



## Wound healing activity of the seed kernels of *Caesalpinia crista* Linn.

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### Abstract

**Objective:** To screen the wound healing activity of different extracts of seed kernels of *Caesalpinia crista* Linn in excision, incision and dead space wound models in albino rats. **Materials and methods :** Seed kernels of *Caesalpinia crista* Linn was defatted completely with petroleum ether (60-80°C) and then marc was subjected to continuous extraction using alcohol. The concentrated alcoholic extract was subjected to fractionation by solvent ether, ethyl acetate butanone and butanol in succession. Petroleum ether extract, alcoholic extract and four fractions were tested for various preliminary phytoconstituents and screened for wound healing activity in excision, incision and dead space wound models in albino rats. **Results:** Ethyl acetate fraction of seed kernel of *Caesalpinia crista* Linn has shown better wound healing activity in incision, excision and dead space wound models as compared to alcoholic extract and solvent ether fraction. While petroleum ether extract, butanol fraction and butanone fraction has shown the least effective wound healing activity. **Conclusion:** From the result obtained it can be concluded that ethyl acetate fraction of seed kernel of *Caesalpinia crista* Linn has significant wound healing property.

**Key words:** *Caesalpinia crista* Linn, wound healing, soxhlet extraction.

### 1. Introduction

*Caesalpinia crista* Linn belonging to family Leguminosae is shrub found throughout the hotter parts of India, Burma and Ceylon particularly along the seacoast. The seeds are almost globular in shape, gray hard with a smooth shiny surface. The shell, which is thick and brittle encloses a yellowish white bitter fatty kernels. The seeds are useful for dispersing swellings restraining

haemorrhage and keeping off infectious diseases. They are also given internally in leprosy. This nut was used as tonic, antipyretic and anthelmintic. The emollient fixed oil from seed kernels removes the freckles of the face and stops discharge from the ear. A decoction of the roasted seed is used in the asthma [1,2]. Seeds are also used to treat inflammations [3].

## 2. Materials and methods

Seeds of *Caesalpinia crista* Linn were collected from Pragati Pharm, Belgaum and were authenticated by Mr. A.P. Kore, Asst. Professor, K.L.E.S's College of Science, Belgaum and a voucher specimen (KLEP-241) has been deposited in the college herbarium.

### 2.1 Extractions and fractionation

The seed coat was broken and kernels (2 kg) were powdered to coarse form. The powdered materials was loaded in soxhlet's extractor and defatted with petroleum ether (60-80°C) in 5 batches (16 cycles each batch). The marc was dried and extracted with rectified spirit in a Soxhlet's apparatus in 5 batches (18 cycles each batch). When the petroleum ether extract was distilled off left a dark fixed oil (200 ml); similarly when alcohol was distilled off the alcoholic extract left behind a sticky residue (325 g).

### Fractionation of alcoholic extract

A 25 g of sample (alcoholic extract) reserved for pharmacological study and remaining dry sticky alcoholic residue (300 g) was suspended in the distilled water (200 ml) with the help of mechanical stirrer. This suspension was extracted with solvent ether. The solvent ether was washed with small quantity of water and then dried over anhydrous sodium sulphite. The solvent was distilled off to get the sticky mass.

The aqueous remnant was then fractionated with ethyl acetate, butanol and butanone. Each fraction was washed with water, dried over anhydrous sodium sulphite and concentrated to a small volume under the reduced pressure and then evaporated to dryness. The petroleum ether extract, alcoholic extract and fractions were subjected to pharmacological studies. The yields of fractions obtained were recorded.

### 2.2 Acute toxicity studies

Healthy adult Wister albino rats of either sex weighing 150-200 g were selected. The study protocol was approved by the Animal Ethics Committee of the institution (CPCSEA Registration No 221). Animals were depilated at desired site before wounding. They were housed individually with free access to food and water, the basal food intake and body weights to the nearest gram were noted.

The animals were starved overnight were divided into 6 groups (n=2) and were fed with increasing doses (10, 30, 100, 300, 1000, 2000 mg/kg) of the alcoholic extract and its fractions. The animals were continuously observed for mortality and behavioral responses for 48 h and thereafter once daily for 14 days after administration.

### 2.3 Wound models

The animals were starved for 12 h prior to wounding. Studies were carried out using ether-anaesthetized rats. Animals were divided in to seven groups of six animals each. Animals were depilated at the dorsal thoracic region before wounding. The first group served as control second, third, fourth, fifth, sixth and seventh groups received petroleum ether extract, solvent ether, ethyl acetate, butanol, butanone fractions and alcohol extract by oral route at a dose of 100 mg/kg body weight daily for 10 consecutive days in the incision and dead space wound model and for 20 days in the excision wound model.

#### 2.3.1 Excision wound

It is inflicted in rats as described by Morton and Malone under light ether anaesthesia [4]. A circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats in semiseptic condition. The parameters studied were wound closure, epithelization time and scar features.

Table 1.  
Influence of *Caesalpinia crista* Linn seed kernel extracts and fractions on wound healing models

Extracts and Fractions	DOSE mg/kg	Percentage of closure of excision (Days)					Incision wound		Dead Space wound	
		4 <sup>th</sup>	8 <sup>th</sup>	12 <sup>th</sup>	16 <sup>th</sup>	20 <sup>th</sup>	Epithelization Time in days	Scar area Sq. mm	Tensile Strength g	Tensile Strength g
Control	100	11.96 ±0.326	24.416 ± 0.254	38.225 ±0.716	66.983 ±0.273	77.446 ±0.406	24.66 ±0.211	15.5 ±1.52	144.29 ±8.052	157.035 ±8.765
Petroleum ether extract	100	8.055*** ±0.117	25.30* ±0.220	42.49** ±0.924	72.71*** ±0.374	83.22*** ±0.377	23.33*** ±0.211	13.16*** ±0.479	169.73* ±16.893	225.08*** ±7.89
Solvent ether fraction	100	16.75*** ±0.176	31.48*** ±0.984	61.933*** ±1.397	81.223*** ±0.614	94.662*** ±0.417	19.00*** ±0.366	10.66*** ±0.3346	280.67*** ±4.213	289.8*** ± 4.351
Ethyl acetate fraction	100	21.21*** ±0.301	47.773*** ± 1.425	76.45*** ± 0.766	86.55*** ±0.717	99.60*** ±0.186	17.66*** ±0.516	6.00*** ± 0.161	285.08*** ±3.487	304.5*** ±4.631
Butanol fraction	100	15.38*** ±0.170	25.45* ±1.082	49.31*** ±0.237	74.42*** ±0.255	85.44*** ±0.310	21.83*** ±0.167	12.33*** ±0.422	181.28** ±5.487	214.06*** ±21.30
Butanone fraction	100	14.80*** ±0.217	27.335* ±0.970	58.89*** ±0.904	78.685*** ±0.588	87.815*** ±0.618	21.5*** ±0.224	11.83*** ±0.479	178.83*** ±6.270	280.73*** ±0.926
Alcohol extract	100	17.74*** ±0.150	35.751*** ± 0.657	66.423*** ±0.827	83.535*** ±0.7188	97.225*** ±0.170	18.33*** ±0.211	9.83*** ±0.479	299.83*** ±5.446	378.56*** ±5.520

All values are in mean ± SEM, (n=6) \*P>0.05 Non significant, \* \*P<0.05 Less significant, \*\*\*P<0.01Significant.

The observation of the percentage wound closure were recorded on 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup> and 20<sup>th</sup> post wounding day and also for epithelization and size and shape of scar area.

### 2.3.2 Incision wound

The method of Ehrlich and Hunt [5] was adopted. Under light ether anaesthesia, two paravertebral incisions of 6 cm were made through the entire thickness of the skin on either side of the vertebral column with the help of a sharp blade. The incisions were sutured using 4-0 silk threads with the help of straight round-bodied needle. To the animals were housed individually, the oral dose was given once a day. On eighth post wounding day, sutures were removed and the breaking strength was determined on 10<sup>th</sup> post wounding day by continuous constant water flow technique of Lee [6].

### 2.3.3 Dead space wound

In rats, subcutaneous implantation of sterilized cotton pellets or gross pith (2.5 cm x 0.3 cm) were done in the axilla and groin by making a pouch through a small nick in the skin. The wounds were sutured and mopped with an alcoholic swab. Excision of the granulomas from the surrounding tissue was performed on the 10<sup>th</sup> post-wounding day under light ether anesthesia. The granuloma surrounding the grass pith were excised and slit open.

The tensile strength of granuloma tissue was measured by the methods of Lee [6].

### 2.4 Statistical analysis

All the results were analyzed by Repeat measure ANOVA followed by Dunnett's test and the level of significance was set at  $P < 0.05$ .

## 3. Results and discussion

As shown in Table-1, the percentage closure of excision wound area was significantly increased in ethyl acetate fraction, alcoholic

extract and solvent ether fraction as compared to the control. The time required for complete epithelization and scar area on complete epithelization were significantly decreased by ethyl acetate fraction, alcoholic extract and solvent ether fraction respectively.

In incision wound studies, there was a significant increase in tensile strength of ten days old wound due to treatment with alcoholic extract, ethyl acetate fraction and solvent ether fraction respectively as compared to the control. In dead space wounds, there was significant increase in tensile strength by alcoholic extract, ethyl acetate fraction and solvent ether fraction respectively.

Ethyl acetate fraction of seed kernel of *Caesalpinia crista* Linn has shown the more significant wound healing activity in incision, excision and dead space wound models as compared to alcoholic extract and solvent ether fraction. While petroleum ether extract, butanol fraction and butanone fraction had shown the least effective wound healing activity.

Wound healing involves different phases such as contraction, epithelization, granulation, collagenation and so on. These findings indicate the possibility of wound healing potential of these seeds in clinical practice.

The results of study show that the ethyl acetate fraction of seed kernel of *Caesalpinia crista* Linn possesses a definite prohealing action. This is demonstrated by a significant increase in the rate of skin breaking strength, which is supported by gain in granuloma breaking strength. Which indicates increased collagen maturation by increased cross-linking [7]. From the study carried out, it may be concluded that the seed kernel of *Caesalpinia crista* Linn is endowed with significant wound healing activity, there by justifying its use in the indigenous system of medicine.

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