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Evaluation Of Wound Healing and Anti-inflammatory Activities of Leaves of *Ziziphus oenoplia*

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Abstract

The wound curing activity of aqueous and alcoholic extracts of *Ziziphus oenoplia* leaves was evaluated by excision and incision wound models on rats. The study was carried out by topical application of 5% w/w ointment of aqueous and alcoholic extracts was prepared with PEG 400 and PEG 4000 as an ointment base. The evaluated parameters were rate of wound contraction, epithelialization period, and tensile strength of tissue and compared results with Neosporin ointment as standard. The results revealed a significant decrease 8 in epithelialization time, a significant increase in tensile strength in the animals treated with aqueous and alcoholic extracts of leaves of *Ziziphus oenoplia*. In addition, significant activity was found in alcoholic followed by aqueous extracts then compared to control. The study concluded that both the extracts were found to possess significant wound healing activity.

Keywords

Ziziphus oenoplia, Epithelialization, Neosporin, tensile strength, and PEG 400 and PEG 4000.

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INTRODUCTION:

India has a long history of plant-based expertise in the field of healthcare. In India, a wide variety of plants/plant extracts/decoctions/pastes are utilized for the treatment of cuts, wounds, and burns by tribes and folklore traditions in the same way as modern medicine [1].

There are a number of plants that were used traditionally and by the tribal people which have not been validated or not been evaluated, keeping the traditional claim in mind [2]. *Ziziphus oenoplia* is distributed in central and south India and almost all the three regions of Andhra Pradesh. It has a traditional claim for its wound healing activity [3].

Since there was no scientific data available and no documentation was carried out on this medicinally important plant. Pain and inflammation are interlinked with that of the wound healing activity and if the extract shows an anti-inflammatory property. So, it is worthwhile to carry out Wound healing and Anti-inflammatory activities of *Ziziphus oenoplia* leaf extracts. As a result, an effort is being made to conduct our research on the wound healing and anti-inflammatory properties of *Ziziphus oenoplia* in the current study [4].

Today, nearly 80% of the global population turns to plant-derived medicines as their first line of defense for maintaining health and combating diseases. Worldwide, one hundred nineteen secondary plant metabolites produced from plants are used as medicines. Chemical investigations have been conducted on 15 per cent of all angiosperms, with 74 per cent of the plant-derived components shown to be pharmacologically active. People in Asia

and India are now incorporating plants into their daily healthcare regimens [5, 6].

We can say for certain that the future discovery of novel therapeutic agents will only come from plants. Our dependence on plants and the knowledge of the use of plants for medicine will increase in the course of time. This increasing medicinal interest highlights the importance of proper conservation of the biodiversity and cultural diversity of the ecosystem in order to safeguard and perpetuate our interdependence of plants as a source of medicine [7, 8].

MATERIAL AND METHODS:

Collection of plant material:

Ziziphus oenoplia plant collected in Kesamudram village of Warangal District, T.S, and botanically identified and authenticated by Prof. Azmera Ragan, Department of Botany, Kakatiya University, T.S, India.

SAMPLE PREPARATION AND EXTRACTION:

Following a thorough washing with tap water, the leaves were dried under shade for about one week and then sealed to prevent deterioration. Next, the shade dried leaves were grounded made into powder. Then this leaf powder was weighed and subjected to extraction. The extraction was done by maceration method4. The extraction was done with different solvents like ethanol, water at room temperature in a glass container for three days [9].

To achieve adequate extraction, the material was agitated periodically during the process.

After three days, the contents of the container were filtered, and the filtrate was concentrated under reduced pressure below 50 until a soft mass was obtained and then preserved in a desicator [10].

Preliminary Phytochemical screening:

The amount of the different extracts was weighed, and they were submitted to preliminary phytochemical screening using industry-standard procedures [11].

Detection of Alkaloids [12, 13]:

Dragendorff's test:

Two drops of Dragendorff's reagent (Potassium bismuth iodide solution) were applied to 1 ml of test filtrate, and the formation of a noticeable reddish-brown precipitate was noticed.

Mayer's test:

1 ml of test filtrate was taken into a test tube and added two drops of Mayer's reagent (Potassium mercuric iodide solution) along the sides of the test tube and observed for white or creamy precipitate.

Wagner's test:

To 1 ml of a test, the filtrate was taken into a test tube, added two drops of Wagner's reagent (Iodine-Potassium iodide solution) along the sides of the test tube and observed for reddish-brown precipitate [14].

Hager's test:

Two drops of Hager's reagent (picric acid) were applied to 1 mL of filtrate, and the presence of a conspicuous yellow precipitate was noted.

Detection of Carbohydrates

Molisch's test:

Two drops of alcoholic solution of α– naphthol (Molisch's reagent) were added to 1 ml of the test solution. After shaking the mixture, 1 mL of concentrated H_2SO_4 was gently added from the test tube's sides. The test tube was placed in cold water and allowed to cool before being used. The test tubes were next examined for the development of a violet ring at the junction.

Fehling's test:

1 ml of test filtrate was boiled on a water bath with a mixture of 1 ml each of Fehling's solutions A and B and allowed to boil for 1min and observed for the formation of a red precipitate.

Benedict's test:

Benedict's reagent (0.5 mL) was added to 0.5 mL of filtrate. The mixture was heated for 2 minutes in a boiling water bath, and the development of a yellow, green, or red coloured precipitate was noticed.

Barfoed's test:

To 1 ml of test filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes and observed for the formation of red precipitate.

Detection of Proteins and Amino acids

Biuret test:

To 3 ml of test filtrate, two drops of 4% NaOH was added and treated with two drops of 1% $CuSO_4$ solution and observed for the Formation of pink colour.

Ninhydrin test:

Three drops of 5 percent Ninhydrin reagent were added to 3 ml of test filtrate and reheated in a boiling water bath for 10 minutes, resulting in the formation of a distinctive purple colour8.

Detection of Steroids and Terpenoids [15, 16]**:**

Salkowski test:

To the test filtrate, m of chloroform and 2 ml of concentrated sulphuric acid were added, shaken well and observed the colouration of chloroform and acid layers. The chloroform layer has a reddish-yellow fluorescence, whereas the acid layer has a greenish-yellow fluorescence.

Liebermann – Burchard's test:

2 ml acetic anhydride and 2 ml chloroform were added to the test filtrate and heated to boiling before cooling. Then, along the walls of the test tube, 1 ml of concentrated sulphuric acid was poured, and the junction was examined for the development of colour.

Detection of Phenolic compounds and Tannins [17]:

Ferric chloride test:

The test filtrates were taken and added two drops of neutral 5% ferric chloride solution and observed for blue, green or violet colour.

Lead acetate test:

The test filtrates were taken, and to this, 3 ml of 10% lead acetate solution were added and observed for the formation of bulky white precipitate.

Bromine water test:

The test filtrates were taken, and 1ml of bromine water was added and observed for discolouration of bromine water.

Detection of Glycosides:

Test for cardiac glycosides [18, 19]**:**

Legal test:

The test filtrates were taken and added few drops of pyridine and one drop of 2% sodium nitroprusside, and a drop of 20% sodium hydroxide solution was added and observed for the formation of deep red colour.

Keller - Killiani test:

The test filtrates were taken and added 2 ml of glacial acetic acid and two drops of 5% ferric chloride solution and mixed. Then one ml of sulphuric acid was added. The reddish-brown colour appears at the junction of the two liquid layers, and the upper layer appears bluish green colour.

Test for anthraquinone glycosides

Borntrager's test:

3 ml chloroform was added to 2 ml filtrate and agitated, the chloroform layer was separated, and 10 per cent ammonia solution was added, and the development of pink colour was seen.

Test for saponin glycosides:

Foam test:

Filtrates were taken, and 20 ml of distilled water was added and shaken for 15 min in a graduated cylinder and observed for the formation of a layer of stable foam.

***In - vivo* Screening:**

1. Anti-inflammatory activity

Carrageenan induced rat paw oedema

2. Wound healing activity

- **Establishment of herbal ointment**
- **Excision method**
- **Incision method**
- **Tensitometer**

Animals

The research utilised male Albino Swiss mice measuring 25-30 grammes and male Wistar rats weighing 150–200 grammes. The animals were housed in polypropylene cages in a room with temperature and humidity controls. The animals were given a normal feed and had unlimited access to clean drinking water [20]. The Vaagdevi Pharmacy College Institutional Animal Ethics Committee accepted the experimental procedure (Protocol approval number: VPC/IAEC/2017/04).

Requirements:

Plethysmometer, Tensiometer, Diclofenac sodium, carrageenan, Neosporin ointment.

Anti-inflammatory activity:

Acute inflammation:

Acute inflammation is induced in animals by the injection of an irritant in the hind paw of the rat to produce oedema. Many phlogistic (irritants) agents have been used, such as formaldehyde, dextran, egg albumin, sulfonated polysaccharides like carrageenan etc.

Many acute inflammatory models involve the injection of irritants into the hind paw of the rat to induce oedema—a famous and good irritant known as carrageenan.

Using a plethysmometer, the volume of the hind paws was measured before and after the inflammation was induced. The quantity of inflammation in untreated and test drug-treated mice was assessed. The medication is said to have anti-inflammatory action if the inflammation in treated animals is lower than in untreated animals.

The Plethysmometer technique is based on the principle of mercury displacement. It is a simple apparatus that consists of two glass arms containing mercury in one of the arms and a scale. The mercury displacement due to the dipping of the paw can be directly read from the scale attached to the mercury column. The net oedema volume can be calculated by subtracting paw volume before the induction of oedema from the paw volume after the inflammation [21, 22].

In the present investigation, the anti-inflammatory activity of the alcoholic and aqueous extract was tested by the carrageenan-induced rat paw oedema method using diclofenac sodium as standard. Adult male Wistar rats weighing 180-220 grammes were used, which are acclimatized to the laboratory conditions and maintained on standard laboratory rat feed and clean water. Rats were fasted 12 hrs prior to the experiment while allowing access to water throughout the experiment. Rats have been divided into six groups, with each group containing six animals. The animals were administered control, standard or test extracts as shown in the Table.

Table 1: Grouping of animals for anti-inflammatory activity by Carrageenan induced rat paw oedema:

Groups(n=6)	Treatment
Group I	CMC
Group II	Diclofenac sodium 50 mg/kg b.w.
Group III	Alcoholic extract 100mg/kg b.w.
Group IV	Alcoholic extract 250mg/kg b.w.
Group V	Aqueous extract 100mg/kg b.w.
Group VI	Aqueous extract 250 mg/kg b.w.

The control and standard samples were prepared in normal saline. A mark was made on the right hind paw just beyond the tibiotarsal junction so that every time the paw is dipped in the mercury column up to the marked level to ensure constant paw volume. After 1 hr of administration of the test and standard samples, 0.05 ml of 1% carrageenan suspension was injected into the dorsal region of a sub-plantar surface of the hind paw of the rat subcutaneously with the help of 26 G needle. The initial paw volume of each rat was recorded before drug administration. The paw volumes were measured at the end of 0.5, 1, 2, 3 and 4 hrs using a plethysmometer. Any change in paw volume of rats was obtained by subtracting initial paw volume from the paw volumes at different time intervals. The average value of oedema was calculated by taking the average of each group at different hours.

Percentage inhibition of oedema was calculated for each group with respect to its control group using the formula:

$$Percentage\ \ reduction = \frac{V_0 - V_t}{V_0} \times 100$$

Where, V_O = volume of the paw of control at time't'
V_t = volume of the paw of test at time't'

ESTABLISHMENT OF HERBAL OINTMENT:

Formulation of Herbal Ointment:

The present study selected water-soluble base (WSB) after performing stability studies for different ointment bases.

WSB

Polyethylene Glycol 400..... 60%
Polyethylene Glycol 4000..... 40%

Procedure for preparation:

On a heated plate, the PEG 400 and PEG 4000 were melted together. It brought the mixture up to approximately 65 degrees Celsius. Using tongs, carefully lift the mixture off the hot plate and stir it until it congealed.

Additives in Ointment:

Preservatives: Herbal ointment undergoes contamination deterioration by bacteria and fungi. To prevent this contamination of ointment base, the additions of preservatives are necessary. E.g., Methylparaben, benzalkonium chloride, tween80, benzoic acid etc.

Incorporation of extract into the ointment base:

The medicated ointment was prepared by incorporating 5% extract was prepared ointment base. The fusion method was employed in the preparation of medicated ointment. The required quantity of the ointment base was weighed and melted at a temperature of about 70oC in a water bath. The designated quantity of the extracts was added to the melted base at 40oC and stirred gently and continuously until a homogeneous dispersion was obtained.

To incorporate the extract into the bases, pulverized the extract with a mortar and pestle. Wetted the extract with a levigating agent and incorporated it into the ointment base. Generally, the amount of the extract to be incorporated into the baseless that of the amount the base. In other words, a small amount of the extract will be incorporated into a large

amount of the ointment base. To create a herbal ointment, mix properly weighed alcoholic and aqueous extracts with an ointment base to generate a smooth paste with 2 or 3 times its weight in the base; progressively incorporate an additional base until a homogeneous ointment was produced, which was then transferred to a suitable container.

Evaluation of ointment:

1. **pH:** The pH meter was calibrated using different buffer solutions; the pH measurements of formulations were done by using a digital type of pH meter by dipping the glass electrode completely into the formulation so as to cover the electrode the measured.
2. **Spread ability**: One of the criteria for the cream or ointment or gel to meet ideal qualities is that it should possess good spreadability is a term expressed to denote the extent of being to which the cream readily spreads on application to the skin or the affected part. The therapeutic efficiency of a formulation also depends upon its spreading value. Hence the determination of spreadability is very important in evaluating ointment characteristics.
3. **Penetration:** Weighed quantities of the ointments were rubbed over definite areas of the skin for a given length of time. Thereafter the unabsorbed ointment was collected from the skin and weighed. The difference between the weight roughly represents the amount absorbed**.**
4. **Irritant effects:** This test was performed to ensure the effect of ointment on the skin. The test for irritancy can be carried out on the skin and rabbits (or) the skin of human beings. Lesions on the cornea, iris, and conjunctive are used for judging the irritancy of the eyes.
5. **Stability of ointments:** The ointment should remain stable from preparation to the time when the whole of it is consumed. The formulations were subjected to stability studies for the period of 45 days with an interval of 15 days at various temperatures 40^0C, room temperature and 45^0C and observed for change in colour, odour and pH, phase separation, consistency, spreadability. In the study present, after performing different stability studies, the herbal ointment was established, and the results of different stability studies were tabulated.
6. **Wound healing activity:** Male albino rats weighing 150-180 grammes were used in both the incision and excision wound models in this research. With six animals in each group, the animals were divided into four main groups: control, standard, and test. The control group received a water-soluble base, whereas the standard group received Neosporin. The test groups were given ointments containing 5% methanol and aqueous extracts mixed into the WSB ointment base.
7. **Excision wound model:** Under moderate ether, animals in each group were anaesthetized an entire thickness of the circular excision incision (about 500mm2) and a 2mm depth on the shaved back. The day of the wounded was counted as day 0. In an open setting, rats were left naked. The areas of the wounds were promptly measured by tracing the area of the wound with a transparent polythene graph paper over it. Then, topical application of the ointments as indicated above was used to treat the wounds until they were fully healed.

Table 2: Grouping of animals for excision wound method

Groups (n=4)	Treatment
Group-I- control	ointment base 5%
Group-II- standard	Neosporin ointment
Group-III	5% Alcoholic extract ointment
Group-IV	5% Aqueous extract ointment

The wounds were monitored, and the area of the wound was measured on 4, 6, 8, 10, 12, 16 post-wounding days, and the mean % wound closure is reported.

Wound healing rate:

$$\%\ of\ \ wound\ \ closure = \frac{Wound\ \ area\ on\ day\ 0 - Wound\ \ area\ on\ day\ n}{Wound\ \ area\ on\ day\ 0} \times 100$$

Where, n =number of days 4th, 8th, 12th and 16th day. Results were tabulated in Table 9.

The period of epithelialization: The period of epithelialization was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound in the excision wound model.

8. Incision wound model:

Each set of rats was anaesthetized, and on each side of the depilated back of the rat, one para long vertebral incision was made through the skin and cutaneous muscles at a distance of approximately 1.5 cm from the midline. Throughout the trial, no full aseptic precautions were performed, and no local or systemic antimicrobials were utilized. All of the groups received the same treatment as the excision wound model. Stitching was done without the use of ligatures. The divided skin was held together and sewn with black silk at 0.5cm intervals after the incision was created, using surgical thread (No. 000) and a curved needle (No. 11). For a proper wound closure, the continuous threads on both wound edges were tightened. The wound was not treated at all.

Table 3. Grouping of animals for incision wound method

Groups(n=4)	Treatment
Group-I- control	ointment base 5%
Group-II- standard	Neosporin ointment
Group-III	5% Alcoholic extract ointment
Group-IV	5% Aqueous extract ointment

The sample extract was applied topically once daily for nine days, along with a basic ointment (control) and the usual medication; after the wounds were completely healed, the sutures were removed on the tenth day, and tensile strength was determined using a locally manufactured Tensiometer.

Tensile strength:

The tensile strength of a wound indicates how well it is healing. Wound–healing agents usually induce an increase in tensile strength. On the ninth day after wounding, the sutures were removed, and the tensile strength was evaluated on the tenth day. The mean tensile strength of the two paravertebral incisions on both sides of the animals was used to determine the wound tensile strength for each animal. Different extracts of ointment-treated wounds were compared to control groups in terms of tensile strength.

Tensiometer

In the present experiment, a local made Tensiometer was used, which consists of a wooden board to which four nails was fixed. To one end, the nail thread tied which is fixed, whereas to another end, easy movement of the thread was allowed with the help of pulley, to the edge of thread weighing balance was attached. Two clamps were tied to the thread on each side. The rats were anaesthetized individually and were placed on a wooden board between nails. The clamps were then gently applied to the skin on both sides of the incision at a distance of 0.5 cm. The weighing scale was loaded with analytical weights, which were gradually increased until the healed incision opened.

Extraction by maceration method

RESULTS AND DISCUSSION:

The extracts of *Ziziphus oenoplia* were macerated with alcohol. The percentage yield, phytochemical investigation, and anti-inflammatory and wound healing properties of the extracts are given in the tables below.

Table 4: Percentage yield of *Ziziphus oenoplia*

S.No	Extraction	Percentage yield
1	Maceration (ethanol)	3.9%
2	Maceration (water)	3.1%

Preliminary Phytochemical screening:

Table 5: Preliminary Phytochemical screening of the ethanolic extracts of *Ziziphus oenoplia*

Test for carbohydrates	Ethanol extract	Aqueous extract
Molisch's test	+ve	+ve
Fehiling's test	+ve	+ve
Barfoed's test	+ve	+ve
Test for alkaloids	Alcoholic extract	Aqueous extract

Dragendroff's test	+	_
Mayer's test	+	_
Wagner's test	+	_
Hager's test	+	_
Test for steroids and terpenoids	Alcoholic extract	Aqueous extract
Salkowski test	+	_
Libermannbechard test	+	_
Test for glycosides	Alcoholic extract	Aqueous extract
Borntrager's test	_	_
Keller killiani test	_	_
saponins test	_	_
Test for phenolics and tannins	Alcoholic extract	Aqueous extract
Ferric chloride test	+	+
Lead acetate test	+	+
Bromine water test	+	+
Test for flavonoids	Alcoholic extract	Aqueous extract
Shinoda test	+	+

When compared to the remaining extracts, the percentage yield of both alcoholic and aqueous extracts was greater. Because the extracts of Ziziphus oenoplia showed good findings for alkaloids, flavonoids, and phenolic substances in different chemical tests, both alcoholic and aqueous extracts were collected for future research.

Anti-inflammatory activity:

Table 6: Effect of Anti-inflammatory activity of *Ziziphus oenoplia* by carrageenan-induced paw oedema in rats:

Group	Mean paw volume in ml				
	0 min	1 hr	2 hr	3 hr	4 hr
Control	0.26±0.05	0.43± 0.05	0.58± 0.07	0.65± 0.05	0.68 ±0.04
Standard	0.15±0.05**	0.20±0.06***	0.31±0.07***	0.36±0.05***	0.30±0.07***
ALNG 100mg/kg	0.23±0.05	0.35±0.05	0.43±0.05**	0.50±0.08**	0.48±0.07***
ALNG 250mg/kg	0.21±0.04	0.33± 0.05*	0.41± 0.09**	0.45±0.10***	0.43±0.05***
AQNG 100mg/kg	0.20±0.06	0.30±0.06**	0.38±0.07***	0.41±0.07***	0.38±0.07***
AQNG 250mg/kg	0.16±0.05*	0.25±0.05***	0.33±0.05***	0.38±0.07***	0.33±0.09***

All values were expressed as Mean ± S.D., *p<0.05, **p<0.01 and ***p<0.001 in response to control.

Fig.1: Effect of Anti-inflammatory activity of *Ziziphus oenoplia* by carrageenan induced method

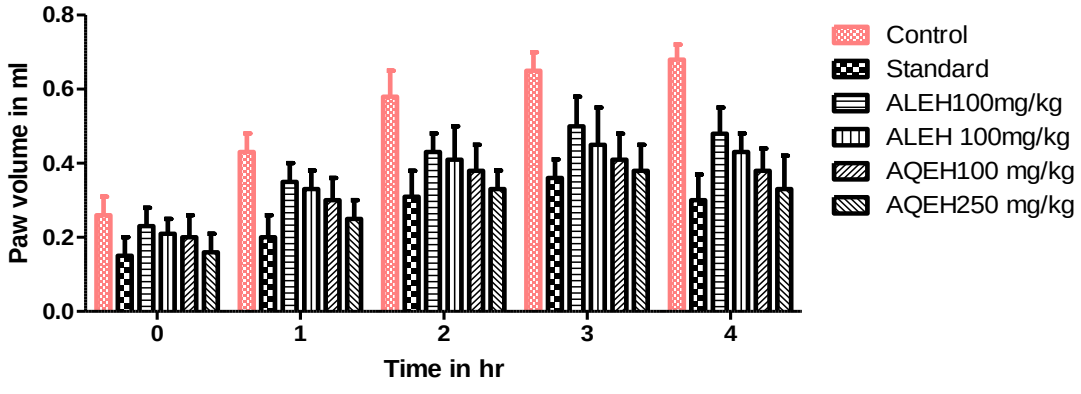


Table 7: Percentage inhibition of carrageenan-induced paw oedema in rats by *Ziziphus oenoplia*

Group	% of Inhibition paw oedema				
	0 min	1 hr	2 hr	3 hr	4 hr
Standard	42.3	53.4	46.5	44.6	55.8
Al extract 100mg/kg	11.5	18.6	25.8	23	29.4
Al extract 250mg/kg	19.2	23.09	29.3	30.7	36.7
Aq extract 100mg/kg	23.07	34.4	34.4	36.9	44.1
Aq extract 250mg/kg	38.4	41.8	43.1	39.7	51.8

The most commonly used primary test for screening novel anti-inflammatory drugs assesses a compound's ability to decrease local oedema produced in a rat paw by injecting an irritant. This oedema depends on the participation of kinins and polymorphs nuclear leukocytes with their pro-inflammatory factors, including prostaglandins. The development of oedema in the paw of the rat after the injection of carrageenan has been a biphasic event. The initial phase, observed around one hour, is attributed to the release of histamine and serotonin; the second, accelerating phase of swelling is due to the release of prostaglandin-like substances. It has been reported that the second phase of oedema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents. In the present study, significant activity was observed in the suppression of the first and second phases of carrageenin-induced inflammation may be due to inhibition of the release of the early mediators such as histamine, serotonin and kinins. The action in the second phase may be due to an inhibition of cyclooxygenase, a prostaglandin derivative. Both the extracts are alcoholic and aqueous extract showed a significant reduction of inflammation in both phases in dose-dependent manner.

Wound healing activity:

Table 8: Effect of Wound healing activity of *Ziziphus oenoplia* by Excision wound method.

Post wound day	Wound Area in mm^2 (% wound contraction)			
	Control	Standard	Alcoholic extract	Aqueous extract
0	500.76±20.86 (0)	517.48±24.64 (0)	497.31±20.44 (0)	500.76±20.86 (0)
2	455.60±14.24 (9)	406.81±24.32 (21.3)	428.58±15.57 (13.8)	398.06±29.47 (20.4)
4	395.08±28.23 (18.5)	216.35±19.04 (58.2)	236.26±16.23 (52.4)	227.18±14.38 (54.6)
6	256.41±21.95 (42.8)	133.05±14.42 (74.2)	152.45±16.20 (69.3)	125.55±17.28 (74.9)
8	249.88±11.95 (50.1)	77.85±14.81 (84.9)	96.2±87.8 (80.5)	76.65±16.65 (84.7)
10	229.30±10.06 (54.2)	43.4±6.71 (91.6)	60.28±7.18 (87.8)	35.4±9.34 (92.9)
12	207.58±13.37 (58.5)	14.33±3.01 (97.2)	26.20±6.49 (94.7)	11.81±4.34 (97.6)
14	152.35±12.68 (69.5)	5.01±2.83 (99)	14.33±3.51 (97.1)	4.65±2.64 (99)
16	131.1±7.65 (74.8)	2.75±1.59 (99.4)	5.81±2.31 (99)	1.781.4 (99.6)

Wound healing activity of *Ziziphus oenoplia* excision method

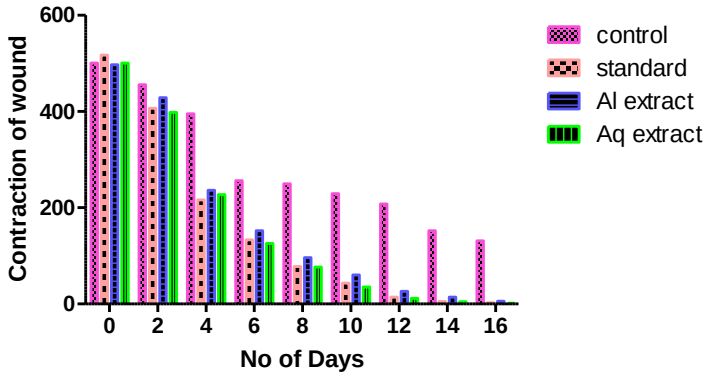
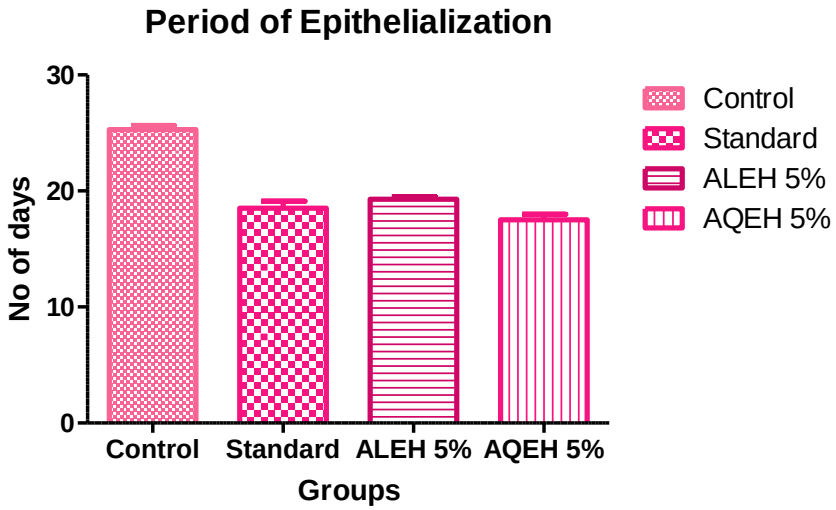


Fig. 2: Wound healing activity of *Ziziphus oenoplia* excision method:

Table 8: Effect of Period of Epithelization in excision wound model treated by *Ziziphus oenoplia* ointment

Group	Period of epithelialization (Days)
Control	25.3±0.81
Standard	18.5±1.51
5% alcoholic extract Ointment	19.3±0.51
5% aqueous extract Ointment	17.5±1.22

Fig 3: Period of epithelialization:



In order to study the wound healing abilities of *Ziziphusoenoplia*, an attempt has been made by employing the topical treatment of extracts on the excised wounds. We have clearly observed an enhanced wound contraction induced by the alcoholic and aqueous extract ointments. This could be attributed to the enhanced contraction property of myofibroblast resulting in the increase of epithelialization, especial in alcoholic extract ointment. It has been reported that topical application of compounds with free radical scavenging properties in patients has been shown to improve wound healing significantly and protect tissues from oxidative damage. The presence of phenolics also supports these results as phenolic compounds are known for their free radical scavenging property.

Table 9: Effect wound healing activity of *Ziziphus oenoplia* by Incision wound method

Group	Tensile strength (gm)
Control	349.5±14.90
Standard	697.50±14.74
5% alcoholic extract Ointment	528.33±12.11
5% aqueous extract Ointment	710.83±15.62

Ziziphus oenoplia has wound healing and anti-inflammatory properties. It was harvested, authenticated, dried, and powdered. Then they were macerated in ethanol and water to remove them. The aqueous and methanolic extracts were discovered to be rich in phytoconstituents such as alkaloids, flavonoids, and glycosides, and the percentage yield for both extracts was also relatively high. So these two extracts were chosen for further screening and total flavonoid and phenol content estimation. Carrageenan produced paw oedema in rats was used to test for anti-inflammatory efficacy. Aqueous extract at 250 mg/kg was shown to be effective in reducing inflammation. The excision and incision wound models in rats were used to screen wound healing activities. These were administered as 5% w/w topical ointments made in water-soluble base U.S.P. The wound healing efficacy of 5% aqueous extract ointment was shown to be considerable.

CONCLUSION:

In conclusion, administering aqueous extract and alcoholic extract to the experimental animals significantly affected all the treated models. As a consequence of the aforementioned findings, it can be inferred that the anti-inflammatory and wound-healing properties of aqueous extract and alcoholic extract were appropriate at a higher dosage of 250 mg/kg. The presence of phytoconstituents like flavonoids and phenolic compounds may be responsible for the above-stated activities. The possible mechanisms were also discussed in the results and discussions session. So, further investigations are needed to isolate the individual pure phytoconstituents responsible for the activities.

REFERENCES

1. Devi N. Indian tribe's and villager's health and habits: Popularity of apocynaceae plants as medicine. International Journal of Green Pharmacy (IJGP). 2017 Jul 20;11(02).
2. Pullaiah T, Rani SS, Murthy KS, Karuppusamy S. Floristic and ethnobotanical studies in andhra pradesh. Indian Ethnobotany: Emerging Trends. 2016 Jul 1:138.
3. Ghosh PK, Gaba A. Phyto-extracts in wound healing. Journal of Pharmacy & Pharmaceutical Sciences. 2013 Dec 24;16(5):760-820.
4. Singh P, Tiwari M, Yadav S, Dubey SP. Comparative Study of the roots of the plants Ziziphus oenoplia and Ziziphus jujuba. Journal of Pharmacognosy and Phytochemistry. 2014;3(1):211-7.
5. Chandukishore T, Samskrathi D, Srujana TL. Examination of in vitro wound healing antimicrobial activity of extract of selected medicinal plants. Asian J Pharm Clin Res. 2020;13(2):127-30.
6. Goyal M. Jujubes and Health Benefits: Ethnomedicinal Uses and Pharmacological Activity. InChinese Dates 2016 Jul 6 (pp. 131-143). CRC Press.
7. Chan K. Some aspects of toxic contaminants in herbal medicines. Chemosphere. 2003 Sep 1;52(9):1361-71.
8. Samy RP, Ignacimuthu S, Raja DP. Preliminary screening of ethnomedicinal plants from India. Journal of Ethnopharmacology. 1999 Aug 1;66(2):235-40.
9. Trease GE, Evans WC. Pharmacognosy13th Edition. London: Bailliere Tindall. 1989.
10. Panthong A, Norkaew P, Kanjanapothi D, Taesotikul T, Anantachoke N, Reutrakul V. Anti-inflammatory, analgesic and antipyretic activities of the extract of gamboge from Garcinia hanburyi Hook f. Journal of ethnopharmacology. 2007 May 4;111(2):335-40.
11. Salawu OA, Chindo BA, Tijani AY, Adzu B. Analgesic, anti-inflammatory, antipyretic and anti-plasmodial effects of the methanolic extract of Crossopteryx febrifuga. Journal of Medicinal Plants Research. 2013 Sep 28;2(9):213-8.
12. Methal BM. 1997. A text book of pharmaceutical formulation. vallabhprakashan, New Delhi, Pp. 236-247.
13. Werner S, Breeden M, Hübner G, Greenhalgh DG, Longaker MT. Induction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. Journal of Investigative Dermatology. 1994 Oct 1;103(4):469-73.
14. Kokane DD, More RY, Kale MB, Nehete MN, Mehendale PC, Gadgoli CH. Evaluation of wound healing activity of root of Mimosa pudica. Journal of ethnopharmacology. 2009 Jul 15;124(2):311-5.
15. Kumar MS, Kirubanandan S, Sripriya R, Sehgal PK. Triphala promotes healing of infected full-thickness dermal wound. Journal of Surgical Research. 2008 Jan 1;144(1):94-101.
16. Nayak BS, Anderson M, Pereira LP. Evaluation of wound-healing potential of Catharanthus roseus leaf

extract in rats. Fitoterapia. 2007 Dec 1;78(7-8):540-4.

17. Singh M, Govindarajan R, Nath V, Rawat AK, Mehrotra S. Antimicrobial, wound healing and antioxidant activity of Plagiochasma appendiculatum Lehm. et Lind. Journal of Ethnopharmacology. 2006 Aug 11;107(1):67-72.
18. Saha K, Mukherjee PK, Das J, Pal M, Saha BP. Wound healing activity of Leucas lavandulaefolia Rees. Journal of Ethnopharmacology. 1997 Apr 1;56(2):139-44.
19. Reddy BS, Reddy RK, Naidu VG, Madhusudhana K, Agwane SB, Ramakrishna S, Diwan PV. Evaluation of antimicrobial, antioxidant, and wound-healing potentials of Holoptelea integrifolia. Journal of Ethnopharmacology. 2008 Jan 17;115(2):249-56.
20. Ammor K, Mahjoubi F, Bousta D, Elhabbani R, Chaqroune A. In vitro litholytic activity of extracts and phenolic fractions of some medicinal plants on urinary stones. Mediterranean Journal of Chemistry. 2020 Jan 10;9(6):468-77.
21. Jisha N, Vysakh A, Vijeesh V, Latha MS. Anti-inflammatory efficacy of methanolic extract of Muntingia calabura L. leaves in Carrageenan induced paw edema model. Pathophysiology. 2019 Sep 1;26(3-4):323-30.
22. Lodhi S, Jain AP, Rai G, Yadav AK. Preliminary investigation for wound healing and anti-inflammatory effects of Bambusa vulgaris leaves in rats. Journal of Ayurveda and integrative medicine. 2016 Mar 1;7(1):14-22.