

WOUND HEALING ACTIVITY AND ANTI ULCER ACTIVITY OF ETHANOLIC EXTRACT OF PEELS OF ANANAS COMOSUS

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ABSTRACT

Aim: To study the “wound healing” & “anti ulcer” activity of ethanolic extract of peels of *Ananas comosus*.

Objectives: Prepare ethanolic extract of peels of *Ananas comosus*, to perform phyto chemical screening studies & evaluation of wound healing, anti-ulcer activities. **Methods:** The wound healing & anti-ulcer efficacy of ethanolic extract of peels of *Ananas comosus* was evaluated in excision wound model and aspirin induced ulcer method. The rats were divided into 4 groups with 6 rats in each group. Group 1 rats were treated with vehicle. Group 2 rats were treated with standard drug. Group 3 and 4 rats were treated with extract at 100 and 200mg/ kg bd wt respectively.

Results: The percentage healing with *Ananas comosus* peel extract was significantly higher than standard from the 2nd day of treatment and continued till the completion of the treatment. The extract has a tremendous potential as a natural potent and safe alternative to conventional antiulcer treatment. **Conclusion:** The ethanolic extract of peels of *Ananas comosus* showed significant wound healing & anti-ulcer activity which is evident by the data obtained.

KEYWORDS: Wound healing; anti-ulcer; ethanolic extract; *Ananas comosus* peel.

1. INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The plants are indispensable to man for his life. The 3 important necessities of life: food, clothing, shelter and a post of other useful products are supplied to him by the plant kingdom. The human being is to be effected with more diseases than any other animal species. In the past all the medicines were used from plants; the plant being man's only chemist for ages. The history of herbal medicine is as old as human civilization.^[1]

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to define against attack from predators such as insects, fungi and herbivorous mammals. At least 12000 such components have been isolated so far; a number established at least 10% of the total.^[2,3] Chemical compounds in plants mediate their effect on the human

body through processes identical to those already well understand for the chemical compounds in conventional drugs; thus herbal medicines does not differ from conventional drugs in terms how they differ.^[2,5]

1.1 WOUND HEALING

A break in the cellular and anatomical architecture of body tissue including the skin mucus membrane, deep lying tissues or surface of internal organs ranging from incision, laceration, abrasion, puncture and closed wounds such as confusion, hematoma and crush injuries is termed as wound.^[4] Due to poor hygienic conditions, wound infection has become common disease in recent years.^[6] Wound healing begins from the time of injury and can continue for varying periods of time, depending on the degree of wounding. Wound healing process can be diversified into 3 stages namely inflammatory, proliferative and lastly the remodeling phase which determines the potency and appearance of the healed tissue.^[7]

Table no-1. Phase of healing ,Time post injury, Cells involved in phase, Function or activity

Phase of healing	Time post injury	Cells involved in phase	Function or activity
Hemostasis	Intermediate	Platelets	Clotting
Inflammation	Day 1-4	Neutrophils, macrophages	Phagocytosis
Proliferation [granulation & contraction]	Day 4-21	Macrophages, lymphocytes, angiocytes, neurocytes, fibroblasts, keratinocytes	re-establish skin function closure
Remodelling	Day 21-2 years	Fibrocytes	Develop tensile strength

1.2 ANTI-ULCER ACTIVITY

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue.^[8,9]

Ulcers are most common on the skin of the lower extremities and in the gastro-intestinal tract, although they may be encountered at almost any site. There are many types of ulcers such as mouth ulcer, esophagus ulcer, peptic ulcer and genital ulcers. Of these, peptic ulcer is seen among many people. The peptic ulcers are erosion of lining of stomach or the duodenum.^[10] The two most common types of peptic ulcer are (1) Gastric ulcer – located in the stomach, characterized by pain; common in old age group.^[12] (2) Duodenal ulcer – found in the beginning of small intestine & characterized by severe pain with burning sensation In upper abdomen that awakens patients from sleep.^[11] In some cases, peptic ulcer can be life threatening with symptoms like bloody stool, severe abdominal pain and cramps along with vomiting blood.

1.3 PLANT

Ananas comosus (pineapple) belongs to the order Bromeliales; family Bromeliaceae; sub-family Bromelioidea. It is a herbaceous perennial, which grows about 2.5 to 5 ft high with a spread of 3 to 4 ft. Native to southern Brazil and Paraguay area, the pineapple was apparently domesticated by the Indians and carried by them up through South and Central America to Mexico. This plant has too many possible beneficial herbal uses to fully enumerate. A few of its uses include: as a uterine tonic, as a carminative, as a laxative and as a diuretic, as an adjuvant in gastric irritability and as a cholagogue in jaundice. The unripe fruits are sour, cooling, appetizer, digestive, anthelmintics, cathartics, cardiotonic, sudorific, styptic, lithontripic, antiscorbutic, febrifuge and tonic and are useful in vitiated conditions of pitta, strangury, dyspepsia, flatulence, colic, hyperacidity, cardiac debility, haematemesis, haemoptysis, renal and vesical calculi, scabies, pruritus, fever and general debility. The leaves are anthelmintics, abortifacient and emmenagogue and are useful in abortion, helminthiasis, amenorrhoea, dysmenorrhoea and whooping cough. Since bromelian, a phytochemical constituent of pineapple is expected to be one of the agents responsible

for faster wound, so it was thought to carry out wound healing action of the fruits for scientific validation.^[13]



2. METHODS AND MATERIALS

2.1. COLLECTION AND AUTHENTICATION OF PLANT SPECIMEN

Large numbers of fruits of *Ananas comosus* were collected from the local area, Guntur, Andhra Pradesh. Before their use they were carefully identified and authenticated by Prof. Dr. P. Satyanarayana raju, Dept of Botany and Microbiology, Acharya Nagarjuna University, Guntur.

2.2 PREPARATIONN OF EXTRACT

Large numbers of peels of *Ananas comosus* were collected. The peels were washed with water, carried out shade drying and powdered using a mechanical stirrer. The coarse material was extracted separately with ethanol by soxhalation method.

Table no-2. PRELIMINARY PHYTO-CHEMICAL SCREENING

Name of the constituent	Name of the test	Observation
1. Carbohydrates	Molisch's test	+ve
2. Glycosides	Borntrager's test	+ve
3. Fixed oils & fats	Saponification test	+ve
4. Proteins and free amino acids	Millon's test; Biuret test	+ve +ve
5. Saponins	Foam test	+ve
6. Phenolic compounds	Dilute ferric chloride solution	+ve
7. Phytosterol	Libermann-burchard's test	+ve
8. Alkaloids	Dragand roff's test	+ve
9. Flavonoids	Shinoda's test	+ve

2.3 SELECTION OF ANIMALS (for both Wound healing and Anti-ulcer activity)

Healthy adult rats of Wistern strain of either sex, weighing 180-220gms were selected for the study. The

experimental protocol was subjected to the scrutiny of the Institutional Animals Ethics Committee and was cleared by same before beginning the experiment.

2.4 MAINTENANCE OF ANIMALS (for both Wound healing and Anti-ulcer activity)

The animal house was well ventilated; animals and the temperature was kept between 19-20°C. The animals were housed in large spacious hygienic cages during the course of the experimental period. The animals were fed with rat pellets feed and purified filter water. As the wounded animals were susceptible to infection hygienic conditions were maintained.

2.5 EXPERIMENTAL DESIGN

The animals were numbered, weighed and then divided into four groups with 6 animals in each as follows

Group 1: Served as vehicle control & applied simple ointment

Group 2: Povidone- iodine ointment (100mg)

Group 3: Extract (100mg)

Group 4: Extract (200mg)

Animals were anaesthetized prior to and during creation of the wounds. The rats were inflicted with wounds. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of 2.5cm (circular area = 300mm²) in length and 0.2 cm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open. Then the ointments were applied (as stated above). Calculated as percentage reduction in wound.

$$\% \text{ wound contraction} = \frac{\text{total area} - \text{healed area}}{\text{total area}} \times 100$$

The wound closure rate was assessed by tracing the wound on days 0, 3, 6, 9 and 12 using transparency paper and a permanent marker. The wound areas recorded were measured using a graph paper.

Number of days required for falling of eschar without any residual raw wound gave the period of epithelization.

2.6 ACUTE TOXICITY STUDIES

The rats were divided into five groups comprising of six rats in each group in each as follows:

Group 1: Healthy control animals

Group 2: Disease control animals, ulcer was induced with 2000mg/kg-bw of Aspirin

Group 3: Ulcer induced rats treated with the extract (100mg/kg-bw)

Group 4: Ulcer induced rats treated with the extract (200mg/kg-bw)

Group 5: Drug control animals- Aspirin induced ulcerated animals treated with Ranitidine (50mg/kg-bw).

DETERMINATION OF ULCER IN THE STOMACH

The stomach's were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted.

SCORING OF ULCER WILL BE MADE AS FOLLOWS

Observations on stomach	Ulcer score
Normal colored stomach	0.0
Red coloration	0.5
Spot ulcer	1.0
Hemorrhagic streak	1.5
Deep ulcers	2.0
Perforation	3.0

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows.

Ulcer index (UI) was measured by using following formula

$$UI = (UN + US + UP) \times 10^{-1}$$

Where,

UI = Ulcer Index.

UN = Average number of ulcers per animal.

US = Average number of severity score.

UP = Percentage of animals with ulcers.

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of ulceration} = \frac{(U_{sc} - U_{st})}{U_{sc}} \times 100.$$

Where,

U_{sc} = Ulcer surface area in control.

U_{st} = Ulcer surface area in treated animals.

STATISTICAL ANALYSIS

The values of wound healing were calculated as mean \pm S.E.M. The significance of the difference of the mean value with respect to control group was analyzed by one way ANOVA followed by student t-test using Statistical 8.0. P<0.05 or above was considered to be significant.

All the values of anti ulcer activity were tabulated and presented in the tables and were expressed as mean + standard error mean (SEM of five animals. Significant difference among the means were calculated at the level of P a<0.001, b<0.01, c<0.05 when compared with controls. The statistical significance was calculated using students 't' test.

3. RESULTS

Table-3: Phyto-chemical screening of *Ananas comosus* peel.

Metabolite	Present/Absent
Carbohydrates and Glycosides	+
Oils	+
Protein and Aminoacids	+
Saponins	+
Phenolic compounds	+
Phytosterols	+
Alkaloids	+
Flavanoids	+
Tannins	+

NOTE: '+' - Presence of secondary metabolites.

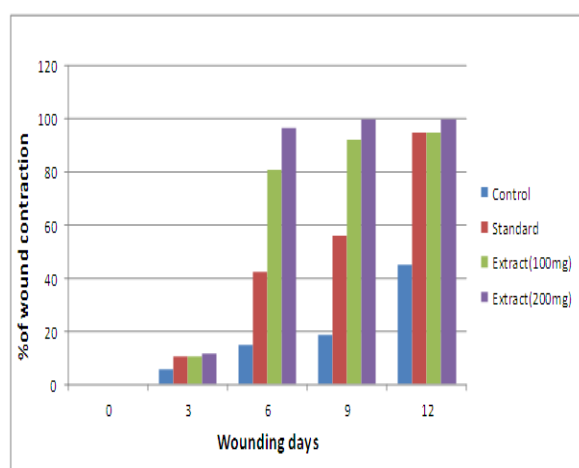
Table- 4: Effect of *Ananas comosus* peel extract on excised wound in rats.

S.no	Animal Treatment	Surface area of excised wound in mm ² on corresponding days				
		0	3	6	9	12
1	Control	300±.565	282.6±0.497	255.44±0.865	244.14±0.616	165.11±0.429
2	Standard (povidone ointment)	300±0.403	269.34±0.676	172.62±0.794	132.84±0.585	10.52±0.505
3	Extract (100mg)	300±0.403	269.07±0.874	57.75±0.585	24.12±0.497	10.52±0.505
4	Extract (200mg)	300±0.344	265.14±0.585	10.68±0.616	0.00	0.00

Values are expressed in Mean ± SEM of 6 animals.

Table-5: Effect of *Ananas comosus* peel extract at different dose levels on the percent of wound contraction with respect of zero day of excised wound in rats.

S.no	Wounding days	Percentage of wound contraction (%)			
		Control	Povidone ointment	Extract (100mg)	Extract (200mg)
1	0	0	0	0	0
2	3	5.8	10.22	10.31	11.62
3	6	14.85	42.46	80.75	96.44
4	9	18.62	55.72	92	100
5	12	45	95	95	100



Graph 1: Effect of *Ananas comosus* peel extract at different dose levels on the percent of wound contraction.

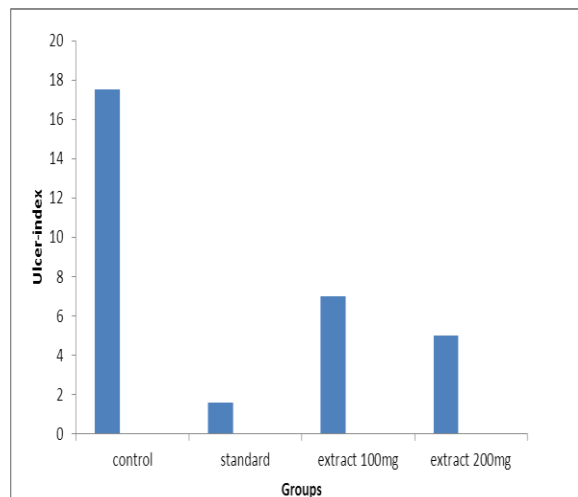


Figure 1: The treatment of excised wound on Day 6 & 12.

Table 6: Effects of *Ananas comosus* peel extract in Aspirin induced ulcers in rats.

S.no	Group	Ulcer index	% Ulcer healing
1	Control	17.5±1.1	-
2	Ranitidine	1.6±0.8a	90.8%
3	Extract 100mg	7.0±1.2b	60%
4	Extract 200mg	5.0±1.08a	71.4%

Values are mean ±SEM of five animals in each group
 $P < 0.001$ Vs Control, $b < 0.01$ Vs control, $c < 0.05$ Vs Control, using students 't' test.

**Graph 2: Effects of *Ananas comosus* peel extracts, control and standard group on ulcer index in aspirin induced model.**

4. DISCUSSION

In our day to day life we are prone to some accidents which cause formation of wounds. To avoid the toxic effect of currently used wound healing agents, we have taken an attempt to search for better drug from plant. Therefore there is a need for prototypes, new templates to use in the design of potential wound healing agent. Traditional Indian system of medicine has many plants with versatile medicinal properties which require detail investigation for effective drug development. With the desire to find new wound healing agents and to open new areas of productivity research, we selected *Ananas comosus* peel (family bromeliaceae); a plant with established medicinal properties for testing its potential wound healing activity.

Peptic ulcer is one of the major ailments effecting humans and develops because of imbalance between aggressive factors (acid, pepsin, *H.pylori*, bile salts) and defensive factors (mucous, bicarbonates, blood flow, epithelial cell restoration and prostaglandins). There are several risk factors that may contribute to formation of ulcer in human beings such as stress, chronic use of anti-inflammatory drugs, continuous alcohol ingestion, *H.pylori* infection, Zollinger-Ellison syndrome etc. An effective anti-ulcer drug should act either by reducing the aggressive factors on gastro-duodenal mucosa or by

increasing mucosal resistance against them. The treatment of peptic ulcer is mainly aimed at reducing the hydrochloric acid secretion, increasing gastric cytoprotection, eradication of *H.pylori* or curing Zollinger Ellison syndrome. So far, several plants have been screened for anti-ulcer activity and many formulations have been developed by combining extracts of these plants. Since aspirin induces gastric ulceration by blocking prostaglandin production, in the present study treatment of *Ananas comosus* peel extracts were able to prevent formation of ulcers. It has also been reported that the presence of phyto-chemical constituent's like tannins, terpenoids, sterols and flavonoids may be responsible for anti-ulcer activity which is in agreement with our findings.

5. CONCLUSION

Wound healing is a complex phenomenon involves various phases. eg. Coagulation, inflammation, wound contraction and epithelization. While the phases between coagulation to collagenation are intimately interlinked, the phases of wound contraction and epithelization are independent to each other and run concurrently.

In the present study ethanolic extract is prepared from the peels of *Ananas comosus* and its wound healing properties were studied in-vivo models in albino rats of Wister strain. However wound healing effect of this peel has not been tested by standard methods. Our results definitely indicate that extract of peels of *Ananas comosus* is able to promote and accelerate the rate of wound healing. The conclusion come from the faster contraction of wounds treated with *Ananas comosus* peel extract compared to control and untreated wound. *Ananas comosus* peel extract was more potent than standard which is commercially available for clinical use. The course of healing by *Ananas comosus* peel extract was 9 days which is 2 days shorter than standard which is considerable advantage for this extract.

The ethanolic extract of peels of *Ananas comosus* fruit showed significant anti-ulcer activity which is evident by the data obtained. The extract having a tremendous potential deserves a special attention of the scientific fraternity to emerge as a milestone for medical science of this millennium due to its safety profile and can be a potent natural and safe alternative to conventional anti-ulcer treatment. However there is a shortage of clinical trial regarding its potency and efficacy.

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