Wound Medicine 28 (2020) 100176

Contents lists available at ScienceDirect

Wound Medicine

journal homepage: www.elsevier.com/locate/wndm

Evaluation of in vivo wound healing activity of *Plumeria obtusa* L. (Champa) spray in rats

Tanay Bihani[a,*], Nilesh Mhaske[b]

[a] Department of Pharmacy, Dr Vithalrao Vikhe Patil Foundation's College of Pharmacy, Vilad ghat, Ahmednagar 414001, Maharashtra, India
[b] Department of Pharmaceutics, Dr Vithalrao Vikhe Patil Foundation's College of Pharmacy, Vilad ghat, Ahmednagar 414001, Maharashtra, India

ARTICLE INFO

Keywords:
Champa
Plumeria obtusa
Wound contraction rate
Tensile strength
Spray

ABSTRACT

Wound healing is a general repair response or process of the body immediately after the disruption of the skin integrity. However wound healing is a complex process in which body repairs itself. The aim of this research is to study the wound healing activity of ethanolic extract of *Plumeria obtusa* L. leaves and to study the efficacy of formulated 2.5%, 5% and 10% *Plumeria obtusa* sprays in in-vivo wound healing models. The spray was prepared in three formulations F1, F2 and F3 containing 2.5% (50 mg/kg BW), 5% (100 mg/kg BW) and 10% (200 mg/kg BW) extract of *P. obtusa* leaves respectively. The wound healing activity was studied by invivo method. In excision model the % wound closure rate on epidermal skin of white Wistar albino rats was studied and was compared with standard Hansaplast Wound spray. The tensile strength or skin breaking strength was measured on the 10th post wounding day in anesthetized experimental rats in incision model. The results obtained by 3 formulations F1, F2 and F3 were studied and compared with the control group. The formula F3 shows complete wound healing on day 17, while the F1 and F2 showed complete healing on day 21 and 19 respectively in excision wound model. In incision wound model the tensile strength was found to be high about 404 gm by application of formula F3 spray. Formula F1 and F2 showed 354 gm and 385 gm tensile strength respectively. The conclusion is ethanolic extract of the leaves of plant *P. obtusa* showed promising wound healing activity as studied by the invivo models. The study shows that Formula F3 with 10% (200 mg/kg BW) *P. obtusa* extract spray shows better and efficient wound healing activity than formula F1 with 2.5% (50 mg/kg BW) and formula F2 with 5% (100 mg/kg BW) extract spray.

1. Introduction

Wound healing is a complex process by which the skin or other body tissues repair or heal itself after trauma. Wound healing is very orderly and highly controlled process characterised by four distinct phases as Hemostasis, Inflammation, Proliferation and Maturation or Remodeling. The repair process needs the co-ordination of various cells, growth factors and cytokines [13]. *Plumeria* belongs to the family *Apocynaceae* which consists of a wide variety of 300 Genera and about more than 1400 Species throughout the world [1]. The plant is grown for its ornamental purpose and consists of sweetly fragrant flowers. The commonly known species having medicinal importance are *Plumeria rubra* L., *Plumeria obtusa* L. and *Plumeria alba* L. [2,6]. *Plumeria obtusa* L. is commonly known as Champa (Hindi), Chaempae (Konkani), Arali (Tamil), Kathgolop (Bengali) in India and White Frangipani (Australia), Melia (Hawaii), Araliya (Sri Lanka), Temple Tree (United Kingdom), Hong Ji Dan Hua (China). It is also called as the Singapore flower or the Singapore graveyard flower [19,26]. *P. obtusa* is native to Northern Central America, Antilles and the Southeastern Mexico. They are prolific in Hawaii [19]. The flowers are white in colour and are having small brilliant yellow centre [7]. The plant can be distinguished from the other species in genus *Plumeria* as basically it is a small tree and the leaves are rounded at the tip or the end [32].

P. obtusa is used in traditional medicine as healers to diabetes mellitus. Leaves are used as purgative, treat headache, skin defects, abortifacient, healing wrap. Roots are used in treatment of skin and liver diseases, leprosy, tumours, ulcers, etc. The plant is also reported to have anticancer properties. The latex is used as diuretic and purgative. Flowers are used as ornamental purpose, perfumes, etc [1]. In Asia, decoction of leaves of the plant is used for treating wounds and skin

Abbreviations: BW, body weight; Conc, concentrated; HCl, hydrochloric acid; H_2SO_4, sulphuric acid; NaOH, sodium hydroxide; $FeCl_3$, ferric chloride; UV, ultraviolet; USP, United states Pharmacopeia; IP, Indian Pharmacopeia; OECD, Organisation for Economic Co-operation and Development; inj, injection

* Corresponding author at: 101/A, Prabhuangan, Vrundavan colony, Sarasnagar, Ahmednagar 414001, India.
E-mail address: tanaybihani1996@gmail.com (T. Bihani).

https://doi.org/10.1016/j.wndm.2019.100176
Received 3 March 2018; Received in revised form 13 September 2019; Accepted 8 November 2019
Available online 12 November 2019

diseases [4,38]. Vietnamese people believed that the plant has healing powers and also used it to treat various skin diseases. The extract of leaves are reported to show the following phytochemicals flavonoids, tannins, glycosides, cardiac glycosides, alkaloids, saponins, terpenoids, phenols, etc present [7]. Various phytochemicals have also been isolated from the different parts of the plant *P. obtusa* [23,34].

Wound dressings such as plasters and bandages protect the wound but also cause discomfort to the patient as the pain associated during dressings and removal process is a bit more. Aerosols are a new drug delivery system in wound healing which minimises the efforts and discomforts caused to the person and hence increases their acceptance and patient compliance. Sprays are easy to use and cause lesser contamination and discomfort as that of dressing [17]. The aim of this research is to study the wound healing property of *P. obtusa* leaves extract spray.

2. Material and methods

2.1. Plant material collection and identification

The fresh leaves of plant *Plumeria obtusa* L. were collected and submitted for plant authentication to the Western regional centre, Botanical Survey of India, Pune and the voucher specimen (BSI/WRC/100-1/Tech./2017/16) was deposited and preserved in the library. The fresh plant leaves collected were washed with water and then shade dried at room temperature. The dried leaves were then grinded in local grinder machine. The powder was then subjected for further process.

2.2. Preparation of extract

The extraction process was carried out by maceration technique. About 100 gm of grinded leaf powder was soaked in 600 ml of ethanol for not less than 7 days at room temperature. The mixture was shaken after every 5 hr during this period. After 7 days the powder mixture was filtered using Whatman filter paper grade 1 and extract was evaporated with rotary vacuum evaporator at 60 °C. The percentage yield of the extract was calculated and is showed in Table 1.

2.3. Phytochemical analysis

The extract of the plant leaves was studied for the presence of various phytochemical classes. For tannins, in 2 – 3 ml of plant extract ferric chloride (5%) was added and blue-black or blue-green colour shows presence of tannins. For determination of flavonoids, shinoda test was performed. In this to 0.5 gm of extract, 5 ml of 95% ethanol was added. To this, few drops of Conc. HCl and 0.5 gm of magnesium turnings were added. Orange, pink, red to purple colour shows the presence of flavonoids, dihydro-derivatives and xanthenes. Flavanols shows a deep red to magenta colour while flavanones and flavonols give weak pink to magnetic colour. The presence of alkaloids was determined by adding 1 gm of plant extract to 10 ml dilute HCl, filter and Mayer's reagent was added. Formation of precipitate shows positive test for alkaloids. Saponins were determined by the foam test & the hemolytic test. In hemolytic test the plant extract was added to one drop of blood placed on a glass slide. The appearance of hemolytic zone under microscope indicates presence of saponins present. Cardiac glycosides were determined by Keller-Killani test. In this 2 ml of extract was added to glacial acetic acid to this about one drop of 5% $FeCl_3$ and H_2SO_4 was added. Appearance of reddish-brown colour at junction of two liquid layers and the upper layer slightly bluish green in colour shows positive test for cardiac glycoside. Anthraquinones were determined by the Borntrager's test. In this 3 ml of extract was added with dilute H_2SO_4. The resulting solution was boiled and filtered and to it equal volume of benzene or chloroform was added. It was shaked well and the organic layer was separated, to it ammonia was added. Appearance of pink or red colour ammonical layer shows presence of anthraquinones. Steroids were determined by Liebermann-Burchard reaction. 2 ml of extract was added to chloroform. 1 – 2 ml acetic anhydride and 2 drops of Conc. H_2SO_4 from side of test shows positive test of steroids. For coumarins, moistened dry powder of extract was taken in test tube. The test tube was covered with filter paper soaked in dilute NaOH solution. After sometimes the filter paper was exposed to UV light, yellowish to green fluorescence indicates presence of coumarins [3,15]

Table 1
Isolation of crude extract from the leaves of *Plumeria obtusa* L. by maceration process.

Weight of powder (gm)	Solvent used for extraction (ml)	Extract obtained (gm)	% yield of crude extract
P. obtusa leaves 100 gm	Ethanol- 600 ml	13.46	13.46%

2.4. Preparation of spray

The *P. obtusa* spray was prepared in three formulations- F1, F2 and F3 containing 2.5% (50 mg/kg BW), 5% (100 mg/kg BW) and 10% (200 mg/kg BW) *P. obtusa* extract as active ingredient. The solvent used for preparation of all three sprays is ethanol 60%. In all formulations propyl paraben was added as preservative quantity sufficient and rose oil as a perfume. The formulations were then filled into suitable container and caped. No change or no reaction occurs in the formulation i.e. formulation remains stable with the excipients propyl paraben and rose oil. These excipients do not interfere with the therapeutic activity of the test *P. obtusa* sprays. The quality control parameters were studied for the following three formulations F1, F2 and F3 sprays.

2.5. Quality control evaluation for sprays

2.5.1. Delivery rate

The delivery rate of *P. obtusa* spray was evaluated according to standard procedure stated in USP [31,37]. 3 aerosol containers from each formulation F1, F2 and F3 were taken individually and the valve was pressed for 3 sec at temperature 25° ± 1 °C. The test was repeated three times and the average delivery rate was calculated in gm per second [9].

2.5.2. Delivery amount

The delivery amount was calculated according to the procedure in USP [37]. About 3 containers from each formulation F1, F2 and F3 were taken individually and the valve was pressed continuously for 4 sec at temperature 25° ± 1 °C. The initial weight and the final weight after pressing the valve were recorded. The difference or the weight loss was calculated from each container and the deliverable amount was estimated in percentage [9].

2.5.3. Minimum fill

Three filled containers from each formulation F1, F2 and F3 were selected and weighed individually. The contents were removed from each container. The packages were opened or dismantled and any residue found was removed by washing with suitable solvent, distilled water and finally with few portions of methanol. The container, the valve and all associated parts were collected and allowed to fully dry. The weight of the container together with the corresponding parts was noted and the difference between the weight of the filled and the empty container was calculated and noted as the net weight of the content [9,37].

2.5.4. Leakage test

The leakage test was conducted according to the method in IP and

Table 2
Effect of *P. obtusa* sprays on wound contraction/closure rates and epithelization period in excision wound model.

Groups	% of Wound healing				Period of Epithelization (Days)
	3rd day	7th day	11th day	16th day	
Control	14.87 ± 2.04	31.62 ± 1.89	52.16 ± 1.21	74.28 ± 1.37	21
P. obtusa 2.5% Spray	18.24 ± 1.56**	35.36 ± 2.4*	56.38 ± 2.16**	77.64 ± 2.08*	20
P. obtusa 5% Spray	23.38 ± 2.24*	41.64 ± 1.92*	62.57 ± 1.34**	82.19 ± 1.62**	19
P. obtusa 10% Spray	28.42 ± 2.17*	47.6 ± 2.06**	70.9 ± 1.57*	93.57 ± 1.46*	17
Hansaplast Wound Spray	30.23 ± 1.91*	51.05 ± 2.13**	77.66 ± 1.23*	99.33 ± 1.74**	16

Values are expressed as mean ± SE, (n = 5 animals), where * P = < 0.05 and ** P = < 0.01 when compared to control.

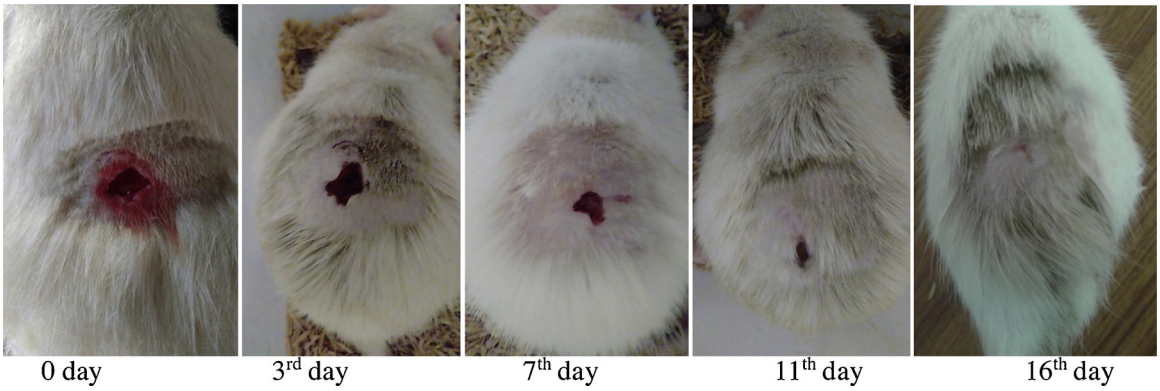

Fig. 1. Effect of 10% *P. obtusa* extract spray on the excision wound model in rats measured on 0, 3rd, 7th, 11th and 16th day.

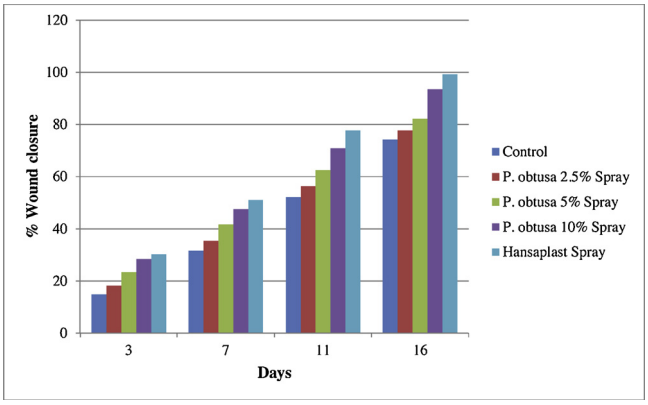

Fig. 2. Wound contraction or closure rate on 3, 7, 11 and 16 days of different groups of animals treated by the *P. obtusa* test 2.5%, 5%, 10% spray and standard Hansaplast wound spray. Values are mean of ± SE, (n = 5 animals), where *P = < 0.05 and **P = < 0.01 when compared to control.

Table 3
Effect of *P. obtusa* sprays on the tensile strength and epithelization period in incision wound model.

Group	Epithelization period (days)	Tensile strength (gm)
Control	17.12	330 ± 2.74
P. obtusa 2.5% Spray	16.23	354 ± 2.36*
P. obtusa 5% Spray	14.68	385 ± 3.8
P. obtusa 10% Spray	12.34	404 ± 3.54*
Standard	11.2	435 ± 2.14*

Values are expressed as mean ± SE, (n = 5 animals), where * P = < 0.01 when compared to control.

the USP [12,37]. Three containers were selected from each formulation F1, F2 and F3 individually and the date and time was recorded. Each container was weighed to the nearest mg and weight was recorded as W1. The containers were allowed to stand in upright position for not less than 3 days at temperature of 25° ± 2 °C and the final weight was recorded as W2. The leakage rate was calculated and expressed as mg per year for each container by the formula-

Leakage rate = 365 × 24/T × (W1 − W2),

where T = test period in hours.

2.5.5. Flammability test

The flammability test for the formulated sprays was determined from the method in Sciarra and Cutie [31] and Sanders [29]. In this if the flame projects more than 18 inch with the valve fully opened then the sample is flammable or if the flame flashes back and burns towards the valve with any degree of valve opening [9].

2.5.6. Spray pattern

The formulations were sprayed onto absorbent paper for 2 sec and the distance between the container and the paper was kept constant as 10 cm. The spray pattern obtained was recorded, which is also shown in Fig. 4 [29,31]

2.6. Toxicological study

2.6.1. Acute toxicity study

The healthy albino Wistar rats were starved for 3 − 4 hr and were subjected to acute toxicity studies as per OECD 423 guideline [28]. The rats were observed continuously for 2 hr for behavioral and neurological changes. Further no death was observed at the highest dose of 2000 mg/kg BW of the test ethanolic extract of *P. obtusa* used.

2.6.2. Acute dermal toxicity

The acute dermal toxicity study was performed for about 14 days on experimental rats which were used previously for acute toxicity study. The different formulations prepared from the *P. obtusa* plant extract F1, F2 and F3 with concentrations 2.5%, 5% and 10% respectively were tested for the dermal toxicity.

Table 4
Evaluation of Delivery rate and Delivery amount of different formulations of *P. obtusa* extracts sprays.

Formulation	Delivery rate (gm/sec)	Delivery amount (%)
F1- *P. obtusa* 2.5% extract Spray	0.19 ± 0.02	97.5 ± 0.5
F2- *P. obtusa* 5% extract Spray	0.19 ± 0.04	97.55 ± 0.5
F3- *P. obtusa* 10% extract Spray	0.20 ± 0.03	97.4 ± 1.1

Values are expressed as mean ± SE, (n = 3 containers), where P = < 0.05.

Table 5
Evaluation of Leakage and Minimum fill of different formulations of *P. obtusa* extracts sprays.

Formulation	Leakage (%/year)	Minimum Fill (%)
F1- *P. obtusa* 2.5% extract Spray	0.13 ± 0.1	100.1 ± 0.1
F2- *P. obtusa* 5% extract Spray	0.13 ± 0.1	100.12 ± 0.2
F3- *P. obtusa* 10% extract Spray	0.13 ± 0.1	100.12 ± 0.1

Values are expressed as mean ± SE, (n = 3 containers), where P = < 0.05.

2.7. Experimental animals

Swiss albino Wistar rats of either sex weighing about (200–250 gm) were used for animal studies for wound healing. The experimental protocol and procedure was approved by the Committee for the purpose of control and supervision of experiments on animals (CPCSEA) by Ref no. DrVVPF's COP/IAEC/Avish/2016/1. Animals were divided in four groups of five each. The animals were housed in proper polypropylene cages under controlled conditions of temperature (20–25 °C) and were given normal food and diet.

2.8. In vivo wound healing models

2.8.1. Excision wound model

The animal was anaesthetized by using ketamine hydrochloride inj. (10 mg/100 g BW-IP). Half an hour prior to administration of ketamine inj. hairs of animals were removed about 2 × 2 cm square from the back of rat which has been untouched before. The wound was then made on the shaved back of rat about 350 mm square and 2 mm deep [22]. The wound area was cleaned with cotton soaked in water. The animals were closely observed for any bacterial infection and if observed they were separated. The animals were divided into five groups comprising of five animals each. Group 1 was considered as Control. Group 2 was treated with Standard drug Hansaplast Wound spray. Group 3 was treated with Formula F1. Group 4 was treated with formula F2 and group 5 was treated with Formula F3. The three formulations containing *P. obtusa* extract and Standard drug i.e. Hansaplast Wound spray was applied daily on the wound area till complete healing was observed. The wound area or the contraction rate was measured on the 3, 7, 11, 16 & 17 post wounding days [24,27]. The percentage of wound contraction/closure rate was calculated by the formula- [16,36]

% of Wound closure = (Initial wound size – Specific day wound size)/ Initial wound size.

2.8.2. Incision wound model

The rats were anesthetized with ketamine hydrochloride inj. (10 mg/100 gm BW- IP). The hairs on its back were removed by electric clipper. Para vertebral incision of about 5 cm long was made on the skin of its back leaving about 1.5 cm^2 from the either side of the back of rat as described by 'Ehrlich and Hunt' [8]. The wound was then cleaned with cotton soaked in normal saline. After hemostasis wound was closed by giving intermittent sutures about 1 cm apart with help of surgical thread and curved needle no 16. Animals were divided in 5 groups consisting of 5 animals each similarly as that of the excision wound model discussed above. Formula F1, F2 and F3 were applied twice daily on the wound area for about 8 days. On the 9^{th} post wounding day all the sutures given were removed. The skin breaking strength or the tensile strength was estimated on the 10^{th} post wounding day in anaesthetized rats by the method described by 'Lee' [18]. In this a line was drawn on either side of the incision line 3 mm away from the wound. Two forceps were firmly applied on the line facing each other. One forcep was fixed and other was connected to the freely suspended lightweight polypropylene graduated container through a string run over to a pulley. Standard weights were put slowly and very carefully into the container. The point where a gradual increase in weight further pulls the wound edges apart. As and when the wound is opened up, the weight was stopped and recorded [14,16,36].

Tensile strength = Total breaking load/Cross sectional area.

3. Statistical analysis

The results of the study were reported as mean ± SEM. Statistical comparison was performed using either unpaired *t*-test or one way analysis of variance (ANOVA) followed by Dunnett's test performed for multiple comparisons. In the Study value of P < 0.05 was considered statistically significant in the experimental data analysis.

4. Result

4.1. Phytochemical analysis

The crude extract of the plant shows positive test for the presence of following phytochemical- tannins, flavonoids, alkaloids, saponins, cardiac glycosides, terpenoids and steroids. Whereas presence of coumarins and anthraquinones showed negative in the crude extract.

4.2. Toxicity study

Acute toxicity study of *Plumeria obtusa* L. with doses 250, 500, 1000 and 2000 mg kg^{-1} body weight administered via oral route showed no mortality up to 72 hr. Similarly no dermatological toxicity was observed on the application of different formulations F1, F2 and F3 of *P. obtusa* extract continuously for 14 days with concentration of 2.5%, 5% and 10% respectively.

4.3. Quality control evaluation for spray

4.3.1. Delivery rate and delivery amount

The delivery rate and the delivery amount of the *P. obtusa* extract

Table 6
Evaluation of flammability test conducted on different formulations prepared by *P. obtusa* leaf extract.

Formulation	Flame Projection (Inch)	Flame Flash back (Inch)
F1- *P. obtusa* 2.5% extract Spray	22	3
F2- *P. obtusa* 5% extract Spray	22	3
F3- *P. obtusa* 10% extract Spray	22	3

Values are expressed as mean ± SE, (n = 3 containers), where P = < 0.01.

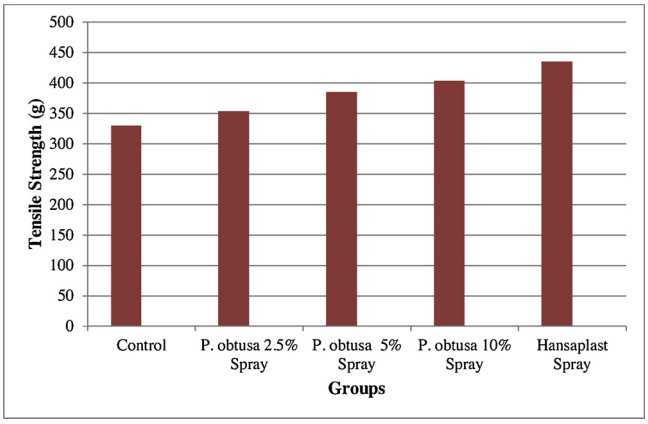

Fig. 3. Tensile strength of different groups of animals treated by the *P. obtusa* test 2.5%, 5%, 10% spray and standard Hansaplast wound spray. Values are mean of ± SE, (n = 5 animals), where * P = < 0.01 when compared to control.

spray was calculated in gm per second and percentage respectively and the results obtained are shown in Table 4.

4.3.2. Minimum fill and leakage test

The minimum fill was calculated in percentage and the leakage test was calculated percentage per year. The evaluated data is shown in the Table 5.

4.3.3. Flammability test and spray pattern

The result of flammability test is shown in Table 6. All *P. obtusa* sprays with different formulations are flammable as they contain ethanol used as a solvent. The spray patterns for different formulations are shown in the Fig. 4. All spray patterns and size are same because all formulas F1, F2 and F3 comprise of the same type of valve. The type of valve affects the spray pattern.

4.4. Normal healing

In excision wound model the control rats took about 21–22 days to heal completely. While in incision wound model the tensile strength or the skin breaking strength of the control group of rats was found to be 330gm. The control groups of the rat in both experimental studies were not provided with any treatment.

4.5. Excision wound model

The wound contraction rate and period required by different groups of experimental animals for complete epithelization of the wound by excision wound model is shown in Table 2. The wound contraction/closure rate measured was found to be 28.42, 47.6, 70.9 and 93.57 on the days 3, 7, 11 and 16 respectively by application of formula F3 and 23.38, 41.64, 62.57 and 82.19 by the application of formula F2. Whereas the wound closure rate was found to be 18.24, 35.36, 56.38 and 77.64 on days 3, 7, 11 and 16 respectively by application of formula F1 (with $p < 0.05$). The photos of the wound are shown in the Fig. 1. According to the results complete wound healing was observed on day 21 in control group. Formula F1 and F2 shows complete wound healing on the 20 and the 19 day respectively. Whereas formula F2 showed complete wound healing on day 17 and the standard drug i.e. Hansaplast Wound spray showed complete wound healing on day 16 (Fig. 2).

4.6. Incision wound model

The tensile strength of the skin in rats was measured on the 10^{th} post wounding day. The wound healing effect of *P. obtusa* sprays in incision wound model is shown in Table 3. The skin breaking strength was found to be 354 gm by application of formula F1 and 385 gm by the application of formula F2. The skin breaking strength was found high about 404 gm by the application of formula F3, where $p < 0.01$. The control group of rats left untreated showed skin breaking strength of about 330gm. The standard spray showed skin breaking strength about 435gm. Hence formula F3 showed significant skin breaking strength as compared to the results shown by control group (Fig. 3).

5. Discussion

Wound occurs when the integrity of any tissue is compromised and wound healing is a general repair response or process of the body immediately after the disruption of the skin integrity. Wound healing is a physiological process and generally does not require much help in treating if it's a small cut or small wound but wounds cause distress, a lot of pain and are susceptible to infection or may lead in formation of chronic wounds. Whereas some diseases like diabetes, ischemia, local infection, malnutrition and ageing also causes delay in wound healing process. Hence use of further wound healing modulators or agents which facilitate the healing process are indicated [20,33].

In our present study the wound healing activity of ethanolic extract of *Plumeria obtusa* L. leaves was evaluated and the wound healing potency of formula F1, F2 and F3 was compared in vivo studies by both excision and incision models. The results obtained were compared with the standard marketed Hansaplast Wound spray. Our aim was to formulate an herbal wound healing spray and evaluate its activity for checking the potency of the formulation.

The preliminary phytochemical study or analysis of the plant *P. obtusa* shows presence of flavonoids, tannins, alkaloids, phenols, saponins and terpenoids present. These phytochemicals act by different mechanisms as tannins, saponins facilitate wound healing by antimicrobial and astringent activity where as flavonoids possess potent antioxidant and free radical scavenging activity and promote wound healing activity [35]. The extract of plant leaves also possess antibacterial activity and this also in turn may facilitate the wound healing

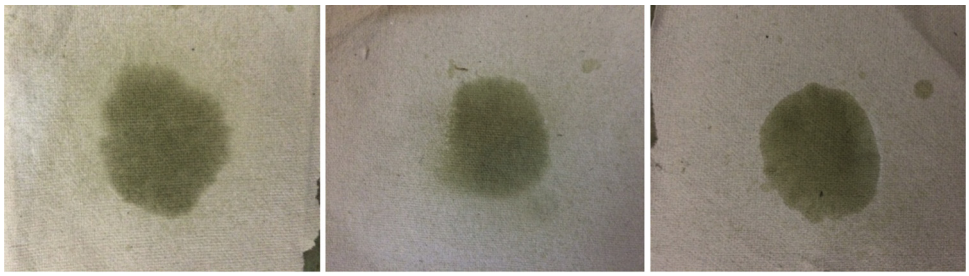

Fig. 4. Spray pattern of different formulations F1, F2 and F3 containing *P. obtusa* extract.

process [25].

6. Conclusion

The complete wound healing was observed on day 20, 19 and 17 by the application of formula F1, F2 and F3 in excision wound model. The tensile strength required for the breaking of skin in rats was also found more in formula F3 as compared to F1 and F2. So it is concluded that the ethanolic extract of leaves of *Plumeria obtusa* L. shows promising wound healing activity as studied by above invivo models and 10% (200 mg/kg BW) *P. obtusa* spray shows potent, better and faster wound healing activity as compared to 2.5% (100 mg/kg BW) and 5% (50 mg/kg BW) *P. obtusa* extract sprays. Hence *P. obtusa* spray can provide a safe and effective application to reduce the patient suffering and improve quality of life.

Funding source

This research did not receive any specific grant from funding agencies in public, commercial, or not-for-profit-sectors.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Acknowledgements

The authors are thankful to the Dr Vithalrao Vikhe Patil Foundation's College of Pharmacy, Vilad Ghat, Ahmednagar for providing all the necessary facilities, chemicals and support to carry out this research work.

References

[1] N. Ali, M. Junaid, D. Ahmad, M. UrRahman, G. Katzenmeier, Antibacterial and antifungal activity of solvent extracts from *Plumeria obtusa* Linn, Trop. Biomed. 31 (2014) 607–615.
[2] All Things Frangipani, About Frangipanis, (2016) http://www.allthingsfrangipani.com/frangipanis.html.
[3] B.A. Baviskar, S.S. Khabadi, S.L. Deore, Experimental Phytopharmacognosy: A Comprehensive Guide, second ed., Nirali Prakashan, India, 2013.
[4] I.H. Burkill, A Dictionary of the Economic Products of the Malay Peninsula vol. 2, Crown Agents for the colonies, London, 1935.
[6] M. Choudhary, V. Kumar, S. Singh, Phytochemical & pharmacological activity of genus *Plumeria*: an updated review, Int. J. Biomed. Adv. Res. 5 (2014) 266–271.
[7] Devprakash, R. Tembare, S. Gurav, S.G.P. Kumar, T.T. Mani, An review of phytochemical constituents & pharmacological activity of *Plumeria* species, Int. J. Curr. Pharm. Res. 4 (2012) 1–6.
[8] H.P. Ehrlich, T.K. Hunt, Effects of cortisone and vitamin A on wound healing, Ann. Surg. 167 (1968) 324–328.
[9] Febriyenti, A.M. Noor, S.B. Bai, Physical evaluations of Haruan spray for wound dressing and wound healing, Int. J. Drug Deliv. Technol. 3 (2011) 115–124.
[12] IP, Inhalation Preparations, Indian Pharmacopoeia vol. 2, The Indian Pharmacopeia Commission, Ghaziabad, 2007, pp. 20–30.
[13] C.W.T. Jacqueline, M.L. Kit, L.L. Cheuk, H.T. Ming, F.K. Hin, K.L. Knok, P.L. Ching, H.K. Khun, C.L. Ping, P.F. Kwok, B.S.L. Clara, The in vivo and in vitro diabetic wound healing effects of a 2-herb formula and its mechanisms of action, J. Ethnopharmacol. 134 (2011) 831–838.
[14] R.K. Jha, N. Garud, R.K. Nema, Excision & incision wound healing activity of flower head alcoholic extract of *Sphaeranthus indicus* Linn. in albino rats, Glob. J. Pharmacol. 3 (2009) 32–37.
[15] K.R. Khandelwal, Practical Pharmacognosy, Techniques and Experiments, nineteenth ed., Nirali Prakashan, Pune, 2008, pp. 149–156.
[16] D. Kumarasamyraja, M. Swamivelmamickam, Evaluation of in vivo and in vitro wound healing activity of aqueous extract of *Acalypha indica*, Int. Res. J. Pharm. Pharmacol. 6 (2015) 57–61.
[17] L. Laila, Febriyenti, S.M. Salhimi, S. Baie, Wound healing effect of Haruan (*Channa striatus*) spray, Int. Wound J. (2011) 1–8.
[18] K.H. Lee, Studies on mechanism of action of salicylates II, retardation of wound healing by aspirin, J. Pharm. Sci. 57 (1968) 1042–1043.
[19] T.K. Lim, Edible Medicinal and Non Medicinal Plants, Vol. 7- Flowers, Springs, (2014), pp. 87–93.
[20] P. Mallefet, A.C. Dweck, Mechanisms involved in wound healing- focusing on injuries, Biomed. Sci. (2008) 609–614.
[22] J.J. Morton, M.H. Malone, Evaluation of vulnerary activity by an open wound procedure in rats, Arch. Int. Pharmacodyn. Ther. 196 (1972) 117–126.
[23] S. Muhammad, A. Nasim, R. Naheed, A. Muhammad Shaiq, J. Abdul, Isolation and characterization of Secondary metabolites from *Plumeria obtusa*, J. Asian Nat. Prod. Res. 13 (2011) 1122–1127.
[24] P.K. Mukherjee, R. Verpoorte, B. Suresh, Evaluation of in-vivo wound healing activity of *Hypericum patulum* (family: Hypericaceae) leaf extract on different wound model in rats, J. Ethnopharmacol. 70 (2000) 315–321.
[25] S. Murthy, M.K. Gautam, S. Goel, V. Purohit, H. Sharma, R.K. Goel, Evaluation of in vivo wound healing activity of *Bacopa monniera* on different wound model in rats, Biomed Res. Int. (2013) 1–9.
[26] P. Narwariya, J. Nabi, Lalit, Preeti, Comprehensive overview of *Plumeria obtusa*, World J. Pharm. Res. 6 (2017) 664–676.
[27] S. Nayak, P. Nalabothu, S. Sandiford, V. Bhogadi, A. Adogwa, Evaluation of wound healing activity of *Allamanda cathartica* L. and *Laurus nobilis* L. extract on rats, BMC Complement. Altern. Med. 6 (2006) 12.
[28] OECD, Guidance for Testing of Chemicals, Acute Oral Toxicity-Acute Toxic Class Method. 423, (2001), p. 17.
[29] P.A. Sanders, Handbook of Aerosol Technology, second ed., Van Nostrand Reinhold Company, New York, 1979.
[31] J.J. Sciarra, A.J. Cutie, Pharmaceutical aerosols, in: L. Lachman, H.A. Lieberman, J.L. Kanig (Eds.), The Theory and Practice of Industrial Pharmacy, third ed. Indian ed., Varghese Publishing House, Bombay, 1986, pp. 589–618.
[32] P.R. Shinde, P.S. Patil, U.A. Bairagi, Phytopharmacological review of *Plumeria* species, Scholars Acad. J. Pharm. 3 (2014) 217–227.
[33] A. Shukla, A.M. Rasik, G.K. Jain, R. Shankar, D.K. Kulshrestha, B.N. Dhawan, In vitro and in vivo wound healing activity of asiaticoside isolated from *Centella asiatica*, J. Ethnopharmacol. 65 (1999) 1–11.
[34] B.S. Siddiqui, I. Firdous, M. Rasheed, S. Begum, Chemical constituents of leaves and stem bark of *Plumeria obtusa*, Phytochemistry 65 (2004) 2077–2084.
[35] H. Soni, A.K. Singhai, A recent update of botanicals for wound healing activity, Int. Res. J. Pharm. Pharmacol. 3 (2012) 1–7.
[36] M. Subalakshmi, A. Saranya, M.U. Maheswari, A. Jarina, S. Kavimani, R. Murali, An overview of the current methodologies used fort the evaluation of drugs having wound healing activity, Int. J. Exp. Pharmacol. 4 (2014) 127–131.
[37] USP, Physical Test and Determination, United States Pharmacopoeia, United states Pharmacopeial Convention, Rockville, 2000, pp. 1895–1896 1959.
[38] S.K. Wong, Y.Y. Lim, N.R. Abdullah, F.J. Nordin, Antiproliferative and phytochemical analyses of leaf extracts of ten *Apocynaceae* species, Pharmacogn. Res. 3 (2011) 100–106.