

Evaluation of Antioxidant and Cytotoxic Potentials against the EAC Cell Line of Aqueous Extract of *Annona reticulata* Linn. Leaves

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Recent studies have focused on the pharmacological potentials of natural compounds as potent medicinal agents. *Annona reticulata* Linn. (Family: *Annonaceae*) has long been used as a remedy for a wide range of ailments by many tribes and ethnic groups worldwide. According to reports, the plant has a variety of medicinal properties, such as anthelmintic, antipyretic, antihyperglycemic, antiulcer, and antinociceptive activities. In addition to evaluating the plant's antioxidant, cytotoxic, and anti-cancer potentials, the current study used GC-MS analysis to identify the compounds causing these effects. Six established methodologies were utilized to evaluate the antioxidant potential. Good antioxidant properties were demonstrated by the extract in DPPH and nitric oxide radical scavenging tests, with IC₅₀ measurements of 96.15 µg/ml and 124.85 µg/ml. In both DPPH and nitric oxide radical scavenging tests ascorbic acid was used as standard; the observed IC₅₀ values for ascorbic acid in these two tests were 14.38 µg/ml and 67.31 µg/ml. In reducing power assay, the extract's antioxidant activity increased with increasing concentration. 136.4 ± 6.35 mg/gm Ascorbic acid equivalents was the total antioxidant capacity value that was determined. The aqueous extract demonstrated total flavonoid content value of 89.15 ± 0.4 mg/gm Quercetin equivalents and total phenol content value of 61.48 ± 0.17 mg/gm Gallic acid equivalents. Good cytotoxic potential was demonstrated by the extract in the brine shrimp lethality test. This test yielded LC₅₀ value of 93.37 µg/ml for the aqueous leaf fraction and 1.63 µg/ml for standard vincristine sulphate; while the obtained LC₉₀ values for the aqueous leaf extract and standard vincristine sulphate were 516.65 µg/ml and 7.17 µg/ml. Additionally, the plant extract exhibited notable anti-cancer potential against the EAC cell line in in-vivo study. The groups of mice treated with 400 mg/kg dose of aqueous leaf fraction and 20 mg/kg dose of 5-FU demonstrated tumour weight and EAC cell number values of 3.00 ± 0.38 g and 7.40 ± 0.81; 5.08 ± 0.35 g and 9.20 ± 0.86, respectively. Therefore, the values obtained from aqueous extract were less than those of the standard medication 5-FU.

Keywords: Antioxidant effect, Anti-cancer activity, Bullock's heart, Cytotoxic effect, Extract, Leaves, Tumor.

It is well known that oxidative stress is one of the main causes of a large number of degenerative and chronic illnesses. Antioxidants are the most efficient method of removing free radicals, which are the source of oxidative stress. By scavenging free radicals or encouraging their breakdown, antioxidants—natural or synthetic—can effectively inhibit the development of such illnesses. The use of herbal resources as natural antioxidants is currently gaining popularity.¹

Cancer, which is a collective term for a group of malignant illnesses, remains a major global health concern. The etiology of cancer involves varying degrees of control. Normal cells undergo genetic and epigenetic modifications during this process, which leads to unchecked cell division and, ultimately, cancer. It is also recognised that reactive oxygen species (ROS) can cause DNA mutations, immune system evasion, the tumour microenvironment, metastasis, and angiogenesis, all of which can aid in the development of cancer. The ability of cancer cells to avoid dying due to unidentified mutations is one of their main problems; this causes a buildup of cells that then move to other parts of the body.²

Clinically significant anticancer chemicals are known to be produced by plants. Plants were used to make several of the chemotherapeutic medications that are currently in use.³ Numerous methods are employed to identify plants that might harbour novel physiologically active chemotherapeutic chemicals. One method in use is the ethnomedical data approach, which bases the choice of a plant on what is currently known about the plant's traditional medicinal usage.³

Furthermore, GC-MS analysis is frequently conducted on plant extracts to identify and quantify volatile and semi-volatile organic components. A thorough chemical profile of the plant is obtained by comparing its chemical profile with an existing library of compounds. It facilitates the identification of plant constituents and the comprehension of the biological roles played by the substances derived from plants.⁴⁻⁵

The current study focused on *A. reticulata*. because numerous studies have indicated that this herbal medicinal plant has cytotoxic, anti-inflammatory, antioxidant, and anti-cancer properties. Over 100 species make up the

genus *Annona*, and *A. reticulata* belongs to the *Annonaceae* family. *A. reticulata* is said to have originated naturally in West Indies, South and Central America. Known by most as the custard apple, *A. reticulata* is a tiny tree. On average, it reaches a height of 5 to 10 meters. Spreading crown, deciduous, oblong or narrow-lanceolate leaves, and a trunk that can be 25–35 cm thick are the tree's distinguishing features.⁶ Also known as Bullock's heart, numerous illnesses have been treated using this traditional plant, such as epilepsy, bacterial infections, worm infestations, fever, ulcers, haemorrhage, dysuria, digestive issues, and more. Indians, Filipinos, and people from a few other countries have also utilised this plant as an anti-inflammatory, anti-stress, and anti-helminthic remedy.⁷ Therefore, decision was taken to look into the antioxidant, cytotoxic, and anti-cancer properties of this traditional herb, *A. reticulata*.

MATERIALS AND METHODS

Preparation of plant extract

A. reticulata leaves were gathered from Savar, Dhaka, Bangladesh. In order to prepare it for grinding, the plant material (leaves) was sun-dried separately. After that, drying was conducted in Gallenkamp (Size 1) oven at lower than 50°C temperature. Grinding mill was used to convert the plant components into coarse powders. Dark-colored flasks containing 538 grams of processed dry plant material were sealed with 10.76 liters of water (solvent). Then the container was kept at room temperature. 24 hours later, Whatman No. 1 filter paper was used to filter the infusions. Then, equivalent amount of solvent (water) was utilized to further extract any leftover residue. The procedure was repeated 48 hours later. Using a rotary evaporator set to 40°C, the combined supernatants were vacuum-dried. The extraction process was considered complete when it was determined that the plant materials were depleted. After extraction, the plant material was dried off and submerged in one liter of deionized water. The plant parts were kept in a sealed container filled with water and shaken and stirred occasionally for a period of seven days. A fresh cotton bed filter was used for each extract. A sticky crude extract concentration was obtained by drying the filtrates

at a temperature of $40 \pm 2^\circ\text{C}$. After being extracted, the materials were labeled appropriately and stored at 4°C in sterile sample containers.⁸

GC-MS Analysis of Aqueous Extract from *A. reticulata* Leaves

The Shimadzu GCMS-TQ8040 was used to conduct the GC-MS analysis. As the carrier gas, helium was employed. With hold times of 1, 2, and 7 minutes, respectively, the column oven was initially set at temperature of 50°C , then increased to 200°C and finally reached at 300°C , in accordance with the GC settings. At 250°C , the injection was performed in splitless mode, with a sampling duration of one minute, a flow control mode set to pressure at 53.5 kPa, and a total flow of 11 ml/min. Under MS circumstances, the temperature of the interface was 250°C , whereas the temperature of the ion source was 230°C . By comparing with library compounds, this approach provides excellent sensitivity and specificity for identifying and quantifying volatile and semi-volatile compounds in complicated plant extracts.

In-vitro Studies

In-vitro Evaluation of Antioxidant Activity

DPPH Radical Scavenging Assay

Serial dilution of stock solution was conducted to obtain different concentrations (400 $\mu\text{g/ml}$, 200 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$). 1 ml of each concentration, appropriately labelled, was contained in test tube. Addition of 2 ml of DPPH solution was done for reaching volume of 3 ml. Incubation (25°C) for 30 min was then conducted. Next, absorbance of diluted extract solution in the solvent was done at 517 nm. Percentage of scavenging activity and IC_{50} were measured.⁹⁻¹⁰

Nitric Oxide Scavenging Assay

Test tubes were filled with 4.0 ml of test fraction and the standard (ascorbic acid) in varying concentrations (400 $\mu\text{g/ml}$, 200 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$). The test tubes received 1.0 ml of a Sodium nitroprusside (5mM solution added to them). To finish the reaction, the test tubes were incubated at 30°C for two hours. After taking 2.0 ml of the solution out of the mixture, 1.2 ml of Griess reagent was added. Next, a spectrophotometer was used to assess the solutions' absorbance at 550 nm in comparison to a blank. The blank was distilled water.¹¹

Reducing Power Assay

2.0 ml of standard solutions of various concentrations or plant extract was taken in different test tubes. Then, 2.5 ml potassium ferricyanide solution (1%) was added. After that, incubation for 10 minutes at 50°C was conducted and then filled with 2.5 ml of trichloroacetic acid solution (10%). Centrifugation for 10 minutes at 3000 rpm of the mixture was performed. After mixing with 2.5 ml of distilled water, extraction of 2.5 ml of the supernatant solution was done. Then 0.5 ml of ferric chloride solution (0.1%) was added. The absorbance of the solutions was measured at 700 nm using a spectrophotometer. The absorbance of the blank solution at 700 nm was measured in relation to the solvent that was used to make it. Greater reducing power was demonstrated by the reaction mixture's increased absorbance.

Determination of Total Antioxidant Capacity

Test fraction or standard (300 μl) in various strengths was combined with reagent solution (3 ml). Incubation (95°C) for 90 minutes was conducted. Absorbance of the solution was measured at 695 nm against blank. Antioxidant activity was measured and expressed in amount of ascorbic acid equivalents.¹²

Determination of Total Phenolics Content

Gallic acid solutions at various concentrations or plant extract (200 $\mu\text{g/ml}$) both in volume of 1.0 ml were taken and kept in test tubes. Folin-Ciocalteu reagent solution (5 ml) that was diluted 10 times was added. After the addition of 4 ml sodium carbonate solution (7.5%), the test tubes were well mixed. The reaction was completed by incubating test tubes containing extract solutions for one hour at the same temperature (20°C). The solutions' absorbance was measured at 765 nm by using spectrophotometer. A standard blank solution contained ethanol, which dissolved the plant extract.¹³⁻¹⁴ Utilizing standard formula; the total phenolic contents of the plant fraction was measured and expressed as gallic acid equivalents (GAE).¹⁶

Determination of Total Flavonoids Content

One ml of reference solution in various concentrations or plant extract (200 $\mu\text{g/ml}$) was taken in test tubes. 3 ml of chloroform was placed in test tubes. Then, eventually added aluminium chloride solution (200 μl), potassium acetate

solution (200 μ l) and purified water (5.6 ml). Then for completion of the reaction 30 minutes incubation period at room temperature was carried out. Utilizing a spectrophotometer the absorbance was measured at 415 nm and compared to a blank. Chloroform served as the blank.¹⁵⁻¹⁷ To calculate total amount of flavonoid components standard formula was used and represented in quercetin equivalents.

In-vitro Evaluation of Cytotoxic Potential Brine Shrimp Lethality Bioassay for Assessing Cytotoxic Activity

38 gm of iodine-free sea salt was dissolved in 1 liter distilled water and the mixture was filtered for creation of transparent seawater solution. *Artemiasalina* leach served as the test organism. Seawater was then poured in small tank and its one side was covered after shrimp eggs were added. It took two days for the shrimps to hatch and become nauplii. During the whole hatching stage, oxygen was continuously supplied. Due to hatching shrimp's phototaxis (attraction to light), nauplii free of egg shells were gathered from the tank's lit section. To enhance vision, the nauplii were taken out of the fish tank and diluted with fresh, clear seawater using a pipette. Ten nauplii were then gently removed using a micropipette. The weight of each test sample was 32 mg. It was dissolved in 200 μ l of pure DMSO and then mixed with 20 ml of saltwater. Therefore, 1600 μ g/ml was the stock solution's concentration. By performing serial dilution with sea water the concentration was converted to 800, 400, 200, 100, 50, 25, 12.5, and 6.25 μ g/ml. Vincristine sulphate was used in this experiment. Vincristine was tested at extremely low concentrations (10, 5, 1, 0.5, 0.25, 0.125, and 0.06 μ g/ml) because of its high cytotoxicity. Three pre-marked test tubes containing 4.95 ml of simulated sea water and ten shrimp nauplii intended to serve as control groups were each filled with 50 μ l of DMSO. Magnifying glass was used to examine the test tubes 24 hours later. Then, quantity of survivors was computed.

Microsoft Excel was utilized to analyse concentration-mortality data and calculation of the proportion of mortality was carried out. LC₅₀ measurement demonstrates the efficacy of plant products. Plotting the percentage mortality against the corresponding log of concentration, the linear regression technique estimates the chemical

concentration at which 50% of test participants die after a specified exposure time. Additionally, the LC₉₀ values for each fraction and standard cytotoxic drug vincristine sulphate were also calculated similarly.¹⁸⁻²⁰

In-vivo Assessment

Experimental Animals

In this investigation, Swiss albino mice having weight of 25–30 g were employed. The mice were taken from Jahangirnagar University's pharmacy department's animal home. Pharmacological studies were conducted on *A. reticulata* leaf extracts in aqueous form at the Jahangirnagar University's Pharmacology Laboratory. The animals were housed in polypropylene cages with temperatures of 25 \pm 2°C, a suitable dark-light cycle, and relative humidity (RH 55 \pm 5%) in regular laboratory settings. The mice were fed pelletised mouse feed from ICDDR, B., and they had unrestricted access to water. Every action that involved handling animals was carried out by following the rules set forth by Jahangirnagar University's animal ethics committee [Ref No: BBEC, JU/M2024/11 (137)].

Assessment of In-vivo Anti-cancer Property against Ehrlich's Ascites Carcinoma (EAC) Cell Line in Mice

Collection of Tumor Cells

For transplantation, Ehrlich Ascites Carcinoma (EAC) cells were collected from Jahangirnagar University in Savar, Dhaka. To keep EAC cells alive, Swiss albino mice were given intraperitoneal transplants of 2 \times 10⁶ EAC cells every 10 days.

Transplantation of Tumor Cells

The initial step was to use ketamine hydrochloride to make the cancerous mice unconscious. After that, the ascitic fluid was collected using a 1 ml syringe. Following two dilutions with 0.9% NaCl saline solution and a final dilution with phosphate buffer solution, it was ready for cell counting under an electron microscope. After that, tumour cells were injected intraperitoneally into the mice in each group. Each mouse received 0.5 ml of treated ascitic fluid containing 2 \times 10⁶ tumour cells intraperitoneally.²¹⁻²³

Treatment Protocol

Four groups of five mice each were created from the animals. EAC cells were transplanted intraperitoneally into each mice

group. This day of implantation was considered as the first day. Treatment started from the next day. Each group received the following treatments in due order: negative control (distilled water, orally), positive control (5-Fluorouracil, 20 mg/kg, intraperitoneally), two doses of aqueous extract (200 and 400 mg/kg, by oral route). Treatment was continued for the following fourteen days. Over the course of the treatment, the mice's body weight was closely observed.²²

Evaluation of Anti-cancer Activity

When the treatment period was over, mice were undergone through overnight fasting and dissected the following day by cervical dislocation. The extraction of ascitic fluid was conducted from peritoneal cavity and blood was collected via the retro-orbital route. Several anti-tumor parameters were meticulously documented, along with some hematological and biochemical markers.²³⁻²⁴

Anti-cancer Parameters

Tumor weight

To ascertain the tumour weight, mice were weighed both prior to and following the ascitic fluid collection.²³

Tumor Cell Count

From the mice's peritoneal cavity, the ascitic fluid was extracted. The fluid was then diluted 100 times using phosphate buffer solution, and a WBC pipette was used to remove the diluted fluid. In order to determine the number of cells,

placement of droplet of cell solution (diluted) on Neubauer's counting chamber was conducted.²⁵ For counting the cells, the formula mentioned below was utilized:

$$\text{Cells}/\mu\text{L} = [(N_c \times D_c) \div (N_s \times V_s)]$$

Here, N_c = Number of cells

D_c = Correction for dilution

N_s = Number of squares counted

V_s = Volume of one square

Hematological Parameters

The blood drawn from each mouse was used to estimate the number of red blood cells (RBC), white blood cells (WBC), and platelets and haemoglobin (Hb%).

Biochemical Parameters

Using the technique outlined by Kumar *et al.* (2015), the measurement of serum biochemical markers like alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin and creatinine, were done. Every analysis was conducted using commercially available kits.

The SPSS 27.0 software was used to examine the observed data from in-vivo investigations. Using Mean \pm SEM, the experiment's results were displayed. Utilising ANOVA, statistical significance was assessed and contrasted with the control at various P value thresholds (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

GC-MS Analysis Report of Aqueous Extract of *A. reticulata* Leaves

GC-MS Analysis Report of Aqueous Extract of *A. reticulata* Leaves

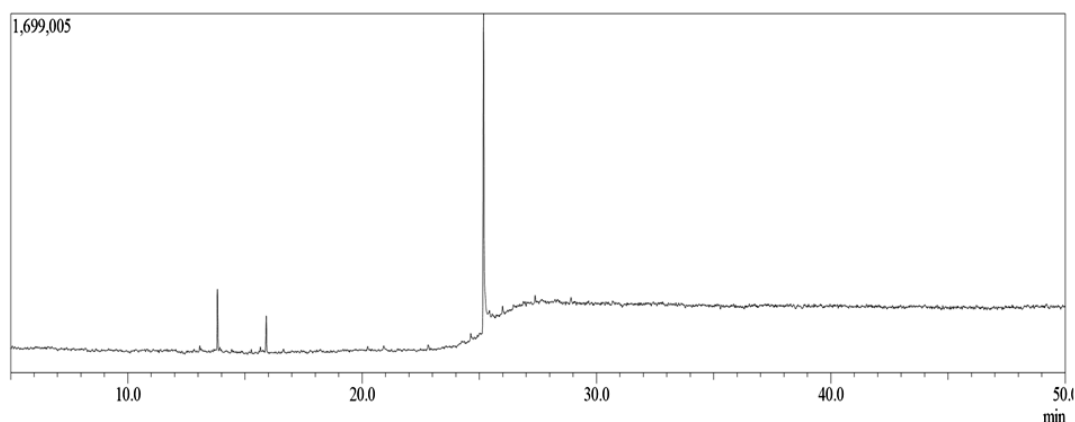


Fig. 1. Total ionic chromatogram of *A. reticulata* crude Aqueous extract

Table 1. Constituents detected by GC-MS analysis in *A. reticulata*'s crude aqueous extract

Serial No.	Compound Name	Molecular Formula & Molecular Weight	Area
1	Cyclopropylcarbinol	C ₄ H ₈ O; 72.10 g/mol	17830
2	Thiophene-3-ol, tetrahydro-, 1,1-dioxide	C ₄ H ₈ O ₃ S; 136.17 g/mol	17830
3	Octodrine	C ₈ H ₁₉ N; 129.24 g/mol	17830
4	1-(5-Bicyclo[2.2.1]heptyl)ethylamine	C ₉ H ₁₉ N; 141.25 g/mol	17830
5	1-Methyldecylamine	C ₁₁ H ₂₅ N; 171.32 g/mol	17830
6	2-Heptanamine, 5-methyl-	C ₈ H ₁₉ N; 129.23 g/mol	17830
7	Benzeneethanamine, 2,5-difluoro-.beta.,3,4-trihydroxy-	C ₈ H ₁₂ F ₂ NO ₃ ; 208.18 g/mol	17830
8	Cystine	C ₆ H ₁₂ N ₂ O ₄ S ₂ ; 240.32 g/mol	17830
9	Acetic acid, [(aminocarbonyl)amino]oxo-	C ₃ H ₆ N ₂ O ₃ ; 118.10 g/mol	17830
10	4-Fluorohistamine	C ₅ H ₈ FN ₃ ; 129.14 g/mol	17830
11	Cyclobutanol	C ₄ H ₈ O; 72.10 g/mol	9329
12	1-Methyldecylamine	C ₁₁ H ₂₅ N; 171.32 g/mol	9329
13	Pentanal	C ₅ H ₁₀ O; 86.13 g/mol	9329
14	D-Alanine	C ₃ H ₇ NO ₂ ; 89.10 g/mol	9329
15	Eicosanoic acid, methyl ester	C ₂₁ H ₄₂ O ₂ ; 326.55 g/mol	56805
16	Triacontanoic acid, methyl ester	C ₃₁ H ₆₂ O ₂ ; 466.81 g/mol	56805
17	Docosanoic acid, methyl ester	C ₂₃ H ₄₆ O ₂ ; 354.60 g/mol	56805
18	Tridecanoic acid, 12-methyl-, methyl ester	C ₁₅ H ₃₀ O ₂ ; 242.39 g/mol	56805
19	Octadecanoic acid, 17-methyl-, methyl ester	C ₂₀ H ₄₀ O ₂ ; 312.52 g/mol	56805
20	1,2-Ethanediamine, N-(2-aminoethyl)-	C ₄ H ₁₃ N ₃ ; 103.17 g/mol	15238
21	2-Methylaminomethyl-1,3-dioxolane	C ₅ H ₁₁ NO ₂ ; 117.15 g/mol	15238
22	Alanine	C ₃ H ₇ NO ₂ ; 89.10 g/mol	15238
23	1,2-Ethanediamine, N-methyl-	C ₃ H ₁₀ N ₂ ; 74.13 g/mol	15238
24	Propanamide	C ₃ H ₇ NO; 73.10 g/mol	22359
25	2-Propanamine, 1-methoxy-	C ₄ H ₁₁ NO; 89.14 g/mol	22359
26	dl-Alanine	C ₃ H ₇ NO ₂ ; 89.10 g/mol	22359
27	Hydroxyurea	CH ₄ N ₂ O ₂ ; 76.06 g/mol	22359
28	1-Propanol, 2-amino-, (+/-)-	C ₃ H ₉ NO; 75.11 g/mol	22359
29	2-Hexanamine, 4-methyl-	C ₇ H ₁₇ N; 115.22 g/mol	22359
30	Ethanol, 2-(methylamino)-	C ₃ H ₉ NO; 75.11 g/mol	10141
31	1,3-Dioxolane-4-methanol	C ₄ H ₈ O ₃ ; 104.10 g/mol	5571
32	N-dl-Alanylglycine	C ₅ H ₁₀ N ₂ O ₃ ; 146.15 g/mol	27186
33	1-Octadecanamine, N-methyl-	C ₁₉ H ₄₁ N; 283.53 g/mol	27186
34	dl-Alanyl-dl-norleucine	C ₉ H ₁₈ N ₂ O ₃ ; 202.25 g/mol	27186
35	(S)-(+)-1-Cyclohexylethylamine	C ₈ H ₁₇ N; 127.23 g/mol	27186
36	1-Methyldecylamine	C ₁₁ H ₂₅ N; 171.32 g/mol	27186
37	Benzeneethanamine, 2-fluoro-.beta.,3-dihydroxy-	C ₈ H ₁₀ FNO; 155.17 g/mol	27186
38	Propanamide, N-methyl-2-amino-	C ₄ H ₉ N ₂ O; 101.13 g/mol	5151
39	2-Methylaminomethyl-1,3-dioxolane	C ₅ H ₁₂ NO ₂ ; 118.16 g/mol	33954
40	Formamide, N,N-dimethyl-	C ₃ H ₇ NO; 73.10 g/mol	17803
41	Propanediamide, 2-amino-	C ₃ H ₁₀ N ₃ ; 88.14 g/mol	17803
42	L-Alanine, TMS derivative	C ₉ H ₂₅ NO ₂ Si ₂ ; 235.48 g/mol	17803
43	1,3-Dioxolane-4-methanol	C ₄ H ₈ O ₃ ; 104.10 g/mol	17803
44	2-(Methylamino)ethanol, O-trimethylsilyl	C ₆ H ₁₈ NOSi; 148.30 g/mol	17803
45	13-Docosenamide, (Z)-	C ₂₂ H ₄₃ NO; 337.57 g/mol	506528
46	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsilyl)trisiloxane	C ₁₁ H ₃₀ O ₃ Si ₄ ; 322.71 g/mol	6749
47	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis(trimethylsiloxy)	C ₁₂ H ₃₆ O ₄ Si ₅ ; 384.86 g/mol	6749

48	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13	$C_{16}H_{48}O_6Si_7$;533.17 g/mol	6749
49	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl	$C_{13}H_{24}OSi_2$;252.50 g/mol	6749
50	Hexestrol, 2TMS derivative	$C_{24}H_{40}O_2Si_2$;416.74 g/mol	6749
51	3-Butoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsilyl)trisiloxane	$C_{13}H_{36}O_3Si_4$;352.78 g/mol	6749
52	Methyltris(trimethylsiloxy)silane	$C_{10}H_{30}O_3Si_4$;310.70 g/mol	6749
53	1-Isopropoxy-5-propyl-2,3-bis(trimethylsilyl)benzene	$C_{18}H_{38}OSi_2$;326.66 g/mol	6749
54	Thymol, TMS derivative	$C_{13}H_{22}OSi$;222.40 g/mol	6749
55	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenyl]	$C_{13}H_{24}Si$;208.41 g/mol	6749
56	Trimethylsilyl-di(trimethylsiloxy)-silane	$C_9H_{27}O_2Si_4$;279.67 g/mol	21459
57	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsilyl)trisiloxane	$C_{12}H_{34}O_3Si_4$;338.75 g/mol	21459
58	1,4-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$;222.48 g/mol	21459
59	Ethyl homovanillate, TMS derivative	$C_{13}H_{20}O_4Si$;268.38 g/mol	21459
60	1,2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$;222.48 g/mol	21459
61	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsilyl)trisiloxane	$C_{11}H_{32}O_3Si_4$;324.73 g/mol	29787
62	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]	$C_{12}H_{36}O_4Si_5$;384.86 g/mol	29787
63	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenyl]	$C_{13}H_{24}Si$;208.41 g/mol	29787
64	1-Isopropoxy-5-propyl-2,3-bis(trimethylsilyl)benzene	$C_{18}H_{35}OSi_2$;323.64 g/mol	29787
65	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsilyl)trisiloxane	$C_{12}H_{34}O_3Si_4$;338.75 g/mol	13013
66	Methyltris(trimethylsiloxy)silane	$C_{10}H_{30}O_3Si_4$;310.70 g/mol	13013
67	4-Hydroxyphenyllactic acid, ethyl ester, di-TM	$C_{17}H_{28}O_4Si_2$;352.57 g/mol	20582
68	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$;310.70 g/mol	20582
69	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	$C_{14}H_{24}O_3Si_2$;296.51 g/mol	20582
70	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodeca-	$C_{12}H_{36}O_5Si_6$; 428.95 g/mol	18997
71	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxaheptadecane	$C_{17}H_{36}O_6$;336.46 g/mol	18997
72	(R)-(-)-Phenylephrine, bis(trimethylsilyl) ether	$C_{15}H_{29}NO_2Si_2$;311.57 g/mol	22043
73	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$;296.59 g/mol	22043
74	3-Methylsalicylic acid, 2TMS derivative	$C_{14}H_{24}O_3Si_2$;296.51 g/mol	22043
75	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-octamethyl-	$C_{16}H_{48}O_7Si_8$;577.26 g/mol	25366
76	Methyltris(trimethylsiloxy)silane	$C_{10}H_{30}O_3Si_4$;310.70 g/mol	25811
77	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$;310.70 g/mol	25811

RESULTS

The library search report indicated that the crude aqueous extract of *A. reticulata* leaves included 77 components.

Antioxidant Activity

DPPH Radical Scavenging Assay

IC₅₀ value obtained for aqueous fraction from *A. reticulata* leaves was 96.15 µg/ml; while

for ascorbic acid observed IC₅₀ value was 14.38 µg/ml. The aqueous leaf fraction was found to have a notable level of DPPH radical scavenging function. Aqueous leaf fraction demonstrated dose-dependent DPPH radical scavenging as ascorbic acid (standard) (Figure 2).

Nitric Oxide (NO) Radical Scavenging Assay

Aqueous leaf fraction showed good scavenging (observed IC₅₀ value 124.85 µg/ml) in

comparison to ascorbic acid, the standard used in this investigation (obtained IC_{50} value 67.31 $\mu\text{g/ml}$). Test fraction demonstrated dose-dependent NO scavenging, as ascorbic acid (standard) (Figure 3).

Reducing Power Assay

The conversion of ferric to ferrous state indicates the reduction activity, which is obtained from the creation of complex having Perl's Prussian blue colour, was measured spectrophotometrically

to evaluate the extracts' reducing power. The fraction showed good reducing power. As the extract concentration rose, it was discovered that the reduction power also rose (Figure 4).

Total Antioxidant Capacity

Phosphomolybdenum method was utilized to determine total antioxidant capacity of test fraction, and represented in ascorbic acid equivalents. Total antioxidant capacity was ascertained by utilizing ascorbic acid standard

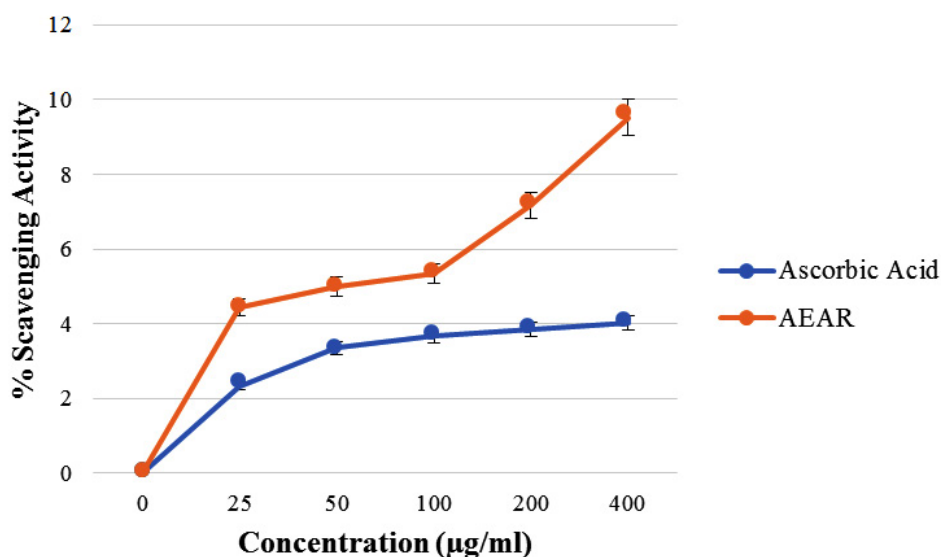


Fig. 2. DPPH radical scavenging activity of the aqueous extract of *A. reticulata* leaves

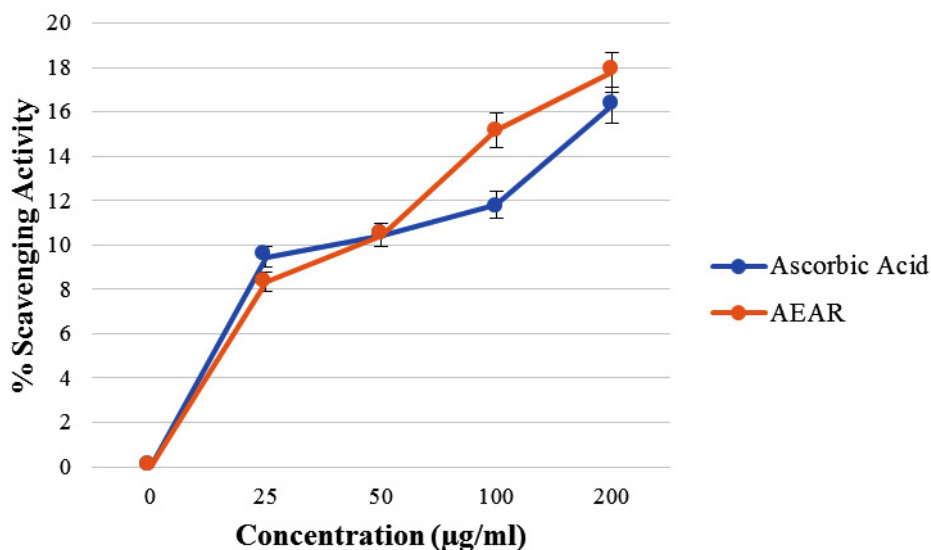


Fig. 3. Nitric oxide (NO) radical scavenging activity of aqueous extract of *A. reticulata* leaves.

curve ($y=0.0182x+0.4455$; $R^2= 0.9784$) (Figure 5). Total antioxidant capacity obtained for aqueous leaf fraction was 136.4 ± 6.35 mg/gm, ascorbic acid equivalents.

Determination of Total Phenolics Content

Gallic acid equivalents (GAE) per gram of fraction were used to express the total phenolic content of the aqueous extract of *A.*

reticulata leaves, which was determined using the Folin-Ciocalteu reagent. Utilizing gallic acid standard curve, the total phenolic contents of the test extract was determined ($y=0.0194x+0.1148$; $R^2=0.9997$) (Figure 6). In the aqueous extract of *A. reticulata* leaves, phenols were found to be plentiful, with a numerical value of 61.48 ± 0.17 mg/gm, Gallic acid equivalents.

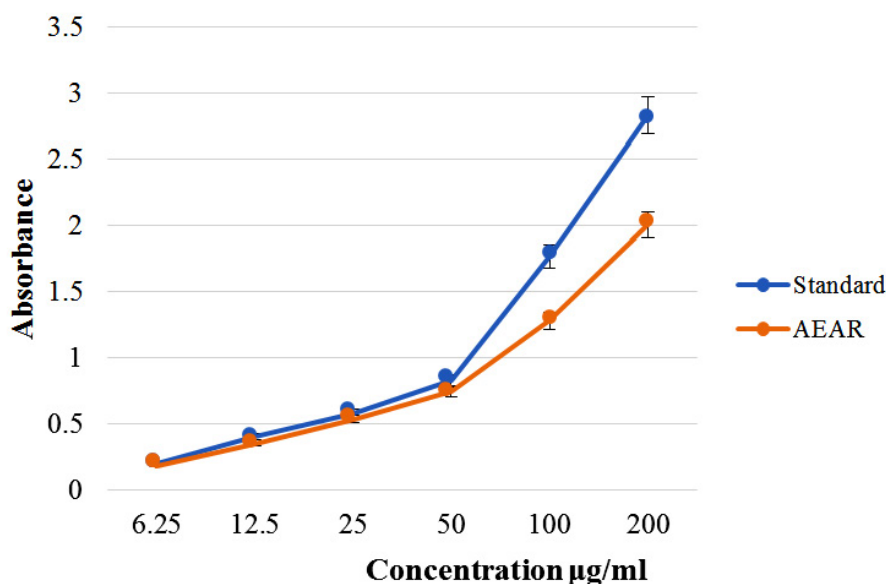


Fig. 4. Reducing power of the aqueous extract of *A. reticulata* leaves

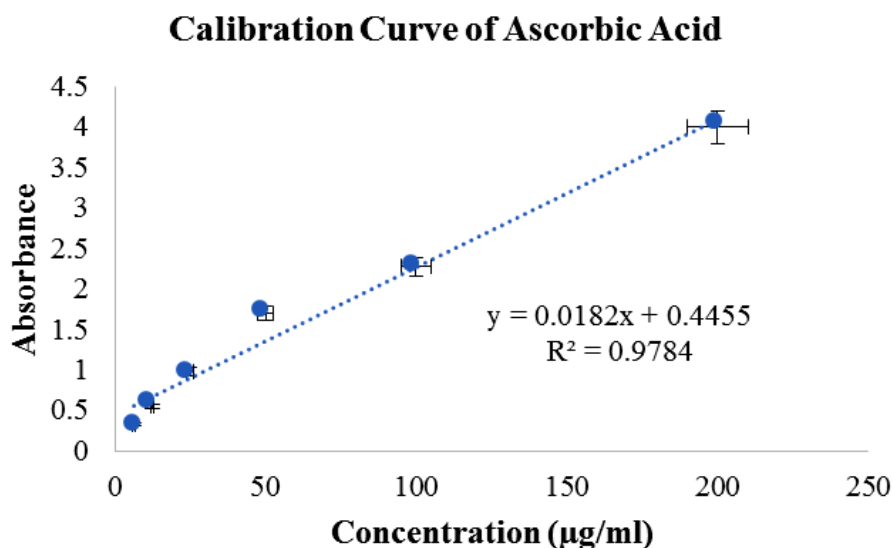


Fig. 5. Calibration Curve of Ascorbic Acid

Determination of Total Flavonoid Content

A. reticulata aqueous leaf fraction was tested for total flavonoid content using the aluminium chloride colorimetric technique. Quercetin standard curve ($y=0.019x+0.0683$; $R^2=0.9738$) (Figure 7) was utilised to calculate quercetin equivalents (QE) per gram of plant fraction in order to ascertain the overall flavonoid content. The aqueous extract of *A. reticulata* leaves had a quercetin equivalents value of 89.15 ± 0.4

mg/gm of extract, indicating a high quantity of flavonoids.

Cytotoxic Activity

In-vitro Brine Shrimp Lethality Bioassay for Cytotoxic Activity

The potential cytotoxic effect of the extract was investigated using Brine Shrimp Lethality Bioassay. The anticancer medication vincristine sulphate demonstrated LC_{50} and LC_{90} values of 1.63 μ g/ml and 7.17 μ g/ml, while the

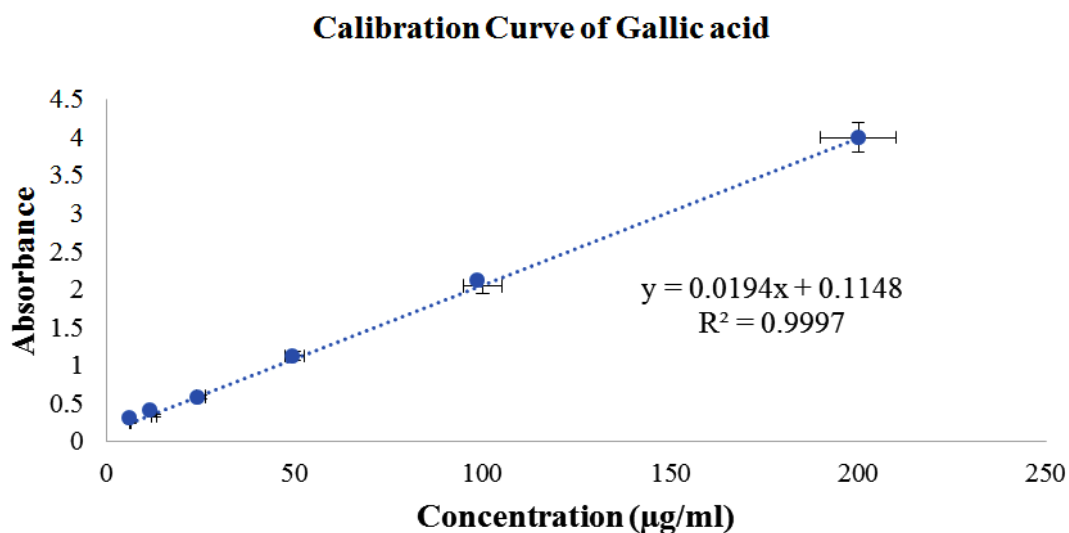


Fig. 6. Calibration Curve of Gallic Acid

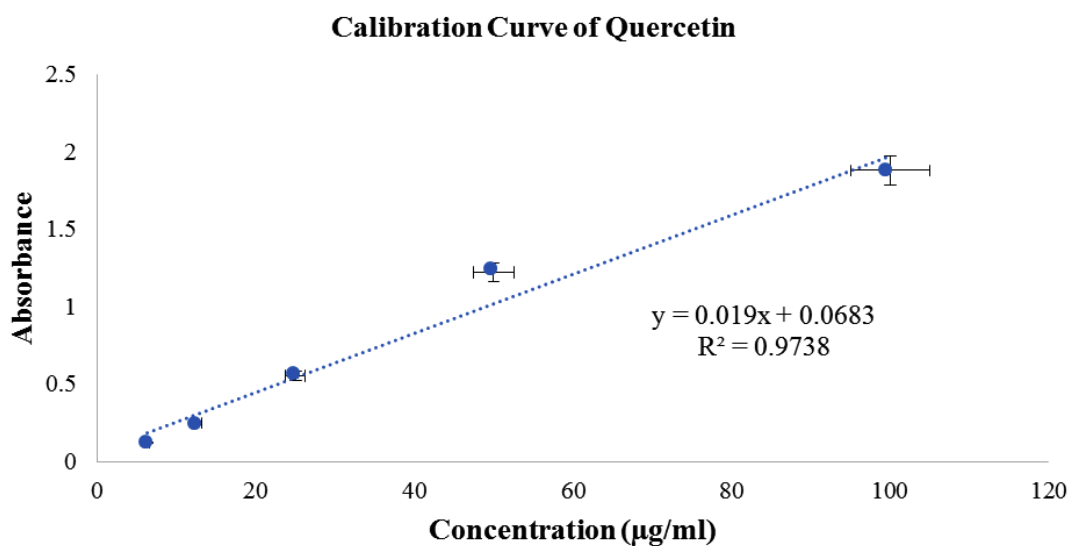


Fig. 7. Calibration Curve of Quercetin

aqueous leaf extract was shown to be extremely poisonous to brine shrimp nauplii, with LC_{50} and LC_{90} values of 93.37 μ g/ml and 516.65 μ g/ml (Figure 8).

Evaluation of In-vivo Anti-cancer Potential against Ehrlich's Ascites Carcinoma (EAC) Cell Line-

Result of Anti-cancer study:

Result of Hematological Parameters in Anti-cancer Study:

Result of Liver and Kidney Markers in Anti-cancer Study:

DISCUSSION

GC-MS Analysis

Among the identified 77 compounds, some compounds were discovered to have good antioxidant, cytotoxic, and anti-cancer

activities. For example, 2,4,6-Cycloheptatrien-1-one,3,5-Bis-Trimethylsilyl- and Thiophene-3-ol, tetrahydro-, 1,1-dioxide were identified in the extract; both of these compounds are reported to possess antioxidant property.³⁵⁻³⁶ Moreover, some components with good cytotoxic activity were also identified, including ethanediamine derivative, docosanoic acid-methyl ester, and Thiophene-3-ol, tetrahydro-, 1,1-dioxide.³⁹⁻⁴⁰ Furthermore, cyclopropylcarbinol; thiophene derivative; cyclobutanol; docosanoic acid methyl ester; 1,2-ethanediamine, n-methyl; propanamide and octadecanoic acid 17-methyl-methyl ester were also found in the extract. All these substances are reported to have good anti-cancer activity.⁴⁹⁻⁵³

Evaluation of Antioxidant Activity

There are several ways to evaluate the antioxidant activity of plant extracts. Free radicals have a well-established role in a variety

Table 2. Effect of Standard and AEAR on the changes in body weight and tumor weight and No. of EAC cell in EAC induced tumor in mice

Group	Body Weight Changes (g) (Mean \pm SEM)	Tumor Weight (g) (Mean \pm SEM)	No. of EAC Cell (Mean \pm SEM)
Disease Control	8.20 \pm 0.66	8.72 \pm 0.46	22.20 \pm 2.08
Standard	16.20 \pm 0.38***	5.08 \pm 0.35***	9.20 \pm 0.86***
AEAR 200	3.60 \pm 0.51***	3.16 \pm 0.84***	8.20 \pm 0.86***
AEAR 400	4.60 \pm 0.81**	3.00 \pm 0.38***	7.40 \pm 0.81***

* $p < 0.05$ (Significance); ** $p < 0.01$ (Highly significance); *** $p < 0.001$ (Very highly significance) against control; Standard is 5-Fluorouracil.

Here, AEAR = Aqueous extract of *A. reticulata*.

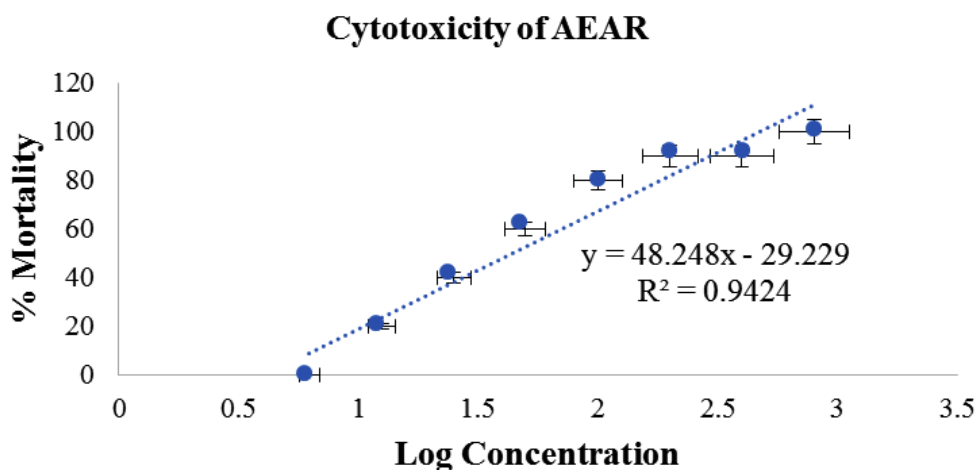


Fig. 8. Graphical representation of cytotoxic potential of aqueous extract of *A. reticulata* against Brine Shrimp nauplii

of clinical manifestations. Because they fight free radicals, antioxidants protect us from a number of diseases. By protecting the antioxidant defences or by inhibiting the formation of reactive oxygen species, they function. It is possible to evaluate the ability of natural products to donate electrons by employing purple-colored solution bleaching with 2,20-diphenyl-1-picrylhydrazyl radical (DPPH). Scavenging DPPH is accomplished by introducing an antioxidant or radical species that decolorises the DPPH solution. The amount of colour change depends on the antioxidants' potency and effectiveness. A significant reduction in the absorbance of the reaction mixture indicates that the tested molecule possesses potent free radical scavenging properties.²⁶⁻²⁸The superoxide

radical is believed to be one of the primary biological sources of reactive oxygen species. Although the superoxide anion is a weak oxidant, it generates strong, dangerous hydroxyl radicals and singlet oxygen, both of which worsen oxidative stress.²⁹Whether the yellow colour of the test solution turns green in a reducing power assay depends on the reducing power of the test specimen. Using reductants in the solution, the Fe^{3+} /ferricyanide combination is reduced to the ferrous form. Fe^{2+} can therefore be tracked using an absorbance measurement at 700 nm.³⁰ Plant-based phenolic compounds are important due to the scavenging effect of their hydroxyl groups. Plant phenolic compounds fall into several categories, but flavonoids are the most common because

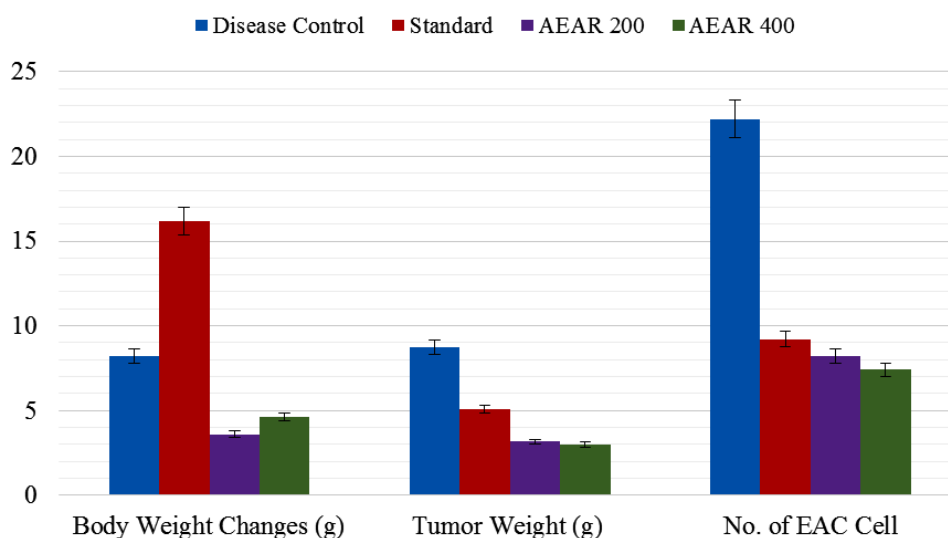


Fig. 9. Effect of Standard and AEAR on the body weight and tumor weight and No. of EAC cell in EAC induced tumor in mice

Table 3. Effect of Standard and AEAR on the hematological parameters in EAC induced tumor in mice

Group	Hb (g/dl) (Mean±SEM)	RBC (10 ⁶ /cmm) (Mean±SEM)	WBC (10 ³ /cmm) (Mean±SEM)	Platelet (10 ⁶ /cmm) (Mean±SEM)
Disease Control	8±0.4	3.6±0.2	15.3±0.6	131.2±5.1
Standard	10.4±0.3*	2.3±0.1***	13.2±0.5*	88±2.3***
AEAR 200	11.9±0.5***	4.5±0.2**	9.3±0.3***	166±4.3***
AEAR 400	12.6±0.5***	4.9±0.1***	8.4±0.2***	150.2±2.1*

*p<0.05 (Significance); **p<0.01 (Highly significance); ***p<0.001 (Very highly significance) against control; Standard is 5-Fluorouracil.

Here, AEAR = Aqueous extract of *A. reticulata*.

of their potent antioxidant qualities. Naturally occurring in plants, flavonoids are thought to enhance human health. Numerous investigations on flavonoid derivatives have shown that they have antibacterial, antiviral, anti-inflammatory, anticancer, and antiallergic activities. The bulk of oxidising molecules, including singlet oxygen, and other free radicals connected to various diseases have been shown to be effectively scavenged by flavonoids.³¹The plants are capable of synthesizing a wide variety of physiologically important compounds. Antioxidant compounds found in many plants shield cells from the damaging effects of reactive oxygen species (ROS), which can harm cells.³²*A. reticulata*'s aqueous leaf extract was tested for antioxidant capacity using

six previously mentioned methods. According to the researches, *A. reticulata* was reported to have antioxidant potential.³³⁻³⁴ Additionally, the components contained in the aqueous extract were identified using GC-MS analytical analysis. The two potent antioxidants 2,4,6-Cycloheptatrien-1-one,3,5-Bis-Trimethylsilyl- and Thiophene-3-ol, tetrahydro-, 1,1-dioxide were discovered in the aqueous extract of *A. reticulata*.³⁵⁻³⁶ 2,4,6-Cycloheptatrien-1-one,3,5-Bis-Trimethylsilyl, has antioxidant properties because it may donate electrons and hydrogen atoms, scavenging free radicals and lowering oxidative stress. Its antioxidant qualities are largely dependent on its structure, especially the tropone ring.³⁷ Because of the thiophene ring's ability to scavenge free

Table 4. Effect of Standard and AEAR extract on the ALT, AST, Bilirubin & Creatinine level in EAC induced tumor bearing mice

Group	ALT (IU/L) (Mean±SEM)	AST (IU/L) (Mean±SEM)	Bilirubin (mg/dl) (Mean±SEM)	Creatinine (mg/dl) (Mean±SEM)
Disease Control	13.9±1.4	135.6±12.5	4.4±0.7	1.9±0.05
Standard	17.2±1.5	90±2.9***	8.1±0.4***	1.3±0.04***
AEAR 200	18.9±0.7*	87.5±1.2***	8.5±0.2***	0.9±0.07***
AEAR 400	20.9±0.5**	84.3±0.8***	8.9±0.3***	0.8±0.05***

*p<0.05 (Significance); **p<0.01 (Highly significance); ***p<0.001 (Very highly significance) against control; Standard is 5-Fluorouracil.

Here, AEAR = Aqueous extract of *A. reticulata*.

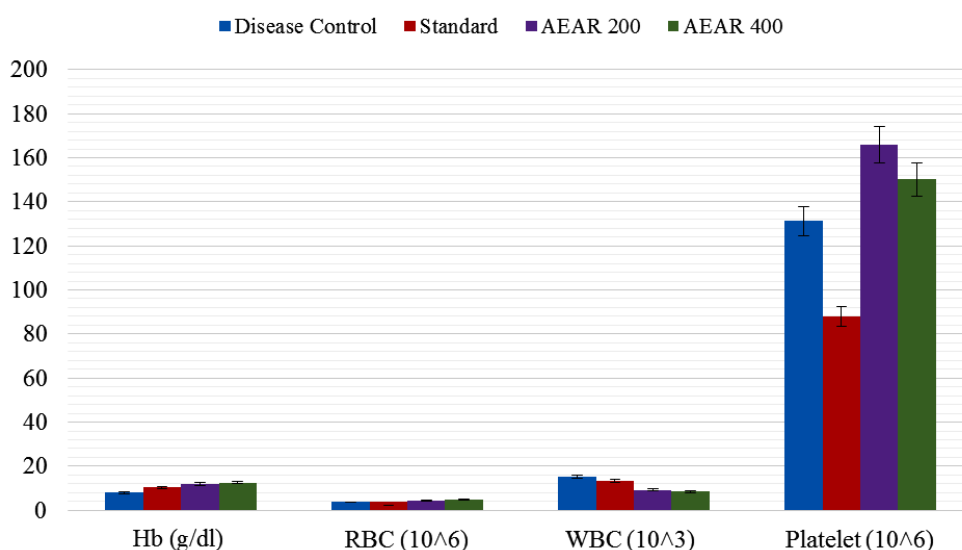


Fig. 10. Effect of Standard and AEAR on the hematological parameters in EAC induced tumor in mice

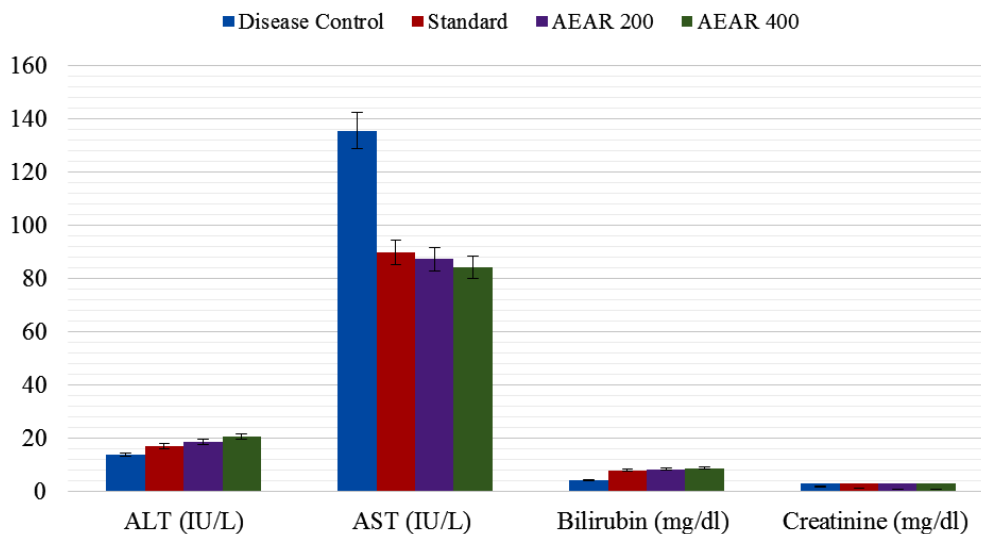


Fig. 11. Effect of Standard and AEAR on the ALT, AST, Bilirubin & Creatinine level in EAC induced tumor in mice

radicals, Thiophene-3-ol, tetrahydro-, 1,1-dioxide can have antioxidant properties. The particular substituents on the thiophene ring affect this action; in general, electron-donating groups improve antioxidant qualities.³⁸

Brine Shrimp Lethality Bioassay for Cytotoxic Activity

Plant fractions that demonstrate LC_{50} values below 1000 μ g/ml are deemed bioactive in this investigation. The outcome shows how toxic *A. reticulata*'s aqueous leaf extract is to brine shrimp nauplii. The extract's lethality was shown to be exactly proportional to its concentration. The percentage of Brine Shrimp nauplii mortality increased in a concentration-dependent manner, indicating the existence of cytotoxic properties. In the Hoechst 33258 fluorescence assay, flavonoids and phenolics have also been demonstrated to exhibit cytotoxicity. As the outcomes of total phenolic content and total flavonoid content determination studies demonstrated that, aqueous leaf extract contains considerable amount of phenol and flavonoid contents, this can be a reason behind the extract's cytotoxic potential. Furthermore, the results of the GC-MS analysis of aqueous fraction from *A. reticulata* discovered that certain cytotoxic components were present. Potent cytotoxic agents, including ethanediamine derivative, docosanoic acid-methyl ester, and

thiophene derivative, are reported to exist in the aqueous fraction.³⁹⁻⁴⁰ The cytotoxic activity of several extracts from *A. reticulata* has also been confirmed by Biswas *et al.* (2012), Pham *et al.* (2020), and other researchers.⁴¹⁻⁴² Because of their capacity to interact with and harm DNA, obstruct cell division, or interfere with cellular functions, ethylenediamine derivatives may have cytotoxic effects. These substances, which are frequently employed as ligands in metal complexes, have the ability to cause apoptosis, or cell death, in a variety of cancer cell lines, which makes them promising candidates for the development of anticancer drugs.⁴³ Docosanoic acid methyl ester is a fatty acid methyl ester that has the potential to be toxic to cells, especially cancer cells, due to its cytotoxic activity. This activity is probably caused by the compound's capacity to cause reactive oxygen species generation and/or cell membrane disruption.⁴⁴ By causing apoptosis, interfering with the course of the cell cycle, and preventing microtubule assembly, thiophene derivative can demonstrate cytotoxic action. Cancer cells may be destroyed as a result of these activities.⁴⁵

Anti-cancer Study

Experimental animals' body weight changes during the course of treatment are closely observed during the assessment of the extract's anti-cancer potential. For ascertaining the anti-

cancer property of test materials, the tumor weight and quantity of EAC cells in ascitic fluid are computed for each group.²²⁻²⁴ Table 2 makes it evident that the standard 5-FU treated group's body weight change outcome was quite concerning. The change in body weight for the disease control group was also noteworthy. However, the body weight change outcomes for the groups treated with aqueous extract (both for 200 mg/kg and 400 mg/kg dosages) were lower than those for the disease control and standard groups. It is notable that the body weight change values further lowers with increasing concentration of the test extract. It was found that the tumor weight values obtained from the test extract and 5-FU treatment groups were lower than those obtained from the disease control group. It is also apparent that tumor weight reduces when the aqueous extract dosage increases. Additionally, it was discovered that the quantity of EAC cells observed decreased in both the standard and test extract treated groups; moreover, it was noticed that the amount of EAC cells decreased as the dose of aqueous leaf extract increased. The test extract's 400 mg/kg body weight dosage produced the strongest anticancer effect, surpassing that of the common medication 5-FU. It is clear from the explanation above that the extract's anti-cancer activity rises as the dosage does (Figure 11).

Hematological Parameters in Anti-cancer Study

As the body's homeostasis is severely disturbed during cancer treatment, animals with cancer usually have alterations in the number of red blood cells, white blood cells, and platelets. However, these changes can be reduced with good anti-cancer treatment. Mice typically have hemoglobin content value of 0.08-26.8 g/dL, and their RBC, WBC, and platelet counts are $0.016-14.3 \times 10^6/\text{cmm}$, $2-10 \times 10^3/\text{cmm}$, and $14-1670 \times 10^6/\text{cmm}$, respectively.⁴⁶ Following dissection, blood was drawn from the test animals' bodies and analysed to ascertain the haemoglobin content as well as the quantity of red blood cells, white blood cells, and platelets. Table 3 shows that aqueous leaf extract at 200 and 400 mg/kg body weight was able to maintain haemoglobin, RBC, WBC, and platelet values within the normal range. This is a very positive indication because many chemotherapy drugs are unable to do so. Thus, it may be concluded that the extract successfully

preserved haematological parameters throughout the entire therapeutic duration.

Liver and Kidney Markers in Anti-cancer Study

As vital organs for the body's overall metabolism and excretion, the liver and kidney may be impacted during cancer treatment. Because cancer changes the body's normal physiological state, it might impact the kidney and liver. To determine whether test samples can lessen the harmful effects of cancer on these essential organs, various significant liver and kidney markers' levels were calculated from the serum extracted from the blood of animals receiving treatment. ALT, AST, bilirubin, and creatinine levels in mice are generally 17-77 IU/L, 54-298 IU/L, 8-33 mg/dl, and 0.2-0.9 mg/dl, respectively.⁴⁷ Table 4 shows that the kidney marker values were altered in the disease control group, whereas the liver marker values were slightly higher. Nevertheless, the creatinine level was not maintained with the standard medication 5-FU. Aqueous leaf extract, however, efficiently maintained the levels of both liver and kidney indicators at dosages of 200 and 400 mg/kg body weight (the bilirubin level fluctuated very little at the 200 mg/kg dosage). In light of this, it can be concluded that the extract may benefit the kidney and liver when treating cancer.

Overall Discussion about Anti-cancer Study

Ehrlich Ascites Carcinoma is a highly aggressive mammary adenocarcinoma that arises spontaneously in mice (EAC). They are distinguished by fast proliferation, undifferentiation, 100% malignancy, and a shortened life span. These traits make EAC comparable to human malignancies. EAC is used in chemotherapeutic studies because, in addition to its resemblance to human tumours, it is highly susceptible to chemotherapy.⁴⁸ In this study, the extract exhibited strong anti-cancer property against the Ehrlich Ascites Carcinoma cell line. The extract included many anti-cancer compounds, according to the GC-MS analytical report. The aqueous extract contained the following compounds: cyclopropylcarbinol; thiophene derivative; cyclobutanol; docosanoic acid methyl ester; 1,2-ethanediamine, n-methyl; propanamide and octadecanoic acid 17-methyl-methyl ester. According to reports, each of these substances has potent anti-cancer activity.⁴⁹⁻⁵³ The special reactivity of the cyclopropane ring allows

cyclopropylcarbinol, and more generally, compounds containing cyclopropane, to have anticancer action. Strongly electrophilic, the stretched three-membered ring reacts easily with biomolecules like DNA, causing damage to the molecule and ultimately cell death.⁵⁴By focussing on several cellular mechanisms implicated in the initiation and spread of cancer, thiophene derivatives demonstrate anticancer efficacy. They can cause cell cycle arrest and apoptosis, interfere with signalling pathways, block particular proteins linked to cancer, and even make cancer cells more sensitive to other treatments.⁵⁵Cyclobutanol can have anticancer effects by improving the stability, water solubility, and DNA binding affinity of platinum complexes, especially when it is added to them. The shape, characteristics, and interactions of the molecule with biological targets can be influenced by the distinct structure of the cyclobutane ring, which includes its puckered conformation and bond angles. This could result in enhanced anticancer efficacy.⁵⁶ Methyl behenate, another name for docosanoic acid methyl ester, has anticancer properties mainly due to its capacity to stop cancer cells from proliferating and to cause them to undergo apoptosis. In terms of regulating the proliferation of breast cancer cells (MCF-7), it has been demonstrated to be more effective than other fatty acid esters, such as hexadecanoic acid ethyl ester.⁵⁷Through a variety of methods, including interactions with biological components such as DNA and proteins, 1,2-ethanediamine, N-methyl can demonstrate anticancer activity. These mechanisms can result in cell cycle arrest, apoptosis, and the prevention of tumour growth.⁵⁸By causing apoptosis, preventing the growth of cancer cells, and interfering with biological functions including mRNA processing, propanamide derivatives can have anticancer effects. In particular, it has been demonstrated that certain propanamide derivatives are cytotoxic to cancer cells but have little impact on healthy cells, indicating the possibility of targeted cancer treatment.⁵⁹By causing apoptosis (programmed cell death) and preventing the proliferation of cancer cells, octadecanoic acid 17-methyl-methyl ester, has demonstrated possible anticancer action.⁶⁰Some researchers have investigated a number of *A.reticulata* extracts for anti-cancer property.⁶¹⁻⁶³The anti-cancer potential of *A.*

reticulata's aqueous leaf fraction was, however, assessed for the first time in it.

CONCLUSION

The investigation highlights that aqueous leaf fraction from *A.reticulata* has considerable pharmacological potentials such as antioxidant, cytotoxic, and anticancer activities. GC-MS analytical data indicates the compounds which lead to these bioactivities. Outcomes of six in-vitro antioxidant studies demonstrated the presence of good antioxidant properties in the test extract. Brine shrimp lethality bioassay illustrated LC₅₀ and LC₉₀ values for both test extract and standard drug; by comparing the values it can be said that the aqueous leaf extract has strong cytotoxic potentials. In-vivo assessment of anti-cancer activity against EAC cell line demonstrated that, aqueous leaf extract has notable anti-cancer activity as it lowers the values of tumor weight and tumor cell count even more than the standard drug 5-FU. However, isolating the bioactive compounds from the extract and testing them, may help clarify the mechanism of action that led to the various bioactivities and may open the door to drawing a firm conclusion about the current findings.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

Every action that involved handling animals was carried out by following the rules set forth by Jahangirnagar University's animal ethics committee [Ref No: BBEC, JU/M2024/11 (137)].

Informed Consent Statement

This study did not involve human

participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author Contributions

Md. Sohel Rana: supervision(lead), investigation (lead), conceptualization (lead), formal analysis (supporting), funding acquisition (equal), writing – review and editing (equal). TasniaBinte Bari Kabbo: data curation (lead), formal analysis (lead), funding acquisition (equal), investigation (lead), methodology (lead), project administration (equal), resources (lead), software (lead), validation (equal), visualization (lead), writing – original draft (lead), writing – review and editing (equal). PriteshRanjanDash: supervision(equal), formal analysis (supporting), investigation (equal), data curation (equal), writing – review and editing (equal). Adila Jahan Anika: data curation (equal), formal analysis (equal), Najiha Tarannum Timon: data curation (equal), formal analysis (equal), Tasnova Nowrin: data curation (equal), formal analysis (equal), ShammiBinte Bashar: data curation (equal), formal analysis (equal).

REFERENCES

- Souri E, Amin R, Jalalizadeh H, Barezi S. Screening of thirteen medicinal plant extracts for antioxidant activity. *Iranian Journal of Pharmaceutical Research*. 2008;7(2):149-154
- Canga I, Vita P, Oliveira I, Castro A, Pinho C. In Vitro Cytotoxic Activity of African Plants: A Review. *In Molecules*. 2022;27(15):4989.
- Deniz U, Güne^o H, Gülne^o F, Mammadov R. Cytotoxic activities of certain medicinal plants on different cancer cell lines. *Turkish Journal of Pharmaceutical Sciences*. 2017;14(3): 222–230.
- Mastovska K, Dorweiler K, Lehotay S, et al. Pesticide multiresidue analysis in cereal grains using modified QuEChERS method combined with automated direct sample introduction GC-TOFMS and UPLC-MS/MS techniques. *Journal of Agricultural and Food Chemistry*. 2010;58(10):5959-5972.
- Ponnamma S, Manjunath K. GC-MS Analysis of phytochemicals in the methanolic extract of *Justicia wynaadensis* (nees). *Int J Pharm Bio Sci*. 2012;3(3):570-576.
- Reddy P, Shantha R, Bharathi V, Kumar K, Venkateshwarlu G. Pharmacognostical evaluation on the Medicinal & Nutritive fruits of Raamaphal-*Annona reticulata* L. *Journal of Pharmacognosy and Phytochemistry*. 2015;4(1):21-28.
- Wen W, Lin Y, Ti Z. Antidiabetic, Antihyperlipidemic, Antioxidant, Anti-inflammatory Activities of Ethanolic Seed Extract of *Annona reticulata* L. in Streptozotocin Induced Diabetic Rats. *Frontiers in Endocrinology*. 2019;10:716.
- Stankovic S, Niciforovic N, Mihailovic V, Topuzovic M, Solujic S. Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of *Teucrium polium* L. subsp. *polium*. *Acta Societatis Botanicorum Poloniae*. 2012; 81(2):117–122.
- Gulcin Y, Alwaseel H. DPPH Radical Scavenging Assay. *In Processes*. 2023;11(8):2248.
- Benslama A, Harrar A. Free radicals scavenging activity and reducing power of two Algerian Sahara medicinal plants extracts. *International Journal of Herbal Medicine*. 2016;4(6):158–161.
- Parul R, Kumar S, Saha P. In-Vitro Nitric Oxide Scavenging Activity of Methanol Extracts Of Three Bangladeshi Medicinal Plants. *The Pharma Innovation Journal*. 2013;1(12):83-88.
- Kamath D, Samshuddin S, Arunkumar D, Avinash G. Determination of total phenolic content and total antioxidant activity in locally consumed food stuffs in Determination of total phenolic content and total antioxidant activity in locally consumed food stuffs in Moodbidri, Karnataka, India. *Advances in Applied Science Research*. 2015;6(6):99-102.
- Pathak K, Das A. Assessment of antioxidant activity of different extracts of *Annona reticulata* (L). *International Journal of Pharmaceutical Sciences and Research*. 2018;9(6):2431-2437.
- Benslama A, Harrar A. Free radicals scavenging activity and reducing power of two Algerian Sahara medicinal plants extracts. *International Journal of Herbal Medicine*. 2016;4(6):158–161.
- Lallianrawna S, Muthukumaran R, Ralte V, Gurusubramanian G, Kumar S. Determination of total phenolic content, total flavonoid content and total antioxidant capacity of *Ageratina adenophora* (Spreng.). *King & H. Science vision*. 2013;13(4):149-156.
- Khan S, Rafatullah M, Sani M, Shaheen M, Sani M, Razlan, M. Determination of Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity of Various Organic Crude Extracts of *Licuala spinosa* Leaves from Sabah, Malaysia. *ASM Science Journal*. 2018;11(3):53-

- 58.
17. Khatiwora E, Kulkarni M, Adsul B, Kulkarni M, Deshpande R, Kashalkar V. Spectroscopic determination of total phenol and flavonoid contents of *Ipomoea carnea*. *International Journal of ChemTech Research*. 2010;2(3):1698-1701.
 18. Waghulde S, Kale K, Patil V. Brine shrimp lethality assay of the aqueous and ethanolic extracts of the selected species of medicinal plants. *In Proceedings*. 2019;41(1):47.
 19. Tawaha A. Cytotoxicity evaluation of Jordanian wild plants using brine shrimp lethality test. *Jordan journal of applied science natural sciences*. 2006;8(1):12.
 20. Olowa F, Nuñez M. Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City, Philippines. *International Research Journal of Biological Sciences*. 2013;2(11).
 21. Prosanta P, Chakraborty M, Karmakar I, et al. Evaluation of Anticancer Activity of Methanol Extract of *Monstera deliciosa* in EAC Induced Swiss Albino Mice. *International Journal of Toxicological and Pharmacological Research*. 2015;7(4):165–170.
 22. Raju A, Christina A, Murali A. Antitumor activity of ethanolic and aqueous extracts of *Drosera Burmannii* in EAC Bearing Mice. *Spatula DD*. 2012;2(2):83.
 23. Lavakumar V. Anticancer and antioxidant effect of *Acanthoporphoricifera* against EAC induced carcinoma in mice. *Journal of Pharmacy Research*. 2012;5(3):1503–1507.
 24. Reddy N. Evaluation of anticancer activity of *Ruellia tuberosa* on eac induced solid tumor. *International Journal of Phytopharmacology*. 2013;4(5):340-346.
 25. Haldar K, Kar B, Bala A, Bhattacharya S, Mazumder K. Antitumor activity of *Sansevieria roxburghiana* rhizome against Ehrlich ascites carcinoma in mice. *Pharmaceutical Biology*. 2010; 48(12):1337–1343.
 26. Nunes X, Silva F, Guedes J, Almeida S. Biological oxidations and antioxidant activity of natural products. *Phytochemicals as nutraceuticals-Global Approaches to Their Role in Nutrition and Health*. 2012;278.
 27. Umamaheswari M, Chatterjee K. In vitro antioxidant activities of the fractions of *Cocciniagrandsis* L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicines*. 2008;5(1):61-73.
 28. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. *Food and bioproducts processing*. 2011;89(3): 217-233.
 29. Alves Q, Jorge D, Juceni P, et al. Methods for determination of in vitro antioxidant activity for extracts and organic compounds. *Química Nova*. 2010;33:2202-2210.
 30. Saeed N, Khan R, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC complementary and alternative medicine*. 2012;12:1-12.
 31. Ao C, Li A, Elzaawely A, Xuan D, Tawata S. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. extract. *Food control*. 2008;19(10): 940-948.
 32. Padmaja M, Sravanthi M, Hemalatha J. Evaluation of Antioxidant Activity of Two Indian Medicinal Plants. *Journal of Phytochemistry*. 2011;3(3).
 33. Jayaprakash A. Phytochemicals, antimicrobial and antioxidant properties of *Annona reticulata* Linn. *JAIR*. 2017;6(6):90-95.
 34. Mondal K, Saha P, Mondal N, Mazumder U. Free radical scavenging property of *Annona reticulata* leaves. *Oriental Pharmacy and Experimental Medicine*. 2008;8(3):260–265.
 35. Devi B, Barkath N, Vijayaraghavan P, Rejiniemon S. GC-MS analysis of phytochemical from *Psidium guajavalinn* leaf extract and their in vitro antimicrobial activities. *Int. J. Pharma Biol. Sci*. 2018; 8:583–589.
 36. Shah R, Verma K. Therapeutic importance of synthetic thiophene. *Chemistry Central Journal*. 2018;12(1).
 37. Charlton C, Mastuygin M, Török B, Török M. Structural Features of Small Molecule Antioxidants and Strategic Modifications to Improve Potential Bioactivity. *Molecules*. 2023; 28(3):1057.
 38. Tavadyan A, Manukyan H, Harutyunyan H, Musayelyan V, Sahakyan D, Tonikyan G. Antioxidant Properties of Selenophene, Thiophene and Their Aminocarbonitrile Derivatives. *Antioxidants (Basel)*. 2017;6(2):22.
 39. Musiliyu A, Veera D, Badisa M. Cytotoxic Activity of N, N'-Bis (2-hydroxybenzyl) ethylenediamine Derivatives in Human Cancer Cell Lines. *Anticancer Research*. 2014; 34:1601-1608.
 40. Nisa S, Bibi Y, Masood S, et al. Isolation, Characterization and Anticancer Activity of Two Bioactive Compounds from *Arisaema flavum* (Forssk.) Schott. *Molecules*. 2022;27(22):7932.
 41. Biswas S, Shahriar M, Khanam A, Ahsan R. Investigation of Antioxidant, In-vitro Cytotoxic, and In-vivo Antitumor Effects of Leaf Extracts of *Annona Reticulata*. *Bangladesh Journal of Microbiology*. 2012;29(2):70-74.

42. Pham V, Le Q, Le T, Vo Q, Ho V. Phytochemical constituents of *Annona reticulata* and their cytotoxic activity. *Letters in Organic Chemistry*. 2020;17(3):206-210.
43. Bracci L, Schiavoni G, Sistigu A, Belardelli F. Immune-based mechanisms of cytotoxic chemotherapy: implications for the design of novel and rationale-based combined treatments against cancer. *Cell Death Differ*. 2014;21(1):15-25.
44. Santos J, Calzada F, Camacho Y, et al. Evaluation of the Anti-Toxoplasma gondii Efficacy, Cytotoxicity, and GC/MS Profile of Pleopeltiscrassinervata Active Subfractions. *Antibiotics (Basel)*. 2023;12(5):889.
45. Santos A, Pereira C, Oliveira B, Junior B, Lima A, Pitta R, Rêgo B, Pitta G. Anticancer properties of thiophene derivatives in breast cancer MCF-7 cells. *Anticancer Drugs*. 2018;29(2):157-166.
46. O'Connell E, Mikkola M, Stepanek M, et al. Practical murine hematopathology: a comparative review and implications for research. *Comp Med*. 2015;65(2):96-113.
47. Santos W, Oliveira C, Hastreiter A, et al. Hematological and biochemical reference values for C57BL/6, Swiss Webster and BALB/c mice. *Brazilian Journal of Veterinary Research and Animal Science*. 2016;53(2):138-145.
48. Elsherbiny M, Al-Gayyar H. Anti-tumor activity of arjunolic acid against Ehrlich Ascites Carcinoma cells in vivo and in vitro through blocking TGF- β type 1 receptor. *Biomedicine & Pharmacotherapy*. 2016;82:28-34.
49. Dembitsky M. Naturally occurring bioactive Cyclobutane-containing (CBC) alkaloids in fungi, fungal endophytes, and plants. *Phytomedicine*. 2014;21(12):1559-1581.
50. Nisa S, Bibi Y, Masood S, et al. Isolation, Characterization and Anticancer Activity of Two Bioactive Compounds from *Arisaema flavum* (Forssk.). *Schott. Molecules*. 2022; 27(22):7932.
51. Musa A, Badisa L, Latinwo M. Cytotoxic activity of N, N'-Bis (2-hydroxybenzyl) ethylenediamine derivatives in human cancer cell lines. *Anticancer Research*. 2014;34(4), 1601-1607.
52. Zhang J, Hao G, Xin W, Yang F, Zhu M, Zhou H. Design, synthesis, and structure-activity relationship of 7-propanamide benzoxaboroles as potent anticancer agents. *Journal of Medicinal Chemistry*. 2019;62(14):6765-6784.
53. Mathai V, Sar K, Mitra C, Jindal K, Wang F. Ethanolic extraction and GC-MS analysis of antioxidant and anticancer bioactive compounds from *Mentha arvensis* and *Aegle marmelos*. *Natural Product Research*. 2024:1-7.
54. Ali E, Aziz A, Bräse S, et al. Unveiling the Anticancer Potential of a New Ciprofloxacin-Chalcone Hybrid as an Inhibitor of Topoisomerases I & II and Apoptotic Inducer. *Molecules*. 2024;29(22):5382.
55. Pathania S, Chawla A. Thiophene-based derivatives as anticancer agents: An overview on decade's work. *Bioorganic Chemistry*. 2020;101:104026.
56. Lichota A, Gwozdziński K. Anticancer Activity of Natural Compounds from Plant and Marine Environment. *Int. J. Mol. Sci*. 2018;19(11):3533.
57. JóŹwiak M, Filipowska A, Fiorino F, Struga M. Anticancer activities of fatty acids and their heterocyclic derivatives. *European journal of pharmacology*. 2020;871:172937.
58. Altaher M, Adris A, Aliwaini H, Awadallah M, Morjan Y. The Anticancer Effects of Novel Imidazo[1,2-a]Pyridine Compounds against HCC1937 Breast Cancer Cells. *Asian Pac J Cancer Prev*. 2022;23(9):2943-2951.
59. Hansen T, Ausbacher D, Zachariassen G, Anderssen T, Havelkova M, Strøm B. Anticancer activity of small amphipathic α 2, 2-amino acid derivatives. *European journal of medicinal chemistry*. 2012;58:22-29.
60. Mostofa G, Reza A, Khan Z, et al. Apoptosis-inducing anti-proliferative and quantitative phytochemical profiling with in silico study of antioxidant-rich *Leea equata* L. leaves. *Heliyon*. 2024;10(1).
61. Khan N, Kumar N, Ballal A, Datta D, Belle S. Unveiling antioxidant and anti-cancer potentials of characterized *Annona reticulata* leaf extract in 1, 2-dimethylhydrazine-induced colorectal cancer in Wistar rats. *Journal of Ayurveda and Integrative Medicine*. 2021;12(4): 579-589.
62. Rao P, Shenoy V, Nayak G, et al. Evaluation of the merit of ethanolic extract of *Annona reticulata* as an anti-cancer agent in human colon cancer cell lines (HCT-116). *F1000Research*. 2024;12:1571.
63. Suresh H, Shivakumar B, Shivakumar S. Phytochemical Potential of *Annona reticulata* Roots for Antiproliferative Activity on Human Cancer Cell Lines. *Advances in Life Sciences*. 2012;2(2), 1-4.