

Research Article

Evaluation of Phytochemicals of *Cassia tora* Linn. and its Cytotoxicity Assay using Brine Shrimp

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ABSTRACT

Cassia tora Linn. is a well known medicinal herb found as a rainy season weed throughout India. Various studies have been conducted in order to find out the applications of *Cassia tora* since many years and found various phytochemical present in the plant, contribute to the use of traditional medicine. Cytotoxicity of the plant is found due to some phytochemical present in the plant and thus this plant has found numerous applications in the medical field. Research Objective: The main aim of the research was to assess the effect of cytotoxicity of the plant extracts against *Artemia salina*. Brine shrimp lethality assay was used in this study to carry out the cytotoxicity assay. Thus, this research paper emphasizes on the screening of phytochemicals present in the various parts of the plant along with the cytotoxicity assay against *Artemia salina*. Results and Conclusion: The present study showed that the presence of phytochemicals vary with the plant parts and the effect of cytotoxicity on Brine shrimp was also observed with the LD₅₀ value less than 100 µg/ mL which can be beneficial in the treatment of various cancers and tumors.

Keywords: *Cassia tora* Linn., phytochemical, brine shrimp, cytotoxicity.

INTRODUCTION

The WHO Traditional Medicine Strategy 2014 - 2023 estimated that the use of traditional medicines in developing nations will increase by 20% till 2020. *Cassia tora* Linn. (Caesalpinaceae) is a well known medicinal herb found as a rainy season weed throughout India. Various studies have been conducted in order to find out the applications of *Cassia tora* since many years. Due to various phytochemicals such as anthraquinone glycosides, naphthopyrone glycosides, phenolic compounds, flavonoids, sennosides, rubrofusarine triglycoside, etc. present in the plant significantly contributes to the diverse biological functions such as antioxidant, hepatoprotective, hypolipidemic, antibacterial, antifertility, antimutagenic, antitumor, antiinflammatory, antifungal, and antihelmintic activities (Pawar and D'mello, 2011; Das et. al., 2011). This research paper focuses on the evaluation of phytochemicals present in the *Cassia tora* Linn samples along with the effect of lethality on Brine Shrimp.

MATERIALS AND METHODS

Collection and Identification of plant materials

The fresh plant samples were collected from Bhiwandi and Murbad. The plant was identified and authenticated as *Cassia tora* Linn. from the Department of Botany, Birla College, Kalyan. All the parts (leaves, roots, flowers, pods and stem) of the plants were separated. Each part of the plant was subjected to washing with distilled water to rinse all the dust and soil, followed by

drying at 60°C in hot air oven for 2 -3 days. The fine powder of these dried leaves, roots, flowers, pods, and stem obtained using a grinder and sieving was stored in airtight container in dry place till further analysis. All analyses were conducted in triplicates.

Preparation of Plant Extract

Soxhlet Extraction or Hot Extraction Method was used for the preparation of the different plant part extracts. Petroleum ether, n - hexane, ethyl acetate, chloroform,

Phytochemical	Test
Alkaloids	Mayer's Test; Wagner's Test; Dragendroff's Test
Anthraquinone	Nitric acid test; Benzene test.
Carbohydrates	Molisch's Test; Benedict's Test; Fehling's Test
Flavonoids	Gelatin test; Lead Acetate test
Glycosides	Modified Bontrager's test; Cardiac Glycoside test
Lignin	Toluidine blue test; Phloroglucinol test
Phenols	Ferric chloride test; Lead Acetate test
Phlobatannins	
Proteins and Amino acids	Xanthoproteic test; Ninhydrin test
Saponins	Foam test
Tannins	Alkaline reagent test
Terpenoids	Salkowski's test; Libermann Burchard's test

Table 1: Phytochemistry of the *Cassia tora* Linn leaves.

Test/Sample	n-Hexane	E.A.	P.E.	Chloroform	Methanol	Aqueous
Alkaloids						
1) Dragendroff's test	+	+	+	+	+	+
2) Wagner's test	+	+	+	+	+	+
3) Mayer's test	+	+	+	+	+	+
Carbohydrates						
1) Molisch's test	-	-	-	-	-	-
2) Benedict's test	+	-	-	-	-	+
3) Fehling's test	-	-	-	+	-	+
Glycosides						
1) Modified Bront.	+	+	+	+	-	+
2) Cardiac glyc. Test	-	-	+	+	-	+
Saponins						
1) Foam test	+	+	+	+	-	+
Terpenoids						
1) Salkowski test	-	-	-	-	-	+
2) Lieberman test	+	+	-	+	+	+
3) Cu Acet. Test	+	+	+	+	+	+
Phenols						
1) Ferric Chloride	-	+	-	-	-	-
2) Lead Acetate	-	+	-	-	-	-
Tannins						
1) Alkaline reagent	-	-	-	-	+	+
Flavonoids						
1) Gelatin test	-	+	-	-	-	-
2) Lead Acetate	-	+	-	-	-	-
Protein & Amino Acid						
1) Xanthoproteic	+	+	+	-	+	+
2) Ninhydrin	+	+	-	-	-	+
Phlobatannins						
1) Anthraquinone	-	-	-	-	-	-
1) Nitric acid	+	+	+	-	+	+
2) Benzene	+	+	-	+	-	+
Lignin						
1) Toluene	-	-	-	-	-	-
2) Phloroglucinol	-	-	-	-	-	-

methanol, and distilled water were used as solvents for the hot extraction method. In case of cytotoxicity assay, distilled water and hydroalcohol were used for the extraction.

Phytochemical Screening Assay

Standard protocols of the phytochemical analysis were followed in order to carry out the phytochemical screening of the *Cassia tora* Linn plant extracts. The following phytochemical tests were conducted for various phytochemicals (R. Sathish et. al., 2016) as per the following table.

Cytotoxicity Assay

The Brine Shrimp Lethality Bioassay technique was carried out for the evaluation of cytotoxicity of the aqueous extracts of *Cassia tora* Linn plant parts i.e. leaves, stem, flower, pod and roots as per the standard protocol (Meyer et. al., 1982; Islam et. al., 2003; and Moshi et. al., 2010). The Brine Shrimp Lethality Assay (BSLA) has been widely used assay for evaluating the cytotoxicity of the plant extracts towards Brine shrimp. In this technique, *Artemia salina* (Brine shrimp) eggs, were

hatched in the artificial seawater prepared as per the protocol given by Kester et. al., 1967. The pH of the solution was adjusted to 7.5 ± 0.5 . Brine shrimp egg was added to artificial seawater in a glass chamber and kept under constant aeration. The chamber was kept illuminated at 1000 lux. After 48 h incubation at room temperature (26 - 30°C), the larvae (nauplii) were attracted to one side of the vessel using a light source (phototactic migration effect) and were collected with a Pasteur pipette. Thirty larvae (nauplii) were drawn through a glass capillary and placed in each injection vial containing sea water and maintained at room temperature for 24 h under the light and surviving larvae was counted. Experiments were conducted with control and the different concentrations ($\mu\text{g/ml}$) of the plant extracts. The test was performed in triplicates. Mortality rate, survival rate, and Lethal Dose 50 (LD_{50}) were calculated.

RESULTS AND DISCUSSIONS

Phytochemical Screening

Table 2: Phytochemistry of *Cassia tora* stem.

Test/Sample	n-Hexane	E.A	P.E	CHCl ₃	Methanol	Aqueous
Alkaloids						
1) Dragendroff's test	+	+	+	+	+	+
2) Wagner's test	+	+	+	-	+	+
3) Mayer's test	-	-	+	+	+	+
Carbohydrates						
1) Molisch's test	-	-	-	-	-	-
2) Benedict's test	-	-	-	-	-	-
3) Fehling's test	-	-	-	-	-	-
Glycosides						
1) Modified Bront.	-	-	-	-	-	-
2) Cardiac glyc. Test	+	+	-	-	+	-
Saponins						
1) Foam test	-	+	+	+	+	+
Terpenoids						
1) Salkowski test	-	-	+	+	+	+
2) Lieberman test	-	-	+	-	+	+
3) Cu Acet. Test	+	+	+	+	+	+
Phenols						
1) Ferric Chloride	-	-	-	-	-	-
2) Lead Acetate	-	-	-	+	+	+
Tannins						
1) Alkaline reagent	-	-	-	-	+	+
Flavonoids						
1) Gelatin test	-	-	-	-	-	+
2) Lead Acetate	-	-	-	+	+	+
Protein & Amino Acid						
1) Xanthoproteic	+	+	+	+	+	+
2) Ninhydrin	+	+	+	-	-	-
Phlobatannins						
1) Anthraquinone	-	-	-	-	-	-
1) Nitric acid	+	+	+	+	+	+
2) Benzene	-	-	-	-	-	+
Lignin						
1) Toluene	-	-	-	-	-	-
2) Phloroglucinol	-	-	-	-	-	-

Preliminary phytochemical screening was carried out using six different solvents i.e. n-hexane, ethyl acetate, petroleum ether, chloroform, methanol and distilled water. The results obtained from qualitative test for phytochemicals in the plant extracts are given in the following tables (Table 3.1, 3.2, 3.3, 3.4, and 3.5).

The overall results of the phytochemical analysis obtained in this study are much similar to the results obtained by Kavya et. al., 2015. The alkaloid tests were highly positive in the leaves in all the six solvents, i.e. n-hexane, petroleum ether, ethyl acetate, chloroform, methanol and aqueous extract, compared to other plant parts. Carbohydrates were present in the n-hexane, chloroform and aqueous extract of leaves. In case of pod sample, carbohydrate tests showed a positive result only in chloroform extract. Furthermore, carbohydrates were also positive in the methanol and aqueous extract of flowers and in the aqueous extract of the root. The glycosides tests were highly positive in the leaves and pod extract compared to other parts since it showed the slight presence of glycosides. The results showed that the

saponins were present in maximum amount in all the plant parts extract of *Cassia tora* in all the six solvent.

Terpenoids were present in maximum amount in the pod and flower extract in each solvent. Leaves, stem and root also showed the presence of terpenoids. Phenols were mostly present in the flower, pod and root extract, minimum amount in the leaf extract. Thus, phenolic compounds are one of the main sources of natural antioxidants (Ali et. al., 2008). Tannins were present in the aqueous and the methanol extract of leaves, pod, and flower whereas it was present in n-hexane, petroleum ether, and ethyl acetate extracts of the stem. Flavonoids were present in high amounts in flowers and root extracts. In leaves it was present in the ethyl acetate, while it was also present in the aqueous and the methanol extract of stem and pod. Proteins were present in maximum quantities in the stem and leaf extract, but in flower it showed moderate presence as compared to the pod and root extract which showed the minimum amount of protein. The methanol extract of pod showed the presence of phlobatannins. Anthraquinone were highly present in

Table 3: Phytochemistry of *Cassia tora* pod.

Test/Sample	n-Hexane	E.A	P.E	CHCl ₃	Methanol	Aqueous
Alkaloids						
1) Dragendroff's test	+	+	+	+	+	+
2) Wagner's test	-	+	+	-	+	+
3) Mayer's test	+	-	-	+	-	+
Carbohydrates						
1) Molisch's test	-	-	-	-	-	-
2) Benedict's test	-	-	-	+	-	-
3) Fehling's test	-	-	-	-	-	-
Glycosides						
1) Modified Bront.	+	+	-	+	-	-
2) Cardiac glyc. Test	+	+	-	-	+	+
Saponins						
1) Foam test	+	+	+	+	+	+
Terpenoids						
1) Salkowski test	+	+	+	+	+	+
2) Lieberman test	+	+	+	+	+	+
3) Cu Acet. Test	+	+	+	+	+	+
Phenols						
1) Ferric Chloride	-	-	+	-	-	-
2) Lead Acetate	-	-	+	+	+	+
Tannins						
1) Alkaline reagent	+	+	+	-	-	+
Flavonoids						
1) Gelatin test	-	-	-	-	-	-
2) Lead Acetate	-	-	+	+	+	+
Protein & Amino Acid						
1) Xanthoproteic	-	-	-	+	-	+
2) Ninhydrin	-	-	+	-	-	-
Phlobatannins						
1) Nitric acid	-	-	+	+	-	+
2) Benzene	+	+	-	-	-	+
Lignin						
1) Toluene	-	-	-	-	-	-
2) Phloroglucinol	-	-	-	-	-	-

Table 4: Phytochemistry of *Cassia tora* flower.

Test/Sample	n-Hexane	E.A	P.E	CHCl ₃	Methanol	Aqueous
Alkaloids						
1) Dragendroff's test	+	+	+	+	+	+
2) Wagner's test	+	+	+	+	+	+
3) Mayer's test	-	-	-	-	+	+
Carbohydrates						
1) Molisch's test	-	-	-	-	+	+
2) Benedict's test	-	-	-	-	+	+
3) Fehling's test	-	-	-	-	-	-
Glycosides						
1) Modified Bront.	+	-	+	-	+	+
2) Cardiac glyc. Test	-	+	-	-	+	+
Saponins						
1) Foam test	+	+	+	+	+	+
Terpenoids						
1) Salkowski test	+	+	+	+	+	+
2) Lieberman test	-	-	-	-	+	+
3) Cu Acet. Test	+	+	+	+	+	+
Phenols						

1) Ferric Chloride	-	-	-	-	-	-
2) Lead Acetate	+	+	+	+	+	+
Tannins						
1) Alkaline reagent	-	-	-	-	+	+
Flavonoids						
1) Gelatin test	-	-	-	-	+	+
2) Lead Acetate	-	+	+	+	-	+
Protein & Amino Acid						
1) Xanthoproteic	+	+	+	+	+	+
2) Ninhydrin	-	-	-	-	-	-
Phlobatannins						
Anthraquinone						
1) Nitric acid	+	+	+	+	+	+
2) Benzene	-	+	+	+	+	+
Lignin						
1) Toluene	-	-	-	-	-	-
2) Phloroglucinol	-	-	-	-	-	-

Table 5: Phytochemistry of *Cassia tora* root.

ROOT						
Test/Sample	n-Hexane	E.A	P.E	CHCl ₃	Methanol	Aqueous
Alkaloids						
1) Dragendorff's test	-	+	+	-	+	+
2) Wagner's test	-	+	-	-	+	+
3) Mayer's test	-	+	-	-	-	+
Carbohydrates						
1) Molisch's test	-	-	-	-	-	-
2) Benedict's test	-	-	-	-	+	+
3) Fehling's test	-	-	-	-	-	-
Glycosides						
1) Modified Bront.	+	+	-	-	-	+
2) Cardiac glyc. Test	-	-	+	-	-	+
Saponins						
1) Foam test	-	+	+	+	+	+
Terpenoids						
1) Salkowski test	+	+	+	+	-	+
2) Lieberman test	-	-	+	-	-	+
3) Cu Acet. Test	+	+	+	+	+	+
Phenols						
1) Ferric Chloride	-	-	-	-	-	-
2) Lead Acetate	+	+	+	-	-	+
Tannins						
1) Alkaline reagent	-	-	-	-	-	-
Flavonoids						
1) Gelatin test	-	-	-	-	-	-
2) Lead Acetate	+	+	+	-	+	+
Protein & Amino Acid						
1) Xanthoproteic	+	-	-	-	+	+
2) Ninhydrin	-	-	-	-	-	-
Phlobatannins						
Anthraquinone						
1) Nitric acid	+	+	-	-	+	+
2) Benzene	+	+	+	-	-	+
Lignin						
1) Toluene	-	-	-	-	-	-
2) Phloroglucinol	-	-	-	-	-	-

Keys: E.A - Ethyl acetate; P.E - Petroleum ether, CHCl₃ - Chloroform, (+) – Present, (-) - Absent

the flower extracts, while leaves, stem and root extract

Table 6: % Mortality of Brine shrimp on different parts of *Cassia tora*.

Cassia Extracts	<i>tora</i>	Concentration	Number of Nauplii initially	Number of added	Number of Survival		% Mortality Rate	
					Number of Nauplii after 24 hrs	Survival	Aq	HA
Leaves of <i>Cassia tora</i>		Control	30		12	15	60%	50%
		Control 1	30		13	13	57%	57%
		Control 2	30		0	0	100%	100%
		50 µg/ml	30		11	13	63%	57%
		100 µg/ml	30		10	8	67%	73%
		150 µg/ml	30		6	11	80%	63%
		200 µg/ml	30		2	8	93%	73%
Stem of <i>Cassia tora</i>		Control	30		27	21	10%	30%
		Control 1	30		21	23	30%	23%
		Control 2	30		0	2	100%	93%
		50 µg/ml	30		22	18	27%	40%
		100 µg/ml	30		15	11	50%	63%
		150 µg/ml	30		11	10	63%	67%
		200 µg/ml	30		9	1	70%	97%
Pods of <i>Cassia tora</i>		Control	30		6	7	80%	77%
		Control 1	30		17	23	43%	23%
		Control 2	30		19	1	37%	97%
		50 µg/ml	30		23	21	23%	30%
		100 µg/ml	30		16	16	47%	47%
		150 µg/ml	30		6	19	80%	37%
		200 µg/ml	30		8	12	51%	60%
Flowers of <i>Cassia tora</i>		Control	30		8	17	73%	43%
		Control 1	30		10	19	67%	37%
		Control 2	30		0	0	100%	100%
		50 µg/ml	30		8	16	73%	47%
		100 µg/ml	30		7	17	77%	43%
		150 µg/ml	30		5	16	83%	47%
		200 µg/ml	30		3	8	90%	73%
Roots of <i>Cassia tora</i>		Control	30		23	28	23%	7%
		Control 1	30		27	28	10%	7%
		Control 2	30		0	0	100%	100%
		50 µg/ml	30		26	6	13%	80%
		100 µg/ml	30		25	8	17%	73%
		150 µg/ml	30		23	1	23%	97%
		200 µg/ml	30		21	0	30%	100%

Key: Aq. - Aqueous; HA - Hydroalcoholic

have shown moderate amount of anthraquinone compared to pod extract which had shown the least amount of anthraquinone.

Thus, methanol and aqueous extract of the *Cassia tora* plant parts such as leaves, stem, pod, flower and root showed the excess amount of phytochemicals compared to other solvents. Phytochemicals were moderately present in petroleum ether, ethyl acetate and chloroform extract as compared to n-hexane which showed the minimum amount of phytochemicals present in the plant extracts.

Cytotoxicity Assay

The Brine shrimp lethality assay represented a rapid, inexpensive and simple bioassay for testing plants extract bioactivity which correlates reasonably well with cytotoxic and antitumor properties. The degree of lethality was found to be directly proportional to the concentration of the extract. The following results were obtained as shown in the Table 3.6.

Aqueous of leaf extracts showed the highest mortality rate followed by aqueous extract of flower. Furthermore, aqueous extract of pods and stem showed a moderate mortality as compared to the aqueous root extract which showed less mortality rate. Hydroalcohol extract of root showed the highest mortality rate as compared to the aqueous extract of the root which showed less mortality rate, thus it may be possible that the brine shrimp were more sensitive towards the hydroalcoholic extract of the root. Hydroalcoholic extract of pods showed a less mortality rate than that of hydroalcoholic leaves and flower extract which corresponds to equal mortality rate. In the lethality assay, 50% of the deaths were seen in the aqueous extracts of leaf and flower; while pod extracts showed the 50% death when 100 µg/ml concentration was added. The aqueous root extract showed no death less than 50%. In hydroalcoholic root extract, 100% deaths of brine shrimp were seen. As the concentration of the dose increased there was an increase in the lethality of

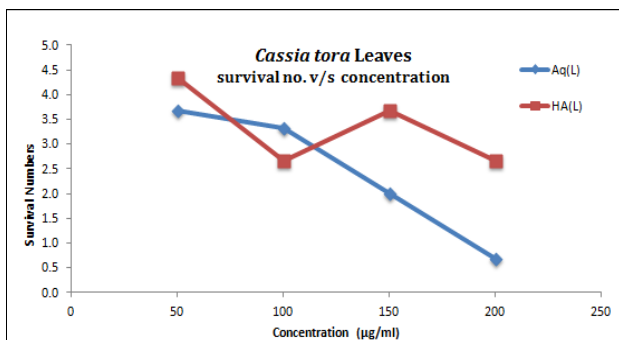


Fig.2.1- Graphical representation of survival rate in *Cassia tora* leaves (Brine shrimp Bio-lethality Assay)

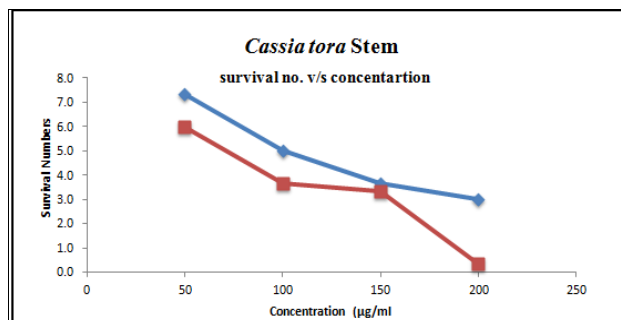


Fig. No 2.2- Graphical representation of survival rate in *Cassia tora* stem- (Brine Shrimp Bio-lethality Assay)

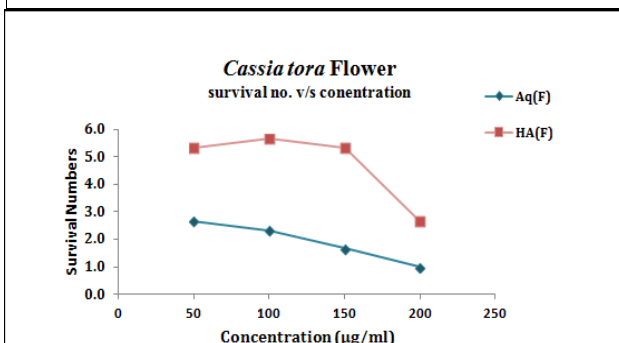


Fig. 2.4- Graphical representation of survival rate in *Cassia tora* flower- (Brine Shrimp Biolethality Assay)

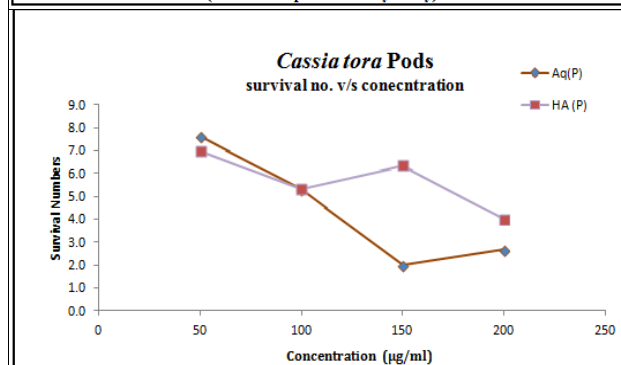


Fig. 2.3- Graphical representation of survival rate in *Cassia tora* pod- (Brine Shrimp Biolethality Assay)

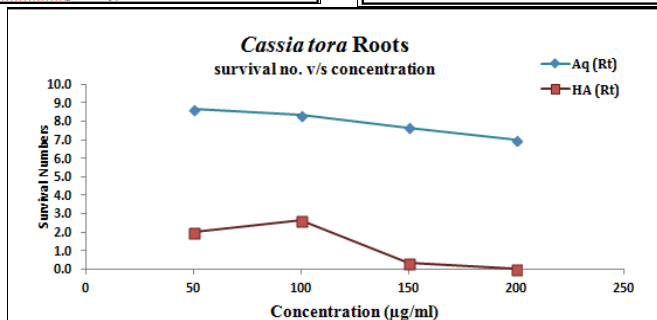


Fig. 2.5- Graphical representation of survival rate in *Cassia tora* root- (Brine Shrimp Biolethality Assay)

Graphical Representation of number of Brine Shrimp survived versus concentration of plant extracts.

the brine shrimp. The adequate amounts of bioactive chemicals that were present in the *Cassia tora* plant parts may be responsible for the lethality of the brine shrimp.

The percentage lethality was determined by comparing the mean surviving nauplii of the test and control tubes. The degree of lethality was found to be directly proportional to the concentration of the extract. Maximum mortalities took place at a concentration of 200µg/ml, whereas least mortalities occurred at 50 µg/ml concentration. However, other parts of the *Cassia tora* showed no significant differences in the percentage mortalities in different concentrations. The LD50 values of the several parts of the plants were obtained from a plot of the percentage of the shrimp nauplii killed against the concentration of the extracts.

The significant lethality of the plant part extracts against brine shrimp is an indicative of the presence of potent

cytotoxic phytochemicals which needs further investigation.

CONCLUSION

The medicinal value of the plant lies in some phytochemicals that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, carbohydrate, saponins, glycosides, proteins, tannins, phenols, anthraquinone, terpenoids etc. Thus, it can be concluded that the *Cassia tora* Linn has numerous phytochemical compounds which can be beneficial in various ways to combat many diseases and also to boost the immune system. The confirmatory phytochemical characterization with advanced techniques of the extracts, the identification of responsible bioactive compounds and quality standards are necessary for future studies. The Brine shrimp bio-lethality assay proved to be a

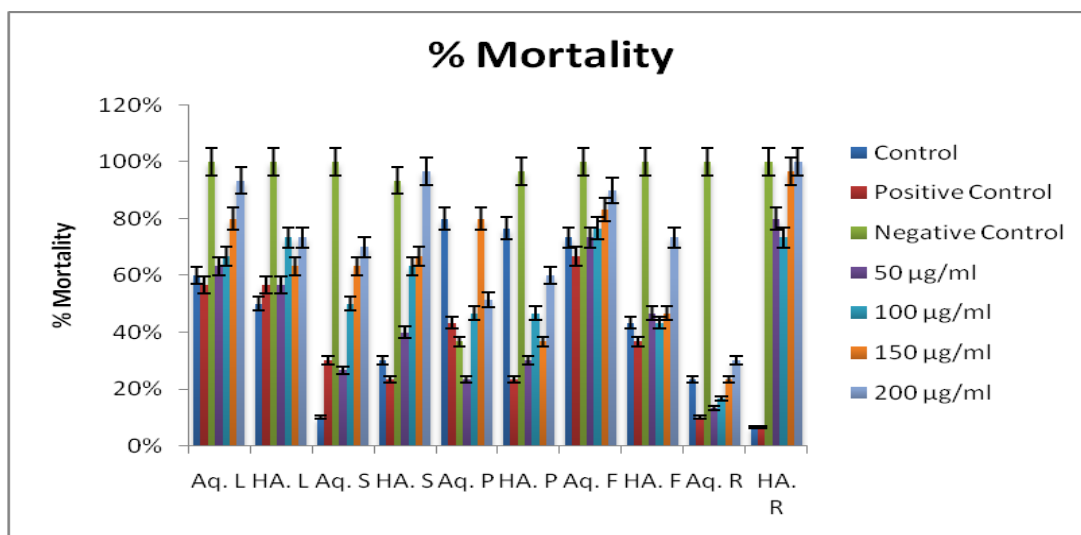


Figure : Graphical representation of % mortality of different *Cassia tora* plant part extracts.

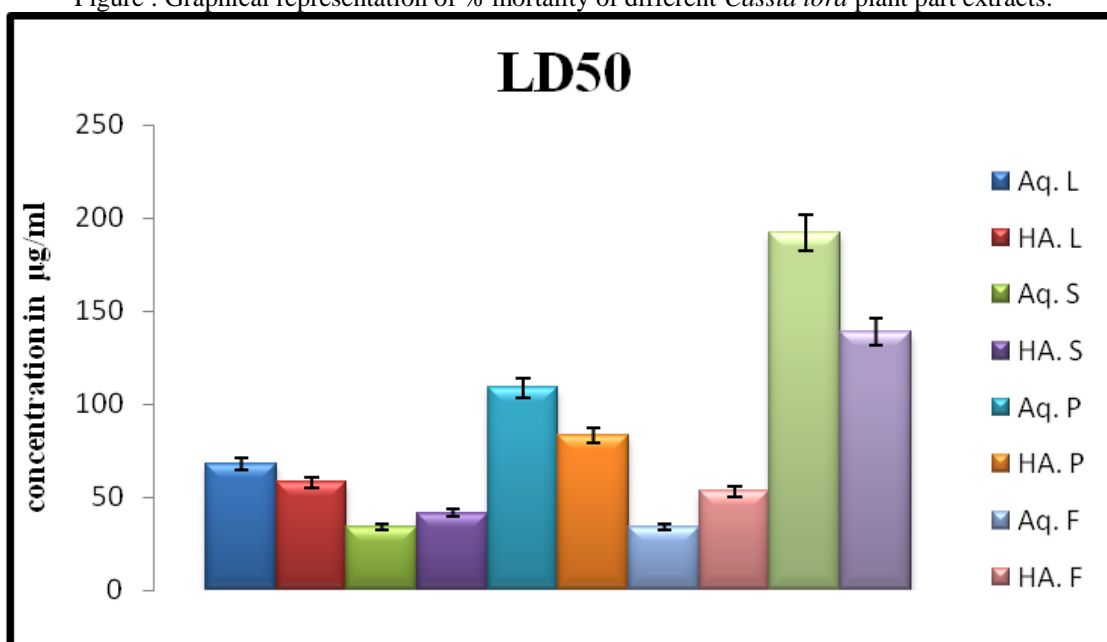


Figure : Graphical Representation of LD₅₀ of different parts of *Cassia tora*.

convenient cytotoxicity assessing system for evaluating the biological activities of several plant parts. Some parts of the plant *Cassia tora* Linn. showed the value of LD₅₀ less than 100 µg/ mL, thus further research is needed to evaluate the mechanism of action of this cytotoxic activity of *Cassia tora* Linn in order to treat cancer and tumors.

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