Biomedical & Pharmacology Journal, December 2024. Vol. **17**(4), p. 2437-2444

Formulation of Antioxidant and Antibacterial Cream Containing *Amaranthus Spinosus* Leaf Extract

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https://dx.doi.org/10.13005/bpj/3036

(Received: 16 August 2024; accepted: 21 October 2024)

Herbal medicine therapy is an ancient practice to treat various health issues, including viral and non-infectious skin diseases. At present time, people are running behind natural or herbal sources because of their fewer side effects and add-on benefits as compared to allopathy medicines. In the Indian traditional system of medicine (Ayurveda), the *Amaranthus spinosus* (Amaranthaceae) plant is used for analgesic, antipyretic, laxative, diuretic, digestible, antidiabetic, anti-snake venom, antileprotic, blood diseases, bronchitis, piles, and anti-gonorrheal. The study's objective was to perform extraction and phytochemical studies of A. spinosus leaves, perform the in-vitro antibacterial and antioxidant activity of the extract, and formulate a topical cream. The ethanol water leaf extract exhibited higher antibacterial efficacy than ciprofloxacin against *Staphylococcus aureus* at 15 and 30 μg, while ciprofloxacin was more effective at 60 μg. However, the extract showed more effectiveness than ciprofloxacin against *Escherichia coli* at all tested concentrations. The study revealed that the leaf extract showed good antibacterial and antioxidant activity. The physical evaluation of the formulated cream showed that the cream was stable with potential application to reduce skin infection with consequent health benefits.

Keywords: Antibacterial agent; Antioxidant; A. spinosus; Cream; Herbal medicine; Leaf extract.

Exploring new bioactive substances derived from natural sources has recently attracted much attention because of their possible therapeutic uses in various fields, including pharmaceuticals and cosmetics. Plants are an eminent source of bioactive substances with a wide range of pharmacological and phytochemical characteristics. Developing topical formulations like cream using plant extracts offers various pharmacological properties, including antibacterial, antioxidant, and anti-inflammatory[1,2]. Topical creams are a perfect vehicle for maximizing the medicinal potential of plant extracts because of their safe, non-greasy texture, ease of application, and a good vehicle to deliver the substances to the skin[2,3]. It has long been known that plants are an excellent source of bioactive chemicals with antioxidant and antibacterial properties, which makes them a good choice for creating topical treatments to treat skin diseases[2,4].

Since ancient times, herbal sources like plants have been used to prevent and treat many diseases. In India and other countries, medicinal plants have been used without any checking

parameters as traditional healers[5]. These plants have different medicinal properties that help the body by boosting protective enzymes and strengthening its antioxidant system. When the body produces too many reactive oxidative species (ROS), it can damage cells and tissues and start inflammation. By controlling ROS levels, these plants can potentially treat various diseases and show biological activities including antimicrobial, antioxidant, anti-cancer, anti-inflammatory, etc.[6]

Antibacterial activity or agents describe compounds that either eradicate or inhibit the development of bacteria. Although scientists have found many antibiotics that can fight different infections well, some bacteria are becoming resistant to other antibiotics, making it hard to treat infections. This is becoming a challenge for human health. Also, antibiotic resistance increases mortality rates and the costs of treating diseases. To minimize or overcome the problem, researchers focus on plant-based or natural antibacterial agents. Additionally, phytoconstituents have diverse chemical structures, which reduce the probability of bacteria developing resistance compared to conventional antibiotics. Furthermore, using antibiotics produced from plants has several benefits, such as their natural abundance, comparatively cheap extraction costs, and the possibility of using environmentally friendly manufacturing techniques. Also, many of these compounds have been conventionally used in herbal medicine for their antimicrobial properties, indicating their safety for human consumption [7]. *A. spinosus* Linn. (Amaranthaceae) is commonly known as Katakhutura (figure 1) in Assamese culture. About 20 species are found or cultivated in India and worldwide four hundred Amaranthus species occur in tropical, subtropical, and temperate climate zones[8–10].

A. spinosus is used as a vegetable in different regions of India. According to Indian tradition, this plant treats leprosy, blood disease, leucorrhoea, bronchitis, rheumatic pain, eczema, gastrointestinal diseases, boils, burns, abscesses, colic menorrhagia, etc. The plant is also an expectorant, digestive, diuretic, antipyretic, antileprotic, anti-gonorrhoeal, antidiabetic, antimalarial, antiviral, anti-inflammatory, etc. The boiled roots and leaves of *A. spinosus* are given to children as emollients, laxatives, and for burns and boils[4,11]. The juice of *A. spinosus* was used in a village area of Assam, India, to prevent swelling around the stomach, while the leaves were boiled without salt and consumed for 2-3 days to cure jaundice. *A. spinosus* is also used, as reported, for its anti-inflammatory, antimalarial, immunomodulatory, anti-diabetic, anti-hyperlipidemic, antioxidant, and spermatogenic activities[1,3,12,13]. Phytoconstituents like alkaloids, glycosides, flavonoids, steroids, phenolic acids, amino acids, saponins, glycosides, lipids, terpenoids, tannins, betanin, b-sitosterol, stigmasterol, linoleic acid, rutin, and beta-carotene[8,9,14].

Studies showed that *A. spinosus* extract has antimicrobial activity in different microbial strains like *Staphylococcus aureus*, *Aspergillus flavus*, and *Escherichia coli*. The plant extract has restored the level of oxidative free radicals in rats, which has helped in the fast recovery of wounds. The phytochemical studies confirmed the presence of terpenoids, alkaloids, and saponins. Different studies confirmed that *A. spinosus* can be used as a substitute treatment for infectious or non-infectious wounds[15–17].

The major objective of the present study is to formulate an antioxidant and antibacterial cream with the extract of *A. spinosus* leaves. Also, evaluating the physical properties, such as color, odour, pH, viscosity and spreadability of the formulated cream is another objective. The ultimate goal is to create a cream with extract from *A. spinosus* that offers a safe, natural option for treating bacterial skin infections.

MATERIALS AND METHODS

Collection, authentication, and extraction of Plant

The flowering plants were collected in the winter season from the village area of Dhubri district, Assam, India. The plant was authenticated at the Botanical Survey of India, Shillong, Meghalaya, India. For the extraction, the leaves were first separated, dried under a shed, and powdered using a grinder. The powdered leaves were sieved through a #40 sieve to get the uniform size of the powdered leaves. The extraction was performed using digestion with different solvents like petroleum ether, ethyl acetate, acetone, ethanol, and hydroalcoholic (ethanol: water). About 25

grams of powdered leaves were mixed with 100 ml of solvent and kept in a water bath for 30 minutes. The extract was separated and dried using filtration and evaporation methods.

Preliminary Phytochemical Screening of Leaf Extract

Using standard methods, the phytochemical screening of the ethanolic extract of *A. spinosus* was conducted to determine the presence of phytochemical constituents[18].

Antibacterial Activity: The antibacterial activity of leaf extract was performed using the agar disk diffusion method. Solid agar media plates are used for the growth of test organisms in this method. The extract is loaded into the paper discs and placed on the solid agar plate after the development of the test organism. Once the extract diffuses into the agar plate, it will stop the growth of bacteria up to a particular area, known as the zone of inhibition, and it will be measured using an antibiotic zone reader. *Staphylococcus aureus* and *Escherichia coli* are the two most commonly used bacterial strains used for this antibacterial study, for the solid agar plates were prepared by dissolving the required quantities mentioned in table 1 of peptone, beef extract, sodium chloride, and agar in water with heat. Sterilization was performed using autoclaving for the liquid agar media and after that, it was allowed to solidify in Petri dish.

A stock solution was prepared in 1ml of dimethyl sulfoxide (DMSO) by dissolving 0.06 gm of extract. The sterilized paper discs were dipped into the solution to make 60 µg/disc concentrations. Further test samples of 30 µg/disc and 15 µg/disc concentrations were prepared by diluting the stock solution. The paper discs loaded with extract, Ciprofloxacin as a standard drug, and control DMSO were placed in the agar plates and incubated for up to 24 hours. The zone of inhibitions was measured and recorded [6,7].

Antioxidant Activity

The activity was performed using radical scavenging of the unchanged 2,2' diphenyl-1-picrylhydrazyl (DPPH) assay for the plant extract. The standard solution was an ascorbic acid with 1 to 10 µg/ml concentrations. The assay was carried out using a UV visible spectrophotometer, with an ascorbic acid solution ranging from 1 to10 µg/ml used as the standard solution.

The sample solution was prepared using a 0.005% DPPH solution in ethanol, where 2 ml of extract solution was mixed. The samples were incubated at room temperature for 30 mins. A UV spectrophotometer measured the optical density at 517 nm for every sample in triplicate [16,17]. The antioxidant activity was determined by using the following equation:

$$\% \text{ inhibition: } [(A_{control} - A_{sample})/ A_{control}) \text{ X100}]$$

Fig. 1. *Amaranthus spinosus* Plant

Table 1. Composition of nutrient agar

Nutrient Agar	Quantity (g)
Peptone	5
Beef Extract	3
Sodium chloride	5
Agar	15
Distilled water	1000 ml

Table 2. Composition of cream with *A.spinosus* extract

Ingredients	Amount(g)
Stearic acid	1
Spermaceti	0.5
Cetyl alcohol	0.5
Glycerine	0.5
Triethanolamine	0.2
Benzyl alcohol	0.2
Water	7
Leaf Extract of *A. spinosus*	0.1

Where A control is the absorbance of control at the moment of solution preparation and A sample is the absorbance of the sample after 45 min.

Formulation of cream-containing extract

The oil phase and water phase were prepared separately by melting the required ingredients mentioned in table 2. Both phases were mixed slowly with continuous stirring at 70 °C. After getting the required consistency, the leaf extract was added to the cream. For a smooth and stable cream preparation, the ingredients are added slowly and step-by-step [3,4,19].

Evaluation of prepared cream: The following evaluation tests are performed to measure the quality of formulated cream. These evaluation parameters help in ensuring the suitable compatibility of cream with skin [19,20].

1. Homogeneity: the prepared cream was observed visually to check the homogeneity.
2. Determination of pH: A digital pH meter was used to check the pH of the prepared cream.
3. Organoleptic test: properties like appearance, odour and texture were evaluated by applying a small portion of cream to the skin.
4. Consistency: to check the consistency of the prepared cream, a cone was dropped from a 10 cm fixed height into a measuring cylinder. The measuring cylinder was filled with prepared cream.
5. Spreadability: The test was performed by observing how easily and quickly the cream spreads over the area after application.

Table 3. Percentage yield of extract

The solvent used for extraction leaves	Weight of Extract (gm)	% of Yield
Pet. Ether	0.87	3.48
Ethyl acetate	1.2	4.8
Acetone	2.1	8.4
Ethanol	2.8	11.2
Ethanol: water (1:1)	3.9	15.6

Table 4. Phytochemical constituent present in the extract of *A. spinosus* leaf

Name of the constituents	Pet. ether	Ethyl acetate	Acetone	Ethanol	Ethanol: water (1:1)
Alkaloids	-	-	+	+	+
Glycoside	-	-	-	-	-
Flavonoids	-	-	+	+	+
Steroids	-	-	-	-	-
Tannins and phenolic Compound	-	-	+	+	+

**+ = Present the phytochemical constituent and - = Absent the phytochemical

Fig. 2. Zone of inhibition against *E.coli* and *S.aureus* bacteria

6. Emolliency: The emollient and greasiness effects of cream were observed by applying it to the skin.
7. Skin irritation test: The test was performed by applying a small portion of cream to an area of skin and being observed for 15-20 minutes to see if any irritation occurred.
8. Stability Study: Prepared cream was kept for three months at three different temperatures, such

Table 5. Antibacterial activity test of *A. spinosus* leaf against bacterial strains

Compounds	Concentration	Zone Of Inhibition (mm)	
		S.aureus	*E.coli*
Leave extract of *A.spinosus* in ethanol:water (1:1)	60 µg	13	16
	30 µg	12	12
	15 µg	10	9.5
Ciprofloxacin	60 µg	15	13
	30 µg	11	11
	15 µg	-	-

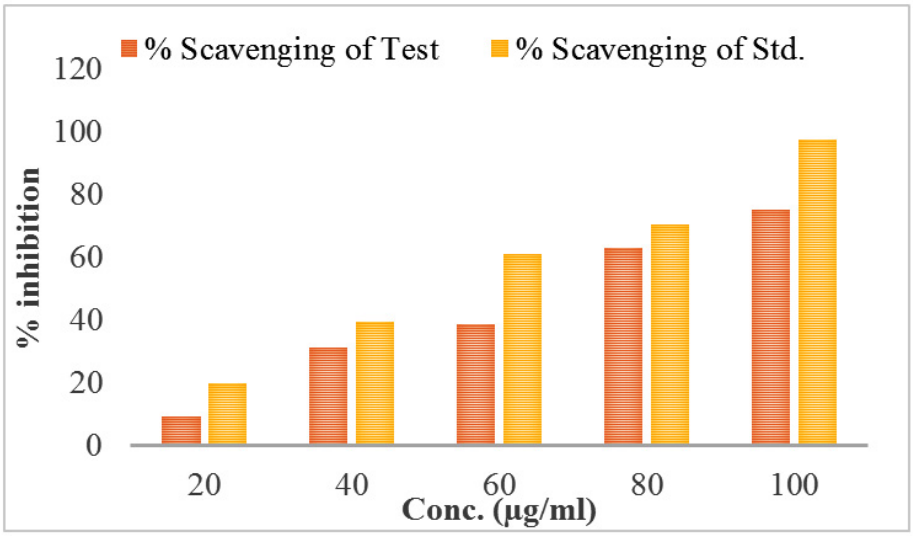

Fig. 3. DPPH scavenging activity

Fig. 4. Prepared cream containing *A. spinosus* extract

Table 6. Results of evaluation of prepared cream

Parameter	Leaf extract cream
Colour	Light green
PH	5.6
Homogeneity	Excellent
Consistency (60 sec)	5mm
Skin Irritation	Nil
Viscosity	1375 Cp
Spreadability (g.cm/sec)	36

Table 7. Evaluation of stability study

Parameter	Room temperature (at 25^0C ± 2)			Accelerated temperature (at 40^0C ± 2)		
	1 month	2 months	3 months	1 month	2 months	3 months
Colour	Light green	Light green	Light green	Light green	Light green	Light green
pH	5.6	5.7	5.7	5.5	5.6	5.7
Homogeneity	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent
Consistency (60 sec)	5mm	5mm	6mm	6mm	5mm	6mm
Skin Irritation	Nil	Nil	Nil	Nil	Nil	Nil
Viscosity	1375 Cp	1410 Cp	1457 Cp	1390 Cp	1426 Cp	1498 Cp
Spreadability (g.cm/sec)	36	37	36	36	38	37

as at 4^0C, 20-30^0C and 40-50^0C. Other parameters were noted.

RESULTS AND DISCUSSION

Percentage Yield of Extract: After extracting powdered leaves, extracts were dried using the evaporation method, and the yield values as a percentage were determined. The table 3 displays the results. The highest percentage yield was found when ethanol and water were used for extraction in a 1:1 ratio.

Preliminary Phytochemical Analysis: After performing the preliminary phytochemical study, it was found that alkaloids, flavonoids, tannins, and phenolic compounds were present in the extract of acetone, ethanol, and ethanol: water. Details of the results are shown in table 4.

Antibacterial Activity

After performing the antibacterial test of ethanol water leaf extract by disk diffusion method, it was shown that at concentrations of 15 and 30 microgram, the extract showed somewhat higher efficiency against *S. aureus* bacteria than Ciprofloxacin. On the other hand, ciprofloxacin showed better effectiveness at a concentration of 60 microgram than extract. The extract showed more effectiveness in all three concentrations than ciprofloxacin against *E.coli* bacteria.

The results of the disc diffusion experiment, which are presented in the accompanying table 5 and figure 2, highlight the significant antibacterial qualities of the leaf extract of *A. spinosus*.

Antioxidant Activity

The IC50 value represents the concentration of a substance required to inhibit a particular biological or biochemical function by 50%. In this study, the IC50 values indicate the efficiency of the test substance and the standard in scavenging free radicals. The IC50 value for the test substance was 69.28 µg/ml. The IC50 value for the standard substance was 49.55 µg/ml. A lower IC50 value signifies a higher potency of the substance in scavenging free radicals. Therefore, the standard substance is more effective in free radical scavenging compared to the test substance (shown in the figure 3). However, the test substance also shows significant scavenging activity, making it a potential candidate for further investigation and development as an antioxidant agent. It also opens up the possibility of exploring the plant further and isolating the compound responsible for the antioxidant activity shown by the plant.

Formulation and evaluation of antibacterial cream

The cream containing *A.spinosus* extract was formulated (shown in the figure 4) with the specified ingredients in the table 2. After the formulation of antibacterial cream, a comprehensive evaluation was conducted to assess its quality and performance. The evaluation test included observations about colour, viscosity, and spreadability and several tests, such as a skin irritation test, homogeneity analysis, and pH measurement. The results are shown in the table 6. The result of the evaluation parameters ensured the suitability of the cream for topical application.

Stability Study of cream

The stability test of the cream was carried out at room temperature and accelerated temperature for at least three months. The results of evaluation parameters were listed in the table 7 and it was observed that the cream was stable.

CONCLUSION

The study concludes by indicating *A. spinosus* potential as a useful source of antioxidant antibacterial compounds for topical therapy formulation. The study started with collecting and authenticating *A. spinosus* plants from the Dhubri area in Assam, India, and then extracting the leaves using different solvents. The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, and phenolic compounds in the leaf extracts, which are known for their antimicrobial properties. Using the disk diffusion technique, the antibacterial activity of the *A. spinosus* leaf extract was evaluated against two common bacterial strains: *E. coli* and *S. aureus*. The antibacterial study revealed that the leaf extracts showed better efficacy when compared to the Ciprofloxacin. The DPPH antioxidant confirmed the leaf extract has good antioxidant property. The antioxidant and antibacterial activity results revealed that the plant *A. spinosus* can be a natural substitute for treating bacterial and other diseases like wound healers, aging, etc. A topical cream formulated to administer the drug easily and efficiently on a required body site. The prepared topical cream was evaluated for physical parameters. The evaluation study confirmed that the cream was stable and suitable for topical application.

However, it is important to acknowledge certain limitations and areas for further exploration. Although *A. spinosus* extract's antibacterial activity was shown in vitro, more research is necessary to confirm its effectiveness in vivo and evaluate its safety profile over an extended period. More investigation is necessary to clarify the mechanisms of action of phytoconstituents and maximize their effectiveness for therapeutic use. Translating these discoveries into real-world applications also requires addressing issues including formulation development, regulatory approval, and standardization of extraction procedures. Furthermore, optimizing the cream formulation, including concentration adjustments and incorporating other synergistic ingredients, could enhance its efficacy and stability.

ACKNOWLEDGEMENT

We want to thank the Institutional research committee of Pratiksha Institute of Pharmaceutical Sciences, Panikhaiti, Chandrapur road, Guwahati, Kamrup (M), Assam, pin: 781026 for their contribution to experimental work, chemicals, instrument and equipment.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author contributions

Methodology and Investigation: Ratnali Bania, Sofiqul Islam, Nekibur Rahman; Writing- Original draft preparation: Ratnali Bania Debabrata Nath, Madhusmita Kumari, Abhishek Parasar; Conceptualization: Ratnali Bania; Validation: Satyendra Deka; Software: Ratnali Bania, Debabrata Nath; Reviewing and Editing: Madhusmita Kumari, Ratnali Bania

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