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Research Article

Studies on phytochemical constituents, antioxidant and anti-inflammatory activities of *Annona reticulata* peel extracts

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Abstract

In the present work, *Annona reticulata* fruit peel extracts were investigated for antioxidant and anti-inflammatory activities. The peel extracts of *Annona reticulata* were acquired by using different solvent systems. Total phenolic contents were estimated by Folin–Ciocalteau method and flavonoid content were measured by aluminum chloride method. Proximate analysis such as total protein, carbohydrate, ash, and moisture contents were also carried out by standard methods. The plant extracts were analyzed by GC-MS method and simultaneously antioxidant activity was investigated by DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay. Anti-inflammatory activity was also explored. Among five different solvents used, the maximum antioxidant activity and anti-inflammatory activity was found in ethanol extract followed by others. The total phenol and flavonoid content in peel extract were found to be 20.14 mg gallic acid equivalents (GAE)/g and 5.58±1.98 mg Quercetin Equivalent (QE)/g respectively. Thus, *Annona reticulata* fruit peel could serve as best lead composite for manipulating a persuasive anti-oxidant and anti-inflammatory drug.

Keywords: *Annona reticulata* fruit peel; Extraction; Antioxidant and anti-inflammatory activity; Compound identification.

Introduction

India is a rich and heritage of herbal medicine. Many plants have been used to treat numerous diseases. *Annona reticulata* is one the plant belongs to Plantae Kingdom, commonly cultivated in Nilgiris, Tamil Nadu. Parts of *Annona* species are widely as natural medicine and exhibited excellent antioxidant activity [1]. Recently, *Annona* species are used to treat parasitic worms and ulcers. The unripe fruits parts of *Annona*species are used for the treatment of diarrhea and dysentery [2]. The leaves extract of *Annona* species showed anticancer activity [3] and anti-inflammatory activity [4] and antinociceptive activity [5]. Ethanolic extract of *Annona reticulata* leaves confirmed the presence of compounds like alkaloids, steroids, terpenoids, coumarins and tannins. Methanolic extract of *A. reticulata* leaves showed antibacterial activity against *Pseudomonas putida*, *Escherichia coli* and *Lactobacillus acidophilus* [6].

Occurrence of alkaloids and flavonoids of the plant extracts contributes vital role in antioxidant activities of plant [7,8]. Antioxidants are noteworthy towards reducing oxidative stress which can distress and damage biological particles [9]. Extraction using organic solvents is one of the essential stages in the route of phytochemical processing for the detection of bioactive elements from plant extracts [10] and also influence of various organic solvents on extraction of plant materials on quantitative level of total phenolic contents of plant extracts were previously studied [11].

In the present work, qualitative and quantitative methods were investigated for analyzing the phytochemical composition and antioxidant and anti-inflammatory activities of different extracts of *Annona reticulata* fruit peel. The effect of the varying concentration of

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phytochemicals in the extracts on their antioxidant and reducing activities was determined.

Materials and methods

Collection of Annona reticulata

The healthy fruits of *Annona reticulata* were collected from agricultural land around Mettupalayam, Tamil Nadu. The peel of the fruit was separated and allowed to dried, and pulverized to powder was acquired by using a mechanical grinder.

Preparation of plant extracts

Plant extract were prepared using ethanol, acetone, chloroform, aqueous and petroleum ether and 1g of peel powder of *Annona reticulata* plant materials was taken in 100 ml of each solvent and mixed well for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature [12]. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated by using vacuum evaporation at 40 °C. The dissolving rate of the crude extracts was maintained approximately 100 %. These solutions were stored at -20 °C until reuse.

Phytochemical screening of plant extract from Annona reticulate

The phytochemical screening of plant extract was carried out according to recent studies [13-15]. The presence of compounds like tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids were analyzed.

Proximate analysis

The proximate analyses (moisture, ash, proteins and carbohydrates) of plant extracts were determined [16]. The moisture and ash were determined using weight difference method. The protein content in the peel extract of *Annona reticulata* was estimated by following the lowry's method [17]. The carbohydrate content of the peel extract of *Annona reticulata* was estimated by following the method as described by Dubois et.al.,(1956) [18].

Antioxidant activity

The antioxidant activity of plant extracts of *Annona reticulata* was carried out by method prescribed by Brand-William et al., (1995) [19]. DPPH solution (0.1 mM) was mixed with various concentrations of the extracts and incubated at room temperature for 30 min. Using UV- Vis spectrophotometer, absorbance was recorded at 517 nm. Ascorbic acid was used as control. Free radical scavenging activity was calculated by using eq. (1).

$$\%\ of\ scavenging = \left\{\frac{[absorbance\ of\ control - absorbance\ of\ sample]}{absorbance\ of\ control}\right\} * 100 \quad ...(1)$$

Quantification of total phenolic contents

Total phenolic content present in the extracts were analyzed by the method prescribed by Rajkumar et al., (2011) [20]. Different concentration of extracts, 1/10 dilution of Folin-Ciocalteau reagent and 7.5% Na_2CO_3 were added and incubated for 15 min at 45 ^oC. Absorbance was recorded at 765 nm using UV-Vis spectroscopy and gallic acid was used as standard.

Determination of total flavonoid content

Total flavonoid content in extract was analyzed by using aluminum chloride colorimetric method [21,22]. 1 ml of the plant extracts was mixed with 0.2 ml of aluminum chloride (10%) (w/v), 0.2 ml of potassium acetate (1 M), 3 ml of methanol and 5.6 ml of distilled water. Quercetin was used as standard. The solution was incubated for 30 min at room temperature. Absorbance was recorded at 415 nm with a UV-Vis spectrophotometer. The total content of flavonoid compounds in the plant extracts was calculated by the eq. (2) [23].

$$C = (c * V)/w \quad (2)$$

Where; C = total content of flavonoid compounds in mg of quercetin equivalent/gm plant extract, c = concentration of quercetin obtained from the calibration curve in mg/ml, V = volume of extract in ml and w = the weight of crude plant extract in gm.

Gas Chromatography-Mass Spectroscopy analysis

Plant extracts were subjected to Gas Chromatography-Mass Spectroscopy (GC-MS) analysis for the identification of bioactive components in peel extracts. GC-MS analysis was carried out on a GC-MS -5975C Agilent system comprising an auto sampler and a gas chromatograph interfaced to a mass spectrometer

instrument, employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm ID × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1.51 ml/min and an injection volume of 1µL was employed (split ratio of 10:1) injector temperature 240°C; ion-source temperature 200°C. The oven temperature was programmed from 700°C (isothermal for 2 min), with an increase of 10°C /min, to 300°C /min, ending with a 9 min isothermal at 300°C. Mass spectra were taken at 70eV; with a scan range 40-1000 m/z. Solvent cut time was 5 min; MS start time being 5 min; MS end time being 35 min; Ion source temperature set to 200°C and interface temperature being 240°C.

Anti-inflammatory activity

The anti-inflammatory activity of plant extract was studied by using inhibition of albumin denaturation technique [24]. Plant extract was mixed with 1% aqueous solution of bovine albumin and were incubated at 37 ºC for 20 min and then heated to 51 ºC for 20 min, absorbance was measured at 660 nm after cooling. Percentage inhibition of protein denaturation was calculated by using eq. (3).

$$Percentage\ inhibition = \{[Absorbance\ of\ control - Absorbance\ of\ test\ sample] * 100\}/\ Absorbance\ of\ control \quad \ldots(3)$$

Statistical data analysis

All the analysis was carried out in triplicates. Data were presented as mean ± standard deviation (SD). Statistical analysis was performed by one-way ANOVA. Microsoft Excel 2007 (Roselle, IL, USA) was used for the statistical evaluations. Significant differences between groups were determined at $p<0.05$. To evaluate relationships between experimental parameters, results were investigated for correlation and tested for significance by Student's t-test.

Results and discussion

Yield of extracts and phytochemical analysis of various extracts of A. reticulata peel

The *A. reticulata* plant peel powder (50 g) yielded 8.56 g of aqueous, 4.52 g of ethanolic, 5.54 g of acetonic, 3.12 g of chloroformic and 6.12 g of petroleum ethercrude extracts after sequential extraction. The potential of plants against diseases like inflammatory, cancer, diabetes, cardiovascular had been proven through the study of polyphenols present in the plants [25]. Table 1 represents the presence of polyphenols including tannin, saponin, flavonoid, quinone, alkaloid, cardiac glycosides, terpenoid, phenol, steroid, coumarin and betacyanins.

Table 1. Phytochemical screening of various extracts of *A. reticulata* peel

Phytochemical tests	Aqueous extract	Ethanolic extract	Acetonic extract	Chloroformic extract	Petroleum ether extracts
Tannins	+	++	+	-	-
Saponins	++	-	-	+	+
Flavonoids	+	+	+	+	-
Quinones	+	++	++	+	+
Alkaloids	+	+	+	-	-
Glycosides	-	-	-	-	-
Cardiac glycosides	++	+	+	+	-
Terpenoids	++	+	+	+	+
Phenols	++	++	++	+	+
Steroids	++	+	++	+	+
Coumarins	+	+	+	-	-
Anthocyanins	-	-	-	-	-
Betacyanins	+	+	+	-	-

+Mild presence, ++ Strong presence, − Absence.

Total phenolic contents

Phenolic compounds are represented as proficiently known for antioxidant, anti-mutagenic and anti-tumor activities [26]. Table 2 represents the differences in the quantity of totalphenolic contents of peel extracts in different concentrations. As a result we concluded that ethanol extract has the maximum total phenolic contents about 29.05±2.12GAE at 400 µg concentration. George et al., (2015) reported that presence of total phenolic contents of *Annona* species were high in methanolic extract when compared to aqueous extract [15]. All the extracts have significantly well constituents of total phenolic contents with various concentrations of different extracts.

Table 2. Total phenolic contents of aqueous, ethanol, acetone, chloroform, petroleum ether extracts of *A. reticulata* peel

Concentration (µg)	Gallic acid equivalence (GAE) ± SD (µg)				
	Aqueous extract	Ethanol extract	Acetone extract	Chloroform extract	Petroleum ether extracts
25	0.75± 0.10	1.52±0.21	0.9±0.1	1.21± 0.11	0.81±0.21
50	1.15±0.15	4.12±0.18	2.54±0.75	2.15± 0.27	1.22±0.25
100	4.12±0.54	8.15±0.45	5.74± 0.45	4.44± 1.22	6.78± 0.45
200	8.05±0.83	17.05± 1.12	10.11±0.79	16.12± 0.91	8.47±0.74
400	11.01±1.45	29.05± 2.12	17.04± 1.12	18.01±0.81	12.05±0.54

Total flavonoid contents

Total flavonoid contents present in various extracts of *A. reticulata* peel were investigated by Aluminum chloride colorimetric method. Ethanol extract of *A. reticulata* peel was found to contain 5.58 ± 1.98 mg of quercetin equivalent per gram of crude extract. Table 3 represents Flavonoid contents in various extract of plant *A. reticulata* peel and were found to decrease in the following order: Ethanol extract> Chloroform extract> Acetone extract>Aqueous extract>Petroleum ether extracts. Similar results reported by Biswas et al., (2012) but total flavonoid contents were at high methanol extract of *Annona* species plant leaf [27].

Table 3. Total flavonoid contents in various extracts of *A. reticulata* peel

Extracts	Total flavonoid contents (mg of quercetin equivalent per gram of crude extract)
Aqueous extract	3.75 ± 0.98
Ethanol extract	5.58 ± 1.98
Acetone extract	4.23 ± 1.52
Chloroform extract	4.58 ± 1.57
Petroleum ether extracts	3.34 ± 1.23

Proximate analysis

The proximate composition of *A. reticulata* peel is given in table 4. The peel contains more of carbohydrates and ash, but no significant amount of fat or proteins. The proximate investigation demonstrates adequate results with respect to moisture content, total ash, protein and carbohydrate values [28].

Table 4. Proximate composition of *A. reticulata* peel

Constituents	Quantity (g/100g)
Moisture	0.23627%
Total ash	31.42%
Protein	66 mg/g
Carbohydrate	25 mg/g

Antioxidant activity of extracts of A.reticulata peel

Different concentrations from 100–500 µg/ml of various extracts of *A. reticulata* peel were analyzed for antioxidant activity by DPPH radical scavenging activity. Concentration dependent manner was observed for radical scavenging activity. From the results listed in table 5, ethanol extract of *A. reticulata* peel showed highest 88.18% inhibition. Other extracts like acetone extract, aqueous extract, chloroform extract and petroleum ether extracts of *A. reticulata* peel showed 84.54, 70.07, 64.56,

58.26% inhibition respectively at 500 µg/ml. Baskar et al., (2007) reported that ethanolic extracts of *Annona muricata* leaf had highest inhibition % in antioxidant activity [1]. Recently, plant extract having antioxidant activity was evidenced due to the sufficient presence of total phenolic and flavonoid content in the plant extract [29,30].

Table 5. Effect of various extract of *A. reticulata* peel on antioxidant model

Concentration (µg/ml)	DPPH Inhibition %				
	Aqueous extract	Ethanol extract	Acetone extract	Chloroform extract	Petroleum ether extracts
100	53.54	62.45	54.45	22.13	11.54
200	55.41	69.89	65.23	44.54	24.57
300	62.45	71.15	72.45	59.21	36.45
400	65.78	80.15	79.45	62.12	47.21
500	70.07	88.18	84.54	64.56	58.26
IC_{50} (µg/ml)	75	68	80	92	150

Gas Chromatography-Mass Spectroscopy analysis

The mass spectra (Fig. 1) of the compounds found in different fractions prepared from ethanol extract of *A. reticulata* peel were matched with the National Institute of Standards and Technology library (NIST) and listed in table 6.

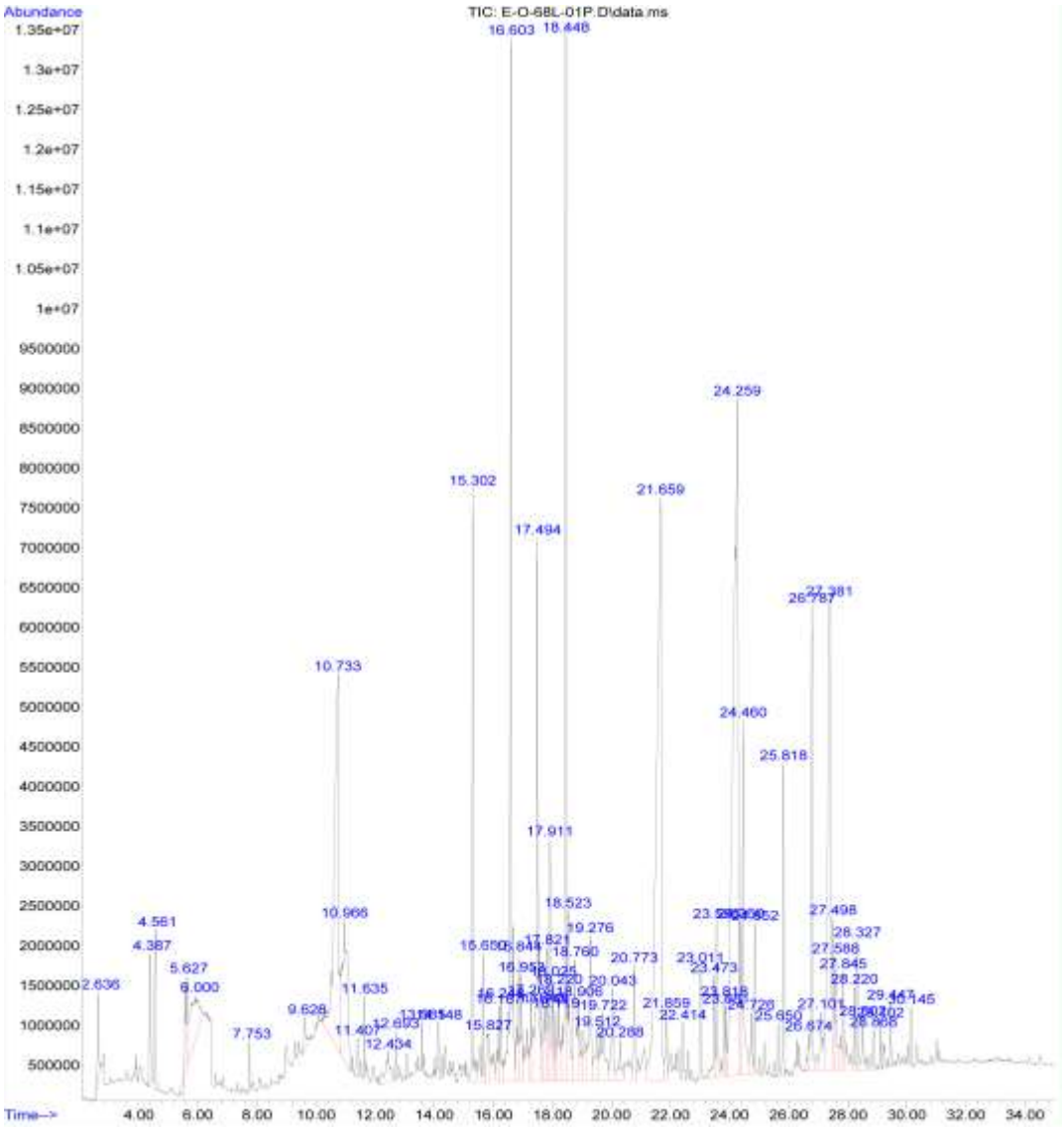

Fig. 1. GC-MS chromatogram of *A. reticulata* peel ethanol extract

Table 6. GC-MS Analysis of Peel extract of *A. reticulata*

Sl. No.	Retention time, min	Area, %	Compound	Molecular weight (g/mol)	Molecular formula
1	2.633	0.40	Acetic acid	60.05	CH_3COOH
2	4.384	0.77	2,3 Butanediol	90.12	$C_4H_{10}O_2$
3	4.563	0.99	2,3 Butanediol	90.12	$C_4H_{10}O_2$
4	5.626	0.62	Sulphuric acid, Dimethyl ester	90.07, 46.07	H_2SO_4,C_2H_6O
5	6.000	1.80	Sulphuricacid, Dimethyl ester	90.07, 46.07	H_2SO_4,C_2H_6O
6	7.751	0.14	2,4 Dihydroxy 2,5-dimethyl-3(2H)-furan-3-one	144.12	$C_6H_8O_4$
7	9.629	0.25	Glycerin	92.09	$C_3H_8O_3$
8	10.736	6.86	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	144.12	$C_6H_8O_4$
9	10.968	3.26	Glycerin	92.09	$C_3H_8O_3$
10	11.409	0.27	Myrtenol	152.23	C10H16O
11	11.634	0.26	Bicyclo[3.1.1]hept-3-en-2-one,6-trimethyl-	150.21	$C_{10}H_{14}O$
12	12.434	0.58	Massoilactone	168.24	$C_{10}H_{16}O_2$
13	12.696	0.15	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (IS-endo)-	196.28	$C_{12}H_{20}O_2$
14	13.587	0.15	.alpha.-cubebene	204.35	$C_{15}H_{24}$
15	14.148	0.24	1H-Cyclopenta[1,3] cyclopropa [1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-,[3aS-(3a.alpha., 3b.beta., 4.beta.,7.alpha.,7aS*)]-	204.35	$C_{15}H_{24}$
16	15.300	0.46	Benzene, 1-(1,5-dimethyl-4-hexynyl)-4-methyl	202.33	$C_{15}H_{22}$
17	15.652	0.46	.beta.-curcumene	204.35	$C_{15}H_{24}$
18	15.824	0.57	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-	204.35	$C_{15}H_{24}$
19	16.190	0.51	Dodecanoic acid	200.31	$C_{12}H_{24}O_2$
20	16.250	0.45	Isolongifolene, 9,10-dehydro-	202.33	$C_{15}H_{22}$
21	16.602	6.52	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.alpha., 4a.alpha.,7.beta.,7a,beta.,7b.alpha.)]-	220.35	$C_{15}H_{24}O$
22	16.841	0.84	Phenol,4-[[(dimethylamino)sulfonyl]methylamino]-	331.36	$C_{21}H_{17}NO_3$
23	16.954	0.71	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	220.35	$C_{15}H_{24}O$
24	17.268	0.42	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-ethylene-	204.35	$C_{15}H_{24}$
25	17.492	3.80	Ar-tumerone	216.31	$C_{15}H_{20}O$
26	17.642	0.51	.alpha.-guaiene	204.35	$C_{15}H_{24}$
27	17.747	0.48	Cycloheptane,4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	204.35	$C_{15}H_{24}$
28	17.821	0.68	6-Isopropenyl-4,8adimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	220.35	$C_{15}H_{24}O$
29	17.911	1.37	Curlone	218.33	$C_{15}H_{22}O$
30	18.023	0.53	1,3,5-trimethyl-2-(2,2,2-trifluoro-ethoxy)-benzene	175.05	$C_8H_8Cl_2$

Anti-inflammatory analysis

The ability of plant extract to inhibit protein denaturation was investigated to study the mechanism of the anti-inflammation activity. It was observed that maximum inhibition of 89.2 % at 5 mg/ml and obtained data were listed in table 7. Leelaprakash et al., (2011) reported that Methanol extract of whole plant of *Enicostemma axillare* showed the maximum inhibition of 71 % [31].

Table 7. Effect of ethanol extract of *A. reticulata* peel on heat induced protein denaturation.

Concentration (mg/ml)	% Inhibition of protein denaturation
0.3125	18.09
0.625	27.4
1.25	49.6
2.5	68.3
5.0	89.2

Conclusions

Results indicate that the ethanol extracts of *Annona reticulata* fruit peel possess the antioxidant and anti-inflammatory properties. These activities may bed ue to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. This study also suggest that the compound of the plant *Annona reticulata* fruit could be used as lead composite for manipulating a persuasive anti-oxidant and anti-inflammatory drug which could be served for treatment of various diseases such as aging, cancer, neurological disorder and inflammation.

Conflicts of Interest

The authors declare no conflict of interest.

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