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Research Article

To Study In - vitro Anticancer activity (Brine shrimp Lethality) and Anticoagulant Activity of Tribulus terrestris L. Seeds

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ABSTRACT

The ethanol extract and aqueous extract from that study. The Tribulus terrestris Seed was then subjected to phytochemical testing, which revealed pharmacological effects such as anticancer activity as measured by the brine shrimp Lethality Bioassay and anticoagulant activity. Aqueous extract has been shown to be more active than ethanol extract in comparison studies. Tribulus terrestris extracts were effective against brine prawns with LC50 values of coc.100, 200, 400, and 800 (g/mL), according to in vitro anticancer activity cytotoxicity evaluation results. The in vitro anticoagulant effects of various Tribulus terrestris extracts in different concentrations of 25, 50, 100, and 200 mg/ml were examined using plasma, obtained from blood samples of healthy individuals. Saline in distilled water was used as a positive control and EDTA was used as a negative control when measuring PT.

INTRODUCTION

The brine shrimp is a straightforward and efficient animal test in biological sciences and in toxicology due to the availability of the eggs, the simplicity with which they may be hatched into larvae, the speed with which the nauplii grow, and the relative ease with which populations can be maintained under laboratory settings. The brine shrimp test offers a bioassay that can be quick, easy, bench-top, and more significantly, affordable and repeatable when used in conjunction with a reference standard. Blood coagulation was first thought of in the 1960s. There are intrinsic and extrinsic pathways in the coagulation system. The extrinsic pathway begins with the exposure of tissue factor and is activated in response to tissue stress. Less is known about the intrinsic pathway's function. Strokes, transient ischemic attacks, deep vein thrombosis, and pulmonary embolism are all conditions that call for the use of anticoagulants.[1]

MATERIALS AND METHOD

Authentication and collection of plant

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In the month of December 2022, the Tribulus terrestris L. plant was harvested from the Sahyadri College of Pharmacy campus in Methawade, Sangola Dist. Solapur. For roughly a month at room temperature, the plant material was shade dried. The plant was verified by the botany department at Sangola College in Sangola, headed by Tembhurne Sir. The dry material was physically ground into a coarse powder with the aid of a grinder, put through a 22mesh sieve, and kept in an airtight container until use.

Preliminary Phytochemical studies:

The *Tribulus terrestris* Seed powder Was extracted with Ethanol and Aqueous. These extracts were tested for presence of different phytoconstituent by qualitative. The results of phytochemical analysis [2,3,4]

***In vitro* anticancer Activity**

A hot air dryer was used to dry 1 ml of each of the aqueous and ethanol extracts at a low temperature (20°C). Next, a digital balance was used to weigh the dry material. It was the mg of dry material present in each extract's ml that made the difference. Measurement of the Concentration of the Extract [5,6,7,8,9]

Materials

1) Anaemia salina Leach. (Brine eggs), Sea salt (NaCl)
2) Small tank
3) Lamp to attract Shrimps
4) Pipettes (5, 10ml) and Micropipette (5-50nl), (10-100ul)
5) Glass vials, Magnifying glass (Asaduzzaman, Md. et.al 2015)

Preparation of seawater

38 gm sea salt (without iodine) was weighed, dissolved in one litre of distilled water and filtered off to get clear solution.

Bioactivity Studies:

Brine Shrimp Lethality Tests (BST):

Brine prawns (Artemia salina Leach) eggs were put to one side of the divided tank and this side was covered after the seawater was collected in a separate tank. Through the hatching, a continuous oxygen supply was provided. It took the eggs two days (48 hours) to hatch and develop into nauplii (larvae). Through the perforations in the dam, the newly hatched shrimps were drawn to the lamp on the opposite side of the divided tank. We collected these nauplii for bioassay.

Preparation of test solutions with samples of experimental plants

Then each vial containing 10 brine prawn nauplii had 100, 200, 400, and 800 g/ml concentrations of the test ingredients injected to it, along with 5 ml of seawater. For each concentration, three vials were used, and a control vial containing 10 nauplii in 20 ml of seawater and 100 l of solvent was employed. For the purpose of conveniently counting the nauplii, a magnifying glass was used. The vials were examined after 24 hours, and the number of surviving in each vial was counted and recorded. The proportion of nauplii mortality at each concentration was estimated using this data. Following that, 2.5 ml of a plant extract solution was mixed to 2.5 ml of seawater that contained 10 nauplii.

Abbot's formula was used to calculate the percentage

mortality:

% Mortality = (Sample−control/control) *100 **[10]**

B) *In vitro* Anticoagulant Activity:

Blood samples from my devoted group members were utilised to evaluate Tribulus terrestris' anticoagulant properties. Participants ranged in age from 20 to 25. The following criteria were used to select them for the study: normal prothrombin time, absence of diabetes or cardiovascular disease (hypertension, congestive heart failure, coagulation disorders like haemophilia A or B), absence of recent NSAID use, absence of obesity or smoking, and absence of dyslipidaemia disorders.

Collection of blood samples:

Using sterile syringes, blood samples from healthy people were taken from veins in their right arms and deposited separately in containers containing tri-sodium citrate to halt the scavenging process. To acquire Pure Platelet Plasma (PPP) for the prothrombin time test, blood cells were separated from plasma using centrifugation (15 minutes at a speed of 3000 rpm). Each person's plasma sample was collected, pipetted separately into individual containers, and stored at room temperature.

Prothrombin time test

In a clean fusion test tube, 0.2 ml of plasma, 0.1 ml of plant extract at various concentrations, and 0.3 ml of Cacl2 (25 mM) were combined. The second test tube was filled with 0.1 ml of 0.9% saline, 0.2 ml of plasma, and 0.3 ml of Cacl2 (25 mM) as a negative control. The third test tube was filled with 0.1 ml of warfarin, 0.2 ml of plasma, and 0.3 ml of Cacl2 (25 mM) as a positive control. Every test tube was incubated in a water bath at 37°C. By tilting the test tubes every 5 seconds, the clotting time was measured using a stopwatch. The prothrombin time is the period when blood begins to clot. Every experiment was run twice, and the average scavenging time was recorded. [11,12]

Plasma sample divided in to four group

- Group 4: 0.2ml plasma + 25mg/ml of Plant extract + 0.3ml of $cacl_2$
- Group 3: 0.2ml plasma + 50mg/ml of Plant extract + 0.3ml of $cacl_2$
- Group 2: 0.2ml plasma + 100 mg/ml of Plant extract + 0.3ml of $cacl_2$
- Group 1: 0.2ml plasma + 200 mg/ml of Plant extract + 0.3ml of $cacl_2$
- Group Negative Control: 0.2ml plasma + 0.1ml of 0.9%saline water + 0.3ml of $cacl_2$

Group positive Control: 0.2ml plasma + 0.1ml of 50mg /ml EDTA+ 0.3ml of $Cacl_2$.

RESULT

Sr.no	Phytochemical	Test	Ethanolic Extract	Aqueous Extract
1	Alkaloids	Hanger Test	++	++
		Mayer's Test	+	-
2	Glycosides	Legal's Test	-	++
		Bontrager's Test	+	-
3	Flavonoids	Lead Acetate Test	++	++
		Alkaline Test	++	+++

(+++: strongly present, ++: Moderate present, +: weakly present, - negative)

Table No 1: Qualitative Test

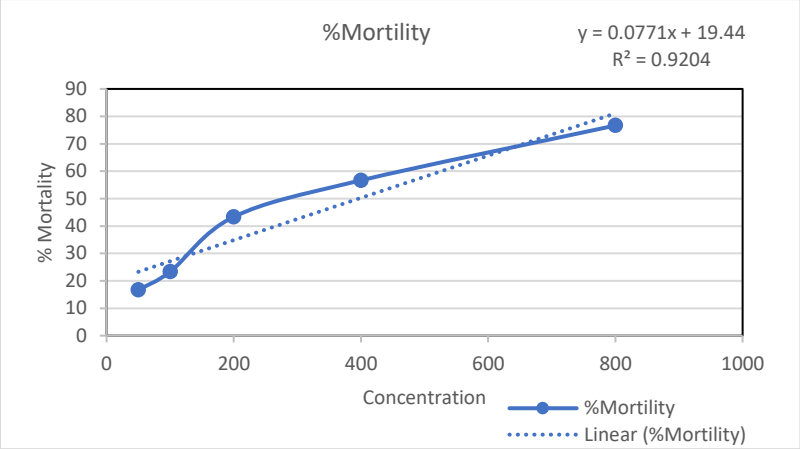
Result of *In -vitro* Anticancer activity

Concentration	No. of Nauplii Dead			Dead Nauplii out of 30	Live nauplii out of 30	%of molarity (mean ±Sem)	LC_{50}
	T1	T2	T3				
50	2	1	2	5	25	16.66666667	331.9
100	3	2	3	8	22	26.66666667	
200	4	4	3	11	19	36.66666667	
400	7	8	8	23	7	76.66666667	
800	8	9	8	25	5	83.33333333	

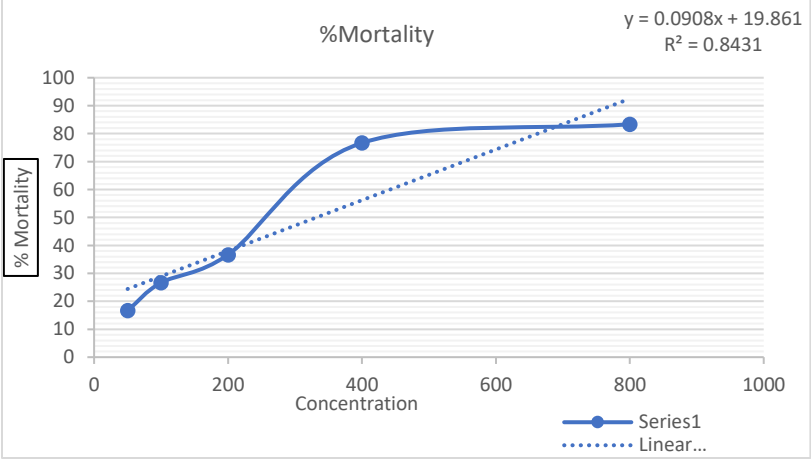
Table 2: Ethanol extract

Concentration	No. of Nauplii Dead			Dead Nauplii out of 30	Live nauplii out of 30	%of molarity (mean ±Sem)	LC_{50}
	T1	T2	T3				
50	2	1	2	5	25	16.66666667	396.36
100	3	2	2	7	23	23.333333	
200	5	4	4	13	17	43.3333333	
400	6	5	6	17	13	56.6666666	
800	7	8	8	25	7	76.6666666	

Table. No 3 Aqueous Extract



Ethanol Extract



Aqueous Extract

In-vitro Anticoagulant activity:

Plant extract	Sample -4 (200mg/ml)	Sample -3 (100mg/ml)	Sample – 2 (50mg/ml)	Sample- 1 (25mg/ml)
Ethanol	1min:25s	5min:9s	11min:15s	18min:15s
Aqueous	2min:38s	7min:9s	14min:15s	25min:15s
Group1(-ve control)	1min:4s	-	-	-
Group2(+ve control)	00min:00s/NA	-	-	-

Table No: 4 In-vitro Anticoagulant activity

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