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in vitro Haemolytic and Thrombolytic Activities of *Launaea cornuta*

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Launaea cornuta or bitter lettuce is a herbaceous perennial plant having a creeping root system that produces a basal rosette of leaves with a central stem that is usually up to 1 m tall, occasionally to 1.5 m. As a result of spread by rhizomes, a single plant can acquire a large area. The current study was designed and carried out to appraise the heamolytic and thrombolytic potential of the plant's extract. Result indicated that the methanolic extract of *L. cornuta* (at dose 20 µg/mL) possess minimum haemolytic activity (2 ± 0.001 %) whereas ethyl acetate extract (at dose 100 µg/mL) possess highest haemolytic activity (9 ± 0.35). Haemolytic percentage was found to be increasing with increase in dose. In this study, ethyl acetate extract was found to possess the maximum thrombolytic activity *i.e.* 64 ± 0.18 % at a concentration of 100 µg/mL and it is due to the presence of some phytochemical constituents.

Keywords: *Launaea cornuta,* Heamolytic, Thrombolytic.

INTRODUCTION

Launaea cornuta have its place in the family asteraceae (Compositae) which is one of the eight families of the order campanulales. Asteraceae is known to be the principal family of the flowering plants and encompasses about 960 genera and some 13,000 species [1]. Compared with some large families such as leguminosae, the number of important economic products derived from this family is relatively small.

Chemical research in recent years has augmented medical interests in the family and there is a superior data of many almost-discarded folk remedies as well as un-investigated plants. The later include some having antitumor or antibacterial activity and others forming commercial sources of rubber latex [1]. Stevioside, anent-kaurene glycoside from Stevia rebaudiana is used as a sweetener for soft drinks in a number of countries and also as antidepressant.

Launaea cornuta is an erect perennial herb with milky juice and hollow stems ranging 1.5 m height and skulking rhizomes. Leaves are deeply divided at the bottom, alternative on the stem, stemless, up to 2.5 cm long, 3 cm widespread, entire or with 2 to 3 couples of parts. Inflorescence huge, prolix with many yellow flower heads on peduncle about 2.5 cm long involucres up to 10 cm long by 4 mm cross, smooth or shortly juvenile, phyllares in 2 to 3 rows, 2-4 mm long exterior, up to 10 mm long anterior. Florets 10-25, yellow up to 15 mm long, ligules frequently rose-coloured, pale brown outside seeds, elongated, corrugated 2-4 mm, long with white pappus 5 mm long [2,3]. The herb is native to Africa and commonly known as wild or bitter lettuce, moleita and merlot (Sudan), muthunga (Kikuyu) muthunga (Meru and Embu) mchunga (Swahili), Mnyinya (Taita) and Achak (Luo). It happens on muddy earths in cultivated lands, including watered crops, on edges of the road, near rivers and bush vegetation. A single plant can cover a large area because of spreaded rhizomes. It is the commonest species of *Launaea* around Nairobi, Kenya [2,3].

The biological activities of *L. cornuta* that have been reported recently are the hypoglycemic activity and safety of the ethyl acetate extract of *L. cornuta* shrub [4,5]. Ethnomedically *L. cornuta* is used as a wild vegetable in African communities for instance Kilifi, the coastal region of Kenya and Nigeria as a source of vitamin C [6]. In East Africa the decoction is used to treat disease like typhoid, the leaves juice is dripped in the ear to stop pain and the herb is boiled with water and the extract used to wash the body for the treatment of measles. In Tanzania the leave decoction is used in the treatment of gonorrhea, ascariasis, stomach pains and fresh roots are chewed to cure swollen testicles [7]. In Kenya, its good browse for goats and rabbits. The roots are used for the treatment of warts and also administered orally in the management of chronic joint pains

[8] and the concoction used to manage breast cancer and benign prostate hyperplasia diabetes [9].

In vitro heamolytic activities are becoming a new area of research in drug lead discovery. Researchers are exploring ethno botanically important plants to find out potential natural products with anti-aggregant action. These studies are important because some patients have become resistant to the already existing drug *e.g.* aspirin [10] and/or conventional medication (warfarin, aspirin) in association with medicinal plant formulations. This is posing a serious problem to the society. Moreover the constant use of synthetic drugs is leading the society to face great danger. In recent years, many antiplatelet aggregating agents have been isolated from plants and have demonstrated potent activity.

Similarly, blood clot formation has been a severe concern of blood circulation. Thrombus or embolus obstructs the blood flow by blocking the blood vessel therefore making tissues scanty of normal blood flow and oxygen. These concerns yield necrosis of the tissue in that area. Thrombin makes blood clot from fibrinogen and is lysed by plasmin, which is activated from plasminogen by tissue plasminogen activator (tPA). The purpose of a fibrinolytic drug is to dissolve thrombin in acutely blocked coronary arteries thereby to restore blood supply to ischemic myocardium, to limit necrosis and to mend scenario [11].

The objective of this study was to carry out *in vitro* haemolytic and thrombolytic activity of *L. cornuta* aqueous and organic solvent extracts.

EXPERIMENTAL

The whole plant was collected from "Bakka Khel" FR Bannu area of Pakistan. The plant material was identified and authenticated by a plant taxonomist at Botany Department, University of Science and Technology, Bannu, KPK, Pakistan.

Preparation of extract: Fresh plant material was washed under running tap water and then with deionized water. The whole plants were air dried and then homogenized to fine powder. 200 g of powder was successively extracted with six different solvents (methanol, ethyl acetate, chloroform, hexane, ethanol and aqueous extracts). The yield was found to be 10, 12, 9, 10, 8, 5 w/v, respectively.

Haemolytic assay: The haemolytic activity of the extract was determined using agar diffusion technique on blood agar plate. Blood agar was prepared and well measuring 5 mm were made on the agar using cork borer. The wells were filled with 20 µL of different concentration of plant extracts solution. The plates were then incubated at 37 °C for 5 h.

Thrombolytic assay: Whole blood (6 mL) was collected from the healthy volunteers in KTH hospital Peshawar (Medical OPD). Blood sample (1 mL) was distributed in pre weighed sterile micro centrifuge tubes and incubated at 37 °C for 90 min for clot synthesis. After clot formation, the serum was completely aspirated without disturbing the clot and the tubes were again weighed to determine the clot weight (clot weight = weight of the tube containing clot – weight of the empty tube). To the each Eppendorf tube containing pre weighed clot, 20, 40, 60, 80 and 100 µL of extracts were added. 50 µL of sterile distilled water and streptokinase was used as a negative and positive control. All the tubes were incubated at 37 °C for 18 h and observed for clot lysis. The fluid obtained after the incubation was removed carefully and the tubes were weighed again to observe the difference in weight after clot disruption

Streptokinase (SK): Streptokinase (15, 00,000 I.U.,) used as a standard was obtained from Highnoon Laboratories (Pvt.) Ltd., Pakistan. 5 mL sterile distilled water was added to streptokinase vial and mixed properly. From this suspension100 µL (30,000 I.U) was used for *in vitro* thrombolytic assay [26].

RESULTS AND DISCUSSION

Haemolytic activity: Haemolytic activity of *L. cornuta* (methanol, ethyl acetate, chloroform, hexane, ethanol and aqueous extracts) with different concentration was screened against normal human erythrocytes. Haemolytic activity of extracts is expressed in diameter of the zone of haemolysis (mm). Result indicated that methanol extract of *L. cornuta* (at dose 20 mg/mL) possess minimum haemolytic activity (2 ± 0.001 %) whereas ethyl acetate extract (at dose 100 µg/mL) possess highest haemolytic activity (9 ± 0.34). Haemolytic percentage was found to be increasing with increase in dose (Table-1). Heamolytic activity can be a result of pore formation in the cell membranes thus changing membrane permeability or it can be due to the alteration of sodium–potassium and calcium–magnesium ATPase activities. Sometime some reduced compounds like phenols can cause oxidation of hemolglobin producing metahemoglobin thus finally causing hemolysis. Measuring heamolytic activity is important as it is an indicator for cytotoxicities [12].

TABLE-1
HAEMOLYTIC ACTIVITY OF *L. cornuta* EXTRACTS AT DIFFERENT CONCENTRATION

Extract	Zones of haemolysis (mm)				
	Concentration of extract (µg/mL)				
	20	40	60	80	100
Methanol	2±0.001	6±0.021	6±0.018	7±0.003	7±0.023
Ethyl acetate	6±0.011	7±0.012	8±0.017	8±0.018	9±0.350
Water	4±0.010	4±0.003	5±0.017	5±0.024	6±0.250
Ethanol	5±0.020	5±0.020	5±0.230	7±0.010	7±0.420
Chloroform	5±0.050	4±0.230	7±0.010	6±0.240	7±0.110
Hexane	5±0.210	5±0.320	7±0.170	7±0.330	7±0.180

Values are represented as mean ± SD of triplicate in each experimental set.

The ethyl acetate extract of *L. cornuta* shows maximum haemolytic activity compare to all the other solvent extracts. The activity of the extract to lyse the blood cell can be linked with bioactive components. It is well documented that flavonoids and the polyphenolic compounds which showed potential beneficial effects on human health and possess antiviral, anti-inflammatory, antitumor, antihaemolytic and antioxidative activity [13]. The zone of haemolysis was directly proportional to concentration of the extract.

Thrombolytic activity: From primordial time, human dependency on plants to treat many diseases is documented. These days phytopharmacological exploration has generated a new field in order to discover plant derivative drugs, which

are proved to be effective in the remedy of certain illnesses and converted the attention of researchers in herbal medicines. It is assessed that nearly 30 % of the pharmaceutical products are synthesized from plants derivatives [14,15]. A number of studies have been carried out on order to discover the plants and natural food sources and their supplements having anti-thrombotic as well as haemolytic potential. It is also investigated that utilizing such food materials can leads to prevent coronary episodes and stroke [16-19]. Although there are several thrombolytic and haemolytic drugs with those synthesized by recombinant DNA technology, but side effects related to some of these drugs that lead to further complications have been testified [20-23].

Platelets play a momentous part in the progress of atherothrombosis as well as harm the areas of endothelial surface (produced by ROS). The stimulated platelets form platelets to platelets bonds, adhere to leucocytes carrying them into a complex process of plaque development and progress [24]. Plasmin is a natural fibrinolytic agent, breaks clot by flouting down the fibrinogen and fibrin present in a clot. An enzyme, streptokinase forms a 1:1 stoichiometric complex with plasminogen that can convert additional plasminogen to plasmin [25]. Moreover, phlorotannin (extracted and isolated from marine brown algae) have a distinct characteristic in development of termination of intravascular blood clot through antiplasmin inhibition [26]. In this study, ethyl acetate extract was found to provide the maximum thrombolytic activity. The phytochemical constituents present in the ethyl acetate extract of *L. cornuta* could participate for its clot lysis activity [27].

Table-2 shows the thrombolytic activity of the *L. cornuta* extracts in various concentrations. The *in vitro* thrombolytic activity study showed that methanol, ethyl acetate, aqueous, ethanol, chloroform and hexane extract showed 57, 64, 49, 54, 41 and 37 % clot lysis respectively for 100 µg/mL and compared with the negative control.

TABLE-2
EFFECT OF *L. cornuta* EXTRACTS ON *in vitro* CLOT LYSIS

Extract	Clot lysis (%)				
	Concentration of crude extract (µg/mL)				
	20	40	60	80	100
Methanol	24±0.020	31±0.001	34±0.001	47±0.017	57±0.130
Ethyl acetate	17±0.017	34±0.028	41±0.012	60±0.14	64±0.180
Water	12±0.020	18±0.020	23±0.001	45±0.067	49±0.032
Ethanol	26±0.010	35±0.030	41±0.020	53±0.120	54+0.180
Chloroform	22±0.030	28±0.120	35±0.410	43±0.110	41±0.140
Hexane	20±0.180	26±0.150	33±0.320	39±0.160	37±0.120

Values are represented as mean ± SD of triplicate.

Percentage of clot lysis was determined with the following formula:

$$\text{Clot lysis (\%)} = (\text{wt. of released clot/clot wt.}) \times 100$$

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