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Research Article

Phytochemical Profiling, Quantification, and Antioxidant Potential of *Annona reticulata* Linn.

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ABSTRACT

The current study examined the phytochemical makeup and antioxidant capacity of *Annona reticulata* leaf extracts in ethanol and water. Phenolics (98.14 mg/g), alkaloids (78.34 mg/g), flavonoids (53.71 mg/g), terpenoids (79.21 mg/g), and saponins (24.69 mg/g) were found to be more abundant in the ethanolic extract than in the aqueous extract (76.83, 56.74, 44.21, 4.21, and 21.49 mg/g, respectively), according to quantitative analysis. Both extracts exhibited concentration-dependent ferric reducing power and free radical scavenging, with the ethanolic extract exhibiting the highest antioxidant activity as measured by the 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power experiments. Several bioactive phytoconstituents that are responsible for the observed antioxidant effects were confirmed to be present by liquid chromatography–mass spectrometry profiling.

Key words: *Annona reticulata* Linn, 2,2-diphenyl-1-picrylhydrazyl antioxidant assay, Ferric reducing antioxidant power antioxidant assay, liquid chromatography–mass spectrometry analysis, Phytoconstituents.

1. INTRODUCTION

Plants are known for their fragrance and medicinal properties. Extracts from different plant parts have therapeutic qualities and are utilized in many pharmaceutical formulations as an ingredient, coloring agent, preservative, and sweetener [1]. Plants generate secondary metabolites called phytochemicals, such as flavonoids, tannins, terpenoids, saponins, and alkaloids, which are believed to provide the plants with their therapeutic qualities [2,3]. The ability of phenolic compounds to scavenge-free radicals has led to their association with antioxidant activity. A class of compounds known as antioxidants can help prevent cancer and other conditions that can cause diseases such as diabetes, heart disease, atherosclerosis, Alzheimer's, and Parkinson's disease [4-6].

Although they contain many secondary metabolites, plants are thought to be the main source of chemicals with medicinal effects [7]. Plants have been effectively used to create cosmetics and toiletry preparations in addition to therapeutic formulations [8-10]. Regular use of synthetic drugs can result in addiction, while plant-based medications do not have the same side effects and are generally safer than synthetic ones [11,12]. In addition, commercial pharmaceutical companies employ plants as a source for the manufacture of synthetic chemicals. According to the World Health Organization, about 85% of poor nations employ plants or their extracts as the active ingredient [13]. The majority of people in poor nations get their primary medical care from plant-based traditional medicine. Ayurveda, the traditional Indian medical system, is similarly based on plants. Plant-based medicines serve as the body's first line of defence and aid in health restoration [14].

About 2400 known plant species make up the *Annonaceae* family, of which *Annona reticulata* is a member. The tree is tiny, 8–10 m tall, and can be either semi-deciduous or semi-evergreen [15]. In English, *A. reticulata* is frequently referred to as custard apple or bullock's heart; however, it has other regional names. "Bullock's heart" refers to the fruit's peculiar heart-shaped form [16]. Although it is grown for its fruit, the plant is primarily recognized for its many therapeutic applications [17]. Different components of this plant, including the leaf, stem, bark, root, and immature fruit, are used to cure various illnesses in traditional medicine [18]. The infusion and paste of A. reticulata leaves have been used to treat ulcers, abscesses, and vermifuges [19]. A bark infusion and dried unripe fruit are used as treatments for dysentery and diarrhea. The leaves and seed extract of A. reticulata are used to make insecticides [20].

Numerous studies have demonstrated that this plant possesses various beneficial effects, including antibacterial, antipyretic, antioxidant, antiproliferative, anticancer, anthelmintic, and antihyperglycemic properties. Furthermore, A. reticulata exhibits anti-inflammatory effects [21]. It is found growing spontaneously in tropical and subtropical regions. In rural areas, different parts of the plant, such as leaves, bark, seeds, and roots, are used in traditional medicine to treat a wide range of ailments [22]. Various extracts from this plant have shown anti-hyperglycemic, cytotoxic, and recombinant caspase inhibitory properties. They also exhibit antinociceptive, analgesic, central nervous system depressive, anti-inflammatory, tumor-inhibiting, and antiproliferative properties [23]. In addition, the plant contains a rich array of phytochemical compounds, including vitamins, terpenoids, phenolic acids, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, and amines, all of which are known for their significant antioxidant activity [24].

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The present study shows the extraction of *A. reticulata* leaves by aqueous and ethanol by the Soxhlet extraction method. After that, quantitative phytochemical analysis was performed to determine the quantity of phytoconstituents, and the antioxidant activity of each extract was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays to exhibit free radical scavenging percentage.

2. MATERIALS AND METHODS

2.1. Collection

Mature leaves of *A. reticulata* collected from the village of Nagpur during the winter season were identified and authenticated by the Department of Botany at Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur. Shade dried the leaves for 4–5 days. By using the grinder, the dried leaves were ground into coarse powder. And stored the powder in an air-tight container for further use.

2.2. Preparation of Extract

Aqueous extract of *A. reticulata* leaves was prepared by using Soxhlet extraction method, 20 g of powder of leaves was taken in a thimble and 250 mL of distilled water was added to the 500 mL round bottom flask, then on heating mantle assembly was continued at temperature 80–90°C for 20 h The sample eluted in round bottom flask was collected and distilled off by simple distillation method and the prepared extract stored in air tight container for further use. Similarly, for the ethanolic extract of *A. reticulata* leaves, the process was continued at a temperature of 60–70°C for 18–20 h. prepared ethanolic extract stored in an air-tight container for further use.

2.3. Quantitative Phytochemical Analysis

2.3.1. Total phenolic content

To determine the total phenolic content, 12.5 µL of plant extract was combined with 12.5 µL of Folin-Ciocalteu reagent in a 300 µL reaction container. This mixture was allowed to stand at room temperature for 10 min. Next, 125 µL of 7% sodium carbonate was added to the solution. The mixture was then incubated in the dark at 37°C for 90 min. After incubation, the reaction volume was adjusted to 300 µL using distilled water. The optical density was measured at 760 nm using a spectrophotometer. A standard calibration curve for gallic acid (20–100 µg/mL) was used to determine the concentration of phenolics.

2.3.2. Total flavonoid content

One hundred microliters of plant extract and one hundred microliters of 2% aluminum chloride were incubated at room temperature for 10 min. A spectrophotometer was then used to measure the optical density at 367 nm. A standard quercetin calibration curve, which ranges from 20 to 100 µg/mL, was used to determine the flavonoid content.

2.3.3. Total terpenoid content

A mixture was prepared by combining 150 µL of a 5% w/v vanillin-glacial acetic acid solution, 500 µL of perchloric acid, and 100 µL of plant extract. The samples were heated at 60°C for 45 min and then cooled in an ice bath. The absorption coefficient was measured at an intensity of 548 nm. To determine the concentration of terpenoids, a standard calibration curve using Ursolic acid (ranging from 20 to 100 µg/mL) was employed.

2.3.4. Total alkaloid content

The reaction was carried out in a 1.5 mL microcentrifuge tube using 100 µL of 0.05 M phenanthroline solution, 100 µL of plant extract, and 100 µL of 0.025 M $FeCl_3$ produced in 0.5 M HCL.

Distilled water was used to lower the final volume to 1 mL. The reaction mixture had been submerged in a water bath at 70°C for 30 min. At 510 nm, the optical density was still recorded. To make comparisons easier, several doses of colchicine (20–100 mg) were utilized as a reference. The total alkaloid content was calculated using the calibration curve.

2.3.5. Total saponin content

500 mL of 72% $H2SO_4$ are combined with 50 mL of plant extract and 50 mL of 8% vanillin to create this reaction mixture. Following this, the reaction mixture was incubated for 10 min at 60°C in a water bath. At a wavelength of 544 nm, absorbance was measured after cooling. The calibration curve was used to determine the concentration of total saponin. For reporting the results, the unit of measurement was milligrams of diosgenin equivalent per gram of fresh or dried material.

3. LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY (LC-MS) ANALYSIS

Metabolite screening of the crude extract was done by using LC-MS in positive and negative modes. The instrument used for this was an Agilent Quadrupole Time- of -Flight G6540B connected to an Agilent 1260 Infinity II high-performance liquid chromatography. The column used was agilent eclipse extra densley bonded-C18, 3*150 mm, 3.5 µ, and the temperature was 40°C. Two mobile phases used: Mobile phase A and mobile Phase B, in which mobile one is 0.1% Formic acid in water, and mobile phase B is 0.1% Formic acid in acetonitrile. The mobile phase flow rate was 0.3 mL/min.

Nebulizer Gas temperature was 300°C, Sheath Gas temperature 350°C, Drying Gas was 81/min, Nebulizer Gas was 35 pounds per square inch gauge, Sheath Gas Flow 111/min, capillary Voltage 3500 V, Nozzle Voltage 1000 V.

4. BIOLOGICAL ACTIVITY

4.1. Antioxidant Activity

4.1.1. DPPH assay

With minor adjustments, the technique outlined by Aquino *et al*. [25] was used to assess the DPPH radical scavenging activity. After adding 10 µL of plant extract solution to the well, 190 µL of DPPH. The plate was placed in the dark for a period of 20 min. The wavelength of absorbance was 517 nm. Through a comparison of absorbance with that of a blank solution (DPPH without sample), the free radical scavenging activity of each fraction was ascertained. One positive control that was employed was ascorbic acid. Utilizing the following formula, the capacity to scavenge the DPPH radical was represented as a percentage inhibition:

$$\text{DPPH Scavenging activity } (\%) \frac{\left[(A0 - A1)\right]}{A0} \times 100$$

Where A0 = Absorbance of control
A1 = Absorbance of the test sample.

4.1.2. FRAP assay

The FRAP assay method previously reported [26] was used, with minimal modifications, to examine the ability to decrease ferric ions. The reaction mixture is made up of 290 mL of 0.3 M acetate. buffer, 5 mL of 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine, 5 mL of 20 mM $FeCl_3$, and 10 mL of plant extracts (1 mg/mL). Absorbance at 595 nm was determined after the reaction mixture was incubated at 37°C for 15 min. The antioxidant potential based on the capacity to reduce ferric ions was reported as mmol ascorbic acid equivalents per gram of plant extract. Ferric reduction was computed using the linear calibration curve of ascorbic acid.

5. RESULTS AND DISCUSSION

5.1. Content of Phenolic

In *A. reticulata* leaves, the total content of phenolics is 76.8306 ± 0.035 mg gallic acid equivalent (GAE) g^{-1} in aqueous extract, while in ethanolic extract it is 98.1420 ± 0.024 mg GAE/g^{-1} [Table 1 and Figure 1]. On comparing these values highest phenolic content is in the ethanolic extract of leaves than in the aqueous extract. These values are determined based on the values of the calibration curve (GAE = R^2 = 0.9904).

5.2. Total Alkaloid Content

Aqueous and ethanolic extracts have a total alkaloid content of 56.74 ± 0.006 mg to 78.34 ± 0.014 mg, which is equal to the amount of colchicine g^{-1} of plant material. The ethanolic extract of dried leaves has the highest quantity of alkaloids.

Table 1: MS ion source: Dual Agilent Jet Stream Electrospray Ionization.

Time (min)	Percentage of mobile phase A	Percentage of mobile phase B
0	95	5
2	95	5
25	5	95
28	5	95
28.1	95	5
30	95	5

5.3. Total Flavonoid Content

In aqueous extract of *A. reticulata* leaves, the amount of flavonoid is 44.2111 ± 0.023 mg equivalent to rutin $g^{-1}$and the ethanolic extract has a flavonoid content is 53.7111 ± 0.009 mg equivalent to rutin g^{-1} From that, it is concluded that the ethanolic extract of dry plant material has the highest flavonoid content.

5.4. Total Terpenoid Content

Fresh aqueous extracts have a lower terpenoid concentration than methanolic extracts. This implies that terpenoids dissolve better than water. Comparable to ursolic acid g^{-1} plant material was the methanolic extract with the highest terpenoid content was 79.2159 ± 0.005 mg/g. The content of the aqueous extract was low 4.2159 ± 0.003 mg/g.

5.5. Total Saponin Content

The saponin content of *A. reticulata* leaves extract was found to be high in methanolic extract, which was 24.6925 ± 0.008 mg equivalent to diosgenin g^{-1} and the aqueous extract shows 21.4886 ± 0.013 mg/g. By comparing the values of both extracts, a high amount of saponin was found in the methanolic extract.

5.6. DPPH Assay

The antioxidant activity of *A. reticulata* Linn. was performed by DPPH assay, and the % of inhibition varies from 9.16 ± 0.02 to 69.17

Table 2: Quantitative phytochemical analysis (Values are mean±standard error of the mean) *n*=3.

Solvent	Phenolic content GAE (mg/g)	Alkaloid content CE (mg/g)	Flavonoid content RE (mg/g)	Terpenoids content UAE (mg/g)	Saponins content DE (mg/g)
Aqueous extract	76.8306±0.035	56.74±0.006	44.2111±0.023	4.2159±0.003	21.4886±0.013
Ethanol extract	98.1420±0.024	78.34±0.014	53.7111±0.009	79.2159±0.005	24.6925±0.008

GAE: Gallic acid equivalent, UAE: Ursolic acid equivalent, CE: Colchicine equivalent, DE: Diosgenin equivalent, RE: Rutin equivalent

Table 3: DPPH scavenging activity.

Concentration of Polyethylene (µg/mL)	Percentage of DPPH scavenging in aqueous extract	Percentage of DPPH scavenging in ethanolic extract	Percentage of DPPH scavenging in ascorbic acid
20	9.16±0.02	23.39±0.004	20.36±0.912667
40	13.35±0.004	35.02±0.002	37.70±0.714
60	36.47±0.005	50.41±0.003	53.46±0.533333
80	51.25±0.005	60.76±0.006	69.02±0.355
100	69.17±0.003	77.55±0.004	82.05±0.205667

DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 4: FRAP assay.

Concentration of Polyethylene (µg/mL)	FRAP mM/g in Aqueous extract	FRAP mM/g in Ethanolic extract	FRAP mM/g in Ascorbic acid (standard)
20	21.1627±0.004	22.2480±0.002	20.4263±0.089
40	30.8527±0.005	37.5193±0.002	40.1937±0.259
60	38.6431±0.004	52.0155±0.002	59.1860±0.422
80	63.9922±0.005	71.2790±0.004	78.1007±0.585
100	78.0620±0.004	90.5814±0.004	101.1627±0.783

FRAP: Ferric reducing antioxidant power

Table 5: LC-MS analysis of positive mode of aqueous extract.

S. No	Name of compound	Category	Molar mass	RT	Activity
1	Swainsonine	Alkaloids	173.1061	3.047	
2	2-amino-8-oxo-9,10-epoxy-decanoic acid	Organic compound	215.1167	3.962	Anticancer, Neurotoxic, and Immunologic effects
3	Plantagonine	Polyphenolic glycosides	177.0785	6.741	Antioxidant, Antimicrobial, Enzyme inhibitor
4	3-Oxo-carbofuran	Carbofuran	235.085	9.956	Antioxidant, Antimicrobial, Anti-inflammatory, Neuroprotective
5	Mycalamide B	Secondary metabolites	517.2868	11.005	Pesticidal property
6	Convolamine	Alkaloids	305.163	12.093	Antimicrobial, Anticancer, and Antiviral
7	p-Coumaroylputrescine	Phenolic compound	234.137	12.18	Antimicrobial, Anticancer, and Antiviral
8	1,2-Dehydroreticuline	Alkaloids	328.1546	13.182	Anticholinergic, Antioxidant, Antimicrobial, Anti-inflammatory
9	4,4'-dihydroxy-3,5-dimethoxydihydrostilben	Stilbene derivative	274.1206	13.71	Antioxidant
10	Isocorydine (+)	Alkaloids	341.1622	13.51	Analgesic and sedative effect
11	Phyllalbine	Alkaloids	291.1473	13.688	Anticancer, Neuroprotective, Antioxidant
12	Supinine	Alkaloids	283.1788	13.875	Analgesic, CNS effect, Antioxidant
13	Variotin	Alkaloids	291.1834	14.002	Antioxidant, Antimicrobial, Anti-inflammatory, Cytotoxicity
14	Betavulgaroside IV	Glycosides	291.1834	19.762	Antioxidant, Antimicrobial, Anti-inflammatory, Cytotoxicity
15	Octhilinone	Biocides	291.1834	20.402	Antioxidant, Antimicrobial, Anti-inflammatory
16	Neosaxitoxin	Alkaloids	315.1293	2.669	Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer
17	Mycalamide B	Bioactive compound	517.2868	9.984	Fungicides, Antimicrobial, Preservative
18	Lunacridine	Alkaloids	305.163	12.093	Antimicrobial, anticancer
19	p-Coumaroylputrescine	Phenolic amide	234.137	12.18	Antioxidant
20	(S)-Edulinine	Alkaloids	291.148	12.342	Antimicrobial, Antioxidant, Antidiabetic
21	Convolvine	Alkaloids	291.148	12.342	Anti-inflammatory, Antimicrobial, Antioxidant, Immunomodulatory,
22	Litcubinine	Alkaloids	314.1376	12.836	Cardiovascular drug
23	(S)-Corytuberine	Alkaloids	328.1546	13.182	antipsychotic
24	(S)-Scoulerine	Alkaloids	327.1468	13.182	Neurotransmitter activity
25	Norcorydine	Alkaloids	327.1468	13.182	Anti-inflammatory, Antimicrobial
30	Coreximine	Alkaloids	327.1468	13.182	Anti-inflammatory, Antimicrobial, Neuropharmacological
31	Norisocorydine	Alkaloids	327.1468	13.182	Antioxidant, Antinociceptive activity
32	Laurotetanine	Alkaloids	327.1468	13.182	Antioxidant, Antimicrobial, Antiplasmodial, and Anti-asthmatic activity
33	Salutaridine	Alkaloids	327.1468	13.182	analgesic
34	Boldine	Alkaloids	327.1468	13.182	Hepatoprotective, Anti-inflammatory, Anticancer, Antidiabetic, Neuroprotective
35	Bracteoline	Alkaloids	327.1468	13.182	Calcium channel inhibition, Cytotoxic
36	Cularidine	Alkaloids	327.1468	13.182	Calcium channel inhibition, Cytotoxic
37	Cularimine	Alkaloids	327.1468	13.182	Antitumor activity
38	(S)-Isoboldine	Alkaloids	327.1468	13.182	Anti-inflammatory Antimicrobial,
39	Gossyvertin	Flavonoids	274.1206	13.195	Anti-inflammatory, antimicrobial, antioxidant
40	Sinomenine	Alkaloids	329.1612	13.471	Analgesic, Anti-inflammatory, Neuroprotective, Immunosuppressive, anti-hypertensive
41	Cinnamoylcocaine	Alkaloids	329.1612	13.471	CNS effects, Anaesthetic effects

(Contd...)

Table 5: *(Continued)*

S. No	Name of compound	Category	Molar mass	RT	Activity
42	Isobavachalcone	Flavonoids	324.1357	13.51	Neuroprotective, Anti-inflammatory, Antimicrobial, Antioxidant, Anticancer
43	Convolvine	Alkaloids	291.1473	13.688	Vasodilator, hypotensive, Anti- anti-inflammatory, analgesic
44	Phyllalbine	Alkaloids	291.1473	13.688	Cardiovascular, Vasodilator, Anti-inflammatory, Antioxidant
45	Supinine	Alkaloids	283.1788	13.875	Anti-inflammatory, Antioxidant, Neuroactive, Antimicrobial

LC-MS: Liquid chromatography–Mass spectrometry, CNS: Central nervous system, RT: Retention time

Table 6: LC-MS analysis of the negative mode of aqueous extract.

S. No.	Compound name	Category	Molar mass	RT	Activity
1	7-acetoxyobacun- 9(11)-ene	Terpenoid	496.2106	12.45	Antifungal, Antiviral, Antioxidant
2	Triamterene	Diuretics	253.1072	13.13	Used for blood pressure and edema
3	Triamcinolone diacetate	Corticosteroid	478.2006	14.21	Anti-inflammatory, immunosuppressive
4	Macrozamin	Alkaloids	384.1379	14.2	Used cardiovascular, Antiarrhythmic, Antioxidant, Antimaterial
5	Rheidin C	Flavonoids	538.1266	14.3	Antioxidant, Anti-inflammatory, Antimicrobial, Anticancer
6	Kuwanon Z	Flavonoids	594.1522	14.67	Antioxidant, Anti- inflammatory, Anticancer, Antimicrobial
7	Triamcinolone diacetate	Glucocorticoids	478.2006	14.83	Anti-inflammatory, Immunosuppressants
8	Dinoseb acetate	Nitrophenyl ester	282.0863	14.86	Herbicides and pesticides
9	β-methasone dipropionate	Glucocorticoid	504.2508	15.53	Anti-inflammatory, Immunosuppressives
10	(Z)-5-[(5-Methyl-2-thienyl) methylene]- 2 (5H)-furanone	Furanone	192.025	12.34	Antioxidant, Anti-inflammatory, Antibacterial
11	Sofalcone	Flavonoids	450.2051	12.45	Used in gastric ulcer
12	Agaritine	Hydrazine derivative	267.1227	13.13	Carcinogenic properties
13	Pyricarbate	Organocarbamate	253.1071	13.13	Pesticidal property
14	Acetohexamide	Sulfonyl urea	324.115	13.29	Antidiabetic property
15	Eremopetasitenin D2	Terpenoid	478.2009	14.83	Antioxidant, anti-inflammatory, Antibacterial
16	Eremopetasitenin C2	Terpenoid	478.2009	14.83	Antioxidant, Anti-inflammatory, Antibacterial
17	AS pulvinone H	(Terpenoids) Secondary metabolites	432.1951	14.83	Antimicrobial, anticancer, and antifungal
18	Stypandrol	Steroidal compound	430.1432	14.56	antidepressant
19	S-Furanopetasitin	Natural compounds	432.1952	14.21	Antioxidant, Anti-inflammatory, Antibacterial, Cytotoxic
20	2-Carboxy-4- dodecanolide	Lactone compound	1 242.1507	14.41	Antioxidant, Anti-inflammatory, Antibacterial, Anticancer
21	(3S,5R,6R,7E)-3,5,6-Trihydroxy-7- megastigmen-9-one	Terpenoids	1 242.1507	14.41	Antioxidant, Anti-inflammatory, Antibacterial, Used in cosmetics and in agriculture
22	Tyromycic acid	Bioactive compound antibiotic)	452.3306	14.56	Antioxidant, Anti-inflammatory, Antibacterial, Anticancer, In flavors and aroma
23	Alpha-L-Rhamnopyranosyl-(1->2)-beta-D-Galactopyranosyl	Glycosides	502.1536	14.6	Antioxidant, Anti-inflammatory, Antibacterial, Anticancer, Used in agriculture and cosmetics
24	S-Furanopetasitin	Furanone	432.1953	14.83	Antioxidant, Antifungal, antimicrobial
25	Portulacaxanthin III	Xanthophyll	268.0706	14.86	Antioxidant, Anti-inflammatory, detoxification, Antimicrobial
26	Acetohexamide	Antibiotic	324.115	13.29	Antioxidant, Anti-inflammatory, Antimicrobial
27	Lofepramine	Antidepressant	418.1799	13.91	Herbicides and Pesticides, Antioxidants, Anti-inflammatories

LC-MS: Liquid chromatography–Mass spectrometry, RT: Retention time

Table 7: LC-MS analysis of the positive mode of the ethanolic extract.

S. No.	Name of compound	Category	RT	Mass	Activity
1	Laurolitsine	Alkaloids	12.521	313.1306	Antioxidant, Anti-inflammatory, Neuroprotective, Antimicrobial, Anticancer
2	Variotin	Antibiotic	12.681	291.1829	Antibacterial, antifungal, cytotoxic
3	α-Santonin	Sesquiterpene lactone	12.943	246.1258	Cytotoxic, anticancer, anthelmintic
4	Nemertelline	Indole alkaloids	13.308	310.1216	Antibacterial, antifungal, cytotoxic, neuroactive
5	Isosinomenine	Isoquinoline alkaloids	13.536	329.1623	Anti-inflammatory, Neuroprotective, Immunomodulatory, Cardiovascular effects
6	Variotin	Polyketides	14.029	291.1837	Antibacterial, Neuroprotective, and Cytotoxic effects
7	Convolamine	Alkaloids	14.213	305.1641	Analgesic, Anti-inflammatory, Neuroprotective
8	N-(Heptan-4- yl) benzo [d][1,3] dioxole-5-carboxamide	Benzodioxole-derived carboxamides	14.275	263.1526	Neurological disorder, Anti-inflammatory, Antimicrobial
9	Xylopinine	Benzylisoquinoline alkaloids	14.532	355.1773	Anti-inflammatory, Antioxidant, Analgesic, Antifungal, Neuroactive, and CNS effects
10	Flutolanil	Fungicides	14.787	323.1133	Antifungal in agriculture
11	Tigloidine	Tropane alkaloids	14.822	223.1577	Anticholinergic and Neuroactive effects
12	Triangularine	Benzylisoquinoline alkaloids	14.928	335.1727	Antimicrobial, Vasodilator, and Neuroactive
13	Alpha-Eucaine	Synthetic alkaloids	15.536	333.1938	Local anaesthetic
14	Pterostilbene	Stilbenoid (polyphenol compound)	16.494	256.1105	Antioxidant, Anti- Anti-anti-inflammatory, Neuroprotective Agent
15	Dinobuton	Dinitrophenyl carbonate ester	17.026	326.1128	Organic pesticides, Acaricides
16	Paraoxon	Organophosphate	17.392	275.0563	Potent neurotoxins
17	Dihydro-betaerythroidine |:}	Alkaloids	19.465	275.1521	Neuroprotective, Nicotine addiction research, Muscle relaxant
18	PI (16:0/18:1 (11Z))	Phosphatidylinositol lipids	27.845	836.5405	Cell signalling, Metabolism, and Membrane dynamics
19	Crassostrea secocarotenoid	Carotenoid	28.208	616.412	Antioxidant, Anti-inflammatory, Immunomodulatory, Photoprotective
20	Allysine	Amino acids	2.412	145.0738	Role in lysin metabolism, in diabetes, and Cardiovascular disease
21	Thalassemine	Alkaloids	2.566	298.1029	Antimicrobial, Antiviral, Anticancer, Neuroprotective
22	Cinnamyl formate	Ester	3.318	162.0685	Antioxidant, Antimicrobial, Fragrance and Flavour industry
23	Cassiastearoptene	Terpenoids	3.318	162.0685	Antioxidant, Antimicrobial, Fragrance, and Flavour industry antifungal
24	Mycalamide B	Polyketides	9.975	517.2874	Antitumor, Antiviral
25	Arginyl-Tryptophan	Dipeptides	11.313	360.1906	Antioxidant, Anti-inflammatory, Immunomodulatory
26	(S)-Edulinine	Alkaloids	11.487	291.1474	Dopaminergic, Antioxidant, anti-inflammatory
27	Convolvine	Tropane alkaloids	11.487	291.1474	Neurological and anti-inflammatory application
28	Phyllalbine	Aporphine alkaloids	11.487	291.1474	dopaminergic, serotonergic, and anti-inflammatory activities.
29	Lenacil	Herbicides	12.334	234.1376	Used in agriculture
30	Laurolitsine	Benzylisoquinoline alkaloids	12.521	313.1306	vasorelaxant, anti-inflammatory, and potential neuroprotective effects.
31	Laurelliptine	Benzylisoquinoline alkaloid	12.521	313.1306	neuroactive, cardiovascular, and antioxidant properties.
32	Muricinine	Alkaloids	12.521	313.1306	Neuroprotective and CNS Effects, Antioxidant and Anti-inflammatory activity
33	N-Feruloyltyramine	Phenolic amide	12.521	313.1306	Anti-hypertensive and Cardioprotective Effects, Antioxidant and Anti- inflammatory activity, Potential neuroprotective

(Contd...)

Table 7: *(Continued)*

S. No.	Name of compound	Category	RT	Mass	Activity
34	Acetylcaranine	Aporphine alkaloids	14.213	313.1306	Antioxidant and Anti- inflammatory activity, Euroactive, Cardiovascular effects
35	Kresoxim-methyl	Fungicides	12.521	313.1306	Antifungal activity, Crop protection
36	Dasytrichone	Flavone	12.521	296.1041	Antioxidant and Anti- inflammatory, antimicrobial
37	Calophyllin B	Xanthons	12.521	296.1041	Antioxidant and Anti- inflammatory, Antimicrobial
38	Mesembrinol	Alkaloid	291.18	291.1829	Mood-enhancing and Anxiolytic properties, Antioxidant, and Anti-inflammatory
39	Lyconnotine	Diterpenoid alkaloids	291.18	291.1829	Neurotoxicity, Cardiotoxicity, Analgesic

LC-MS: Liquid chromatography–Mass spectrometry, CNS: Central nervous system, RT: Retention time

Table 8: LC-MS analysis of the negative mode of the ethanolic extract.

S. No.	Name of component	category	Molar mass	RT	Activity
1	Decarbamoylsaxitoxin	Alkaloids	256.1285		Neurotoxins
2	Mangostenone B	Xanthons	462.2052	16.684	Anticancer, Antimicrobial, Antioxidant, anti-inflammatory
3	4-Methoxycinnamoyloleanolic acid methyl ester	Triterpenoid ester	630.4275	25.895	Anticancer, Antioxidant, Anti-inflammatory, Hepatoprotective
4	Decarbamoylsaxitoxin	Alkaloids	256.1285	12.971	Neurotoxins
5	Tyromycic acid	Triterpenoids	452.3307	14.402	Antitumor, Antimicrobial, Antioxidant, Anti-inflammatory
6	3-Oxo-12,18-ursadien-28-oic acid	Triterpenoids	452.3307	14.402	Antitumor, Antimicrobial, Aphotochentioxidant, Anti-inflammatory, Hepatoprotective
7	Mangostenone B	Xanthons	462.2052	16.684	Anticancer, Antimicrobial, Antioxidant, Anti-inflammatory, antifungal, Neuroprotective
8	DU 122290	Pyrroles	362.1667	21.633	Antipsychotics
9	Mutatoxanthin	Xanthophyll Carotenoid	584.4221	25.895	Antioxidant, Anti-inflammatory
10	Prenigroxanthin	Xanthophyll Carotenoid	584.4221	25.895	Antioxidant, Photoprotective
11	Triphasiaxanthin	Xanthophyll Carotenoid	584.4221	25.895	Antioxidant, Photoprotective
12	Antheraxanthin A	Xanthophyll Carotenoid	584.4221	25.895	Antioxidant, Photoprotective, Epoxide reactivity
13	(3S,3'R,4xi)-beta, beta-Carotene-3,3',4-triol	Carotenoid	584.4221	25.895	Antioxidant, Photoprotective
14	Cryptoxanthin 5,6:5',8'-diepoxide	Carotenoid	584.4221	25.895	Antioxidant, Photoprotective, Epoxide reactivity, Vitamin A precursor
15	Cryptochrome	Flavoproteins	584.4221	25.895	Photoreceptor in plants
16	Antheraxanthin	Xanthophyll Carotenoid	584.4221	25.895	Photoprotection, Nonphotochemical quenching, Light adaptation
17	Capsanthin	Xanthophyll Carotenoid	584.4221	25.895	Antioxidant, Photoprotective, anticancer, Anti-inflammatory
18	Flavoxanthin	Xanthophyll Carotenoid	584.4221	25.895	Antioxidant, Photoprotective, anticancer, Anti-inflammatory
19	Myxol	Carotenoid	584.4221	25.895	Antioxidant, Photoprotective, and Health benefits

LC-MS: Liquid chromatography–Mass spectrometry, RT: Retention time

± 0.003 in aqueous extract, while in ethanolic extract it was 23.39 ± 0.04 to 77.55 ± 0.004 [Table 2 and Figure 1], from which the standard ascorbic acid shows the highest % of inhibition at 100 µg/mL, which was 82.05 ± 0.205. As compared with the standard ethanolic extract, it shows the highest % of inhibition, which was 77.55 ± 0.004, and in the aqueous extract, it was found to be 69.17 ± 0.003, from which it was concluded that the ethanolic extract shows the highest % of inhibition.

5.7. FRAP Assay

The FRAP test was used to measure the antioxidant activity of *A. reticulata's* ethanolic and aqueous extracts [Figure 3]. Regular R2 calibration curve values (0.998) were used to calculate FRAP estimates (ascorbic acid). Fe^{2+} equivalent values in mg/g of plant material. Aqueous extract of *A. reticulata* leaves has a high ferric reduction capacity of 78.06202 ± 0.004, and ethanolic extract has a maximum ferric reduction

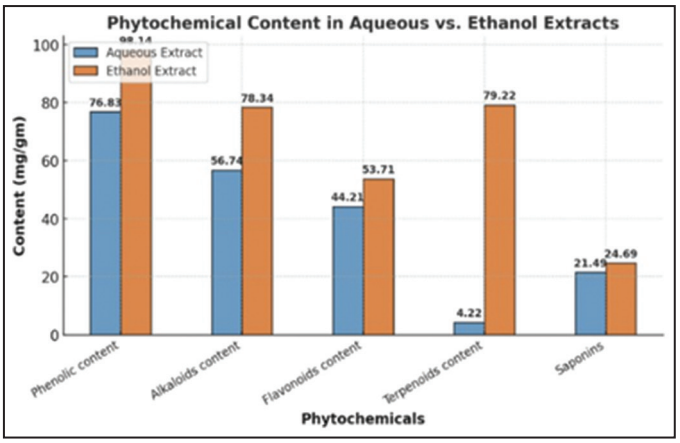

Figure 1: Quantitative phytochemical screening.

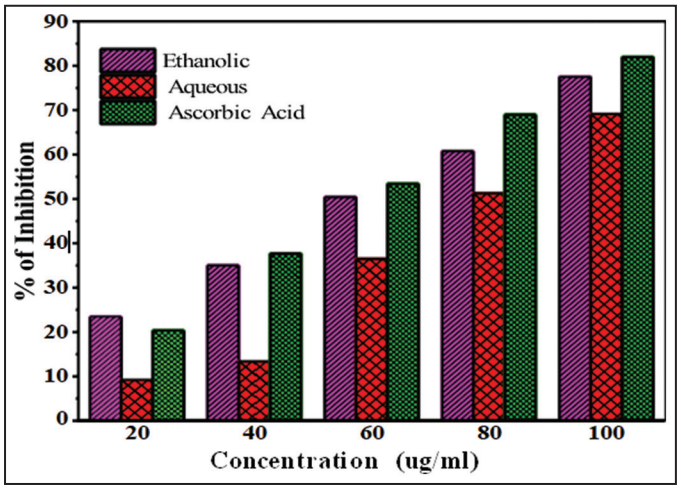

Figure 2: 2,2 diphenyl-1-picrylhydrazyl scavenging activity of *Annona reticulate*.

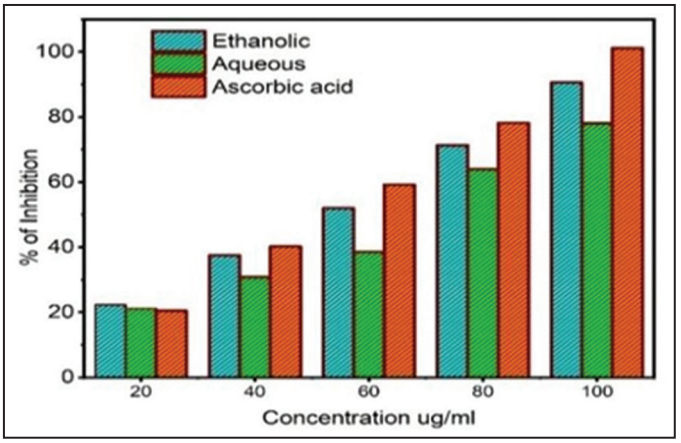

Figure 3: Ferric reducing antioxidant power assay of *Annona reticulata*

capacity of 90.5814 ± 0.004. By comparing with the standard, the highest FRAP was found to be in the ethanolic extract of *A. reticulata* leaves.

5.8. LC-MS Analysis

LC-MS analysis of aqueous and ethanolic extracts of *A. reticulata* leaves revealed the diversity of compounds such as alkaloids, phenolic compounds, saponins, terpenoids, and flavonoids (as shown in Tables 5,6 for aqueous extract and Tables 7,8 for ethanolic extract). Other important components are polyketides, fungicides, esters, lipids, glycosides, organophosphates, dipeptides, herbicides, lactones, antibiotics, etc., which show different biological properties. They have different molar masses and different retention times.

6. CONCLUSION

The present study it is concludes that the aqueous and ethanolic extract of leaves of *A. reticulata* contains a variety of phytoconstituents such as polyphenols, alkaloids, flavonoids, saponins, terpenoids, and glycosides. After that, quantitative phytochemical analysis of the plant extract was done. From that, it is concluded that phytoconstituents are found more in the ethanolic extract compared to the aqueous extract. LC-MS analysis indicates the presence of phytoconstituents responsible for antioxidant properties, as well as some other essential compounds such as xanthones, fungicides, herbicides, and xanthophylls. Which are responsible for various biological activities? By antioxidant assays such as DPPH assay and FRAP assay for the antioxidant activity of the plant extract. As shown in Tables 3 and 4, it is observed that aqueous extracts show 69.71 ± 0.003 and in ethanol, 77.05 ± 0.004 in the DPPH assay. While in FRAP, the ethanolic extract was found to be 90.5814 ± 0.004, in the aqueous extract, it was 78.0620 ± 0.004; from that, it was concluded that the ethanolic extract has more antioxidant activity than the aqueous extract.

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