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Anti-burn Activity of *Syzygium Cumini*

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Abstract

Introduction: Burns are one of the most common and devastating forms of trauma. Burns are damage to the skin or injury to flesh caused by heat, electricity, chemicals, light, radiation or friction. **Materials:** Syzygium cumini was collected from cultivator, chopped and dried in the shade. Later was size reduced, extracted with ethanol, the extract obtained was dried and used for preclinical studies. 24 albino male Sprague Dawley rats weighing between 120-150grams were selected. **Method:** The heating nail method was used to produce burn wounds to anesthetized animals after shaving the fur. First degree burns were induced using heated nail of surface area 2cm^2 with contact time of 20sec. The nail was preheated in 100°C boiling water for 10 mins and then was applied perpendicularly to the shaved skin for a period of 20sec. After formation of burn wounds, the formulations were repeatedly applied (one application every day) to the burned areas for 10 days. The tensile strength of the wound is measured as parameter of evaluation. **Result:** Syzygium cumini was found to have antioxidant activity and antiburn activity with $P<0.05$ and rejected null hypothesis. It also showed quick burn healing, improving the tensile strength of healed wounds. This anti-burn wound healing property is due to the presence of suitable phytochemical constituents. **Conclusion:** Syzygium cumini has significant antiburn potential and antioxidant potential. Futher studies can be performed on isolating the fraction to identify the main active ingredient responsible for its potential.

Keywords
Antiburn activity, Burns, Syzygium cumini, Wound healing, Kala jamun.

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INTRODUCTION

"Burns are damage to the skin or injury to flesh caused by heat, electricity, chemicals, light, radiation or friction." [1]

Burns are one of the most common and devastating forms of trauma. The thermal injury creates a breach in the surface of the skin. Unlike most forms of trauma, burn injury is something the vast majority of the population can claim to have some experience of, even if in a very mild form. It perhaps also gives us some inclination as to the pain and suffering the involvement of larger area of skin must generate. Indeed, in the severest forms, burn injury is felt to be most severe form of trauma that is survivable. If survived, such an injury alters an individual's life in all aspects; their appearance, their ability to function independently in society and consequently their psychological wellbeing. Burns are one of the most widespread injuries in accidents and remain a global public health issue. The burn wound is a continuous and severe threat against the rest of the body due to invasion of infectious agents, antigen challenge, and repeated additional trauma caused by wound cleaning. Although many advances have been made in our understanding and care of burn injuries, there are still many burns healed with scar formation, resulting in significant aesthetic disfigurement and dysfunction.

One of the surveys conducted by the WHO reports that more than 80% of the world's population still depends upon the traditional medicines for various diseases.[2] Approximately one-third of all traditional medicines in use are for the treatment of wounds

and skin disorders, compared to only 1-3 % of modern drugs.

Thermal burns to the skin produce a remarkably different healing response due to their effects on the viability of cells and tissue. Thermal burns create an extensive zone of frank necrosis that includes dead cells and denatured connective tissue. Beyond the area of total destruction, a zone of coagulation necrosis exists, in which denaturation of plasma and cellular proteins leads to the obstruction of blood vessels and lymphatics. This effect, in turn, induces nutrient starvation of involved tissue.

MATERIALS

PLANT: Syzygium cumini plant was collected commercially from cultivator. The plant was chopped and dried in shade. It was the subjected to size reduction by mechanical grinder. The powdered material was subjected to soxhlet extraction with ethanol; the extract obtained was dried and used for preclinical studies.

CHEMICALS: All the chemicals Ethanol (China-Changshu Yanguan Chemical) and drugs Silver Sulphadiazine 1%w/w (Super Formulations Pvt. Ltd.), Xylazine (Sigma Aldrich) and Ketamine (Sigma Aldrich) used for the study were of analytical grade.

ANIMALS: Twenty-four (24) albino male Sprague Dawley rats weighing between 120-150 grams were used for the study. They were kept in polypropylene cages and allowed to acclimatize to the environment for two weeks before the commencement of the experiment. The animals were fed with standard animal pellet grower mash and allowed access to water ad libitum.

METHODOLOGY

HEATING NAIL METHOD

Adult Sprague-Dawley strain weighing 120-150g were used. They were divided into four groups of six each. The animals were fasted overnight. Anesthesia was administered on the dorsal area, with Ketamine (70mg/kg, IP) or Xylazine (5mg/kg, IP). The animals were shaved on the dorsum from neck to tail longitudinally and transversally in such a way as to spare the ventral surface of thorax and abdomen using electronic clipper. First degree burns were induced using heated nail of surface area 2cm^2 with contact time of 20sec. The nail was preheated in 100°C boiling water for 10 mins and then was applied perpendicularly to the shaved skin for a period of 20sec. (Meyer T.N. et al,1999).

After formation of burn wounds, the formulations were repeatedly applied (one application everyday) to the burned areas for 10 days. They were placed in individual cages for recovery and had free access to food and water. The experimental room was maintained between 25°C and 28°C with natural light and dark cycles. Regular observation of healing patterns are to be done from day one to ten and parameters to measure wound healing are tensile strength, percentage wound contraction and period of epithelialization.

ARRANGEMENT OF TENSIOMETER

Two stands were arranged facing opposite to each other. Thread was attached to both the stands. Stand L was kept stationery. Artery forceps was attached to stand L. Stand R was allowed to move and artery forceps was attached to it with the help of thread. On the other side of the thread a cup was hanged in which weights were placed accordingly.

PLANT SELECTION: Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. In the search of natural agents useful in the treatment of burn and to justify the use of *Syzygium cumini* seeds in the folklore system of medicine for burns, the anti-burn activity of *Syzygium cumini* seeds have been investigated. Literature review was done on the number of articles published on the anti burn activity of medicinal plants in science direct, springer link, Indian journal of pharmacology etc., more than 250 articles were found. These figures demonstrate the increased interest for this type of research among that portion of the scientific community dedicated to the investigation of the medicinal properties of plants. In the studies themselves one finds a wide range of criteria. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds such as alkaloids, flavonoids, diterpenes, triterpenes or naphthoquinones, among others. Based on these articles the plant was selected and the extraction was carried out.

HERBARIUM: The plant obtained from the cultivator was send to botanist for authentication and was identified as *Syzygium cumini*.

Fig 1.: Dried fruits of syzygium cumini

SIZE REDUCTION: The plant was procured from commercially from cultivator. The plant was chopped and dried in shade to ensure that the volatile components are not lost. It was then subjected to size reduction by mechanical grinder. The powdered material was subjected to soxhlet extraction with ethanol; the extract obtained was dried and used for preclinical studies.

Fig 2: Powder of dried fruits of syzygium cumini

HOT CONTINUOUS EXTRACTION (SOXHLET):

In this method, the finely ground crude drug was placed in a porous bag or "thimble" made of strong filter paper, which was placed in extraction chamber of the Soxhlet apparatus. The extracting solvent in flask was heated, and its vapors condensed in condenser. The condensed extract dripped into the thimble containing the crude drug, and extracted it by contact. When the level of liquid in extraction chamber rose to the top of siphon tube, the liquid contents of extraction chamber siphoned into flask. This process was continuous and was carried out until a drop of solvent from the siphon tube did not leave residue when evaporated. The advantage of this method is that large amounts of drug can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

STEPS OF EXTRACTION: The following steps were sequentially involved in the extraction procedure.

Soaking of the powdered plant materials: 50grams of the powdered drug was weighed accurately & taken in a beaker. It is moistened with a little volume of the solvent such that the entire material is uniformly moistened by the solvent.

Packing in the thimble: A thimble was prepared to accommodate the previously wetted powder. The thimble is prepared in such a way that its height is prepared in such a way that its height is about 2/3rd of the height of the body so as to ensure proper evaporation & condensation of the solvent.

Addition of solvent: The solvent was added in such a way that the entire thimble is soaked by the first cycle of solvent. Two such cycle of solvent was taken so as to ensure complete soaking of the thimble & its content.

Process of extraction: The extraction process was started & is continued till the whole of the solvent become colorless & a semi solid extract is obtained.

Fig 3: Extraction process

SOLVENT EVAPORATION:

Trace amount of the solvent may be present in the semi-solid extract obtained. This solvent remnant was removed by subjecting it to desiccation. A dessicator with activated silica gel was used for this purpose. This is known as Open-Dish Evaporation. Solvent can be evaporated by placing the solution in an open container (an Erlenmeyer, evaporating dish, beaker, vials).

The problem with open-dish evaporation is that the solvent is released into the air. Open-dish evaporation should always be done in a hood if the solvent is anything other than water. Even in a hood, however, vapors are released into air. If the solvent is a hazardous compound (for instance, methylene chloride), it is probably better to choose another method of solvent removal.

PHYTOCHEMICAL PROFILE:

Phytochemical prole of syzygium cumini is done for carbohydrates, proteins, amino acids, fats and oils, steroids, volatile oils, glycosides, alkaloids, tannins, phenolic compounds, enzymes, organic acids and vitamins.

YIELD CALCULATION:

Yield calculation is done by using the dry weight basis. When sample results are to be calculated on a dry weight basis, a second portion of sample should be weighed at the same time as the portion used for analytical determination. Immediately after weighing the sample for extraction, weigh 5-10g of the sample into a teared crucible. Dry this aliquot overnight at 105°C. Allow to cool in a desiccator before weighing. The percentage yield of the obtained extract was calculated for the sample by the following way. The empty weight of the RBF (round bottomed flask) was initially taken as W_1 gram.

The weight of the RBF + extract after desiccation was taken as W_2 gram.
The weight of the extract is hence $W_1 - W_2 = W_3$ gram.
Calculate the % dry weight as follows:

$$\%dry\ weight = \frac{wt.of\ dry\ sample}{wt.of\ sample} \times 100$$

ANTIOXIDANT ACTIVITY

Diphenyl-2-picrylhydrazyl (DPPH) scavenging effect:

Diphenyl-2-picrylhydrazyl (DPPH) forms a stable molecule on accepting an electron or a hydrogen atom and thus has applications in the determination of radical scavenging activity of natural products.

Antioxidant reacts with DPPH and converts it into 1, 1-diphenyl-2-picrylhydrazine free radical. The degree of decolourisation indicates the scavenging potential of the antioxidant drug. 0.1 mM solution of DPPH in ethanol was prepared and 2ml of this solution was added to 1ml of extract solution in ethanol at different concentrations i.e. 10, 20, 40, 80, 100µg/ml. After 20 min absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

PRECLINICAL STUDY MODEL

HEATING NAIL METHOD

Adult male Wistar or Sprague-Dawley strain weighing 120-150g were used. They were divided into four groups of six each. The animals were fasted overnight. Anaesthesia was administered on the dorsal area, with Ketamine (70mg/kg, IP) or Xylazine (5mg/kg, IP).

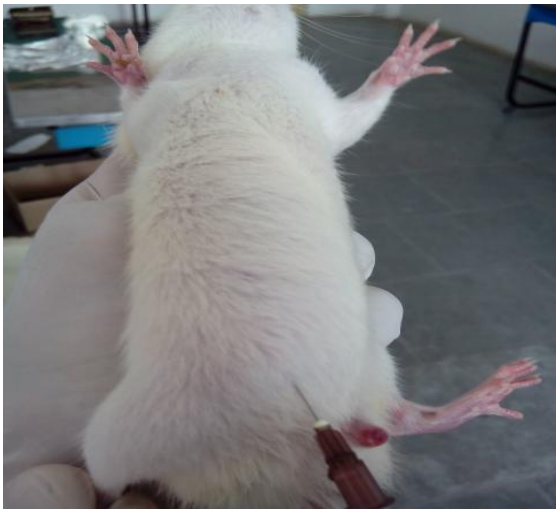


Fig 4: Administration of Anaesthesia

The animals were shaved on the dorsum from neck to tail longitudinally and transversally in such a way as to spare the ventral surface of thorax and abdomen using electronic clipper.

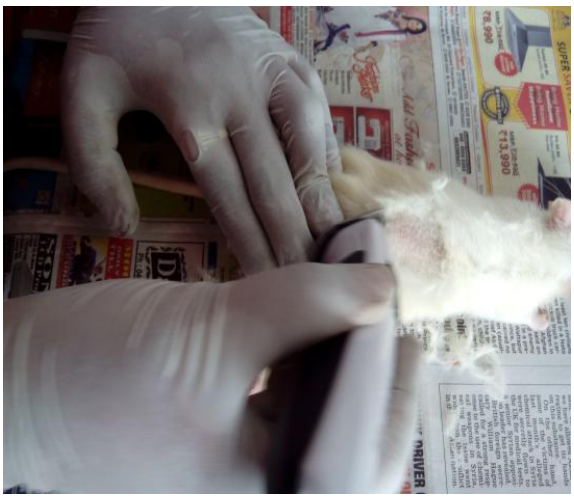


Fig 5: Shaving using electronic clipper

First degree burns were induced using heated nail of surface area 2cm^2 with contact time of 20sec. The nail was preheated in 100°C boiling water for 10mins and then was applied perpendicularly to the shaved skin for a period of 20sec.[3] After formation of burn wounds, the formulations were repeatedly applied (one application everyday) to the burned areas for 10 days.

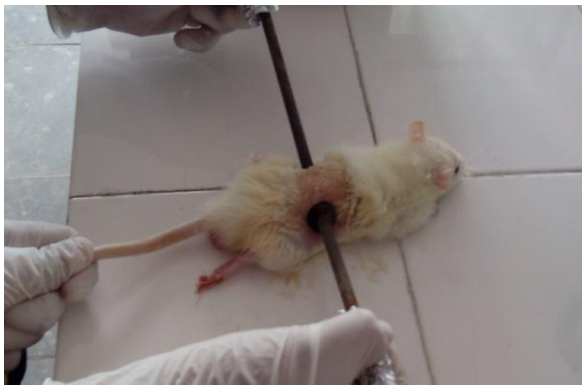


Fig 6: Induction of first-degree burn

The randomized animals are grouped and divided into:
Group A: **Untreated group**, the animal with **no burn and no vehicle.**

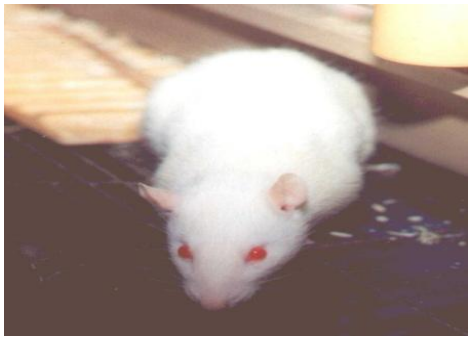


Fig 7: Healthy animal

Group B: **Control group burned** rats with **first degree burn and normal saline.**

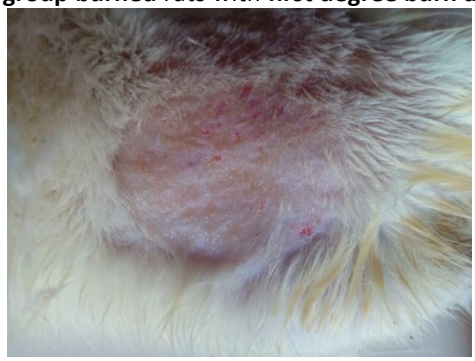


Fig 8: Untreated animal

Group C: **Standard group** rats with **first degree burn and standard drug (1% silver sulfadiazine).**

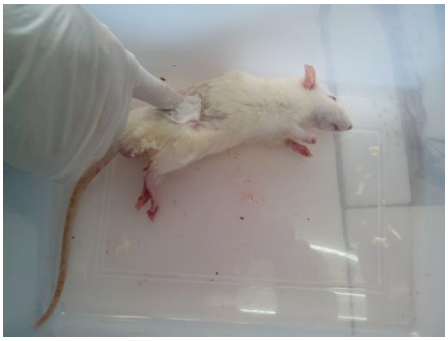


Fig 9: Treated animal

Group D: **Test group** rats with **first degree burn and test drug (ethanolic extract of *Syzygium cumini*).**

Fig 10: Extract treated animal

They were placed in individual cages for recovery and had free access to food and water. The experimental room was maintained between 25°C and 28°C with natural light and dark cycles. Regular observation of healing patterns were done from day one to ten and parameters to measure wound healing are tensile strength, percentage wound contraction and period of epithelialization.

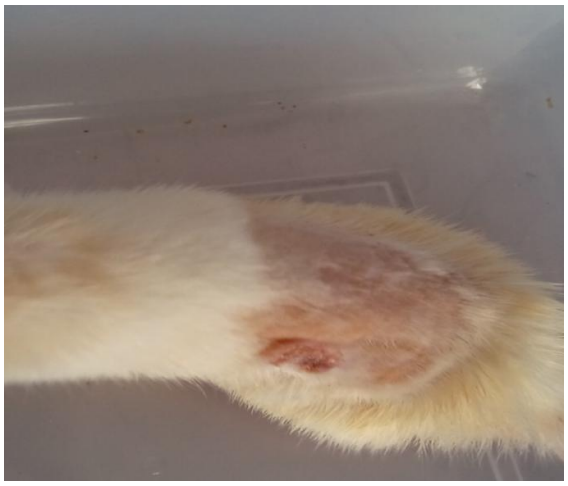


Fig 11: Induced First degree burn

MEASUREMENT OF TENSILE STRENGTH:

Two stands were arranged facing opposite to each other. Thread was attached to both the stands. Stand L was kept stationery. Artery forceps was attached to stand L. Stand R was allowed to move and artery forceps was attached to it with the help of thread. On the other side of the thread a cup was hanged in which weights were placed accordingly.[5] The arrangement was setup as shown in Fig 12.

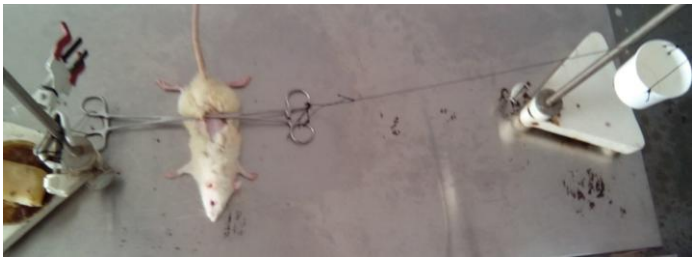


Fig 12: Tensiometer during measurement of tensile strength

Both the artery forceps were clipped to the either side of the burn wound. Weights were added in the cup until wound dehiscence takes place. These weights determine the tensile strength of the burned skin.

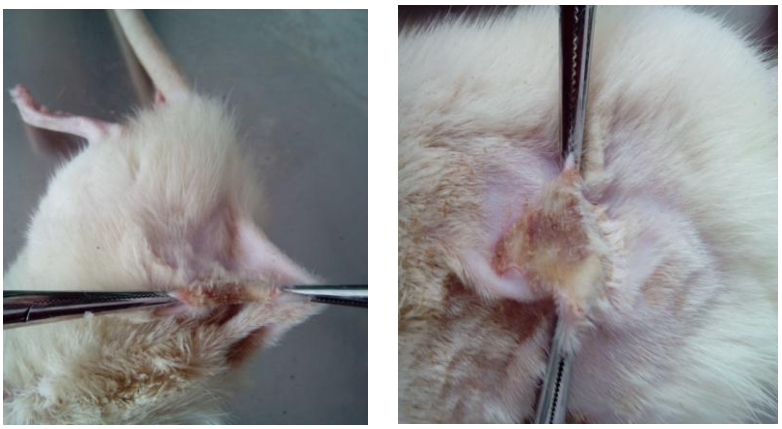


Fig 13: Dehiscence of burn wound

RESULTS

YIELD CALCULATION: Calculate the % dry weight as follows:

W_1 = 500 gms, W_2 = 40 gms

% Dry weight = $\frac{W_1 - W_2}{W_1} \times 100$

= 500-40/500 ×100

% YIELD = 92 %

PHYTOCHEMICAL EVALUATION

The preliminary phytochemical investigation of extract of *Syzygium cumini* shows presence of carbohydrates, tannins, alkaloids, saponins and flavonoids in ethanolic extract. The presence of Carbohydrates was confirmed by Molisch's test, Fehling's test & Barfoed's test; while, Alkaloids by Mayer's test, Wagner's test & Dragendroff's test. The presence of Flavonoids was confirmed by Shinoda test & Lead acetate test. While of Glycosides by Borntrager's test; Phenolics & Tannins by Bromine water test; Saponins by Foam test (reported by Khandelwal). As, ethanolic extract shows presence of most of these compounds, its extract was selected for this study (Table 1).

TABLE 1: PHYTOCHEMICAL INVESTIGATION OF SYZYGIUM CUMINI

Sr. No.	NAME OF COMPOUNDS	ETHANOLIC EXTRACT OF SYZYGIUM CUMINI
1.	Alkaloids	+++
2.	Carbohydrates	+++
3.	Glycosides	+
4.	Saponins	+
5.	Fats and Oils	+
6.	Flavonoids	+++
7.	Tannins	+++
8.	Steroids	+
9.	Proteins	+
10.	Volatile oils	-

+ = slightly present; ++ = moderately present; +++ =highly present;
- = Absent.

ANTIOXIDANT ACTIVITY

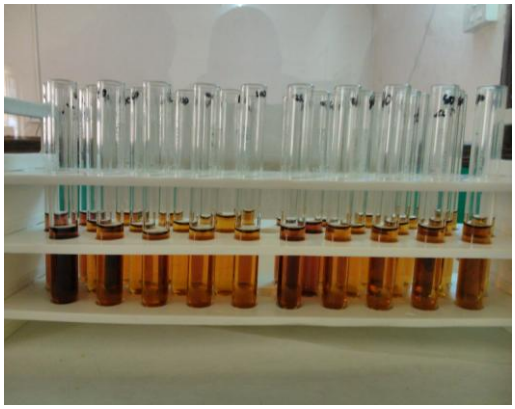


Fig 14: Discoloration DPPH with increased concentration of extract.

TABLE 2: MEASUREMENT OF ANTIOXIDANT ACTIVITY

Sr. No.	Volume(µl)	Concentration (mg)	Abs 1	Abs 2	Abs 3	Abs 4	Abs 5	Abs 6	Mean	Std. Dev.	Std Err	% Abs	% Inhibition
1	1	0.1	0.154	0.215	0.206	0.551	0.546	0.224	0.316	0.001	0.00	14.72	85.27
2	2	0.2	0.139	0.211	0.228	0.588	0.559	0.217	0.318	0.001	0.00	14.81	85.18
3	4	0.4	0.131	0.21	0.627	0.546	0.513	0.215	0.414	0.003	0.001	19.29	80.7
4	6	0.6	0.131	0.203	0.614	0.544	0.573	0.194	0.376	0.001	0.00	17.52	82.47
5	8	0.8	0.136	0.206	0.57	0.521	0.551	0.212	0.366	0.001	0.00	17.05	82.94

6	10	1	0.141	0.189	0.545	0.521	0.194	0.207	0.299	0.001	0.00	13.93	86.06
7	20	2	1.444	1.368	1.422	1.404	1.348	1.405	1.398	0.06	0.02	65.14	34.85
8	40	4	0.903	0.91	0.94	0.877	0.88	0.863	0.895	0.01	0.005	41.7	58.29
9	60	6	0.798	0.797	0.791	0.779	0.772	0.771	0.784	0.006	0.003	36.53	63.46
10	80	8	0.784	0.783	0.944	0.768	0.771	0.788	0.806	0.008	0.003	37.55	62.44
11	100	10	0.771	0.77	0.769	0.765	0.765	0.771	0.768	0.006	0.002	35.78	64.21

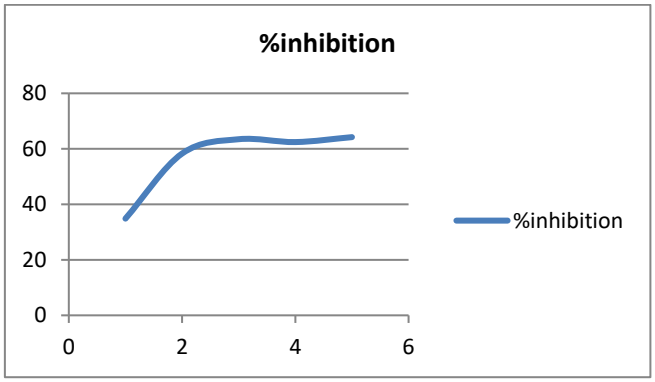


Fig 15: % Inhibition of *Syzygium cumini* extract

The IC_{50} value of the *Syzygium cumini* extract is 160µg/ml.

TABLE 3: STANDARDISATION & % INHIBITION OF ASCORBIC ACID

S.NO	CONC (µg)	MEAN ± S.D	%INHIBITION
1	1	1.226 ±0.173	42.87
2	2	1.247 ±0.178	41.89
3	4	1.638 ±0.101	23.67
4	6	1.259 ±0.196	41.33
5	8	1.191 ±0.251	44.5

IC_{50} value of the standard Ascorbic acid is 1.2µg/ml.

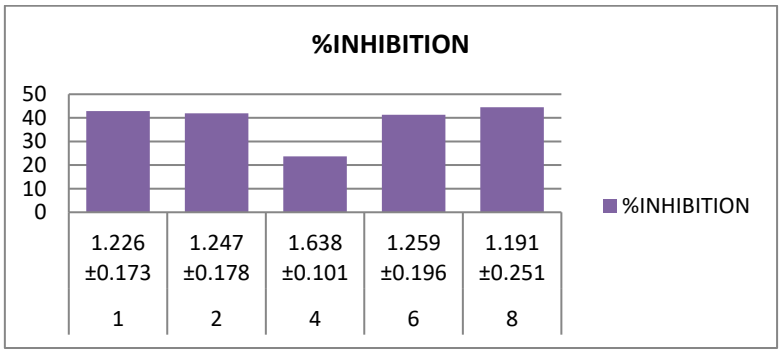


Fig 16: % Inhibition of Ascorbic acid

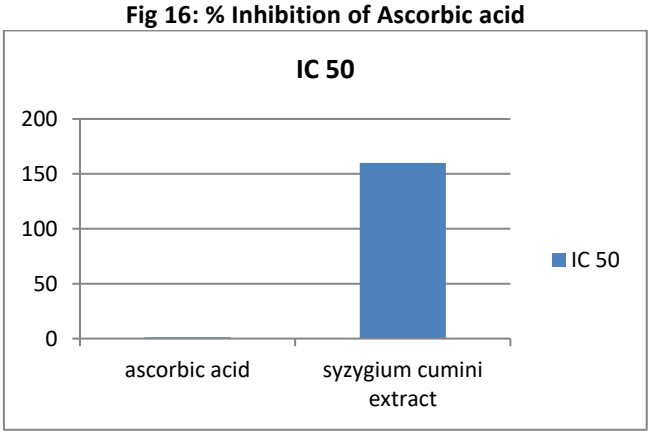
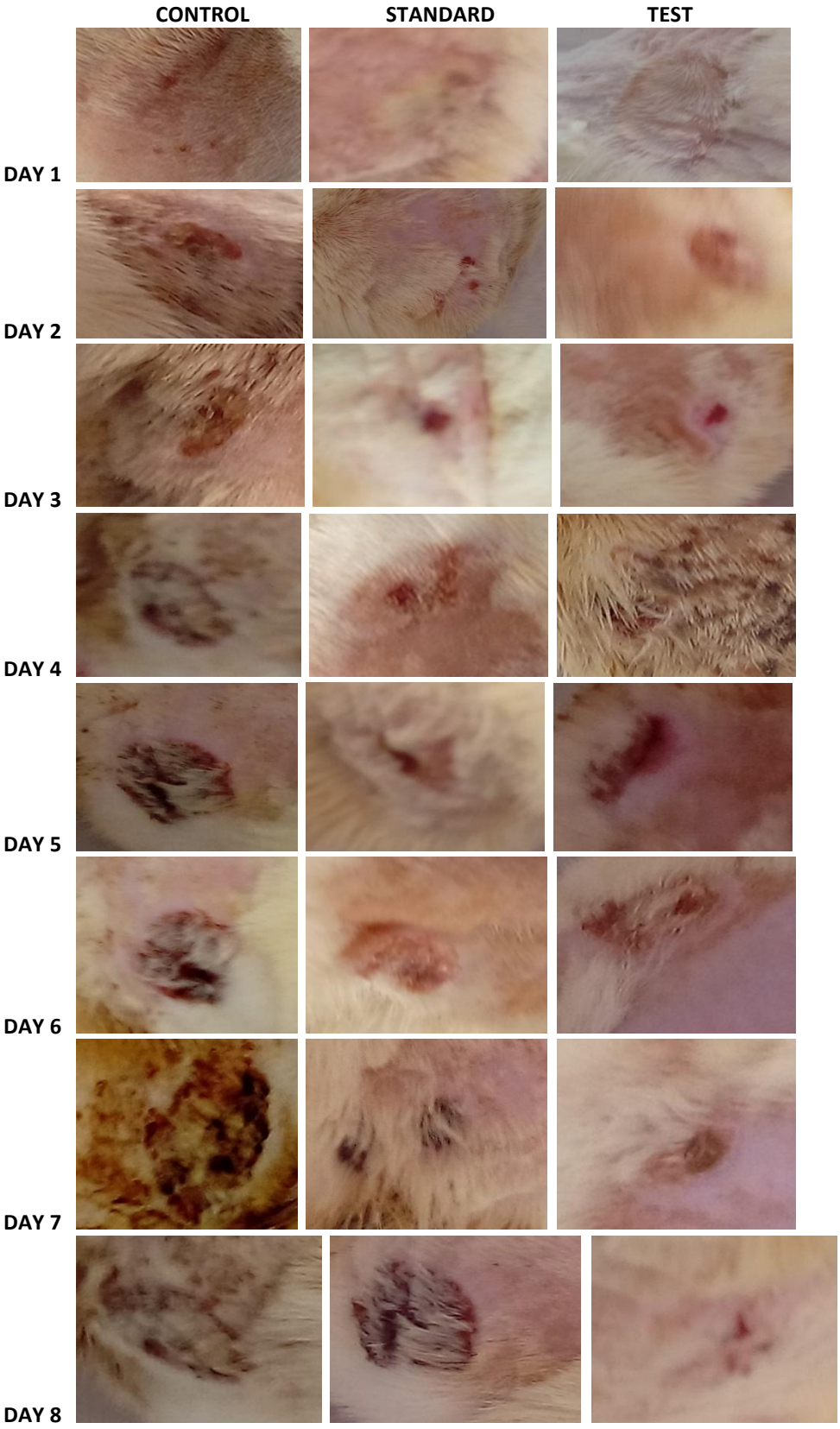


Fig 17: Comparison of IC_{50} values of Ascorbic acid and *Syzygium cumini* extract

HEALING PATTERNS



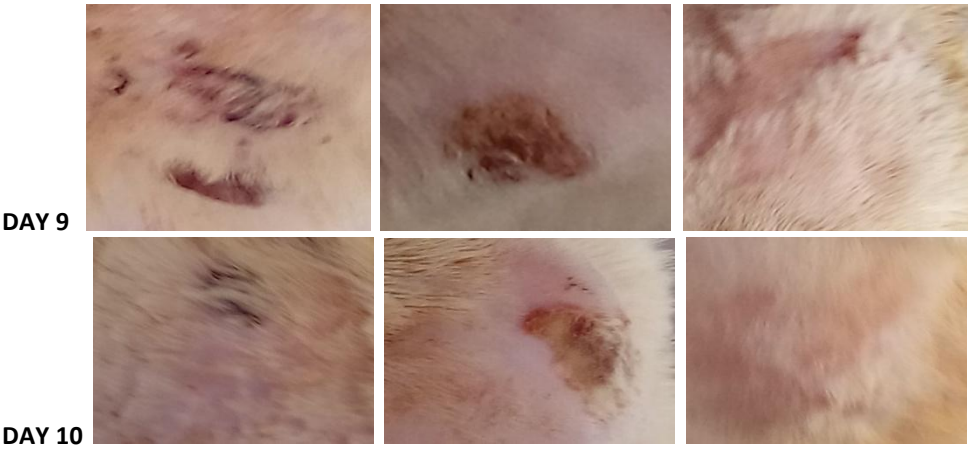


Fig 18: Healing patterns

TABLE 4: MEASUREMENT OF TENSILE STRENGTH

Sr. No.	UNTREATED (gms)	STANDARD (gms)	TEST (gms)
(1.)	230	350	230
(2.)	210	350	220
(3.)	200	350	200
(4.)	215	350	210
(5.)	220	350	200
(6.)	225	350	220

No tearing of wound was observed above 350gms.

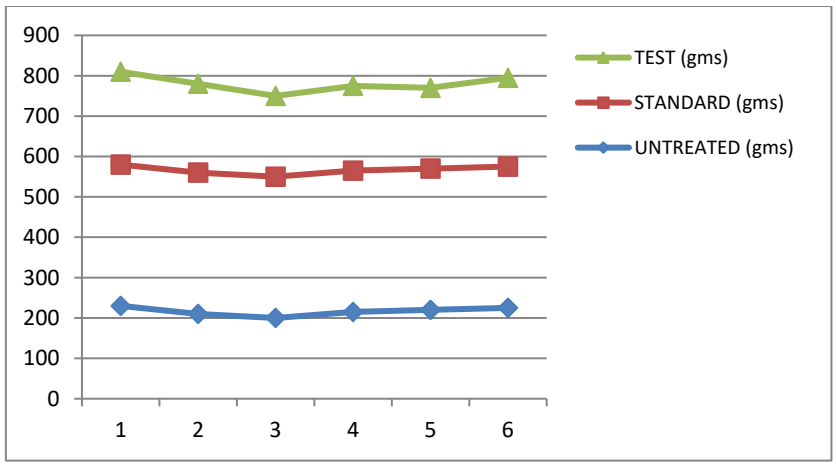


Fig 19 : Comparison of tensile strength of Test, Standard & Untreated groups

TABLE 5: SIGNIFICANT DIFFERENCES BETWEEN ALL THE GROUPS

GROUPS	N	MEAN	STANDARD DEVIATION	STANDARD ERROR
UNTREATED	6	216.67	10.801	4.410
STANDARD	6	213.33	12.111	4.944
TEST	6	350.00	0.000	0.000
TOTAL	18	260.00	66.088	15.577

There is significant difference in test, untreated and standard, but there is no difference between untreated & standard. Independent samples of distribution of tensile strength across the group using Kruskal-Wallis test was found to be 0.002 & rejected the null hypothesis. Asymptomatic significance is displayed. The significance level is P<0.05.

DISCUSSION

The ethanolic extract of *Syzygium cumini* belonging to the family Myrtaceae was found to be beneficial in treatment of first degree burns in experimental animals.

In the preliminary phytochemical evaluation of the ethanolic extract of *Syzygium cumini* dried fruits powder, showed the presence of alkaloids, phytosterol, phenolic compounds and tannins, flavanoids and lignin. Flavanoids, tannins and anthraquinones are the major phytoconstituent present in this plant which may be responsible for the burn wound healing action. The burn wound healing property of the extract of the *Syzygium cumini* dried seeds appears to be due to the presence of its active principle which accelerated the healing process and enhances tensile strength to the healed burn wound.

Thermal injury of the skin is an oxidation process, associated with biological and metabolic alterations; thermal injury generates free radicals from various cellular populations through many pathways; and the modulation of generated free radical activity with antioxidants seems to be an important part in pharmacological treatment of burns. The antioxidant activity of *Syzygium cumini* dried fruits extract was investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assays. It showed that flavonoids present in the extract of dried fruits of *Syzygium cumini* exhibited the free radical scavenging activity which might have helped in the healing of burn wounds in experimental animals. Some medicinal plants were able to accelerate the burn lesion healing rate - an effect related to these plants' containing antioxidant agents.

Heating nail method was used in induction of first-degree burns. This method is the safest method which helps producing superficial burns and avoids deeper injuries as it is heated only till 100°C. Epidermal regeneration of a wound is a complex process that the residual epithelial cells proliferate in an integrated manner to form an intact epidermis. There was significant burn wound healing observed in standard group which was treated using silver sulphadiazine, when compared to control group. The burn wound was completely healed in test group which was treated with ethanolic extract of *Syzygium cumini*, when compared to the standard group animals. Wound contraction, re-epithelialisation and hair growth was effectively observed in the test animals than control and standard groups.

There were significant differences between groups in the reduction of weight. The average weight of animal in all groups increased after the treatment period. Although, hair growth was very significant in extract treated group since day 4 and reference standard group when compared to control.

Tensile strength was measured on day 10^{th}. The test animals (extract) exhibited greater tensile strength than the standard group (silver sulphadiazine).

CONCLUSION

The present study aimed to investigate the burn wound healing potential of ethanolic extract of dried fruits of *Syzygium cumini* by using heating nail model in Sprague-dawley rats. The burn wound healing activity was conducted to evaluate the unexplored herbal plant material and to provide scientific evidence of pharmacological properties. The burn wound healing activity of the plant may be due to phytochemicals like Flavonoids, Tannins, Alkaloids, Carbohydrates and Saponins. One of these compounds may be responsible for this activity. Heating nail model is one of the simulatory modules of post-operative care which replicates the true clinical conditions. This method is highly reliable, versatile and effective.

The ethanolic extract has shown a greatly significant result when compared to control and standard. Burn wound healing properties were remarkable and highly reproducible. The tensile strength was nearly equal to healthy skin and also in comparison with the standard and control. The extract-treated wounds were found to epithelialize faster, and the rate of wound contraction was significantly increased as compared to control wounds. There was a significant increase in tensile strength, and decrease in epithelization period in extract-treated group as compared to control and standard drug-treated groups. It can be concluded from the present findings that extract of dried fruits of *Syzygium cumini* could be efficiently used in the treatment of burns.

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