JOURNAL OF PHARMACOLOGY AND BIOMEDICINE
E-ISSN: 2456-8244
www.rbscience.co.in

Evaluation of wound healing action of Annona squamosa bark extract

Priyanka Ojha*, Sandeep Jain

IPS College of Pharmacy, Gwalior, Madhya Pradesh, India

*Corresponding Author

Email ID – priyankajh658@gmail.com

Abstract

Successive solvent extraction of the defatted bark of *Annona squamosa* was perfomed using soxhlet method and the extraction abilities of different solvents for recovering extractable components from leaves followed the order: ethanol>ethylacetate>water. The findings of preliminary phytochemical screening suggest the presence of alkaloids, saponins, phenolics, tannins, terpenoids, sterols, and flavonoids in the bark of the plant. The acute toxicity study reveals a LD_{50} of more than 2000 mg/Kg for the phytochemical rich ethanolic extract of the bark of AS. The ethanolic extract of *Annona squamosa* bark was evaluated for the *in vivo* wound healing effect by the excision model. The topical application of 1 and 2 % w/w of the *Annona squamosa* extract resulted in an accelerated wound healing capability. The plant extract (ASBE 2%) exhibited 86.15 % contraction of wound on the 15th day which was more than the povidone iodine used as the standard that exhibited 78.1% contraction of the wound. The control animals with ointment base were found to contraction 69.42 % while ASBE 1% healed 78.1% of wound by the 15th day.

Keywords: *Annona squamosa*, wound, bark extract, phytochemical, phenolics, extraction

Received 12/08/2021; Revised 22/08/2021; Accepted 23/08/2021

Scan QR Code to visit Website

Introduction

According to the World Health Organization (WHO), about 80% of people in the developing countries rely on traditional medicine, for which the demand is increasing dramatically (Lakshmi et al., 2012). Thus, a wide attention has been made on plants in recent years, in order to explore the active principles, which are contributing to their pharmacological effects. Several plant secondary metabolites such as carotenoids, tocopherol and polyphenolic compounds have been suggested to serve as alternative agents to treat various diseases that are caused by oxidative stress (Gulcin, 2012; Shabbir et al., 2013).

Annona squamosa is a popular tree that is widely cultivated for its edible fruit. The species has been found to contain uvaricin, 4-deoxyannoreticuin, (2,4-cis and trans)- squamoxinone, squamostanin-C, squamostanin-D, Rollicosin and Squamostolide. Annona has been associated with various pharmacological activities like antihyperthyroidic, analgesis, anti-inflammatory, antiulcer, antioxidant, antilipidemic, antibacterial, cytotoxic, hypoglycemic, vasorelaxant, hepatoprotective, larvicidal, insecticidal and anthelmintic (Gajalakshmi et al., 2011).

The literature has specified the antioxidant potential of root extracts of Annona squamosa. Oxidation has been linked inflammation and wound and anti oxidant molecules could be beneficial in initiating wound healing. The objective of the present investigation was to evaluate the wound healing ability of the bark of *Annona squamosa*.

Material and Methods

The chemical and reagent used in the present study were procured from various scientific suppliers and were used as obtained without any further purification. The instruments used were available in the laboratory of the institution and were used without calibration.

Collection and authentication of plant material

The bark of *Annona squamosa* (AS) was collected from the surrounding regions of Gwalior, Madhya Pradesh in the month of March**.** The authenticated bark was dried in shade and coarsely powdered for use.

Solvent extraction of phytoconstituents

Powdered bark of AS (750 g) was defatted with hexane at room temperature for 24 h. The marc was dried and packed in the extractor of the soxhlet apparatus and extracted successively with ethyl acetate and methanol by hot continuous extraction process for about 72 h. The aqueous extraction was performed by cold maceration process after completion of the solvent extraction process. The extracts were filtered and the solvents were evaporated on water bath. The extracts obtained were collected and placed in desiccator to get rid of the excess moisture content**.** The dried extracts were stored in desiccator for phytochemical screening and pharmacological evaluation.

Phytochemical Screening (Singh and Singh, 2017)

In order to determine the type of phytoconstitutents present all the extracts were evaluated by phytochemical qualitative reactions. The screening was performed for triterpenes/steroids, proteins, coumarins, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color cahnge or the precipitate formation was used as analytical responses to these tests. A small quantity of the extracts were dissolved in 5 mL of distilled water and filtered. The filtrate was tested to detect the presence of various phytochemical constituents in the sample.

Table 1 Preliminary phytochemical testing

Phytoconstituents	Test	Observation
Tannins (Braymer's Test)	2ml extract + 2ml water + 2-3 drops Ferric chloride (5%)	Green precipitate
Flavonoids	1ml extract + 1ml lead acetate (10%)	Yellow coloration
Terpenoids	2ml extract + 2ml acetic anhydride+ 2-3 drops conc. sulfuric acid	Deep red coloration
Steroids (Salkowski Test)	2ml extract + 2ml chloroform + 2ml sulfuric acid (conc.)	Reddish brown ring at the junction
Phlobatannins (Precipitate Test)	2ml extract + 2ml Hydrochloric acid (1% v/v) + heat	Red precipitate
Carbohydrates (Molisch's Test)	2ml extract + 10ml water + 2 drops Ethanolic α-naphthol (20%) +2ml sulfuric acid (conc.)	Reddish violet ring at the junction
Saponins (Foam Test)	5ml extract + 5ml water + heat	Froth appears
	5ml extract + Olive oil (few drops)	Emulsion forms
Glycosides (Liebermann's Test)	2ml extract + 2ml chloroform + 2ml acetic acid	Violet to Blue to Green coloration
Coumarins	2ml extract + 3ml NaOH (10%)	Yellow coloration
Alkaloids (Hager's Test)	2ml extract + few drops of Hager's reagent	Yellow precipitate
Proteins (Xanthoprotei c Test)	1ml extract + 1ml sulfuric acid (conc.)	White precipitate
Emodins	2ml extract + 2ml ammonium hydroxide + 3ml Benzene	Red coloration
Anthraquinones (Borntrager's Test)	3ml extract + 3ml Benzene + 5ml ammonia (10%)	Pink, Violet or Red coloration in ammonical layer
Anthocyanins	2ml extract + 2ml hydrochloric acid (2N) + ammonia	Pinkish red to bluish violet coloration
Leucoanthocyanins	5ml extract + 5ml Isoamyl alcohol	Organic layer into Red

Pharmacological Evaluation

Animals

Male Wistar rats (8-10 month old) weighing 180-250g were used for the study of wound repair activity. The animals were housed in cages (6 per cage) during the course of experimental period and maintained at 12 h light and dark cycle with a temperature [17-26°C] maintained throughout the study. The animals were fed with standard rodent pellet feed and water *ad libitum*. The animals were fasted 12 hours before the experiment with free access to only water.

Acute Toxicity Study

A total of three animals were used which received a single oral dose (2000mg/kg) of the ethanolic extract of *Annona squamosa*. Animals were observed for the first 30 min after dosing; at regular intervals during the first 24 h and daily thereafter for duration of 14

days. The observations were made for changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, perspiration, urinary incontinence, and defecation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was also observed over the duration of observation.

Preparation of test samples and standard drugs

The test samples were prepared by formulating the dried ethanol free plant extract in simple ointment base (cetostearyl alcohol, liquid paraffin, yellow soft paraffin, and hard paraffin) as a 1% and 2% w/w ointment. Commercially available Povidone Iodine Ointment (5 %) was used as standard drug for comparison of action.

Preparation of simple ointment base (Gaur et al., 2009)

The hard paraffin (50g) and cetostearyl alcohol (5ml) were taken in a china dish and allowed to melt in a hot water bath. Yellow soft paraffin (5g) and liquid paraffin (2g) were then incorporated to this mixture. The mixture was stirred until all the ingredients were melted completely, followed by cooling. The ointment prepared was mixed with the ethanolic extract of AS bark at 1% and 2% w/w concentration.

Evaluation of wound healing by in vivo excision model

Experiment Design

The animals were divided into 4 groups, each consisting of 6 rats. Wound was made in the animals of all groups. Group 1 served as control, group 2 served as standard, to which povidone iodine ointment was applied topically, groups 3 and 4 served as test, to which extract-ointments (1% and 2% w/w) were applied topically.

Table 2 Experimental design for excision model

Group	Nomenclature	Treament
Group I	Vehicle Control	Simple ointment base
Group II	Standard	Povidone iodine ointment (5% w/w)
Group III	Test 1	AS bark extract (1% w/w)
Group IV	Test 2	AS bark extract (2% w/w)

All the test samples; vehicle and standard drug samples were applied topically on the wound of each of the animals daily, under sterile conditions.

Creation of excision wound (Samanta et al., 2016)

Excision wound was made as described by Morton and Malone (1972). On the day of inducing wound, each animal was anesthetized using short exposure to diethyl ether. The hair (fur) on the dorsal region of each rat was removed by using an electric shaver. The area of the wound to be created was marked on the back of the animals with methylene blue. A square wound of about 1.5cm (width) x 0.2cm (depth) was created along the markings using toothed forceps, a surgical blade and pointed scissors. The surgical procedures were carried out under sterile condition. After 24 h of wound creation, the ointments were applied gently to cover the wounded area once daily

until complete healing. Wound area and wound contraction, were monitored on each day.

Measurement of wound contraction

The progression of wound healing was judged by the periodic assessment of the contraction of excision wounds. The wound contraction was studied by tracing the raw wound area subsequently on 0th, 3rd, 6th, 9th, 13th and 15th day. Scar residue, area and time of complete epithelialization were also measured. All animals in each group were monitored until complete healing of wounds occurred and the day at which each wound healed was recorded.

$$\text{Percent wound contraction} = \frac{Initial\ wound\ area - wound\ area\ on\ measurement\ \ day}{Initial\ wound\ area}\ X\ 100$$

Results and Discussion

The extraction abilities of different solvents for recovering extractable components from leaves followed the order: ethanol>ethylacetate>water (Table 3).

Table 3 Extraction yield of various solvents

Solvent	Yield	Appearance	Texture
Ethylacetate	18.7	Green	powder
Ethanol	36.9	Dark brown	sticky
Aqeuous	16.1	Dark brown	sticky

Phytochemical Screening

The findings suggest the presence of alkaloids, glycosides, phenolics, terpenoids, sterols, and flavonoids in the bark of the plant. The presence of alkaloids anonaine, asim; diterpenoid Annosquamosin in the bark of AS has also been reported (Mittal, 2016; Ma et al., 2017).

Table 4 Phytochemical screening of *Annona squamosa* bark extracts

Phytoconstituents	Ethyl acetate extract	Ethanolic extract	Aqueous extract
Tannins (Braymer's Test)	+	+	+
Flavonoids	-	+	+
Terpenoids	-	+	-
Steroids (Salkowski Test)	-	+	-
Phlobatannins (Precipitate Test)	-	+	-
Carbohydrates (Molisch's Test)	+	-	-
Saponins (Foam Test)	-	-	-
	+	+	+
Glycosides (Liebermann's Test)	-	+	+
Coumarins	-	+	-
Alkaloids (Hager's Test)	-	+	+
Proteins (Xanthoproteic Test)	-	-	+
Emodins	-	+	-
Anthraquinones (Borntrager's Test)	+	-	-
Anthocyanins	+	+	+

Leucoanthocyanins	-	+	-

The ethanolic extract contained the maximum type of phytoconstituents and hence it was considered for evaluation of the wound healing potential.

Acute Toxicity Study

The acute toxicity test was performed by using the dried ethanolic extract at concentration of 2000 mg/kg to the test animal, administered orally. No abnormal observations were visible and none of the animals died and hence the dose of upto 2000 mg/Kg was considered to be safe.

Wound Healing action

The ethanolic extract of *Annona squamosa* bark were evaluated to determine the *in vivo* wound healing effect by the excision model (n=6). The topical application of 1 and 2 % w/w of the *Annona squamosa* resulted in an enhanced and statistically significant wound healing activity *in vivo*. The wound area measurements and the percent wound contraction results of the progressive healing of the excision wounds for the vehicle control; standard reference drug and plant extract are presented in table 5.3. From the results it can be clearly seen that the ethanolic extract of the plant had an excellent wound healing potential with almost complete closure of the wound of the animals by 15th day. The plant extract (ASBE 2%) exhibited 86.15 % contraction of wound on the 15th day which was more than the povidone iodine used as the standard that exhibited 78.1% contraction of the wound. The control animals with ointment base were found to contraction 69.42 % while ASBE 1% healed 78.1% of wound by the 15th day. It is apparent that the *Annona squamosa* ethanolic extract of the bark showed good activity.

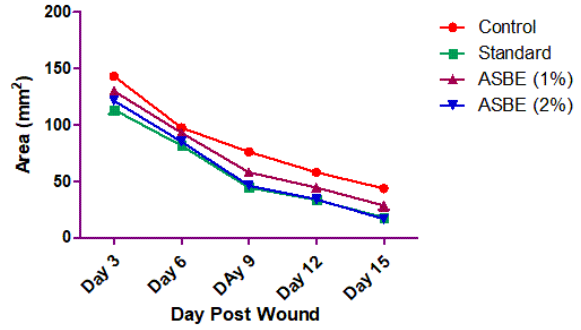

Figure 1 Wound healing efficacy of *Annona squamosa* by *in vivo* excision model

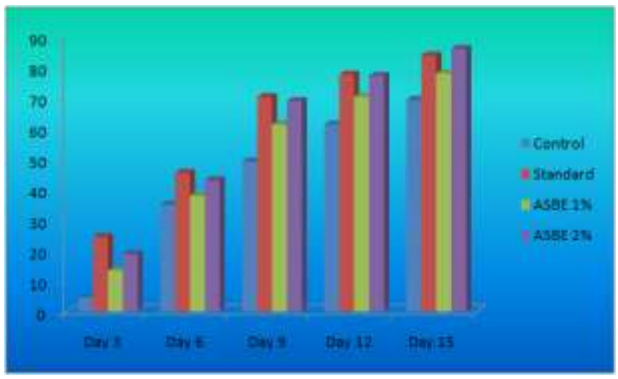

Figure 2 % contraction of wound exhibited by *Annona squamosa* by *in vivo* excision model

Studies pertaining to wound healing activity of plant extracts have been carried out by several scientists. A significant reduction in wound size was observed with the ethanolic extract of *Tridax procumbens* when compared to standard drug and aqueous extract (Talekar et al., 2012). Kodati et al. (2011) showed good increase in the rate of wound contraction and

earlier re-epithelialization using methanolic extract of *Plumbago zeylanica* root in Wistar albino rats.

Conclusion

The outcome of the present investigation has opened up several promising avenues for future research. The present investigation had thrown light on the remarkable potential of commonly available plant *Annona squamosa* in terms of its pharmacological benefits it offers. The ethnaolic extract of the bark of *Annona squamosa* was found to be effective in the functional recovery of the wound. The bioactive components in the extract can be isolated, purified and their structure can be elucidated.

Acknowledgement

The authors are thankful to the management of IPS College of Pharmacy, Gwalior for providing necessary facilities to carry the research work.

References

Gajalakshmi S, Divya R, Divya Deepika V, Mythili S, Sathiavelu A. (2011) Pharmacological activities of Annona squamosa: A review. International Journal of Pharmaceutical Sciences Review and Research 10(2): 24-29.

Gaur R, Azizi M, Gan J., Hansal P, Harper K, Mannan R, Panchal A, Patel K, Patel MK, Patel N, Rana J, Rogowska A. (2009). Simple ointment: Formulated preparations. British Pharmacopoeia. 3.

Gulcin, I. (2012) Antioxidant activity of food constituents : An overview, Arch Toxicol, 86, 345-391.

Kodati, DR, Burra S, Goud PK. (2011) Evaluation of wound healing activity of methanolic root extract of Plumbago zeylanica L. in Wistar albino rats. Asian Journal of Plant Sciences and Research 1: 26-34.

Lakshmi MS, Rani KS, Reddy TU. (2012) A review on diabetes mellitus and the herbal plants used for its treatment, Asian Journal of Pharmacy and Clinical Research 5: 15-21.

Ma C, Chen Y, Chen J, Li X, Chen Y. (2017) A Review on Annona squamosa L.: Phytochemicals and Biological Activities. The American Journal of Chinese Medicine 45 (5): 1–32

Mittal AS. (2016) Phytochemical Investigation Of Annona Squamosa Bark. Bulletin of Environment, Pharmacology and Life Sciences 5(11)

Samanta R, Pattnaik AK, Pradhan KK, Mehta BK, Pattanayak SP, Banerjee S. (2016) Wound healing activity of Silibinin in mice. Pharmacognosy Research 8(4): 298-302.

Shabbir M, Khan MR, Saeed N (2013) Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of Maytenus royleanus leaves, BMC Complementary and Alternative Medicine doi:10.1186/1472-6882-13-143.

Singh N, Singh M. (2017) An Evaluation of Phytochemicals in Maceration and Infusion Extracts of Cyperus rotundus Linn Leaves in Two Different Solvents. Journal of Pharmacology and Biomedicine 1(2): 77-84

Talekar YP, Das B, Paul T, Talekar DY, Apte KG, Parab PB. (2012) Evaluation of wound healing potential of aqueous and ethanolic extracts of Tridax procumbens Linn. in Wistar rats. Asian Journal of Pharmaceutical and Clinical Research 5: 141-145.