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Phytochemical analysis and evaluation of haemolytic potential of methanolic extracts of root of *Chrysopogon zizanioides* and rhizome of *Acorus calamus*

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Abstract

Many plants and plant-derived products have been used in the preparation of traditional medicine to prevent and treat various diseases. Even though medicinal plants are rich sources of bioactive compounds, analysis of their composition is a prerequisite along with an evaluation of their cytotoxicity. The haemolytic test determines the ability of any compound to destroy red blood cells, leading to the release of haemoglobin. Plants Chrysopogon zizanioides and Acorus calamus have been reported to exhibit several medicinal properties. Hence this study was designed to elucidate the medicinal plant extracts by phytochemical analysis and to evaluate the haemolytic potential of methanolic extracts of the root of Chrysopogon zizanioides and rhizome of Acorus calamus, towards the human erythrocytes, using spectrophotometer. Phytochemical analysis revealed the presence of alkaloids, tannins, terpenoids, steroids and carbohydrates in the crude extract of both plants. From the results of this study, it is clear that the percentage of haemolysis increases with an increase in the concentration of crude extract of the root of Chrysopogon zizanioides and rhizome of Acorus calamus treatments. However, in both the treated groups, the haemolysis percentage was very less; indicating that these extracts are safe for human erythrocytes.

Keywords: *Phytochemicals, haemolysis, Chrysopogon zizanioides, Acorus calamus*

Medicinal uses of various plants and plant-derived products have been recorded in traditional medicine from time immemorial (Keerthika *et al*., 2022). It is strongly believed that herbal medicines are natural and hence without significant side effects and less likely to cause dependency. Investigations for exploring natural antioxidants have experienced rapid growth due to the harmful effects of synthetic antioxidants (Hashemi *et al*., 2018; Casarotti and Jorge, 2012; Dong *et al*., 2017; Gouvinhas *et al*., 2014).

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Despite the popular usage of plants as food and medicine (Kataria *et al*., 2011; Arshath *et al*., 2022) even now the information on the composition, exact formulation and quantity of plant material used for the treatment is not standardised. Hence, accurate scientific assessment of herbal medicine is a prerequisite for the global harmonization of ethnomedical claims, to attain their full therapeutic potential (Mahady, 2001).

In recent years, the exploration of natural compounds from plants against a variety of pharmacological targets has been an important concern in many laboratories. Advanced scientific techniques have brought a revolution in the traditional medicine industry and now the focus is on bioactive molecules (Ghosh *et al*., 2018). Findings of phytochemical analysis in recent decades indicate the presence of several compounds with significant bioactivity (Cheng *et al*., 2012; Asakawa, 2001).

Identifying new compounds with desirable activity and developing new drugs from natural sources is complex and time-consuming. Hence, a study of the toxic effect of any active molecule is very much necessary while drug designing, for which haemolytic activity is very useful (Eric *et al*., 2004).

In the present study two different species of plants *Chrysopogon zizanioides* and *Acorus calamus*, with reputed medicinal properties among the local practitioners which are popular in traditional therapy were selected. It is reported that bioactive compounds from these plants are mainly phenolics (Asha Devi and Deepak, 2011) which have the potency not only as medicine but also as food additives to prevent lipid oxidation (Raja *et al*., 2009; Balakumbahan *et al*., 2010). As the accumulation of compounds varies in different parts of the plant (Suhartati Djarkasi *et al*., 2019), the root of C*hrysopogon zizanioides* and rhizome of *Acorus calamus* were selected for the study.

Hence, the current study aimed to explore the medicinal plant extracts by phytochemical analysis and to evaluate the haemolytic potential of different concentrations of root of *Chrysopogon zizanioides* and rhizome of *Acorus calamus* plant crude extracts towards the human erythrocytes.

Materials and methods

Plant samples and preparation of extraction

Chrysopogon zizanioides (Vetiver in Tamil) and *Acorus calamus* (Vasambu in Tamil) are the two samples used in the present study. Both the plant samples were collected from Cuddalore located along the eastern coastal region in Tamil Nadu, India. Crude extracts of *Chrysopogon zizanioides* root and rhizome of *Acorus calamus* plant were prepared using methanol. For this 20 gm of dried sample powder was dissolved in 100 ml of methanol in a beaker with aluminium foil covered on it. Then the beaker was kept in a hot water bath at 50º C for 4 hours. After the incubation period, the extract was filtered with Whatman filter paper and the filtrate was collected in a 50 ml beaker. The residue present over the filter paper was discarded and filtrate was taken for further study. Then the filtrate was kept at 50º C for a few hours until the extract got completely dried and turned into semisolid form. This semi-solid sample was weighed and the yield was noted.

Elucidation of the medicinal plant extracts by phytochemical analysis

Test for Alkaloids (Dragendroff's test): 0.2 ml of sample was taken and 0.2 ml of HCl was added. To this 2-3 drops of Dragendroff's reagent were added and the appearance of orange or red precipitate and turbid solution indicates the presence of alkaloids.

Test for Carbohydrates (Molisch's test): 0.2 ml of sample was mixed with a few drops of Molisch's reagent (α- naphthol dissolved in alcohol). 0.2 ml of sulphuric acid was added along the sides of the test tube and observed for the appearance of a purple colour ring for a positive test.

Test for Tannins (Braymer's test): 0.2 ml of plant extract was mixed with 2 ml water and heated in a water bath for 10 minutes. The mixture was

filtered and ferric chloride was added to the filtrate and observed for a dark green solution which indicates the presence of tannin.

Test for Terpenoids (Salkowki's test): 0.2 ml of plant extract was taken in a test tube with 0.2 ml of chloroform. To this, concentrated sulphuric acid was added carefully to form a layer. The presence of reddish-brown colour at the interface would show the presence of terpenoids.

Test for Glycosides (Liebermanns test): 0.2 ml of sample was mixed with 0.2 ml of chloroform and 0.2 ml of acetic acid was added to this solution and the mixture was cooled on ice. Sulphuric acid was added carefully and the colour change from violet to blue to green indicates the presence of a steroidal nucleus (aglycone portion of glycoside).

Test for Steroids (Lieberman Burchardt test): 0.2 ml of sample was mixed with 0.2 ml of chloroform. To this 0.2 ml of concentrated sulphuric acid was added. The appearance of red colour in the lower layer of chloroform indicates the presence of steroids.

Test for Saponins (Foam test): To 0.2 ml of extract was added 0.6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Flavonoids (Alkaline reagent test): 0.2 ml of plant extract was taken in a test tube and mixed with a dilute sodium hydroxide solution. To this diluted hydrochloric acid was added. Observation of a yellow solution that turns colourless later would indicate the presence of flavonoids.

Test for Glycoprotein (Mucilage test): 0.2 ml of extract was taken in a test tube and 0.2 ml of absolute alcohol was added and allowed to try. If the precipitation occurs then mucilage is present.

Test for Volatile oil: 0.2 ml of extract was treated with a few drops of dilute hydrochloric acid. The appearance of a white precipitate indicates the presence of volatile oils.

Test for phenols (Ferric chloride test): To 0.2 ml of extract 0.4 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. The formation of blue or green colour indicated the presence of phenols.

Evaluation of the haemolytic potential of plant extracts

Five ml of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 4º C. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a micropipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4. Washed erythrocytes were stored at 4º C and used within 6 h for haemolytic assay (Vinjamuri *et al*., 2015). Erythrocytes suspension (50 µl of 1/10 dilution in 1X PBS) was taken into 2 ml Eppendorf tubes and 100 µl of different concentrations (6.25, 12.5, 25, 50, 100, 200 µg/ml) of test samples (plant extracts) were added. 100 µl of 1X PBS was used as negative control and 100 µl of 1% Triton X-100 was used as positive control. The reaction mixture was incubated at 37° C for 60 minutes. The volume of the reaction mixture was adjusted to 1 ml by adding 850 µl of 1X PBS. Finally centrifuged at 300 rpm for 3 min and the resulting supernatant was measured at 540 nm in a spectrophotometer to determine the concentration of haemoglobin at different concentrations of the samples (Mebrahtom 2012; Mehboob *et al*., 2013; Ibrahim *et al*., 2006; Henkelman *et al*., 2009). Test samples were compared to reference materials (1% Triton X-100 and 1% SDS) and haemolysis was assessed by the following formula.

$$\% \text{ Haemolysis} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Results and discussion

Phytochemical analysis

Results of the phytochemical analysis revealed the presence of alkaloid, tannin, terpenoid, steroid, carbohydrate and flavonoids in *Chrysopogon zizanioides* root and *Acorus*

calamus plant rhizome crude extract. The presence of saponin was reported only in *Acorus calamus*. But glycoside, glycoprotein and starch were not detected in the samples. The results of the phytochemical analysis of the root crude extract of both the selected medicinal plants are given in table 1.

Table 1. Results of phytochemical analysis

Sl. No.	Types of tests	*Chrysopogon zizanioides*	*Acorus calamus*
1	Alkaloid	+	+
2	Carbohydrate	+	+
3	Tannin	+	+
4	Terpenoid	+	+
5	Glycoside	-	-
6	Steroid	+	+
7	Saponin	-	+
8	Flavonoid	+	+
9	Glycoprotein	-	-
10	Starch	-	-

+ = Positive, - = Negative

Haemolytic potential of plant extracts

The haemolytic activity of the extracts among the tested samples was found to be more in *Acorus calamus* than *Chrysopogon zizanioides*. The sample of *C. zizanioides* did not show any significant haemolytic activity. The obtained data has negligible haemolysis and the sample screened can be considered for pharmaceutical studies. Sample *A. calamus* has shown toxicity up to 57.85% at its highest test concentration.

It is clear from the result summarised in Table 2 and Fig. 1, that the percentage of haemolysis increased with an increase in the concentration of samples in both *A. calamus* and *C. zizanioides* treatments. But both the treated groups exhibited a very less percentage of haemolysis. Hence these two samples could be considered safe for human erythrocytes.

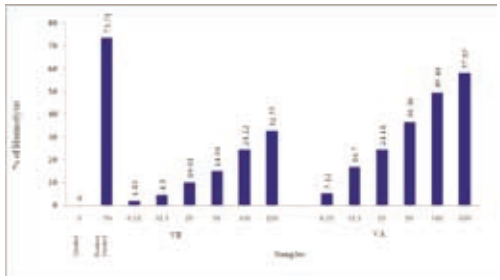

Fig. 1. Graphical representation of haemolysis assay

Many plants are useful sources of various bioactive compounds and hence people have been making use of them as traditional medicine (Tanmay *et al*., 2018). Nevertheless, many herbs can be toxic especially in higher quantities and with frequent use. Besides,

Table 2. Results of the haemolytic potential of plant extracts

Sample	Conc in µg/ml	Absorbance at 540 nm	% of Haemolysis
Control (PBS)	0	0.2676	0
Positive Control (SDS)	1%	0.0709	73.51
Chrysopogon zizanioides	6.25	0.2627	1.83
	12.5	0.2561	4.30
	25	0.2408	10.01
	50	0.2277	14.91
	100	0.2028	24.22
	200	0.1805	32.55
Acorus calamus	6.25	0.2539	5.12
	12.5	0.2229	16.70
	25	0.2029	24.18
	50	0.1703	36.36
	100	0.1353	49.44
	200	0.1128	57.85

herb-drug interactions should be monitored to promote the effective utilization of herbal medicine (Fong, 2002).

Therefore, it is very important to know any plant's composition before being used for various purposes. Hence, various parts of *Chrysopogon zizanioides* and *Acorus calamus* known as traditional medicinal plants needed further analysis for better utilization. This is because even the quality and the quantity of the bioactive compounds vary in different parts of the same plant.

Generally, bioactive compounds are present in all parts of the plants like fruit, seeds, leaves and roots (Shahidi and Naczk, 1995). As people have been using the roots of these plants for treating various problems in the traditional medicinal system, *Chrysopogon zizanioides* root and *Acorus calamus* rhizome were selected for the study. Hence in this experiment in addition to phytochemical analysis, different concentrations of crude extracts of root of *Chrysopogon zizanioides* and *Acorus calamus* rhizome were screened for haemolytic activity towards the human erythrocytes. Treatment of crude extract of both plants showed very less haemolytic activity which indicated low toxicity to human blood cells and the extract probably did not contain any components that have haemolytic potential if used *in vivo*.

Conclusion

The present study focused on the phytochemical evaluation of crude extract of *Chrysopogon zizanioides* and *Acorus calamus* root which revealed the presence of alkaloid, tannin, terpenoid, steroid and carbohydrate in both samples. The results of the haemolytic activity indicated that even though the percentage of haemolysis is directly proportional to the concentration of samples in both *Chrysopogon zizanioides* and *Acorus calamus* treatments, the haemolysis percentage is very less and these samples could be regarded as safe for human erythrocytes.

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Conflict of interests

The authors declare no conflict of interest.

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