Wound Healing Properties of Annona Squamosa Leaf Extract: A Scientific Herbal Gel

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Abstract: **Annona squamosa, commonly referred to as custard apple, has a variety of therapeutic uses. Toxins can be eliminated from the human body by using Annona squamosa. The leaves are used as medicine to cure skin conditions like boils, bug bites, and malignant tumors. Crushed leaves are used to wounds and illnesses, and their scent is used to ease weariness and restlessness. The custard apple, or Annona squamosa L., is a significant tropical fruit that is grown in the West Indies, South and Central America, Ecuador, Peru, Brazil, India, Mexico, the Bahamas, Bermuda, and Egypt. It is a member of the Annonaceae family. The health advantages of custard apple plant leaves, which are ascribed to a significant variety of phytochemicals, have been investigated. Due to the tannins and vitamin C found in A. squamosa leaves, which have anti-inflammatory and insecticidal properties, the Indian custard apple is believed to aid in wound healing. Hydrogel formulations' capacity to cure wounds was assessed using a number of metrics, including tensile strength and wound contraction. The outcome shows the decrease in wound area for each group over the course of the fourth, twelve, and sixteenth days. When animals were given H2 and H3 formulations, it was seen that wounds healed. The percentage of wound contractions at day 20 was 80.5% and 83.9%, respectively.**

Keywords: **Annona squamosa, wound healing, Leaf Extract, Gel formulation, Anti-bacterial.**

INRODUCTION

These substances shown cytotoxicity towards a Physical injuries known as wounds cause the skin to open up or break, and proper wound care is crucial to restoring the skin's interrupted anatomical integrity and functional status.(1) The manufacture of medicine formulations and the extraction of active ingredients are complex processes that demand high profits. The tiny, semi-deciduous Annona squamosa Linn. (Annonaceae) tree, which ranges in height from 3 to 7 m, with an open, broad crown or branches that spread irregularly. It is found throughout India's several agroclimatic zones. Different plant components, such as fruit, leaves, bark, and roots, are utilized to treat a variety of illnesses and are becoming more widely recognized for their therapeutic and nutraceutical benefits.(2) Around the world, ethnopharmacological uses have been found for a variety of A. squamosa L. plant components, including the bark, roots, leaves, fruit pomace, peel, and even the seeds. Historically, the plant and its fruit were used to cure tumors, bleeding, fever, diarrhea, epilepsy, and as an anti-cold remedy.(3) The best way to get rid of and lessen the effects of FR that induce oxidative stress is through antioxidant defense systems [8]. They are FR's natural scavengers, which are known to harm cells and result in age-related illnesses.(4) The phenolic chemicals, alkaloids, peptides, amino acids, sterols, tannins, flavonoids, polysaccharides, and tocopherols that are found in A. squamosa seed extract are primarily responsible for the biological activities that it exhibits. Remarkably, annonaceous acetogenins that are isolated from custard apple seeds have antitumor and anticancer properties range of cancer cell types.(5)



HYTOCHEMICAL PROFILE

Up to 28% sugar, consisting of sucrose 2.53%, dextrose 5.05%, and laevulose 0.04%, along with fragrant flavors, makes up the extremely sweet custard apple.

Vitamin C, iron, calcium, thiamine, potassium, amino acids, carotene, riboflavin, niacin, ascorbic acid, and dietary fibers are all present in considerable amounts. The custard apple has a moderate glycemic load and a low glycemic index while having a high sugar content. Aliphatic ketones, such as palmitone, are among the particular compounds that were isolated. GC-MS study of leaf oil produced 59 chemicals, including organic acids such as hexanoic and octanoic acid and purines 20. The primary constituents included isoquinoline alkaloids, ι-cadinene (6.7%), α-murlen (5.5%), α-cadinol (4.3%), and βcaryophyllene (31.4%), a naturally occurring bicyclic sesquiterpene. It was discovered that two acetogenins that were separated from the leaves, annoreticuin and isoannoreticuin, were specifically cytotoxic to specific human tumors.(6)

MATERIALS AND METHODS

(1)COLLECTION OF LEAVES:

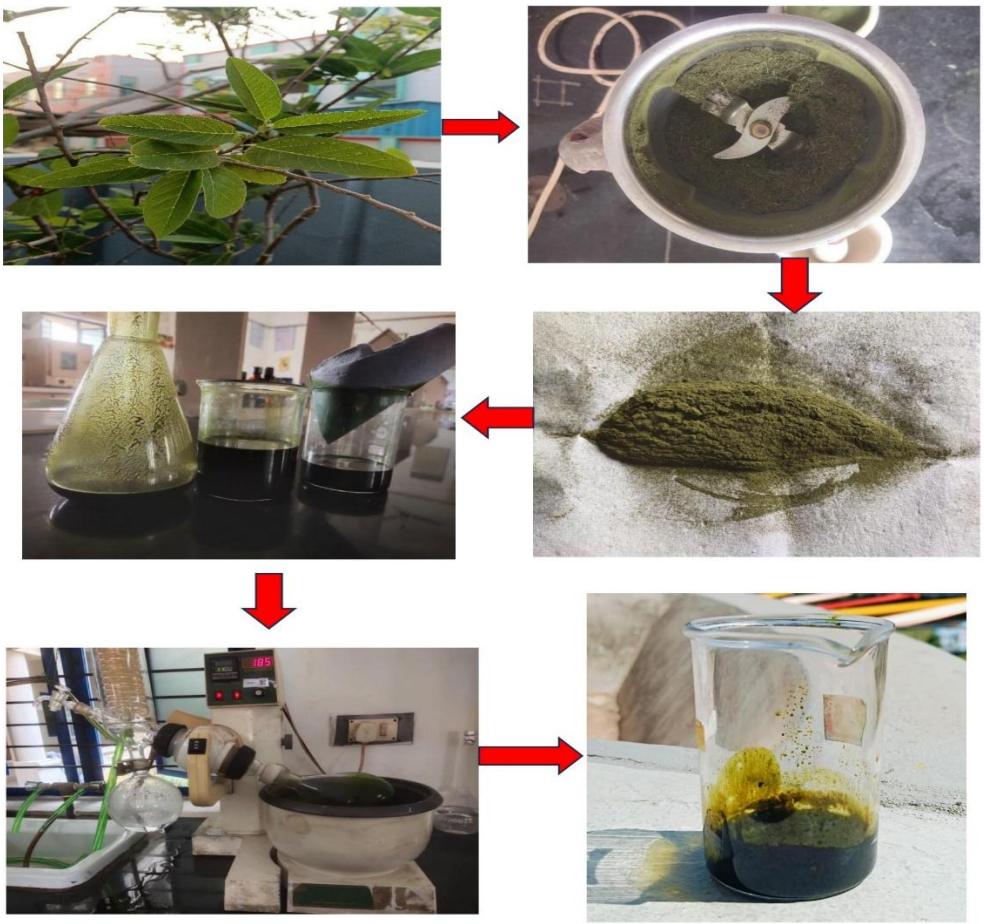
Fresh Annona squamosa leaves were gathered from Thube farm. The leaves were then cleaned with tap water to get rid of the dirt and soil after gathering. They have spent four to five days drying in the shade. Using a mechanical grinder, dried leaves were ground into a powder.

The dried powder that was produced was kept in an airtight container.(7)

(2)LEAF EXTRACTION:

The soxhlet extraction assembly's extractor was loaded with 100g of precisely weighed leaf powder, which was then extracted using 90% ethanol as the extracting solvent. The extraction process was carried out for around eight hours, or until the extraction solvent was clear. A Buckner funnel was used to filter the solvent extractive while it was hot and under vacuum. A rotating vacuum evaporator was used to dry out the extract, which was then kept in an airtight container until it was needed.(8)

Extraction Procedure



BIOLOGICAL/PHARMACOLOGICAL ACTIVITIES

(1)ANTIBACTERIAL ACTIVITY:

According to Shenoy et al. (2009) [63], the plant's custard apple leaves were thoroughly extracted using a Soxhlet apparatus using a variety of solvents, including petroleum ether, solvent ether, chloroform, alcohol, and chloroform water, in ascending order of polarity. The cup plate method was used to screen each of the five extracts for microorganisms. The highest zone of inhibition was displayed by the petroleum ether, alcoholic, and chloroform water extracts. Thus, these extracts were used to promote wound healing. All models that used Annona squamosa leaf petroleum ether extracts produced noteworthy outcomes. When compared to the control group, all of the results were significant for various wound healing activity criteria.(9)

(2)ANTIMICROBIAL ACTIVITY:

Four metal extracts were used to assess antimicrobial activity. The antibacterial activity was assessed using the agar diffusion method. For analysis, two gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and two gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) were chosen. /ml). The investigation's findings demonstrated that the methanolic extract against E. coli had the highest limit of inhibition, followed by the ether oil extract against A. coli (MIC: 165 µg/ml) and the methanolic extract against Aeruginosa (MIC: 130 µg/ml). Three distinct solvent extracts of Annona squamosa and Annona reticulate leaves were used in a different investigation to assess the antibacterial activity. Three Gram-positive (Bacillus subtilis) bacteria were used to investigate the antibacterial activity of the broth dilution and agar cup procedures.(10)

(3)ANTICANCER:

One special characteristic of human tumors is their capacity to avoid apoptosis, which can lead to efficient tumor development and cancer progression. One of the main causes of treatment failure is the strong resistance of cancer cells to apoptosis in response to a relevant stimulation. As a result, several cancer treatment approaches, such as chemotherapy and radiation therapy, rely heavily on the death of cancer cells [38]. The creation of natural pharmaceutical pharmaceuticals has been prompted by a long history of using natural items as ethnomedicines, which are cheap and have little side effects, as opposed to expensive synthetic treatments that have harmful effects [39]. Natural bioactive substances that induce apoptosis have attracted a lot of attention in the field of anticancer drugs in recent decades.(11)

(4)ANTIDIABETIC:

The main characteristics of diabetes, an endocrine and metabolic disease, are insulin resistance, insulin insufficiency, and high blood sugar levels. The International Diabetes Federation (IDF) reports that diabetes mellitus (DM) is becoming more commonplace worldwide. It is projected that 642 million people will have diabetes by 2040, up from 415 million in 2016 [57]. In addition to insulin, a variety of oral hypoglycemic medications are available for the treatment of diabetes mellitus, including sulfonylurea (glimepiride), thiazolidinedione (rosiglitazone), and biguanides. However, fake pharmaceutical ingredients are dangerous for expectant mothers and have caused serious problems for patients.(12)

(5)HEPATOPROTECTIVE:

It was discovered that Annona squamosa leaf methanol extract was hapatoprotective. It reduces the hepatotoxicity caused by isoniazid-rifampicin in rats, as evidenced by improvements in total protein and decreased glutathione levels, as well as restoration in the elevated liver enzymes ALT, AST, GGT, and ALP, serum bilirubin, and TBARs [83,84]. Similarly, at dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg (extract per body weight) for a week, Annona squamosa hydroalcoholic seed extract is shown to be hepatoprotective against CCl-4-induced hepatotoxicity.(13)

(6) WOUND HEALING ACTIVITY:

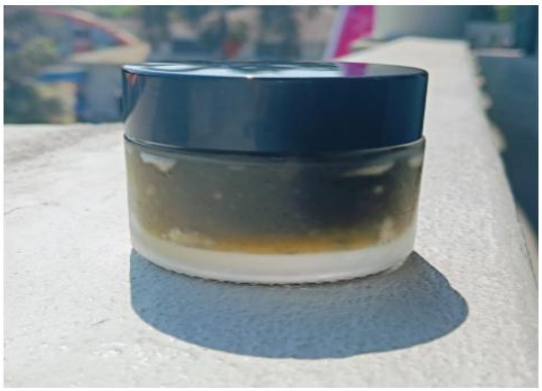
Applying an ethanolic extract of Annona squamosa leaves topically promotes wound healing by boosting the production of collagen, glycosaminoglycan, and cells at the areas of injury [95, 96]. This validates the traditional topical application of Annona squamosa leaves for ulcers and wounds (14).

(7) ANTIOXIDANT:

As previously reported by Narasimhan et al, the antioxidant activity of A. squamosa extracts was assessed in vitro using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical's scavenging activity. First, 1 mL of plant extracts in varying quantities (10–100 μg/mL) was combined with 0.1 mM DPPH (1 mL). Following 30 minutes of dark incubation, the absorbance at 517 nm was measured (15).

METHOD OF PREPARATION

In order to prepare the gel formulation, propyl paraben, glycerine, carbopol 940, and Guar gum were first dissolved in distilled water and left overnight. Take Annona squamosa leaf extract in propylene glycol, and then add it to leftover dispersion. After adding the remaining amount of water, triethanolamine was added to neutralize the pH to 7 whiles being continuously stirred for ten minutes.



Formulation

TABLE 1: FORMULATION OF HERBAL GELL:

INGRIDIENTS	QUANTITY
Carbopol 940	2gm
Guar gum	1.0gm
Propyl paraben	0.5gm
Glycerine	1gm
Propylene Glycol	1.5gm
Triethanolamine	0.5gm
Distill Water	q.s

PHYTOCHEMICAL TESTS:

(1) Alkaloid Test:
Concentrated hydrochloric acid was added to 2 milliliters of plant extract. Following that, a few drops of Mayer's reagent were added. Alkaloids were indicated by the presence of green color.(16)

(2) Saponin Test:
A solution of 5% ferric chloride was added to 1 milliliter of plant extract. The development of greenish black suggested that tannins were present.(17)

(3) Flavonoid Test:
A fraction of the plant extract's aqueous filtrate was mixed with 5 milliliters of diluted ammonia solution, and then concentrated sulfuric acid was added. The presence of flavonoids was shown by the appearance of yellow coloring.(18)

(4) Phenol Test:
A few drops of 10% ferric chloride were added to 1 milliliter of the extract after 2 milliliters of distilled water. When a green color formed, it meant that phenols were present.(19)

EVALUTION TESTS:

IN-VITRO TESTS:

(1) PHYSICAL PROPERTIES OF ANNONA SQUAMOSA GEL:

S.NO:	PHYSICAL PROPERTIES	GEL FORMULATION
1.	Colour	Green Colour
2.	Odour	Characteristic
3.	State	Semi solid
4.	Texture	Smooth

(2) PH READING:

In order to encourage wound healing and prevent bacterial growth, the PH of ANNONA SQUAMOSA Wound Healing Gel was determined to be 4.9+/-2.

(3) SPREADABILITY TEST:

The Spreadability was recorded as less than 15 seconds, indicating excellent spreadability and ease of application.

CONCLUSION

Wound healing gels containing Annona squamosa (custard apple) leaf extract can hasten wound healing by stimulating epithelialization, wound contraction, and collagen synthesis, as well as lowering oxidative and inflammatory stress in the wound site. Topical treatment of ethanolic extract of A. squamosa enhances active GAG production and collagen maturation during wound healing. Topical treatment of ethanolic extract of A. squamosa enhances active GAG production and collagen maturation during wound healing. This, in turn, is a good sign for better wound healing.

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