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"Pharmacognostical Investigation and In-Vitro Cytotoxicity Study of Helicteris Isora Fruit"

[1]Mr. Shubham Swami, [2]Mr. Utkarsh Joshi, [3]Ms. Rashmi Gurav, [4]Ms. Prajakta Karambelkar, [5]Ms. Aishwarya Thakur*, [6]Dr. Vijay Jagtap

Yashwantrao Bhonsale College of Pharmacy, University of Mumbai, Sawantwadi - 416510, Maharashtra, India.

Corresponding Author: Ms. Aishwarya L.Thakur, Yashwantrao Bhonsale College of Pharmacy Sawantwadi - 416510, Maharashtra, India.

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ABSTRACT: The present study is to focus on the cytotoxicity of the hydro-alcoholic extract of the Indian medicinal fruit Helicteris isora (Murudsheng) belonging to family Malvaceae. Among several plant Helicteres isora is an important medicine possessing remarkable nutritional and therapeutic activities. Such as it have Antioxidant activity, Antimicrobial activity, Anticancer Activity, Antdiabetic activity. The brine shrimp bioassay method is very useful for assessment of toxicity of plant extract. Brine shrimp lethality bioassay method was used for the present study and the cytotoxicity was reported in terms of lethality concentration (LC50). The shrimp were hatched and active shrimp were collected and used for assay. Then the active shrimp added to 2.5 ml test solution and kept for 24 hrs. and lethality concentration (LC50) was assessed. 24 h of observation all the shrimp were survived in the control. Even though, maximum mortalities were observed at a concentration of 10000µg/ml and1000ug/ml and least mortality 100ug/ml concentrations. It was observed that in higher concentration of treatment extracts, the shrimps were start dying only after 6 h and after 24 h all the shrimps died. The result on the lethality of Helicteres isora fruit (hydro-alcoholic extract) is with LC50 value.199.2ug/ml. against brine shrimps. The hydro-alcoholic extracts of Helicteris isora exhibited potent brine shrimp lethality (LC50). The present study supports that brine shrimp bioassay is simple, reliable and convenient method for assessment of bioactivity of medicinal plant. Also investigate Pharmacognostical properties of the Helicteris isora fruit. For an example, organoleptic properties of drug, physical evaluation like moisture content, total ash value etc.

KEY WORDS: Helicteris isora, Brine shrimp lethality, Lethality Concentration 50, Hydro-alcoholic extract.

Fig 1. HELICTERES ISORA FRUIT.

I. INTRODUCTION

Plants are put to medicinal use all over world since time immemorial. The importance of medicinal plant and traditional health system in solving health care problem of work gaining increase attention Because of this resurgence of interest, the research on plant of medicinal importance is growing tremendously. The essence of present study is to focus on the cytotoxicity of the alcoholic extract of indian medicinal plant Helicteres isora belonging to family Malvaceae. Helicteres isora fruit is medicinal plant of indian origin possess a plenty of therapeutic compounds useful for treating various diseases. Most of these compounds are highly nutritious and rich source of antioxidants. Many plants and herbs contain an excellent composite of nutritive and medicinal properties which are easily available, cost effective and safe for long term use. Considering these facts and taking into account a broad spectrum of their usage, focus of research has been to find lead molecules in herbal resources. Experimental and clinical studies from our laboratory as well as that

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from other researchers have provided strong evidences of relation between bioactive compounds and reduced risk of cancer and other disorders. Among several ancient medicinal plants, Helicteres isora is an important medicinal plant possessing remarkable nutritional and therapeutic activities. It is a tropical south-east Asian shrub cultivated throughout India. Different parts of the plant are traditionally used in indian system of medicine to cure various diseases. The H. isora was a rich source of bioactive compounds such as polyphenols, tannins and alkaloids that exhibit therapeutic effects. Moreover, H. isora is to be a good source of carbohydrate, proteins, fiber, calcium, phosphorus and iron. Based on extraction and characterization studies have shown the presence of some antioxidant compounds such as ascorbic acid, flavonoids and phenolic. Phytoconstituents of Helicteres isora Linn. Fruits The essential chemical constituents of Helicteres isora Linn. are Isoscutellarein and their derivatives; Helisterculins A and B, Helisorin, Gallic acid, Caffeic acid, vanillin, p-Coumaric acid. Some researchers isolated three new compounds which are 49-O-b-D-glucopyranosyl rosmarinic acid , 4,49-O-di-b-Dglucopyranosyl rosmarinic acid and 2R-O-(49-O-b-D-glucopyranosyl caffeoyl)-3-(4hydroxyphenyl), lactic acid named as 49-O-b-Dglucopyranosyl isorinic acid were isolated together with rosmarinic acid from the fruit of H.isora (Sterculiaceae), anIndonesian medicinal plant. The structures of these compounds, including the absolute stereochemistry of ,were elucidated by spectroscopic analysis and chemical means compound had greater scavenging activity against superoxide anion produced with xanthine and xanthine oxidase than rosmarinic acid. Now-a-days brine shrimp (Artemia salina) lethality assay is commonly used to check the cytotoxic effect of bioactive chemicals. These eggs are really cysts which, if they are kept dry, can remain dormant for years before hatching. As soon as the eggs are exposed to water, the hatching process begins. Artemia salina the brine is a component of the fauna of saline aquatic and marine ecosystem it play an important role in the energy flow of the food chain. And it can be used in a laboratory bioassay in order to determine the toxicity by the estimation of the medium lethality concentration LC50 which have been reported for a series of toxin and plant extract. Brine Shrimp Lethality Assay is the most convenient system for monitoring biological activities of various plant species. This method is very useful for preliminary assessment of toxicity of the plant extract.Only a few species were bioactive against the brine shrimp bioassays at 10 and 100 µg de extract per ml, and a low relationship between the brine shrimp bioassays and the cytotoxicity assays was found. However, most of the invertebrates presented toxicity in some of the bioassays at 1000 µg/ml in a way that was consistent with the cytotoxicity results With respect to the effect of the time of exposure, in the hatchability test the highest percentage of toxicity was detected at 24hr or 30 hr of exposure, and significant changes in toxicity were not detected in subsequent times of exposure. The very low hatching rate detected after the 12 hr treatment was probably due to an alteration in the development of Artemia embryos. Cytotoxic (cyto=cell, toxic=damage) drugs work by targeting and damaging cells that grow at a rapid rate. Brine shrimp lethality bioassay is a proper design ofexperiment as many other medical researchers ,organic chemicals are generally used as drug vehicle or carriers in brine shrimp lethality bioassay ,however these compounds often have toxicity or even the pleiotropy in vitro or in vivo hence researches must be aware of their desirable or undesirable effect and proper control experiments should be carefully design otherwise , bias may introduce in bioassay and effect of solvent can be attributed to the tested medicine falsely. In brine shrimp lethality bioassay both positive and negative group should be design .solvent is very important player in brine shrimp lethality bioassay.[1][4]

II. METHODS[10]

1. COLLECTION AND AUTHENTICATION OF RAW MATERIAL :The fruit Helicteres isora we bought from the Ayurvedic shop that is in South Konkan region means Shiroda , Taluka: Vengurla.After collection we dried it under the sunlight for 2 days. And then dried fruit of Helicteres isora stored in air tight well closed container to prevent from external moisture. After 2 / 3 days of drying process, the powder is assemble by using a Grinder.

2. ORGANOLEPTIC (MORPHOLOGICAL) EVALUATION

- Colour-Green (Raw) , Brown (Dried)
- Odour Characteristics
- Shape- Twisted with a screw at its pointed end
- Taste- Tasteless

3. EXTRACTION OF PLANT MATERIAL

Coarsely powdered plant material was subjected to cold maceration with 70% ethanol with occasional

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shaking. Extract was filtered through Whatman filter paper and the filtrate was collected in Clean glass container. Marc was dried and subjected to Soxhlet extraction using 95% ethanol (till the colored extract become colorless) at 40 °C. Extract was filtered immediately under hot condition and filtrate was mixed with filtrate of cold maceration. Obtained filtrate was dried and stored in air tight container as an extract.

4. PHYSICOCHEMICAL INVESTIGATION: The powdered material of Helicteres isora fruit was subjected to physico-chemical investigation to assess the qualitative chemical composition through following standard methods.[2]

1. Loss of drying :

The mass is lost due to heat expressed in percent w/w. Weigh accurately 10grams of sample was poured on petri dish and kept in hot air oven at 105ºC for 5 hours. Loss on dying of sample with reference to initial volume was calculated.

2. Ash values

a) Total ash: Weighed 2 grams of sample and incinerated in a crucible at a temperature 500-600ºC in a muffle furnace until carbon free ash was obtained. Then it cooled, weighed and percentage of total ash was calculated with reference to the air dried drug.

b) Acid-insoluble ash: The above obtained ash was used to boil for 5min with 25ml of (70 g/l) hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retains on filter paper was washed with hot water and the filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash was calculated with reference to air dried drug.

c) Water - Soluble ash: The above obtained ash was boiled for 5min with 25ml of water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450ºC in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powdered.

CYTOTOXICITY STUDY BY BRINE SHRIMP LETHALITY BIOASSAY (BSLA) METHOD[3],[5],[6]

1. Preparation of Sea Water

Weighed 38 gm of rock salt and Dissolved in 1L of water. Add Pinch of yeast to maintain the pH. Stirred it to dissolved and keep it for 15 min.

2. HATCHING OF BRINE SHRIMP

Artemia salina [brine shrimp eggs] collected from Amazon was used as a test organism. Seawater was taken in the small tank and shrimp eggs are added to one side of the tank and then this was covered. Two days wew allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. The hatching shrimp was attracted to the light and so nauplii free from eggs shell was collected from tank. The nauplii was taken from the tank by a dropper and diluted with fresh sea water to increase visibility.

Fig 2. HATCHING ASSEMBLY

3. Putting Shrimp on a Microscope Slide

Take a clean glass slide and gently rub it for dry and shiny. Put a drop of sea water with shrimp onto the slide. Suck the any extra water so that shrimp is confined in a blob of water. This must not dry up, so add just one drop of water every minute. Then place a slide beneath a hand lens i.e. (10x) or under the a low power microscope (40x) you can observed much detailed.

4. Preparation of Test Solution with Sample of Helicteres isora Fruit Extract

Clean 4 test tube were taken and label it. Plant extract of 10mg was pipette out. Then stock solution was prepared by dissolving 10mg plant extract and dissolved in 2% of pure Dimethyl sulfoxide (DMSO). Thus the concentration of stock solution was 10 mg/ml. Then the solution was diluted 10000, 1000, 500, 100 ug/ml with sea water containing 10 nauplii.

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Fig 3. PREPARATION OF TEST SOLUTION

5. Preparation of Control Group

Control groups were used in cytotoxicity study to validate the test method and ensure that the result obtained were only due to the activity of the test agent And effects of the other possible factor were nullified.

6. Counting of Nauplii

After 24 hrs, the test tube were inspected using magnifying glass against a black background and the number of survived nauplii in each test tube counted. From this data the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

III. RESULT AND DISCUSSION

1. Organoleptic Properties:

The colour of helicteres isora fruit is brownish grey and characteristic odour. shape of helecteris isora is screw like fruit and it is tasteless.

2. Physicochemical Properties

Table no 1:

Sr.no	Physico-chemical parameter (%w/w)	Result for Tinospora cordifolia (%w/w)
1	Total ash	14 ± 0.05
2	Acid soluble ash	9.0 ± 0.07
3	Water soluble ash	7.06 ± 0.04
4	Loss on drying	8.0 ± 0.017

Ash values: It is useful in determining the authenticity and purity of the samples. The samples were found within the range of 14 ± 0.05, 9.0 ± 0.07 and 7.06 ± 0.04 respectively.

Loss on drying: The major factor responsible for the deterioration of the samples is moisture. Low moisture content is always desirable for higher stability of drugs. The samples were found within the range of 8.0 ± 0.017 .

3. Cytotoxicity Study (BSLA Method)

Table no.2:

Conentration	No. of mortile shrimp	No. of shrimp dead	Percentage of dead
10000	6	4	90%
1000	5	5	70%
500	3	7	50%
100	1	9	40%

Tableno.3

Concentration	Log 10 concentration	Percentage of dead	Probit value
100	2	40	4.87
500	2.6989	50	5.13
1000	3	70	5.67
10000	4	90	6.64

Very important evaluation for ensuring purity of the drug. The brine shrimp lethality assay (BSLA) is a simple and inexpensive bioassay used for testing the efficacy of phytochemical present in the plant extracts. The present study determined that the extent of lethality was directly proportional to the concentration of the extract. After 24 h of observation all the shrimp were survived in the control. Even though, maximum mortalities were observed at a concentration of 10000µg/ml and1000ug/ml and least mortality 100ug/ml concentrations. It was observed that in higher concentration of treatment extracts, the shrimps were start dying only after 6 h and after 24 h all the shrimps died.

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The result on the lethality of Helicteres isora fruit (hydro-alcoholic extract) is with LC50 value.199.2ug/ml. against brine shrimps. The presence of alkaloids, tannins, and flavonoids could be accounted for its cytotoxic properties. Thus, the results shows that the Helicteres isora fruit can exhibit cytotoxicity against brine shrimp with mortality rate .

IV. CONCLUSION

The hydro-alcoholic extract of Helicteres isora plant species (fruit) could exhibit cytotoxicity against brine shrimp lethality assay consisting of 100ug/ml concentration and with Lc50 of 199.2ug/ml. Although other concentrations viz, 10000 ug/ml and 1000ug/ml. This could be due to the presence of different bioactive compounds present in the plant species. Although BSLA is inadequate in determining the mechanism of action of bioactive substances in the plant, it is very useful in providing the preliminary screening that can be supported by a more specific bioassay once the active compound has been isolated. Thus some useful drugs of therapeutic importance May develop out of research work.

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