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ANTIOXIDANT AND CYTOTOXICITY STUDY ON THE SUCCESSIVE EXTRACTS OF LEAVES, STEMS, AND ROOTS OF THE MEDICINAL PLANT *GARCINIA COWA* GROWING IN BANGLADESH

Matiur Rahman[1], Ananta Kumar Das[2]*, Md. Farid Uddin[1] and Koushik Saha[1]

[1]Department of Chemistry, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

[2]Department of Pharmacy, Gono Bishwabidyalay, Nolam, Savar, Dhaka-1344. Bangladesh.

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***Corresponding Author**
Ananta Kumar Das
Department of Pharmacy,
Gono Bishwabidyalay,
Nolam, Savar, Dhaka-1344.
Bangladesh.

ABSTRACT

Garcinia cowa is a significant medicinal plant for its traditional therapeutic purposes and a valuable source of novel pharmaceuticals. The leaves, stems, and roots of *G. cowa* were extracted with hexane, chloroform, ethyl acetate, and methanol to inspect the antioxidant and cytotoxicity. The total phenolic content, total flavonoid content, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, and total antioxidant capacity experiments to determine antioxidant activity. Brine shrimp lethality bioassay for cytotoxicity. The total phenolic content study of root and stem extracts in methanol and ethyl acetate exhibit greater phenolic content. But in the total flavonoid content assay, ethyl acetate, methanol & chloroform extracts of root, and ethyl acetate extract of the stem have higher flavonoid content. The DPPH free radical scavenging assay of the extracts exhibited that n-hexane & ethyl acetate extracts of root and n-hexane extract of stem showed good free radical scavenging capacity with IC_{50} values 20.80, 24.9 and 37.5 µg/mL, respectively, as compared to standard ascorbic acid 7.25 µg/mL. These results are also similar to total antioxidant capacity determination experiment. The antioxidant study revealed that the stem and root extracts of *G. cowa* have good antioxidant properties. Brine shrimp lethality bioassay study of the extracts exhibited that ethyl acetate extract of leaf, chloroform extract of root, and n-hexane extract of stem showed moderate cytotoxicity.

KEYWORDS: *Garcinia cowa*, Antioxidant, Phenolic content, Flavonoid content, DPPH, Cytotoxicity.

INTRODUCTION

Modern medications utilized as standard treatments in developed countries are produced using traditional knowledge of medicinal plants.[1] The number of vascular plant species used as medicines range from 350,000 to 500,000, or 10% of all vascular plant species.[2] All human populations have employed medicinal plants for centuries. They serve as the basis for numerous pharmaceutical, vitamin, and nutritional supplements products applied nowadays.[3] Since the beginning of civilization, people have used homeopathic treatments made from herbal plants.[4] Additionally, organic substances derived from natural sources have been and continue to be used to treat various diseases. These substances are employed both in their natural state (as pure drugs or phytomedicine) and as building blocks for the creation of synthetic and semi-synthetic analogues with enhanced drug ability.[5] For more than 50 years, natural products have been acknowledged as useful in the fight against acute diseases, including cancer. [6] Cancer is brought on by altered gene expression and faulty gene function, loss of normal cell growth, development, and control, problems with apoptosis, angiogenesis start, and metastasizing to other healthy tissue or organs.[7] 9.6 million people died from cancer in 2018, and 18.1 million additional cases were reported.[8] Cancer has 36 different forms and mostly affects men and women in different ways, including colorectal, liver, lung, stomach, and prostate cancers in men and breast, cervix, colorectal, lung, and thyroid cancers in women.[9] Plants are a rich source of phytochemicals with biological effects that can be utilized to make anticancer medications. The demand for herbal products as a complementary therapy is rising. According to statistics from WHO, traditional medicine is used by 80% of the population in underdeveloped nations.[10]

Garcinia cowa Roxb. (Family Clusiaceae), a tree with edible fruits and leaves, often known as cowa fruit, grows in tropical Asia, Africa, Polynesia, and Indonesia.[11] The antioxidant activity of a bioactive compound refers to the ability to remove free radicals effectively, suppress lipid peroxidation events, and stop other types of oxidative damage.[12] According to reports, the phenolic components of *Garcinia cowa* roxb exhibit a wide range of biological and pharmacological capabilities, including cytotoxic, antibacterial, and antioxidant actions.[13] More importantly, antioxidant activity has been linked to the protection of several chronic diseases, including cancer, diabetes, and cardiovascular disease.[11] The phytochemical examination of *G. cowa* revealed the presence of different chemicals in the plant's fruits, leaves, stems, and roots, including depsidones, flavonoids, xanthones, phloroglucinol, terpenes and steroids, and other random substances, including palmitic acid

and hydroxybenzoic acid.[14] The secondary metabolites found in *Garcinia* species include prenylated and oxygenated xanthones, which have biological effects such as antifungal, anti-inflammatory, antitumoral, antioxidant, and HIV-inhibitory characteristics.[15] The first and sole depsidone from *G. cowa*, exhibited cytotoxicity against the cancer cell lines NCI-H187 and MFC-7. Morelloflavone-7-O-glucoside demonstrated strong antioxidant activity. Nine known phloroglucinols, including Cambogia, guttiferone K and oblongifolins, champagne, and garcicowins, show specific cytotoxicity against the healthy colon cells and the cancer cell lines HT-29 and HCT-112 (CCD-18Co). The phloroglucinols discovered in *G. cowa* have a benzoyl group as well as substituent groups made up of geranyl and polyprenyl units.[16]

MATERIALS AND METHODS

Collection and Plant Identification

In April 2022, *Garcinia cowa* leaves, stems, and roots were collected in a rural area of Cumilla, Bangladesh. A taxonomist from the National Herbarium in Dhaka identified the specimen and assigned it the DACB Accession Number 78797.

Extraction of Crude Samples of the Plant *Garcinia cowa*

The roots and stems were divided into little pieces and dried thoroughly underneath the shed. Under the shed, the leaves had likewise been well dried. The dried leaves, stems, and roots were ground into powder using a grinder. The dried powder of the leaves, stems, and roots were then successively extracted at room temperature with n-hexane, $CHCl_3$, ethyl acetate, and methanol and completely dried on a rotary evaporator followed by a desiccator to yield the extracts. The extracts are denoted as GCL-1: n-hexane extract of leaf, GCL-2: $CHCl_3$ extract of leaf, GCL-3: EtOAc extract of leaf, GCL-4: methanol extract of leaf, GCS-1: n-hexane extract of stem, GCS-2: $CHCl_3$ extract of stem, GCS-3: EtOAc extract of stem, GCS-4: methanol extract of stem, GCR-1: n-hexane extract of root, GCR-2: $CHCl_3$ extract of root, GCR-3: EtOAc extract of root, GCR-4: methanol extract of root.

Test for Determination of Antioxidant Activity

Total Phenolic Content (TPC)

The TPC of extracts was calculated by a colorimetric reaction based on the Folin-Ciocalteu reagent (FCR).[18] The FCR is a phosphomolybdate colorimetric determination of phenol and polyphenolic antioxidants.[19] In test tubes, 1 mL of plant extract (200 µg/mL) or standard of various concentrations (200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL) was prepared. Later, 5 mL of FCR and 4 mL of 7.5% Na_2CO_3 were introduced and thoroughly mixed. The reaction

mixture took about 30 minutes for standard, while it took one hour at room temperature to extract the solution. The absorbance at 765 nm was measured using a UV-VIS spectrophotometer. A calibration curve was created using gallic acid as a standard. Gallic acid equivalents (GAE) were used to evaluate the phenolic content of extracts in mg. The following equation was used to determine the total amount of phenolic compounds present in plant extracts as GAE: C = (c x V)/m,

Where; C is the total amount of phenolic compounds in GAE as mg/g of plant extract. c is the gallic acid concentration, measured in mg/mL, as determined by the calibration curve. V is the extract's volume in milliliters, and m is the gram weight of the raw plant extracts.

Total Flavonoids Content (TFC)

The colorimetric approach using aluminum chloride was used to determine the TFC concentration in plant extracts.[20] This procedure placed 1 mL of plant extract (200 µg/mL) or standard in the test tube at different concentrations. In the previously described test tube, 3 mL of methanol, 200 µl of 10% aluminum chloride, and 1M potassium acetate solution were added. Finally, the reaction mixture was combined with 5.6 mL of water. After incubating for 30 minutes, the solution's absorbance was recorded at 415 nm using a spectrophotometer against a blank. The TFC of extracts was estimated as mg of QE (quercetin equivalents)/g of extract using quercetin as a reference standard. The following equation was used to determine the TFC present in plant extracts as QE: C = (c x V)/m,

Where; C is the quantity of flavonoid components in total per gram of plant extract as QE. c is the measurement of QE in milliliters as determined by the calibration curve. V is the extract's volume in milliliters, and m is the gram weight of the raw plant extract.

Total Antioxidant Capacity (TAC)

Prieto et al.'s phosphomolybdenum method was used to calculate the extract's TAC. [21] This method involved filling separate test tubes with 300µL of each extract or standard solution (200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL). Then, each test tube received 3 ml of phosphomolybdate reagent. The reaction took place in the test tubes after 90 minutes of 95°C incubation. A spectrophotometer was then used to measure the absorbance at 695 nm. The following equation was used to compute the TAC in plant extracts, which is given as ascorbic acid equivalents (AAE): C = (c x V)/m

Where; C is the TAC measured in AAE per gram of plant extracts. c = the AAE concentration determined from the calibration curve in mg/ml, V = the extract volume in ml, and m = the weight of the raw plant extract in gm.

DPPH Free Radical Scavenging Assay

The activity of neutralizing free radicals was determined by measuring the absorbance using the DPPH technique.[17] A reactive radical called DPPH acts as an electron acceptor and causes other substances to oxidize. Antioxidants counteract DPPH by oxidizing themselves. A dark-coloured crystalline powder known as DPPH is made up of reactive free radicals in solution. Ascorbic acid at various concentrations (1 ml) and Garcinia cowa leaf, stem, and root extract (1 ml) solutions were separately mixed with 2 ml of 0.004% DPPH solution. The combinations were maintained in the dark for 30 minutes before measuring absorbance at 517 nm using a UV-Visible spectrophotometer. The DPPH free radical is scavenged when the rich violet colour fades to light yellow or colourless.

$$\text{Calculation: \% Inhibition} = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ Control}\right) \times 100$$

The IC_{50} concentration is the level at which 50% of all DPPH free radicals are neutralized.

Cytotoxicity Study as Brine Shrimp Lethality Bioassay (BSLT)

The brine shrimp bioassay method was used to test the cytotoxic effect to see if it killed the shrimp. In this approach, 16 mg of each test sample was obtained and dissolved in 100 µl of purified dimethyl sulfoxide (DMSO) before being reduced to 20 ml with seawater (800 µg/ml). Then, seawater was used to progressively dilute the solution to 800, 400, 200, 100, 50, 25, 12.5, and 6.25µg/mL and standard vincristine sulfate as 10 to 0.313 µg/mL. Ten nauplii were harvested in the plant extracts solutions for 24 hours at room temperature after being combined with 5 ml of seawater. In order to count the remaining nauplii in each test tube, the test tubes were magnified and inspected against a dark backdrop. This information was used to calculate the percentage of brine shrimp nauplii for each concentration. The mortality estimate was obtained using the formula.

$$\%\ Mortality = \frac{No.of\ nauplii\ taken - No.of\ nauplii\ alive}{No.of\ nauplii\ taken} \times 100$$

A median lethal concentration (LC_{50}) is frequently used to describe the effectiveness of a plant product's concentration mortality relationship. By using the linear regression technique to plot percent mortality against the matching dosage log, it can be determined that this

chemical concentration causes death in 50% of the study participant's exposure period. Vincristine sulfate was used in this experiment as a positive control to contrast the cytotoxicity of test substances.

RESULTS AND DISCUSSION

Total Phenolic Content

Using the FCR, the total phenolic content of the *Garcinia cowa* extracts was calculated and represented as GAE per gram of plant extract. The total phenolic contents of the extracts are calculated using the standard curve of gallic acid (y =0.0048x + 0.2589; R^2 = 0.9817). The result of phenolic content is given in table 1 and figure 1.

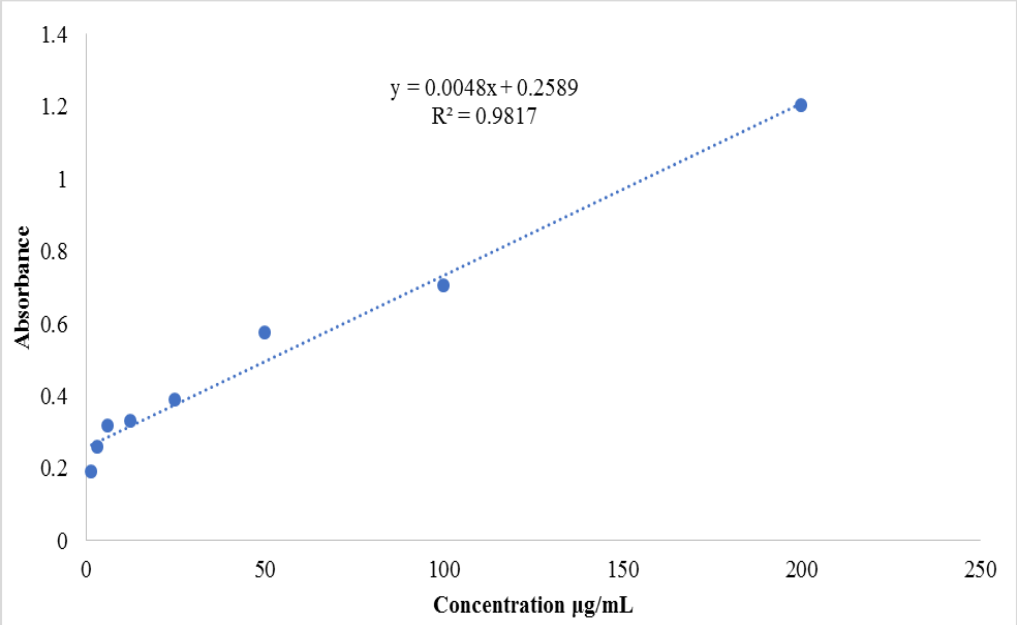

Figure 1: Calibration curve of gallic acid.

Table 1: Total phenolic content of the *Garcinia cowa* extracts.

Sample Name	Abs.	Plant Extracts Wt. (g/mL)	Conc. (C) as GAE µg/ml	Conc. (C) as GAE mg/ml	c*V (mg)	A=(c*V)/m TPC as GAE, (mg/g)	Mean ± SD (mg/g)
GCL-1	0.287	0.0002	5.854	0.006	0.006	29.27	28.23 ± 1.47
	0.285	0.0002	5.437	0.005	0.005	27.19	
GCL-2	0.345	0.0002	17.938	0.018	0.018	89.69	90.73 ± 1.47
	0.347	0.0002	18.354	0.018	0.018	91.77	
GCL-3	0.322	0.0002	13.146	0.013	0.013	65.73	65.21 ± 0.74
	0.321	0.0002	12.938	0.013	0.013	64.69	
GCL-4	0.36	0.0002	21.063	0.021	0.021	105.31	107.40 ± 2.95
	0.364	0.0002	21.896	0.022	0.022	109.48	
GCS-1	0.304	0.0002	9.396	0.009	0.009	46.98	50.63 ± 5.16
	0.311	0.0002	10.854	0.011	0.011	54.27	

GCS-2	0.408	0.0002	31.063	0.031	0.031	155.31	160.52 ± 7.37
	0.418	0.0002	33.146	0.033	0.033	165.73	
GCS-3	0.544	0.0002	59.396	0.059	0.059	296.98	296.46 ± 0.74
	0.543	0.0002	59.188	0.059	0.059	295.94	
GCS-4	0.665	0.0002	84.604	0.085	0.085	423.02	424.06 ± 1.47
	0.667	0.0002	85.021	0.085	0.085	425.10	
GCR-1	0.332	0.0002	15.229	0.015	0.015	76.15	68.85 ± 10.31
	0.318	0.0002	12.313	0.012	0.012	61.56	
GCR-2	0.412	0.0002	31.896	0.032	0.032	159.48	162.08 ± 3.68
	0.417	0.0002	32.938	0.033	0.033	164.69	
GCR-3	0.561	0.0002	62.938	0.063	0.063	314.69	315.73 ± 1.47
	0.563	0.0002	63.354	0.063	0.063	316.77	
GCR-4	0.783	0.0002	109.188	0.109	0.109	545.94	548.54 ± 3.68
	0.788	0.0002	110.229	0.110	0.110	551.15	

The results of the present study showed that the methanol extract of root (GCR-4), methanol extract of stem (GCS-4), ethyl acetate extract of root (GCR-3) and ethyl acetate extract of stem (GCS-3) have higher phenolic content with 548.54±3.68, 424.06±1.47, 315.73±1.77 & 296.46±0.74 mg/g, respectively as equivalent to standard GAE. Higher phenolic content indicates good antioxidant properties.

Total Flavonoids Content

The aluminum chloride colorimetric method was used to measure the total flavonoid contents (TFC) of the extracts of the *Garcinia cowa*. The TFCs given as QE/g of the plant extract were estimated using the standard quercetin curve (y=0.0057x + 0.0186; R^2 = 0.9801). The flavonoid content results are shown in table 2 and figure 2 below.

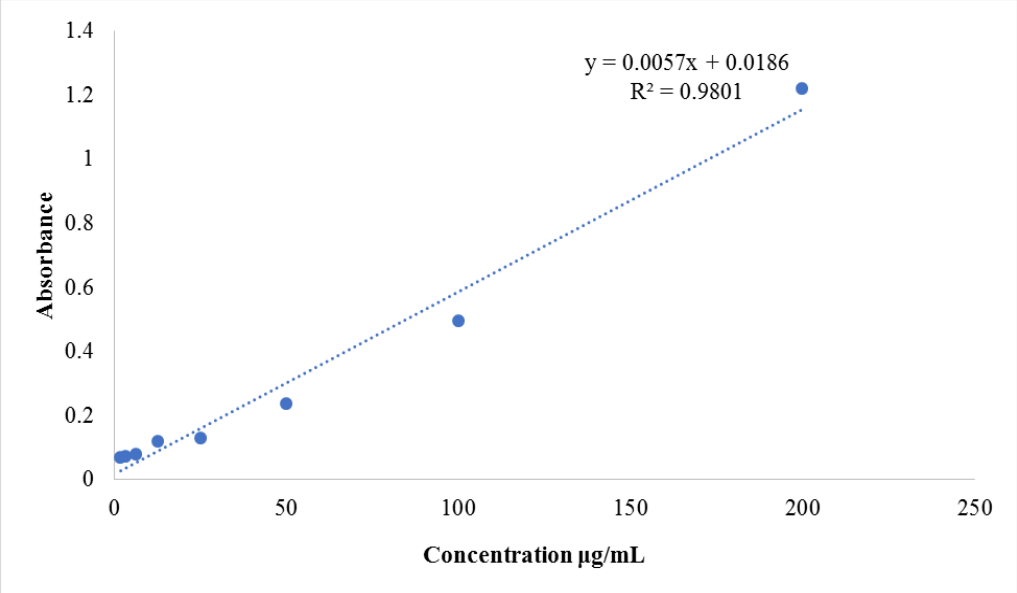

Figure 2: Quercetin calibration curve.

Table 2: *Garcinia cowa* extracts total flavonoid contents.

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	QE Conc. (C) µg/ml	QE Conc. (C) mg/ml	c*V (mg)	A=(c*V)/m TFC as QE, (mg/g)	Mean ± SD (mg/g)
GCL-1	0.257	0.0002	41.825	0.042	0.042	209.12	218.77 ±13.65
	0.279	0.0002	45.684	0.046	0.046	228.42	
GCL-2	0.343	0.0002	56.912	0.057	0.057	284.56	289.39 ± 6.82
	0.354	0.0002	58.842	0.059	0.059	294.21	
GCL-3	0.286	0.0002	46.912	0.047	0.047	234.56	239.82 ± 7.44
	0.298	0.0002	49.018	0.049	0.049	245.09	
GCL-4	0.227	0.0002	36.561	0.037	0.037	182.81	188.95 ± 8.68
	0.241	0.0002	39.018	0.039	0.039	195.09	
GCS-1	0.233	0.0002	37.614	0.038	0.038	188.07	192.89 ± 6.82
	0.244	0.0002	39.544	0.040	0.040	197.72	
GCS-2	0.221	0.0002	35.509	0.036	0.036	177.54	179.30 ± 2.48
	0.225	0.0002	36.211	0.036	0.036	181.05	
GCS-3	0.391	0.0002	65.333	0.065	0.065	326.67	327.11 ± 0.62
	0.392	0.0002	65.509	0.066	0.066	327.54	
GCS-4	0.265	0.0002	43.228	0.043	0.043	216.14	216.14 ± 0.00
	0.265	0.0002	43.228	0.043	0.043	216.14	
GCR-1	0.3	0.0002	49.368	0.049	0.049	246.84	238.95 ±11.16
	0.282	0.0002	46.211	0.046	0.046	231.05	
GCR-2	0.364	0.0002	60.596	0.061	0.061	302.98	307.81± 6.82
	0.375	0.0002	62.526	0.063	0.063	312.63	
GCR-3	0.661	0.0002	112.702	0.113	0.113	563.51	559.56 ± 5.58
	0.652	0.0002	111.123	0.111	0.111	555.61	
GCR-4	0.408	0.0002	68.316	0.068	0.068	341.58	341.14 ± 0.62
	0.407	0.0002	68.140	0.068	0.068	340.70	

The present study of total flavonoids contents in the extracts of *Garcinia cowa* showed that ethyl acetate, methanol & chloroform extracts of root, and ethyl acetate extract of the stem have higher flavonoids content 559.56±5.58, 341.14±0.62, 307.81±6.82 & 327.11±0.62 mg/g, respectively of flavonoids as QE. The higher flavonoid content is allied to good antioxidant action.

Total Antioxidant Capacity

When supplied at low concentrations compared to those contained in an oxidizable substrate (polypeptides, triglycerides, carbohydrates, and DNA), any molecule that considerably slows down or stops that substrate from oxidizing is referred to be an antioxidant.[22,23] Antioxidants' primary purpose is to shield the organism against the harm that free radical damage might cause.[24] Phenolic molecules are primarily responsible for antioxidant action. The phospho-molybdenum approach is based on the creation of a green phosphate/Mo (V) complex with maximal absorbance at 695 nm and the lowering of Mo (V1) to Mo (V) by the

antioxidant component. The amount of ascorbic acid equivalents per gram in plant extracts represents the total antioxidant capacity of such extracts (y=0.004x + 0.0516; R2 =0.9984). The results of the total antioxidant study showed in table 3 and figure 3.

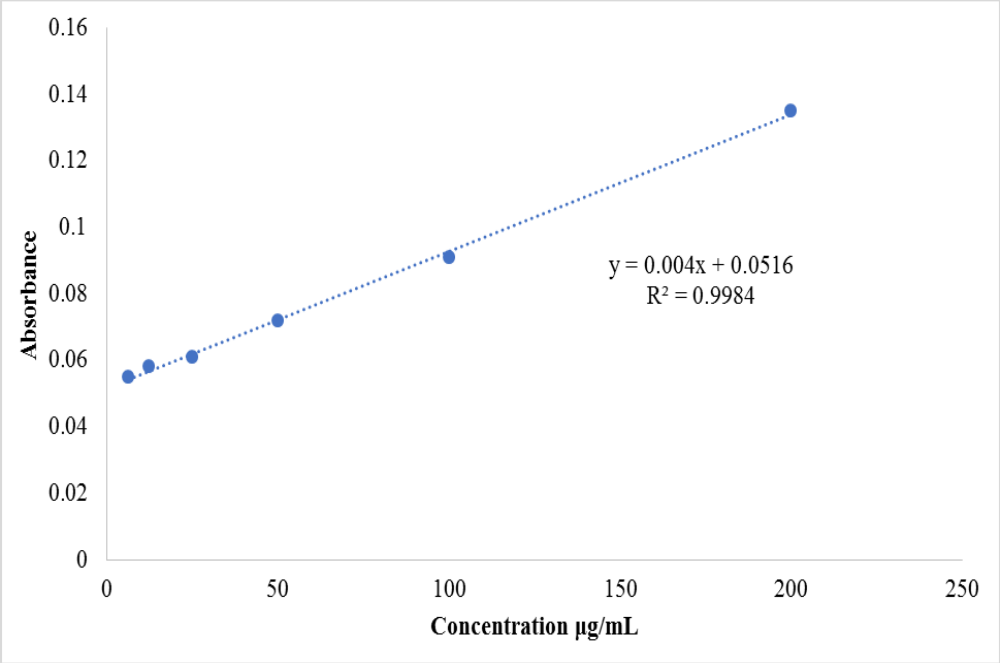

Figure 3: Calibration curve for ascorbic acid.

Table 3: Total antioxidant capacity of the extracts of *Garcinia cowa.*

Sample ID	Abs.	Plant Extracts Wt. (g/mL)	Conc. (C) as AAE µg/ml	Conc. (C) as AAE mg/ml	c*V (mg)	A=(c*V)/m TAC as AAE, (mg/g)	Mean ± SD (mg/g)
GCL-1	0.312	0.0002	65.100	0.065	0.065	325.50	326.75 ± 1.77
	0.314	0.0002	65.600	0.066	0.066	328.00	
GCL-2	0.289	0.0002	59.350	0.059	0.059	296.75	296.13 ± 0.88
	0.288	0.0002	59.100	0.059	0.059	295.50	
GCL-3	0.318	0.0002	66.600	0.067	0.065	333.00	330.50 ± 3.54
	0.314	0.0002	65.600	0.066	0.066	328.00	
GCL-4	0.245	0.0002	48.350	0.048	0.048	241.75	243.63 ± 2.65
	0.248	0.0002	49.100	0.049	0.049	245.50	
GCS-1	0.412	0.0002	90.100	0.090	0.090	450.50	451.75 ± 1.77
	0.414	0.0002	90.600	0.091	0.091	453.00	
GCS-2	0.347	0.0002	73.850	0.074	0.074	369.25	366.75 ± 3.54
	0.343	0.0002	72.850	0.073	0.073	364.25	
GCS-3	0.148	0.0002	24.100	0.024	0.024	120.50	118.63 ± 2.65
	0.145	0.0002	23.350	0.023	0.023	116.75	
GCS-4	0.341	0.0002	72.350	0.072	0.072	361.75	366.13 ± 6.19
	0.348	0.0002	74.100	0.074	0.074	370.50	
GCR-1	0.455	0.0002	100.850	0.101	0.101	504.25	506.13 ± 2.65
	0.458	0.0002	101.600	0.102	0.102	508.00	

GCR-2	0.322	0.0002	67.600	0.068	0.068	338.00	336.75 ± 1.77
	0.32	0.0002	67.100	0.067	0.067	335.50	
GCR-3	0.427	0.0002	93.850	0.094	0.094	469.25	471.13 ± 2.65
	0.43	0.0002	94.600	0.095	0.095	473.00	
GCR-4	0.084	0.0002	8.100	0.008	0.008	40.50	39.88 ± 0.88
	0.083	0.0002	7.850	0.008	0.008	39.25	

TAC study of the extracts of *Garcinia cowa* showed that n-hexane & ethyl acetate extracts of root and n-hexane extract of the stem have a significant total antioxidant capacity with 506.13±2.65, 471.13±2.65 and 451.75±1.77 mg/g, respectively AAE. Again, chloroform & methanol extracts of the stem, chloroform extract of root, and ethyl acetate extract of the stem showed moderate total antioxidant capacity with 366.75±3.54, 366.13±6.19, 336.75±1.77 and 330.50±3.54 mg/g, respectively. Other extracts also showed a considerable amount of total antioxidant capacity compared to the standard.

DPPH Free Radical Scavenging Assay

When DPPH receives an electron provided by an antioxidant chemical, it decolorizes, which may be quantified by alterations in absorbance. The results and IC_{50} values of the extracts of the *Garcinia cowa* are presented in Table 4 & Table 4.

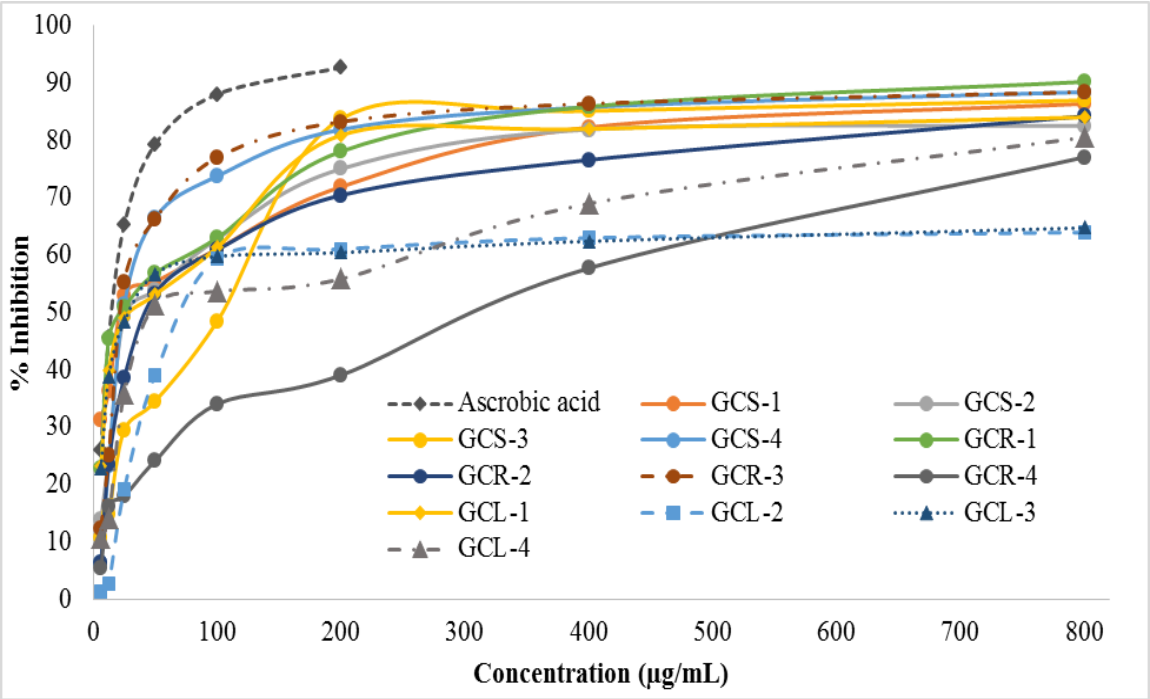

Figure 4: Comparative % of inhibition VS concentration of standard and extracts.

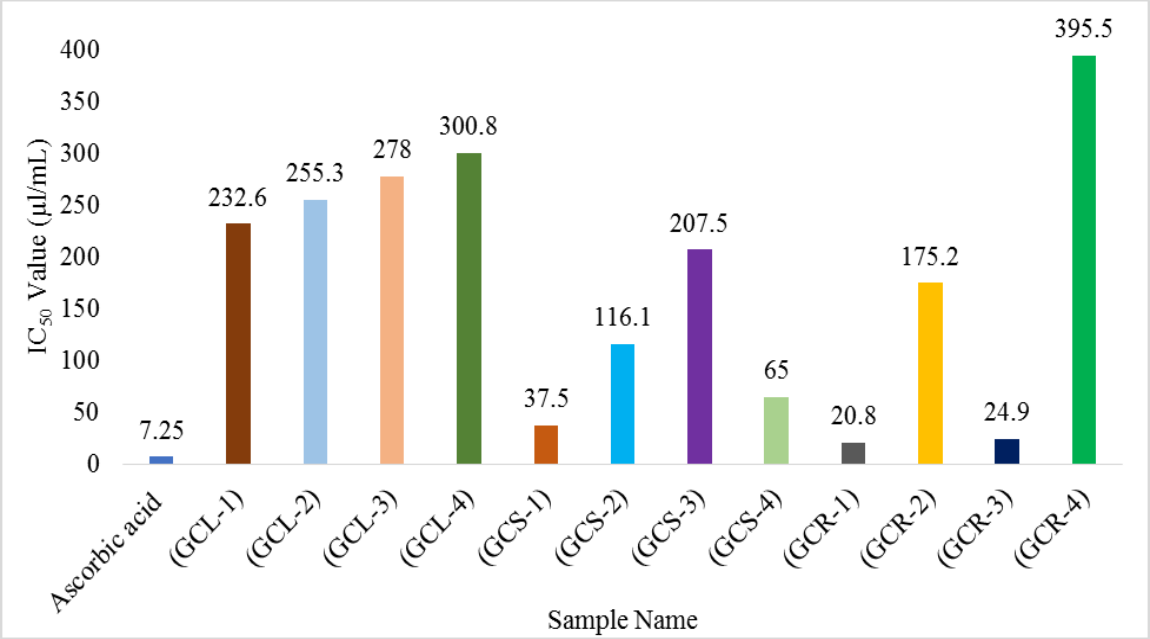

Figure 5: IC_{50} Values of standard and extracts of *Garcinia cowa.*

The DPPH free radical scavenging technique is a well-liked method for evaluating the antioxidant or free radical scavenging properties of plant extracts. When reduced by hydrogen or electron transfer, the stabilized nitrogen-centered free radical (DPPH) colour changes from violet to yellow.[22] A lower IC_{50} value indicates that the decolorization impact is more pronounced the stronger the antioxidant activity. The DPPH radical scavenging assay of the extracts exhibited that, n-hexane (GCR-1) & ethyl acetate (GCR-3) extracts of root and n-hexane extract of stem (GCS-1) showed significant free radical scavenging capacity with IC_{50} value 20.80, 24.9 and 37.5 µg/mL, respectively as compared to standard ascorbic acid with IC_{50} value 7.25 µg/mL. Again, methanol (GCS-4) and chloroform (GCS-3) extracts of stem showed moderate free radical scavenging capacity with IC_{50} value 65.0 and 116.1 µg/mL, respectively. Other extracts showed slightly free radical scavenging capacity compared to standard.

Brine Shrimp Lethality Bioassay

Lethality of Brine Shrimp Cytotoxic activity bioassay (BSLT) is a general bioassay that appears to identify a wide range of bioactivity found in crude extracts. It seems that BSLT is predictive of cytotoxicity and pesticide activity and is widely used in the bioassay for bioactive compounds.[25] The Brine Shrimp Lethality Bioassay was used to measure the cytotoxic activity of each extract. The results of the cytotoxicity study are presented in the following table 5-17 and figure 6,7.

Table 5: % mortality at different concentration & LC_{50} values of vincristine sulphate.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
0.313	-0.50446	10	0	10	0	**0.544**	**3.501**
0.625	-0.20412	10	0	10	0		
1.25	0.09691	13	2	8	15		
2.5	0.39794	10	5	5	50		
5	0.69897	13	7	6	54		
10	1	10	8	2	80		

Table 6: % mortality at different concentration & LC_{50} values of GCL-1.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	0	10	0	3.1155	1304.67
12.5	1.097	10	1	9	10		
25	1.398	10	2	8	20		
50	1.699	10	2	8	20		
100	2.000	10	3	7	30		
200	2.301	10	3	7	30		
400	2.602	10	4	6	40		

Table 7: % mortality at different concentration & LC_{50} values of GCL-2.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	0	10	0	3.545	3507.52
12.5	1.097	10	1	9	10		
25	1.398	10	1	9	10		
50	1.699	10	2	8	20		
100	2.000	10	2	8	20		
200	2.301	10	2	8	20		
400	2.602	10	4	6	40		

Table 8: % mortality at different concentration & LC_{50} values of GCL-3.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	1	9	10	1.43413	27.17
12.5	1.097	10	3	7	30		
25	1.398	10	5	5	50		
50	1.699	10	7	3	70		
100	2.000	10	9	1	90		
200	2.301	10	10	0	100		
400	2.602	10	10	0	100		

Table 9: % mortality at different concentration & LC_{50} values of GCL-4.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	0	10	0	3.0672	1167.35
12.5	1.097	10	0	10	0		
25	1.398	10	0	10	0		
50	1.699	10	1	9	10		
100	2.000	10	1	9	10		
200	2.301	10	3	7	30		
400	2.602	10	5	5	50		

Table 10: % mortality at different concentration & LC_{50} values of GCS-1.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	1	9	10	1.6681	46.57
12.5	1.097	10	2	8	20		
25	1.398	10	4	6	40		
50	1.699	10	6	4	60		
100	2.000	10	6	4	60		
200	2.301	10	7	3	70		
400	2.602	10	10	0	100		

Table 11: % mortality at different concentration & LC_{50} values of GCS-2.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	0	10	0	2.8068	640.91
12.5	1.097	10	0	10	0		
25	1.398	10	0	10	0		
50	1.699	10	1	9	10		
100	2.000	10	2	8	20		
200	2.301	10	4	6	40		
400	2.602	10	5	5	50		

Table 12: % mortality at different concentration & LC_{50} values of GCS-3.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	0	10	0	2.4214	263.88
12.5	1.097	10	0	10	0		
25	1.398	10	1	9	10		
50	1.699	10	2	8	20		
100	2.000	10	3	7	30		
200	2.301	10	5	5	50		
400	2.602	10	6	4	60		

Table 13: % mortality at different concentration & LC_{50} values of GCS-4.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	0	10	0	2	100
12.5	1.097	10	1	9	10		
25	1.398	10	1	9	10		
50	1.699	10	3	7	30		
100	2.000	10	5	5	50		
200	2.301	10	7	3	70		
400	2.602	10	8	2	80		

Table 14: % mortality at different concentration & LC_{50} values of GCR-1.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	0	10	0	1.799	62.95
12.5	1.097	10	1	9	10		
25	1.398	10	2	8	20		
50	1.699	10	4	6	40		
100	2.000	10	6	4	60		
200	2.301	10	8	2	80		
400	2.602	10	10	0	100		

Table 15: % mortality at different concentration & LC_{50} values of GCR-2.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	2	8	20	1.578	37.84
12.5	1.097	10	4	6	40		
25	1.398	10	4	6	40		
50	1.699	10	5	5	50		
100	2.000	10	7	3	70		
200	2.301	10	7	3	70		
400	2.602	10	9	1	90		

Table 16: % mortality at different concentration & LC_{50} values of GCR-3.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	0	10	0	2.027	106.41
12.5	1.097	10	2	8	20		
25	1.398	10	3	7	30		
50	1.699	10	3	7	30		
100	2.000	10	4	6	40		
200	2.301	10	6	4	60		
400	2.602	10	8	2	80		

Table 17: % mortality at different concentration & LC_{50} values of GCR-4.

Conc. (µg/ml)	Log Conc.	(N_0) No. of taken nauplii	No. of dead nauplii	(N_1) No. of alive nauplii	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
6.25	0.796	10	0	10	0	2.043	110.41
12.5	1.097	10	1	9	10		
25	1.398	10	1	9	10		
50	1.699	10	2	8	20		
100	2.000	10	3	7	30		
200	2.301	10	6	4	60		
400	2.602	10	10	0	100		

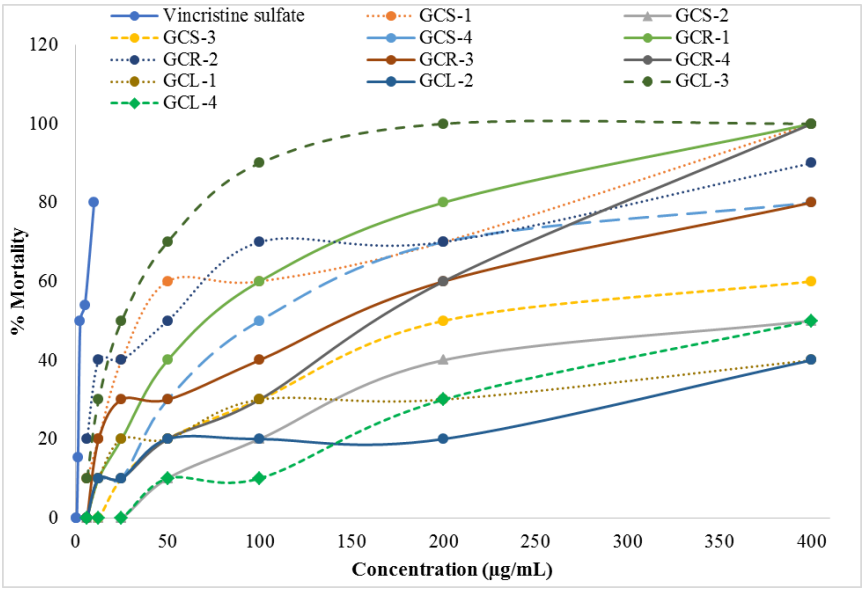

Figure 6: Comparative % of mortality VS concentration of standard and extracts.

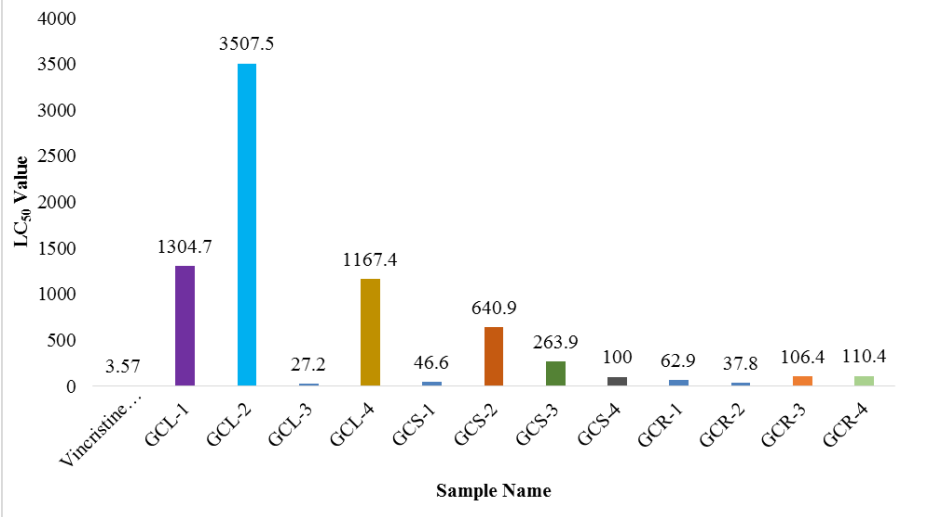

Figure 7: LC_{50} Values of extracts of *Garcinia cowa* and standard.

The cytotoxicity study of the extracts exhibited that ethyl acetate extract of the leaf (GCL-3) and chloroform extract of root (GCR-2), and n-hexane extract of the stem (GCS-1) showed significant cytotoxicity with LC_{50} value 27.17, 37.84 and 46.57 µg/mL, respectively compared to the standard vincristine sulfate with LC_{50} value 3.501µg/mL. Again, the n-hexane extract of root (GCR-1), methanol extract of the stem (GCS-4), ethyl acetate (GCR-3), and methanol (GCR-4) extracts of root showed moderate cytotoxicity compared to the standard. Ethyl acetate extract of the stem (GCS-3) showed minor cytotoxicity compared to the standard. Other extracts showed nonsignificant cytotoxicity compared to the standard.

CONCLUSION

Antioxidant studies in different methods exhibited that n-hexane & ethyl acetate extracts of root and n-hexane extract of stem showed significant free radical scavenging capacity compared to standard ascorbic acid. The cytotoxicity study of the extracts exhibited that ethyl acetate extract of leaf, chloroform extract of root, and n-hexane extract of stem showed moderate significant cytotoxicity. The findings of our study suggested that the leaves, stems, and roots of *Garcinia cowa* growing in Bangladesh have potent natural therapeutic agents for antioxidants.

CONFLICT OF INTEREST

Each author confirmed that they had no contesting interests.

AUTHORS DECLARATION

The authors affirm the originality of the material presented in this article and agree to accept any duties or rights associated with its content.

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