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TOTAL PHENOLS, ALKALOIDS, TANNINS AND ANTIOXIDANT ACTIVITY OF WILD *PRUNUS ARMENIACA* L. SEED COLLECTED FROM GARHWAL REGION OF UTTARAKHAND, INDIA

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Keywords:

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ABSTRACT: Plants are the important constituents of folk and traditional medicines and fulfill nutrition and dietary needs from ancient times. The aim of this study to evaluate phytochemicals and antioxidant activity of seed oil of wild apricot (*Prunus armeniaca* L). Thenon-cultivated seed of wild *Prunus armeniaca* L. from the Garhwal, Uttarakhand, India was analyzed for its phytochemicals *e.g.*, total phenols, tannins, and total alkaloids by Folin-Ciocalteau reagent, Folin-Denis reagent, and solvent extraction method, respectively. Antioxidant activity of seed oil was determined by DPPH, reducing power assay and nitric oxide scavenging method. Results obtained showed that seeds of *P. armeniaca* contain tannins (6.47 %), total phenols (0.27%), a total alkaloid (0.22 %). *P. armeniaca* seed oil showed significant antioxidant activity when analyzed by DPPH, reducing power assay, and nitric oxide free radical scavenging method. Moreover, the IC_{50} value of seed oil from DPPH, reducing power and nitric oxide assays were 0.1, 0.5, and 0.5 mg/ml and % scavenging activity range 24.12-84.82% respectively. These studies clearly show that *P. armeniaca* seed oil has significant potential as a natural antioxidant.

INTRODUCTION: Plants are the natural source of medicines mainly due to the presence of secondary metabolites and have been used as medicine in crude extract form. They have also been used to isolate the bioactive compounds in modern medicine and herbal medicine systems [1, 2].

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Interest towards antioxidants has been growing due to their protective roles in pharmaceutical and food products against oxidative deterioration [3].

Plants rich in phytochemicals like tannins, phenol-lics, alkaloids, flavonoids, isoflavonoids are having multiple biological effects, including antioxidant activity of the same potential as by synthetic antioxidants *e.g.*, ascorbic acid, vitamin E, carotenoids without any side-effects [4-6]. Biologically active components, like polyphenols and carotenoids, have in the last decades attracted much interest due to their antioxidant and antimicrobial properties and their ability to protect

against chronic diseases [8-12]. Due to the high demand for medicinal plants, it is recommended by several conservation groups for research and cultivation of wild varieties. Apricots *(Prunus armeniaca* L.*)* are one of the most popular fruit belongs to the family Rosaceae and subfamily *Prunoidea*is a hardy, moderate size tree approximate 10-11 meter tall borne in the dry temperate climate zones [13], commonly found in Kamad area of the south-west part of district Uttarkashi (Uttarakhand, India) with an altitude of 1800 m above sea level [14]. The bark is reddish in colour with broad and roundish leaves, pointed apex, margins smooth, finely serrated.

Pinkish white, singly borne sessile flowers. Fruits are 5-9 cm in size, round-shaped, yellow with redness, fleshy and juicy texture [15,] and yellow colour is due to the presence of carotenoids [16-18]. Apricot has abundant bioactive compounds, including polyphenols, carotenoids, volatiles, minerals, sugars, and vitamins [19-21] *e.g.* Quercetin 3-rutinoside, kaempferol 3-rhamnosyl-hexoside, quercetin 3-acetyl-hexoside, cyanidin 3-rutinoside, cyaniding 3-glucoside, quercetin-3-O-rhamno-glucoside, kaempferol 3-rutinoside, aglycons (+)-catechin, (-)-epicatechin, kaempferol, chlorogenic, neochlorogenic, caffeic, p-coumaric and ferulic acids [22-25]. Chlorogenic acid was the most abundant compound, accounting for a percentage of the total amount of polyphenols in apricot [26]. A main cyanogenic glucoside known as Amygdalin was isolated from *Prunusdulcis* and also identified in *P. armeniaca* of about 3–4% by weight[27]. *P. armeniaca* seeds rich in various elements, and seed oil contains oleic acid (73.58%) and linoleic acid (19.26%) [28-29].

Apricot roots contain proanthocyanidinent – epiafzelechin -3-O-phydroxybenzoate, entepia-fzelechin, afzelechin, and glycoside as 4-O-glycosyloxy-2-hydroxy-6-methoxyacetophenone [30]. Previous studies revealed that biochemical composition, nutritional characteristics, and biological activity of apricots depend on various factors cultivation area, climatic conditions, cultivation time, and ripening stage [8, 17, 31-32]. There were also some recent studies on triacylglycerols profiles, fatty acid profile, biodiesels production and essential oil application *P. armeniaca* kernel [33-37]. To best of our knowledge, no report is available on the total phenols, alkaloids, tannins content, and antioxidant activity of wild *P. armeniaca* seed collected from the Garhwal region of Uttarakhand, India; therefore this study is taken into account

MATERIALS AND METHODS:

Chemicals: Folin-Ciocalteau reagent and Folin-Denis reagent, potassium nitrate, ethanol, carbon tetrachloride, potassium acetate, ferric chloride, sodium carbonate, potassium iodide, methanol, ascorbic acid, catechol, gallic acid, caffiene, methanol,2,2-Diphenyl-1-Picryl-hydrazyl (DPPH, potassium ferricyanide, trichloroacetic acid, curcumin, sodium nitroprusside. All reagents are of analytical grade.

***Prunus armeniaca* Seeds:** *Prunus armeniaca* seeds collected from Kandaee village lies in Pauri district of Garhwal region between the range of Latitude 29° 45′ to 30° 15′ and E Longitude 78° 24′ to 79° 23′. Dried fruits collected in polythene bags and brought to the laboratory. The voucher specimen (GEU/chem. no. 36) was deposited, and seeds were identified/ authenticated by the national institute of science communication and information resources, New Delhi.

Extraction of Seed Oil: 1.5 to 2 g of powdered seed weighed into cellulose thimble covered with glass wool to prevent floating and attached with a weighed dried flat-bottom flask with the help of soxhlet assembly for oil extraction. 250 to 300 ml of Pet ether (40°-60 °C) added in this flask and kept at refluxing assembly for 8 h till complete extraction after complete extraction flask removed and the solvent evaporated by a rotary evaporator then oven-dried at 105 °C to remove any traces of solvent and oil % calculated by difference [38].

Determination of Phytochemicals: (Total phenolics, Tannins, Total alkaloids) approximately 2 gm of powdered seed mixed with 10ml of 80% ethanol, and the solution was stirred approximately for 3 h on a magnetic stirrer. The extract was centrifuged atrpm of 10,000 for 15 minutes to obtain clear supernatants for the testing of phytochemicals [39-41]. Phenolic content was determined by the Folin-Ciocalteau reagent. One ml sample was mixed with 3ml distilled water and 0.5 ml of colour developing reagent. After 2 min 20 % sodium bicarbonate reagent ($NaHCO_3$) was added, and the resulting

solution was boiled for 1 min. The absorbance was recorded at 650 nm on UV-VIS Spectrophotometer (Shimadzu) after the development of blue colour against reference standard catechol and calculated as per formula.

Total Phenolics mg/ 100 gm (y) = mx+c (by standard linearity curve)

Y-absorbance, m-instrument sensitivity, x-concentration to be calculated, c-correction

For determination of tannin content**,** in 1 ml sample,7.5 ml distilled water,0.5 ml Folin Denis reagent, and 1 ml of 35% $NaHCO_3$ solution was added and mixed properly, and the absorbance was recorded by UV-visible double beam spectro-photometer (Shimadzu) at 700 nm against a reference standard gallic acid, calculated as per the formula

Tannins, mg/100 gm (y) = mx+c (by standard linearity curve)

Y-absorbance, m-instrument sensitivity, x-concentration to be calculated, c-correction For alkaloid content estimation, 1.5, gm seed powder was taken into a separating funnel and 10 ml of distilled water, 1 ml of 20% aqueous Na_2CO_3 solution was added and extracted with 10 ml of carbon tetrachloride three times. The non-aqueous carbon tetrachloride layer collected in a clean 50 mL volumetric flask and diluted to 50 mL with the carbon tetrachloride. The absorbance of the sample was measured on a UV-Vis spectrophotometer at 270 nm in reference to caffeine's standard. Alkaloid content calculated as per formula given

Alkaloids, mg/100gm (y) = mx+c (by standard linearity curve

Y-absorbance, m-instrument sensitivity, x-concentration to be calculate-correction

All the tests are done in triplicate and the mean values are taken for result interpretation.

Determination of Antioxidant Activity of Seed:

DPPH Radical Scavenging Activity: 2,2-Diphenyl-1-picrylhydrazyl, commonly known as DPPH dark coloured crystalline powder composed of stable free radicals used as an indicator for antioxidant evaluation. In reaction, blue colour dye changes light colour to colourless depending on antioxidant capacity of the material. This activity of seed oil is determined based on Cuendet method [42] with some modifications. Standard ascorbic acid dissolved in methanol at different concentrations (mg/ ml) for comparison with methanol diluted seed oil. One ml of 0.1mM freshly prepared methanolic DPPH was added in standard and sample and mixed thoroughly. The resulting solution was incubated at room temperature for 30 min, and absorbance was measured at 517 nm against control and % Free radical scavenging activity (FRSA) calculated as per equation mentioned below.

$$\%FRSA = [(A_{DPPH}-A_{EXTR)} / A_{DPPH}] \times 100$$

Where $A_{DPPH\ is}$ the absorbance value of the control sample, and A_{EXTR} is the absorbance of seed oil.

Reducing Power Assay: In this method, compound to be tested for reduction potential with potassium ferricyanide to potassium ferrocyanide, according to the method of Oyaizu [43] by using Ascorbic acid as standard. Serial concentrations of standard and seed oil were mixed with 2.5 ml of 0.2M phosphate buffer (pH6.6), 2.5 ml of 1% potassium ferricyanide solution, mixed and heated on a water bath for 20 min at 50 °C. In cooled solution, 2.5 ml of 10%w/v trichloroacetic acid solution was mixed. Total sample centrifuged for 10 min at 3000-4000 rpm. Now 2.5 ml of supernatant liquid was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% w/v ferric chloride solution mixed properly, absorbance taken at 700 nm by UV-VIS double beam spectro-photometer. Increasing in absorbance with concentrations of seed oil correlates with their significant reducing potential

% reducing power = (Test OD - Control OD / Test OD) × 100

Nitric Oxide free Radical Scavenging Activity: Nitric oxide free radical scavenges oxygen to stop chain reaction by reducing nitrite ions production. This activity of seed oil was determined on the method of Sreejayan [43] by a reagent known as Griess reagent with some modifications.

Curcumin was used as a reference; both the standard and extracted seed oil were diluted with methanol at different concentrations. One ml of Sodium nitroprusside (10 mg) in Phosphate buffer saline and 2 ml methanol added to the standard and sample and mixed vigorously.

The resulting solution was incubated at 25 ± 2 °C temperatures for 150 min, and after incubation 5 ml of Griess reagent added. The absorbance of the resulting solution was taken at 546 nm in the UV-visible double beam spectrophotometer (Shimadzu) against blank without sample. Reduction % and IC_{50} of oils are calculated as follows.

% Antiradical activity = (Control absorbance- Sample absorbance/ Control absorbance) × 100

All the tests are done in triplicate, and the mean values are taken for result interpretation.

RESULTS AND DISCUSSION: Phytochemicals, *e.g.*, phenolics, tannins, alkaloids, are chemical compounds that occur naturally in plants. Even though they are non-essential nutrients, means not required sustaining life, but they prolong the life of plant species because of their significant physiological effects. They act as antimicrobial, antioxidants, enzyme and hormones stimulators, helps in DNA replication, binding to cell walls, therapeutic effects on diseases such as cancer, cardiovascular and other chronic problems. Tannins are bitter polyphenolic compounds with an astringent taste, having a very important role in the protection of plants from the attack of insects, predators due to their astringency characteristics [44].

Polyphenolics work as a natural antioxidant against damage caused by free radicals formed in common metabolic processes. Polyphenols help in improving the sensitivity of the nervous system, gene regulation, inflammatory enzyme activity [45-46] Alkaloids the phytochemicals that composed of heterocyclic nitrogen compounds. In ancient times alkaloids extract used as an ingredient in liquid medicines and in the treatment of various diseases. Codeine and morphine as an analgesic (painkiller), quinine as antimalarial, atropine as an antidote to nerve gas poisoning, Caffeine as central nervous system stimulant, nicotine as insecticides, sanguinarine as antibacterial showing antiplaque activity, used in the oral rinse and toothpaste [47].

Phytochemicals in *P. armeniaca* Seed Oil: Screening of seed oils of wild apricot revealed that it contains an appreciable amount of phytochemicals, responsible for various physiological effects in which one most important is antioxidant activity.

TABLE 1: (A) ABSORBANCE OF TANNINS CONTENT (B) ABSORBANCE OF TOTAL PHENOLICS (C) ABSORBANCE OF TOTAL ALKALOIDS

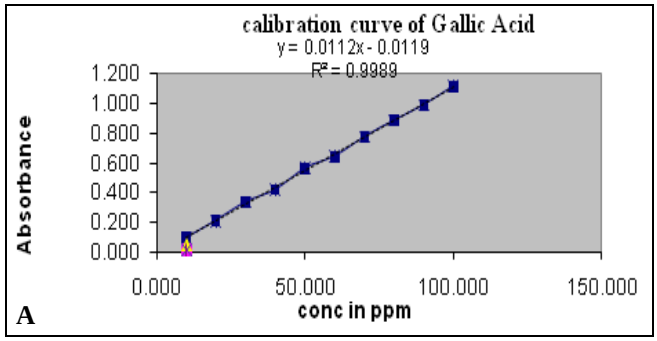
S. no.	Conc. in ppm	Absorbance at 700 nm	*P. armeniaca*
1	10.0	0.098	0.085
2	20.0	0.214	
3	30.0	0.339	
4	40.0	0.421	
5	50.0	0.567	
6	60.0	0.647	
7	70.0	0.778	
8	80.0	0.889	
9	90.0	0.992	
10	100.0	1.121	

B

S. no.	Conc. in mg\ml	Std (Catechol) Absorbance (650nm)	*P. armeniaca*
1	10	0.089	0.0212
2	20	0.186	
3	30	0.244	
4	40	0.356	
5	50	0.396	
6	60	0.489	

C

S. no.	Conc. in mg\ml	Abs. at 270 nm	*P. armeniaca*
1	5	0.054	0.0112
2	10	0.086	
3	15	0.121	
4	20	0.187	
5	25	0.201	
6	30	0.254	



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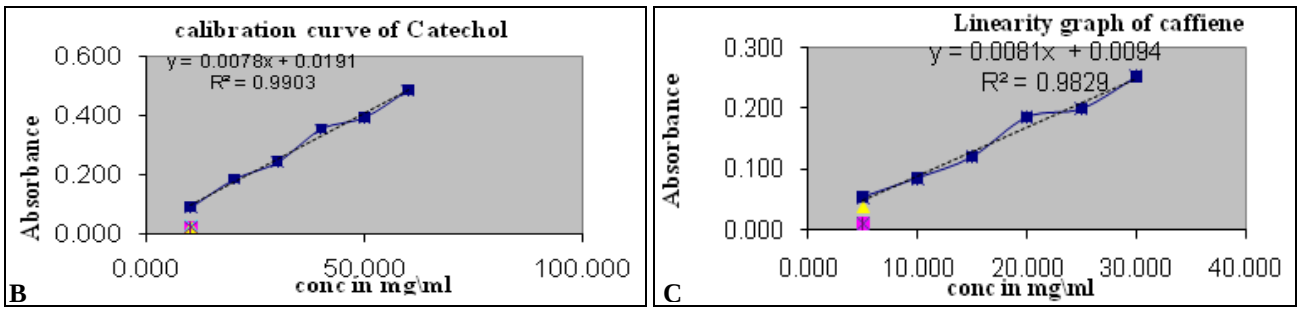

FIG. 1: CALIBRATION CURVE OF STANDARD (A) GALLIC ACID (B) CALIBRATION CURVE OF CATECHOL (C) CALIBRATION CURVE OF CAFFEINE

TABLE 2: PHYTOCHEMICAL CONTENT OF SEEDS

S. no.	Phytochemical Contents	*Prunus armeniaca*
1	Total tannin content, %	6.47
2	Total phenolics content, %	0.27
3	Total alkaloid content, %	0.22

P. armeniaca seed contains 6.47% tannin that is supported by the data obtained in previous research on apricot that is (0.0038%) which differ in concentration but gives strong evidence of the presence of tannin content [48]. Alkaloid content present in seeds of *P. armeniaca* (0.22%) while phenolic content was 0.27%. Total phenolic content (TPC) of apricot kernel grown in Ladakh region was found to be 92.2 to 162.1 mg gallic acid equivalent/100 g (0.092% to 0.016%) [49]. The total phenolic compound found in fresh apricot samples was in range of 4452.7 mg GAE 100 g−1 (4.45%) Catechin was the predominant phenolic compound in all apricot samples under study followed by quercetin 3-rutinoside and epicatechin, which belong to the flavonoids group [50].

Antioxidant Activity of Seed Oil: Antioxidant are those substances which can delay or inhibit oxidation of oxidizable substrate by neutralizing free radicals, released as unwanted by-product continuously during the essential aerobic metabolism. The anti-oxidant potential of *P. armeniaca* seed oil was determined by three methods and the outcome is listed below.

DPPH Scavenging Activity: Free radical scavenging activity of the seed oil is highest at the concentration of 1.0 mg/ml, against ascorbic acid as reference standard. IC_{50} value is 59.95 % at 0.1 mg/ml.

Reducing Power Assay: Potential antioxidant activity of any compound correlates with its reducing capacity. The sample reduces the Fe^{3+} (ferric) / tripyridy-ltriazine complex to Fe^{2+} (ferrous) form which can be observed visually by the change of colour% [51].

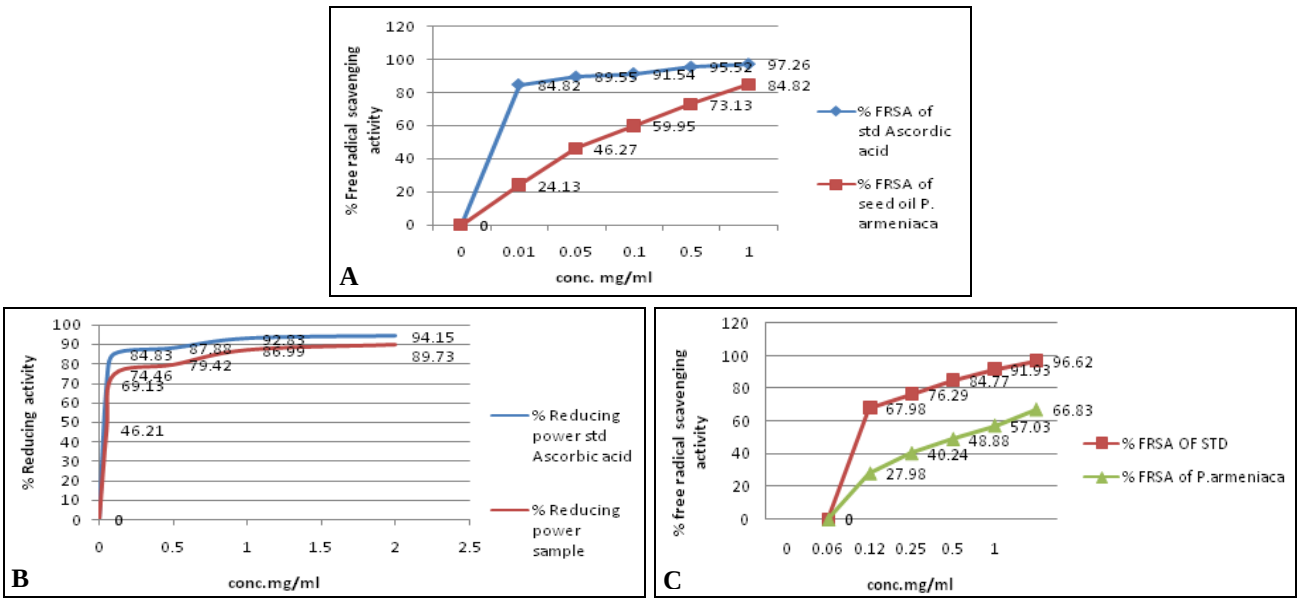

FIG. 2: THE ANTIOXIDANT ACTIVITY OF *PRUNUS ARMENIACA* SEED OIL WAS EVALUATED WITH SEVERAL ESTABLISHED METHODS (A) DPPH RADICAL SCAVENGING ASSAY (B) REDUCING POWER ASSAY (C) NITRIC OXIDE SCAVENGING METHOD

P. armeniaca seed oil is standardized for its antioxidant capability against ascorbic acid, 50% reducing power obtained at a concentration of 0.5 mg/ml.

TABLE 3: THE ANTIOXIDANT ACTIVITY PERCENTAGE CALCULATIONS

Con.mg/ml	DPPH Method		Reducing Power Assay		Nitric Oxide free Radical Scavenging Activity	
	Ascorbic Acid (Standard)	*P. armeniaca* Oil	Ascorbic Acid (Standard)	*P. armeniaca* Oil	Ascorbic Acid (Standard)	*P. armeniaca* Oil
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.01	84.82	24.12	69.13	15.47	67.98	27.98
0.05	89.55	46.26	84.83	26.8	76.29	40.25
0.1	91.54	59.95	87.88	51.03	84.77	48.88
0.5	95.52	73.13	92.83	56.17	91.93	57.03
1.0	97.26	84.82	94.15	77.81	96.62	66.83

Nitric Oxide Free Radical Scavenging Activity: Nitric oxide radical scavenging of seed oil was evaluated and results are in the range of 27.98 - 66.83% at variable concentrations listed in **Table 3.** IC_{50} values are 0.5 mg/ml, at which oil showed more than 50% free radical scavenging efficiency. IC_{50} value summarised in table 4 observed by all three methods for the estimation of antioxidant property of seed oil. IC_{50} is commonly known as "half-maximal inhibitory concentration indicates the concentration required to inhibit a biological or biochemical function by half means effective for removal of 50% free radicals or oxidative species, which are responsible for degenerative oxidation of biological system and causes various diseases. Previous references are available regarding the antioxidant activity of seed oil of *P. armeniaca.*

A study by Stryjecka *et al.* (2019) [37] on antioxidant activity of five apricot (*P. armeniaca*) cultivars grown in Poland by FRAP assay (ferric reducing antioxidant power) showed that seed oil of *P.armeniaca* has good antioxidant activity. Zhou *et al.* (2018) [52] reported that oil extracted from roasted apricot kernels at 120 °C to 180 °C temperatures for 10 min showed improved oxidative stability and antioxidant activity due to the production and transference of alcohol-soluble phenolics into the oil. Data obtained from research done in Turkey also showed that total antioxidant capacity, total carotenoid and total phenolic contents were highest in most of wild apricot fruits [53]. Different studies are also available which showed the antioxidant activity of seed and fruit of *P. armeniac* [8, 22, 36, 49, 52].

TABLE 4: COMPARISON OF IC_{50} VALUES OF SEED OILS WITH STANDARD

IC_{50} Value (mg/ml)	*P. armeniaca*	Std IC_{50} Value (mg/ml)
DPPH Scavenging method	0.1	0.06
Reducing power Aassay	0.5	0.05
Nitric oxide Scavenging	0.5	0.06

CONCLUSION: The phytochemical and anti-oxidant studies of seed and seed oil revealed the presence of medicinally active constituents like alkaloids, tannins, phenolics and various biological activities which has also supported by earlier research and literature. Therefore, extracts and oils from these plants could be seen as a good source for useful drugs and health benefits.

This research also confirms the antioxidant capacity of seed oils which is comparable to those of the standard compounds ascorbic acid, curcumin and compared with previous results, therefore be proposed as new potential sources of natural additives for the pharmaceutical industries.

The present work is not only for commercial interest, may have a social, economic and ecological impact also. Wild seed oils, according to their chemical compositions, physiochemical properties can be used as a source of raw materials in different industries related to, food processing, medicinal, cosmetics, and biofuels.

These research data showed that commercialization of these wild oilseeds has a significant economic impact on local communities and there is potential for promoting wild apricot fruit from specific geographical regions because they contained a higher amount of natural phytochemicals and antioxidant compounds.

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