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Ethyl Acetate Extract of *Annona reticulata* Linn.: An Assessment of its Cytotoxicity and Antioxidant Properties

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Abstract:

Annona Reticulata a little evergreen tree of the Annonaceae family, linn is grown for its fruits all throughout India. Its varied components are also utilized in traditional medicine to cure a variety of ailments. It is a plant that is widely used in the Ayurvedic medical system to treat a wide range of illnesses. A. reticulata parts are utilized in both industrial and medicinal goods. It has a number of therapeutic benefits, including anthelmintic, analgesic, antipyretic, anti-inflammatory, and cytotoxic actions. Phytochemicals such as tannins, alkaloids, phenols, glycosides, flavonoids, and steroids are abundant in it. Therefore, the phytochemicals, antioxidant, and cytotoxic properties of *Annona reticulata* L.'s ethyl acetate extract will be the main topics of this review. The study's findings indicate that Annona reticulata Linn. leaves possess a considerable capacity for both cytotoxicity and antioxidants. Based on the results of this study, extensive pharmacological and phytochemical research is needed to eventually identify bioactive molecules as potential lead compounds.

Keywords:

Annona Reticulata; Acetate Extract; Phytochemical; DPPH

1. Introduction: Plants are a major source of medication and have a major impact on global health. It has long been recognized that medicinal herbs and plants can provide significant therapeutic benefits or healing agents [1]. Any plant that has compounds in one or more of its organs that have medical value or that serve as building blocks for the creation of effective medications is considered medicinal [2-4]. Over the past 10 years, the demand for medicinal plants for use in both developing and industrialized countries' traditional medicine (TM) and contemporary and alternative medicine (CAM) has expanded substantially [5, 6]. When it comes to using a spectrum of biochemicals, the kingdom of plants offers a high degree of structural diversity. The discovery of several novel pharmacophores has resulted from phytochemical investigations on medicinal plants. Pharmacophores have been essential in the search for new drugs.

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Table 1: List of medicinally important plant-derived drugs [7].

Sr. No.	Drug	Botanical source	Therapeutic uses
1.	Artemisinin	*Artemisia annua* L.	Antimalarial drug
2.	Galantamine	*Galanthus woronowii* Losinsk	Anti-Alzheimer's drug
3.	Taxol	*Taxus brevifolia*	Anticancer drug
5.	Opium alkaloids	*Papaver somniferum*	Analgesic, antitussive
6.	Vinca alkaloids	*Catharanthus roseus*	Anticancer
7.	Reserpine	*Rauvolfia serpentina*	Antihypertensive
9.	Quinine, Quinidine	*Cinchona spp.*	Antimalarial
10.	*Digitalis* glycosides	*Digitalis purpurea, Digitalis Lanata*	Cardiotonic glycosides
11.	Sennosides A and B	*Cassia angustifolia*	Laxative
12.	Pervilleine A	*Erythroxylum pervillei*	Anticancer
13.	Silvestrol	*Aglaia foveolata*	Cytotoxic

In Africa, Asia, Latin America, and the Middle East, traditional medicine (TM) still provides primary care for between 70 and 95 percent of the population. It is believed that 100 million people in the European Union (EU) alone use herbal, complementary, or traditional medicine; in some countries, this number can reach 90% of the total population [8]. Plants produce a large number of secondary metabolites, which are believed to be the primary source of compounds of medicinal value. Not all plants have pharmacological function, even if they all have some kind of chemical component. When chemical components have the capacity to exert pharmacological effects on the physiological systems of an animal body, they are referred to as active chemical constituents, or simply constituents [6, 9, 10]. For their health, around 75% of people on the planet primarily rely on plants and plant extracts. Plants are currently the source of more than 120 prescription medications that are clinically beneficial. The majority of these were created due to their application in conventional medicine.

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The use of medicinal plants has a long history; however, the use of the whole plant or raw materials for treatment or experimentation has many drawbacks, including changes in the plant's compounds in different climates, simultaneous development of synergistic compounds that lead to adverse effects of antagonists, or other unexpected changes in bioactivity, and changes or loss of bioactivity due to the variability and accumulation, storage and preparation of raw materials; therefore, advancing towards the isolation of compounds and the use of pure substances with bioactivity, instead of the plant benefits, has certain benefits including convenient examination of therapeutic effects and determination of toxic doses to control the quality of the therapeutic formulation. The beginning of the development of herbal medicines was concurrent with the development of chemistry and isolation, purification, and determination of plant compounds [11].

The seed of this plant is reported to contain acetogenins mainly cis and transisomurisolenin [12], annoreticuin, bullatacin, squamosine and rolliniastatin (Maeda et al., 1993). Leaf and roots contain Sesquiterpenes mainly spathenelol, muurolene, copacne, e- desmol [12]. Numerous other ethnomedical studies have been conducted nationwide to determine the traditional uses of *Annona reticulata* Linn.

2. Methods and Materials:

2.1 Collection: At first with the help of a comprehensive literature review Annona reticulata L. from Annonaceae family was selective for this investigation. The leaves were collected from Barishal and identified by the taxonomist of National Herbarium of Bangladesh, Mirpur Dhaka.

2.2 Preparation of plant extract: The plant part (leaves) was sun-dried separately and subsequently dried in a hot air oven (Size 1, Gallenkamp) at a lower temperature (not more than 50°C) to make it acceptable for grinding purposes. Then, in the Department of Pharmacy at Jahangirnagar University, plant parts were ground into coarse powders using a high capacity grinding mill. These powders were then stored in airtight containers with the necessary markings for identification and kept in a cool, dark, and dry place for the investigation.

2.3 Extraction procedure: The powdered plant materials (200 gm) were used for extraction by Soxhlet apparatus at elevated temperature (65°C) using Ethyl acetate (1000 ml of solvent). After each extraction the plant material was dried and used again for the next extraction. Extraction was considered to be complete when the plant materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. After Ethyl acetate extract was complete the plant materials were dried and soaked into distilled water (IL). The plant materials were kept in water for 7 days in sealed container accompanying occasional shaking and string. The extract were filtered individually through fresh cotton bed. The filtrates obtained were dried at temperature of 40±2°C to have gummy concentrate of the crude extract. The extract was kept in suitable container with proper labeling and stored in cold and dry place.

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2.4 Phytochemical screening: Phytochemical Screening Procedure were carried out on basis of Molisch's General test for Carbohydrates, Fehling's test, Test for combined Reducing Sugar, Liebermann- Burchard's Test, Lead acetate test.

2.5 Determination of Total Phenolics content: The content of total phenolic compounds in plant methanolic extracts was determined as described previously using the Folin-Ciocalteu Reagent (FCR). The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent.

2.6 Determination of Total Flavonoids Content: The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids. The wavelength 415 nm is chosen for absorbance measurement.

2.7 Determination of Total Antioxidant Capacity: The phosphor-molybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids. The phosphor-molybdenum method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acid pH. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI) and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm.

2.8 Ethical consideration: All experimental animals were used according to predefined ethical consideration and following the guideline of research ethics committee of Jahangirnagar University.

2.9 Data analysis: $P< 0.05$, $P< 0.01$ and $P< 0.001$ were considered statistically significant, highly significant and very highly significant respectively. Independent samples T test was performed to analyze this data set. SPSS version 20.0 and Microsoft excel software is used to analyze data.

3. Result and discussion:

3.1 Phytochemical screening: Preliminary phytochemical screening of the methanol extract of leaf of Annona reticulate Linn revealed the presence of different kind of chemical groups that are summarized in table 2.

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Table 2. Result of chemical group test of the methanolic extract of leaves of Annona reticulata Linn

Extract	**Carbohydrate**		**Glycoside**		
	Molisch's test [General test for Carbohydrates)	Fehling's Test for reducing sugar)	General test for glycoside		
EAEAR	+	+	+		
Extract	**Alkaloid**	**Steroids** (Liebermann-Burchard&'s Test)	**Tannin(**Lead acetate test)	**Flavonoids**	
EAEAR	+	-	+	+	

[+Presence, - Absence]

[EAEAR= Ethyl Acetate Extract of Annona reticulata Linn.]

Ethyl acetate extract of Annona reticulata Linn. leaves have been shown to possess phytoconstituents including carbohydrates(monosaccharides), combined reducing sugar, alkaloid, glycosides, tannins, and flavonoid, and steroids was detected.

3.2 Phenol content determination: Total phenolic content of the Ethyl acetate extract of leaf of Annona reticulata Linn, was determined by using the Folin-Ciocalteu reagent and were expressed as Gallic acid equivalents (GAE) per gram of plant extract (Figure 1).

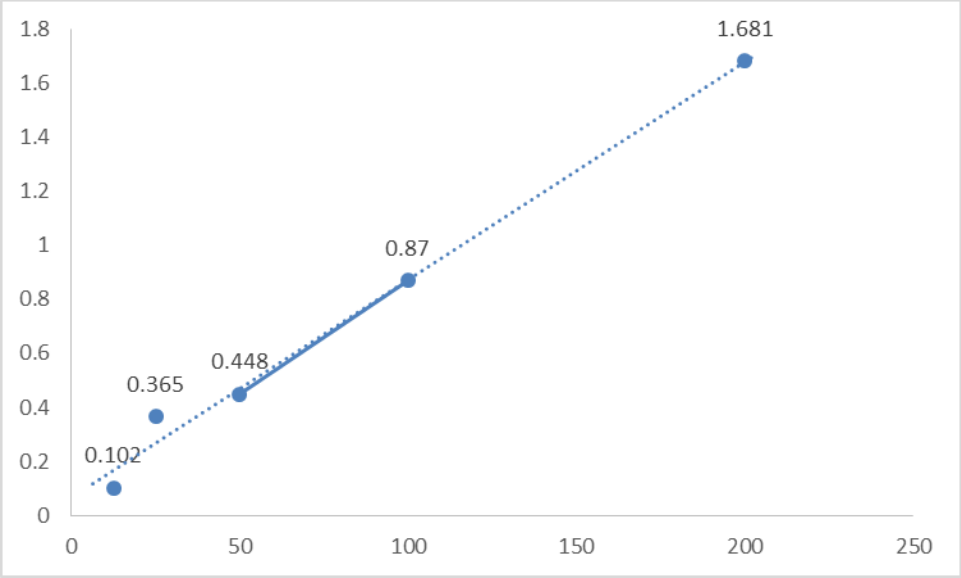

Figure 1. Calibration Carve of Gallic acid

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Phenolics are a class of chemicals found frequently in plants and include flavonoids (flavones, isoflavones, and flavonones), anthocyanins, and catechins for example. They have the ideal structural chemistry for their free radical scavenging action. The antioxidative properties of polyphenols come from their high reactivity as hydrogen or electron donors, which can stabilize and delocalize the unpaired electron (a function that breaks chains), and their ability to chelate metal ions (which stops the Fenton process) [13, 14]. Low plasma concentrations of ascorbate, tocopherol, and B-carotene have been linked to cardiovascular diseases in epidemiological studies, and oxidation processes have been proposed as a major factor in atherogenesis. Furthermore, there is an inverse correlation between quercetin ingestion and plasma LDL cholesterol levels [15].

It has also been shown that polyphenols have anticarcinogenic properties through their ability to regulate the enzyme systems responsible for converting pro- or carcinogens into genotoxins by converting them into less reactive compounds prior to their interaction with DNA. Polyphenols have been shown to lower the creation of reactive intermediates because the Cytochrome Paso superfamily of enzymes converts a large number of pro-carcinogens to reactive chemicals before they interact with DNA and trigger malignant transformation [16]. Additionally, it has been shown that several polyphenols (quercetin, flavones, flavonones, antioxidant, and tangeretin) can raise the activity of glutathione reductase in rats. Generally speaking, the stimulation of this enzyme results in an increase in cellular defense, which guarantees that potential poisons are conjugated and removed from the body faster. Additionally, it has been shown that they inhibit the activities of lipoxygenase and cyclooxygenase, which reduces platelet aggregation and thrombotic tendency. Despite being a crude extract, the A. reticulata Linn. leaf's Ethyl acetate extract demonstrated excellent potency in the phenolic content determination experiment. It also contains a sizable number of chemicals that are gallic acid equivalents (polyphenolic compounds).

3.3 Flavonoid content determination: Aluminum chloride colorimetric method was used to determine the total flavonoid contents of the Ethyl acetate extract of leaf of A. reticulata, (Figure 2).

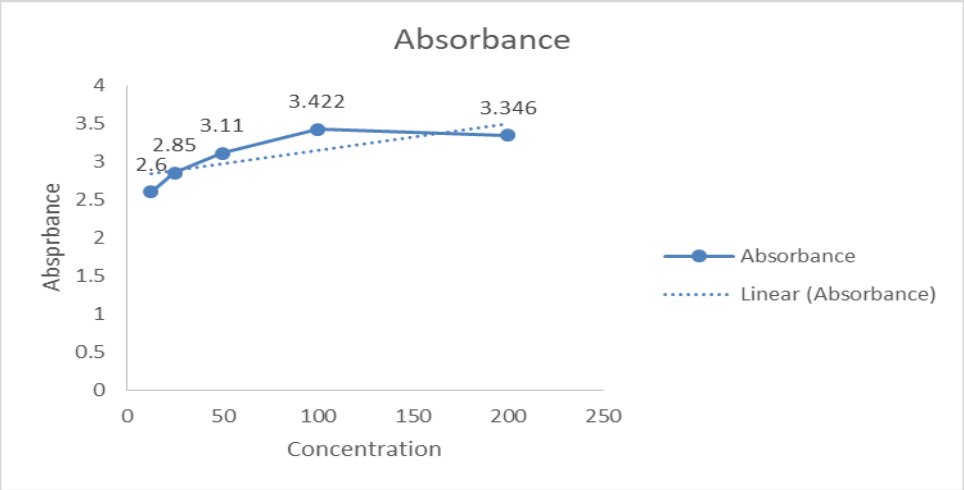

Figure 2. Calibration curve of Quercetin

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Flavonoids have a major impact on plants' antioxidant systems. The scavenging of free radicals, the inhibition of enzymes that create free radicals, and the chelation of metal ions like iron and copper are only a few of the various mechanisms that contribute to flavonoids' antioxidant properties [17]. Depending on their structural composition, flavonoids can scavenge almost all known ROS. The ethyl acetate extract of A. reticulate leaves has been discovered to have a high amount of flavonoids, despite its crudeness.

3.4 Antioxidant capacity: Total antioxidant capacity of the ethyl acetate extract of A. reticulata leaves was evaluated by the phosphor-molybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract.

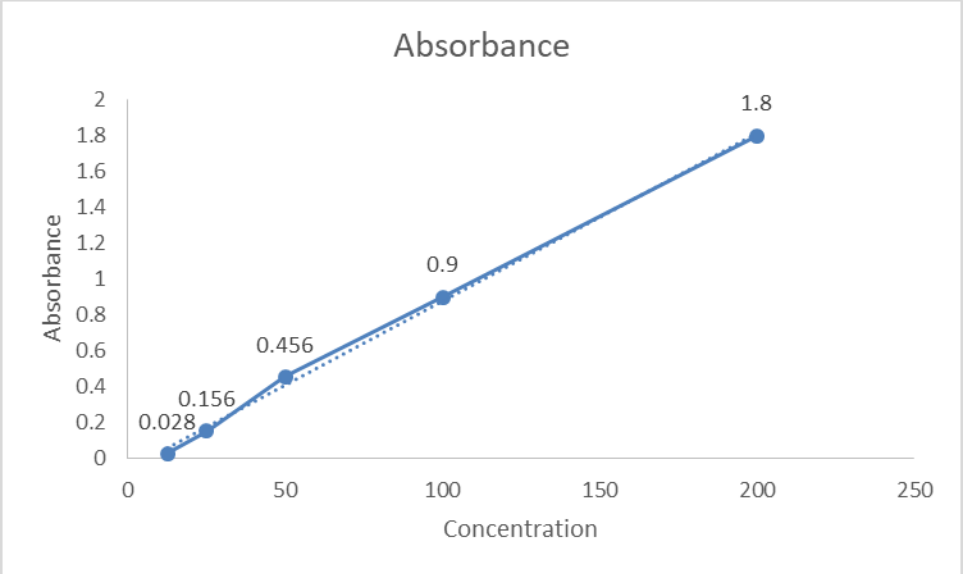

Figure 3. Antioxidant activity determination curve

The total antioxidant activity of the A. reticulata leaf ethyl acetate extract (Figure 3) was assessed by measuring its capacity to reduce the phosphate/Mo(VI) complex to phosphate/Mo (V). Since the assay is simple to perform and unrelated to other commonly used antioxidant assays, it was chosen to be expanded to plant extract. It has been used to correctly quantify the quantity of vitamin E in seeds [18]. Since the antioxidant activity [19], is expressed in terms of ascorbic acid equivalents, the question is also quantitative. According to recent studies, there is a tendency for total phenols and antioxidant activity to be significantly positively correlated in many plant species. The current study confirms the assertion by demonstrating a substantial overall antioxidant capacity in the ethyl acetate extract of A. reticulata leaves (measured in terms of ascorbic acid equivalent).

When an antioxidant chemical donates an electron to DPPH, the DPPH undergoes decolorization, which can be quantified by changes in absorbance at 517 nm. The ICso values

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for the Ethyl acetate extract of A. reticulata leaves. The ethyl acetate extract of A. reticulata leaves was shown to have a 5.% scavenging of DPPH radical that increased with concentration, with this extract showing a high level of scavenging.

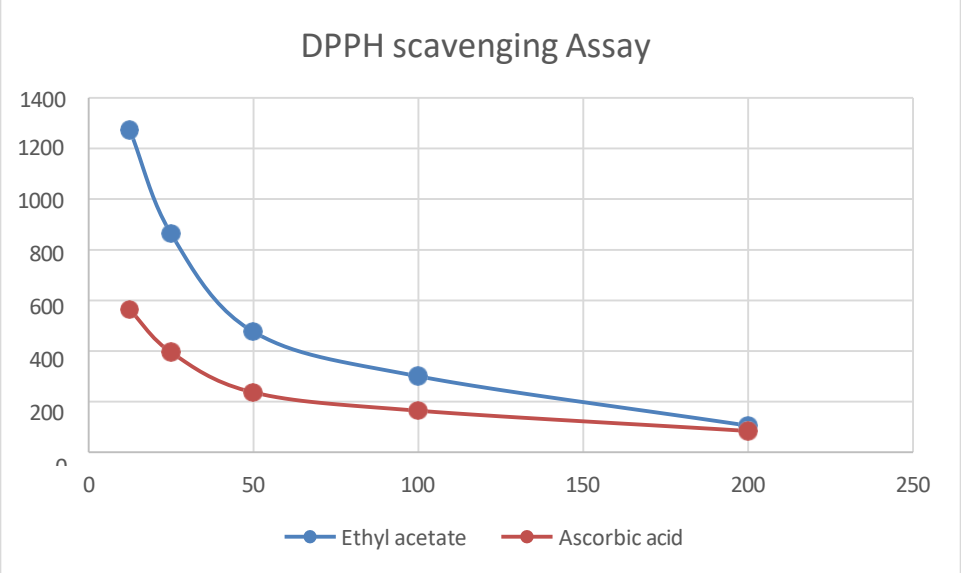

Figure 4. DPPH scavenging assay curve

The crude extract of A reticulata demonstrated dose-dependent scavenging of DPPH radicals in DPPH radical scavenging experiments in a manner akin to that of the reference antioxidant ascorbic acid (Figures 4). A popular and reliable method for assessing a compound's capacity to scavenge free radicals or the antioxidant capacity of a plant extract is called DPPH radical scavenging [20]. The DPPH antioxidant assay is based on the fact that 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a persistent free radical, can decolorize when antioxidants are present [21]. The absorbance between 515 and 517 am as well as the apparent deep purple color are caused by the odd electron that the DPPH radical possesses. The amount of decolorization caused by DPPH accepting an electron given to it by an antioxidant chemical can be calculated from changes in absorbance. The ability to contribute an electron to neutralize the DPPH radical is demonstrated by the ethyl acetate extract of A. reticulata leaves.

4. Future of medicinal plant research: Since the dawn of time, human cultures have maintained a strong relationship with their surroundings and have utilized natural resources to produce food and medicine. Through trial and error, people became aware of the use of plants to create food and medicine, and eventually they were able to fulfill their own requirements by using their environment [22, 23]. With the emergence of civilizations and the development of new infrastructure, information about medicinal plants has long been progressively passed down from generation to generation. Nearly every culture uses medicinal plants as a resource for health. Herbal medicines can contribute to the birth of a new age in healthcare by standardizing and assessing the health of active substances originating from plants, which can

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be used to treat human ailments in the future. The exploration and finding of natural plant resources can be greatly aided by an awareness of traditional knowledge and therapeutic plants.

Because the human body is incapable of producing them, phytochemicals, which are produced by all plants, are advantageous to human health. Many plants have been found to have pharmacological effects as a result of their metabolites. Plant-metabolites are organic substances that fall into two categories: primary and secondary metabolites. Organic substances known as primary metabolites, such as glucose, starch, polysaccharides, protein, lipids, and nucleic acid, are good for the body's growth and development. Secondary metabolites, such as alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, tannins, and volatile oils, are produced by plants. These secondary metabolites are what give plants their medicinal value in treating a wide range of illnesses. Medicinal plants can be employed as a cheap, safe, and efficient kind of treatment [24].

5. Conclusion: The phytochemical components of Annona reticulata leaf extracts, such as phenols, flavonoids, saponins, and others, were screened for. These phytochemicals are what give the plants their therapeutic properties. Leaf extracts were made for this purpose using a solvent containing ethyl acetate. The results showed the rich presence of majority of phytochemical constituents which can be correlated with the possible significant medical potential of the plant. Over the last several years plants have been recognized as an imperative source of medicines. Exploration of phytochemicals derived from different plant parts as potential bioactive agent has become a fascinating strategy. The goal of the current study is to concentrate on several facets of A. reticulata Linn. It was once used to treat a variety of illnesses. It has a diverse array of minerals and secondary metabolites that may have varying medicinal effects. According to its phytopharmacological characteristics, A. reticulata Linn. may be essential for treating a variety of medical disorders. This study determines the usefulness of the A. reticulata Linn. plant, which may be important for the creation of novel plant-based medications.

Conflict of interest: None

Data availability: Not applicable.

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