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Research Article

Antioxidant and cytotoxicity assessment of the methanolic extract of *Annona reticulata* Linn

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ABSTRACT

The research and application of plants in food supplements and drugs have attracted great interest. This study aimed to examine the efficiency of several solvents for the extraction of the main compounds from Annona reticulata leaves and to evaluate the antioxidant, antibacterial, and anticancer activities of these extracts. The methanol extract of leaves was used to conduct preliminary phytochemical group tests as part of the phytochemical study. The methanolic extract of the plant's leaf contained a variety of phytoconstituents, such as carbohydrate, glycoside, alkaloid, steroids, and tannin. Antioxidant potential was evaluated using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, reducing power assessment, total antioxidant capacity, total phenol and total flavonoid contents determination assays. In DPPH radical scavenging methods, a dose dependent scavenging of DPPH radical was observed by the methanol extract. High DPPH radical scavenging was demonstrated by the methanol extract of leaf with ICs value of 74.93 µg/ml whereas ICs value of standard ascorbic acid was noted as 16.01 ug/ml. Also the plant part was found to possess high amounts of phenolics expressed as galic acid equivalent (72 mg/gm GAE). The extract was reported to possess moderate amounts of flavonoids expressed as quercetin equivalent (81.5 mg/gm QE). In reducing power assay, the extret were found to exhibit concentration dependent reducing power. In addition, the methanolic extract of Annona reticulata Linn. Leaves displayed remarkable total antioxidant capacity expressed as ascorbic acid equivalent (148 mg/gm AAE). In Brine Shrimp Lethality Bioassay, the extract produced dose dependent cytotoxicity effect to brine shrimp nauplii with methanol extract of leaf exhibiting high toxicity having LC's value 39,67 ug/ml where standard anticancer drug vincristine sulphate had the LC50 value of 0.69 µg/ml. In conclusion, A. reticulata leaves extracts may contribute to the development of new remedies as an alternative source of antibiotics or for colon cancer therapies.

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INTRODUCTION

Medicinal plants are globally valuable sources of herbal products, and they are disappearing at a high speed. This article reviews global trends, developments and prospects for the strategies and methodologies concerning the conservation and sustainable use of medicinal plant resources to provide a reliable reference for the conservation and sustainable use of medicinal plants. We emphasized that both conservation strategies (e.g. in situ and ex situ conservation and cultivation practices) and resource management (e.g. good agricultural practices and sustainable use solutions) should be adequately taken into account for the sustainable use of medicinal plant resources. We recommend that biotechnical approaches (e.g. tissue culture, micropropagation, synthetic seed technology, and molecular marker-based approaches) should be applied to improve yield and modify the potency of medicinal plants [1, 2]. Medicinal plants are globally valuable sources of new drugs. There are over 1300 medicinal plants used in Europe, of which 90 % are harvested from wild resources; in the United States, about 118 of the top 150 prescription drugs are based on natural sources. Furthermore, up to 80 % of people in developing countries are totally dependent on herbal drugs for their primary healthcare, and over 25 % of prescribed medicines in developed countries are derived from wild plant species. With the increasing demand for herbal drugs, natural health products, and secondary metabolites of medicinal plants, the use of medicinal plants is growing rapidly throughout the world [3, 4]. The presence of active substances like alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarin compounds in the organs of medicinal plants influences their therapeutic properties. These substances have a physiological impact on both human and animal bodies or are biologically active in relation to the diseases they are used to treat [5]. Plants can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity. Medicinal plants are considered as a rich resources of ingredients which can be used in drug development either pharmacopoeial, non-pharmacopoeial or synthetic drugs. Along with medicinal formulations plants have been successfully utilised for the development of cosmetics and toiletry preparations [6]. Plant-derived products have an imperative biological role against certain pathogenic organisms and were considered to be a major source of modern drugs. Rural people residing in developing countries are relying on traditional herbal medical system due to their strong believe and minimum access to allopathic medicines. Hence, ethnomedicinal knowledge is useful for the maintenance of community's based approaches under this medical system. Present study was carried out in an unexplored remote tribal area of Pakistan to investigate and document the existing ethnomedicinal knowledge on local flora. Plant-derived products have an imperative biological role against certain pathogenic organisms and were considered to be a major source of modern drugs. Rural people residing in developing countries are relying on traditional herbal medical system due to their strong believe and minimum access to allopathic medicines. Hence, ethnomedicinal knowledge is useful for the maintenance of community's based approaches under this medical system. Present study was carried out in an unexplored remote tribal area of Pakistan to investigate and document the existing ethnomedicinal knowledge on local flora [7]. This is proven by the fact that of the total number of publications analyzed, more than 100,000, only 11% are in the Agricultural and Biological Sciences category, while more than 50% are grouped in the Pharmacology, Toxicology and Pharmaceutics category and Medicine. This study

highlights the scarce research from the agronomic perspective regarding domestication, production or genetic or biotechnological research on breeding of medicinal plants [8]. Plants contain abundant amount of secondary metabolites, they are considered to be principal source of therapeutically active compounds. Although all plants contain chemical constituents of one kind or another, all of them are not pharmacologically active. The chemical constituents, which are capable of influencing the physiological systems of the animal body by exerting some pharmacological actions, are designated as active chemical constituents of simply constituents [9]. These medicinally active chemical substances are generally produced in the plants as by-products or side products during the synthesis and metabolism of the primary metabolites and are not used or utilized by the plants for their normal life activities. They are termed as secondary metabolites. These compounds may be distributed in all parts of the plant body or may present in higher amount in some specific part [9].

Table 1. Chemical constituents of some medicinal plants [9].

Species	Plant Part	Pure Compound	Use(S)
Aconitum Napellus	Tuber	Aconitin	Pain
Ammi Majus	Fruit	Furanocoumarins	Psoriasis
Ananas Comosus	Fruit	Bromelain	Inflammation
Artemisia Annua	Herb	Artemisinin	Malaria
Atropa Belladonna	Herb	Hyoscyamine, Atropine	Parasympathomimetic
Camptotheca Acuminata	Leaves, Seeds	Camptothecin	Chemotherapy
Cannabis Sativa	Flowering Tips	Tetrahydrocannabinol	Pain
Carica Papaya	Milky Latex	Papain	Inflammation
Species	**Plant Part**	**Pure Compound**	**Use(S)**
Catharanthus Roseus	Herb	Vincristine, Vinblastine, Vindesine, Vinorelbine	Chemotherapy
Chondrodendron Tomentosum	Juice Of Above-Ground Parts	Tubocurarine	Muscle Relaxant
Cinchona Pubescens	Bark	Quinine, Quinidine	Malaria, Irregular Heartbeat
Cinnamomum Camphora	Wood	Camphor	Colds
Citrus Spp.	Fruit Peel	Hesperidin, Diosmine	Inflammation
Claviceps Purpurea	Sclerotia (Spore Bodies)	Ergot Alkaloids, Ergotamine, Ergometrine	Obstetrics, Migraine
Coffea Arabica	Seeds	Caffeine	Stimulant
Colchicum Autumnale	Seeds	Colchicine	Gout
Cytisus Scoparius	Stems	Sparteine	Irregular Heartbeat
Daucus Carota	Root	Carotene	Antioxidant
Digitalis Lanata, D. Purpurea	Leaves	Cardiac Glycosides; Digitoxin, Digoxin	Heart Insufficiency
Dioscorea Villosa	Rhizome	Diosgenin	Synthesis Of Steroidal Hormones
Ephedra Spp.	Stems	Ephedrine	Respiratory Ailments
Erythroxylum Coca	Leaves	Cocaine	Pain

Euphorbia Peplus	Leaves	Ingenol Mebutate	Actinic Keratosis
Fagopyrum Esculentum	Herb	Rutin	Capillary And Venous Disorders, Antioxidant
Galanthus Woronowii	Bulb	Galanthamine	Alzheimer's Disease
Gloriosa Superba	Bulb	Colchicine	Gout
Guaiacum Officinale	Wood	Guajacol	Inflammation, Antioxidant
Justicia Adhatoda	Leaves	Vasicine	Bronchitis
Lobelia Spp.	Herb	Lobeline	Asthma
Species	**Plant Part**	**Pure Compound**	**Use(S)**
Lycopodium Clavatum	Herb	Huperzine A	Alzheimer's Disease
Nicotiana Tabacum	Leaves	Nicotine	E-Cigarettes
Papaver Somniferum	Latex From Unripe Fruit	Morphine (And Aporphin) , Codeine, Papaverine, Noscapine	Pain, Cough, Cramps
Paullinia Cupana	Seeds	Caffeine	Stimulant
Pausinystalia Johimbe	Bark	Yohimbine	Aphrodisiac
Physostigma Venenosum	Seeds	Physostigmine	Alzheimer&'S Disease
Pilocarpus Jaborandi	Leaves	Pilocarpine	Glaucoma
Podophyiham Peltatum	Rhizome	Podophyllotoxin (For Synthesis Of Etoposide)	Chemotherapy
Psychotria Ipecacuanha	Root	Emetine	Anti-Amoebic, Emetic
Rauvolfia Serpentina	Root	Reserpine, Ajmaline, Ajmalicine	Hypertension
Sanguinaria Canadensis	Rhizome	Sanguinarine	Oral Hygiene
Silybum Marianum	Fruit	Silymarin	Liver Ailments
Strophanthus Gratus	Seeds	Ouabain	Heart Insufficiency
Styphnolobium Japoni-Cum	Flower Buds	Rutin	Inflammation, Antioxidant
Syzygium Aromaticum	Flower Buds	Eugenol	Dentistry
Taxus Baccata	Leaves, Bark	Paclitaxel (Taxol)	Chemotherapy
Theobroma Cacao	Seeds	Theobromine, Theophylline	Stimulant, Asthma
Vanilla Planifolia	Cured Fruit	Vanillin	Spice
Vinca Minor	Leaves	Vincamine	Cerebral Circulatory Disorders

Table 2. Some medicinal plants of Bangladesh and their traditional uses [10]

Scientific Name	Local Name (Bangla)	Traditional Uses
Adhatoda Vesica	Basak, Basakpata	Cough, Asthma, Bronchitis, Pneumonia, Rheumatism
Aegle Marmelous	Bael	Dysentery, Diarrhea, Constipation
Aloe Barbadensis	Ghritakanchan	Constipation, Peptic Ulcer, Diabetes, Asthma, Burns
Andrographis Paniculata	Kalmegh, Kalomegh	Liver And Spleen Disorders, Constipation, Diarrhea, Dysentery, Dyspepsia

Asparagus Racemosus	Shatamuli	Urinary Disorders, Diabetes, Jaundice, Dyspepsia, Diarrhea
Azardirchata Indica	Neem	Inflammation Of the Gum, Gingivitis, Fevers, Small Pox
Bacopa Monniera	Brahmishak	Epilepsy, Mental Illness, Indigestion, Ulcers, Asthma, Diabetes, Anemia, Infertility
Centella Asiatica	Thankuni	Skin Problems, Digestive Disorders, Leprosy, Dysentery
Eclipta Alba	Kesuti, Kalokeshi	Liver And Eye Diseases, Skin Diseases, Itching, Bronchitis, Asthma, Hermias
Emblica Fficinalis	Amloki, Amla	Cold, Cough, Scurvy, Palpitation, Hemorrhoids, Diarrhea, Jaundice.
Euphorbia Hirta	Bara-Keru	Cough, Bowel Problems, Asthma, Bronchitis.
Lawsonia Inermis	Mehedi, Mendi	Skin Diseases, Dandruff, Sore Throat, Diarrhea, Dysentery.
Ocimum Sanctum	Tulsi	Cough, Bronchitis, Diarrhea, Gastric Disorder, Itching, Leprosy, Earache.
Rauvolfia Serpentine	Sarpagondha	Hypertension, Insomnia, Anxiety, Insanity, Epilepsy
Ricinus Communis	Bherenda	Constipation, Rheumatism, Paralysis, Asthma, Dropsy
Terminalia Arjuna	Arjun Gach	Hypertension, Liver Cirrhosis, Asthma, Dysentery
Wthania Somnifera	Ashwagondha	Headache, Convulsions, Insomnia, Cough, Rheumatism, Dropsy
Xanthium Indicum	Ghagra	Urinary Problems, Sores of Mouth

The Annona genus (Annonaceae) consists of about 119 species, most of which are shrubs and trees widely distributed in the tropical and subtropical regions, including the Southeast Asia countries such as Malaysia, Indonesia, Thailand, Cambodia, Laos, and Vietnam [11]. A. reticulate L. is widely distributed in tropical and subtropical regions. The plant is indigenous to the West Indies. In India it is widely cultivated and naturalized as a fruit consuming plant and deciduous tree. It is distributed in Bengal, Burma and Southern regions of India. It is native to tropical regions of America, particularly in West Indies and South America. The plant is widely cultivated in Bangladesh and Pakistan [12]. Several species of Annona bear highly prized fruits. The alligator apple (4. glabra) of tropical America and western Africa, also known as pond apple and corkwood, is a 12-metre (40-foot) evergreen tree with 18- cm- (7-inch-) long oval leaves and fragrant yellowish flowers. It bears smooth, gnarled, yellowish fruits, 5-10 cm long, which are edible but of poor flavour. Its roots are used to make bottle corks and fishing floats and as rootstock for grafting less hardy species of Annona. A. reticulata is a small semideciduous tree reaching 6.0-7,5 m tall. It contains numerous lateral branches. It is a small tree with glabrous branches. The stems are cylindrical having lenticels and very short coffee-coloured hairs.

Figure 1. Leaves And Fruits Of Annona Reticulata

Annona reticulata Linn is a versatile tree and its fruits are edible. It possesses several medicinal properties such as anthelmintic, analgesic, anti-inflammatory, antipyretic, wound healing and cytotoxic effects. Traditionally the plant has been employed for the treatment of epilepsy, dysentery, cardiac problem, parasite and worm infestations, constipation, haemorrhage, bacterial infection, dysuria, fever, ulcer and as insecticide. Bark is a powerful astringent and used as a tonic whereas leaves used for helminthiasis treatment. The bark of the plant A. reticulata L. is a powerful astringent and given as tonic. The plant has been used as an anti-inflammatory agent in wound healing, anti-anxiety, anti-stress, anti- mutagenic, and spasmolytic agent. Leaf and stem extract shows inotropic, positive chronotropic and spasmolytic activities [13].

METHODS AND MATERIALS:

Collection and Identification:

First, a member of the Annonaceae family, Annona reticulata L., was chosen for this inquiry with the aid of a thorough literature review. The leaves were collected from Narail district, Bangladesh and identified by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. For future reference, the voucher specimens of the plants have been kept at the herbarium.

Plant Material Preparation:

To make the plant part (leaves) acceptable for grinding, they were first dried in the sun separately and then at a lower temperature (no higher than 50°C) in a hot air oven (Size 1, Gallenkamp). After that plant parts were ground into coarse powders in the Department of Pharmacy. Jahangirnagar University using high capacity grinding mill which were then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

Extraction Procedure:

The Soxhlet equipment was used to extract the 200 g of powdered plant material using methanol at a temperature of 65 °C (1000 ml of solvent). The plant material was dried and used once more for the subsequent extraction after each extraction. In cycles of colorless liquid siphoning in the Soxhlet apparatus, it was confirmed that extraction was complete when the plant materials became depleted of their contents. The plant components were dried when the methanol extract was finished, then steeped in distilled water (IL). For seven days, the plant materials were kept in water in a sealed container with string and sporadic shaking. Each individual extract was filtered through a clean cotton bed. The acquired filtrates were dried at 40+2°C to produce a sticky concentration of the crude filtrate. The crude extract were used for phytochemical and pharmacological (in vitro) evaluation.

Phytochemical screening:

The crude plant extract were subjected to different qualitative tests to find out the presence of chemical constituents. These were identified by characteristic color changes using standard procedure.

Table 3. Phytochemical Screening Process

Constituent	Test Name	Procedure	Observation
Carbohydrate	Molisch's test (General test for Carbohydrates)	A 10% alcoholic solution of a-naphthol was dissolved in two milliliters of water, and two drops of Molisch's reagent were added. To create a layer of acid beneath the aqueous solution, allow 2 ml of concentrated sulfuric acid to trickle down the edge of the inclining test tube.	If a carbohydrate is present, a red or reddish violet ring forms at the intersection of the two layers. A solution that is dark purple is produced when standing or shaking.
	Fehling's test	2 ml of an aqueous extract of the plant material was added to Iml of a mixture of equal volumes of Fehling's solutions present A and B then boiled for a few minutes	A red or brick-red precipitate is formed if a reducing sugar is present.
	Test for combined Reducing Sugar	1 ml of an aqueous extract of the plant material with 2 ml of dilute hydrochloric acid was boiled for 5 minutes. Then it was cooled and neutralized with Sodium hydroxide solution and Fehling's test that stated above was performed	A brick-red precipitate is formed if a combined reducing sugar (e.g. sucrose) is present due to the release of the reducing sugars on hydrolysis.

Constituent	Test Name	Procedure	Observation
Glycosides	General test	A small amount of an alcoholic extract of the fresh or dried plant material was dissolved in 1 ml of water and add few drops of aqueous sodium hydroxide solution.	When glycosides are present, a yellow hue forms.
Steroids	Liebermann-Burchard's Test	A small amount of a petroleum ether extract of the plant material was dissolved in 1 ml of chloroform then 2 ml of acetic anhydride was added with 1 ml of conc. Sulphuric acid.	If a steroid is present, a greenish tint is created, which turns blue when standing.
Tannin	Lead acetate test	5 ml of aqueous extract of the plant material was taken in a test tube and a few drops of a 1%solution of lead acetate was added.	A yellow or red precipitate is formed.
Flavonoids		A few drops of conc hydrochloric acid were added to a small amount of an alcoholic extract of the plant material	Flavonoids are present when an immediate red color develops.

Constituent	Test Name	Procedure	Observation
Alkaloid	General laboratory Tests	About 0.5 g of the extract was Stirred with 5 ml of 1% hydrochloric acid on a steam bath and filtered. I ml of filtered was treated with a few drops of the following reagents separately Turbidity or	** For Mayer's reagent white or creamy while precipitate.

		formations of the respective colored precipitates indicates the presence of alkaloids in the extract ** Mayer's reagent Potassium- mercuric iodide ** Hager's reagent (1% solution of picric acid) **Dragendorff's reagent (Bismuth potassium iodide solution)	** For Hager's reagent yellow crystalling precipitate. ** For Dragender's reagent orange o orange-red precipitate.

Phenol content determination:

Using the Folin-Ciocalteu Reagent (FCR), the total phenolic component concentration in plant methanolic extracts was ascertained as previously described. For the colorimetric testing of phenolic and polyphenolic antioxidants, phosphomolybdate and phosphotungstate are combined to create the Folin-Ciocalteu reagent (FCR), also known as Folin's phenol reagent or Folin-Denis reagent. It functions by calculating the concentration of the material under test that is required to prevent the reagent from oxidizing.

Flavonoids determination:

The colorimetric approach based on aluminum chloride has as its basic premise the formation of acid stable complexes between aluminum chloride and the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. Moreover, aluminum chloride and the ortho-dihydroxyl groups in the A- or B-ring of flavonoids combine to produce acid-labile complexes. The wavelength used to measure absorbance is 415 nm.

Total antioxidant capacity determination: Typically, the phosphor-molybdenum technique finds antioxidants like carotenoids, α-tocopherol, ascorbic acid, and some phenolics. The antioxidant component reduced Mo(VI) to Mo(V), which led to the creation of a green phosphate/Mo(V) complex at an acid pH. This was the basis for the phosphor-molybdenum technique. Essentially, it is thought that the complex facilitates molybdenum reduction more easily, and that reductants and Mo(VI) undergo an electron-transfer reaction that results in the production of a green phosphate/Mo(V) complex with a maximum absorption at 695 nm.

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.

RESULT AND DISCUSSION:

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 4.

Table 4. 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)	Test(for combined Reducing Sugar	General test for glycoside
MEAR	+	+	-	+

Extract	Alkaloid		Steroids (Liebermann-Burchard's Test)	Tannin(Lead acetate test)	Flavonoids
MEAR	+	+	+	+	MEAR

Key: MEAR-Methanolic Extract of Annona reticulata Linn.

[+Presence, -Absence]

There is evidence that the leaves of Annona reticulata Linn. contain a variety of phytoconstituents, including monosaccharides, alkaloids, glycosides, steroids, tannins, and flavonoids [14]. We found no combined lowering sugar. The extract's diverse pharmacological actions may be explained by these phytoconstituents.

Total Phenol Content Determination:

Using the Folin-Ciocalteu reagent, the total phenolic content of the methanolic leaf extract of Annona reticulata Linn was assessed and was expressed as Gallic acid equivalents (GAE) per gram of plant extract.The total phenolic contents of the test fractions were calculated using the standard curve of Gallic acid (Figure 2).

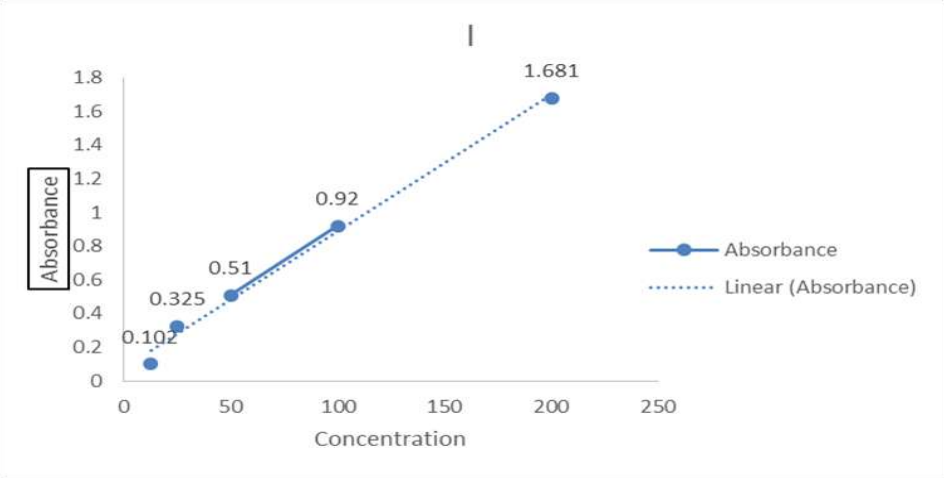

Figure 2. Total Phenol Content Determination

Total flavonoids content determination:

The methanolic extract of A. reticulata leaf was used to determine the total flavonoid content using the aluminum chloride colorimetric technique. The total flavonoid content was calculated (Figure 3) and was expressed as quercetin equivalents (QE) per gram of the plant extract. The methanolic extract of the leaf of A. reticulata was found to contain appreciable amount of flavonoids in this result. The scavenging of free radicals, chelation of metal ions like iron and copper, and inhibition of free radical-generating enzymes are only a few of the methods by which flavonoids exhibit antioxidative characteristics [15].

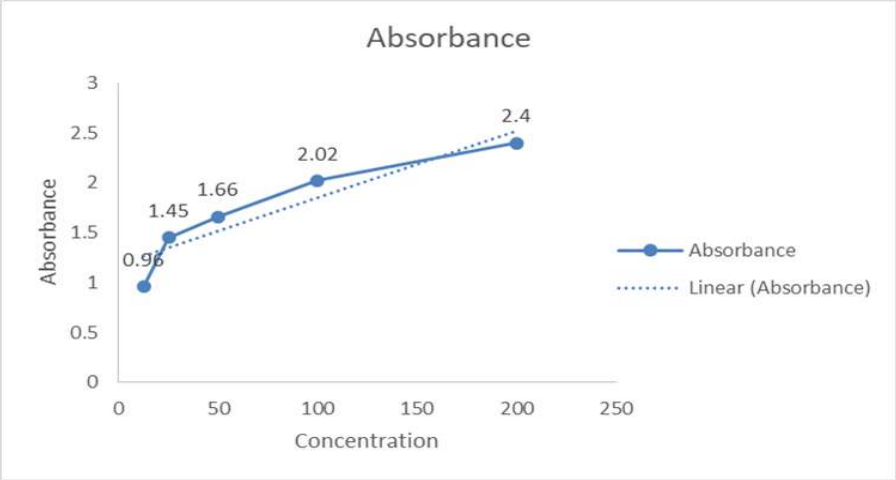

Figure 3. Calibration Curve Of Quercetin

Total Antioxidant Capacity:

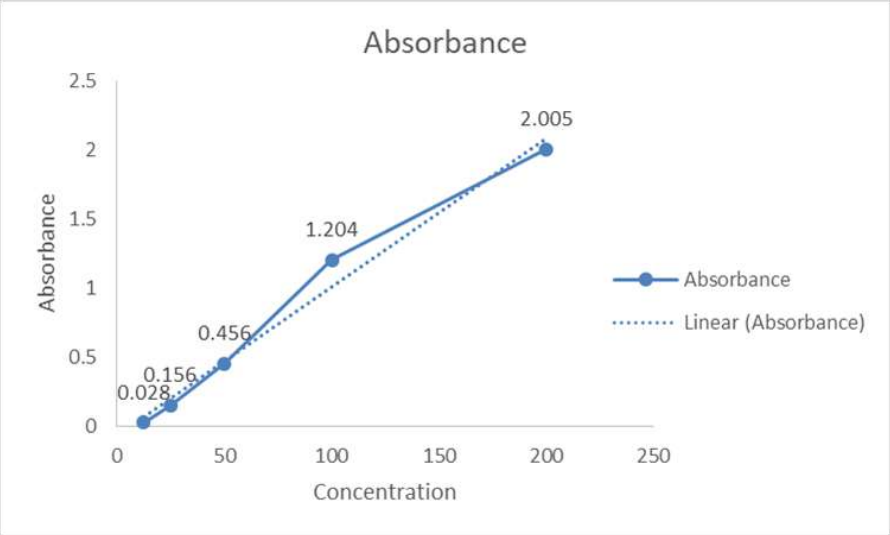

Figure 4. Calibration Curve Of Ascorbic Acid

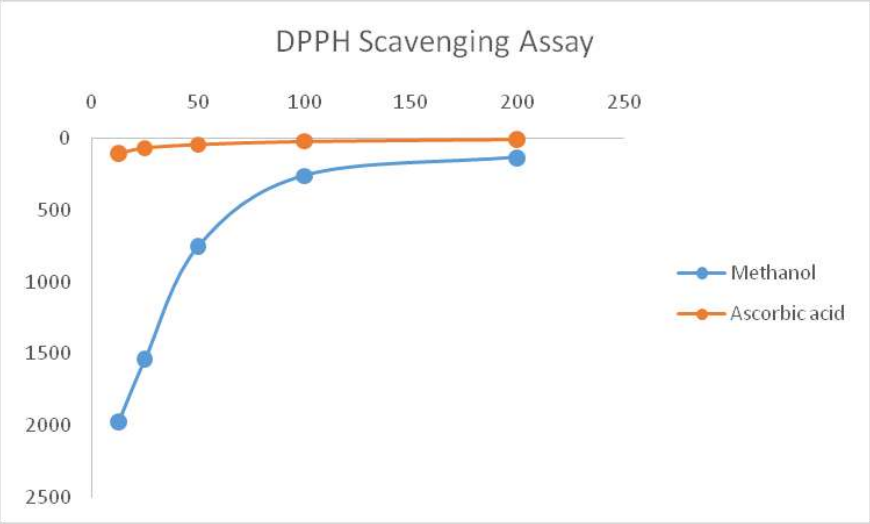

Figure 5. DPPH Radical Scavenging Activity Of The Methanolic Extract Of A. Reticulata

Total antioxidant capacity of the methanolic 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. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid (Figure 4). The methanolic extract of the leaf of A. reticulata was found to possess high total antioxidant capacity. In DPPH radical scavenging assays, the crude extract of A reticulata showed dose dependent scavenging of DPPH radicals in a way similar to that of the reference antioxidant ascorbic acid (Figure 5). DPPH radical scavenging is a popular and reliable method for screening the free radical scavenging activity of compounds or antioxidant capacity of plant extract. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2- picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. Changes in absorbance at 517 nm can be used to measure the amount of decolorization that occurs when an antioxidant chemical donates an electron to DPPH [16, 17]. The methanolic extract of A. reticulata leaves showed dose dependent antioxidant properties. In practically all of the methods, the extract demonstrated good antioxidant activity. The phenolic content and other phytochemical components of the extract may be responsible for the extract's overall antioxidant activity. The results of the current study point to A reticulata as a possible source of natural antioxidants that may be extremely valuable as therapeutic agents in halting or delaying the onset of aging and the accompanying degenerative disorders linked to oxidative stress. Producing antioxidants for the food industry to use as preservatives may potentially have an influence.

Brine Shrimp Lethality Bioassay for Cytotoxic Activity:

The extract was also subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this study, methanol extract of leaf was found to be the most toxic to Brine Shrimp nauplii, with LC5o of 39.67 µg/ml whereas anticancer drug vincristine sulphate showed LCs value 0.699 µg/ml (table 5). The high toxicity of methanolic extract of leaf probably attributed to the alkaloid that is confirmed in phytochemical screening.

Table 5. LC50 of the methanolic extract Brine Shrimp lethality bioassay

Test Sample	Concentration (µg/ml)	Log conc.	%Mortality	Corrected %Mortality	LC50 (µg/ml)	LC90 (µg/ml)
MEAR	12.5	1.09691	0	-11.1111	39.67	457.2
	25	1.39794	20	11.11111		
	50	1.69897	40	33.33333		
	100	2	90	88.88889		
	200	2.30103	100	100		
	400	2.60206	100	100		
	800	2.90309	100	100		
VS	0.06	-1.22185	10	0		
	0.125	-0.90309	20	11.11		
	0.25	-0.60206	30	22.22		
	0.5	-0.30103	40	33.33	.0699	6.33
	1	0	50	44.44		
	5	0.69897	90	88.88		
	10	1	100	100		

In toxicity evaluation of plant extract by Brine shrimp lethality bioassay LC50 values lower than 1000 ug/ml are considered bioactive. The Brine Shrimp Lethality Bioassay also indicates antifungal effects, pesticidal effects, teratogenic effects, toxicity to environment and many more. Table 5 shows the lethality of methanolic extract of A. reticulata leaf to the Brine Shrimp nauplii. The degree of lethality shown by the extractive was found to be directly proportional to the concentration of the extractives ranging from the lowest concentration (12.5 µg/ml) to the highest concentration (800 pg/ml). This concentration dependent increment in percent mortality of Brine Shrimp nauplii produced by the A reticulata indicates the presence of cytotoxic principles in these extractives. Alkaloids and steroids were detected by preliminary phytochemical screening. Therefore, the existence of such chemicals may be the cause of the observed cytotoxic activity. Once more, studies on the function of steroids and alkaloids in the cytotoxic action of plant extract are available.

FUTURE DIRECTIONS

In order to draw a firm conclusion about the results of the current study, further, more sophisticated research is required as all of the experiments that were conducted in it were based on crude extract and are therefore deemed to be preliminary. A thorough phytochemical analysis must be set up in order to identify and maybe isolate the chemical components contained in the crude extract. To prove that a particular chemical constituent is in fact responsible for a certain biological activity, separated phytoconstituents must then undergo all the current tests as well as some additional, more sophisticated in vivo and in vitro pharmacological procedures. Antioxidant testing methods undertaken were all in vitro. In vivo antioxidant testing methods like TBARS (Thiobarbituric acid reactive substance), erythrocyte membrane stabilization assay, measurement of NO and antioxidant enzyme levels in brain, heart and liver samples may confirm the antioxidant activity of the plant parts. Therefore, additional chemical and pharmaceutical investigations using A. reticulata extract for isolating new bioactive compounds and evaluating their precise mode of action and toxicity profile may be the next steps to take in order to discover new lead compounds.

CONCLUSION

To support the antioxidant and cytotoxic properties, methanolic extract of Annona reticulata Linn. (Family: Annonaceae) leaf was subjected to rigorous phytochemical and pharmacological research. The phytochemical screening revealed the chemical elements that form the basis of their pharmacological activity. The brine shrimp lethality test's high toxicity of A. reticulata's extract suggests the presence of bioactive components in the plant. The plant extract showed a possible antioxidants potential. The phytochemical screening revealed chemical constituents that form the foundation of their pharmacological activity.. The high toxicity of A. reticulata's extract in the brine shrimp lethality test implies that the plant contains bioactive components. Potential antioxidant activity was detected in the plant extract. The phytochemical screening revealed chemical constituents that form the foundation of their pharmacological activity. The high toxicity exerted by the extract of A. reticulata in brine shrimp lethality bioassay suggests bioactive principles in the plant. The plant's extract showed potential antioxidant action.

Conflict of interest: None.

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