activity of the sample extracts, as measured by TEAC, ORAC, and FRAP values, was strongly linked with vitamin C and TP (total phenolics contents). Sun and colleagues, in a 2002 study, also observed a direct linkage between antioxidant activity and phenolic content of phytochemical extracts from different fruits. Similarly, Gil and coworkers found in 2002 that TP contributed more significantly to the antioxidant activity than vitamin C and carotenoids. In another investigation by Imeh in 2002, the TP content of sixteen commonly consumed fruits was compared, revealing a wide variation in the phenolic compounds and antioxidant activity among the cultivars analyzed. It has been observed that a wide range of plant fruit extracts and phenolic compounds have anti-inflammatory, anti-carcinogenic, antimicrobial and vasodilatory functionalities. Therefore, it is recommended to evaluate the value of functional fruit for consumption by humans, to further determine the composition of phenolic contents and the activity of antioxidants. The literature revealed a direct relation between antioxidant activity and phenolic contents. Genotypes, fruit tissues and fruit maturity level are the determinant factor composition of phenolics among fruits, while to a smaller extent, it depends on environmental factors. Moreover, the phenolic content may differ within different parts of the same species and even entire fruit species [28]. Colour can also be a causal factor in determining the number of contents present in fruits such as anthocyanin, phenolic contents and antioxidants were observed to be higher in peaches containing red flesh than in peaches containing light-coloured flesh, opposite to the carotenoids concentration that was less in light-coloured flesh than that in yellow-flesh ones. Plums with red flesh have higher phenolic contents and anthocyanin predominantly than the other plums but the capacity of antioxidants still stands greater. The total phenols have a strong association with antioxidants so we can access the phenolic calculations by knowing the total quality and quantity of antioxidants present in the sample mainly plums and peaches [29]. Phenolic compounds and antioxidant capabilities of fruits were strongly linked sometimes as in peaches with yellow flesh. This reveals that genotypic importance is linked with constituents which cause antioxidant capacities such as carotenoids, phenols and vitamin C. The relation between phenols and antioxidant constituents is varied because of differences in progeny. Besides, studying the total phenolic contents (TPC) and antioxidant activity of Chinese Jujube fruit (*Ziziphus jujuba* Mill) observed that TEAC, EC_{50} and FRAP values of pulp and some other fruits like peel developed a strong bond to the TPC ($R= 0.997$, $R= 0.992$ and $R= 0.985$ respectively). Regarding the amount of TP in the peel – it was five to six times higher than in the pulp showing considerable dissimilarities among the parts tested. No reasonable linkage was seen for any other phytochemical trait versus vitamin C. The study showed that the pulp part of the peel contains a higher concentration of three parameters than any other tested fruit. Except in the pulp of the apple, a strong relation between antioxidant and phenolic capacity was observed in all fruits and cultivars which was analyzed for this research. Antioxidant capacity and some phenolic compounds were increased in direct relation in these samples but some variations were also observed because of the different chemical nature of these species. Variation and different natures can also be present due to the presence of different parts of a single or the same species.

Determination of Antioxidant Activity

The total antioxidant capacity, abbreviated as TAC, of variable beverages and plant food, can be accessed by applying different in vivo and in vitro assays. Depending on the technique of measuring their endpoints, these assays differ greatly due to differences in their mode of action and chemistry (generation of different target molecules and radicals). As there are different antioxidant compounds that cannot be evaluated by a single technique, different methods are deployed to calculate food TAC. The principal process through which free radicals can scavenge and oxidation is limited in food by phenols is the radical scavenging mechanism. Therefore, phenolic contents in selected fruits and their antioxidant activity are evaluated

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by in vitro-free scavenging tests. FRAP assay, ORAC (oxygen absorbance capacity), TRAP, inhibition of peroxidation in the linoleic acid system, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), TEAC, bleaching of β- carotene in the linoleic acid system and 2, 2‴-azinobis 3-ethyl benzothiazoline-6-sulfonate (ABTS+) radical scavenging assays are some of the assays use for evaluation and calculation of antioxidant activity [30]. FRAP Assay, which is a ferric ion-reducing antioxidant power, is considered an evident technique for the study of antioxidant activity. Because of the reductive property of phenols, ferrous ions (with +2 charge) are produced from the reduction of ferric ions (+3 charged). The Standard is set as 583 nm. At this wavelength, the reductive property can be observed by monitoring changes in absorption. Trolox Equivalent Antioxidant Capacity (TEAC) reduces ABTS+ to ABTS with water-soluble Trolox (vitamin E analogue). In this way, antioxidant activity is determined by the degree of decolourization. TRAP "total peroxyl radical trapping antioxidant parameter" is based on R-phycoerythrin. During peroxidation (controlled), the presence of antioxidants is judged by fluorescence decay of this lag phase. Results appeared as Trolox (standard antioxidant) in both ORAC and TRAP. Another method is to use synthetic radicals with polar solvents. ABTS 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals are commonly used synthetic radicals but later are most applicable to determine the presence of radicals in samples of interest. The DPPH scavenging is calculated by calorimeter with the use of antioxidants [31]. Then the sample is reacted with it in a solution of methanol. At 515 nm, the radical reduction is evaluated by the change in absorbance of that solution. This analysis is quick as DPPH can report the results within 15-30 minutes. This analysis is reported in the form of IC50 performing the assessment of antioxidants present in guava by comparing the potential of DPPH, ORAC, ABTS and FRAP techniques. They prepared fruit extract in dichloromethane and methanol solvents. ORAC can only work in the solvents prepared by dichloromethane while the rest can work in both solvents.

Phenolic Contents in *Rosaceae* Fruits

Plants have secondary metabolites in the form of phenolic compounds that are derived from phenylalanine. These compounds perform diverse functions such as imparting colour to fruits and leaves, giving protection from herbivores and harmful radiations like UV rays, attracting and repelling insects for pollination etc. Phenols are compounds that contain benzene (aromatic) rings with hydroxyl groups attached to it. These range from phenolic acids which are simple phenolic compounds to tannins, a complex structure with more considerable molecular weight (ever more than 30 thousand daltons) [32]. Plant phenolics have attained scientific interest in recent decades due to their wide array of health-promoting and biochemical properties including antioxidant, antimutagenic, anti-inflammatory and antimicrobial activities. Researchers identified about eight thousand phenolic groups present in the plant kingdom. These are mainly derived from hydroxycinnamic (p-coumaric, sinapic and ferulic) and hydroxybenzoic acid (syringic, p- p-hydroxybenzoic, vanillic and protocatechuic). Due to the difference in the degree and pattern of aromatic ring methoxylation and hydroxylation, these derivatives are divergent (Figure 1).

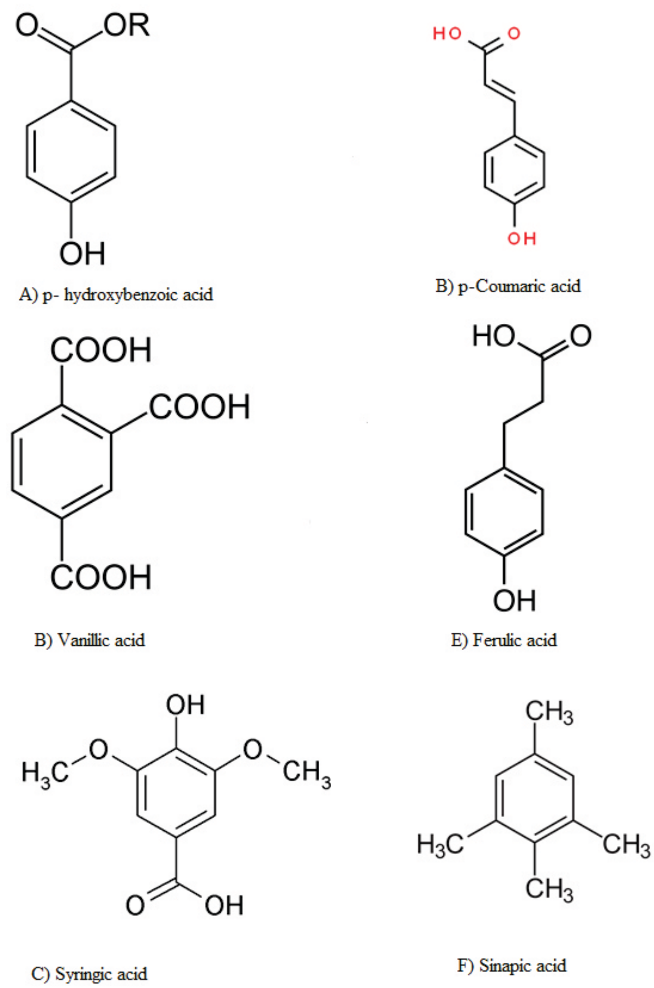

Figure 1: Chemical structure of some phenolic compounds which are the derivatives of (A-C) benzoic acid and (D-F) cinnamic acid

Flavonoids

Flavonoids are another class of phenols which consist of two rings of phenols and are linked through oxygenated heterocyclic pyran rings. A large content of dietary phenolics around 60% is being distributed by these compounds. In the literature, there are approximately forty thousand flavonoid compounds, according to researchers. There are different classes of flavonoids such as

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anthocyanins, flavonols, flavones and flavanones [33]. These are due to differences in groups attached to it like hydroxyl groups, oxygen, methyl groups and sugars (Figure 2).

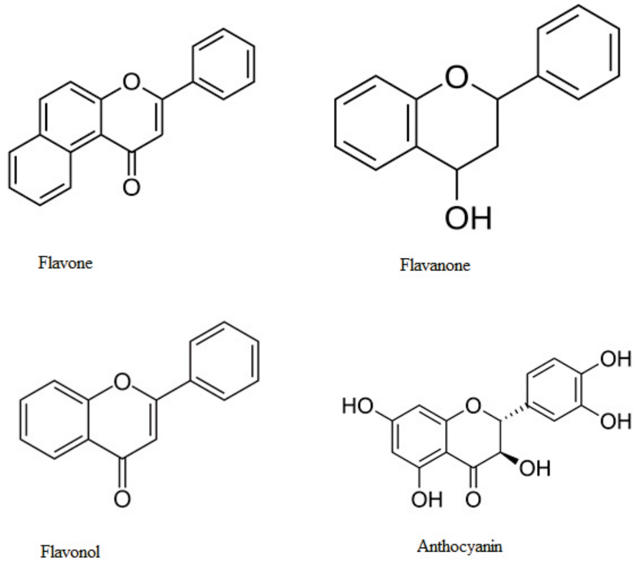

Figure 2: Some well-known and most studied flavonoid compounds

Fruits contain essential nutrients and admirable antioxidant compounds; therefore, many intentions are paid to use them as a functional food. Flavonoids and phenolic acids are mainly studied and well-known phenolic compounds [34]. Moreover, the consumption of fruits and derived ingredients of fruits is beneficial for health and medicinal purposes besides its nutritional aspects.

Extraction of Fruits' Phenolic Compounds

Extraction Solvent

For potential execution as ingredients as well as antioxidants of nutraceuticals and functional food, the first step is the extraction of bioactive forms and phytochemicals of phenols from various fruits. It is highly effective by light and heat so extra attention should be given to protect it from oxidation and degradation. For the isolation and extraction of adequate amounts of antioxidants (active components) without modification and degradation, different techniques with appropriate solvents are utilized. The sample is first processed through drying, milling, grinding, and homogenization before the start of extraction in practice. An accurate extraction technique and suitable solvent should be chosen for the excellent assessment of antioxidants. Phenolic compounds are extracted in alcoholic solvents and concentrated mostly in plant vacuoles [35].

Extraction Technique

Different extraction techniques are deployed for successful antioxidant extraction. For isolation, conventional shaker-type extraction techniques are widely used. Others are also considered such as Soxhlet extraction or percolation for preparing phenolic extracts. This method involves accelerated heat usage which is not compatible with heat-labile substances extraction. Enzymatic techniques are now applied in the replacement of conventional ones to attain polyphenol recoveries. They can act on the polymers of the cell wall, thus improving yields in the food industry. Cellulose and α- amylase distorts bound phenols from materials. Despite high expenses, rapid innovation in biotechnology made it easier to use extraction techniques with better quality, less time and low cost. UAEPU (Ultrasound Extraction Process) is another way for an analyte's extraction in comparatively less time and with better stability. It was applied for the evaluation of efficacy in two strawberry types to test the speed and rate of analyte degradation. MAE (microwave-assisted extraction) uses microwaves as the source of energy and employs phenolic substance extraction and separation from numerous plant species. It is basically a novel extracting approach for heating the sample or solvent with the energy source and transferring the analyte into solvent from the matrix or standards. This technique is superior to conventional extraction processes because it uses the combination of solvent and heat at a time or in a step. Besides these, MAE has the advantages of less process time, low expenses, less usage of solvent and high-quality extraction [36].

Hydrolysis Aid

Ester glycosides, Aglycone and bound phenolics are some forms of phenol, in nature. These have variant degradation susceptibility and extraction degrees from each other. After the process of extraction, some insoluble phenolic compounds are still left behind which is to be removed by applying adequate techniques. These insoluble compounds cannot be extracted before because they are bound to high molecular weight insoluble compounds like proteins and carbohydrates. The process of extraction is usually the systematic release of phenols from matrices in the sequence. In the fresh step, soluble or extractable substances such as glycosides and some esters are extracted by an aqueous organic solution (methanol). In literature, extraction magnitudes have been enlisted including ethanol/water, acetone/water, ethanol-acetone/water, acetified water and methanol/water. While insoluble solvent complexes which are coupled with polymers of the cell walls by glycosidic and ester linkages are difficult to extract by these solvents. For these bound or insoluble phenolic compounds, different techniques such as acid hydrolysis or base hydrolysis are used for extraction [37].

Analysis of Phenolic Compounds

Phenolic contents can be determined by applying methods

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like spectrophotometry. Over time, several methods have been proposed under the condition of chemical reagents for phenolic compound analysis (both qualitative and quantitative) in ordinary plant material. Among the well-known methods, the Folin-Denis Assay is considered to be an acceptable technique usually used to analyze the phenolic content in beverages and plants. In this technique, the reagent reduction of phosphomolybdic-phospho-tungstic acid (Folin-Denis) takes place in an alkaline solution, giving an output of a blue-coloured complex typically in the proximity of phenolics. The progression of colour and the abundance of compounds is determined at 725 nm and propped up by a "standard calibration curve" method. Later, the assay was further amplified by replacing Folin-Denis with a Folin-Ciocalteu reagent. Nevertheless, the replaceable Folin-Ciocalteu reagent has a more significant molybdate compound concentration in the reactive complex – making it a more suitable candidate for reduction [38].

Chromatographic Methods

Chromatographic techniques are applied for the identification, purification and separation of phenolic contents. Some of the known techniques are; High-Performance Liquid chromatography (HPLC), Gas chromatography (GC), Thin-Layer chromatography (TLC), and Paper chromatography (PC). Among all, HPLC is a broadly applied technique for the identification and isolation of phenolic compounds. Reverse-phase HPLC is under extensive use for partitioning the complex mixtures of phenolic compounds, some common mixtures are plant-based and other natural products-based mixtures. The HPLC method was also suggested to extract mixtures from the cranberry juice, particularly the flavonoid and phenolic compounds, for further quantification and separation. The study disclosed that the compounds found in the cranberry case were benzoic acid, primarily a phenolic, while major flavonoids were quercetin and myricetin. Moreover, analysis is also conducted on different other samples – berries, beverages, and fruits and beverages and their phenolic contents was evaluated by applying the HPLC technique following the acidic hydrolysis and alkaline. Saskatoon berry (59 mg 100g), chokeberry (96 mg/100 g), and blueberry (85 mg/ 100g) were found to be the leading phenolic acid sources. Whereas cherry, apple, and dark plum were among the fruits with high phenolic contents (28 mg/ 100g). On the other hand, coffee (97 mg/ 100g) ranked first, following green tea and black tea containing the highest (30-36 mg/ 100g) phenolic content among beverages. Phenolic acid contents of plums were determined by using RP-HPLC (Reverse-phase HPLC) accompanied by a monolithic column. However, to get satisfactory outcomes, the concentration, mobile phase and pH of organic modifiers were revamped. Successful results were obtained under gradient conditions when keeping the mobile phase containing 50 mM solvent A (phosphate buffer at pH 2.2) and Solvent B (acetonitrile) respectively. The UV detection limits were set between 0.098 and 2.05μg/mL for p-hydroxyphenyl acetic and vanillic acid subsequently. The antecedent method was also effectively tested on several plum fruits to analyze their polyphenolic acid content in them. Moreover, Using RP-HPLC few selective berry fruits – the individual flavonoids and phenolic acid components were also quantified. The extracts of phenols are usually filtered and directly inserted on the RP-HPLC or split up beforehand by either liquid-liquid extraction or gel filtration chromatography or even solid phase extraction. Therefore, to filter out the sample or the pre-splitting up of the flavonoids and phenolic compounds solid phase extraction backed by a disposable C18 cartridge analytical approach is suggested. Other approaches for phenol filtration are alkaline, enzymatic, or acidic hydrolysis, which discharges glycosyl and acyl impurities from the phenolic compounds Table 1 [39]. Active hydrolysis may disintegrate the unstable aglycones, yet, it may be challenging in the case of glycosides – to completely hydrolyze them, which might further lead to recurring inaccurate outcomes. Despite being under adequate hydrolysis, the literal phenolic compound content in foods may not give accurate results – the outcomes may prove falsifiable up to 50%. Furthermore, various publications are available addressing the analysis of phenolic compounds using the HPLC methods. HPLC columns are mostly found to be reversed phase (RP), having a range of 100 to 300 mm in length and a diameter of 4.6 mm. Free phenolic acids like protocatechuic, gallic, p-hydroxybenzoic, m-hydroxybenzoic, syringic, vanillic, and caffeic acid along with others are quantified by using the HPLC method. These can be extracted by 10% acetic acid, methanol solutions, and further by ethyl acetate/diethyl ether (1:1) to perform acid hydrolysis. These hydrolyses are calculated to determine phenolic contents present in the sample, especially plant foods. This is a comparatively quick analysis with one exception of ellagic acid, which needs a long time of 20 hours for quantification. With the advancement of technology, new techniques are now developed, such as MALDI and LG-MS (liquid chromatography- Mass Spectroscopy) coupled with Electrospray Ionization (ESI) for phenolic compound qualification and quantification.

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Table 1: Determination of phenolic compounds in plant sources using HPLC

Compounds	Fruits	Column	Mobile phase	Detector
Phenolic Flavonoid [39]	Apple	C18 column (250×4.6 mm with 5 μm packing)	i) 2% acetic acid in water/methanol ii) 0.25 mmol phosphate buffer, pH = 2.5/acetonitrile	UV/Vis detector
Flavonoids Phenolic acids [40]	strawberry and blackcurrant	ODS-Hypersil column	i) Dihydrogen ammonium phosphate ii) orthophosphoric acid and acetonitrile	
Phenolic acids and Flavonoids [41]	Apple and pear	5 mm C (25034.6 mm I.D.)	i) 2% (v/v) acetic acid in water (eluent A) ii) 0.5% acetic acid in water and acetonitrile (50:50, v/v; e luent B).	Poto-diode array detector
Phenolic acid [42]	Bean	C18 Luna column (Phenomenex, 150×4.6 mm; particle size 5 mm)	i) 0.1% formic acid ii)methanol	diode array detector
Phenolic acid [42]	Fruit juices	ODS (5 μm ,250×4.6 mm I. D)	0.1mol/1 HCl and Methanol	Photo-diode array detector
Phenolic acid [43]	Berry fruits	RP-C18 column (250 mm × 4.6 mm, 5 μ particle size)	40% tri-fluoroacetic acid (0.3%), 40% acetonitrile and 20% methanol	Photo-diode array detector
Phenolic acid [44]	Plants	PRP-1 Column (4.1×150mm,5μm)	3.1% methanol(V/V) in 20moml/L K2HPO4	Photo-diode array detector
Flavonoids [45]	Tomatoes	Hypersil-ODS column	i) Acetonitrile ii) phosphate buffer	UV/Vis
Phenolic acid [46]	Walnut fruit	SS (250 ×4.6mm), Hypersil 5 ODS	i) 2% acetic acid in water ii) 0.5 % acetonitrile (50:50%, V/V)	Photo-diode array detector
Phenolic acid Flavonoids	Apple and Pear fruit	Aqua 5μm C18 (250×4.6 mm I. D)	i) 2% acetic acid in water ii) 0.5 % acetonitrile (50:50%, V/V)	Photo-diode array detector
Polyphenol	Apricot fruit	C18(250×4.6mm, 4μm)	i) 3% acetic acid ii) 25%acetonitrile iii) 72% water	UV-VisPhoto-diode array detector
Poly phenolics [47]	Apple and Apple products	RP-column (250mm×4.6mm, 3μm)	i) 10.2 % acetic acid in 2mmol sodium acetate ii) acetonitrile	Diode-array detector
Phenolic acid	Tomatoes	RP-C18 (Phenomenex, 4×3mm, 5 μm)	i) 0.1% formic acid ii) Methanol	Diode-array detector

CONCLUSIONS

Fruits of the *Rosaceae* family offer high food functional values, such as nutritional and medicinal properties and contribute significantly to consumers' diets and to human health in general. According to research, phenolics in food are linked greatly with the protection and prevention of cancer, cardiovascular and other chronic conditions. The after-effects of the current examination demonstrated that the studied *Rosaceae's* natural products are likely wellsprings of high-esteem supplements and antioxidants in Pakistan and in this way can be investigated as significant elements for the nutraceutical business functional food industry.

Authors Contribution

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Conflicts of Interest

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