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Formulation and Phytochemicals Screening of Amaranthus Spinosus

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Abstract: *For many centuries and even today plants have provided mankind with remedies for many diseases. In India there are 47000 plant species of which 15000 are reported to have medicinal properties. These plants play a major role in primary healthcare as therapeutic remedies in India. It is further claimed that these plants have been the bases of treatment and cure for various diseases in India.*

Keywords: *diseases*

I. INTRODUCTION

For many centuries and even today plants have provided mankind with remedies for many diseases. In India there are 47000 plant species of which 15000 are reported to have medicinal properties. These plants play a major role in primary healthcare as therapeutic remedies in India. It is further claimed that these plants have been the bases of treatment and cure for various diseases in India.

Amaranthus spinosus. (A. spinosus.)is one such medicinal plant of India. Belongs to the family of Amaranthaceae, the plant is believed to originate from South and Central America and then introduced into various regions of Africa specially south tropical African countries such as Zimbabwe, Botswana, Malawi, Zambia and Namibia. The plant is also widely distributed in waste places, roadsides and fields in Bangladesh, Ghana, Cambodia, Philippines Maldives, Japan, Sri Lanka, Myanmar, Indonesia, Australia and India. 21 A. spinosus L. grows annually as an erect perennial herb with many branches.

Stems are hard, terete or obtusely angular and greenish to purple in colour. Leaves are alternate, have bitter taste with a characteristic odour. Flowers are numerous, appear throughout the year. Fruit is ovoid shaped. Seed is shiny, black or brownish-black in colour,

Importance of ayurveda:

The importance of Amaranthus spinosus in Ayurveda lies in its multipurpose therapeutic potential, as well as its nutritional and medicinal value.

Ayurvedic Importance of Amaranthus spinosus :

1. Healing Properties (Aushadha Guna)

• In Ayurveda, Amaranthus spinosus is valued for its Shita Virya (cooling potency), which makes it effective in treating conditions caused by Pitta and Vata imbalances.

• Tikta-Kashaya Rasa (bitter-astringent taste) helps in detoxification and drying excess secretions.

• Ruksha Guna (dry quality) supports digestive fire (Agni) and helps manage loose stools or dysentery.

2. Common Ayurvedic Uses

• Shotha (Swelling/Inflammation): Acts as an anti-inflammatory.

• Raktapitta (Bleeding Disorders): Its cooling nature helps manage bleeding tendencies, especially nasal bleeding or excessive menstruation.

• Arsha (Piles): Juice or decoction is used in management of piles due to its astringent and anti-inflammatory effects.

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• Prameha (Urinary Disorders): Works as a diuretic to clear urinary tract infections or painful urination.
• Vrana Ropana (Wound Healing): Fresh leaf paste is applied to wounds, boils, and ulcers to accelerate healing.

3. Rasayana (Rejuvenative) Potential
• It is mildly rejuvenative due to its rich nutritional content—used traditionally as a leafy vegetable in Ayurvedic dietetics for promoting Ojas (vitality).

4. Grain Substitute and Fasting Food
• The seeds of Amaranthus spinosus are considered Satvika (pure) and often used in fasting (Upavasa) diets. They are easy to digest and help maintain energy.

World wide use of Ayurveda :
As of recent estimates, there are approximately 500,000 registered Ayurveda practitioners worldwide. This includes over 420,000 practitioners in India and an estimated 80,000 to 100,000 across other countries, reflecting the global reach and integration of Ayurveda into healthcare systems .

Global Reach of Ayurveda
Ayurveda is legally recognized as a traditional medicine system in over 25 countries, including the United States, Germany, and several nations in Europe and Asia. In Germany alone, there are approximately 200 to 300 treatment centers offering Ayurvedic medical services.

Growth and Recognition
The global market for Ayurvedic products and services has expanded significantly, with the market size increasing from $3 billion in 2014 to $23.3 billion in 2022. This growth is driven by rising consumer interest in natural and holistic healthcare approaches.
The World Health Organization (WHO) acknowledges the use of Ayurveda in 93 member states and is working towards integrating Ayurveda into international health frameworks, including the International Classification of Diseases.

Chromatographic methods
Chromatographic Techniques are methods used to separate and analyze mixtures by exploiting differences in how components interact with a stationary and mobile phase. These techniques are broadly classified based on the physical state of the mobile phase (gas or liquid) and the nature of the stationary phase (e.g., solid, liquid, gel).

Key Chromatographic Techniques:
1. Gas Chromatography (GC): Separates volatile compounds in a gas phase. Liquid Chromatography (LC): Separates compounds in a liquid phase. Gas Chromatography (GC) is an analytical technique used to separate and analyze compounds that can be vaporized without decomposition. It is commonly used in chemistry and biochemistry for identifying substances within a mixture and measuring their concentrations.
2. High-Performance Liquid Chromatography (HPLC): A type of LC using high pressure to improve speed and resolution. HPLC (High-Performance Liquid Chromatography) is an analytical technique used to separate, identify, and quantify components in a liquid mixture. It's widely used in pharmaceuticals, food analysis, environmental testing, and chemistry labs.
3. Thin-Layer Chromatography (TLC): A planar chromatography technique used forqualitative analysis of mixtures. TLC stands for Thin Layer Chromatography, which is a laboratory technique used to separate and identify compounds in a mixture.

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4. Paper Chromatography: Another planar chromatography method, often used for educational purposes. Paper Chromatography is a simple and inexpensive method used to separate and identify mixtures of soluble substances, especially colored compounds like inks, dyes, and plant pigments.
5. Column Chromatography: Separates components in a mixture using a column packed with a stationary phase. Column chromatography is a laboratory technique used to separate and purify individual components from a mixture based on their different interactions with a stationary phase (usually a solid like silica gel or alumina) and a mobile phase (a liquid solvent). The mixture is passed through a column packed with the stationary phase, and different substances in the mixture move through the column at different speeds, allowing them to be collected separately.
6. Ion-Exchange Chromatography: Separates compounds based on their ionic charge. Ion Exchange Chromatography (IEC) is a technique used to separate and purify charged molecules—such as proteins, amino acids, or ions—based on their net charge.
7. Gel-Permeation Chromatography (GPC): Separates compounds based on size. Gel permeation chromatography is a chromatographic technique that separates molecules according to their size as they pass through a column packed with porous gel particles.
8. Affinity Chromatography: Separates components based on specific interactions with a binding agent. Affinity Chromatography is a highly specific separation technique used to isolate a particular molecule (like a protein, enzyme, or antibody) based on its biological interaction with a specific binding partner.

AIM & OBJECTIVE

Aim : Formulation and Phytochemicals screening of Amaranthus spinosus

Objectives:

1) To identify and collect plant species traditionally used for anti-venom and anti-hemorrhoid treatment.
2) To prepare extracts from the selected plant materials using appropriate extraction methods (e.g., maceration, Soxhlet, cold percolation).
3) To perform preliminary phytochemical screening of the extracts to identify active constituents such as alkaloids, flavonoids, tannins, saponins, terpenoids, etc.
4) To evaluate the in-vitro and/or in-vivo anti-venom activity of the plant extracts against specific venom (e.g., snake venom).
5) To evaluate the in-vitro and/or in-vivo anti-hemorrhoid activity of the plant extracts using appropriate animal models and pharmacological tests.
6) To isolate and characterize the major bioactive compounds responsible for anti-venom and anti- hemorrhoid activity using chromatographic and spectroscopic techniques (e.g., HPLC, GC-MS, NMR, FTIR).
7) To assess the toxicity and safety profile of the active extracts/compounds using acute and sub- chronic toxicity studies.
8) To standardize the plant extracts/formulations using modern analytical technologies to ensure consistency, potency, and reproducibility.
9) To compare the efficacy of the plant-based formulations with existing conventional drugs.
10) To develop a prototype formulation (cream, capsule, or tincture) based on the standardized extract for potential therapeutic application.

PLANT PROFILE

AMARANTHUS SPINOSUS

Amaranthus spinosus, commonly known as the spiny amaranths spiny pigweed, prickly amaranth or thorny amaranth, is a plant that is native to the tropical Americas, but is present on most continents as an introduced species and sometimes a noxious weed.It can be a serious weed of rice cultivation in Asia.

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SYNONYM

Hindi (amaranth in hindi) – **चौलाई**, **चौराईकासाग**, **कटैलीचवलाई**

English – **स्पाइनीएमारेन्थ** (Spiny amaranth), **थानीपपगवीड** (Throny pigweed), Prickly Amaranth (**पिक्लीएमारेन्थ**)

Sanskrit – **तण्डुलीय**, **मेघनाद**, **काण्डेर**, **तण्डुलेरक**, **पवषघ्न** Bengali – **काांटानटे** (Kantanate), **काांटामरीस** (Kantamaris)

Marathi – **काांटेमाठ** (Kantemath)

Gujarati – **काांटालोडाांभो** (Kantalodambho), **काांटालोधीम्डो** (Kantalodhimdo) Kannada – **मुल्लुहररवेसोप्पु** (Mulluharivesoppu)

Telugu – **मोलाटोटाकुरा** (Molatotakura) Tamil – **मुलुक्कोरै** (Mullukkore)

Nepali – **वनलुडे** (Vanlude) Malayalam – **मुलेन्चीरा** (Mullanchira)

Biological Source:

The biological source of Amaranthus spinosus refers to the root part of the plant that are used for medicinal or pharmacological purposes.

Botanical Name: Amaranthus spinosus Linn.

Family: Amaranthaceae

chemical constitution Amaranthus spinosus:

1. Phytochemicals

These include:

- Alkaloids – have potential analgesic and antimicrobial activity.
- Flavonoids – like quercetin and rutin, known for antioxidant, anti-inflammatory, and cardio protective effects.
- Tannins – with astringent properties.
- Saponins – with cholesterol-lowering and immune-modulating effects.
- Phenolic compounds – strong antioxidants.
- Terpenoids – known for anti-inflammatory and antimicrobial actions.
- Steroids – may include β-sitosterol.

2. Vitamins and Minerals

- Vitamins: Vitamin A (as beta-carotene), Vitamin C, Vitamin E, folates.
- Minerals: Iron, calcium, magnesium, phosphorus, potassium, and zinc.

3. Amino Acids and Proteins

- High-quality protein content.
- Essential amino acids such as lysine, leucine, and valine.

4. Fatty Acids

- Small amounts of linoleic acid and oleic acid in seeds and leaves.

Uses of amranthus spinosus

Aphrodisiac chronic dysentery digestive system disorders , urinary disorders ,Eczema and skin allergy, Amaranthus spinosus, commonly known as spiny amaranth or thorny amaranth, is a plant widely used in traditional medicine, agriculture, and nutrition. Here's a summary of its main uses:

1. Medicinal Uses

- Anti-inflammatory and analgesic: Used traditionally to treat pain, swelling, and fever.

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- Antidiabetic: Some studies suggest it may help lower blood sugar levels.
- Antioxidant: Rich in compounds that combat oxidative stress.
- Diuretic and laxative: Helps in promoting urination and relieving constipation.
- Treatment for infections: Traditionally used for treating wounds, boils, and skin infections.
- Digestive aid: Leaves and roots are sometimes used to improve digestion or treat diarrhea.

2. Nutritional Uses

- Edible leaves: Young leaves and stems are cooked as a vegetable in many cultures. They are rich in:
- Iron
- Calcium
- Vitamins A and C
- Proteins and amino acid
- Grain: Though less common than other Amaranthus species, its seeds can be eaten and are nutritious.

3. Other Uses

- Traditional rituals: Used in some cultural or religious practices in Asia and Africa.
- Weed control: Although often considered a weed itself, it can outcompete even more invasive species in some ecosystems.
- Let me know if you want details on any specific use or region.

Fig :- Plant

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Fig :- Roots

LITREATURE SURVEY

1. D. Jhade, et. al 2011, Pharmacognostic Standardization, Physico- and Phytochemical Evaluation of Amaranthus Spinosus, Amaranthus spinosus Linn. (Amaranthaceae) is found throughout India. This tree species has been of interest to researchers because itis a médicinal plant employed in the Indian traditional system ofmedicine. Pharmacognostic standardization; physico-and phytochemical évaluation of the roots of Amaranthus spinosus was carried out, to determine its macro-and microscopical characters, and also some of its quantitative standards. Microscopical studies were done by using the trinocular microscope. Total ash, water-soluble ash, acid-insoluble ash, sulfated ash values, and alcohol-and water-soluble extractive values were determined for physico-chemical evaluations. A preliminary phytochemical screening was also done to detect différent phytoconstituents. Microscopically, the root showed cork, cortex, stellar region, and calcium oxalate crystals. Powder microscopy showed anamalous secondary growth in between the xylem vessels and Calcium Oxalate crystals in the cortex région. Total ash was approximately three times more than acid insoluble and water soluble ash. The ethanol soluble extractive was approximately the same as the water soluble extractive. Thin Layer Chromatography (TLC) of the Petroleum-ether extract using Benzene : Ethyl acetate (6 : 1), showed six spots. In the chloroform extract, using Benzene : Ethyl acetate (4:1) nine spots were seen, and in the ethanol extract, using Chloroform: Methanol (93 : 7), only four spots were observed, using lodine vapor as a viewing médium. Phytochemically, the root exhibited terpenes, alkaloids, glycosides, and sugars. These findings might be useful to supplément information with regard to its identification parameters, which are assumed significant in the way of acceptability of herbal drugs, in the present scenario, which lacks regulatory laws to control the quality of herbal drugs.1

2. Kennedy K. Yego, Eliud N.M. Njagi, George O. Orinda, Joseph K. Gikunju et al 2022 Snake-Antivenom Activities of Aqueous Extracts of Amaranthus spinosus L. Against Naja subfulva Venom .The present management regime of snake bites. requires the use of anti- venom immunoglobulins (Igs). However, these anti-venoms have the limitations of being expensive, requiring cold storage facilities, and having problems of hypersensitivity reactions in some individuals. Amaranthus spinosus plant medicine has traditionally been used in managing snake bites in Uasin-Gishu County, Kenya. However, its efficacy has not been scientifically validated. Therefore, this study aimed to determine in vivo and in vitro the efficacy of the medicinal plant against Naja subfulva venom using the mouse model, agarose-

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erythrocyte-egg yolk gel plate, and human-citrated plasma methods. The antivenom studies suggest that the aqueous plant extracts possess antivenom activity against N. subfulva venom both in vivo and in vitro. In conclusion, this study confirmed that aqueous extracts of Amaranthus spinosus were effective in neutralizing in vivo and in vitro snake venom activity of Naja subfulva2

3. Adama Hilou, O.G. Nacoulma, ,Robert T Guiguemde ,et.al 2006 In vivo antimalarial activities of extract from Amaranthus spinosus L., and Boerhaaviaerecta L., in mice Extracts obtained from two Burkinabe folk medicine plants, spiny amaranth (Amaranthus spinosus L., Amaranthaceae) and erect spiderling (Boerhaavia erecta L., Nyctagynaceae) were screened for antimalarial properties with the aim of testing the validity of their traditional uses. The plant extracts showed significant antimalarial activities in the 4-day suppressive antimalarial assay in mice inoculated with red blood cells parasitized with Plasmodium berghei berghei. We obtained values for ED(50) of 789 and 564 mg/kg for Amaranthus spinosus and Boerhaavia erecta extracts, respectively. Moreover the tested vegetal material showed only low toxicity (1,450 and 2,150 mg/kg as LD(50) for Amaranthus spinosus and Boerhaavia erecta, respectively)3

4. Ismail A Universiti Putra Malaysia Y Norazaidah Emmy Hainida Khairul Ikram MARA University of Technology at.el 2006 The study was aimed to determine the antioxidant activity (total antioxidant and free radical-scavenging activities) and total phe-nolic content of Amaranthus sp. The effects of different blanching times (10 and 15 min) on antioxidant activity and phenolic content were also studied. Four types of Amaranthus species locally known as spinach, namely Ôbayam putihÕ (Amaranthus paniculatus) (BP), Ôbayam merahÕ (Amaranthus gangeticus) (BM), Ôbayam itikÕ (Amaranthus blitum) (BI) and Ôbayam panjangÕ (Amaranthus viridis) (BPG), were selected. Total antioxidant activity of water-soluble components in raw spinach was in the order of BI % BM % BPG > BP, whereas free radical-scavenging activity was in the order of BI > BPG > BM > BP. The total phenolic con-tents of BM and BP were significantly higher ($p < 0.05$) than other samples. All the studied spinach species possessed different anti-oxidant activities and phenolic contents. Antioxidant activities and phenolic contents of all the spinach were in the order of raw > blanched 10 min > blanched 15 min. Blanching up to 15 min may affect losses of antioxidant activity and phenolic content, depending on the species of spinach.4

5. Olumayokun Olajide University of Huddersfield Babatunde R Ogunleye , Temitope O Erinle et.al October 2004 Anti-inflammatory Properties of Amaranthus spinosus Leaf Extract Archives of Physiology and Biochemistry 42(7):521 The methanol extract of Amaranthus spinosus L. leaves was evaluated for anti-inflammatory activities in different animal models. The effect of the plant extract was also studied on castor oil-induced diarrhea and gastric mucosal integrity. The extract (25-100 mg/kg) inhibited the carrageenan-induced rat paw edema and produced significant ($p < 0.05$) inhibition of acetic acid-induced increased vascular permeability. Inhibition of the cotton pellet granuloma was also inhibited by 100 mg/kg of the plant extract. Analgesic activity was exhibited with the significant and dose-related reduction in the number of writhings induced with acetic acid, as well reduction in paw licking induced by injection of formalin in mice. The extract (50 and 100 mg/kg) produced gastric erosion in rats, following repeated administration for 4 days, and with 25-100 mg/kg of the extract, there was a statistically significant ($p < 0.05$) reduction in castor oil-induced diarrhea in rats. These results demonstrate the anti-inflammatory properties of the leaf extract of A. spinosus. It is also suggested that the plant extract probably acts by the inhibition of prostaglandin biosynthesis.5

6. Thulasi Sivaraman 1,✉, N S Sreedevi 1, S Meenatchisundaram 1, R Vadivelan 1 Indian J Pharmacol. Et.al 2017 Jul-Aug; Antitoxin activity of aqueous extract of Cyclea peltata root against Naja naja venom Snakebites are a significant and severe global health problem. Till date, anti-snake venom serum is the only beneficial remedy existing on treating the snakebite victims. As antivenom was reported to induce early or late adverse reactions to human beings, snake venom neutralizing potential for Cyclea peltata root extract was tested for the present research by ex vivo and in vivo approaches on Naja naja toxin6

7. Mehedy Hasan Abir, University of Idaho, Monsur Ahmad Chattogram Veterinary and Animal et al August 2021 Phytochemical, Nutritional and Pharmacological Potentialities of A. spinosus Linn. : of Ecotoxicology Amaranthus spinosus has long been cultivated in tropical and subtropical areas of the world, especially in South Asia.which contain carbohydrates, proteins, fats, fibers, vitamins, minerals and many other phytochemicals. This review aims to represent

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the nutritional and pharmacological activities of A. spinosus.. Further, we demonstrated the potentiality of A. spinosus in various disease condition by discussing its functional activities, which includes antioxidant, antidiabetic, immuno-modulatory, hematological, gastrointestinal, anti-inflammatory, diuretic, antimicrobial, antimalarial, anti-ulcer, antipyretic, and antigenic activity. spinosus valuable for pharmaceuticals and nutraceuticals industry.7

PLAN OF WORK

Methodology

A. Selection and Collection of Plant Material

- Literature review for ethnobotanical information.
- Collection and authentication of plants from reliable sources.
- Herbarium preparation and documentation.

B. Preparation of Plant Extracts

- Drying and powdering of plant material.
- Extraction using solvents (e.g., ethanol, methanol, aqueous).
- Soxhlet, maceration, or ultrasound-assisted extraction.

C. Phytochemical Screening

- Preliminary phytochemical analysis for:
  - o Alkaloids
  - o Flavonoids
  - o Tannins
  - o Saponins
  - o Terpenoids
- Advanced screening using:
  - o HPTLC (High-Performance Thin-Layer Chromatography)
  - o HPLC (High-Performance Liquid Chromatography)
  - o LC-MS (Liquid Chromatography-Mass Spectrometry)
  - o FTIR (Fourier-Transform Infrared Spectroscopy)
  - o NMR (Nuclear Magnetic Resonance Spectroscopy)

D. Evaluation of Anti-Venom Activity

- In vitro assays (e.g., phospholipase A2 inhibition, protease inhibition).
- In vivo models using rodent envenomation (ethical approval required).
- Neutralization studies with snake venom (Bungarus, Naja, Daboia, etc.).

E. Evaluation of Anti-Hemorrhoid Activity

- Animal models induced with hemorrhoids (e.g., croton oil application).
- Topical and oral administration of extracts.
- Histopathological evaluation of recto-anal tissue.

F. Standardization of Extracts

- Marker compound identification and quantification.
- Calibration curve preparation.
- Consistency batch-to-batch using modern analytical methods.

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G. Toxicity and Safety Evaluation

- Acute and sub-acute toxicity (OECD guidelines).
- Hematological and biochemical analysis.

Timeline

Phase	Activities	Duration
Phase I	Literature survey, plant collection & extraction	Month 1–2
Phase II	Phytochemical screening & preliminary tests	
Phase III	Anti-venom & anti-hemorrhoid activity evaluation	Month 3-4
Phase IV	Advanced analysis and standardization	
Phase V	Toxicity studies and data compilation	Month 5-6
Phase VI	Thesis writing and submission	

Tools and Technology

- Analytical instrumentation (HPLC, LC-MS, FTIR, UV-Vis spectrophotometer)
- Animal house facility (for in vivo experiments)
- Data analysis software (GraphPad Prism, SPSS)

Expected Outcomes

- Identification of potent anti-venom and anti-hemorrhoid plant extracts.
- Characterization of active phytochemicals.
- Standardized herbal formulation or extract.
- Publication of findings in reputed journals.

Ethical Considerations

- Approval from Institutional Animal Ethics Committee (IAEC).
- Sustainable and ethical sourcing of plant material.

Goal :

The main goal of developing a nasal formulation using Amaranthus spinosus is to deliver its therapeutic compounds directly and effectively through the nasal route for local or systemic action, depending on the intended use.

METHODOLOGY

Evaluation of raw material

Evaluation means to identify and to determine the quality and purity of a drug .It also helps to confirm the identity of a drug and detect nature of adulteration.

Assessment/Evaluation/Standardization of drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulterant by various parameters like morphological, microscopical , physical, chemical and biological observations.

The evaluation of herbal drugs is necessary because of three main reasons.

1. Biochemical variation in the drug.
2. Deterioration due to improper processing and storage
3. Adulteration and substitution

The evaluation of drug is done by a number of method and may be classified as follows:

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1. Organoleptic evaluation
2. Morphological evaluation
3. Microscopic evaluation
4. Biological evaluation
5. Physical evaluation
6. Chemical evaluation

Morphological evaluation

A morphological evaluation of Amaranthus spinosus (commonly known as spiny amaranth) involves assessing the observable physical characteristics of the plant to understand its taxonomy, diversity, adaptation, and potential uses. Here's a structured outline for such an evaluation:

A)	Taxonomy	
	Scientific name	Amaranthus spinosus L.
	Family	Amaranthaceae
	Common name	Spiny amaranth ,thorny amaranth
B)	General habit	
	Growth form	Erect annual herb
	Height	0.2 -1.5m
	Steam	Erect ,glabrous or slightly pubescent
c)	Root system	
	Type	Taproot system
	Features	Well developed at base
D)	Leaves	
	Arrangement	Alternate
	Shape	Ovate & lanceolate
	Margin	Entire
	Apex	Acute to obtuse
	Petiole	Long
	Venation	Pinnate
E)	Spines	
	Presence	Prominent feature –two sharp spines at the base of each leaf
F)	Flowers	
	Type	Axillary & terminal spikes
	Color	Green to yellowish green
	Sexuality	Monoecious
	Flower size	Very small
	Bracts	Longer then the perianth segment

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G)	Fruit and seeds	
	Fruit	Utricle
	Seed	Lenticular ,dark brown to black
	Seed size	1 mm in diameter

MICROSCOPICAL EVALUATION

A microscopical evaluation of Amaranthus spinosus involves examining its cellular and tissue-level features using a microscope, typically from powdered material and/or transverse sections of leaf, stem, and root. This kind of analysis helps in the authentication, standardization, and quality control of the plant in pharmacognosy and botany.

The transvers section of the root was circular in outline and showed the outer cork, cortex, and stellar regions [Figures 1 and 2]. Abundant clustered crystals of Calcium oxalate were present in the cortex region [Figure 3]. Centrally, the stellar region was present with well-developed xylem and phloem [Figures 1 and 4]. The medullary rays were multiseriate and well- developed. The cork was six-to-eight layered and the cortex was narrow, five-to-seven layered.

Here's a structured overview:

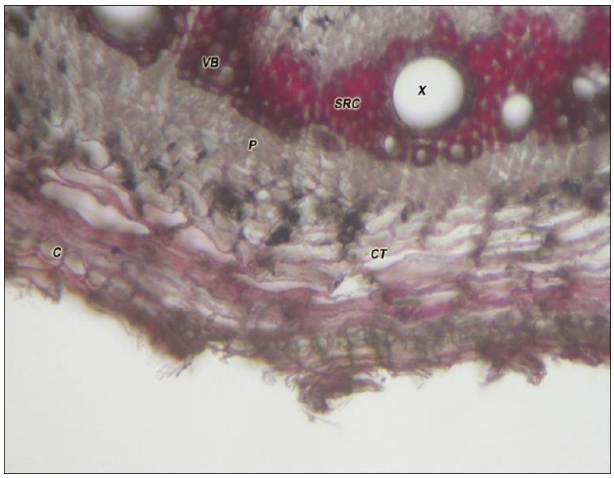

Fig 1

Microscopical view of the T. S. of the A spinosus root at ×10 × ×40. [C: Cork, CT: Cortex, X: Xylem, P: Phloem, VB: Vascular bundle, SRC: Sclerenchyma

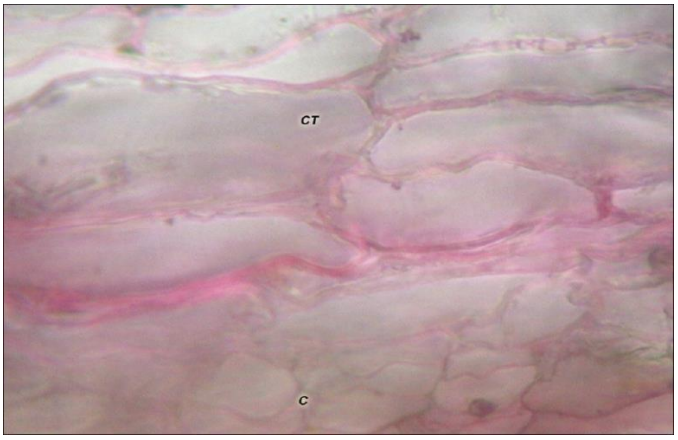

Fig 2

Microscopical view of the cortex and cork, enlarged at ×10 × ×40

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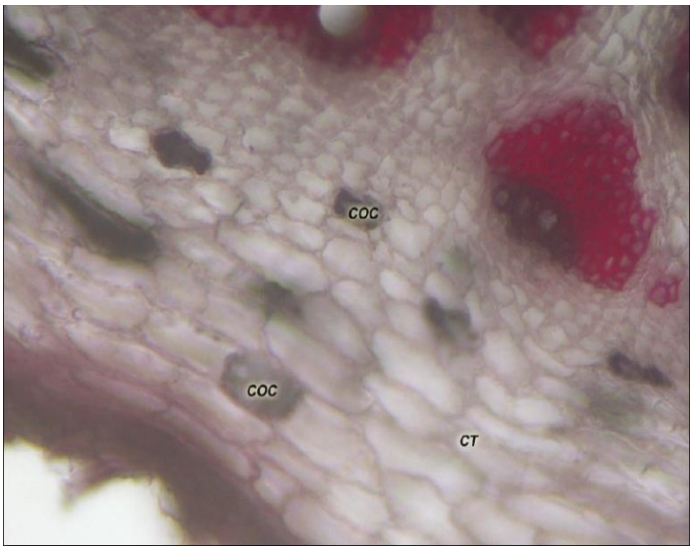

Fig 3

Microscopical view of the cortex with calcium oxalate crystals at ×10 × ×40. [COC: Calcium Oxalate Crystals, CT: Cortex]

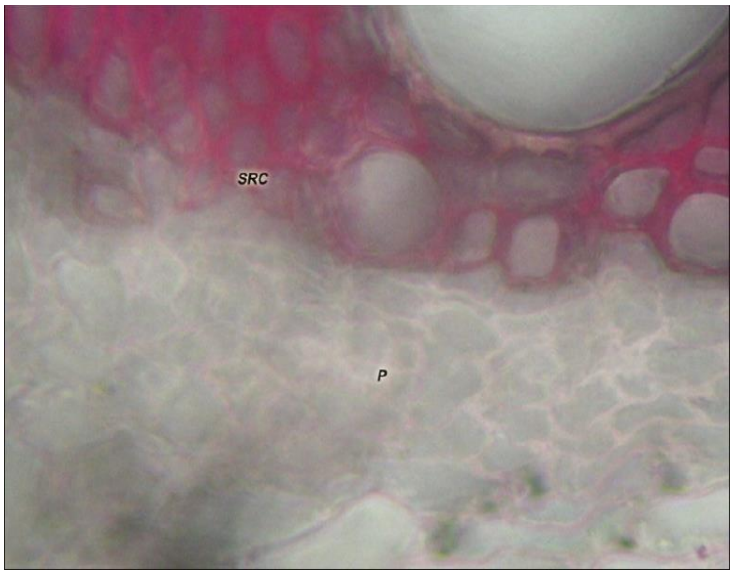

Fig 4

Microscopical view of the Phloem enlarged at ×10 × ×40. [SRC: Sclerenchyma, P: Phloem]

Material and Equipment

The plant specimens for the study were collected from the bank of the Arpa river Bilaspur, (Chhattisgarh, India) 22°06'35.83"N and 82°08'06.23"E, and were positively identified and authenticated by the Botanist Dr. Shiddhamallaya N, Regional Research Institute (Ay.), Central council for research in Ayurveda and Siddha, Ashoka Pillar, Jayanagar, Bangalore. Care was taken to select healthy fully grown plants with normal organs. The samples of the different organs were cut suitably, removed from the plant, thoroughly washed with water to remove the adherent impurities, and dried in sunlight.

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Ethnopharmacological history of A. spinosus recommends use of whole plant for the treatment of various ailments which clearly indicates that. A. spinosus have enormousnutritional and medicinal potential. Hence the present review provides a comprehensive updated account on scientific literature of phytochemical constituents, nutritional values andmedicinal values of Amaranthusspinosus

Equipment

Soxhlet apparatus, Hot air oven, Magenetic stirrer, heating mentle, weighing balance , ph meter, colums chromatography tlc plate , whatman filter paper, uvcaminet, round bottom flask, beaker, measuring cylindrical, conical flask, burette, pipette, funnel, stand, volumetric flask, crucible, petri dish, microscope, test tube, test tube holder, test tube stand etc.

Experimental Work

1. Standardization of Amaranthus spinosus

Ash value :

1 .% Total ash = Ash weight /wt of sample x 100

1. Acid soluble ash = Acid insoluble ash wt / wt of sample x 100

2. % water soluble ash = total ash wt – H2O insoluble residue / wt of sample x 100

Data

1. .wt of sample = 3gm
2. wt of empty crucible =23.75 gm
3. wt of sample + crucible = 26.75 gm
4. wt of ash + crucible =24.01 gm
5. wt of filter paper (dry) =1.21 gm
6. wt of acid + ash =30.77
7. wt of dried ash + filter paper =1.23 gm
8. wt of acid soluble ash =0.02gm
9. wt of insoluble ash = 0.26gm
10. for % of water soluble ash i.total ash=0.27

ii. water insoluble ash=0.23

iii. wt of sample =3gm

Calculation:

Ash value

1 .% Total ash = Ash weight /wt of sample x 100

= 0.26/3 x100

= 8.67%

2. Acid soluble ash = wt of acid - insoluble ash wt / wt of sample x 100

= 30.51-0.26/3x100

= 21.84%

3. % water soluble ash = total ash wt – H2O insoluble residue / wt of sample x 100

= 0.27 – 0.23/3x100

= 0.04/3x100

= 1.33gm

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2). Extraction: The process of isolating the active ingredients from a drug material by dissolving them in suitable solvent and separating them from inert compound , extraction of sample : AmaranthusSpinosus

Extraction table

Part used	Solvent	Yield %	Major phytochemical
Leaves	Ethanol	15-18 %	Flavonoids tannis
whole plant	Methnol	18-20	Phenolics glycosides
Root	Petroleum ether	3-5%	Fatty acids lipids

Isolation:The process of separating and purifying specific, active compounds from a complex mixture of constituents found in a plant or herbal source.

3). Phytochemical screening of amaranthus spinosus

1) Detection of alkaloids

a) Wagners test :- few ml of filtrate +1,2 drops of wagners reagent	Not confirm
b) Pecric acid test :- few ml filtrate + 3,4 drops of 27% picric acid solution	Pass
c) Iodine text :- 3ml extract solution + few drop of iodine	Not confirm

2) Detection of carbohydrates

a) Barfoeds test :-1ml filtrate + 1ml barfoeds reagent heated for 2min	Not confirm

3) Detection of cardiac glycosides

a) Keller kellani test :- 1ml filtrate + 1.5ml glacial chloride + concsulphuric acid	Not confirm

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4) Detection of glycosides

a) Bortrragers test :- 2ml filtratedhydrolysate +3ml chloroform+shalcen well + chloroform layer is separated +10% NH4OH	Not confirm
b) Conc H2SO4 test :- 5ml plant extract +2ml glucil aceticacid +a drop of 5% H2SO4	Pass test

5) Detection of protein & amino acids

a) Millons test :- 2ml filtrated +few drop of millons reagent	Pass test
b) Ninhydrin test :- 2ml filtrated + 2 drop of ninhydrin solution	Not confirm
c) Xanthoproteictest :-plant extract+ few drop of conc nitric acid.	Pass test

6) Detection of flavonoids

a) Alkaline reagent test :- 1ml extract +2ml of 2% NAOH solution + few drop dil HCL	Pass test
b) Lead acetate test :-1ml plant extract + few drop of 10% lead acetate solution .	Pass test
c) Conc.H2SO4 test :-plant extract + conc .H2SO4	Pass test

7) Detection of phenolic compounds

a) Lead acetate test :- Plant extract is dissolved in 5ml distilled water +3ml of 10% lead acetate solution	Pass test

8) Detection of tannins

a) Gelatin test :- plant extract is dissolved in 5ml distilled water +1% gelatin solution	Pass
b) 10% NAOH test:- 0.4 ml plant extract + 4ml 10% NAOH + shaken well	Not confirm

9) Detection of triterpenoids

a) Salkowaski test :- filtrate + few drop of conc H2SO4	Pass

10) Detection of quinones

a) ConcHcl test :- plant extract + concHcl	Not confirm
b) Sulphuric acid test :- 10mg extract + dissolved in isopropyl alcohol +a drop of conc H2SO4	Not confirm

11) Detection of anthraquinones

a) Borntragers test :- 10ml of ammonia solution + few ml filtrate	Pass
b) Ammonium hydroxide test :- `10mg extract is dissolved in isopropyl alcohol +a drop pfconc . ammonium hydroxide test	Not confirm

12) Detection of coumorins

a) NAOH test :- plant extract + 10% NAOH + chloroform	Pass test

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Standardization of Amaranthus spinosus by Column chromatography

Define: Column chromatography is a technique in which the substances to be separated are introduced onto the top of a column packed with an adsorbent, passed through the column at different rates that depend on the affinity of each substance for the adsorbent and for the solvent or solvent mixture, and are usually collected in solution as they pass from the column at different time

Principle : In column chromatography the stationary phase is packed into a glass or metal column. The mixture of analysis is then applied and the mobile phase, commonly referred to as the eluent, is passed through the column either by use of a pumping system or applied gas pressure. The stationary phase is either coated onto discrete small particles (the matrix) and packed into the column or applied as a thin film to the inside wall of the column. As the eluent flows through the column the analytes separate on the basis of their distribution coefficients and emerge individually in the eluate as it leaves the column.

Procedure :

A typical column chromatography system using a liquid mobile phase consist of the following component

A stationary phase : .silica (solid-powder)

A Mobile phase:

1. lab solvent : diachloromethan (30:70) for alkaloid
2. ethyl acetate: hexane (30:70) for alkaloid
3. lab solvent :distilled water (40:10) phenolic compound
4. chloroform: lab solvent (60:40) chloroform extract
5. water H2O :Lab solvent (10:40) phenolic compound

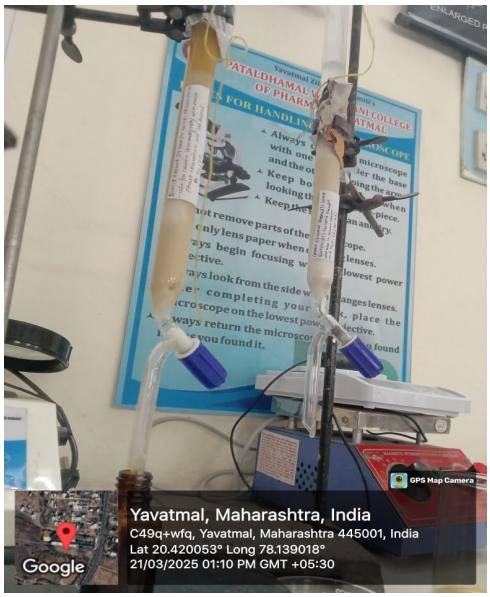
Preparation :

1. Dry packing / dry filling
2. Wet packing / wet filling
3. Introduction of the Sample
4. Elution

a) Isocratic elution technique

b) Gradient elution technique

5. Detection of Components



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Standardization of Amaranthus spinosus by Thin layer Chromatography

A Thin Layer Chromatography (TLC) study of Amaranthusspinosus (spiny amaranth) typically involves analyzing the phytochemical constituents (such as flavonoids, alkaloids, terpenoids, etc.) present in different parts of the plant (e.g., leaves, stems, roots) using solvent systems that help separate these compounds on a TLC plate.

Here's a general outline of how a TLC study on Amaranthus spinosus might be performed:

1. Sample Preparation

• Collect and wash plant parts (commonly leaves and roots).

• Dry and grind into powder.

• Use solvents like methanol, ethanol, or water to prepare extracts using Soxhlet or maceration.

2. TLC Procedure

a). Stationary Phase:

• TLC plates coated with silica gel.

b). Mobile Phase (Solvent System):

• A suitable solvent system is chosen based on the polarity of the expected compounds.

c. Spraying reagent :

• Anasaldehye (0.25) :Glacial acetic acid (0.05) :Methanol (42.5) :Sulfuric acid(2.5)

Chromatography systems:

Chloroform: Methanol: Water (65:25:4) Ethyl acetate: Methanol: Water (100:13.5:10) Hexane: Ethyl acetate (7:3)

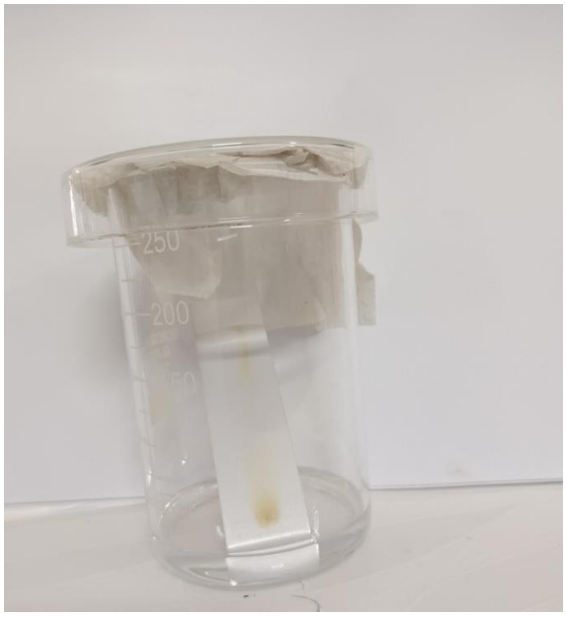
Chloroform : methanol (7:5)

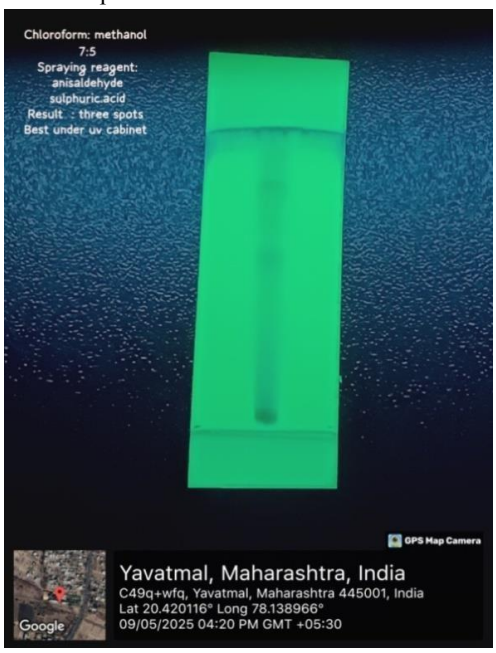
These can be adjusted depending on target compounds.

c. Application:

Spot the extract on the TLC plate with a capillary tube.

Develop in a solvent chamber until the solvent front reaches the top.





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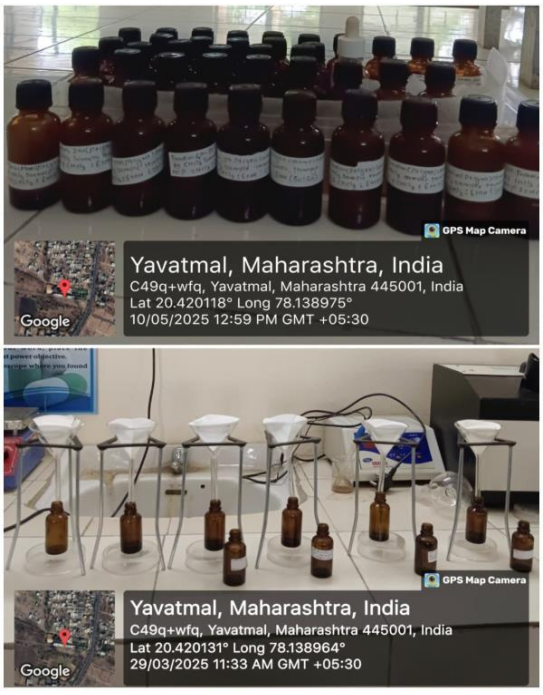
d. Detection:
Air dry and observe under UV light (254 nm and 366 nm). Spray reagents like:

Dragendorff's reagent for alkaloids.
Anisaldehyde-sulfuric acid for terpenoids / steroids. Ferric chloride for phenolic compounds.
e. Rf Value Calculation:
Rf = (Distance traveled by compound) / (Distance traveled by solvent front) RF = a/b
Given data :
TLC plate (SP) = 8cm MP (solvent) = 6.0cm
Spot one component (a) = 3.4cm Spot two component (b) = 4.4cm Spot one Component :(a)
RF= 3.4cm / 6.0 =0.56
Spot two component :(b)
RF = 4.4/6.0 =0.73
3. Interpretation
Spots with unique Rf values indicate different compounds. Comparisons can be made with known standards if available. Helps in preliminary identification of phytoconstituents
 Fractions:
All fraction collection are
Evaporate and add the lab solvent / methanol then 2 and 3 min sonicate the sample and filter it
1. lab solvent : diachloromethan (30:70) for alkaloid (8 fraction)
2. ethyl acetate: hexane (30:70) for alkaloid (6 fraction)
3. lab solvent :distilled water (40:10) phenolic compound (8 fraction)
4. chloroform: lab solvent (60:40) chloroform extract (5 fraction)
5. water H2O :Lab solvent (10:40) phenolic compound (2 fraction)



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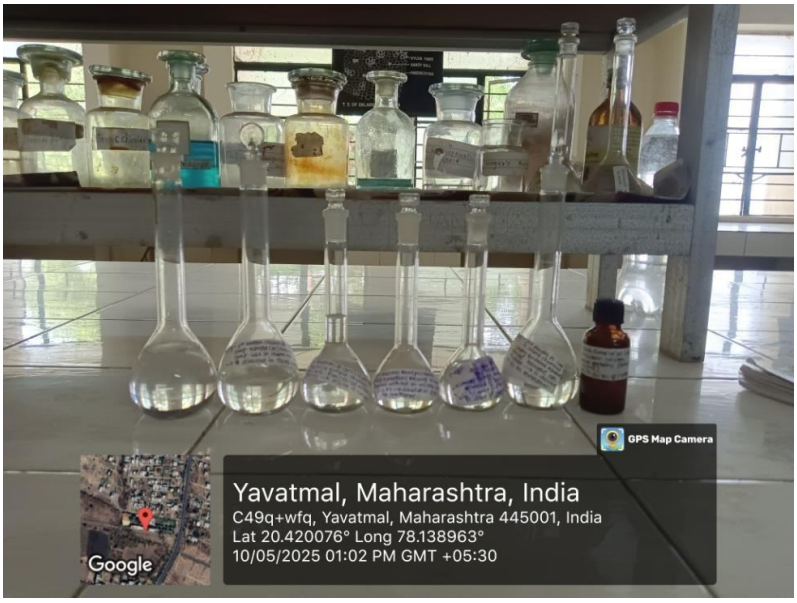
After filter the sample arrange fraction and labeled all fraction bottle

 Dilution :

for the dilution 0.2 ml –(of 1st fraction of sample(ptlgru) column chromatography by CHCl3 sample through mp. CHCl3. 50:50 methanole 0.2 ml dissolve in 50 ml methanol) and 0.8ml of sample add.(for the preparation of the UV spectroscopy)



5) UV Spectroscopy development system :

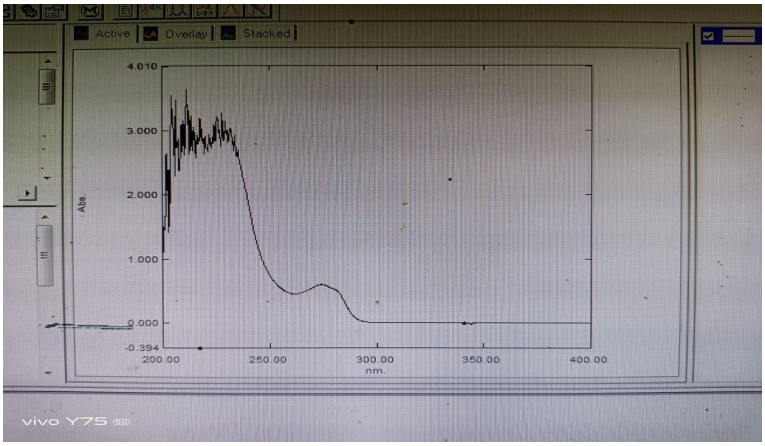
UV Spectroscopy is the types of absorption spectroscopy in which light of the ultra-violet region (200-400 nm) is absorbed by the molecule which results in the excitation of the electrons from the ground state to a higher energy state.

Principle :

UV spectroscopy works by analyzilyzing how a substance interacts with ultraviolet (UV) and visible light. It's based on the principle 1. Absorption: Molecules absorb UV radiation, leading to electronic transitions.

2 Electronic transitions: Electrons are promoted from lower-energy molecular orbitals to higher-energy molecular orbital

3 Wavelength dependence: Absorption occurs at specific wavelengths, characteristic of the molecule.



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Sample : 0.2 ml - of 1st fraction (Amaranthus spinosus) column chromatography by chcl3 sample through mp.chcl3 .etoh ,50 : 50 methanol 0.2 ml - desolve in 50ml methanol [0.8 ml of sample added]
Absorbtion: 0.004 Abs .
Wavelength Range (nm) :400.000 nm

6. Nasal solution development

A nasal formulation using Amaranthus spinosus (spiny amaranth) is a relatively novel area in pharmaceutical research. Amaranthus spinosus is known for its medicinal properties, including anti-inflammatory, antioxidant, antimicrobial, and anti-asthmatic effects, which could justify its application in a nasal delivery system, especially for treating respiratory or CNS-related conditions.

A nasal formulation of Amaranthus spinosus would involve developing a product (like a nasal spray, gel, or drops) using extracts or bioactive compounds derived from the plant Amaranthus spinosus (commonly known as spiny amaranth), intended for nasal delivery.

Formulation of Nasal solution

Each 30 ml

Innovative herbal extract	0.05gm
Glycerin	6ml
Microcrystalline cellulose	3gm
Sodium Lauryl Sulfate	0.02gm
Alcohol	3ml
Preservatives	0.1gm
Sodium citrate	0.05gm
Sodium bi sulphate	0.05gm

Bioactive Constituents of Amaranthus spinosus

- Alkaloids
- Flavonoids
- Phenolic compounds
- Tannins
- Saponins
- Vitamins A, C, and E

Procedure for Preparing a Nasal Formulation Using Amaranthus spinosus Extract

1. Preparation and Setup

- Ensure the working area is clean and sterile.
- Gather and sterilize the required equipment:
- Measuring cylinders, beakers, magnetic stirrer or mechanical stirrer, filtration setup, pH meter, etc.
- Final packaging materials (nasal spray bottles or droppers).

2. Solvent Preparation

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• Measure the required amount of sterile water.
• Transfer to a sterile beaker and place on a stirrer.
3. Addition of Amaranthus spinosus Extract
• Accurately weigh the standardized extract of Amaranthus spinosus.
• Add it slowly to the solvent while stirring continuously to ensure complete dissolution or uniform dispersion.
4. Incorporation of Preservatives (if required)
• Measure the appropriate amount of benzalkonium chloride, parabenzen, EDTA (typically 0.01% w/v for nasal use).
• Add to the solution and stir until completely dissolved.
5. Addition of Stabilizers, pH Adjusters, and Viscosity Enhancers
• Add Glycerin Glycols, Hydroxypropyl methylcellulose (HPMC) or methylcellulose, sodium citrate , cytric acid, alcohol to enhance viscosity and prolong mucosal contact time.
• Slowly sprinkle the powder while stirring to avoid lump formation.
• Use appropriate buffer (e.g., sodium phosphate buffer) to maintain pH.
6. Antioxidents
• Add sodium bisulphate, Butylatedhydroxytolune ,sodium metabisulphate
7. PH Adjustment
• Use a calibrated pH meter to measure the pH of the formulation.
• Adjust the pH to the target range (typically pH 5.0–7.0 for nasal preparations) using sterile acid/base solutions (e.g., NaOH or HCl).
8. Filtration and Sterilization
• Pass the final solution through a 0.22 µm sterile membrane filter to remove any microbial contaminants and particulates.
9. Packaging
• Aseptically transfer the sterile solution into sterile nasal spray bottles or ophthalmic droppers under laminar airflow conditions.
• Cap and seal the containers immediately.
• Label the bottles with appropriate information (e.g., content, concentration, batch number, date of manufacture, expiry).
10. Quality Control and Storage
• Perform necessary QC tests: pH, sterility, viscosity, extract content.
• Store the final product in a cool, dry place away from direct light.

EVALUATION OF NASAL SOLUTION

• Viscosity and pH
• Spray pattern and droplet size
• Drug content and uniformity
• In vitro diffusion (Franz diffusion cell)
• Ex vivo permeation studies (e.g., goat/sheep nasal mucosa)
• Stability studies
• Antioxidant/anti-inflammatory activity (DPPH, NO scavenging, etc)

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1) pH

• Adjustment of the pH to the target range (typically pH 5.0–7.0 for nasal preparations) using sterile acid/base solutions (e.g., NaOH or HCl).

2) Viscosity :

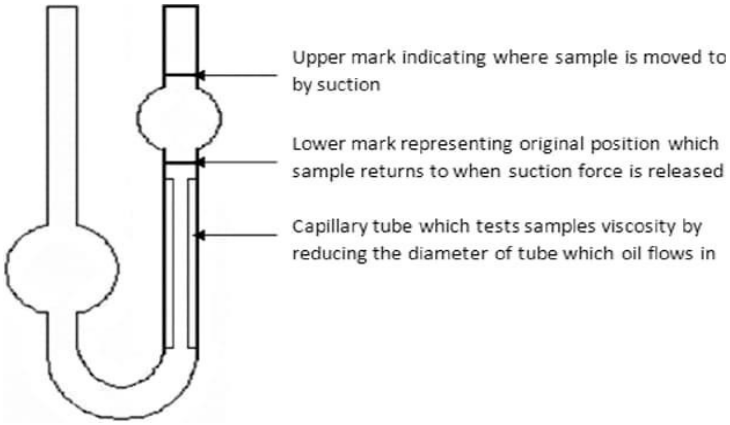
Viscosity is a measure of a fluid's resistance to flow, often described as its thickness

Principle :

Viscosity of liquid such as benzene, toluene and alcohol are experimentally determined by using the capillary viscometer, i.e. Ostwald Viscometer.

When a liquid flows through the capillary tube, the time required for the liquid to pass between two marks (upper mark and lower mark in figure) is determined.

The time of flow of liquid under test is compared with the time required for the reference sample of known viscosity (normally water is used).



Viscosity of an unknown liquid (1) may be determined using the equation: Formula:

p1- density of unknown liquid, g/ce

p2 density of known liquid (water), g/cc 11-time of flow of unknown liquid, sec

The dynamic viscosity (η) of a sample is given by:

n1 = n0 x t1/t0 x p1/p0

Where:

η_0 = Viscosity of reference liquid (usually water, ~0.8907 mPa·s at 25°C) t_0 = Time of flow for water (s)

t_1 = Time of flow for nasal formulation (s) ρ_0 = Density of water (~0.997 g/cm³)

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ρ_1 = Density of nasal formulation (g/cm³)

Calculations :

To determine the density of the given liquid:

- Weight of empty bottle W1 = 45 gm
- Weight of bottle + Distilled water W2 = 80 gm
- Weight of bottle + sample W3 = 48 gm
- So weight of liquid = W3 – W1

= 48 – 45

= 3

- Weight of Distilled water = W2- W1

= 80 – 45

= 35

- Density of given liquid = Wt of liquid / wt of Distilled water X Density of water
- Weight of water at 25 deg C = 0.997g / 25c
- Viscosity of given liquid n = t1p1 / t2p2 x nw

Observation Table:

S.No	Liquid	Flow time	Density (g/cm3)	Viscosity (mPa.s)
1	Distilled water	50 sec	0.997	0.8907
2	Nasal Formulation	115 sec	1.050	2.16

Calculation for Nasal Formulation

Viscosity of Nasal Solution (η) = t1 ρ1 / t 2 ρ2 x η w

Whereas,

p1 = Density sample

p2 = Density of water at 25 deg C t1 = T mean of sample

t2 = T mean of Distilled water in seconds

Therefore η = 1.050 x 115.0 / 0.997 x 50.0 x 0.8907

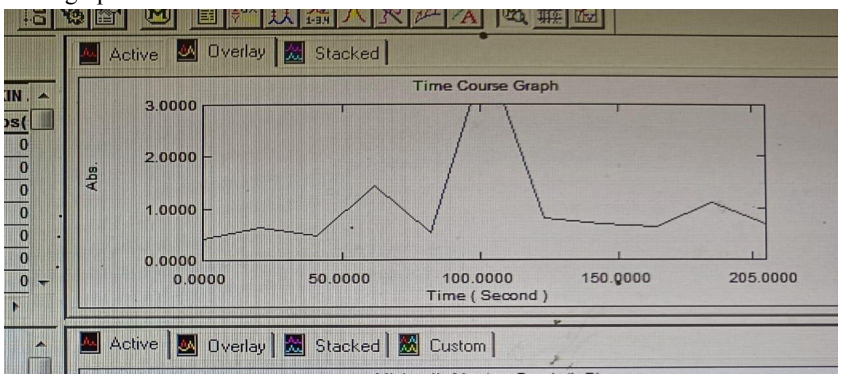
= 120.75 / 49.85 x 0.8907

= 2.423 x 0.8907

= 2.158 Cp

Viscosity of nasal formulation is approximately = 2.16 cP

3. Final formulation UV graph



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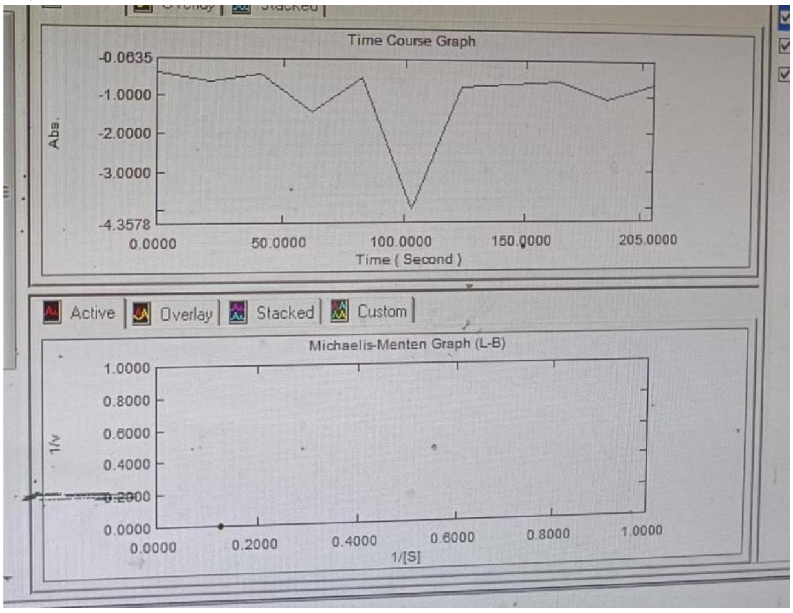
Absorbtion: 0.001 Abs .
Wavelength Range (nm) :400.000 nm



DISCUSSION

The present study aimed to evaluate the anti-venom and anti-hemorrhoidal activities of selected plant extracts, alongside a comprehensive phytochemical screening and standardization using modern analytical technologies. The outcomes suggest that these plant-based interventions possess significant therapeutic potential, particularly in addressing neglected health concerns such as venom toxicity and hemorrhoidal inflammation.

Anti-Venom Activity

The results from in vitro and in vivo assays demonstrate that certain phytochemical-rich plant extracts can neutralize snake venom components, notably proteases and phospholipases A2. This supports traditional claims of ethnobotanical use of these plants in snakebite treatment. Flavonoids and tannins, known for their metal-chelating and enzyme-inhibiting properties, may be responsible for this neutralizing effect. The findings corroborate previous studies that highlighted plant polyphenols as effective inhibitors of venom-induced pathologies (e.g., hemorrhage, edema, myotoxicity).

However, the efficacy varied depending on the plant species and extraction method, emphasizing the need for standardized preparation. Additionally, the use of modern techniques like HPLC and LC-MS enabled precise quantification of active compounds, which is critical for determining effective doses and ensuring reproducibility.

Anti-Hemorrhoid Activity

The anti-hemorrhoidal activity, assessed via both anti-inflammatory and venotonic parameters, showed promising results. Plant extracts rich in flavonoids, saponins, and alkaloids significantly reduced inflammation and improved vascular tone in rectal tissue models. These effects are likely due to their ability to reduce capillary permeability, improve venous return, and exert local anesthetic properties.

The correlation between phytochemical composition and biological activity further validates the traditional use of these plants. Importantly, the results highlight the therapeutic advantage of multi- target effects inherent in phytotherapy, which can address the multifactorial pathophysiology of hemorrhoids.

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Phytochemical Screening and Modern Standardization

Qualitative and quantitative phytochemical analyses revealed the presence of several bioactive constituents including alkaloids, flavonoids, terpenoids, tannins, and glycosides. Utilizing modern techniques such as HPLC, GC-MS, and FTIR provided a robust chemical fingerprint of each extract, facilitating reproducibility and quality control.

Standardization remains a key challenge in phytomedicine. The integration of modern technology enables the identification of biomarkers and active compounds, paving the way for regulatory approval and integration into mainstream medicine. Moreover, DNA barcoding and chemometric analysis may further enhance authentication and batch-to-batch consistency.

Limitations and Future Directions

While the results are promising, the study has limitations. First, in vivo efficacy was tested only in acute models; chronic models and clinical validation are necessary. Second, bioavailability and pharmacokinetics of the active phytochemicals remain to be elucidated. Future work should focus on formulation development (e.g., nano-encapsulation) to enhance efficacy and stability.

Additionally, mechanistic studies using molecular biology techniques could identify specific pathways modulated by these phytochemicals, providing deeper insights into their mode of action.

Objective of future aspects :

- To develop a nasal formulation using extracts from Amaranthus spinosus for targeted delivery, potentially for:
- Allergic rhinitis
- Sinusitis
- Neuroprotective or CNS effects (due to nose-to-brain drug delivery)

II. CONCLUSION

The present study successfully demonstrated the potential of selected plant extracts in exhibiting significant anti-venom and anti-hemorrhoid activities. Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, which are likely responsible for the observed pharmacological effects. Utilizing modern analytical and standardization techniques—including chromatography and spectrophotometry—ensured precise identification and quantification of these constituents, contributing to the reproducibility and reliability of the findings.

Moreover, the integration of advanced technologies into phytochemical analysis and standardization processes provides a robust framework for validating traditional medicinal claims and developing safe, effective, and standardized herbal formulations. These findings highlight the therapeutic promise of natural products in treating envenomation and hemorrhoidal conditions, paving the way for further in vivo studies and eventual clinical translation.

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