Antioxidant, Cytotoxicity and *In vitro* Anti-inflammatory Study on the Different Extracts of Stems and Roots of the Two Medicinal Plants: *Cassia sophera* and *Jatropha curcas*

ABSTRACT

Different extracts of stems and roots of *Cassia sophera* and *Jatropha curcas* were screened for antioxidant, cytotoxic activity and *in-vitro* anti-inflammatory activity using various methods. DPPH free radical scavenging assay of *Cassia sophera* & *Jatropha curcas* showed that methanol extract of root and stem have significant antioxidant activity with IC_{50} value 2.42 & 7.49 µg/ml, respectively in comparison to standard with IC_{50} value 4.38 µg/ml. In total phenolic content determination experiment, the results showed that, for *cassia sophera*, methanol extract of stems have higher phenolic content i.e 273.14±10.69 mg/g as equivalent to gallic acid whereas for *Jatropha curcas* it is ethyl acetate extract of stem with value 154.53±5.76 mg/g. The total flavonoids content study of the extracts of *Cassia sophera* exhibited that, ethyl acetate extract of root has higher flavonoid content i.e. 105.37±5.89 mg/g of flavonoids as equivalent to quercetin whereas for *Jatropha curcas* it is chloroform extract of stem with value 50.28±6.55 mg/g. The cytotoxicity study exhibited that all the extracts have lower cytotoxic property compared to standard for *Cassia sophera* whereas for *Jatropha curcas*, chloroform extract of stem has significant cytotoxicity with LC_{50} value 4.52 µg/ml compared to standard vincristine sulfate with LC_{50} value 3.04 µg/ml. From *in-vitro* anti-inflammatory assay of methanol extracts of stems and roots, the result showed that both plants have strong activity in compared to standard diclofenac sodium.

KEYWORDS: *Cassia sophera*, *Jotropha curcas*, Antioxidant activity, Phenolic content, Flavonoid content, Cytotoxicity, *In-vitro* anti-inflammatory activity.

INTRODUCTION

Plants offer a wide range of possible applications, particularly in traditional medicine and pharmaceuticals. Due to the paucity and expensive expense of orthodox treatment, a sizable segment of the global population relies on traditional medicine.[1] Numerous therapeutic compounds have been produced from medicinal plants.[2] There are many plants that contain a variety of phytopharmaceuticals, which are important in human, veterinary, and agricultural medicine. The discovery of new drug leads for the treatment and prevention of diseases depends heavily on natural products.[3]

The Caesalpinaceae plant *Cassia sophera*, often known as "Kasondi," is a key component in Unani medicine. The plant can be found all over India, Bangladesh[4] and in the majority of tropical nations[5]. It frequently occurs in wastelands, by the sides of roads, and in forests.[6] *Cassia sophera* is a glabrous, three-meter-tall shrub. The compound leaves have eight to twelve pairs of sharp, tapering leaflets, and they yield rachies with a solitary gland at the base. It bears carymbose racemes of yellow flowers. It has also been reported to be helpful for the treatment of symptoms of epilepsy include ascites, piles, skin conditions, jaundice, fever, articular pain, and palpitations.[7,8] In cases of rheumatic and inflammatory fever, it serves as a febrifuge. However, it is also utilized in some homeopathic and ayurvedic immunomodulatory medicine.[9] It has been noted that *Cassia sophera* Linn possesses anti-inflammatory and analgesic properties.[10] Significant liver protection was provided by an aqueous extract of *Cassia sophera* leaves when exposed to ranitidine-induced liver injury.[11,12] Phytochemical studies on this medicinal plant have already been conducted in a number of nations, including Bangladesh, India, Pakistan, Thailand, and other regions of the world. However, no thorough biological and phytochemical investigations on the extracts from this plant's stems and roots have been conducted in Bangladesh. Previous phytochemical screenings also resulted in the isolation of novel anthraquinone sopheranin, as well as β-sitosterol, chrysophenol, physcion,

and emodin[13]. From the seeds of *Cassia sophera*, a brand-new cycloartane triterpene glycoside known as cyclosphoroside A was isolated[14].

Jatropha curcas is a woody shrub belonging to the Euphorbiaceae family and spreads all over the world. It is known as goat nuts or just *Jatropha* in different regions all over the world. It is very much common plant grows in Bangladesh and locally known as Jamal Gota. According to earlier studies, the *Jatropha* plant is indigenous to Central and South America. It is an important medicinal plant for novel pharmaceuticals. The plant has attracted the attention of chemists for its medicinal values. Different parts of the plants are used by the Ayuvedists and Yunans to cure various diseases. It has long been used in different systems of medicine in the treatment of cancer, diabetics, inflammation, dysentery, angiogenesis effects and also potent for anti-bacterial activities.[15,16]

Therefore, the present study was undertaken with an objective to determine antioxidant and cytotoxic activities of the different extracts of stems and roots of *Casia sophera* and *Jatropha curcas*.

MATERIALS AND METHODS

Collection of Plant Material and Identification of the Plant

Stems and roots of *C. sophera* and *J. curcas* were collected from the National Botanical Garden in Mirpur, Dhaka, Bangladesh. The plants were then identified and authenticated by taxonomist of Bangladesh National Herbarium (BNH). Voucher specimen of these two plants was deposited at BNH under the accession number DACB-66332 and 39512 for *C. sophera* & *J. curcas* respectively.

Extraction of Crude Samples from Stems and Roots of the Plants

The stems and roots were cut into small pieces and dried well under the shed. After that, the dried stems and roots were turned into powder separately by using a grinder machine. The dried

powder of stems and roots were then extracted successively with n-hexane, chloroform, ethyl acetate and methanol at room temperature.

Test for Determination of Antioxidant Activity

DPPH Free Radical Scavenging Assay

The free radical scavenging activity was analysed spectrophotometrically by DPPH method[17]. DPPH is a reactive free radical that acts as an electron acceptor and causes the oxidation of other substances. On the other hand, antioxidants act as electron donors. Antioxidants neutralize DPPH by being oxidized themselves. DPPH is found as a dark-coloured crystalline powder composed of stable free-radical molecules and forms deep violet colour in solution. Different concentrations (800, 400, 200, 100, 50, 25, 12.5, 6.25 mg/ml in methanol) of ascorbic acid solution (1 ml) as well as *Cassia sophera* stem and root extract solution (1 ml) were mixed separately with 3 ml of 0.4 mM DPPH solution. The mixtures were kept in dark for 30 minutes to measure absorbance at 517 nm using UV-Visible spectrophotometer where ascorbic acid was used as a positive control. The whole procedure was performed three times for each test solution. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging of DPPH free radical is indicated by the deep violet colour being turned into pale yellow or colourless.

Calculation:

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

IC_{50} is the concentration at which 50% of the total DPPH free radical is scavenged/ neutralized and can be determined by the linear regression method from plotting % inhibition against corresponding concentration. Each of the method was performed three times and the results were averaged.

Determination of Total Phenolic Content

The total phenol content in extracts was determined using Folin-Ciocalteu reagent (FCR) based on colorimetric reaction[18]. The FCR is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants [16]. In brief, 1 mL of plant extract (200 µg/mL) or standard of different concentrations (200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) was taken in test tubes. Then, 5 mL of FCR and 7.5 % Na_2CO_3 were added to it and mixed well. Moreover, test tubes containing standard incubated for 30 minutes to complete the reaction but the test tubes containing extract solution were incubated for 1 hr at room temperature. Absorbance was taken at 765 nm by UV-VIS spectrophotometer. Gallic acid was used as a standard to produce a calibration curve. The phenol content in extracts was expressed as mg of gallic acid equivalents (GAE) /g of extract.

The total content of phenolic compounds in plant extracts in gallic acid equivalents (GAE) was calculated using the following equation:

$C = (c \times V)/m$,

Where; C = total content of phenolic compounds, mg/g plant extract, in GAE.

c = the concentration of gallic acid established from the calibration curve in mg/ml.

V = the volume of extract in ml

m = the weight of crude plant extract in gm.

Determination of Total Flavonoids Content

Total flavonoid content in plant extracts was determined using the colorimetric method involving aluminum chloride[19]. In this method, 1 mL of plant extract (200 µg/mL) or standard of different concentrations (200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) was taken in the test tube. 3 mL of methanol, 200 µl of 10 % aluminum chloride solution and 200 µl of 1M potassium

acetate solution were added to the previously mentioned test tube. Finally, 5.6 ml of distilled water was mixed with the reaction mixture. The reaction mixture was then incubated for 30 minutes at room temperature and the absorbance of the solution was measured at 415 nm using a spectrophotometer against of blank. Quercetin was used as a reference standard and flavonoid contents of extracts were calculated as mg of quercetin equivalents (QE)/ g of extract.

The total content of flavonoid compounds in plant extracts in quercetin equivalents was calculated using the following equation:

$C = (c \times V)/m,$

Where; C = total content of flavonoids compounds, mg/g plant extract, in quercetin equivalents.

c = the concentration of quercetin established from the calibration curve in mg/ml.

V = the volume of extract in ml

m = the weight of crude plant extract in gm.

Determination of Total Antioxidant Capacity

The total antioxidant activity of the extract can be evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*[20]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green PO_4/Mo (V) complex at acidic p^H. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reactants and Mo (VI) and formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. In this method, 300 µl of each plant extracts or standard of different concentration (200, 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) solutions were taken into different test tubes. Then 3ml of phosphomolybdate reagent was added into each of the test tubes. The test tubes incubated at 95°C for 90 minutes to

complete the reaction. Then the absorbance was measured at 695 nm using a spectrophotometer.

The total antioxidant activity in plant extracts is expressed as ascorbic acid equivalents and was calculated using the following equation:

C = (c x V)/m,

Where; C = total content of antioxidant compounds, mg/g plant extract, in ascorbic acid equivalents.

c = the concentration of ascorbic acid established from the calibration curve in mg/ml.

V = the volume of extract in ml

m = the weight of crude plant extract in gm.

Brine Shrimp Lethality Bioassay (Cytotoxicity Study)

The cytotoxic activity was performed by brine shrimp lethality bioassay method[21]. In this method, 32 mg of each of the test samples were taken and dissolved in 200 µl of pure dimethyl sulfoxide (DMSO) and finally, the volume was made to 20 ml with seawater. Thus, the concentration of the stock solution was 800 µl/ml. Then the solution was serially diluted to 400, 200, 100, 50, 25, 12.5, 6.25 µl/ml with seawater. Then 5 ml of plant extract solution was added to 5 ml of seawater containing 10 nauplii and incubated at room temperature for 24 hrs. After 24 hours, the test tube was inspected using a magnifying glass against a black background and the number of survived nauplii in each tube was counted. From this data, the percentage of the brine shrimp nauplii was calculated for each concentration. The mortality was calculated using the formula.

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

The effectiveness of the concentration mortality relationship of plant products is usually expressed as a median lethal concentration (LC_{50}). This represents the concentration of the chemical that produces death in half of the test subjects, after a certain exposure time and is determined by linear regression method from plotting percentage mortality against the correspondent log of concentration. Vincristine sulphate was used as positive control in this assay to compare cytotoxicity of the test samples.

In Vitro Anti-Inflammatory Assays

Inhibition of bovine albumin denaturation

To evaluate the anti-inflammatory effects of the extracts, the standard protocols were used with minor modifications [22,23]. A volume of 1 ml of extracts (aqueous and ethanolic) or of diclofenac sodium at different concentrations (100, 200, 500, and 1000 μg/ml) was homogenised with 1 ml of an aqueous solution of bovine serum albumin (5%) and incubated at 27 °C for 15 minutes. The mixture of distilled water and BSA constituted the control tube. Denaturation of the proteins was caused by placing the mixture in a water bath for 10 minutes at 70 °C. The mixture was cooling inside the ambient room temperature, and each mixture's activity was measured at 660 nm.

Inhibition of egg albumin denaturation

Protein denaturation was performed with slight modifications for reference protocol [24]. Test solution consisting of 1 ml of different concentrations of plant extract ranging from 100-500 µg/ml or standard diclofenac sodium as same concentration was mixed with 1 ml of egg albumin solution (1 mM) and incubated at 27±1 ˚C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 70 ˚C in a water bath for 10 minutes. After cooling, the turbidity was measured spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate, and the average was taken. The following formula was used to calculate the inhibition percentage:

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

DPPH Free Radical Scavenging Assay

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The results and IC_{50} values of the extracts of the *Cassia sophera* and *Jatropha curcas* are presented in the tables 1-3 & figure 1.

Table 1: The percentage (%) of scavenging activity of ascorbic acid (AA)

Concentration (µg/mL)	Absorbance of Sample	% Scavenging of ascorbic acid
12.5	0.921	37.39
25	0.648	55.95
50	0.429	70.84
100	0.284	80.69
200	0.179	87.83

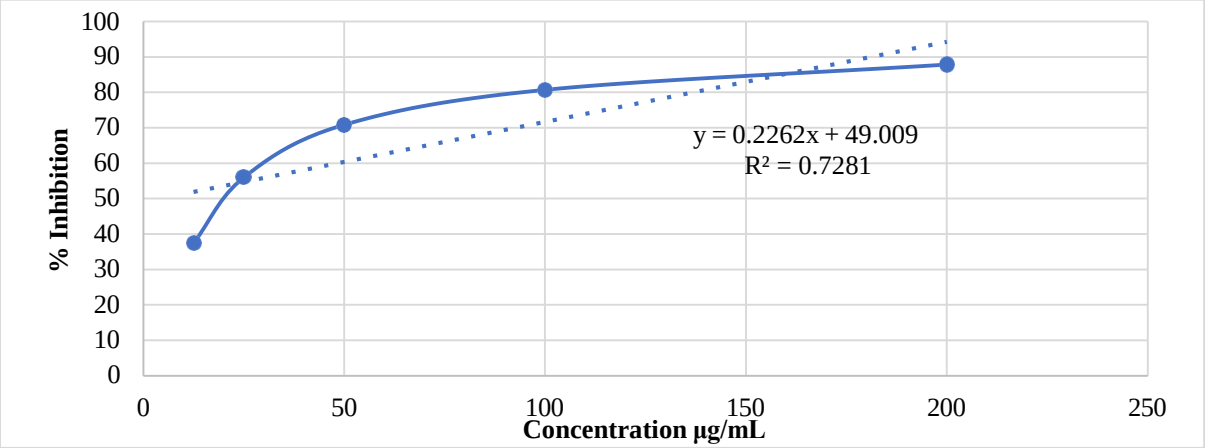


Figure 1: DPPH free radical scavenging assay of ascorbic acid

Table 2: IC_{50} values of standard and extracts for *Cassia sophera*

Sample Name	IC_{50} Value (µg/mL)
Ascorbic acid	4.38
n-Hexane extract of stem (CS-1)	1258.64
Chloroform extract of stem (CS-2)	2403.85
Ethyl acetate extract of stem (CS-3)	56.46
Methanol extract of stem (CS-4)	31.85
n-Hexane extract of root (CS-5)	127.38
Chloroform extract of root (CS-6)	255.06
Ethyl acetate extract of root (CS-7)	21.97
Methanol extract of root (CS-8)	2.42

The evaluation of a compound's ability to scavenge free radicals or the antioxidant capacity of plant extracts is frequently done using the DPPH radical scavenging method[25]. The color of DPPH changes from violet to yellow upon reduction by either the action of hydrogen or electron donation[26]. Higher antioxidant activity, as seen by a lower IC_{50} value, is correlated with increased decolorizing action.

The result of the DPPH free radical scavenging assay showed that methanol extract of root (CS-8) has significant antioxidant activity with IC_{50} value 2.42 µg/ml in comparison to standard with IC_{50} 4.38 µg/ml & ethyl acetate extract of root (CS-7) exhibited good antioxidant property. Again, methanol and ethyl acetate extract of stem (CS-4 & CS-3) showed moderate free radical scavenging activity. Other extracts showed non-significant free radical scavenging activity in comparison to the standard. [**Table-2**]

Table 3: IC_{50} values of standard and extracts for *Jatropha curcas*

Sample Name	**IC_{50} Value (µg/mL)**
Ascorbic acid	4.38
n-Hexane extract of stem (JC-1)	61.33
Chloroform extract of stem (JC-2)	291.66
EA extract of stem (JC-3)	492.71
MeOH extract of stem (JC-4)	7.49
n-Hexane extract of root (JC-5)	53.13
Chloroform extract of root (JC-6)	46.79
EA extract of stem root (JC-7)	223.36
MeOH extract of root (JC-8)	20.88

For *Jatropha curcas,* DPPH free radical scavenging assay showed that, methanol extracts of stem (JC-4) have significant free radical scavenging activity with IC_{50} value 7.49 µg/ml compared to the standard ascorbic acid with IC_{50} value 4.38 µg/ml & methanol extract of root (JC-8) have good antioxidant activity. Again, chloroform & n-hexane extract of root (JC-6 & JC-5) and n-hexane extract of stem (JC-1) showed moderate free radical scavenging activity with IC_{50} value 46.79, 53.13 and 61.33 µg/ml, respectively in compared to the standard. Other extracts showed non-significant free radical scavenging activity.

Total Phenol Content Determination

The total phenolic contents of the methanolic extracts are calculated using the standard curve of gallic acid (y =0.0043x + 0.0926; R^2 = 0.9964).

Table 4: Absorbance found with different concentrations of gallic acid

Concentration (µg/mL)	Absorbance
1.56	0.092
3.125	0.101
6.25	0.118
12.5	0.15
25	0.208
50	0.288
100	0.562
200	0.94

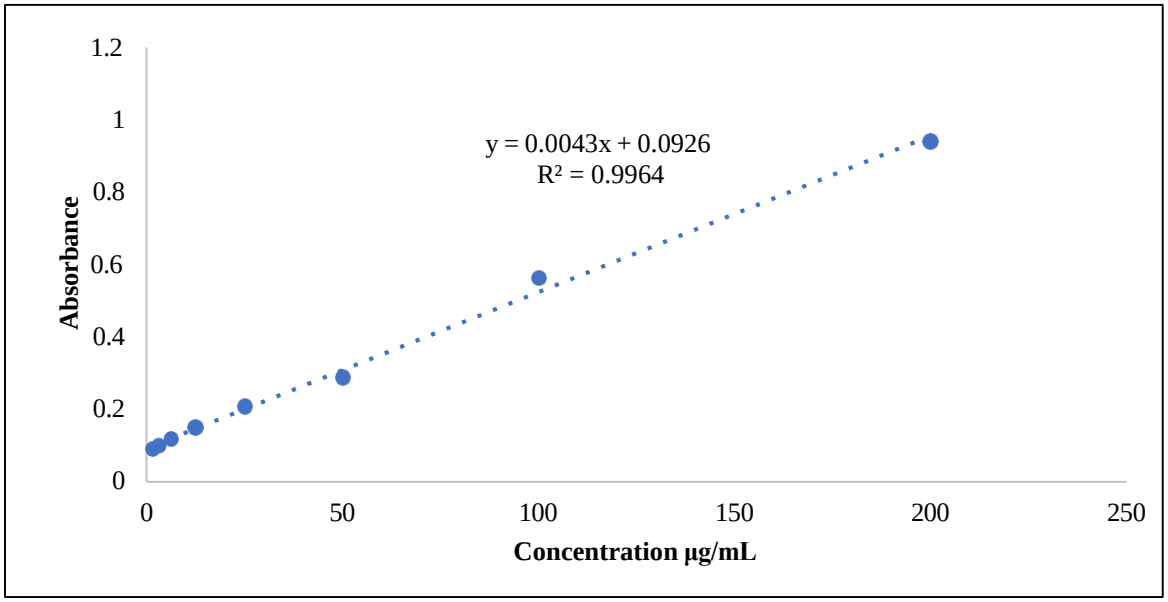


Figure 2: Calibration curve of gallic acid

Table 5: Total phenolic contents present in extracts of *Cassia sophera*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	GAE conc. (C) (µg/ml)	GAE conc. (C) (mg/ml)	V (mL)	TPC as GAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
CS-1	0.104	0.0002	2.651	0.003	1	13.26	10.35±4.11
	0.099	0.0002	1.488	0.001	1	7.44	
CS-2	0.116	0.0002	5.442	0.005	1	27.21	32.44±7.40
	0.125	0.0002	7.535	0.008	1	37.67	
CS-3	0.246	0.0002	35.674	0.036	1	178.37	184.19±8.22
	0.256	0.0002	38.000	0.038	1	190.00	
CS-4	0.213	0.0002	28.000	0.028	1	140.00	137.9±4.11
	0.208	0.0002	26.837	0.027	1	134.19	
CS-5	0.14	0.0002	11.023	0.011	1	55.12	55.7-±0.80
	0.141	0.0002	11.256	0.011	1	56.28	
CS-6	0.185	0.0002	21.488	0.021	1	107.44	99.30±11.51
	0.171	0.0002	18.233	0.018	1	91.16	
CS-7	0.321	0.0002	53.116	0.053	1	265.58	273.14±10.69
	0.334	0.0002	56.140	0.056	1	280.70	
CS-8	0.257	0.0002	38.233	0.038	1	191.16	191.16±0.00
	0.257	0.0002	38.233	0.038	1	191.16	

CS-1: n-Hexane extract of stem, CS-2: $CHCl_3$ extract of stem, CS-3: Ethyl acetate extract of stem, CS-4: MeOH extract of stem, CS-5: n-Hexane extract of root, CS-6: $CHCl_3$ extract of root, CS-7: ethyl acetate extract of root, CS-8: MeOH extract of root.

The high reactivity of polyphenols as hydrogen or electron donors, which can stabilize and delocalize the unpaired electron (chain-breaking function), and their capacity to chelate metal ions (termination of the Fenton process), are what give them their antioxidative properties[27].

Epidemiological studies have revealed a link between cardiovascular diseases and low plasma concentrations of ascorbate, tocopherol and β-carotenes and oxidation reactions have been suggested to play a major role in atherogenesis[28,29].

The results of the present study exhibited higher phenolic content in CS-3, CS-4, CS-7 and CS-8 (184.19±8.22, 137.9±4.11, 273.14±10.69 & 191.16±0.00 mg/g, respectively) as equivalent to gallic acid. Other extracts showed lower phenolic content. The results of phenolic content study revealed that, ethyl acetate and methanol extracts of stems and roots of *Cassia sophera* demonstrate good phenolic content & indicates good antioxidant capacity.

Table 6: Total phenolic contents present in extracts of *Jatropha curcas*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	GAE conc. (C) (µg/ml)	GAE conc. (C) (mg/ml)	V (mL)	TPC as GAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
JC-1	0.098	0.0002	1.256	0.001	1	6.28	8.60±3.29
	0.102	0.0002	2.186	0.002	1	10.93	
JC-2	0.146	0.0002	12.419	0.012	1	62.09	67.91±8.22
	0.156	0.0002	14.744	0.015	1	73.72	
JC-3	0.229	0.0002	31.721	0.032	1	158.60	154.53±5.76
	0.222	0.0002	30.093	0.030	1	150.47	
JC-4	0.151	0.0002	13.581	0.014	1	67.91	68.49±0.82
	0.152	0.0002	13.814	0.014	1	69.07	
JC-5	0.15	0.0002	13.349	0.013	1	66.74	70.81±5.76
	0.157	0.0002	14.977	0.015	1	74.88	
JC-6	0.148	0.0002	12.884	0.013	1	64.42	69.07±6.58
	0.156	0.0002	14.744	0.015	1	73.72	
JC-7	0.164	0.0002	16.605	0.017	1	83.02	86.51±4.93
	0.17	0.0002	18.000	0.018	1	90.00	
JC-8	0.16	0.0002	15.674	0.016	1	78.37	81.28±4.11
	0.165	0.0002	16.837	0.017	1	84.19	

JC-1: n-Hexane extract of stem, JC-2: $CHCl_3$ extract of stem, JC-3: Ethyl acetate extract of stem, JC-4: MeOH extract of stem, JC-5: n-Hexane extract of root, JC-6: $CHCl_3$ extract of root, JC-7: ethyl acetate extract of root, JC-8: MeOH extract of root.

The results of the present study exhibited that higher phenolic content in ethyl acetate extract of stem (JC-3) i.e. 154.53±5.76 mg/g and lower phenolic content in n-hexane extract of stem (JC-1) i.e. 8.60±3.29 mg/g as equivalent to standard gallic acid. Other extracts also showed moderate phenolic content approximately 65-90 mg/g as GAE. Higher phenolic content indicates higher antioxidant properties.

Determination of Total Flavonoid Content

To calculate the total flavonoid concentrations (TFC) of the *Cassia sophera* and *Jatropha curcas* extracts, aluminum chloride colorimetry was used. The total flavonoid contents were calculated using the standard curve of quercetin ($y=0.0054x + 0.0077$; $R^2 = 0.9993$).

Table 7: Absorbance found with different concentrations of quercetin

Concentration (µg/mL)	Absorbance
1.56	0.004
3.125	0.012
6.25	0.021
12.5	0.051
25	0.122
50	0.266
100	0.553
200	1.065

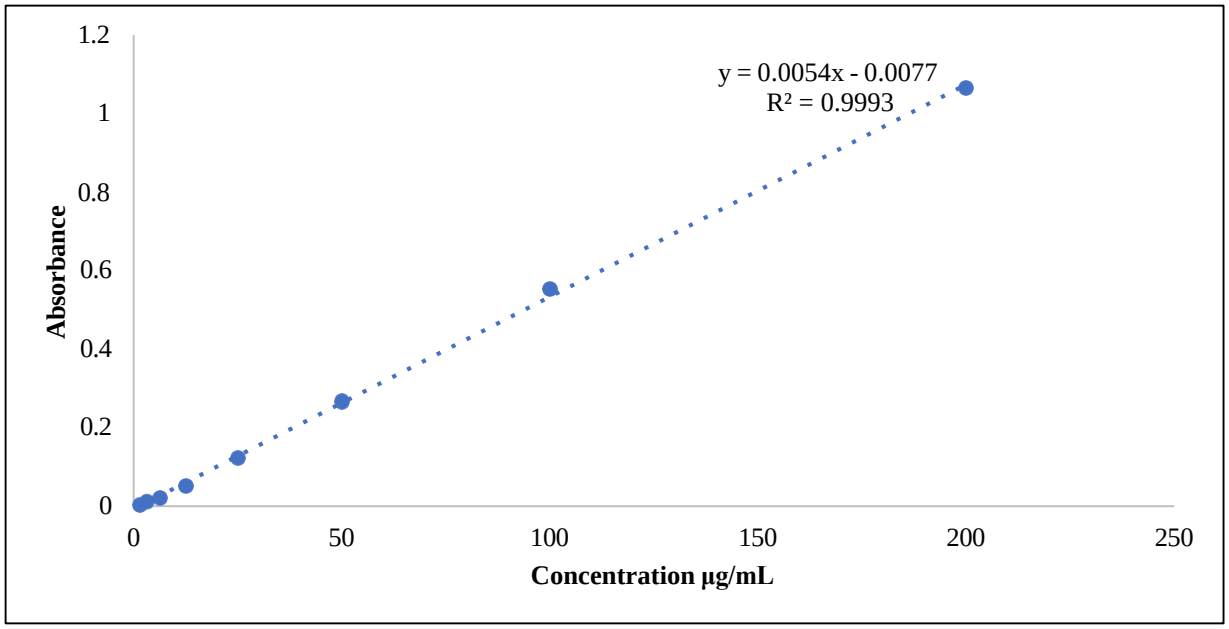


Figure 3: Calibration curve of quercetin.

Table 8: Total flavonoid contents of the extracts of *Cassia sophera*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	QE conc. (C) (µg/ml)	QE conc. (C) (mg/ml)	V (mL)	TFC as QE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
CS-1	0.044	0.0002	6.722	0.007	1	33.61	29.44±5.89
	0.035	0.0002	5.056	0.005	1	25.28	
CS-2	0.035	0.0002	5.056	0.005	1	25.28	26.20±1.31
	0.037	0.0002	5.426	0.005	1	27.13	
CS-3	0.078	0.0002	13.019	0.013	1	65.09	67.41±3.27
	0.083	0.0002	13.944	0.014	1	69.72	
CS-4	0.037	0.0002	5.426	0.005	1	27.13	24.81±3.27
	0.032	0.0002	4.500	0.005	1	22.50	
CS-5	0.042	0.0002	6.352	0.006	1	31.76	29.44±3.27
	0.037	0.0002	5.426	0.005	1	27.13	
CS-6	0.051	0.0002	8.019	0.008	1	40.09	34.54±7.86
	0.039	0.0002	5.796	0.006	1	28.98	
CS-7	0.117	0.0002	20.241	0.020	1	101.20	105.37±5.89
	0.126	0.0002	21.907	0.022	1	109.54	
CS-8	0.055	0.0002	8.759	0.009	1	43.80	38.24±7.86
	0.043	0.0002	6.537	0.007	1	32.69	

CS-1: n-Hexane extract of stem, CS-2: $CHCl_3$ extract of stem, CS-3: Ethyl acetate extract of stem, CS-4: MeOH extract of stem, CS-5: n-Hexane extract of root, CS-6: $CHCl_3$ extract of root, CS-7: ethyl acetate extract of root, CS-8: MeOH extract of root.

The flavonoid content of the plant is of interest for evaluating its antioxidant activities because flavonoids are renowned for their antioxidant qualities. The amount of flavonoids and the ability of plant extracts to serve as antioxidants have been discovered to be positively correlated. The main step of the process is the formation of a complex between the flavonoid and $AlCl_3$ that results in a yellow solution. The presence of flavonoid molecules is subsequently determined by measuring the absorbance spectrophotometrically[30].

The total flavonoids content study of the extracts of *Cassia sophera* exhibited that, ethyl acetate extract of root (CS-7) has higher flavonoid content i.e. 105.37±5.89 mg/g of flavonoids as equivalent to quercetin & other extracts showed low flavonoid content.

Table 9: Total flavonoid contents of the extracts of *Jatropha curcas*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	QE conc. (C) (µg/ml)	QE conc. (C) (mg/ml)	V (mL)	TFC as QE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
JC-1	0.025	0.0002	3.204	0.003	1	16.02	17.87±2.62
	0.029	0.0002	3.944	0.004	1	19.72	
JC-2	0.067	0.0002	10.981	0.011	1	54.91	50.28±6.55
	0.057	0.0002	9.130	0.009	1	45.65	
JC-3	0.012	0.0002	0.796	0.001	1	3.98	3.52±0.65
	0.011	0.0002	0.611	0.001	1	3.06	
JC-4	0.008	0.0002	0.056	0.000	1	0.28	0.74±0.65
	0.009	0.0002	0.241	0.000	1	1.20	
JC-5	0.045	0.0002	6.907	0.007	1	34.54	37.31±3.93
	0.051	0.0002	8.019	0.008	1	40.09	
JC-6	0.019	0.0002	2.093	0.002	1	10.46	9.54±1.31
	0.017	0.0002	1.722	0.002	1	8.61	
JC-7	0.024	0.0002	3.019	0.003	1	15.09	12.31±3.93
	0.018	0.0002	1.907	0.002	1	9.54	
JC-8	0.033	0.0002	4.685	0.005	1	23.43	25.74±3.27
	0.038	0.0002	5.611	0.006	1	28.06	

JC-1: n-Hexane extract of stem, JC-2: $CHCl_3$ extract of stem, JC-3: Ethyl acetate extract of stem, JC-4: MeOH extract of stem, JC-5: n-Hexane extract of root, JC-6: $CHCl_3$ extract of root, JC-7: ethyl acetate extract of root, JC-8: MeOH extract of root.

The present study of total flavonoids contents in the extracts of *Jatropha curcas* showed that chloroform extract of stem (JC-2) has higher flavonoid content i.e. 50.28±6.55 mg/g as equivalent to quercetin. The higher flavonoid content is allied to good antioxidant activity. Again, methanol extract of stem (JC-4) has lower flavonoid content in compared to the standard.

Determination of Total Antioxidant Capacity

A chemical is considered an antioxidant if it "substantially retards or prevents the oxidation of a substrate (proteins, lipids, carbohydrates, and DNA) when supplied at low concentrations relative to those of the substrate"[31,32]. Antioxidants' primary purpose is to shield the body from the damaging effects of free radicals[33]. The antioxidant effect is mainly due to phenolic compounds, such as phenolic acid, and phenolic diterpenes. The phospho molybdenum method

is based on the reduction of M0 (V1) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with maximum absorption at 695 nm. Total antioxidant capacity of plant extracts expressed as the number of gram equivalent of ascorbic acid ($y=0.0021x + 0.0345$; $R^2 =0.9841$)

Table 10: Absorption of ascorbic acid at the different concentrations for quantities determination of total antioxidant activity.

Concentration (µg/mL)	Absorbance
1.56	0.048
3.125	0.048
6.25	0.051
12.5	0.058
25	0.077
50	0.108
100	0.178
200	0.375

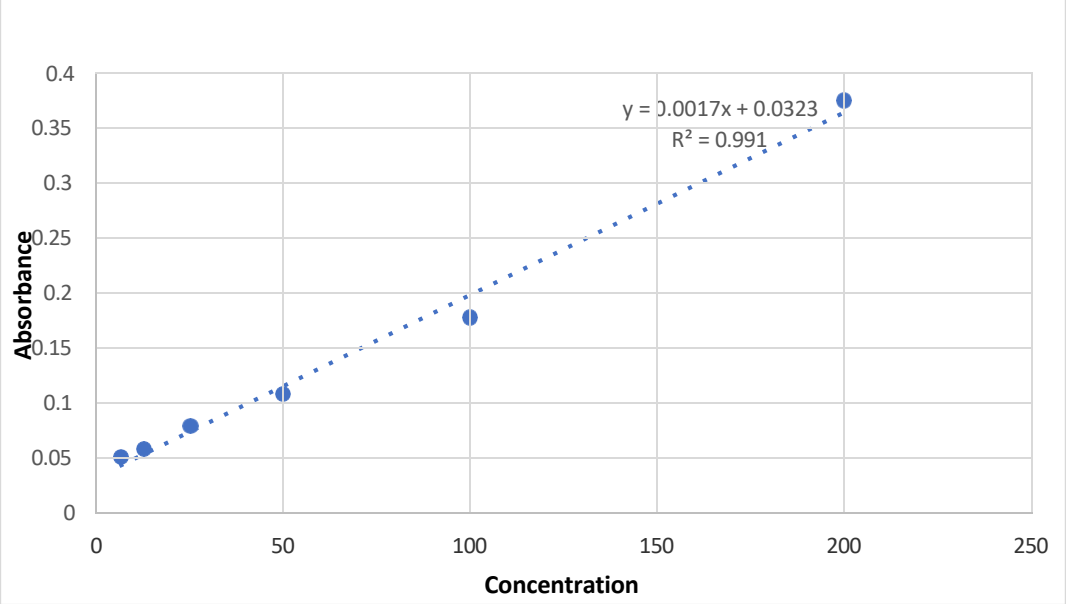


Figure 4: Calibration curve for ascorbic acid

Table 11: Total antioxidant capacity of the *Cassia sophera*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	AAE conc. (C) (µg/ml)	AAE conc. (C) (mg/ml)	V (mL)	TAC as AAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
CS-1	0.061	0.0002	16.882	0.017	1	84.41	78.53±8.32
	0.057	0.0002	14.529	0.015	1	72.65	
CS-2	0.057	0.0002	14.529	0.015	1	72.65	72.65±0.00
	0.057	0.0002	14.529	0.015	1	72.65	
CS-3	0.084	0.0002	30.412	0.030	1	152.06	159.41±10.40
	0.089	0.0002	33.353	0.033	1	166.76	
CS-4	0.08	0.0002	28.059	0.028	1	140.29	144.71±6.24
	0.083	0.0002	29.824	0.030	1	149.12	
CS-5	0.069	0.0002	21.588	0.022	1	107.94	110.88±4.16
	0.071	0.0002	22.765	0.023	1	113.82	
CS-6	0.077	0.0002	26.294	0.026	1	131.47	138.82±10.40
	0.082	0.0002	29.235	0.029	1	146.18	
CS-7	0.137	0.0002	61.588	0.062	1	307.94	299.12±12.48
	0.131	0.0002	58.059	0.058	1	290.29	
CS-8	0.119	0.0002	51.000	0.051	1	255.00	262.35±10.40
	0.124	0.0002	53.941	0.054	1	269.71	

CS-1: n-Hexane extract of stem, CS-2: $CHCl_3$ extract of stem, CS-3: Ethyl acetate extract of stem, CS-4: MeOH extract of stem, CS-5: n-Hexane extract of root, CS-6: $CHCl_3$ extract of root, CS-7: ethyl acetate extract of root, CS-8: MeOH extract of root.

Phenolic substances like phenolic acid and phenolic diterpenes are primarily responsible for the antioxidant effect. Plant extracts have antioxidant properties that go beyond phenolics. Because of their ability to function as reducing agents, hydrogen donors, and singlet oxygen quenchers due to their redox characteristics, phenolics have a significant antioxidant activity[34].

In the present study of total antioxidant activity of the extracts of *Cassia sophera* exhibited that, CS-3, CS-4, CS-6, CS-7 & CS-8 (159.41±10.40, 144.71±6.24, 138.82±10.40, 299.12±12.48 and 262.35±10.40 mg/g respectively as equivalent to ascorbic acid) has good antioxidant capacity. Other extracts showed moderate to lower phenolic content compare to above mentioned

extract. The results of total antioxidant capacity exhibited higher antioxidant property of the polar solvent extracts.

Table 12: Total antioxidant capacity of the *Jatropha curcas*

Sample Name	Abs.	Wt. of Plant Extract (g/mL)	AAE conc. (C) (µg/ml)	AAE conc. (C) (mg/ml)	V (mL)	TAC as AAE, A=(c*V)/m (mg/g)	Mean ± SD (mg/g)
JC-1	0.056	0.0002	13.941	0.014	1	69.71	77.06±6.24
	0.06	0.0002	16.294	0.016	1	81.47	
JC-2	0.067	0.0002	20.412	0.020	1	102.06	100.59±2.08
	0.066	0.0002	19.824	0.020	1	99.12	
JC-3	0.071	0.0002	22.765	0.023	1	113.82	110.88±4.16
	0.069	0.0002	21.588	0.022	1	107.94	
JC-4	0.06	0.0002	16.294	0.016	1	81.47	80.00±2.08
	0.059	0.0002	15.706	0.016	1	78.53	
JC-5	0.082	0.0002	29.235	0.029	1	146.18	140.29±8.32
	0.078	0.0002	26.882	0.027	1	134.41	
JC-6	0.075	0.0002	25.118	0.025	1	125.59	128.53±4.16
	0.077	0.0002	26.294	0.026	1	131.47	
JC-7	0.065	0.0002	19.235	0.019	1	96.18	90.29±8.32
	0.061	0.0002	16.882	0.017	1	84.41	
JC-8	0.095	0.0002	36.882	0.037	1	184.41	187.35±4.16
	0.097	0.0002	38.059	0.038	1	190.29	

JC-1: n-Hexane extract of stem, JC-2: $CHCl_3$ extract of stem, JC-3: Ethyl acetate extract of stem, JC-4: MeOH extract of stem, JC-5: n-Hexane extract of root, JC-6: $CHCl_3$ extract of root, JC-7: ethyl acetate extract of root, JC-8: MeOH extract of root.

The results showed that methanol extract of root (JC-8) have good antioxidant property compared to other extracts. Again, n-hexane and chloroform extract of root (JC-5 & JC-6) have moderate antioxidant property. Other extracts also showed considerable amount of antioxidant property.

Cytotoxicity Study

The brine shrimp lethality bioassay is an all-purpose bioassay that seems to be able to identify a wide range of bioactivity found in crude extracts (BSLT)[35]. In the bioassay for bioactive substances, it seems that BSLT is a reliable indicator of cytotoxicity and pesticide activity[36]. By using a Brine Shrimp lethality test, the cytotoxic activity of each extract was identified. The test tubes were examined and counted for the number of surviving nauplii after the 24-hour period. This information was used to compute the lethality percentage for each concentration. The results of the cytotoxicity study are presented in the following tables and

figure-

Table 13: Data for Bri ne Shrimp lethality bioassay for vincristine sulfate

Conc. (µg/ml)	Log conc.	No. of nauplii taken (N_0)	No. of nauplii alive (N_1)	No. of nauplii dead	Mortality, $M=\frac{N0-N1}{N0}*100$	Log LC_{50} (µg/ml)	LC_{50} (µg/ml)
0.313	-0.50446	10	10	0	0	**0.4829**	3.04
0.313	-0.50446	10	10	0	0		
0.625	-0.20412	10	9	1	10		
1.25	0.09691	11	8	3	27		
2.5	0.39794	10	5	5	50		
5	0.69897	11	3	8	73		

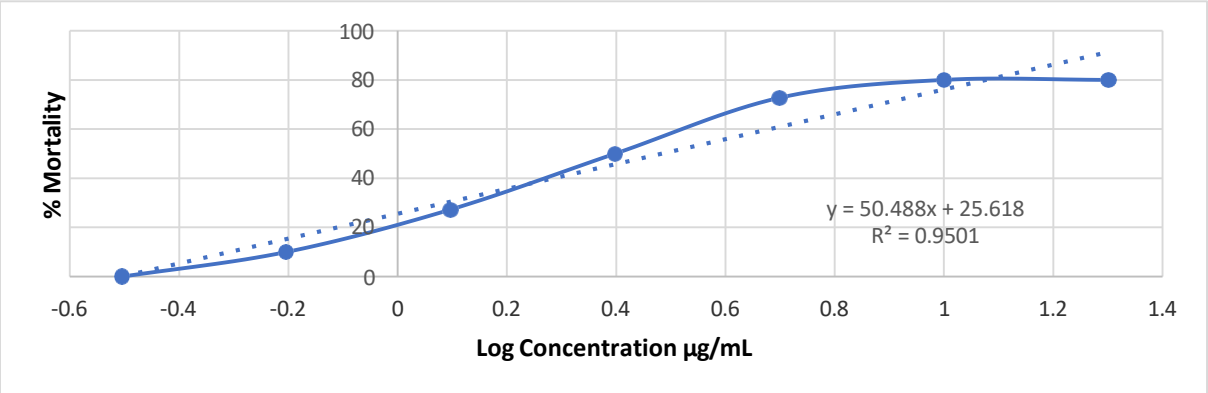


Figure 5: Calibration curve for % mortality vs log concentration of Vincristine sulfate.

Table 14: LC_{50} values of extracts of *extracts* and standard for *Cassia sophera*

Sample name	LC_{50} Value
Vincristine sulfate	3.04
n-Hexane extract of stem (CS-1)	141.86
Chloroform extract of stem (CS-2)	143.57
Ethyl acetate extract of stem (CS-3)	92.4
Methanol extract of stem (CS-4)	180.65
n-Hexane extract of root (CS-5)	108.49
Chloroform extract of root (CS-6)	52.74
Ethyl acetate extract of root (CS-7)	82.83
Methanol extract of root (CS-8)	58.33

The cytotoxicity study of *Cassia sophera* exhibited that all the extracts have lower cytotoxic property compare to standard. In comparative study chloroform extract of root (CS-6) & methanol extract of root (CS-8) extracts showed higher cytotoxicity compared to other extracts and methanol extract of stem (CS-4) has least cytotoxic property.

Table 15: LC_{50} values of extracts of *extracts* and standard for *Jatropha curcas*

Sample name	LC_{50} Value
Vincristine sulfate	3.04
n-Hexane extract of stem (JC-1)	67.29
Chloroform extract of stem (JC-2)	4.52
EA extract of stem (JC-3)	50.09
MeOH extract of stem (JC-4)	40.43
n-Hexane extract of root (JC-5)	58.59
Chloroform extract of root (JC-6)	25.00
EA extract of root (JC-7)	46.3
MeOH extract of root (JC-8)	39.69

The cytotoxicity study of the extracts exhibited that chloroform extract of stem (J-2) has significant cytotoxicity with LC_{50} value 4.52 µg/ml compared to standard vincristine sulfate with LC_{50} value 3.04 µg/ml. Other extracts also showed good cytotoxicity in compared to the standard.

In Vitro Anti-Inflammatory Assays

Inhibition of bovine albumin denaturation

Table 16: In-vitro of bovine albumin anti-inflammatory activity of the std. (Diclofenac Sodium).

Concentration (µg/mL)	Log Concentration (µg/mL)	Abs. Control	Abs. Sample	% Inhibition
25	1.398	1.279	1.019	20.33
50	1.699	1.279	0.752	41.20
100	2.000	1.279	0.527	58.80
200	2.301	1.279	0.258	79.83
500	2.699	1.279	0.091	92.89

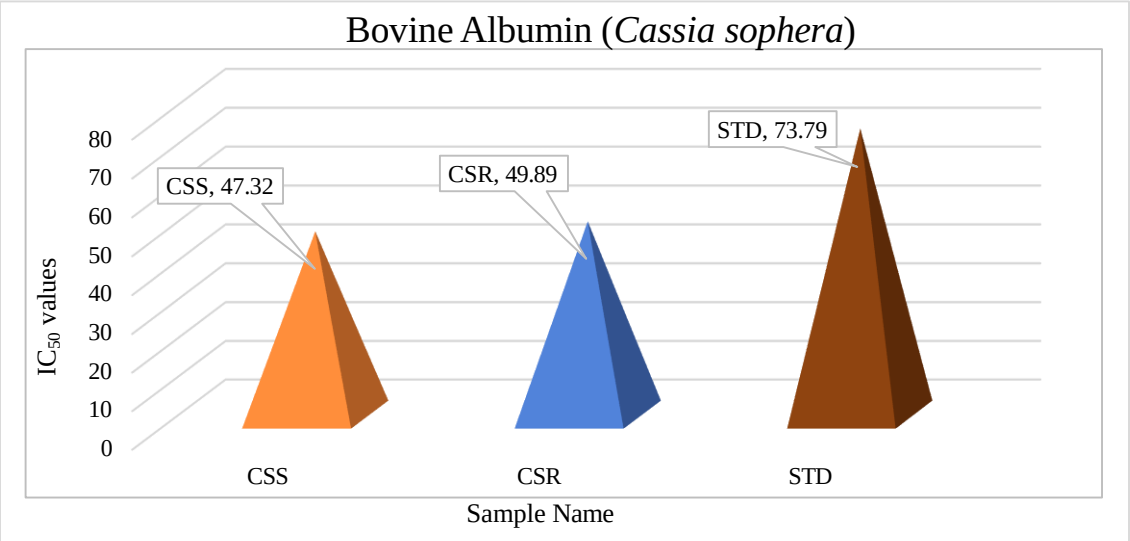


Figure 6: IC_{50} values of the methanol extracts of *C. sophera* and standard diclofenac sodium.

CSS: *Cassia sophera* stems, CSR: *Cassia sophera* roots, STD: Standard

The decrease in IC_{50} values of test samples indicated the stabilization of protein, i.e., inhibition of heat-induced protein (bovine and egg albumin) denaturation by extracting and referencing the drug diclofenac sodium [37]. The results showed that both stem and root of methanol extracts were more active than diclofenac sodium, being effective in lower concentrations.

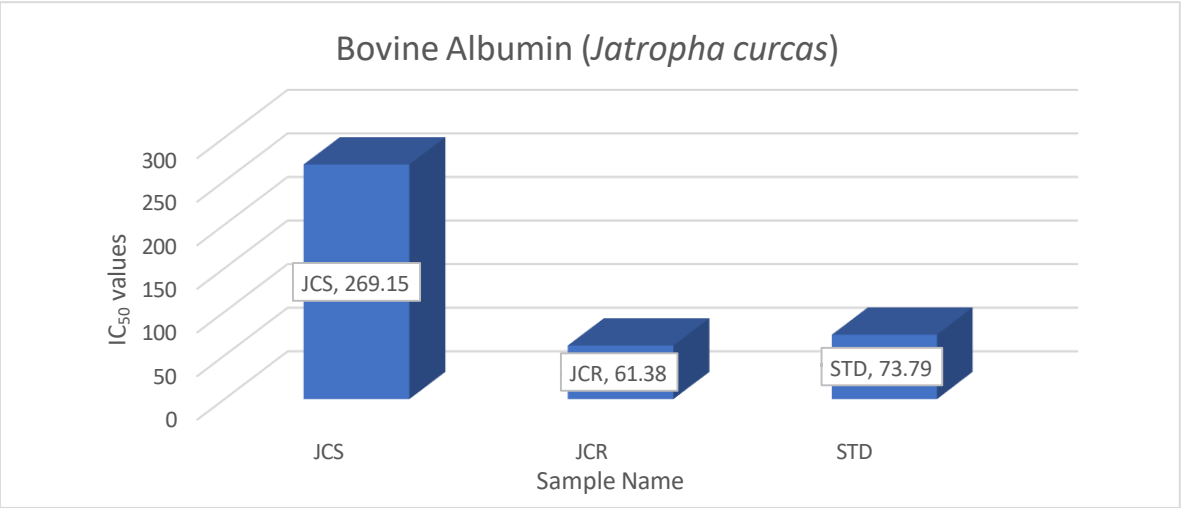


Figure 7: IC_{50} values of Bovine Albumin of the extracts and standard diclofenac sodium.

JCS: *Jatropha curcas* stems, JCR: *Jatropha curcas* roots, STD: Standard

From IC_{50} values, it is evident that root extract of methanol showed better activity than standard diclofenac sodium. On the other hand, stem methanol extract showed minor activity in compared to standard.

Inhibition of egg albumin denaturation

Table 17: In-vitro of egg albumin anti-inflammatory activity of the Std. (Diclofenac Sodium).

Concentration (µg/mL)	Log Concentration (µg/mL)	Abs. Control	Abs. Sample	% Inhibition
25	1.398	0.569	0.287	49.56
50	1.699	0.569	0.194	65.91
100	2.000	0.569	0.163	71.35
200	2.301	0.569	0.121	78.73
500	2.699	0.569	0.079	86.12

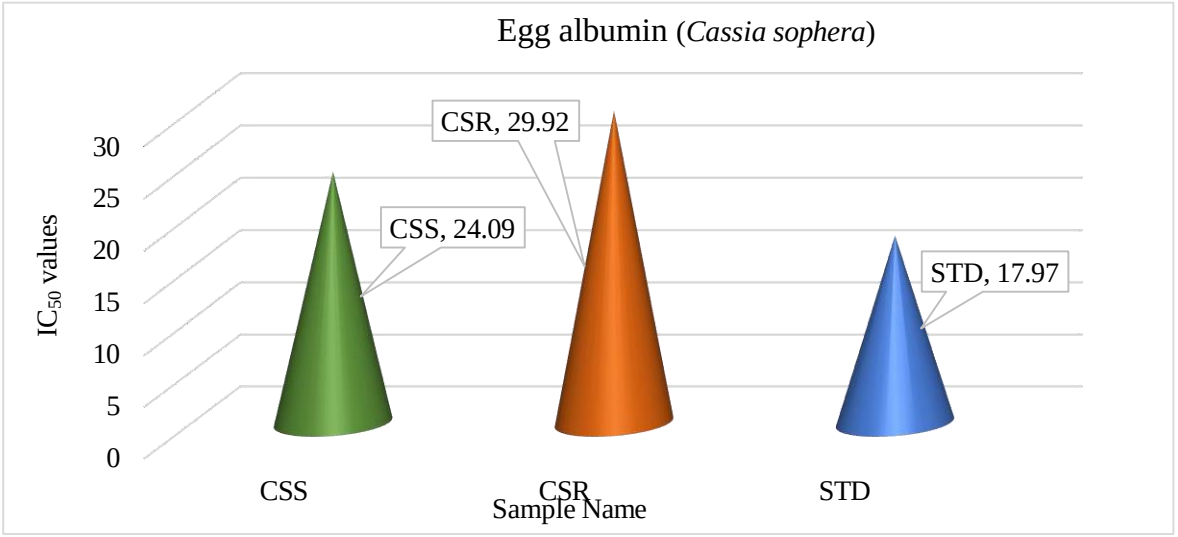
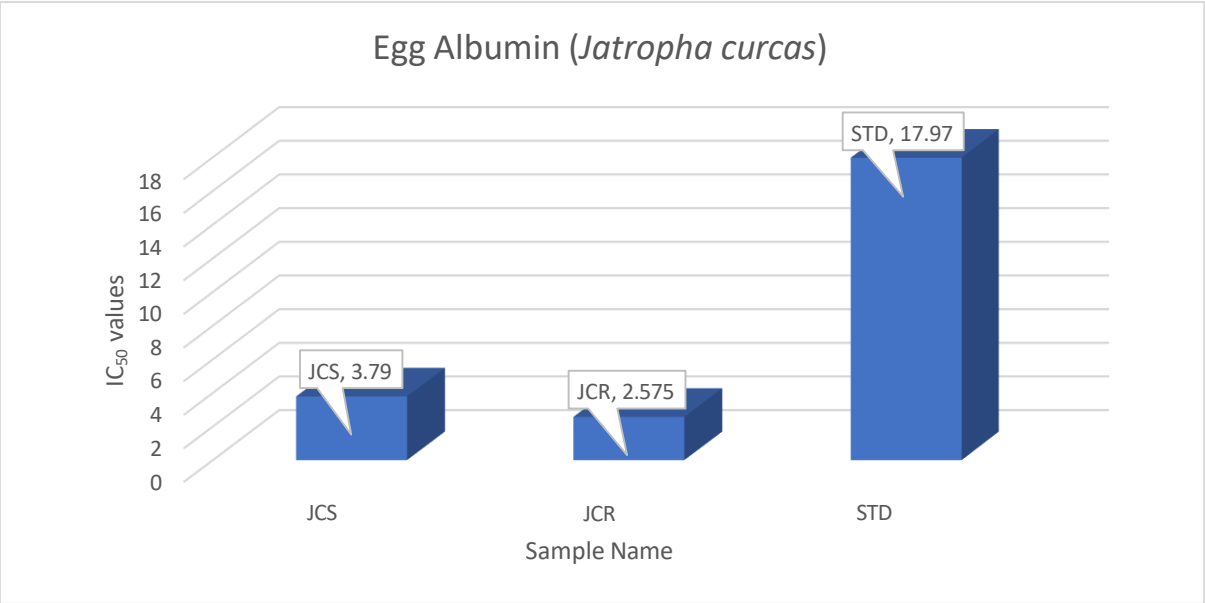


Figure 8: IC_{50} values of egg albumin of the extracts and standard diclofenac sodium.

CSS: *Cassia sophera* stems, CSR: *Cassia sophera* roots, STD: Standard

From IC_{50} values, the results showed that both stem and root of methanol extract have good activity in compared to standard diclofenac sodium.



JCS: *Jatropha curcas* stems, JCR: *Jatropha curcas* roots, STD: Standard

Figure 9: IC_{50} values of Egg Albumin of the extracts and standard diclofenac sodium.

From IC_{50} values it is evident that both stems and roots of methanol extract showed significant activity than standard diclofenac sodium.

CONCLUSION

The result of the DPPH free radical scavenging assay of *cassia sophera* showed that methanol extract of root showed significant antioxidant activity with IC_{50} value 2.42 µg/mL in comparison to standard whereas methanol extract of stem have significant free radical scavenging capacity with IC_{50} value 7.49 for plant *Jatropha curcas*. The cytotoxicity study exhibited that all the extracts have lower cytotoxic property compare to standard for *Cassia sophera* whereas for *Jotropha curcas*, chloroform extract of stem has significant cytotoxicity with LC_{50} value 4.52µg/ml compared to standard vincristine sulfate with LC_{50} value 3.04 µg/ml. From *in-vitro* anti-inflammatory assay of methanol extracts of stems and roots, the result showed that both plants have good activity in compared to standard diclofenac sodium. The findings of our study suggested that stems and roots of *Cassia sophera* and *Jatropha curcas* growing in Bangladesh have potent natural therapeutic agents for antioxidant, cytotoxicity and anti-inflammatory activity.

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