



Haemolytic activity of Indian medicinal plants toward human erythrocytes: an *in vitro* study

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ABSTRACT

In this study aqueous extract of the leaves of *Aerva lanata* Linn., *Calotropis gigantea* Linn. and *Elaeocarpus ganitrus* Roxb were screened for the haemolytic activity towards human erythrocytes. The haemolytic activity was performed by modified spectroscopic method at four different concentrations (125, 250, 500, 1000 µg/ml). The haemolytic activity of the different extracts was found in the following order: *C. gigantea* > *A. lanata* : *C. gigantea* (1 : 1) > *C. gigantea* : *E. ganitrus* (1 : 1) > *A. lanata* : *C. gigantea* : *E. ganitrus* (1 : 1 : 1) > *A. lanata* : *E. ganitrus* (1 : 1) > *A. lanata*. However, all the extracts alone and in combination with each other exhibited very low haemolytic activity. *E. ganitrus* did not exhibit any haemolytic activity at any dilution. Hence, they can be considered as safe to human erythrocytes.

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Introduction

Medicinal plants are the rich source of medicinally important compounds and since ancient time, plants and plant derived products are used as medicine in traditional and folk medicinal system. Initially the herbal drugs were used in the form of dried powder, gums, extracts or formulations of more than one plant products. Advanced scientific techniques brought a revaluation in herbal medicine industry and all focus is concentrate on active principles (bioactive molecule). However, a lot of processing is required to develop a drug from the natural sources. Toxicity of the active molecule is a key factor during drug designing, and haemolytic activity represents a useful starting point in this regard, it provides the primary information on the interaction between molecules and biological entities at cellular level. Haemolytic activity of any compounds is an indicator of general cytotoxicity towards normal healthy cells (Da Silva et al., 2004). Usually, saponins (a group of phytochemical) present in the plants showed haemolytic activity by creating changes in the erythrocyte membrane. In vitro haemolytic assay by spectroscopic method provides an easy and effective method for the quantitative measurement of hemolysis. This method provides the evaluation of the effect of different concentrations of biomolecules on the human erythrocytes.

In this study, *A. lanata* (Amaranthaceae), *C. gigantea* (Apocynaceae) and *E. ganitrus* (Elaeocarpaceae) were screened for the haemolytic activity. Earlier, *A. lanata* has been reported for anti-diabetic activity (Vetrivelvan and Jegadeesan, 2002), anthelmintic activity (Rajesh et al., 2010), antiinflammatory activity, diuretic activity (Vetrivelvan et al., 2000), urolithiasis activity (Rao, 1985), antimicrobial activity (Chowdhury et al., 2002) and hepatoprotective activity (Nevin and Vijayammal, 2005). *C. gigantea* has been reported for analgesic activity (Pathak and Argal, 2007), antibacterial activity (Kumar et al., 2010a; Kumar et al., 2010b), antifungal activity (Kumar et al.,

2010c), cytotoxic activity (Wang et al., 2008), anti-diarrhoeal activity (Chitme et al., 2004), antioxidant activity (Singh et al., 2010), insecticidal activity (Alam et al., 2009), wound healing activity (Deshmukh et al., 2009), CNS activity (Argal and Pathak, 2006) and hepatoprotective activity (Lodhi et al., 2009). *E. ganitrus* has been reported for anticonvulsant activity (Dasgupta et al., 1984), antiinflammatory activity (Singh and Pandey, 1999), antimicrobial activity (Singh et al., 2010; Singh and Nath, 2010), antihypertensive effect (Sakat et al., 2009) and anxiolytic effects (Shah et al., 2010). The aim of this study was to screen the aqueous extract of the leaves of *A. lanata*, *C. gigantea* and *E. ganitrus* for the haemolytic activity towards human erythrocytes.

Material and Methods

Plant material

Leaves of *A. lanata* were collected from Seshachalam Hills, Chittoor, AP, (13°40'N 79°19'E) during December 2008. Leaves of *C. gigantea* were collected from Vellore district, TN, India (12°54'N 79°8'E) in the month of December, 2008. Leaves of *E. ganitrus* were collected from Guwahati, Assam (26°11'N 91°44'E) during January 2009. The plants materials were carried to the Molecular and Microbiology Research Laboratory, VIT University, Vellore. A voucher specimen for each plant was maintained in our laboratory for the future reference.

Processing of plant

The leaves of the plants were collected and washed thoroughly in tap water followed by distilled water. The leaves were shade dried at room temperature. Dried leaves were uniformly grinded using mechanical grinder.

The leaves powder was extracted in distilled water. Ten gram of plant powder was extracted in distilled water using a soxhlet extractor. The extract was concentrated using rotary evaporator and dried using lyophilizer. Dried extract was collect in air tight container and stored at 4°C.

Preparation of erythrocytes suspension

Five millilitres of blood was collected from a healthy individual (blood group O positive) in a tube containing heparin. The blood was centrifuged at 1500 rpm for three minutes in a laboratory centrifuge. Plasma (supernatant) was discarded and the pellet was washed three times with sterile phosphate buffer saline solution (pH 7.2±0.2) by centrifugation at 1500 rpm for 5 min. The cells were resuspended in normal saline to 0.5%.

Haemolytic activity

In vitro haemolytic activity was performed by spectrophotometer method (Yang et al., 2005). A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml of the plant extracts (125, 250, 500 and 1000 µg/ml concentrations in phosphate buffer saline). The mixtures were incubated for 30 min at 37°C in a incubator. The mixture was centrifuged at 1500 rpm for 10 min in a laboratory centrifuge. The free hemoglobin in the supernatant was measured in UV-Vis spectrophotometer at 540 nm. Phosphate buffer saline and distilled water were used as minimal and maximal haemolytic controls. Each experiment was performed in triplicates at each concentration.

The level of percentage hemolysis by the extracts was calculated according to the following formula:

$$\% \text{ Hemolysis} = \frac{A_t - A_n}{A_c - A_n} \times 100$$

Here: A_t is the absorbance of test sample.

A_n is absorbance of the control (saline control)

A_c is the absorbance of the control (water control)

Statistical Analysis

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically by using Microsoft Excel 2007 (Roselle, IL, USA).

Results and Discussion

Since ancient time, plants products been utilized for the treatment of various health problems. Plants are one of the most important sources of drug discovery and development. The plants used in this study have been excessively used in traditional medicine to cure a variety of diseases. *A. lanata* is used to cure helminthic infection, diabetes, inflammation, skin diseases, kidney stone, headache, cough, cholera, dysentery and diarrhea. It is also reported for diuretic properties (Rajesh et al., 2011). *C. gigantea* is used to cure paralysis, swellings, intermittent fevers, asthma, catarrh, anorexia, helminthic infections, inflammations, fever, infections, cough, asthma, bronchitis and dyspepsia (Kumar et al., 2011). *E. ganitrus* is used to cure stress, anxiety, depression, palpitation, nerve pain, epilepsy, migraine, lack of concentration, asthma, hypertension, arthritis and liver diseases (Dasgupta et al., 1984).

In this study, haemolytic activity of the aqueous extract of leaves of *A. lanata*, *C. gigantea* and *E. ganitrus* alone and in combination with each other was screened against normal human erythrocytes. Haemolytic activity of the plant is expressed in percentage hemolysis and reported as mean±standard deviation of three replicates. All the samples exhibited very low haemolytic effect toward human erythrocytes. However, these extracts showed dose dependant increase in haemolytic activity (Figure 1). Based on IC_{50} values, aqueous extract of *C. gigantea* exhibited the maximum hemololytic activity and ranked 1 in the list. Aqueous extract of *E. ganitrus* not exhibited any haemolytic activity towards human erythrocytes and ranked 7 in the list. IC_{50} values of all the

extracts and there combinations are reported in Table 1. The haemolytic activity of the different extracts was found in the following order: *C. gigantea* > *A. lanata* : *C. gigantea* (1 :1) > *C. gigantea*: *E. ganitrus* (1 : 1) > *A. lanata* : *C. gigantea* : *E. ganitrus* (1 : 1 : 1) > *A. lanata* : *E. ganitrus* (1 : 1) > *A. lanata*.

Some other plants also have been studied for the haemolytic activity towards humans or animal erythrocytes. Different solvent extracts of *Syzygium cuminii* seeds and *Crateva nurvula* bark were reported to possess no haemolytic effect on sheep erythrocytes (Mathur et al., 2011). *Achyranthes aspera* was reported to possess very low haemolytic activity towards human erythrocytes (Priya et al., 2010). Aqueous extract of *Lantana camara* and its various solvent fractions were reported to possess moderate haemolytic activity towards human erythrocytes (Kalita et al., 2011). Oliveira1 et al., 2009 screened the haemolytic activity of seventy one extracts from twelve plants. Only three extracts prepared from *E. nuda* showed significant haemolytic activity. Karanja oil from *Pongamia glabra* was reported to exhibite a dose dependent increase in the haemolysis towards the rabbit red blood cells (Gandhi and Cherian, 2000). Mukherjee and Rajasekaran (2010) reported the high haemolytic activity of the different solvent extracts of *Allium stracheyi* Baker towards human red blood cells. Chloroform and aqueous extract of leaves of *Acanthus ilicifolius* were reported to possess significant haemolytic activity towards the chick red blood cells (Thirunavukkarasu et al., 2011).

The results of this study concludes that the aqueous extracts from the leaves of *A. lanata*, *C. gigantea* and *E. ganitrus* alone and in combination with each other, are non/less toxic to the human erythrocytes.

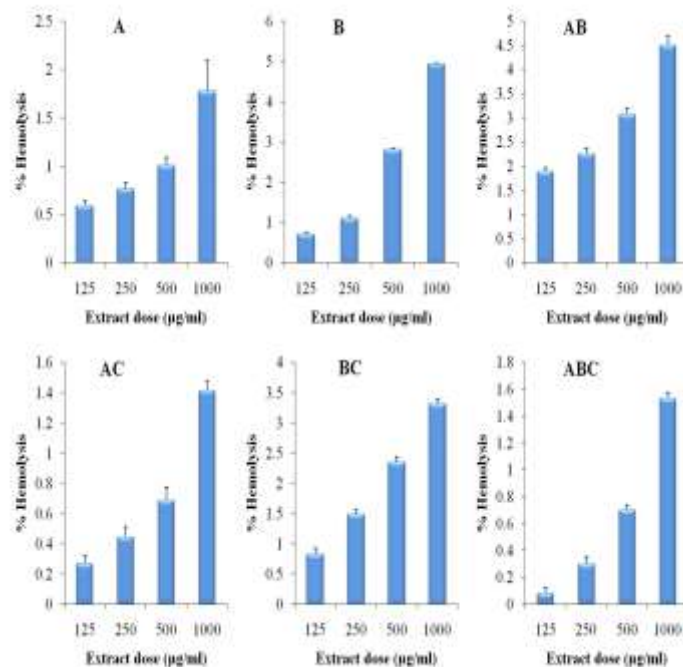


Figure 1: Haemolytic activity of plant extracts against human erythrocytes

Data is represented as mean± standard deviation (n=3)

Here, A: *Aerva lanata*, B: *Calotropis gigantea*, AB: *Aerva lanata* : *Calotropis gigantea* (1 : 1), AC: *Aerva lanata* : *Elaeocarpus ganitrus* (1 : 1), BC: *Calotropis gigantea*: *Elaeocarpus ganitrus* (1 : 1), ABC: *Aerva lanata* : *Calotropis gigantea* : *Elaeocarpus ganitrus* (1 : 1 : 1)

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Table 1: IC₅₀ value of the plant extracts towards human erythrocytes

Sample	IC ₅₀ value (mg/ml)	Rank
<i>Aerva lanata</i>	49.90	6
<i>Calotropis gigantea</i>	12.47	1
<i>Elaeocarpus ganitrus</i>	-	7
<i>Aerva lanata</i> : <i>Calotropis gigantea</i> (1 : 1)	16.15	2
<i>Aerva lanata</i> : <i>Elaeocarpus ganitrus</i> (1 : 1)	49.59	5
<i>Calotropis gigantea</i> : <i>Elaeocarpus ganitrus</i> (1 : 1)	24.64	3
<i>Aerva lanata</i> : <i>Calotropis gigantea</i> : <i>Elaeocarpus ganitrus</i> (1 : 1 : 1)	25.10	4

IC₅₀ = Inhibitory concentration that inhibited 50% of the exposed cells