

# Phytochemical screening and Haemolytic activities of hydroalcoholic extract of *Santalum album* .L leaves

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## Abstract

The present study has been designed to screen the phytochemical constituents present in the hydroalcoholic extract of *Santalum album* linn leaves to ascertain the presence of saponins and further studies carried out on the extract to evaluate its haemolytic activity towards bovine erythrocytes. Haemolytic effect of *S. album* leaf extract was evaluated according to the WHO guidelines in which the varying concentration of the extract was treated with the blood and lysis was determined visually. The result revealed that the *S. album* leaf extract produced lysis of erythrocyte at a minimum concentration of 475µg/ml. These observations will stimulate further research in the field of phytochemistry and also in clinical application of the phytochemical constituents of *S. album*. L.

**Keywords:** Haemolytic activity, erythrocyte, saponins, *Santalum album*.

## 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.<sup>1</sup> Saponins are a class of natural products which are structurally constructed of aglycone and sugars. The name 'saponin' comes from soap as its containing plants agitated in water form soapy lather. Saponins are widely distributed in many plants and are relatively widespread in our food stuffs and herbal preparations. They also exhibit a variety of biological activities, and have been investigated toward the development of new natural medicines and prove the efficacy of traditional herbal medicines. Steroidal saponins can effect haemolysis of red blood cell at high dilution and act as a fish poison.<sup>2</sup> 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.<sup>3</sup>

*Santalum album* is a potent medicinal plant, has been widely used in folk medicine for treatment several disease. The leaves contain variety of bioactive components such as saponins, glycosides, steroids, tannins and flavonoids, which are responsible for several biological and pharmacological properties. Moreover, a plant must have a long history of human use as food stuffs or ingredients of medicines, and their safety should be officially guaranteed.<sup>4</sup> Therefore, the objectives of the present study was screen phytochemical constituents and evaluate the possible haemolytic activity of hydroalcoholic extracts of leaves of *Santalum album* towards Bovin erythrocytes.

## Material and Methods

### Plant material

The fresh leaves of *Santalum album* Linn used for the present studies were collected from Mangalore, in April 2013 and authenticated by Mr. Dinesh Nayak Advisor (Green belt), Mangalore SEZ Limited.

### Processing of plant

The leaves of the plants were collected and washed carefully in tap water followed by distilled water. The leaves were shade dried at room temperature. Dried leaves were uniformly grinded using mechanical grinder. The coarse powder was packed into Soxhlet column and the extracted 70% ethanol for 48 hrs.<sup>5</sup> The solvent was removed using rotatory flash evaporator. The dried extract was stored in airtight container in refrigerator below 10°C.

### Preliminary phytochemical screening<sup>6,7</sup>

The phytochemical screening of the plant extract was carried out according to the standard procedures to determine the various active constituents present.

### **Materials used**

Tri-sodium citrate – 36.5g/l

Blood suspension- 2%v/v

Phosphate buffer pH 7.4

Stock solution(drug) - 100µg/ml.

### **Determination of haemolytic activity**<sup>8</sup>

Haemolytic effect of S.album leaf extract was evaluated according to the WHO guidelines.

### **Preparation of blood suspension**

Glass-stoppered flask was filled to one-tenth of its volume with sodium citrate (36.5 g/l).It was swirled in order to ensure that the flask was completely moistened. Introduced a sufficient volume of blood freshly collected and was shaken immediately. From this 2 ml of citrated blood was transferred to 100-ml volumetric flask and carefully diluted to volume with phosphate buffer pH 7.4.

### **Preparation of sample solution**

Weighed accurately about 100mg of the extract and transferred into a 100ml volumetric flask. made up the volume to 100ml using phosphate buffer pH 7.4

### **Preliminary test**

Prepared serial dilutions of plant extract with phosphate buffer and blood suspension using 4 test tubes as shown in the table no 1.

The tubes were gently inverted in order to mix them. The tubes were then shaken again after 30 minutes and are allowed to stand for 6 hours at room temperature. Examined the tubes and recorded the dilutions at which haemolysis has occurred, indicated by clear red solution without any deposits of erythrocytes.

The total hemolysis is observed in tubes 3 and 4, a two-fold dilution of the original plant material extract was prepared with phosphate buffer pH 7.4.

### **Main test**

Prepared a serial dilution of the plant material extract, undiluted or diluted as determined by the preliminary test, with phosphate buffer pH 7.4 and blood suspension (2%) using 13 test-tubes as shown in table no 2.

Carried out the dilutions and evaluations as in the preliminary test but observed the results after 24 hours. Calculated the amount of medicinal plant material in gram or of the preparation in gram or ml, that produced total hemolysis.

## **Results and discussion**

The phytochemical screening of the extracts of the leaves of santalum album Linn was carried out using hydro alcoholic extracts for the test of glycosides, alkaloids, Steroids, saponins, tannins and flavonoids. The extracts revealed the presence of glycosides, Steroids, saponins, tannins and flavonoids shown in Table no 3.

### **Determination of Haemolytic activity**

#### **Preliminary test**

In preliminary test haemolysis was observed only in the third and fourth test tubes.

#### **Main test**

In main test haemolysis was observed in the 12<sup>th</sup> and 13<sup>th</sup> test tubes.

## **Conclusion**

The leaves of santalum album Linn, contains glycosides, alkaloids, Steroids, saponins, tannins and flavonoids, which were revealed from the phytochemical screening of the leaf extract. The ability of the plant extracts to lyse red blood cells at varying degrees could be recognized to the presence of different types of saponins. Different saponins showed different levels of haemolytic activity. This observation can also be correlated with the findings of that a certain plant and even part e.g. leaves of the same plant may contain different saponins which can differ in biological features. From the main test conducted with the leaf extract of santalum album L, it was found that minimum concentration of 475 µg/ml of the extract produced the lyses of the RBC. However, this haemolytic activity only takes place with parenteral administration. The results of this study have revealed that the leaves of the plant santalum album Linn contained saponins, which were responsible for haemolytic activities against blood.

## **Acknowledgments**

The authors are thankful to the authorities of A.Shama Rao Foundation Mangalore, Karnataka, India for the facility.

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Table no. 1: Preliminary test

Test tube no:	1	2	3	4
Plant material extract 1000 µg/ml (ml)	0.10	0.20	0.50	1.00
Phosphate buffer pH 7.4 (ml)	0.90	0.80	0.50	0.0
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00

Table no 2: Main test

Test tube no:	1	2	3	4	5	6	7	8	9	10	11	12	13
Plant extract (ml)	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
Phosphate buffer pH 7.4 TS (ml)	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10	0.05	-
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table no 3: phytochemical screening

Sr.no	Test	Result
1	Alkaloids	-ve
2	Flavonoids	+ve
3	Glycosides	+ve
4	Saponins	+ve
5	Steroids	+ve
6	Tannins	+ve
+ve = present, -ve = absent		

Table no 4: haemolytic activity of S album leaf extract

Concentration (1000 $\mu$ g/ml)	0.10 ml	0.20 ml	0.50 ml	1.0 ml
haemolysis	nil	nil	Haemolysis observed	Haemolysis observed

Table no 5: haemolytic activity of S album leaf extract

Concentration (1000 $\mu$ g/ml)	0.4	0.45	0.5	0.55	0.6	0.65	0.7	0.75	0.8	0.85	0.9	0.95	1.0
haemolysis	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	lysis	lysis