

Antiinflammatory and Antimicrobial Activity of Anthraquinone Isolated from *Aloe vera* (Liliaceae)

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Aloe vera Linn family Liliaceae is known as wonder herb because of its wide medicinal uses. Cell sap of *Aloe vera* was taken in Petridish and was phytochemically screened for anthraquinone which is phenolic compound found invariably, in sap of fleshy leaves. Anthraquinone 'Emodin' was isolated from the plant sap which show pain killing, anti-inflammatory and antimicrobial activity against *Staphylococcus aureus* strain (MTCC 96).

Key Words: *Aloe vera*, Antimicrobial, Antiinflammatory, Liliaceae, Anthraquinone.

INTRODUCTION

Aloe vera is a short stemmed succulent perennial herb of family Liliaceae. The succulent leaves are crowded on the top of their stems. Grey green and spotted when young having spiny edges and bitter latex inside. The plant is highly valued in the traditional system of medicine in India for treating a variety of diseases, e.g., apply the poultice of jelly over severe open wounds and claim it to be useful in wound healing.

As a result of indiscriminate use of allopathic antibacterial drug there is a high risk of development of resistance to the antimicrobial drug. The plants pertaining antimicrobial, antiinflammatory and wound healing activities have been investigated throughout the country. Basri and Fan¹ have reported antibacterial agent in acetone extract of gall of *Quercus infectoria*. Similarly Balian *et al.*² have reported anti-inflammatory activity of leaf of *Silybum marianum*. In our previous studies Sharma *et al.*³ have reported the antimicrobial activity of saponin isolated from *Achyranthus aspera*. Soni and Saxena⁴, Khare *et al.*⁵ have reported smooth muscle relaxant activity in isolated compound of *Tephrosia purpurea* and *Eclipta alba*, respectively. Nagina *et al.*⁶ have isolated a bioactive saponin from *Tridax procumbens* as antiasthmatic agent. Juneja *et al.*⁷ have reported the anti inflammatory activity of some folklore medicinal plants. Kar⁸ have reported antimicrobial and wound healing properties of stem bark of *Toddalia asiatica*. The present paper reports a bioactive compound isolated from leaf jelly of *Aloe vera* as antimicrobial against *Staphylococcus aureus* (MTCC 96) and albino rats Swiss variety.

EXPERIMENTAL

The plant *Aloe vera* Linn was collected from the local forest area of Vidisha district of Madhya Pradesh, India. The leaves were washed thoroughly with water and disinfectant. The jelly was collected in a glass bowls, which was used for the phytochemical screening and purification of the anthraquinone 'Emodin'. The jelly was extracted using 5 different solvent system in soxhlet apparatus, for 8 h duration each in petroleum ether, chloroform, acetone, ethanol and water.

Preliminary testing of anthraquinone: 100 mg of jelly was added in 25 mL of 2 M HCl, it was heated on water bath for 15 min and then filtered. The filtrate was allowed to cool and shaken well with 20 mL ether. The ethereal layer was separated with 100 mL of ammonia. The aqueous layer become red in colour which shows the presence of anthraquinone. Further 100 mg of the sample (jelly) was added to 50 mL of chloroform and a water bath for 15 min. This was evaporated to dryness. The residue was dissolved in 0.5 mL of chloroform. This gave red colour which confirm the presence of anthraquinone in the jelly.

The extract was spotted on silica gel G coated glass plate which were developed in a solvent system, ethyl acetate:anhydrous formic acid:petroleum ether 25:1:75. The chromatogram was allowed to develop which gave 6 fractions. The R_f value of which was detected in UV chamber and naked eye (Table-1). Loss in drying, total ash soluble, water soluble value was detected which have been mentioned in Table-2.

TABLE-1
TLC SEPARATION OF THE CRUDE EXTRACT

Solvent system	Purified fraction	No. of spots	Colour characterization		R_f value
			Visible light	UV Light	
Ethyl acetate:formic acid:petroleum ether (25:1:75)	Fr. 1	Spot 1	Brown	Invisible	0.10
		Spot 2	Invisible	Yellow	0.25
		Spot 3	Invisible	Blue	0.35
Ethyl acetate:formic acid:petroleum ether (25:1:75)	Fr. 2	Spot 1	Dark yellow	Fluorescent	1.17
		Spot 2	Creamy	Invisible	0.56
		Spot 3	Yellow	Brown	0.18
		Spot 4	Dark yellow	Fluorescent	0.25
		Spot 5	Invisible	Fluorescent	0.12

TABLE-2
PLANT CONTENT AND ASH CONTENT OF *Aloe vera*

Wet weight of plant (g)	4000
Loss in weight after drying (g)	3650
Percentage of loss in weight (g)	91.25
Weight of ash content (g, %)	2.19, 10.95
Loss in weight after burning (g)	17.81
Water soluble (g, %)	1.53, 17.00
Acid soluble (g, %)	0.66, 30.00
Dry weight of plant material at rooms temperature (g)	350

Structural elucidation: On the basis of the spectrum and the preliminary examination, comparing with the authentic samples obtained from Phytolab GmbH & Co. KG Hamburg, Germany an Emodin compound was elucidated finally.

***Aloe emodin*:** m.f. $C_{15}H_{10}O_5$; m.w. 270.24 g/mol; m.p. 170 °C; content: > 86 % HPLC; tested on: 1H NMR ^{13}C NMR, IR, MS, TLC, water content and residual solvent.

Animals: Wister albino rats (*Rattus norvegicus*) were used for antiinflammatory activity. The experimental protocol was conducted as per guidelines of CPCSEA, 804/03/CA/CPSCEA and with approval of IAEC. The rats were orally fed with the extracts in gum acacia at three doses 1, 2 and 4 g/kg body weight and the inflammatory activity was observed after 3 and 6 h duration as per standard method of Winter *et al.*⁹.

RESULTS AND DISCUSSION

Antiinflammatory activity: The preliminary phytochemical screening of leaf gel of *Aloe vera* when tested against Carrageenan induced paw oedema model it was noticed that 100 mg/Kg body wt. cause significant decrease in the paw oedema which produced *ca.* 75 % inhibition in petroleum ether extract, while ethanolic extract at the same dose causes 90 % inhibition. The results were found to be quite significant ($p < 0.01$) Table-3.

TABLE-3
ANTIINFLAMMATORY EFFECT OF LEAF OF PLANT IN
PETROLEUM ETHER AND ETHANOL EXTRACT

Drug dose/route	Carrageenan induced paw oedema		Formalin induced paw oedema	
	Oedema (mL)	Inhibition (%)	Oedema (mL)	Inhibition (%)
Control	1.22±0.20	–	1.18±0.11	–
Aspirin (150 mg/kg oral)	0.26±0.13	78.79	0.16±0.04	86.86
Leaf extract (in petroleum ether) 100 mg/kg/oral	0.32±0.09	74.00	0.17±0.04	85.61
Leaf extract (ethanol) 100 mg/kg/oral	0.07±0.02	93.9	0.10±0.05	91.27
One way Annova	F	7.91	–	37.55
	dt	3.20	–	3.20
	P	<0.01	–	<0.01

Antimicrobial activity: The minimum inhibitory concentration (MIC) of the 2 plant extracts was determined for *Staphylococcus aureus* (MTCC 96) at 10 mg/mL to 0.0/95 mg/mL concentration. The tested extract which was added to the sterile Hinton broth culture media by micropipette to the Petridish. Each extract was assayed in triplicate. The bacterial suspension was used for positive culture and the MIC value was determined. The MIC value of ethanolic and petroleum ether extract of the leaf gel of *Aloe vera* is shown in Table-4. The MIC values for the two extracts *i.e.* petroleum ether and ethonal came to be 0.078 mg/mL and 0.0391 mg/mL against *S. aureus*, respectively. The results were compared with positive and negative control.

TABLE-4
DETERMINATION OF MIC VALUES OF EXTRACTS OF
Aloe vera AGAINST *S. aureus* (MTCC98)

Concentration (mg/mL)	<i>S. aureus</i> (MTCC 96)		Control	
	Petroleum ether	Ethonal extract	Positive	Negative
10.0000	-	-	+	-
5.0000	-	-	+	-
2.5000	-	-	+	-
1.2500	-	-	+	-
0.6250	-	-	+	-
0.3125	-	-	+	-
0.1563	-	-	+	-
0.0781	+	-	+	-
0.0391	+	+	+	-
0.0195	+	+	+	-

- = Absence of growth, positive control: Bacterial in pension and saline.

+ = Presence of growth, negative control: Extracts and broth.

Intraparetonial injection of carrageenan and formalin leads to the inflammation of paw oedema. In the present study, the both plant extracts have shown antagonistic activity to paw oedema as shown in Table-3. The carrageenan induced paw oedema 0.30 ± 0.09 have shown 74 % inhibition with petroleum ether extract where as the ethanolic extract 0.07 ± 0.02 paw oedema gave max. 93.9 % inhibition. In formalin induced paw oedema, the value came to be 86.8 and 91.2 % inhibition in petroleum ether and ethanolic extract of the plant, respectively. It may found in both the cases the ethanolic extract is more effective in reducing paw oedema. Similarly, anti-inflammatory activities of *Lagenaria siceraria* fruit juice extract in albino rat was observed by Ghule *et al.*¹⁰. Dorni *et al.*¹¹ have reported the antiinflammatory activity of *Plambaga capensis* where they have found this activity in the plant due to naphthaquinine in petroleum ether.

The antimicrobial activity of *Aloe vera* leaf extract in both solvents of petroleum ether and ethanol was tested against *S. aureus*. The results showed maximum MIC value in case of ethanolic extract. Nair *et al.*¹² reported antibacterial activity of aqueous and in organic extracts of some medicinal plants against 6 bacterial strains using agar disc diffusion methods. Aqueous extract was found to be more effective. In our previous report, Sharma *et al.*³ have islated saponin from *Achyranthus aspers* which showed minimum inhibitory concentration at 0.15 mg/mL dose. Similarly, Avani and Neeta¹³ reported the antimicrobial activity of *Eliphantopus scaber* which give 50 % inhibition at 2 mg/mL and complete inhibition 4 mg/mL concentration of the acetone extract of the plant.

The present study indicates the potential of herbal drug for formulation of anti-inflammatory and antimicrobial drugs. Further studies are needed to reveal the exact mechanism of antiinflammation by the anthraquinone isolated from *Aloe vera*.

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