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Review

Phytochemicals, Antimicrobial and Antioxidant Properties of *Annona reticulata* Linn.

A. Jayaprakash
Department of Biochemistry, Sacred Heart College (autonomous), Tirupattur-635 601, Vellore District, Tamil Nadu, India
aruljaypee@gmail.com*; +91-9841378323

Abstract

Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used to treat various ailments and compounds from plants continue to play a major role in primary healthcare as therapeutic remedies. There is a general call for new drugs that are highly effective, possess low toxicity, and have a minor environment impact. With increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, interest in the use of biogenic drugs has revived throughout the world. *Annona reticulata* Linn. also known as Sitaphala, Sarifa, Custard- Apple, belongs to the family Annonaceae. *Annona reticulata* Linn. is widely cultivated throughout India as an fruit consuming plant and deciduous tree (Fig. 1). It is a native of South America and West Indies, also cultivated in Bangladesh and Pakistan. The Annona genus consists of about 119 species, most of which are shrubs and trees widely distributed in the tropical and subtropical regions, including South-east Asian countries. *Annona reticulata* is a highly apparent plant in ayurvedic system of medicine for the treatment of various ailments. Hence, this review will focus on the phytochemicals, antimicrobial and antioxidant activity of *Annona reticulata*.

Keywords: *Annona reticulata* Linn., custard apple, phytochemicals, antimicrobial, antioxidant.

Introduction

Natural products play a major role as active substances, model molecules for the discovery and validation of drug targets. Plants are the chief source of natural compounds used for medicine, in which medicinal plants have attracted considerable interest and most attention for their wide variety of bioactive metabolites. It is currently estimated that approximately 420,000 plant species exist in nature. Medicinal plants are defined as those which produce one or more active constituents capable of preventing or curing an illness. These plants contain a range of effective compounds and can produce very different effects according to the way in which the drug is treated. Medicinal plants have been explored therapeutically in traditional medicines to alleviate human ailments for several millennia. According to the World Health Organization, about 80% of the developing countries (e.g., Brazil, China, India, and Thailand) rely on traditional medicines and of those, 85% use plants or their extracts as the active substance. Numerous studies have been carried out to screen extracts from medicinal plants for the presence of novel compounds and an investigation of their biological activities. Plants have been known to be a reservoir of secondary metabolites which are being exploited as source of bioactive substance for various pharmacological purposes. The fact that some of these plants have been used traditionally for centuries and modern scientific studies have shown the existence of good correlation between the traditional or folkloric application of some of these plants further strengthens the search for pharmacologically active compounds from plants. Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity. Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, antibacterial, and antiviral activities. The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing and in recent years, there has been a worldwide trend towards the use of the natural phytochemicals.

Annona reticulata Linn.

The genus 'Annona' is from the Latin word 'Anon', meaning 'yearly produce', referring to the production of fruits of the various species in this genus. Annonaceae, the custard apples are a family of flowering plants consisting of trees, shrubs, or rarely lianas. With about 2300 to 2500 species and more than 130 genera, the family is concentrated in the tropics, with few species found in temperate regions. About 900 species are Neotropical, 450 are Afrotropical, and the other species Indomalayan mainly originated from West Indies, Central and South Africa and it is naturalized in

*Corresponding author

Southeast Asia, India, Taiwan and West Africa. *Annona reticulata* is an erect tree with a spreading crown and 10 to 14 inch (25-35 cm) thick trunk. The leaves are deciduous, alternate, oblong or narrow-lanceolate with visible veins and have a bad smell. The flowers that never fully open, appear in drooping clusters and they are fragrant and slender, with 3 outer fleshy, narrow petals, light-green externally and pale-yellow with a dark-red or purple spot on the inside at the base (Morton, 1987).

Systemics of *Annona reticulata:*

Kingdom	: Plantae
Division	: Angiosperms
Order	: Magnoliales
Family	: Annonaceae
Genus	: Annona
Species	: reticulata
Binomial name	: *Annona reticulata* L.

The height of *A. reticulata* is near about 6-7.5 m. It contains numerous lateral branches. It is a small tree with glabrous branches. The stems are cylindrical having lenticels and very short coffee colored hairs. Leaves are oblong, lancelets, membranous, acute, and rounded or curate at the base. The upper surface of leaves is glabrous and on lower surface it contains few spreading hairs. Two to four flowers may present on lateral pedicel. Fruits are edible, somewhat heart shaped, rough and yellow in color which change to yellowish red on ripening. Fruits are sweet, astringent and useful in blood complaints (Fig. 1). Seeds are smooth and blackish in color (Savithramma *et al.,* 2015). Leaves of *Annona reticulata* are shed at the first on set of cold weather and the tree is dormant throughout winter. It has survived temperatures of 3-2°C without serious harm when fully grown. The species prefers a more humid (Shital *et al*., 2014).

Phytochemicals of Annona

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components. The quantity and quality of phytochemicals present in plant parts may differ from one part to another. Moreover, plant secondary metabolites present chemical and pharmaceutical properties interesting for human health (Raskin *et al.,* 2002; Reddy *et al.,* 2003).

Fig. 1. *Annona reticulata* in its natural habitat.

Compounds belonging to the terpenoids, alkaloids and flavonoids are currently used as drugs or as dietary supplements to cure or prevent various diseases (Raskin *et al.,* 2002) and in particular some of these compounds seem to be efficient in preventing and inhibiting various types of cancer (Watson *et al.,* 2001; Reddy *et al.,* 2003). *Annona reticulata* is a most important medicinal plant in India and their leaves are often screened for phytochemical activity. The shade dried leaves were extracted with hexane, methanol and aqueous solvents. Various parts of plants such as the leaves, fruits, barks, roots and even the seeds are being used for preparation of medicines (Prasad *et al.,* 2016). Some compounds have been isolated and reported from the various parts of the plant *Annona squamosa*. Different chemical constituents like Borneol, Camphene, Camphor, car-3-ene, Carvone, β-Caryphyllene, Eugenol, Farnesol, Geraniol, 16-Hetriacontanone, Hexacontanol, Higemamine, Isocorydine, Limonine from stems, root extracts of *Annona squamosa* Linn. were also reported (Shital *et al*., 2016). It is used for the various ailments and the volatile constituents of *A. squamosa* Linn. bark were identified from the essential oil obtained by the steam distillation and studied by GC/MS (Kaladhar *et al*., 2014).

Leaf extracts of *Annona reticulata* were screened for the presence of phytochemical constituents like terpenoids, phenols, flavonoids, saponins and others. Flavonoids isolated from aqueous extract of *Annona squamosa* Linn. has been showed antimicrobial activity. Bullatacin is one such compound that possessed anti-tumoral and pesticide activity. In another study two major alkaloids have been isolated from *A. reticulata* leaf extract. The name of the compounds was liriodenine and oxoanalobine, both compounds belonged to the group of oxoaporphines and were identified by their spectra. The compounds were isolated from the root extract of the plant (Duddukuri *et al*., 2014).

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Ethanolic *Annona reticulata* leaf extract was found to be more prominent than the aqueous extract and phytochemical screening was carried out to detect the major phytoconstituents. Structures of compounds were identified as N-Nitrosoxylopine, Roemerolidine and Duguevalline. The compounds were subjected for the screening of antimalarial activity, the preliminary phytochemical screening test confirmed the presence of alkaloids, terpenoids, flavonoids, steroids, saponins and glycosides (Gowdhami *et al*., 2014). Florence *et al*. (2014) evaluated the phytochemical constituents in the leaf extract of some members of the family Annoceae. Leaf extract were prepared from the organic solvents and phytochemical screening was performed using standard methods. The screening of the plant extract showed the presence of alkaloids, carbohydrates, coumarins, flavonoid, glycosides, phenolic compounds, phytosterols, proteins, quinones, saponins, steroid and terpenoids. Phytochemical screening of the *Annona reticulata* leaf extract showed the presence of flavonoids, terpenoids, saponins, tannins and reducing sugars (Suresh *et al*., 2006). Phytochemical analysis of the leaf material revealed that the antibacterial activity of the plant material is because of the presence of phenolic compounds. A great range of biotic molecules referred to as secondary metabolites are produced by plants, thereby making them a rich source for diverse forms of medicine. Additionally, the primary advantage of using these naturally derived products include safety for human consumption, possess no harmful effects on the environment and low cost is incurred in treating microbial infections when they are used (Himesh *et al*., 2011).

Antimicrobial activity of Annona

The widespread use of commercially available antimicrobials led to the consequence of emergence of antimicrobial resistant pathogens that ultimately led to the threat to global public health. Since 1980s, the introduction of new antimicrobials has declined due to the huge expense of developing and testing new drugs. All commercially available antibiotics with prolonged use may have negative effect on human health because they kill gut flora, so human beings need to take probiotics to replace the killed gut flora. All the above points make a clear way for herbal antimicrobials. The use of plants for treating diseases is as old as the human civilization. There are many plants which have been in use as traditional medicine, so they are called as medicinal plants. The use of plants for curing diseases was inevitable as is already proven by seeing the problems associated with synthetic antibiotics. Jamkhande and Amruta (2016) in their study evaluated the antibacterial activity of stem bark of *Annona reticulata* Linn. against three gram negative (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) and gram positive (*Staphylococcus aureus*, *Bacillus subtilis* and *B. cereus*) strains of bacteria using nutrient agar media. The antifungal activity of the extract was also carried out against *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme*, *Aspergillus flavus*, *Trichoderma viride* and *Candida albicans* using potato dextrose agar media. Extract exhibited dose dependent scavenging as that of standard, ascorbic acid. Extract was found to have pronounced ability to inhibit *B. cereus* and also exhibited significant activity against all strains of bacteria. Predominant antifungal activity was showed against *T. viride*, and *C. albicans* fungi. The results obtained from this study revealed that root extract of *A. reticulata* had remarkable antimicrobial activity. Rani *et al*. (2013) evaluated the leaves of the plant *Annona reticulata* and extracted using different ranges of polar organic solvents like low (Ethyl acetate), medium (Butanol) and high (Methanol). Qualitative analysis and antimicrobial activity was investigated. The Ethyl acetate and Methanol extracts showed better antibacterial activity, the significant inhibitory effect against *Escherichia coli*, *Pseudomonas putida and Lactobacillus acidophilus*, and thus displayed highest inhibitory zone of 19.5 mm, 19 mm and 19 mm when compared to Butanol. Simon *et al*. (2016) in her study evaluated the antimicrobial activity of dried leaf extracts of *Annona squamosa* (L.) against two gram negative bacterial strains namely *Escherichia coli* and *Pseudomonas aeroginosa* and two clinical fungal pathogens namely *Candida albicans* and *Aspergillus niger* by agar cup method. The leaf extracts of *Annona squamosa* (L.) was found to have high antibacterial activity than anti-fungal activity. The results suggest that the leaves are a rich source of valuable primary and secondary metabolites exhibiting the antimicrobial activity.

Aamir *et al.* (2013) studied the *Annona squamosa* extracts and fractions for antimicrobial activity against standard microbial strains of *Klebsiella pneumoniae* (gram-negative), *Staphylococcus aureus* (gram-positive), *Escherichia coli* (gram-negative), *Salmonella typhi* (gram-negative), *Enterococcus faecalis* (gram-positive), *Pseudomonas aeruginosa* (gram-negative) and *Salmonella paratyphi* (gram-negative) by Agar-Disc diffusion method and minimal inhibitory concentration (MIC) was noted. *Annona squamosa* extract was found to be more effective than *Phoenix dactylifera*. When both extracts were used in combination, they showed strong synergistic effect against all the pathogens tested in the study except for the *P. aeruginosa* and *S. para typhi*. Vidyasagar and singh (2012) carried out an comparative antimicrobial activity of methanolic root, leaf and seed cotyledons extracts of *A. squamosa* against four fungi namely, *Trichophyton rubrum, Aspergillus niger*, *A. flavus* and *Candida albicans* and three bacteria, *Bacillus subtilis* (Gram positive), *Escherichia coli* and *Serratia marcurs* (Gram negative) using agar well diffusion method.

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Maximum inhibition was found with 40 mg/mL concentration of methanolic root and seed cotyledon extracts against all the tested organisms under investigation. Panda *et al.* (2011) in his study noted methanolic leaves extract of *A. reticulata* showing significant activity against *Bacillus subtilis*, *Staphylococcus aureus,* and *Vibrio alginolyticus*. In comparison to the extracts of *A. reticulata*, *A. squamosa* had strong antibacterial activity. Methanolic leaves extract of *A. reticulata* showed significant activity against *Bacillus subtilis*, *Staphylococcus aureus,* and *Vibrio alginolyticus*. In comparison to the extracts of *A. reticulata*, *A. squamosa* had strong antibacterial activity.

Khanja and Joshi (2015) evaluated several plant extracts using solvents such as ethanol, methanol, ethyl acetate, chloroform, hot water and cold water. Agar well diffusion method was used for the assessment of antimicrobial activity. 100% acetone leaf showed an average zone of inhibition of 14.83 mm against *Staphylococcus aureus*, 15.67 mm against *Escherichia coli* and 14.6 mm against *Pseudomonas aeroginosa*. About 100% chloroform leaf showed an average zone of 15.37 mm against *Staphylococcus aureus,* 13.0 mm against *Escherichia coli* and 15.33 mm against *Pseudomonas aeruginosa* and 100% chloroform leaf hot showed an average zone of 14.33 mm against *Candida albicans*. Tetracycline was used as a standard antibiotic against bacterial pathogens. Kaladhar and Rayavarapu (2014) carried out antimicrobial studies on human pathogenic bacteria's and fungi. *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Enterococcus faecalis, Bacillus subtilis* are bacterial species, *Candida albicans, Aspergillus niger* are the fungal species. The maximum zone (29 mm) of antibacterial effect was observed with the crude extract of *Annona reticulata* against the Gram-positive organism *Pseudomonas aeruginosa*. The methanol has shown good activity with tested bacteria and fungi. All the tested extracts were shown good antibacterial activities compared to standard antibiotic (Tetracyclin).

Antioxidant activity of Annona

Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently, there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in re-antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad *et al.,* 2006). They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions (Sudarajan *et al.,* 2006). Jamkhande *et al.* (2016) evaluated the *in vitro* antioxidant activity of *Annona reticulata* leaf extracts. The shade dried leaves were extracted with methanol and aqueous methanolic extract was partitioned successively with n-butanol, chloroform and acetone solvents. Methanolic extract was subjected to antioxidant screening using DPPH free radical scavenging activity and H_2O_2 scavenging activity. The antioxidant activity showed that the extracts exhibited scavenging effect in concentration dependent manner. The radical scavenging activity of *A. reticulata* leaf extract was estimated using stable free radical of 1,1-diphenyl-2-picrylhydrazyl assay (DPPH). There was a noticeable correlation between total polyphenols and free-radical DPPH scavenging activity. Biva *et al.* (2006) in DPPH radical scavenging assay, methanol soluble extract was found to be the most potent with an IC50 value of 103.5 µg/mL. The amount of total phenolics was also found to the highest in the methanol soluble extract (283.16 ± 8.90 mg/g), followed by chloroform soluble extract (216.90 ± 4.48 mg/g). Here BHT and ascorbic acid were used as standards with IC50 values 8.2 µg/mL and 25 µg/mL respectively. Kalidindi *et al.* (2015) evaluated the antioxidant activities of methanol, chloroform, and aqueous extracts of *Annona squamosa* Linn. The antioxidant potential of each extract was determined by free radicals scavenging activity and reducing power property of *A. squamosa* leaves. The free radical scavenging activity and reducing power property of all extracts were found to be concentration dependent, with the methanol extract exhibiting higher antioxidant activity than the chloroform extract, which was more effective than the aqueous extract of *A. squamosa* leaves. Vijayaraghavan *et al.* (2013) evaluated the *Chromolaena odorata* and seed extract of *Annona squamosa* with different solvents. The leaf extracts and seed extracts were evaluated for antioxidant activities by DPPH radical scavenging assay. Among three accessions with different solvents used, maximum antioxidant activity found ethanolic leaf extract from *Chromolaena odorata* and seed extract of *Annona squamosa* followed by others. The present study reveals that these plants are of therapeutic potential due to their high free radical scavenging activity.

Saptarini *et al.* (2015) study aimed to compare the antioxidant activity of extracts and fractions of *Ficus benjamina* and *Annona reticulata* leaves against 1,1-diphenyl-2- picrilhydrazyl. The steps of this study consisted of extraction, fractionation with n-hexane, ethyl acetate and water, phytochemical screening, antioxidant activity determination, and comparing the IC50 values. Percentage scavenging activity of the extracts and fractions against DPPH was calculated to determine the antioxidant activity. The IC50 value of *Ficus benjamina* was 127.86 ppm for ethanolic extract, 94.01 ppm for water fraction, 115.48 ppm for ethyl acetate fraction, and 335.50 ppm for n-hexane fraction. The IC50 value of *Annona reticulata* was 274.31 ppm for ethanolic extract, 211.42 ppm for water fraction, 367.91 ppm for ethyl acetate fraction, and 741.08 ppm for n-hexane

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fraction. The results showed that the *Ficus benjamina* water fraction was the best antioxidant compared to other extract and fraction. *Ficus benjamina* (Moraceaae) and *Annona reticulate* (Annonaceae) are herbal resources that had antioxidant activity. Padmini *et al.* (2014) determined the antioxidant activity and the total phenol content of the ethanolic extract of fruit pulp of the Sri Lankan variety of *A. muricata*. Antioxidant activity was determined by using 2,2-diphenylpicrylhydrazyl free radical scavenging assay, ferrous ion chelating assay, and ferric reducing ability of plasma assay. The total phenol content was determined by Folin-Ciocalteu method using Gallic acid standard. The fruit pulp extract exhibited scavenging activity having an IC50 value of 725 ppm and EC50 value for ion chelating activity as 306 ppm. The ferric reducing ability of 1000 ppm of the fruit pulp extract was equivalent to that of 25 ppm of vitamin C. The total phenol content of the extract of fruit pulp was 139 mg Gallic Acid Equivalent/100 g. The ethanolic extract of Sri Lankan variety of *A. muricata* fruit pulp exhibited moderate antioxidant capacity in terms of redox properties. The DPPH radical scavenging assay is an easy, rapid and sensitive method for the antioxidant screening of plant extracts. DPPH is a stable nitrogen centered free radical, the color of which changes from violet to yellow upon reduction by electron donation. According to regression analysis of percentage Inhibition *vs* vitamin C concentration, R2 was 0.99. Vitamin C has high antioxidant capacity and showed free radical scavenging activity having IC50 value of 3.11 ± 0.04 ppm. According to the regression analysis of percentage Inhibition *vs A. muricata* fruit pulp extract concentration, R2 was 0.97. Fruit pulp extract of *A. muricata* exhibited free radical scavenging activity having IC50 value of 724.98 ± 3.00 ppm. With compared to the IC50 value of Vitamin C, *A. muricata* fruit pulp extract showed the low ability of radical scavenging activity.

Elagbar *et al.* (2016) evaluated the total oil yield and the fatty acid composition in the *Annona muricata* L. fixed oil using organic solvent extraction and GC-FID. The seeds were found to contain about 21.5% of crude fixed oil on a dry weight basis. The crude oil containing fatty acid was converted into methyl esters and analyzed by GC-FID. Fourteen fatty acids were identified using GCFID. The major monounsaturated and saturated fatty acids were oleic acid (39.2%) and palmitic acid (19.1–19.2%), respectively, whereas the α-linolenic acid (1.2%) and linoleic acid (34.9%) were polyunsaturated fatty acid. The other saturated acids were stearic acid (3.3%), arachidic acid (0.4%), myristic acid (0.1%), heptadecanoic acid (0.1%), behenic acid (0.1%), and lignoceric acid (0.1%). Some of the fatty acids have not been reported earlier from the oil of *Annona muricata* L. Fixed oil exhibited significant free radical scavenging activity which was measured using DPPH and is also known to inhibit the gastrointestinal motility significantly. DPPH scavenging activity (IC50) was calculated graphically. The extracted oil (at concentration of 1280 µg/mL) showed 77.5% and 78.0% activity comparable to that of α-tocopherol. The IC50 values ranged from 190.1 ± 2.9 to 202.5 ± 3.1 µg/mL against DPPH. The antioxidant activity may be attributed to the presence of α-tocopherol (12.5 mg/kg), phenolic compounds, and unsaturated fatty acids. The lipid content may change due to oxidation of the oil which in turn affects the antioxidant potential of the oil; hence it is important to determine peroxide value of extracted oil. Essama *et al.* (2015) studied the antioxidant evaluation by the DPPH assay revealed that *Annona muricata* extracts enclosed antioxidant activity. The EC50 of the barks, stems and leaves extracts are respectively 0.09 ± 0.027, 0.116 ± 0.02 and 0.29 ± 0.0078 mg/g of DPPH. Previous studies had shown the influence of organ of plant extracted on yield of total phenolic content and antioxidant activity of the extracts. The higher radical scavenging activity of barks extract could be due to the high content in phenolic compound compared to stems and leaves extracts. Several studies have shown the relationship between the antioxidant activity and total phenolic compounds. Phenolic compounds like flavonoids, due to their chemical structure, are ideal donors of hydrogen to the DPPH radical. Shirwaikar *et al.* (2017) evaluated the free radical scavenging potential of the leaves of the plant *Annona squamosa* Linn. using different antioxidant models of screening. The ethanolic extract at 1000 µg/mL showed maximum scavenging of the radical cation, 2,2- azinobis-(3-ethylbenzothiazoline- 6- sulphonate) (ABTS) observed upto 99.07% followed by the scavenging of the stable radical 1,1 diphenyl, 2- picryl hydrazyl (DPPH) (89.77 %) and nitric oxide radical (73.64%) at the same concentration. However, the extract showed only moderate scavenging activity of superoxide radicals and anti-lipid peroxidation potential, which was performed using rat-brain homogenate. The findings justify the therapeutic applications of the plant in the indigenous system of medicine, augmenting its therapeutic value.

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***Corresponding author**