

Phyto-Chemical Investigation and Therapeutic Evaluation Of *Aloe Barbadensis*

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Abstract

The present study was an endeavor to investigate phyto chemical constituents of *Aloe barbadensis* whole leaf using aqueous, ether and solvent extracts. The extracts were tested for the presence Carbohydrate, Saponins, Flavonoids, Tannins, Alkaloids Anthraquinones and Resins. The gel part of the *Aloe* leaf obtained after peeling of outer rind was used for anti inflammatory activity while the remaining rind part was macerated to get yellow exudates with aqueous media or acetone and crude sap was used to evaluate the antimicrobial activity. The antimicrobial results showed effective inhibitory action against *S. aureus*, *E. coli*, & *C. albicans*. The anti-inflammatory activity was studied in both gel & sap of the leaf by formalin induced paw edema method. Diclofenac sodium ointment was taken as the standard drug. The percentage inhibition of paw edema was observed to be 80-95% (Gel), 49.04 (sap) compared to 88.02% as in case of Diclofenac sodium ointment. The results indicated that gel of *Aloe* leaf possessed significant anti-inflammatory activity while crude sap possessed antimicrobial activity.

Keywords: aloe vera, anthraquinone, antimicrobial, anti-inflammatory

Introduction

Natural products have always been a prolific source of new drugs and drug lead even from the Vedic times. Recent analysis has suggested that about 80% drug molecules are natural products or obtained from them. India is one of the richest countries in world supplied with medicinal plants. The limitations associated with synthetic pharmaceutical products has opened avenues for 'Green Medicine' that is considered to be safe, more accessible and affordable too. To determine the potential and promote the use of herbal medicine, it is essentially required to intensify the research studies on traditional and folklore medicines. *Aloe barbadensis* miller or *Aloe vera* is a very popular household plant; commercially known as Curacao aloe. *Aloe vera* a succulent plant belonging to the family Liliaceae is a tropical to subtropical plant. *Aloe vera* leaf gel contains 75 active ingredients and 200 active compounds including 20 minerals, 18 amino acids & 12 vitamins^{1,2}. The gel part has been reported to be effective against skin ulcer^{3,4}, radiation burn⁵, peptic ulcer⁶ and anti-inflammatory activity due to the presence of anthraquinones, polysaccharides, saponins and flavonoids⁸ etc. The whole plant leaf contains two basic parts latex and gel. Latex is obtained from leaf lining; it is yellowish in colour and bitter in taste. It mainly contains anthraquinone glycosides- aloin, aloe-emodin and barbaloin that are potent stimulant laxatives⁹. In smaller quantity they appear to aid absorption from the gut, are potent antimicrobial agents^{10,11}. For medicinal use, the leaf lining is dried and the residue is used as herbal laxative¹².

Inflammation is the patho-physiological response of mammalian tissues to a variety of hostile agents, and the complex events and mediators involved in inflammation can induce, maintain & aggravate many disorders. NSAIDs are frequently used to overcome or reduce the consequences of inflammation. But the frequent use of NSAIDs presents many side effects; to minimize this present study was undertaken.

The present study aimed to investigate the phytochemical constituents and antimicrobial and anti-inflammatory activity of aloe vera gel and sap.

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Material and Methods

Chemicals-

Petroleum ether, acetone (Loba Chemicals) Diclofenac sodium ointment (Marketed products), paraffin gel and all other reagents were of analytical grade.

Plant Material -

Aloe vera fresh leaves were collected locally and identified and authenticated by Bioscience Dept, Rani Durgawati Vishwavidyalaya, Jabalpur. The leaves were washed and upper rind was scraped with sharp knife and gel was collected in a vessel. The rind was macerated with aqueous, acetone and ether separately and crude extract of leaf was used. The gel was used without any further treatment.

Phytochemical Investigation-

The phytochemical study of the whole plant leaf was carried out using the aqueous, ether and acetone extracts and was tested for the presence of active constituents as shown in Table 1.

Animals-

Wistar albino rats (200-250g) of both sexes were used for anti inflammatory studies. The animal studies were approved by Institutional Animals Ethical Committee and carried out as per the CPCSEA guidelines. The animals were kept in groups of three per cage and fed on standard diet with water ad libitum.

Anti-inflammatory Activity

Formaldehyde induced paw edema method.

Wistar albino rats (200-250) were used for the anti-inflammatory studies. The animals were divided into four groups of 3 each and were treated as under.

Group-1: Served as control. Paraffin gel (2g) was applied locally.

Group-2: Freshly isolated gel (0.5g) was applied on the hind paw 30 minutes before formalin injection.

Group-3: Crude extract of leaf sap (0.5g) was applied on the hind paw 30 minutes before formalin injection on rat's hind paw.

Group-4: Diclofenac ointment (0.1g) was applied on the hind paw.

After 30 minutes of application, rats were challenged with a subcutaneous injection of 0.1 ml of 1% w/v solution of formalin into the sub planter side of the left paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to this mark. The paw volume was measured using plethysmometer immediately after injection at zero hour and followed by every 30 minutes for 3 hrs after injection of phlogistic agent to each group¹⁶.

The percentage inhibition of inflammation was calculated using the formula-

$$\text{Percentage Inhibition} = (1 - V_t/V_c) \times 100$$

Where V_t - Increase in paw volume in test animal

V_c - Increase in paw volume in control group.

The results obtained as mean increase in paw volume (ml) and percentage inhibition in edema are presented in Table

2. Antimicrobial study-

Pure culture of the bacterial and fungal isolated (*Staphylococcus aureus*, *E. coli* and *Candida albicans*) was maintained on the agar slants. Antimicrobial susceptibility testing was determined by cup plate method¹⁷. The *in vitro* screening of antifungal activity was carried out against microorganisms. The cups were made aseptically with cork borer having 6 mm diameter and 0.2 ml of test solution of each extract was

poured in it. The plated were incubated at 37° C and the zone of inhibition measured after 72 hrs of incubation (Table. 3 / Fig-1).

Results and discussion

Phytochemical investigation-

Preliminary phytochemical analysis of Aloe vera in aqueous, ether and acetone extract was done to detect its constituent (Kokate,1990). The chemical test performed in the aqueous extract for anthroquinones, glycosides, reducing sugar and flavonoids were positive in other extract like ether acetone methanol. The chemical test for saponin, carbohydrate, sterol, resins and anthraquinone were positive. In acetone extract, the chemical test performed for sterol and anthraquinone were positive. These test results indicates that the extract of Aloe vera gel have anti-inflammatory activity because of mucopolysaccharides (Manna,1993, Femina, 1999) where as Protein and Tannins were found to be negative in all solvent. The Anthroquinone test confirms the presence of glycosides flavonoids and saponins in leaf extract. (Table-I).The test is positive in leaf rind. The dried whole leaf extract also gives positive anthroquinone test." The specific test for Aloe vera or aloin was done with prepared sample in acidic form with whole plant or leaves unlike other the common constituent. The specific test for aloe vera was positive with whole plant extract. Thus it is confirmed that the whole plant is best for the medicinal use (Satyavati, et al.,1976).

Antimicrobial study-

The antimicrobial results showed that the crude leaf sap and acetone extract both have effective inhibitory effect on *Staphylococcus aureus* with zone of inhibition 22 and 18 mm respectively. *Escherichia coli* was also tested and zone of inhibition was found to be 12 and 11 mm in both crude and acetone extract. Where as the fungi like *Candida albicans* were also inhibited by *Aloe vera* leaf extract. *Candida albicans* is an opportunistic human pathogen causing Candidiasis, which is normally controlled by our immune defence system. Infection of candida on localized area cause obvious recurrent and persistent infections such as vaginitis, oral thrush and diaper rash. The inhibitory effect of *Candida albicans* was 22 and 11 mm in both crude and acetone extract respectively

Anti-inflammatory Activity-

Anti-inflammatory test was done on the albino rat hind paw by mercury displacement method with the help of plethysmometer. This is a better way to measure the oedema induced with the help of formalin. The percentage inhibition of oedema in control rat measured for gel and sap was 80.95% and 49.04 % respectively as compared to petroleum ether (50.47%) and diclophenac sodium ointment (88.02%). It is thus concluded that gel has many constituents, which are helpful as anti-inflammatory agent than the outer rind of *Aloe vera*. The presence of polysaccharide, sterol, salicylic acid and saponin all together help as healing agent, analgesic and anti-inflammatory agent. Thus *Aloe vera* appears to act as a modulatory system towards wound healing and inflammation and is a potentially valuable tool for managing lower extremity conditions.

The plant showed remarkable antimicrobial and anti-inflammatory activity. Conclusively the active constituents present in gel and sap of *Aloe vera* plant have been found to possess astonishing antimicrobial and anti-inflammatory activity making it a highly promising drug.

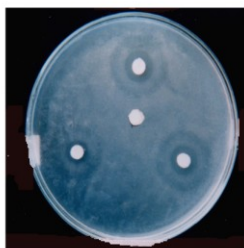
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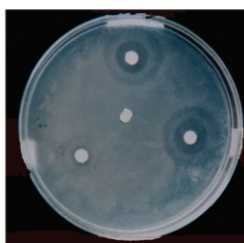
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Table 1. Phytochemical investigation of various extracts of *Aloe vera*

Constituents	Extracts		
	Aqueous	Acetone	Ether
Carbohydrate	+	+	+
Saponins	+	+	-
Flavonoids	+	-	-
Tannins	-	-	-
Alkaloids	-	-	-
Anthraquinons			
Borax Test,	+	+	+
Bromine Test,	+	+	+
Aloin Test	+	-	+
Nitric acid Test	+	+	+
Resins	+	+	+



Staphylococcus aureus



Escherichia coli



Candida albicans

Fig 1 Anti Microbial Activity of *Aloe vera*

Table 2. Anti-inflammatory activity of *Aloe vera*.

Group No.	Treatment	Dose	Increase in paw volume (mean) 3hrs	Percentage inhibition of edema
1.	Control	0.1ml	0.42 ± 0.01	-
2.	Gel	1gm	0.08±0.03	80.95
3.	Sap	1 gm	0.214 ± 0.02	49.04
4.	Petroleum Jelly (Placebo)	1gm	0.208 ± 0.01	50.47
5.	Diclofenac Sodium Ointment	1gm	0.06 ± 0.03	88.02

Table 3. Antimicrobial Activity of *Aloe vera*

S.No.	Microbial Agent	Sample	Zone of Inhibition (Diameter in mm)
Bacteria			
1	<i>Staphylococcus aureus</i>	Gel	11
		Crude sap	22
		Acetone extract	18
2	<i>Escherichia coli</i>	Gel	--
		Crude sap	12
		Acetone extract	11
Fungi			
3	<i>Candida albicans</i>	Gel	--
		Crude sap	22
		Acetone extract	11