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Bioactive compounds and medicinal properties of Aonla (*Phyllanthus emblica* L.)

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Abstract

The phytochemical characterization of five aonla types was examined using RP-HPLC on methanolic, ethanolic, and ethyl acetate extracts. Aonla extracts were subjected to RP-HPLC analysis at 270 nm, which allowed the identification of five compounds: ascorbic acid, gallic acid, ellagic acid, ethyl gallate, and quercetin. The amounts of identified phytochemicals in each variety showed significant differences. Following varieties Kanchan, NA-7, Banarasi, and Chakaiya in order of phytochemical concentration, the greatest amount was found in the methanolic extract of variety Desi. Methanol, one of the solvents, extracted the most phytochemicals, but ethyl acetate, used to extract the different aonla types, produced the least. The two phenolic components that were present in the highest concentration in aonla variety extracts were gallic acid and ellagic acid. The most prevalent phenolic components in extracts of aonla cultivars were gallic acid and ellagic acid. The highest concentrations of ellagic acid (349.51 mg/100 g) and gallic acid (233.49 mg/100 g) were discovered in the methanolic extract of the Desi variety. The current study's findings indicated that aonla is an excellent source of phenolic and flavonoid chemicals.

1. Introduction

The words “bioactive” are made up of the “bio” and the “active.” The derivation of the word “bio” is “bios,” which is derived from the Greek and means “life” and the prefix “active” comes from the Latin word “activist,” which implies to be lively, energetic, or to include action. This activity displays every phenomenon that gives rise to a type of life, a working system, or a process. Growing interest is being shown in bioactive molecules across a variety of fields, including geomedicine, plant science, contemporary pharmacology, agrochemicals, cosmetics, the food industry, nanobioscience, etc. This emerging area of research holds great promise which has resulted in an increase in a number of research projects aimed at diversifying the sources of bioactive chemicals and enhancing their production or salvage routes. Plants generate typical bioactive chemicals. Bioactive compounds are not just found in plants. These compounds are also present in some types of animals and other living things, including bacteria mushrooms, and other microbes. According to Schrezenmeier *et al.* (2000), there are often two limitations to the notion of bioactivity. Positive and negative health consequences have previously been explored; the other calls for the bioactive component to have quantifiable biological effects at physiologically relevant levels. Given the wide spectrum of physiological systems that this bioactivity may impact, completing this task will be challenging and need the simultaneous involvement of several specializations and disciplines.

Aonla, a plant belonging to the Phyllanthaceae family and commonly referred to as Indian gooseberry, is found in abundance in tropical

and subtropical regions of China, India, Indonesia, and the Malay Peninsula. Along with Chinese and Tibetan medicine, aonla is a crucial component of Indian medicine (Ayurvedic Formulation of India-Part-1, 2003). Gallic acid, ellagic acid, tannic acid, quercetin, and other phenolic and flavonoid compounds have been found to have radical scavenging action, analgesic, anti-inflammatory, and immunomodulatory activities. According to Vinayagamoothy (1982), aonla's aqueous extract has antibacterial, antipyretic, and tonic effects. Sachan *et al.* (2013) demonstrated that aonla has nutritional benefits, which will raise awareness of the fruit among educated people. Aonla's phenolic component lessens ascorbic acid loss from oxidation during heating and low-temperature storage. Previous phytochemical studies reported that major bioactive compounds present in the aonla fruits are phenolics such as hydrolyzable tannins (both ellagitannins and gallotannins), anthocyanins, flavonoids, flavanols, and phenolic acids. Aonla fruit has >70 g of carbs per 100 g of dry weight (DW), making it a significant source of carbohydrates. Along with the amounts of protein, fat, and minerals like iron, calcium, and phosphorus that are present (ranging from 2.0 to 4.5, 2.1 to 3.1, and 0.2 to 0.6 g/100 g DW, respectively) fiber is another important element (7.2-16.5 g/100 g DW). Another important component which is found in aonla fruit is ascorbic acid (vitamin C).

Since the beginning of time, various phenolic-containing plant components have been employed to cure a variety of ailments. Prior to clinical application, preclinical research is necessary to evaluate the pharmacological, phytochemical, toxic, and biological properties of any herbal medication. In order to determine its efficacy, a comprehensive qualitative and quantitative investigation of the phenolic component contained in plants using scientific techniques is needed. The primary goal of this work was to identify and measure the primary phytochemicals in aonla.

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The aonla fruit's 100 g of pulp contains 82.2% water, 0.5% protein, 0.1% fat, 14% carbohydrates, and 600 mg of vitamin C. When the aonla fruit juice is taken out, the vitamin C

concentration increases even higher. Vitamin C levels in the dried fruit of aonla range from 2428 to 3470 mg per 100 g, which is a large amount.

Table 1: Nutritional composition of Aonla

Nutrients		Vitamins		Minerals	
Dietary fiber	17%	Vitamin C	46%	Manganese	7%
Carbohydrates	3%	Vitamin A	6%	Potassium	6%
Protein	2%	Vitamin B6	4%	Copper	4%
Calories	2%	Thiamin	3%	Phosphorus	3%

Table 2: Nutritional constituent of Aonla

Variety	Moisture (%)	Carbohydrate (%)	Fiber (%)	Minerals (%)	Protein (%)	Fat (%)	Vitamin (mg/100 g)
NA-10, Balwant, Chakaiya, Hathijhool	81	14	3.2	0.3	1	0.5	720
NA-7, Banarasi, Kanchan, Desi	81.3-84.6	73.8-87.1	7.2-22.4	2.2 to 3.1	2- 3.2	0.4-0.5	193-315
Krishna	85.6-87.7	70.7-73.8	13.9-16.5	2.3-2.8	2.9-3.6	0.5-0.6	421-506

2. Phytochemicals of Aonla

2.1 Hydroxybenzoic acid

4-Hydroxybenzoic acid (PHBA) sometimes also called p-hydroxybenzoic acid (PHBA), is a phenolic derivative of benzoic acid. Hydroxybenzoic acid is a white crystalline solid that's marginally soluble in H₂O and CaCl₂, but much more soluble in polar organic solvents like alcohol and acetone. 4-Hydroxybenzoic acid is also called a starting point for the production of paraben esters which are used as preservatives in cosmetics and some ophthalmic treatments.

2.2 Coumaric acid

The organic chemical coumaric acid has the formula H₉O₂C₆H₄CH = CHCO₂H. It is one of three hydroxycinnamic acid isomers. Coumaric acid is a white substance that is very slightly soluble in water but substantially soluble in ethanol and diethyl ether. P-coumaric acid (PCA) is a phenolic acid with antioxidant, anti-inflammatory, analgesic, and antibacterial effects.

2.3 Syringic acid

Syringic acid is a naturally occurring phenolic chemical and dimethoxybenzene derivative of gallic acid that is 3, 5-dimethyl ether. It functions as a plant metabolite. It belongs to the benzoic acid family and phenol family. Syringic acid syringe conjugate acid.

2.4 Protocatechuic acid

Protocatechuic acid is a dihydroxybenzoic acid which is a form of phenolic acid. It is a key metabolite of antioxidant polyphenols. In *in vitro* and *in vivo* investigations, it has mixed effects on normal and malignant cells. Protocatechuic acid has numerous pharmacological activities, such as antioxidant, anti-inflammatory, neuroprotective, antibacterial, antiviral, anticancer, antiosteoporotic, analgesia, and antiaging properties; protection from metabolic syndrome; and preservation of liver, kidney, and reproductive functions.

2.5 Vanillic acid

The chemical name of vanillic acid (4-hydroxy-3-methoxy benzoic acid) is a flavoring ingredient derived from dihydroxybenzoic acid. It is an oxidized version of vanillin. Vanillic acid is also used as an intermediate in the synthesis of vanillin from ferulic acid. Vanillic acid inhibits the related molecular pathways, resulting in various bioactivity against cancer, diabetes, obesity, neurodegenerative, cardiovascular, and hepatic illnesses. Its derivatives may also be used to treat autoimmune illnesses, as well as fungal and bacterial infections.

2.6 Corilagin

It is a kind of ellagitannin. Corilagin was identified in 1951 from extract and *Caesalpinia coriaria*, thus the molecule's name. It is also present in *Alchorneag landulosa* and *Punica granatum* leaves. It is a mild inhibitor of carbonic anhydrase. Corilagin is an ellagitannin containing a hexahydroxydiphenoyl group that bridges the glucose core's 3-O and 6-O. It functions as an antihypertensive, (peptidyl-dipeptidase A) inhibitor, nonsteroidal anti-inflammatory medication, and antioxidant.

2.7 Quercetin 3O-glucoside

It is a flavonoid oxo anion formed by the deprotonation of the hydroxy group at position 7 of quercetin's flavone molecule.

2.8 Chebulinic acid

It is an ellagitannin that is found in Euphorbia longan seeds, *Terminalia chebula* fruits, and *T. macroptera* leaves.

2.9 Kaempferol

Kaempferol is a kind of flavonoid that is present in a wide range of plants and plant-derived foods such as spinach and broccoli. It is a yellow crystalline solid with a melting point of 276°C - 278°C (529-532 degrees Fahrenheit). Kaempferol is very soluble in hot ethanol, ethers and DMSO is just marginally soluble in water. Engelbert Kaempfer, a 17th-century German naturalist, inspired the term Kaempferol.

2.10 Gallic acid

The chemical name of gallic acid is 3,4,5-trihydroxy benzoic acid is a ($C_6H_2(OH)_3CO_2H$). Gallic acid has been linked to several health benefits such as antioxidant, anti-inflammatory, and anticancer characteristics. This chemical is effective in the treatment of gastrointestinal, cognitive, metabolic, and cardiovascular diseases.

2.11 Flavonols

Flavonols are plant metabolites that can provide health benefits through cell signaling pathways and antioxidant capabilities. Flavonols

have an important role in plant life since they are responsible for color, flavor, lipid oxidation prevention, vitamin and enzyme protection, and protection against ultraviolet (UV) radiations and parasites. The chemical structure of distinct flavonols determines their biological and pharmacological properties which include the presence of hydroxyl groups, functional group substitution, and a double bond between C_2 and C_3 . Flavonols are composed of a 2-phenylchromane nucleus ($C_6-C_3-C_6$). The skeleton of flavonol is composed of two benzene rings (ring A and B) joined by the 3-C chain that creates a closed pyran ring (C ring) that fuses with ring A.

Table 3: Various bioactive compounds in Aonla

Hentriacontane (Kim <i>et al.</i> , 2011)	C31-Saturated fatty acid ester compound	Antioxidative, Protection against UV radiation, Antitumour activity, Anti-inflammatory and Anticancer activity
D. L. α -Tocopherol (Jim Duke, 2015)	Alcoholic compound	Antioxidant property, Anticancer, Antitumor, Anti-mutagenic, Antidiabetic, Anti-infertility, Antiparkinsonian, Antialzheimeran, Antiatherosclerotic, Antistroke, Hepatoprotective, Cardioprotective, Immunomodulator, Vasodilator, <i>etc.</i>
1-Hexacosanol (Ramalakshmi <i>et al.</i> , 2011)	Long chain fatty alcohol	Antioxidant, Antibacterial, and Anticancerous, Neuronal growth stimulators
Octadecanoic acid, Methyl ester (Kumar <i>et al.</i> , 2010)	Unsaturated fatty acid ester compound	Antioxidant, Antibacterial, Antifungal, Anti-inflammatory, Antiarthritic, Antihistimic, Anticoronary

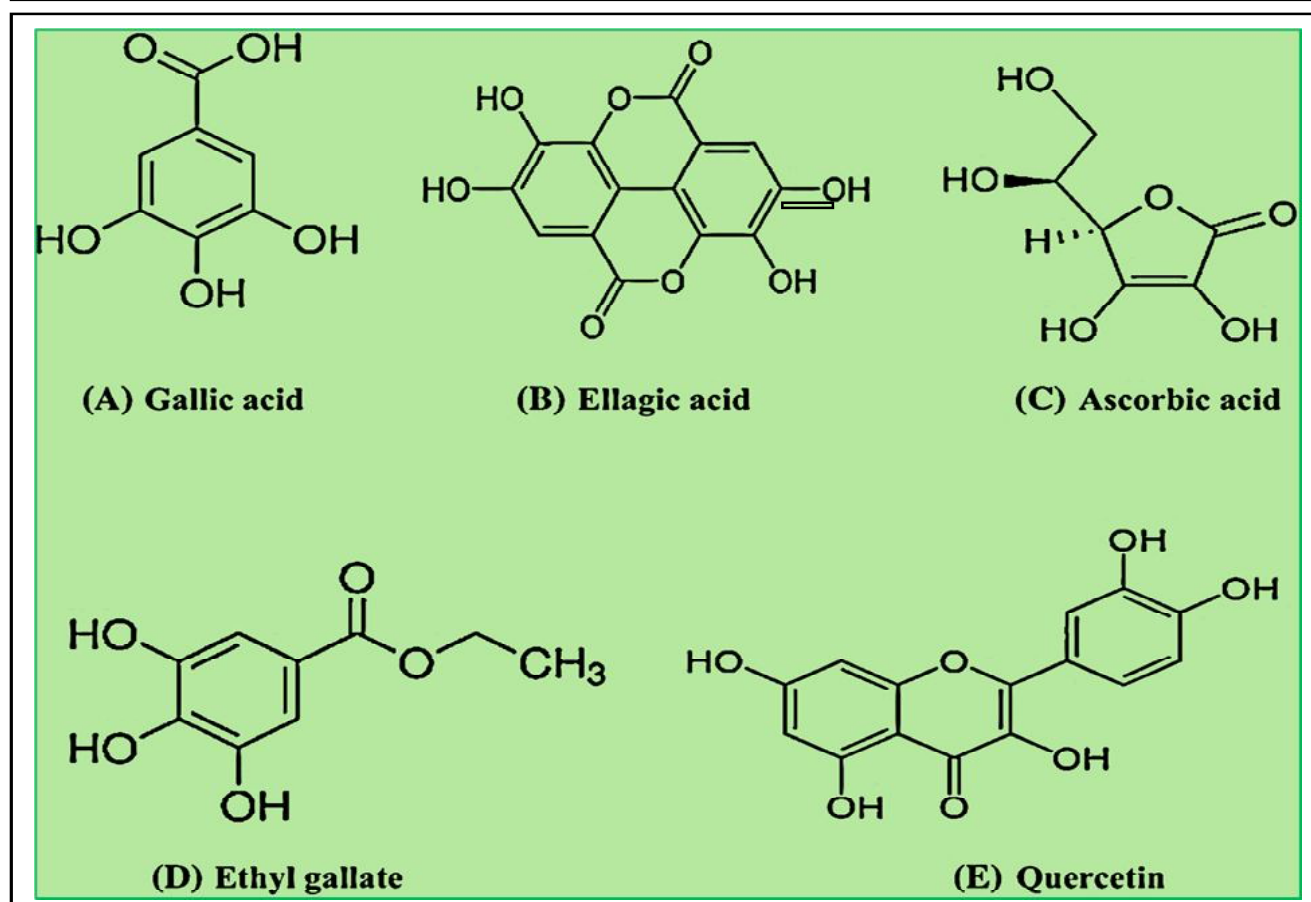


Figure 1: Chemical structure of bioactive compounds present in aonla: (a) Ascorbic acid, (b) Gallic acid, (c) Ethyl gallte, (d) Ellagic acid, and (e) Quercetin.

3. Medicinal uses

It has been used to cure ailments in the traditional medical systems including the Thai medical system, Chinese herbal medicine, Tibetan medicine, Sri Lankan medicine, and the Indian traditional healthcare system. The fruits of aonla are employed in Indian and Unani medical systems due to the availability of phytochemicals such as geraniin and isocorilagin, which have immunostimulatory actions and aid in immune system boosting. Aonla is also used for Ayurvedic treatment. Agadatantra (Ayurveda toxicology), one of Ashtanga Ayurveda's eight divisions, describes numerous antidotes or antitoxic compositions known as Agada (Shastri, 2014). Aonla is also a rich source of nutrients, including amino acids, vitamin C, carbohydrates, alkaloids, and phenolic acids. A variety of pharmacological activities that cure the prevention and treatment of diseases have been described for all aonla components, but mainly the fruit. A promising component for use in natural healthcare goods such as food, health, and cosmetics has been identified as the 1,1519 Aonla branch. The therapy of hyperlipidemia one of the main causes of cardiovascular problems may be helped by aonla bioactive substances. The prevention of cardiovascular illnesses by aonla and/or its constituents has been demonstrated in numerous research. One instance is the research done by Nambiar *et al.* (2015). On the impact of aonla juice which contains the major polyphenols myricetin, gallic acid, and

kaempferol on low-density lipoprotein (LDL) oxidation. According to the authors, LDL cholesterol oxidation was 90 % lessened by limiting the uptake of LDL oxidation in macrophages. According to a different study, amla polyphenols (emblicanin A and B, gluconic, and pedunculagin) prevented the development of fibrosis in mice's cardiovascular tissue after ischemia and reperfusion. Studies examining the effects of aonla supplementation in children from beetle families. Found that the supplemented group had lower levels of LDL, cholesterol, and blood glucose than the control group. This study also showed that *P. emblica* controlled the level of serum electrolytes and activation of the endogenous antioxidant system.

3.1 Antidiabetic activities

The natural components of the *P. emblica* plant are connected to antidiabetic activities. According to *in vitro* research, the key phytochemicals in the aonla, such as ellagic acid and vitamin C block the critical enzymes responsible for the breakdown of glucose (especially amylase and glucosidase). Clinical studies back up the health benefits of aonla phytochemicals for diabetics. Considering an example, following 21 days of trim levels in animals with induced diabetes, daily doses of 3 g of *P. emblica* powder extract lowered blood glucose levels in diabetic persons. In another investigation with diabetic mice, 250 mg/kg and 500 mg/kg ellagic acid-rich *P. emblica* extract dramatically lowered blood sugar levels.

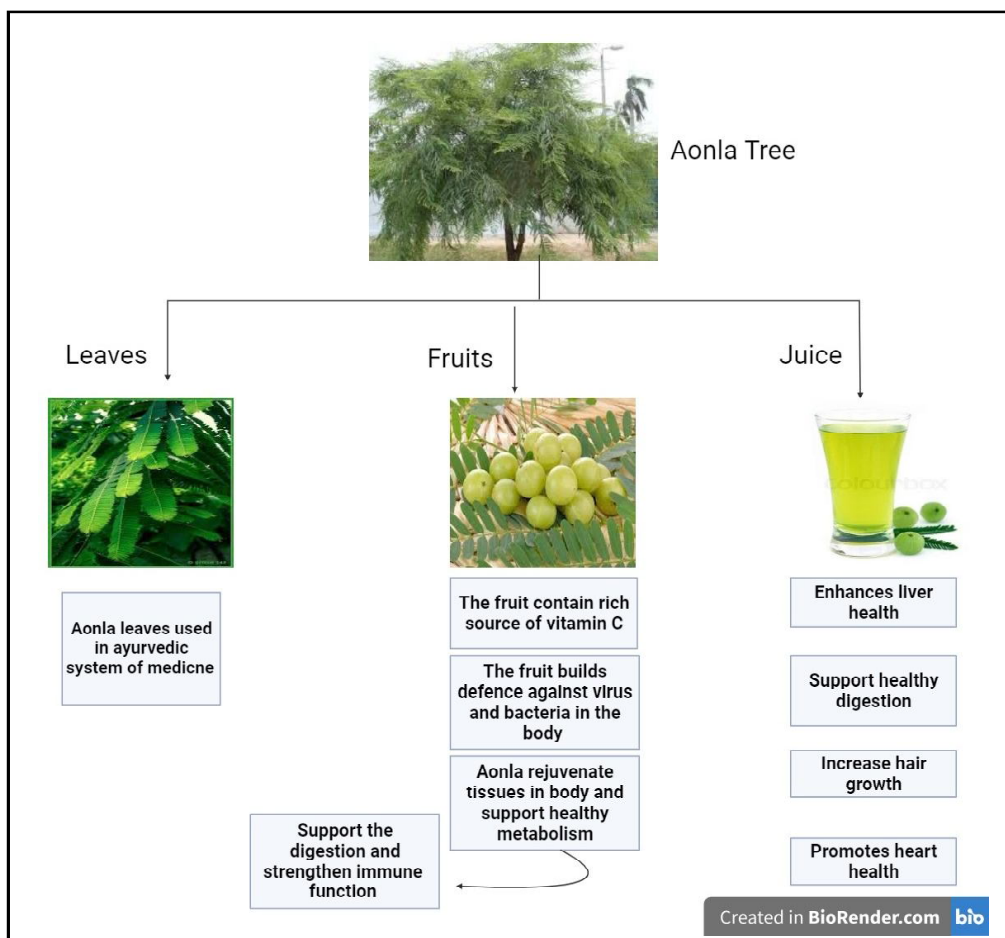


Figure 2: Medicinal uses of Aonla.

3.2 Anticancer activity

Numerous nonclinical and clinical investigations have shown that plant-derived polyphenols help to prevent cancer. Polyphenols, for instance, function in several ways to minimize DNA damage, produce chemicals that increase inflammation, inhibit oxidative stress, and trigger apoptosis. The HeLa cell line demonstrated DNA fragmentation increased caspase-3, 7, and 8 activity and up-regulation of protein in response to aonla extracts indicating the activation of the death

Table 4: Phytochemicals found in Aonla

	Hydroxybenzoic acid	Coumaric acid	Syringic acid
Chemical name	4-Hydroxybenzoic acid	4 hydroxy cinnamic acid	3,5-dimethyl ether
Solubility	soluble in H ₂ O and CaCl ₂ but much more soluble in polar organic solvents like alcohol and acetone	very slightly soluble in water but substantially soluble in ethanol and diethyl ether	Soluble in methanol, chloroform, water, and ethyl alcohol
Uses	Used as preservatives in cosmetics and some ophthalmic treatments	Used as antioxidant, anti-inflammatory, analgesic, and antibacterial effects	Used for the treatment of various diseases like diabetes, cancer, cerebral ischemia, neuro, liver damage, anti-microbial and antiendotoxic activities

3.3 Digestive tract protection

P. emblica polyphenols have been demonstrated to protect the gastrointestinal organs. Because *Helicobacter pylori* is a recognized cause of stomach ulcers, one of the possible effects of aonla bioactive substances is suppression of clarithromycin-resistant *H. pylori* strains *in vitro*. Many digestion-related issues are on the rise as a result of changing food habits and everyday routines. Diverticulitis, ulcerative colitis, microscopic colitis, proctitis, appendicitis, necrotizing enterocolitis, rectal ulcer, haemorrhoids, colorectal cancer, constipation, irritable bowel syndrome, and other functional and structural diseases, infections, and irritations (Amine *et al.*, 2003). Fruit and vegetable eating is expected to save 2.7 million lives worldwide (Chellammal, 2022).

4. Extraction of bioactive compounds

Fresh mature healthy leaves of *P. emblica* were obtained in November from Algarkovil hills near Madurai, Tamil Nadu, India. Dr. P. Jayaraman, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai-05, authenticated the selected plants. To avoid the loss of essential oil, the leaves are shade-dried at room temperature and processed using a grinding machine. The extraction method used was Soxhlet extraction. For 8 h, 200 g of dry powdered plant material was extracted in a Soxhlet device with 250 ml of petroleum ether. Following extraction, the solvent is evaporated using a rotary vacuum evaporator to get a concentrated extract at 60°C in a water bath. It is then aseptically dried with the aid of a drier and subjected to spectroscopic examination. FT-IR analysis petroleum ether leaf extracts of *P. emblica* is analyzed by using (FT-IR) (Thermo Scientific Nicolet 1S5 FT-IR). The spectrum is concentrated in the IR regions 600-4000 cm using the KBr pellet technique. The spectrums of leaf extract were recorded. GC-MS spectroscopy the GC-MS technique is used in this study to identify the phytochemicals present in the extracts; the GC-MS analysis of crude extracts was performed by using a Perkin Elmer system (GC Clarus 600, USA) equipped with an AOC-20i autosampler and gas chromatograph interfaced to mass spectrometer (GC-MS) instrument under the following conditions: Column Elite-5MS fused silica capillary column (30.0 m 0.25 mm

receptor pathway for apoptosis although caspase-9 remained unchanged. The *in vitro* matrigel invasion analysis in this study also found that *P. emblica* inhibited MDA-MB-231 cell invasiveness, although normal lung fibroblasts showed no cytotoxicity (MRC5). The polyphenol pyrogallol from *P. emblica* is also examined in the human lung cancer cell lines H441 and H₃2₀. Pyrogallol reduced cell growth. Proliferation was inhibited by the decrease in cyclin B1, Cdc25c, and Bcl-2 expression as well as the increase in Bax expression.

ID, 250 m d f). The detector was in electron impact mode, with ionization energy at a constant flow rate of 1 ml/min, the energy is 70 eV with helium (99.999 %) as the carrier gas. The injection volume was 1 l of the sample (split ratio of 10:1), and the injector temperature was set at 240°C. The oven temperature is set to 60°C (isothermal for 2 min), with a 10°C/min increase to 300°C/min for 6 min.

4.1 Extraction of flavonoids

Flavonoids 5 ml of weak NH₄ solution was added to aqueous filtrate followed by the concentrated sulphuric acid. The endpoint shows yellow colour which indicates the presence of flavonoids.

5. Conclusion

According to the study's findings, aonla fruits contain phenolic and flavonoid chemicals. Overall, methanol was the best solvent for phenolic and flavonoid extraction. Regardless of the extraction solvent utilized, the Desi variety had the greatest phytochemical content of all the cultivars. Using RP-HPLC, the primary phytochemicals in aonla extract were identified as gallic acid, ellagic acid, ethyl gallate, ascorbic acid, and quercetin. The existence of the recognized phytochemicals was verified by aonla's FTIR spectra. To further understand the aonla fruit as a possible source of phytochemicals, additional research is needed to isolate and identify more bioactive phytochemicals from the crude extract of aonla fruits. Additionally, phenolic and flavonoid compound purification is required to support their medicinal and nutraceutical benefits.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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