

## Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

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4**Pharmacological screening of *Annona reticulata* Linn. Stem bark extracts for wound healing activity**Fulchand V. Kajale<sup>1\*</sup>, Shagufta A. Farooqui<sup>1</sup>, Vaishnavi V. Khandre<sup>1</sup>, Avinash. S. Musale<sup>1</sup>, Shrinivas K. Sarje<sup>2</sup>

Department of Pharmacology, Nanded Pharmacy College, Nanded, Maharashtra-431605, India.

Received: 18.07.2023

Revised: 02.09.2024

Accepted: 10.09.2024

Published: 30.09.2024

**ABSTRACT: Background:** By traditionally plant *Annona reticulata* L. used for the treatment of epilepsy, dysentery, cardiac problem, parasite and worm infestations, constipation, haemorrhage, bacterial infection, dysuria, temperature, ulcer, and as insect repellent. **Aim:** The aim of the current study was to invent out the wound healing effect of Ethyl acetate and Methanol extracts of *A. reticulata* L. (stem bark). **Method:** The wound healing activity of *A. reticulata* L. stem bark extracts were estimated by using *in vivo* excision wound model (in rats) by using Soframycin as a standard drug marketed preparation (group III), various concentration of ointment was prepared (1 and 2% w/w) of each extract (group IV, V, VI, VII) positive control, negative control respectively (group I, II) (n=6). The outcome of healing was calculated by measuring area or by measuring; the length of wound and value compared using software. **Results:** At the end of activity, the test group shows activity similar to standard. Test group V i.e., AR-EAE 2% w/w, showed highly significant difference in wound area when compared with positive control group I, test group V showed that there is no significant difference in wound area when compared with the standard. On comparison of wound area at 21<sup>st</sup> day test group V 2% w/w AR-EAE (0.21±0.05), nearly similar to standard (0.11±0.07). **Conclusion:** Above results of the study concludes that the ointment of plant extract has potential to heal the wound. Furthermore, study can reveal the exact molecular mechanisms, biochemical parameters included in it.

**Corresponding author:**

Mr. Fulchand V. Kajale  
Assistant Professor  
Nanded Pharmacy College,  
Nanded, Maharashtra-431605, India.  
Tel: +91-7066575242  
E. Mail ID: [fulchandk22699@gmail.com](mailto:fulchandk22699@gmail.com)

**INTRODUCTION:**

A wound is defined as the damage or breaking of the cellular and structural or functional durability of living tissue <sup>[1]</sup>. It is classified into four main phases: hemostasis inflammation, proliferation and remodelling <sup>[2]</sup>. The First phase haemostasis occurs instantly after injury <sup>[3]</sup>. To prevent exsanguination, vasoconstriction arises and platelets start, to the adhesion and build-up at the site of injury <sup>[4]</sup>. Next is the inflammatory reaction then by the proliferative phase, which is characterized by angiogenesis, collagen formation, granulation tissue formation, epithelialization and wound contraction <sup>[5]</sup>. The outer cellular matrix and collagen are remodelled in

**Keywords:** *Annona reticulata* L., Ethyl acetate, Methanol extract, ointment, excision wound model.

the ending phase that gives integrity and strength to the healed tissue. The proliferative phase is containing angiogenesis, collagen synthesis epithelialization and wound contraction <sup>[6]</sup>. Angiogenesis contains formation of the new blood vessel growth from endothelial cells. Then epithelial cells crawl across the wound and start to cover it <sup>[7]</sup>. The wound's contraction is done by myofibroblasts, which are contractile web-like fusiforms, which grip to the wound edges and undergo contraction by using a mechanism similar to that of smooth muscle <sup>[8]</sup>. Healing is a complex process that involves a series of biological or chemical and cellular reactions started in response to an injury that retains the function and integrity of injured tissues. Wound healing contains platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, after that angiogenesis and re-epithelization of injured tissue <sup>[9]</sup>. *A. reticulata* Linn is one of the traditionally important for the treatment of various diseases. It belongs to the family *Annonaceae* <sup>[10]</sup>. Leaves cover a wide range of chemicals like kaurenoic acid, amino acids, carbohydrates, alkaloids, steroids, proteins, flavonoids, tannins, glycosides and phenolic compounds <sup>[11]</sup>. The root of this plant contains flavonoids acetogenin, carbohydrates, alkaloid proteins, and tannins. By tradition the plant was used for the treatment of epilepsy, dysentery, cardiac problem, parasite and worm infestations, constipation, hemorrhage, bacterial infection, dysuria, temperature, ulcer and as insect repellent. Bark is astringent and used as a stimulant where leaves are used for helminthiasis treatment <sup>[12]</sup>. The height of *A. reticulata* L. is near about 8 to 10 m <sup>[13]</sup>. It comprises many twigs. It is a small tree with glabrous twigs. Leaves are oblong, lanceolate, membranous, and rounded at the tip. The superior surface of leaves is smooth and the inferior surface encompasses few spreading hairs. About 2 to 4 flowers may be present on the side of the pedicel. Fruit is slightly heart shaped, rough and yellowish in colour which changes into yellowish red on maturation. Fruit is slightly sour, and useful in blood complaints. Seeds are plain and blackish in colour <sup>[14]</sup>. The antioxidant property of this plant is due to polyphenols and flavonoids <sup>[15]</sup>. This plant was also rich in Calcium (Ca), Phosphorus (P), K, Mg, Na, Chlorine (Cl), Sulphur (S), Manganese (Mn), Zinc (Zn), Fe, Cu, Selenium (Se), Cobalt (Co), Nickel (Ni), and Chromium (Cr) <sup>[16]</sup>. This plant has the phytoconstituents like acetogenins anonaine, oxoushinsunine, reticuline, whereas the stem bark can contain anonaine,

oxoushinsunine, liriodenine reticuline, michelalbine, asimilobine, anomontine, etc. Leaves can contain elemol,  $\alpha$ -eudesmol,  $\alpha$ -cadinol, etc. The seed contains fatty oils and n-fatty acyl tryptamines <sup>[17]</sup>. For present study we selected the plant and literature survey revealed this plant has a variety of phytochemicals, which is useful to treatment of wounds, after preparation of extract confirmatory tests was performed. For topical application, ointment of various concentrations was prepared (1 and 2% w/w). *In vivo* model for study i.e., excision wound model was done on rat, results were expressed in standard error of mean (S.E.M).

## MATERIAL AND METHODS:

### Collection and authentication plant material:

The fresh stem bark of *A. reticulata* L. was collected from the local region of Bhusni Maharashtra. This plant (specimen No. H-2) was identified and authenticated by Dr. Shirang S. Bodke, Associate Professor and Head of Botany and Horticulture, Yashwant Mahavidyalaya, Nanded. The fresh stem barks of plant *A. reticulata* L. were kept to shade drying and further crushed to coarse powder, and then the powder passed through mesh No. 14 and stored in airtight flask for further use.

### Preparation of Extracts:

Dried powder of stem bark of *A. reticulata* L. was extracted with petroleum ether, ethyl acetate, and methanol (successive solvent extraction) by Soxhlet extractor apparatus according to the standard method until colorless solution was observed in siphon tube. About 270 g of the powdered plant and 1500 ml Solvents were used for extraction. Later completion of extraction extract was cooled and evaporated by using a super fit rotary evaporator to get the concentrated extract. The extracts were stored in airtight flasks for avoiding contamination and the percentage of yield of extracts was calculated.

### Preparation of Ointment Base:

The composition of ointment base was wool fat (5 g), hard paraffin (5 g), cetostearyl alcohol (5 g), and white soft paraffin (85 g). All above chemicals were mixed gently according to their melting point in a hot water bath. After complete mixing of all chemicals, the solution was cooled and stored into a glass bottle for further use. About 100 g ointment base was prepared.

### Preparation of Ethyl acetate and Methanol extract ointment:

About 1 and 2 g of Ethyl acetate and Methanolic stem bark extract of *A. reticulata* L., were mixed with 99 and

98 g of ointment base respectively to prepare the 1 and 2 % w/w concentrated ointment <sup>[18]</sup>.

#### **Selection and procurement of animals:**

The experiment was performed with the approval of Institutional Animal Ethics Committee (IAEC) following guidelines of CPCSEA.

#### **Animals used**

The male and female Wistar rats with an approximate weight of 150 to 250 g body weight were selected for study.

#### **Housing facilities:**

The animals were selected for experiments maintained with standard procedure of laboratory condition in the animal house of Nanded Pharmacy College approved by the committee for control and supervision on experiments on animals (CCSEA). All animals were placed in 12 h light /dark cycle with maintained temperature condition (22±2 °C), fed with standard pellet diet and water *ad libitum*. All animals for experiment were placed in the maintained animal house of college for at least 1 week before the start of procedure. The experimental procedure for the study was surveyed according to the standards of the Institutional Animal Ethics Committee (IAEC).

#### **Acute toxicity Study:**

For the present study of calculation of safe dose of *A. reticulata* L. was done by referring standard references. Many researchers carried out an acute oral toxicity study of selected plant material and its extracts <sup>[19]</sup>. This method was allowing a judgment with respect to categorizing the test substance to one of a series of toxicity classes defined by fixed LD<sub>50</sub> cut-off values. Researchers evaluated for acute toxicity of plants as per OECD guideline No. 423 (Acute oral toxicity-class method) were considered for calculating experimental safe dose <sup>[20]</sup>.

#### **Acute Dermal Irritation:**

Acute dermal irritation test was carried out on Wistar rats. The ointment of *A. reticulata* L. ethyl acetate, and methanol extracts at various concentrations was applied topically. The animals were observed for any cases of mortality, skin irritation or any toxic effect. Animals were observed for any swelling or oedema or any other local damage to skin. The study was conducted according to the Organization for Economic Cooperation and Development (OECD) guidelines 404 <sup>[21]</sup>.

#### **Study of Wound Healing Activity:**

##### **Excision Wound Model:**

The Excision wound model was the 21 days study <sup>[22]</sup>. Male and female Wistar rats (200 to 250 g) of approximately two months of age were used as experimental animals. Animals were divided into Seven groups of six rats in each group i.e., Positive control, Negative control, Standard (Soframycin) <sup>[23]</sup>, and four extract treated groups (Higher 2 % and lower 1 %) <sup>[24]</sup>. The animals were housed in standard environmental conditions of temperature, humidity, 12 h light/dark cycle. During the experiment the rats were administered a standard pellet diet and water *ad libitum*. The animals were anesthetized by using ketamine (10 mg/kg) <sup>[25]</sup> and with the help of Gillette Guard shaving razor hairs on the back thoracic region of rats were removed <sup>[26]</sup>. The Excision wound 5 cm away from the ear, a circular wound with around 2 cm<sup>2</sup> area and 2 mm depth were created <sup>[27]</sup>. The animals were placed in separate cages. The entire wound was left open. All animals were treated with topical application of ointment. The wound area was measured by scale, and observed day-to-day progress for all groups until completely healing of the wound <sup>[28]</sup>.

#### **RESULTS:**

##### **Wound Healing Activity by Excision wound model:**

The groups were compared using one-way analysis of variance (ANOVA), P < 0.05 was considered significant difference when standard, test compared with positive control in Excision wound model, and P < 0.001 was considered highly significant difference when test compared with positive control in Excision wound model.

On 7<sup>th</sup> day, the group of animals treated with standard ointment i.e., Soframycin cream showed significant decrease in wound area when compared with the positive control group. The test group i.e., AR-EAE 1 %, AR-EAE 2 %, AR-ME 1 % and AR-ME 2 % showed no significant decrease (P < 0.05) in wound area when compared with the positive control group. Test group showed no significant decrease in wound area as compared with the standard.

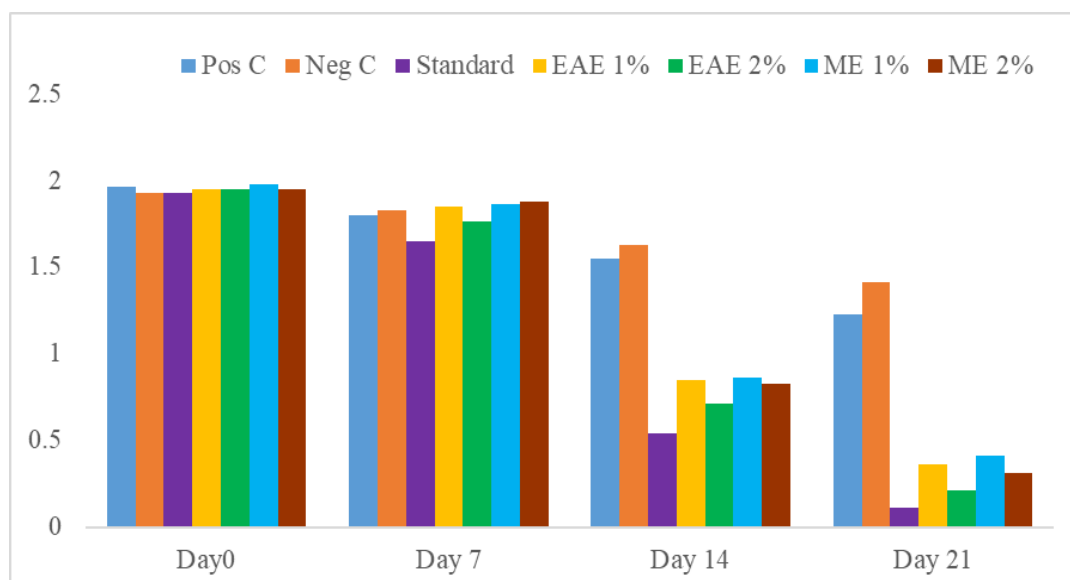
On 14<sup>th</sup> day, the group of animals treated with standard ointment i.e., Soframycin showed significant decrease in wound area when compared with the positive control group. The test group i.e., AR-EAE 1 %, AR-EAE 2 %, AR-ME 1 % and AR-ME 2 % showed significant decrease (P < 0.05) in wound area when compared with

**Table 1. Evaluation of wound healing activity of AR stem bark extracts.**

Groups / Days	Area of wound in cm <sup>2</sup> (X±SD)			
	Day 0	Day 7	Day 14	Day 21
Group I Positive control	1.96±0.04	1.80±0.02	1.55±0.05	1.23±0.05
Group II Negative control	1.93±0.04	1.83±0.02	1.63±0.05	1.41±0.07
Group III Standard	1.93±0.02	1.65±0.02**	0.54±0.05**	0.11±0.07**
Group IV AR EAE 1%	1.95±0.04	1.85±0.02*	0.85±0.05**	0.36±0.05**
Group V AR EAE 2%	1.95±0.04	1.76±0.03*	0.71±0.07**#	0.21±0.05**#
Group VI AR ME 1%	1.98±0.06	1.86±0.03*	0.86±0.05**	0.41±0.07**
Group VII AR ME 2%	1.95±0.04	1.88±0.03*	0.83±0.05**	0.31±0.07**

Each value represents the mean ± S. E. M. (n=6).

\*Significant difference when test & standard compared with positive control (P < 0.05). \*\* Highly significant difference when test compared with positive control (P < 0.001). # Non-significant difference when test compared with standard. Δ Significant difference when test compared with standard but more effective than standard. Data obtained was presented as means ± standard error of mean (S.E.M) for the number of animals in each group (n=6).



**Fig 1. Evaluation Wound Healing Activity of *Annona reticulata* L.**

the positive control group. Test group showed no significant change in wound area when compared with the standard.

On 21<sup>st</sup> day, the standard treated group i.e., Soframycin ointment showed a highly significant decrease in wound area when compared with the positive control group. The test group i.e., AR-EAE 1 %, AR-EAE 2 %, AR-ME 1 % and AR-ME 2 % showed highly significant decrease (P< 0.001) in wound area when compared with the positive control group. Test group showed no significant change in wound area when compared with the standard.

From above observation, it was found that the *A. reticulata* L. extracts exhibit significant wound healing activity and AR-EAE 2 %, and AR-ME 2 % showed highly significant decrease wound area when compared

with positive control group and significant difference when compared with standard but no more effect than standard.

#### DISCUSSION:

The plant was recognized as one of the sources of the medicinal products which are helpful to humans. A variety of plants shows various pharmacological activities. Extracts obtained from various parts of plants have various therapeutic properties. They are also used for preparations of herbal products which do not show any side effects as compared to synthetic drugs. The regular use of the synthetic drugs results in addiction or toxic effects, thus it is safer to use natural medicines than the synthetic drugs. The present study was an attempt to provide traditional claims about the plant *A. reticulata* L.


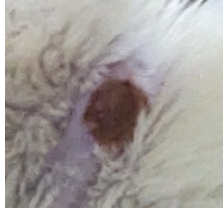

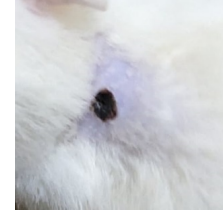


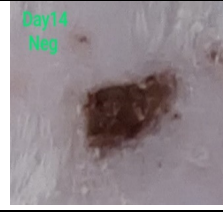







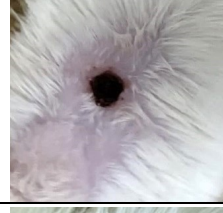
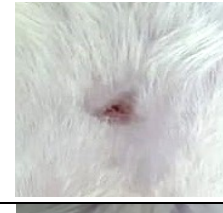



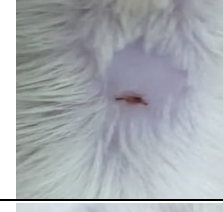


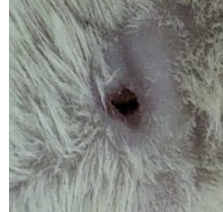




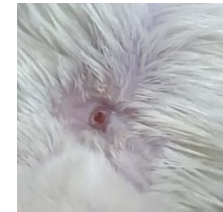
Groups / Days	Day 0	Day 7	Day 14	Day 21
<b>Group I Positive control</b>	 Day 0 Pos			
<b>Group II Negative control</b>			 Day14 Neg	
<b>Group III Standard</b>	 Day 0 std	 Day 7 Std		
<b>Group IV AR EAE 1%</b>				
<b>Group V AR EAE 2%</b>				
<b>Group VI AR ME 1%</b>				
<b>Group VII AR ME 2%</b>				

Fig 2. Wound Healing Activity of *Annona reticulata* L. in Wistar rat.

mentioned in Ayurved and evaluation for its wound healing activities. Acute toxicity study goals at establishing the therapeutic index. Extracts were found safe up to 1000 mg/kg. The method used to check the wound healing potential of *A. reticulata* L. stem bark extracts was excision wound model on albino rats as animal model. The study was done for 21 days and the parameter screened was wound area in cm<sup>2</sup>. In this study, all the extracts of *A. reticulata* L. were found to show promising wound healing effects. On 21-day AR-EAE 2 % showed a highly significant decrease in wound area when compared with positive control. and significant differences when compared with standard.

#### CONCLUSIONS:

The results of study showed that the wound healing activity of *A. reticulata* L. by using Excision wound model in rats. Rats in (IV to VII) group were treated with AR ethyl acetate and AR methanol extracts with the dose of 1 and 2 % ointment Soframycin as standard. In this study, all the extracts of *A. reticulata* L., were found to show promising wound healing effects. However, Ethyl acetate extract shows more effect than methanol extract. Therefore, it was concluded that the *A. reticulata* L. stem bark extract could be one of the herbal remedies for wounds.

#### ACKNOWLEDGEMENT:

Authors wish to thanks to Authority of Nanded Pharmacy College, Nanded, for providing the Laboratory facility for the completion of this research work.

#### REFERENCES:

1. Minakshi C, Mengi S. Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in rats. *Phytother Res*, 2006; 20(9): 799-805.
2. Wilkinson HN, *et al.* Wound healing: Cellular mechanisms and pathological outcomes. *Open Biol*, 2020; 10(9): 200-223.
3. Schultz GS, Chin GA, Moldawer L, *et al.* Principles of Wound Healing. In: FitrIDGE R, Thompson M, editors. *Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists* [Internet]. Adelaide (AU): University of Adelaide Press; 2011.
4. Esimone CO, Nworu CS, Jackson CL. Cutaneous wound healing activity of a herbal ointment containing the leaf extract of *Jatropha curcas* L.

- (Euphorbiaceae). *Int J Appl Res Nat Prod*, 2008; 1(4): 1-4.
5. Nayak BS, Raju SS, Chalapathi Rao A V. Wound healing activity of *Persea americana* (avocado) fruit: a preclinical study on rats. *J Wound Care*, 2008; 17(3): 123-125.
6. Kumar GV, Paliwal SK. Wound-healing activity of ethanolic and aqueous extracts of *Ficus benghalensis*. *J Adv Pharm Technol Res*, 2011; 2(2): 110.
7. Zaal AA, Oran SA, Shakhaneh JM. Effect of *Rhus coriaria* L. methanolic fruit extract on wound healing in diabetic and non-diabetic rats. *J Cosmet Dermatol*, 2022; 21(8): 3567-3577.
8. Abraham F, *et al.* Evaluation of *in vivo* wound healing activity of methanol extract of *Achyranthes aspera* L. *J Ethnopharmacol*, 2012; 143(2): 469-474.
9. Katole R, Gautam J, Mokate D. Phytochemical study of *Annona squamosa* L. and *Annona reticulata* L. *Int J Res*, 2018; 5(12): 156-175.
10. Prathapa RM, *et al.* Pharmacognostical evaluation on the Medicinal & Nutritive fruits of Raamaphal *Annona reticulata* L. *J Pharmacogn Phytochem*, 2015; 4(1): 21-28.
11. Yadav Kashikant. *et al.* Evaluation of anti-ulcer and analgesic activity of methanolic extract of leaves of *Annona reticulata*. *World J Pharm Res*, 2019; 8(6): 981-1004.
12. Bharadwaj R, Haloi J, Medhi S. Topical delivery of methanolic root extract of *Annona reticulata* against skin cancer. *South Afr J Bot*, 2019; 124: 484-493.
13. Khatun, Mst Rokeya, *et al.* Allelopathic activity of *Annona reticulata* L. leaf extracts and identification of three allelopathic compounds for the development of natural herbicides. *Agronomy*, 2022; 12(11): 2883.
14. Jamkhande, Prasad G. *et al.* *Annona reticulata* Linn. (Bullock's heart): Plant profile, phytochemistry and pharmacological properties. *J Trad Complement Med*, 2015; 5(3): 144-152.
15. Subba B, Aryal P. Study of biological activity and chemical constituent of *Annona reticulata*. *J Institute Sci Technol*, 2016; 21(1): 157-163.
16. Chowdhury SS, Tareq AM, Tareq SM. *et al.* Screening of antidiabetic and antioxidant potential along with phytochemicals of *Annona* genus: a review. *Future J Pharm Sci*, 2021; 7: 1-10.
17. Manoj Kumar S, Azamthulla M, Saravanan KS. Pharmacognostical evaluation and anti-convulsant

- property of *Annona reticulata* Linn. (*Annonaceae*) root. *Futur J Pharm Sci*, 2021; 7(1): 173.
18. Ingole Nishikant A, *et al.* Study of wound healing activity of *Parthenium hysterophorus* by excision wound model in rats. *Int J Med Res Professionals*, 2018; 4(2): 308-313.
  19. Vineela P, Josthna P, Naidu CV. Ameliorative effect of *Annona reticulata* L. leaf extract on antihyperglycemic activity and its hepato-renal protective potential in streptozotocin induced diabetic rats. *J Ayurveda Integr Med*, 2021; 12(3): 415-426.
  20. The Organization of Economic Co-Operation and Development (OECD). Paris, France: The OECD Guideline for Testing of Chemicals: 423-Acute Oral Toxicity; 2001.
  21. The Organization of Economic Co-Operation and Development (OECD). Paris, France: The OECD Guideline for Testing of Chemicals: 404-Acute Dermal Irritation/Corrosion; 2002.
  22. Ibrahim M, *et al.* Ointment-based combination of *Dittrichia viscosa* L. and *Marrubium vulgare* L. accelerate burn wound healing. *Pharmaceuticals*, 2022; 15(3): 289.
  23. Kumar PS, Srivastava S, Rao CV. Wound healing activity of ethanolic extract of *Selaginella bryopteris* on rats. *Pharmacogn J*, 2020; 12(2): 335-341.
  24. Sheeba, M, *et al.* Wound healing activity of *Cassia occidentalis* L. in albino Wistar rats. *Int J Integr Biol*, 2009; 8(1): 1-6.
  25. Farooqui SA, Patil SS, Tekale SA, Gunjkar VN. Evaluation of wound healing activity of flavonoids from *Ipomoea carnea* Jacq. *Anc Sci Life*, 2007; 26(3): 45.
  26. Quazi A, Patwekar M, , Patwekar F, Mezni A, Ahmad I, Islam, F. Evaluation of wound healing activity (excision wound model) of ointment prepared from infusion extract of polyherbal tea bag formulation in diabetes-induced rats. *J Evid Based Complementary Altern Med*, 2022; 2022: 1372199.
  27. Zeng Q, Qian Y, Huang Y, Ding F, Qi X, Shen J. Polydopamine nanoparticle-dotted food gum hydrogel with excellent antibacterial activity and rapid shape adaptability for accelerated bacteria-infected wound healing. *Bioact Mater*, 2021; 6(9): 2647-2657.
  28. Boudjelal A, Smeriglio A, Ginestra G, Denaro M, Trombetta D. Phytochemical profile, safety

assessment and wound healing activity of *Artemisia absinthium* L. *Plants (Base)*, 2020; 9(12): 1744.

**Conflict of Interest:** None

**Source of Funding:** Nil

**Paper Citation:** Kajale FV\*, Farooqui SA, Khandre VV, Musale AS, Sarje SK. Pharmacological screening of *Annona reticulata* Linn. Stem bark extracts for wound healing activity. *J Pharm Adv Res*, 2024; 7(9): 2403-2409.