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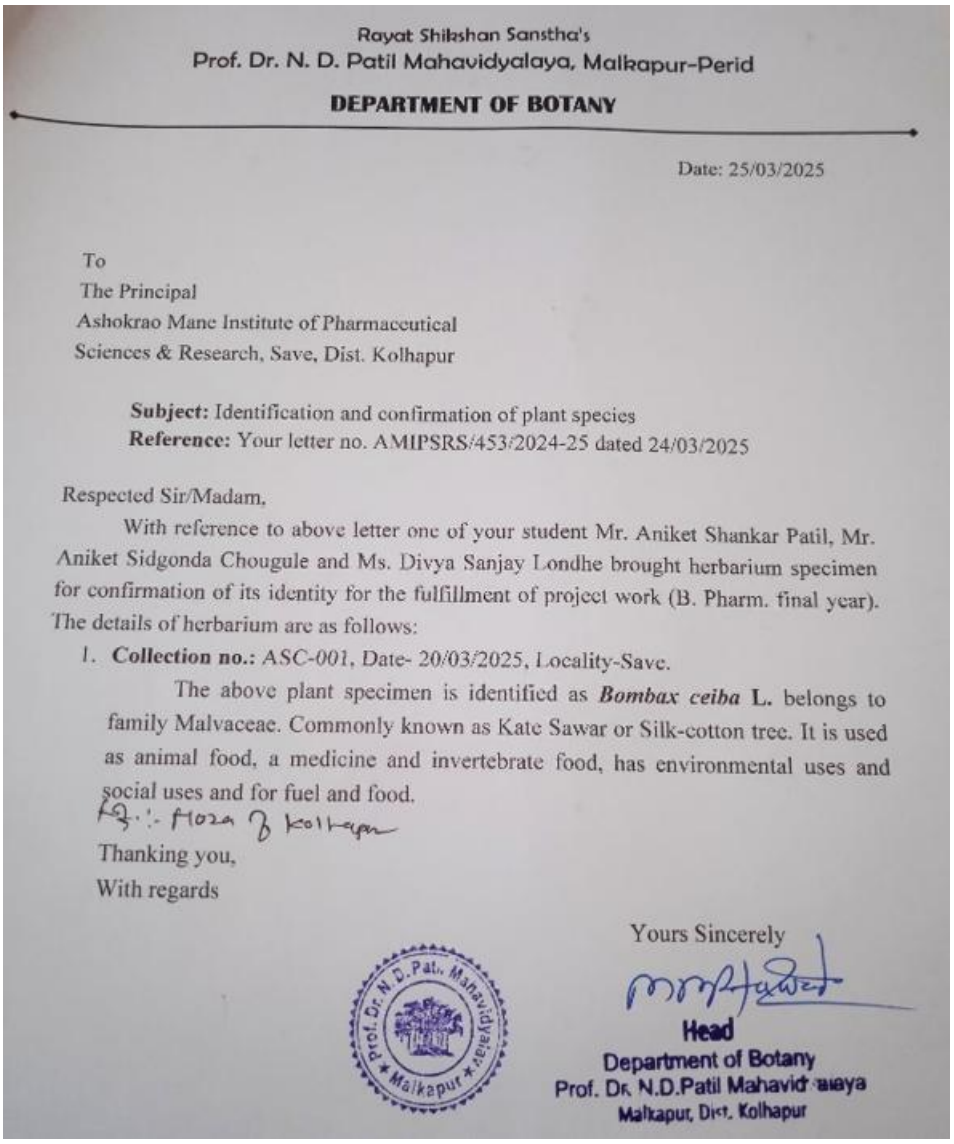
In Vitro Screening of Herbal Extracts for Cytotoxicity in Normal Cell Lines

Aniket Sidhgonda Chougule[1], Aniket Shankar Patil[2], Divya Sanjay Londhe[3], Dr. A. S. Kulkarni[4]

Abstract: ***The research activity has been framed; to study the cytotoxic action of Bombax Ceiba belongs to the family Bombacaceae. A detailed study has been performed by using ethanolic extract of the fruit peel of the plant Bombax Ceiba. The whole cytotoxic activity of the extracts was performed on brine shrimp. The presences of phenolic compound in Ethanolic extract of Bombax Ceiba fruit peel were showed potent Cytotoxicity. The whole preliminary phytochemical screening of the extract showed the presence of carbohydrate, glycosides, alkaloids, protein, amino acid, flavonoids, phenols and tannins. This work provides a scientific support for the high cytotoxic activity of this plant and thus it may find potential applications in the treatment of the diseases.***

Keywords: *Bombax ceiba*, Brine Shrimp, phenolic compound, Cytotoxic activity

Letter of Authentication of Herbal Drug (*Bombax Ceiba*)



Rayat Shikshan Sanstha's
Prof. Dr. N. D. Patil Mahavidyalaya, Malkapur-Perid
DEPARTMENT OF BOTANY

Date: 25/03/2025

To
The Principal
Ashokrao Mane Institute of Pharmaceutical
Sciences & Research, Save, Dist. Kolhapur

Subject: Identification and confirmation of plant species
Reference: Your letter no. AMIPSRS/453/2024-25 dated 24/03/2025

Respected Sir/Madam,

With reference to above letter one of your student Mr. Aniket Shankar Patil, Mr. Aniket Sidgonda Chougule and Ms. Divya Sanjay Londhe brought herbarium specimen for confirmation of its identity for the fulfillment of project work (B. Pharm. final year). The details of herbarium are as follows:

1. **Collection no.:** *ASC-001*, Date- 20/03/2025, Locality-Save.

The above plant specimen is identified as ***Bombax ceiba* L.** belongs to family Malvaceae. Commonly known as Kate Sawar or Silk-cotton tree. It is used as animal food, a medicine and invertebrate food, has environmental uses and social uses and for fuel and food.

Thanking you,
With regards

Yours Sincerely

Head
Department of Botany
Prof. Dr. N.D.Patil Mahavidyalaya
Malkapur, Dist. Kolhapur

1. Introduction

Toxicity: Toxicity refers to undesired harmful effects due to excess dose, i.e., more than therapeutic dose. E.g. Overdose of paracetamol causes hepatotoxicity. If metabolising capacity of body is impaired, drug that is administered in therapeutic doses is not sufficiently metabolised. This accumulation results in toxicity with therapeutic dose. [1]

Cytotoxicity is a general term that can refer to a single toxic effect on any type of cell, including epithelial, endothelial, ormesenchyme cells. If a specific cell type is being targeted,

for instance, the term haemolysis may be used to describe the cytotoxic effect on a red blood cell. Cytotoxicity (including haemolysis) diagnostics are generally performed in the initial stages of drug development and aids in setting appropriate experimental in vitro pharmacology dose parameters. In some situations, such as those for some antimicrobial peptides, cytotoxicity can be a penetrating property, hence a desirable characteristic for efficacy, and the profile does provide therapeutic index information. Other lipid molecules, such as cationic liposomes, LNPs and nanoparticles actively employ their positive charge surface to bind to a cell, or particular receptor on the surface of a cell, and this characteristic makes them useful drug delivery systems. What this also implies is as a matter of principle charged products have the potential to induce unwanted toxic effects, which include membrane permeabilization, unintended cell binding, and/or in the case of erythrocyte binding, red blood cell lysis reviews cationic and ionisable cationic lipids, and how lipids of a cationic nature can induce intracellular and nuclear membrane disruption which can lead to release of lysosome degrading enzymes.

Since the nucleic acids and their precursors are the main target of action and active nucleic acid synthesis occurs during cell division, the majority of cytotoxic medicines are more effective against actively dividing cells. Compared to normal epithelium linings, bone marrow, reticuloendothelial (RE) system, and gonads, the majority of malignancies, and big solid tumors in particular, have a decreased growth fraction (a smaller percentage of cells are in division). The majority of medications specifically affect these tissues in a dose- dependent manner, while individual member sensitivity varies.[2]

Brine Shrimp:

The brine shrimp A group of seven or nine species found in Artemia most likely broke off from a Mediterranean progenitor during the Messinian salinity crisis around five and a half million years ago. The With over 1,700 samples from diverse populations around the globe, the Laboratory of Aqua culture and Artemia Reference Centre at Ghent University is home to the biggest known collection of Artemia cysts.[3]

A very rudimentary Arthropod called Artemia, has a segmented body and broad appendages. The body of this animal typically contains 19 segments of the body, the first 11 bearing pairs of appendages, the next two (often fused) containing reproductive structures, and the final segments constructing the tail. An adult male Artemia will typically be 8–10 millimeters (0.31–0.39 in) in length, a female will be 10–12 mm (0.39–0.47 in), and both sexes and their legs are 4 mm (0.16 in) in width.[4]

The head, thorax, and abdomen makeup Artesia's body structure. The chitin us exoskeleton, a thin, elastic covering that shed periodically, encloses the whole body. Before each ovulation, the female Artemia undergoes a molting. Swimming, reproduction, and digestion are just a few of the functions of brine shrimp that are not controlled by the brain; rather, ganglia in the local nervous system may regulate or synchronize these functions. Local control also governs autotomy, the voluntary loss or dropping of body parts for defense, along the neural system. There are two kinds of eyes in Artemia. On elastic stalks are attached two separate compound eyes. Adult brine shrimps rely on these sophisticated eyes as their main optical sense. Located anteriorly in the center of the head, the median eye, also called the naupliar eye, is the sole visual sense organ in nauplii that continues to operate until adulthood.[5]

Figure 1: BrineShrimp[5]

Life Cycle of Brine Shrimp

As temperatures rise in the spring, large numbers of brine shrimp cysts begin to hatch in Utah's Great Salt Lake. By the end of April, the lake is heavily populated with young brine shrimp larvae called nauplii.[6]

These shrimps undergo 14 to 17 developmental stages before reaching maturity, with each stage separated by a molting process. During each molt, the shrimp shed their old outer shell and grow a new, larger one.[7]

Inideal conditions—such as warm water, ample food, and high oxygen levels—brine shrimp can become adults in just eight days. However, the lake's typically harsh environment slows this process, and it usually takes three to six weeks for them to fully mature.

When the circumstances are right, mature females release developing embryos or free- swimming nauplii into the water. But as the temperature lowers and food becomes limited, the females discharge latent cysts. Inside the cysts, the embryos' development is halted. The outer shell protects them from the outdoors. When the circumstances improve, the embryo restarts development, continuing the life cycle.[8]

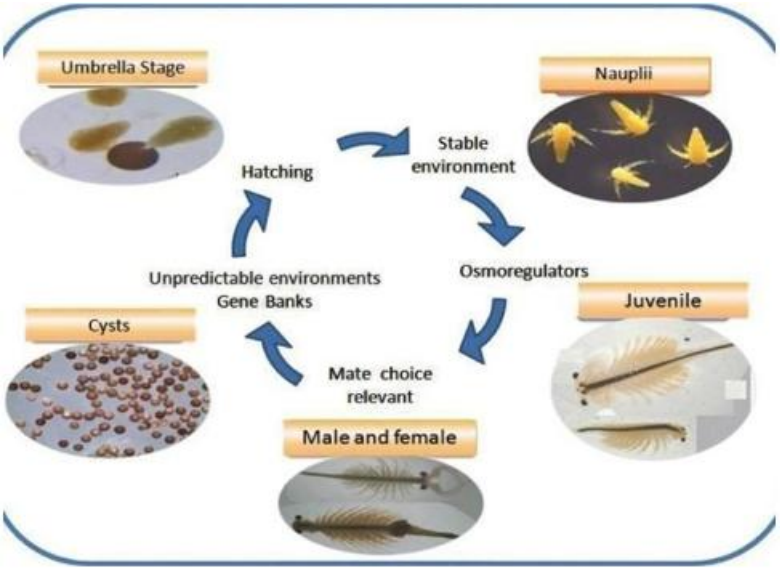


Figure 2: Life Cycle of Brine shrimp[8]

Herbal plants demonstrated cytotoxic activity

Figure 3: Alvera

Figure 4: Ashwagandha

Figure 5: Catharanthus roseus

Figure 6: Taxus brevifolia

Figure 7: Camptotheca acuminate

Figure 8: Podophyllum peltatum

Figure 9: Crucuma longa

Figure 10: Aconitum Carmichaelii

Figure 11: Ephedra distachya

Figure 12: Atracurium besilate

Figure 13: licprice

Figure 14: Azadirachta indica

***BOMBAX CEIBA*:**

Bombax ceiba plant is similar to Kalpataru because each and every part can be used to its medicinal as well as commercial importance.[9] Bombax ceiba plant is' The best god's creation' for the human race and nature's valuable gift since its multi-purposes eco-friendly uses. There are many customs, traditions, rites, folk tales and rituals associated with this plant. Bombax ceibaL. Also known as silk cotton tree, Semal and it is well known among most tribal people.[10] The tree carries medicinal as well as commercial importance but is exploited by means of usage for Holika- dahan. Bombax ceiba in India is known by different names in different languages suchas Semal, Shalmali, Indian Kapok tree, Shimul. [11]

The genus Ceiba is a tall, straight, and stiff deciduous tree that is a member of the Bombacaceae family. It has spikes on its tall, straight trunk to protect it from predators. It has palmate, big, spreading, glabrous, digitate leaves that are 15 to 30 cm long and have a common petiole. In April, ornithophilous large red pentamerous blooms with cup-shaped, thick, meaty sepals appear. The blooms contain a strong perianth with stiff filaments and a well-differentiated ovary. A white-fibered capsule containing smooth, black or gray seeds is present in mature blooms. When trees have no leaves, they develop large, eye-catching blossoms. This plant has socioeconomic and ethno medical relevance since it was used by many Indian tribal culturesas fuel, food, fodder, fibre, and medicine.

Figure 15: Bombax Ceiba

A wide range of ailments, including pulmonary tuberculosis, kidney and bladder ulcers, enteritis, gonorrhoea, diarrhoea, menorrhagia, skin conditions, calculus disorders, chronic inflammation, catarrhal inflammation, chronic cystitis, and pulmonary tuberculosis, have been extensively treated with all parts of Bombax ceiba L. in traditional medicine.[12] B. ceiba pharmacologically exhibits a wide range of biological actions, such as anti-inflammatory, anti- cancer, anti-acne, antipyretic, antimicrobial, antibacterial, anti-obesity, hypotensive, hypoglycaemic, hepato protective, and cardio protective qualities.[13] To the best of our knowledge, the genotoxic and anticancer effects of Bombax ceiba L. flowers have not been extensively studied. As a result, the current study concentrated on assessing two main goals: To assess the ethanol extract from B. ceiba flowers' total polyphenol content and DPPH radical scavenging ability; to look into the extract's cytotoxicity and genotoxicity against liver and lung cancer cells using the MTT assay and comet assay, respectively.

More and more people are turning to traditional medicines to heal ailments.[14] Due to the extensive usage of ethno medicine, the WHO and numerous research-focused organizations have made significant investments in the study of traditional herbal medicine. 76 of the 1562 approved drugs (from 1994 to 2014) were either unaltered natural goods or botanicals, while 654 were derivatives of natural products. [15] This increased focus on traditional medicine led to a surge in study on the scientific validity of medicinal herbs. The research has examined the red silk-cotton tree's scientific validity for a variety of biological functions.[16] *Bombax ceiba* is usually known with different type of names like as Kapok tree, Moca and Semal are members of the *Bombuencere* family. Following numerous scientific studies, its many therapeutic applications have been documented in Indian traditional pharmaceutical systems like Ayurveda, Siddha, and Unani. Africa, Australasia, tropical Asia, and Asia all have abundant supplies of it. At elevations of up to 1500 meters, it is primarily found in India.

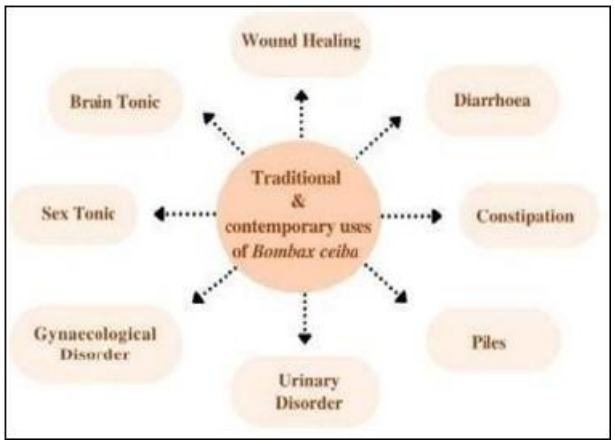


Figure 16: Various traditional and contemporary uses of Bombax ceiba.[16]

Table 1: Taxonomical classification

S. No.	Rank	Scientific Name	Common Name
1	Kingdom	Plantae	Plants
2	Subkingdom	Viridiplantae	Vascular Plants
3	Super division	Spermatophyta	Sea Plant
4	Division	Magnoliophyte	Flowering Plants
5	Class	Magnoliopsida	Dicotyledons
6	Subclass	Dilleniidae	-
7	Order	Malvales	-
8	Family	Bombasaceae	Kapok–Tree family
9	Genus	Bombox L.	Cotton tree
10	Species	BombaxCeiba L.	Red silk cotton tree

2. Need of Study

This work serves to validate its traditional medicinal application against tumour, determines bioactive compounds, and gives an idea about its overall toxicity. Positive finding scan direct further in vitro cytotoxicity research and drug development. Cytotoxicity assays are needed in the market to ensure the safety and efficacy of various products, from drugs and cosmetics to medical devices and industrial chemicals. These assays help determine the potential of a substance to damage or kill cells, which is crucial for identifying potential side effects and ensuring the safety of human use.

3. Objective For the Study

1) Identification of herbal plant
2) Identification tests for evaluation of various chemical constituents.
3) Extraction of *Bombax Ceiba* using ethanol solvent by Soxhlet extraction method
4) To check Cytotoxicity activity

4. Literature Review

1) In the reference book of Pharmacology studied on the cytotoxicity activity of mechanism of actions and side effects and complications.[17]
2) In the reference book of Pharmacognosy investigated on the herbal plants, its identification tests its cultivation and collection. It also provides information about different extraction methods like purification, maceration, decoction etc. It gives pathway for the screening of photochemical nature of plant extract. The plant extract found rich source of carbohydrates, alkaloids, flavonoids, glycosides, tannins etc.[19]
3) In the reference book of Indian medicinal plants vol. 1 2nd edition the list of some of the drugs employed, showing their nature according to native ideas, and also the real use in European medicines.[18]
4) In reference eBook of international journal of pharmacy of and pharmaceutical science vol. 4th antioxidant and anti haemolytic activities of Bombax ceiba pentandra spike and fruit extract. [20]
5) In reference book of European journal of medicinal plant vol. 3 1st edition of the in vitro anti – inflammatory evaluation of crude Bombax ceiba extracts. [21]

5. Material and Methods

5.1 Collection and identification of plan material:

The plant was collected in large quantities from Save, Malkapur, Shahuwadi, Kolhapur District in Maharashtra. The botanical identification and authentication of the plant material were conducted by Department of Botany, Prof. Dr. N. D. Patil Mahavidyalaya, Malkapur- Perid Tal-Shahuwadi, Dist.-Kolhapur Before the authentication process, the entire plant, including Stem, Seed, Flower and Branches, was dried at room temperature until the moisture content was removed.

5.2 Extraction procedures

Preparation of extraction from methanol

The entire plant of *Bombax Ceiba*, was dried at room temperature. After complete drying mechanically ground into powder using a mixer and sieved using a No. 10/44 sieve. The plant powder was packed into 200ml thimbles within a Soxhlet apparatus. Extraction took place over about 45 cycles in 13hours to complete the extraction in five separate 50gm batches, employing ethanol (80%). The concentrated extract was obtained by separating solvent by simple distillation and then extract was packed, weighed and kept in a refrigerator.

5.3 Extraction of crude drug using Soxhlet apparatus:

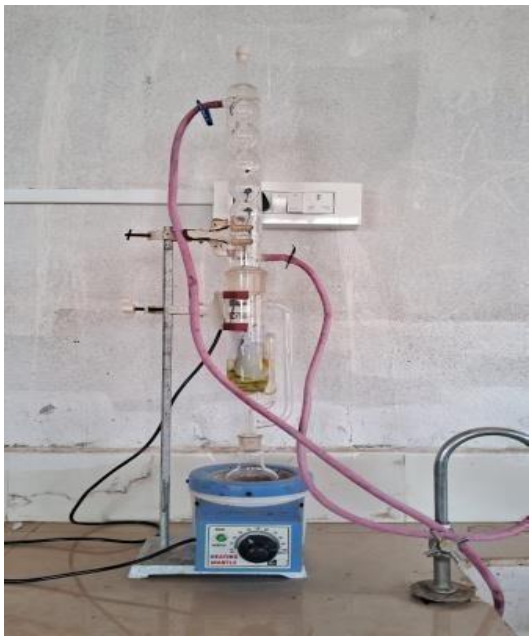


Figure 17: Soxhlet Apparatus

Phytochemical Identification Test

Table 2: Phytochemical screening of *Bombax Ceiba* Plant Extract

S. No.	Phyto-Constituents	Test	Observation	Inference (Ethanolic extract)
1.	Carbohydrates and Glycoside	**1.Fehling's test:** 1ml of each Fehling's A and Fehling's B where mixed and heated with onemin. and Extract were added and heated for 5min. On water bath.	First yellow then brick red colour.	Reducing sugar is present.
		2.Molisch'stest: Ethanolic extract was treated with few drops of a-naphthol in alcohol. To this add H_2SO_4 along the side of the test tube.	No violet ring is formed between the junction of two liquids.	Carbohydrate is absent.
		3.Barfoed'stest: 1ml of extract is heated. with 1ml of Barfoed's reagent on water bath.	Red colour.	Monosaccharide is present.
2.	Alkaloids	**1.Dragendorff'stest:** 1ml of extract treated with few drops of Dragendorff's reagent.	Reddish brown precipitate.	Alkaloids are present.
		2.Mayer'stest: 1ml extract treated with Mayer's reagent.	Cream colour precipitate.	Alkaloids are present.
		3.Wagner'stest: 1ml extract treated with Wagner's reagent.	Reddish brown precipitate.	Alkaloids are present.
		4.Hager'stest: 1ml of extract treated with Hager's reagent.	Yellow precipitate.	Alkaloids are present.
3.	Protein and amino acids	**1.Biurets test:** 2ml extract treated with 2ml Biurets reagent.	No Violet colour.	Protein is absent.
		2.Millon'stest: 2ml extract treated with 2ml of Millon's reagent	No white precipitate.	Amino acid is absent.
4.	Flavones and Flavonoids	**1.Alkaline reagent test:** 1ml extract treated with few drops of 10% NaOH	Yellow colour observed.	Flavonoid is present.
		2.Shinoda test: To the extract add few magnesium turnings and Conc. Hydrochloric acid drop wise.	Pink colour observed.	Flavonoid is present.
		3.Conc.H2SO4	Yellow not colour appears.	Flavonoid is absent.
5.	Phenols and tannins	**1.FeCl3 test:** 1ml extract is treated with ferric chloride solution.	Blue colour appears	Tannins are present.

Cytotoxicity Activity

Brine Shrimp Lethality Assay (BSLA)[22]

Materials Required:

Apparatus:

- Beaker or hatching chamber
- Incubator or lamp (light source, ~25–30°C)
- Pasteur pipette
- Test tubes or vials (labelled)
- Micropipette
- Counting chamber or magnifying lens.

Chemical:

- Brine shrimp (Artemia saline) eggs
- Artificial seawater (3.8 g sea salt in 100mL distilled water)
- Plant extract/compound
- Control (solvent only) Brine Shrimp Lethality Assay
- Dimethyl sulfoxide (DMSO) or ethanol (as solvent)

Step 1: Hatching Brine Shrimp Eggs

Prepare artificial sea water by dissolving 3.8 g sea salt in 100mL distilled water.

↓

Add brine shrimp eggs to the hatching container with sea water.

↓

Provide continuous aeration and light (a desk lamp works fine).

↓

Allow to hatch for 24–48 hours at~28°C.5.After hatching, collect nauplii (larvae) using a pipette (they move toward the light).

Step 2: Sample Preparation

Dissolve plant extract/compound in DMSO or ethanol to prepare a stock solution.

↓

Prepare serial dilutions (e.g.,10,100,1000µg/mL) in sea water.

↓

Ensure final DMSO or ethanol concentration in each tube is <1%.

Step 3: Bioassay

Transfer 10 naupliiin to each test tube/vial (use clean pipette). Add 5 mL of each concentration into separate tubes.

↓

Setup:

- Test samples (various concentrations)
- Negative control (sea water + solvent)
- Positive control (e.g., potassium dichromate or vincristine)

↓

Incubate all tubes at room temperature (~28°C) for 24 hours.[23]

Brine shrimp lethality Assay

Percent mortality = $\frac{\text{(Totalnauplii – Alivenauplii)}}{\text{Total naupliix100}}$

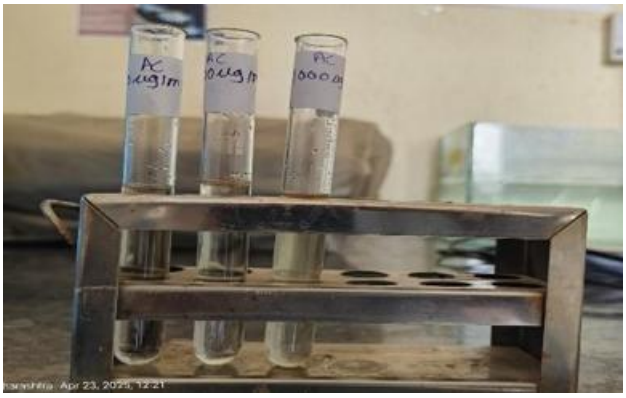


Figure 18: Brine shrimp lethality Assay

6. Result and Discussion

Brine Shrimp Lethality Assay (BSLA) of sample

Table 3: Brine shrimp lethality Assay

Group	Conc. (µg/mL)	Total Shrimp	Survived T1	T2	T3	Total Survived	Dead	% Mortality
Control	-	30	10	10	10	30	0	0%
Sample -AC	10	30	5	4	5	14	16	53.33%
	100	30	3	3	2	8	22	73.33%
	1000	**30**	**1**	**2**	**2**	**5**	**25**	**83.33%**

The extract's Cytotoxicity activity correlated with its overall Polyphenolic and Flavonoid content, the Phenolic compounds and flavonoids play a significant role and high mechanism of action may be due to its high contents responsible for the Cytotoxicity activity of the *Bombax Cebia* extract.

7. Conclusion

The Brine Shrimp Lethality Assay of sample AC

demonstrated a dose-dependent cytotoxic effect. At increasing concentrations of 10, 100, and 1000 µg/mL, the present mortality of brine shrimp was observed to be 53.33%, 73.33%, and **83.33%**, respectively. In contrast, the control group exhibited 0% mortality, confirming that the observed lethality was due to the bioactive compounds present in the sample. These results suggest that sample AC possesses significant cytotoxic potential**.**

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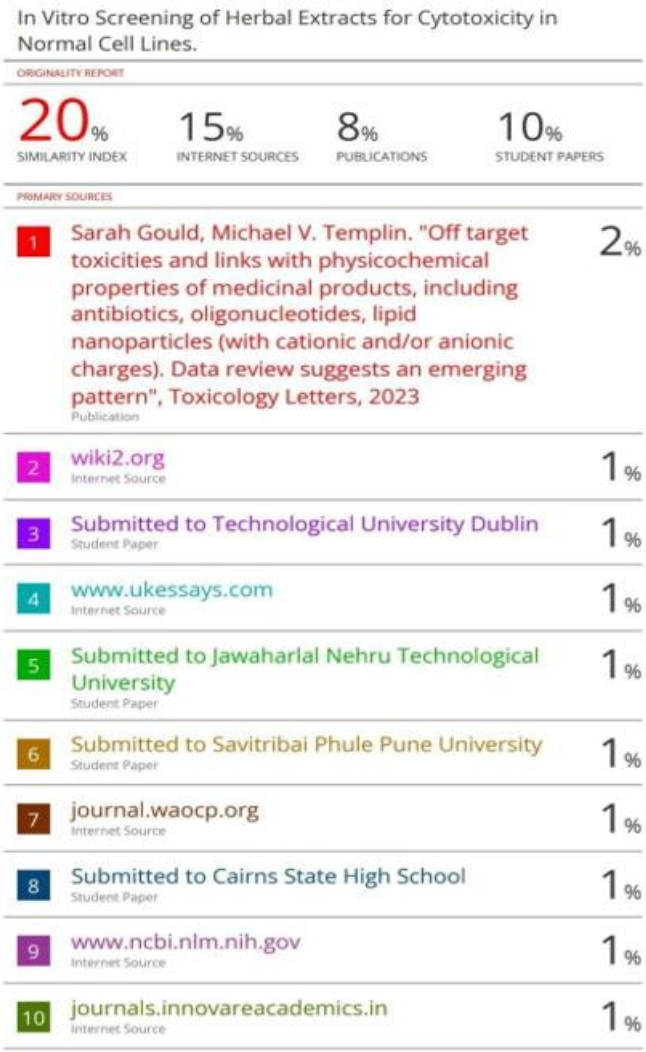

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