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Pharmacology and Phytochemistry of Saponin Isolated from *Aloe vera* for Wound Healing Activity

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Aloe vera Mill. (Family-Liliaceae) water extract showed the presence of saponin with high polarity and maximum percentage yield 21.8 %. Wound healing activities were noticed within 4-8 d of granulation of wound and maximum 82.29 % maturation of collagen was observed in albino rats.

Key Words: *Aloe vera*, Aloegenin, Wound healing activity.

INTRODUCTION

The plant based traditional medicine system continue to provide primary health care to more than three quarters of the world's population. Plant derived traditional medicine, therefore has an important role in the maintenance of health all over the World. Some major categories of plants derived products include personal care products and phyto-cosmetics, herbal medicines, natural health products and phyto-pharmaceuticals[1]. Plants possess several secondary metabolites as the chemicals for their defence. *Aloe vera* contain soapy substances aloegenin which form about 3 % part of its gel and are capable of cleansing having antiseptic properties. These act powerfully as antimicrobial agent[2]. During recent years, Suguna *et al.*[3] have reported the effects of *Centella asiatica* extract on dermal wound healing in rats. Sharma *et al.*[4] have reported antimicrobial activity of saponins isolated from *Achyranthes aspera* against *Staphylococcus aureus*. From the present laboratory, Saxena[5], Choudhary[6], Soni *et al.*[7] have also reported the biologically active compound from *Achyranthes aspera*, *Tephrosia purpuria*, *Euphorbia hirta* and *Dolichos lablab* which have shown antihistaminic and smooth muscles relaxant activities. Looking to the pertaining literature and in view of the necessity, the present study was proposed to be investigated on wound healing activity of *Aloe vera*.

EXPERIMENTAL

Petiole of *Aloe vera* was collected from the local surrounding of Rath Tehsil of Hamirpur district and after proper identification a voucher specimen was procured in the Herbarium record of Pest Control and Ayurvedic Drug Research Laboratory, Vidisha, India, at S.No. 31.

Preparation of extract: The powdered material of 40-60 mesh size were soxhleted in different solvent systems and was evaporated in vacuum evaporator to get semi solid crude. The percentage yield of extract as shown in the Table-1.

TABLE-1
PERCENTAGE YIELD OF *ALOE VERA* BY SOXHLET EXTRACTION METHOD AT 45 ºC

Solvent used	Wt. of powder (g)	Wt. of extract (g)	% Yield crude extract
n-Hexane	200	25.3	12.65
Chloroform	400	30.9	7.72
Ether	200	25.7	12.85
Ethanol	300	28.4	9.40
Water*	200	43.6	21.80

*Water gave highly polar compound.

Saponification: A simple test for Saponin is therefore, to shake up an aqueous alcoholic plant extract in test tube and noted if persistent foams are formed above the liquid surface.

Chemical analysis of crude extract: The biologically active compound was separated from the crude extract by column chromatography[8]. It was followed by TLC using different solvent systems[9]. The plates were run and spots were identified and R_f value was calculated for each spot as shown in Table-2.

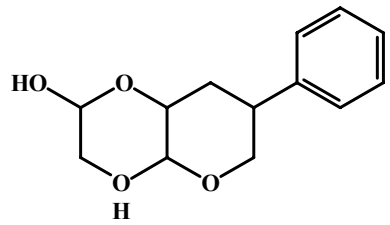
TABLE-2
TLC OF CRUDE EXTRACT OF *Aloe vera*

Solvent system	Purified fractions	No. of spots	Colour characterization		R_f value
			Visible light	UV light	
Benzene:Pet. ether (9:1)	Fr. 1	Spot 1	Brown	Invisible	0.10
		Spot 2	Invisible	Yellow	0.25
		Spot 3	Invisible	Blue	0.35
Benzene:Methanol (9:1)	Fr. 2	Spot 1	Dark yellow	Fluorescent	1.12
		Spot 2	Creamy	Invisible	0.50
		Spot 3	Yellow	Brown	0.18
		Spot 4	Dark yellow	Fluorescent	0.25

Acid hydrolysis of purified fraction: The fractions were hydrolyzed with 10 % H_2SO_4 in MeOH:H_2O (1:1) at 80 ºC for 4 h. The reaction mixture was neutralized with $BaCO_3$ and fractions was evaporated to dryness in vacuum to get a residue and to make crystal for spectral analysis.

Spectral analysis: It was carried out at SAIF, CDRI Lucknow to get spectral data *i.e.* IR, UV, ^{1}H NMR and ^{13}C NMR for structural elucidation. IR displays strong broad O-H stretching bands which were broadened due to hydrogen bonding and a sharp 'non-bonded' peak was noticed at 3399 cm^{-1}. Carbon-carbon double bond stretching bands were observed at 1629 cm^{-1} of medium range. Three short

peaks were identified at 1211, 1151 and 1111 cm^{-1} which represent carbon-oxygen single bonds. In UV, 260 nm peak showed the presence of cyclohexadiene. In ^{1}H NMR, one peak is obtained at 4.845 ppm range which indicate the presence of Saponin like compound. In ^{13}C NMR, one peak is obtained at 50 ppm which indicates the presence of C-OH. Second one very small peak is obtained at the range of 30 ppm which indicates the presence of C-C=C. The compound has the highest molecular mass of the saponin isolated from water. The R_f value of the compound was found between 0.1 to 0.5 which indicates the presence of saponin. On the basis of this analysis a saponin like compound aloegenin was isolated from the whole plant of *Aloe vera*.



Aloegenin

TABLE-3
SHOWING AGE WISE BREAKING STRENGTH (BS) AND PERCENT MATURATION OF COLLAGEN

Age of granulation (d)	BS of un-tanned piece	BS of tanned piece	% Maturation of Collagen
2	142 ± 1	471 ± 2	30.14
4	180 ± 2	459 ± 1	39.21
8	228 ± 2	465 ± 2	48.38
10	220 ± 1	415 ± 1	53.01
12	270 ± 2	440 ± 1	61.36
14	318 ± 1	460 ± 1	69.13
16	362 ± 1	470 ± 1	77.02
18	395 ± 1	480 ± 2	82.29

Study of wound healing activity: The study was based on the work reported by Peacock[10]. From group of wound bearing animals, the harvested granulation tissue was isolated on 4 to 18 wound healing days. The granulation tissue was cut into nearly identical pieces. One of them was soaked in physiological salt solution and the other in 5 % formaldehyde and time of exposure of the tissue was adopted. From breaking strength (BS) value of tanned and un-tanned pieces, the percent maturation of collagen in 4-18 wound healing days was calculated by this formula:

$$\% \text{ Maturation} = \text{BS of Un-tanned piece} / \text{BS of Tanned piece} \times 100$$

RESULTS AND DISCUSSION

Table-1 reports the percentage yield of crude extract in soxhlet at different solvents with increasing order of polarity. Maximum yield 21.8 % was observed in water extract which represents high polarity of this compound. Table-2 reports TLC fractions of *Aloe vera* extract with their R_f value which when compared with authentic marker compounds (Sigma Aldrich Co. Pvt. Ltd, USA) shows the presence of Saponin. Table-3 shows systematic details of age wise breaking strength of tissues and percent maturation of collagen during 4 to 18 d of granulation of wound and 82.29 % maturation of collagen was observed with in 18 d.

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