

# Evaluation of Wound healing activity of Ethanolic extract of *Aristolochia bracteata* and *Cassia tora* on Wistar Albino rats

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**Abstract:** The ethanolic extract of the leaves of *Aristolochia bracteata* and *Cassia tora* were studied for its effect on wound healing in rats excision wound model. Simple ointment (ointment base) was used as a control and nitrofurazone ointment as a reference standard. Ethanolic extracts of both the plants have been shown to possess good therapeutic potential as anti-inflammatory agent and promoter of wound healing due to the presence of active terpenes, alkaloids and flavonoids. It was observed that the wound contracting ability of the extracts were significantly greater than that of the control, which was comparable to that of the reference standard Nitrofurazone ointment.

**Key words:** *Aristolochia bracteata*, *Cassia tora*, Nitrofurazone ointment, simple ointment.

## INTRODUCTION:

The process of wound healing involves a variety of biological responses, such as an acute inflammation, cellular proliferation and a contraction of collagen lattice formed<sup>1</sup>. The wound healing process may be prevented by the presence of reactive oxygen species or microbial infection, since the type of cell to be first recruited to the site of injury is the neutrophil which is produced in response to cutaneous injury<sup>2,3</sup>. Appropriate method for healing of wounds is essential for the restoration of damaged tissue. Research on wound healing agent is one of the developing areas in modern biomedical sciences and many traditional practioner, across the world particularly in countries like India have valuable information of many plants for treating wounds and burns<sup>4</sup>. Medicinal plants occupy a distinct place in the life of human, right from the primitive till today<sup>5</sup>. Use of plants as a source of medicine has been inherited and is an important

component of health care system in India<sup>6</sup>. *Aristolochia bracteata* is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites<sup>7</sup>. This plant belongs to the family Aristolochiaceae. *Cassia tora* (Leguminosae) is a wild crop and grows in most parts of India as a weed. According to Ayurveda the leaves and seeds are acrid,<sup>8</sup> laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardiogenic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders.<sup>9</sup> Chemical component of *Cassia tora* are anthraquinones, chrysophanol, emodin, obtusifolin, obtusin, chryso-obtusin, aurantio-obtusin, and their glycosides. Hence in our study, we aimed to determine wound healing activity of *Aristolochia bracteata* and *Cassia tora*.

**MATERIALS AND METHODS:****Collection of the plant materials:**

The plants *Aristolochia bracteata* and *Cassia tora* is widely found throughout India. For our work the plants were collected in and around Thanjavur, Tamilnadu, India. The leaves were collected and they are dried in room temperature. Then they are crushed in to small pieces and these dried small pieces are finely powdered.

**Extraction:**

The extraction is done by maceration process and the powder material was extracted with ethanol. About one hundred grams of the powdered leaf material was mixed with four hundred ml (1:4) of ethanol and subjected to extraction by cold maceration method. The set up were kept at room temperature for seven days for complete extraction during the period of maceration, the content was shaken occasionally to mix and enhance extraction. After seven days the extract were filtered through muslin cloth. The extract was collected in suitable container and it was subjected to concentration by evaporation.

**Preliminary qualitative phytochemical examination of ethanolic extracts of *Aristolochia bracteata* and *Cassia tora*:**

Ethanolic extract was evaluated for preliminary phytochemical screening for identification of its active constituents like alkaloids, glycosides, phytosterol, fixed oils and fats, saponins, tannins and phenolic compounds. proteins and free amino acids, gums and mucilages, flavonoids, lignin.

**Ethics**

The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee,

and was cleared by same before beginning the experiment

**Materials Required**

Simple ointment, a standard drug for comparing the wound healing potential of the ointment containing *Aristolochia bracteata* (5%w/w) and *Cassia tora* (5%w/w) and nitrofurazone ointment (0.2% w/w) as a reference standard.

**Animals Used**

Wistar Albino rats (150 – 180 gms) were selected for these studies. Six rats were taken for each groups. The rats were used after an acclimation period of 7 days to the laboratory environment. They were provided with food and water.

**Excision wound model**

Four groups with six animals in each group were anaesthetized with ether. The rats were depilated on the back. Cutting away a 500mm<sup>2</sup> full thickness of skin from the depilated area inflicted one excision wound, the wound was left undressed open environment. Then the drugs, i.e., the reference standard (nitrofurazone ointment), Simple ointment (ointment base), the ointment containing *Aristolochia bracteata* and *Cassia tora* were applied once daily till the wound was completely healed. This model was used to monitor wound contraction and a wound closure time. Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day.

**Statistical Analysis**

Data are expressed as Mean± SD was evaluated by student's t test by comparing with the control. Values of < 0.05 were considered statistically significant.

**Table. 1 Phytochemical investigation of *Aristolochia bracteata* extracts**

S. No.	Constituents	Ethanol	Method
1	Alkaloids	+	Mayers reagent, Hagers ,Wagners reagent
2	Carbohydrates	—	Molisch's test
3	Glycosides	—	Borntrager's test
4	Phytosterols	+	Libermann burchard
5	Saponins	+	Agitated with distilled water → foam
6	Fixed oils & fats	+	KOH+phenolphthalein
7	Tannin & phenols	—	Fecl <sub>3</sub> , Lead acetate
8	Protein & Aminoacids	+	Millons reagent
9	Gums & mucilages	—	Alcohol
10	Flavonoids	+	Con H <sub>2</sub> SO <sub>4</sub>
11	Lignin	—	Phloroglucinol HCl acid

**Table. 2 Phytochemical investigation of *Cassia tora* extracts**

S. No	Constituents	Alcohol	Method
1	Alkaloids	+	Drangondroffs reagent
2	Carbohydrates	+	Molisch's test
3	Glycosides	+	Borntrager's test
4	Phytosterols	+	Liebermann burchard
5	Saponins	—	Distilled water
6	Fixed oils & fats	+	KOH+phenolphthalein
7	Tannin & phenols	—	FeCl <sub>3</sub> , Lead acetate
8	Protein & Amino acids	—	Millons reagent Red colour
9	Gums & mucilages	—	Alcohol
10	Flavonoids	+	Con H <sub>2</sub> SO <sub>4</sub>
11	Lignin	—	Phloroglucinol HCl acid

**Table. 3 Effects of ointment containing alcoholic extracts of *Aristolochia bracteata* and *Cassia tora* on excision wound model**

Post Wounding Days	Wound Area in mm <sup>2</sup> (Mean ± SD)			
	Simple Ointment (control)	Nitrofurazone Ointment (0.2%w/w)	5%w/w <i>Aristolochia Bracteata</i> Ointment	5%w/w <i>Cassia tora</i> Ointment
0	530 ± 33.6(0)	516 ± 36.8(0)	520 ± 24.0(0)	514 ± 21.0(0)
2	509 ± 18.6(3.9)	458 ± 36.3(11.2)	463 ± 19.8(10)	372 ± 18.8(27)
4	465 ± 13.8(12.2)	318 ± 12.6*(38.3)	378 ± 17.9*(27)	312 ± 19.9*(39)
6	424 ± 30.1(20.0)	270 ± 14.7*(47.6)	223 ± 13.3*(57)	245 ± 15.3*(52)
8	389 ± 14.8(26.6)	193 ± 11.4**(62.5)	147 ± 14.7**(71)	162 ± 12.5**(68)
10	345 ± 23.6(34.9)	110 ± 8.6**(77.3)	89 ± 10.1**(82)	95 ± 9.6**(81)
12	269 ± 14.3(49.2)	79 ± 6.3**(84.6)	56 ± 6.4**(89)	66 ± 7.4**(87)
14	215 ± 11.3(59.4)	36 ± 1.6**(93.0)	32 ± 4.8**(93)	37 ± 3.5**(92)
16	189 ± 14.3(64.3)	10 ± 1.9**(98.0)	18 ± 1.2**(96)	19 ± 0.8**(96)
18	171 ± 15.1(67.7)	0.0 ± 0**(100)	0.0 ± 0**(100)	0.0 ± 0**(100)

p<0.01\*, p<0.001\*\* Vs control by student's t test.

Values in parentheses indicates the percentage of wound contraction.

## **RESULTS AND DISCUSSION:**

Ethanollic extracts of *Aristolochia bracteata* showed the presence of alkaloids, saponins, phytosterol, fixed oils & fats, proteins and amino acids and flavonoids. Table 1 reveals the presence of various phytoconstituents.

Table – 2, reveals the phytoconstituents of *Cassia tora* in ethanolic extract. Ethanolic extracts showed the presence of alkaloids, carbohydrates, glycosides, phytosterols, fixed oils & fats and flavonoids.

The ethanolic extracts of plant materials were incorporated with simple ointment base (5%) for easy application to the open wound created in Albino rats. The measurement of progress of the wound healing induced by the nitrofurazone ointment (0.2%w/w), ointment containing the plant extracts (5%w/w) and the control group (simple ointment base) in the

excision wound model are shown in the Table 3. It was observed that the wound contracting ability of the extracts were significantly greater than that of the control, which was comparable to that of the reference standard nitrofurazone ointment.

The process of wound healing occurs in four phases: (i) coagulation which prevents blood loss, (ii) inflammation and debridement of wound, (iii) repair, including cellular proliferation and (iv) tissue remodeling and collagen deposition. Any agent, which accelerates the above process, is a promoter of wound healing. Ethanolic extracts of both the plants have been shown to possess good therapeutic potential as anti-inflammatory agents and promoter of wound healing due to the presence of active terpenes, alkaloids and flavonoids. The wound healing property of ethanolic extract of *Aristolochia bracteata* and *Cassia tora*

appears to be due to the presence of its active principles which accelerates the healing process.

## CONCLUSION

We conclude that the formulation containing ethanolic extracts of *Aristolochia bracteata* and *Cassia tora* have remarkable wound healing activity when compared with that of control.

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