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REVIEW ON PHYTOCHEMICAL DETERMINATION AND ANTIVENOM ACTIVITY OF ANDROGRAPHIS PANICULATA

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ABSTRACT

Snakebite envenomation remains a major global health challenge, particularly in tropical regions, where limited accessibility, high cost, and adverse reactions associated with conventional antivenom therapy underscore the need for safer and more effective complementary treatments. *Andrographis paniculata* (Burm. f.) Nees, a medicinal plant widely used in traditional medicine systems, is rich in bioactive phytochemicals—especially diterpenoid lactones such as andrographolide, neo andrographolide, and 14- deoxyandrographolide—that exhibit diverse therapeutic properties. This study focuses on the phytochemical determination and antivenom potential of *A. paniculata*, exploring its role as an alternative or adjunct therapy for snakebite management. Phytochemical profiling reveals significant concentrations of diterpenoids and flavonoids, compounds known for strong antioxidant, anti-inflammatory, and enzyme-inhibitory activities. Evidence from existing in- vitro and in-vivo research demonstrates that extracts of *A. paniculata* can inhibit key venom enzymes—including phospholipase A_2, hyaluronidase, acetylcholinesterase, and proteases— thereby mitigating venom-induced oxidative stress, inflammation, edema, haemorrhage, and tissue necrosis. Additionally, the plant shows potential to enhance the efficacy of commercial antivenom by reducing toxin spread and improving systemic protection. Overall, *A. paniculata* emerges as a compelling natural candidate for developing plant-based antivenom strategies and improving outcomes in venom-related pathologies.

KEYWORDS: Andrographis paniculata, andrographolide, antivenom activity.

INTRODUCTION

Snakebite envenomation is a major public health concern in numerous tropical and tropical regions, particularly in Asia, Africa, and Latin America. The World Health Organization recognizes snakebite as a neglected tropical complaint, with millions of cases and thousands of deaths being annually. Envenomation leads to complex pathologies similar as coagulopathy, neurotoxicity, cytotoxicity, original towel necrosis, inflammation, and oxidative stress. These goods are caused by a admixture of venom factors, including phospholipases A_2, proteases, hyaluronidases, and cytotoxins. Although elixir serum remedy remains the standard treatment, it has notable limitations high cost, defined vacuity in pastoral areas, threat of acuity responses, and inadequate effectiveness against venom- convinced original towel damage. These challenges have encouraged experimenters to explore factory- grounded natural products as reciprocal or indispensable remedial agents.[1]

Traditional medical systems have long employed medicinal shops for treating colorful poisons and poisonous mouthfuls. Andrographis paniculata (Burm. f.) Nees, generally known as “king of bitters,” is a medicinal factory extensively used in traditional Ayurvedic, Unani, and Southeast Asian herbal systems. The factory is rich in different phytochemical ingredients, particularly diterpenoid lactones similar as andrographolide, neo andrographolide, and 14- deoxy andrographolide, which are responsible for its broad diapason of pharmacological conditioning. expansive exploration has demonstrated the factory ‘Santi-inflammatory, anti- oxidant, anti-venom, anti-cancer, anti-diabetic, anti-bacterial, anti-

microbial, anti-pyretic, anti- helminthics, anti-malaria, anti-fertility, hepatoprotective, gastrointestinal, and immunomodulatory parcels, pressing its eventuality as a remedial resource. Their strong free- radical scavenging capability, enzyme- inhibitory eventuality, and anti-inflammatory goods give a scientific base for probing the factory's implicit part in neutralizing venom- convinced toxin.[2]

In recent times, interest has grown in exploring factory-grounded druthers or adjuncts to conventional antivenoms because antiserum curatives frequently face limitations similar as high cost, cold- chain conditions, adverse responses, and limited neutralization of original towel damage. Banes from medically important snakes induce complex pathologies involving proteolytic enzymes, phospholipases, hyaluronidases, and poisons that spark oxidative stress, inflammation, hemolysis, and necrosis. Due to its bioactive composites with strong antioxidant and enzyme- inhibitory parcels, A. paniculata has surfaced as a promising seeker for molarity venom-convinced systematic and original venom.[3]

Phytochemical determination of Andrographis paniculata is essential for relating and quantifying its active ingredients responsible for remedial goods. Detailed phytochemical profiling helps link specific composites to natural conditioning similar as enzyme inhibition, membrane stabilization, and antioxidant action. These mechanisms are applicable to elixir exertion because venom- convinced pathologies involve oxidative stress, proteolytic declination, and seditious responses. probing the antivenom eventuality of A. paniculata not only supports the scientific confirmation of traditional medicinal uses but also offers a foundation for developing safer, factory- grounded remedial agents to round or enhance current snakebite operation strategies. former studies have suggested that factory excerpts rich in antioxidants and enzyme impediments may incompletely neutralize snake venom factors or reduce the inflexibility of venom- convinced towel damage.

Andrographis paniculata[4,5,6]

Synonyms: Kalmegh**,** King of bitter, Green Chiretta, Indian Echinacea.

Biological Source: It is obtained from the whole plant of herbaceous species of Andrographis paniculata (Burm. f.) Nees.

Family: Acanthaceae.

Chemical Constituents: The main chemical constituents of Andrographis paniculata are diterpenoid lactones, such as andrographolide, neo andrographolide and 14-deoxyandrographolide. This plant also contains flavonoids.

Taxonomic Classification[7,8]

Kingdom	Plantae
Phylum	Magnoliophyta (Angiosperms)
Class	Magnoliopsida (Dicotyledons)
Order	Lamiales
Family	Acanthaceae
Genus	Andrographis
Species	Andrographis paniculata

Therapeutic Uses

Neutralization Of Lethality: In beast studies, the excerpts have been shown to significantly increase the survival time of mice challenged with murderous boluses of snake venom, though they may not help death entirely when used alone.

Enzyme Inhibition: The factory's active ingredients, similar as andrographolides and flavonoids, work by modifying or inhibiting the conduct of poisonous venom enzymes, including.

Phospholipase A2(PLA2): This enzyme causes wide towel damage, neurotoxicity, and cardiotoxicity; A. paniculata effectively inhibits its exertion.

Hyaluronidase: This enzyme acts as a" spreading factor" for venom within the victim's body; inhibition helps decelerate the systemic spread of poisons.

Acetylcholinesterase (Ache): Inhibition of this enzyme is particularly applicable to negativing the neurotoxic goods of cobra venom, which can lead to respiratory palsy.

Protease, Phosphomonoesterase, And L- Amino Acid Oxidase: Other poisonous enzymes present in snake venom are also inhibited.

Amelioration Of Original Goods: The excerpts help to neutralize original pharmacology goods of envenomation, similar as haemorrhage(bleeding), edema(lump), and coagulation abnormalities.

Adjuvant Therapy: Research suggests that A. paniculata excerpt may be most effective when used as a supplement(adjuvant) to conventionality-snake venom (ASV) serum. Combining the factory excerpt with lower boluses of ASV can enhance the overall effectiveness and potentially reduce the quantum of ASV demanded, which is important because conventional ASV can beget severe side goods like anaphylactic shock and serum sickness in about 20 of cases.

Current Status: While traditional healers have long used A. paniculata for snakebites, it's presently the

subject of ongoing scientific study, primarily in in- vitro and in- vivo(beast) models. farther mortal clinical studies are needed to completely understand its implicit and medium of action for use in mortal cases. The World Health Organization (WHO) has not yet championed any herbal product as a relief for conventional ASV.[9,10]

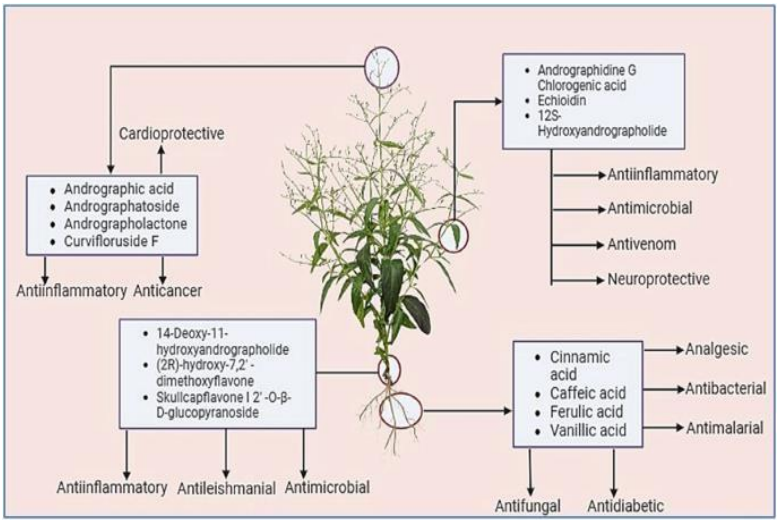

Figure 1: Andrographis paniculata (pharmacological effect).

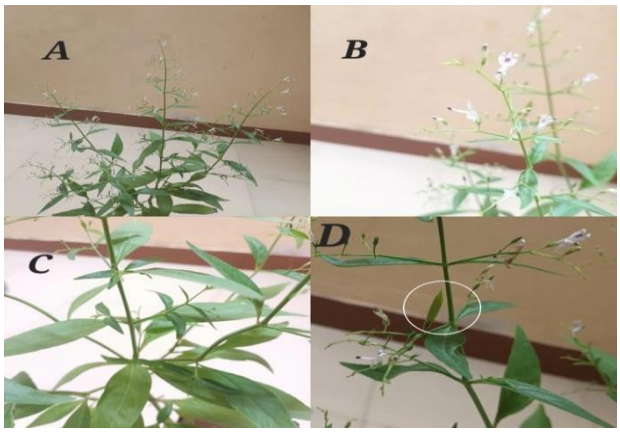

Figure 2: Andrographis paniculata (A- whole plant, B-Flower, C-Leaves & D-Fruit).

Plant Morphology[11,12]

1. Habit

A. paniculata is an erect, annual, herbaceous species attaining 30–110 cm in height. The plant exhibits a simple to moderately branched habit, with branching more pronounced in open, high- light environments.

2. Root System

It possesses a well-developed taproot with several lateral rootlets. Roots are slender, pale brown, and moderately woody at maturity. The root system is efficient in nutrient uptake from poor soils, supporting its adaptability.

3. Stem

The stem is quadrangular (four-angled), a typical feature of many Acanthaceae members. It is green, glabrous, and slightly swollen at nodes. Internodes are long and smooth, while the basal region becomes sub-woody in later stages. The stem's bitter latex contains diterpenoid lactones, including andrographolide.

4. Leaves

Leaves are simple, opposite, and decussate.

- **Shape:** Lanceolate to ovate-lanceolate
- **Size:** 2-8 cm *1-3 cm
- **Margin:** Entire
- **Apex;** Acute to acuminate
- **Surface:** Thin, glabrous, dark and green
- Leaves posses a strong bitter taste and are a rich source of bioactive compounds, including andrographolide and related diterpenes.

5. Inflorescence

Inflorescences are terminal and axillary panicles; a defining characteristic reflected in the species epithet *paniculata*. The panicles are slender, elongated, and bear numerous small flowers. Flowering typically occurs from late monsoon to winter, depending on regional climate.

6. Floral Morphology

Flowers are small, zygomorphic, and bisexual.

- **Calyx:** Five narrow, lanceolate sepals

- **Corolla:** Tubular and bilabiate; predominantly white with purplish-violet spots or streaks on the lower lip
- **Androecium:** Two stamens, often with hairy anthers
- **Gynoecium:** Superior ovary, bicarpellary and syncarpous; slender style with bifid stigma

The floral structure supports entomophilous pollination, primarily by small insects.

7. Fruit

- The fruit is a linear-oblong capsule, measuring 1.5–3.0 cm in length. It is initially light green and turns yellowish-brown upon maturation. The capsule dehisces longitudinally along both sutures to release seeds.

8. Seeds

- Seeds are numerous, minute, yellow-brown, and subquadrate. They exhibit a slightly wrinkled surface and lack elaborate dispersal structures. Seed viability is highest soon after maturity, and germination is epigeal.

Pharmacological Activities[13,14,15]

1. Anti-inflammatory activity

- Andrographolide (a diterpene lactone) is a key anti-inflammatory agent.
- pathways (e.g., NF-κB) and reduces production of pro-inflammatory cytokines.
- Extracts of *A. paniculata* (aqueous, methanol, etc.) have shown anti-inflammatory effects in animal models (e.g., carrageenan-induced paw edema).

2. Antioxidant activity

- Flavonoids and diterpenoid lactones in the plant contribute to its antioxidant properties.
- In experimental models, *A. paniculata* enhances antioxidant defenses by increasing the activity of enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase, and by scavenging free radicals.
- These antioxidant effects support its hepatoprotective (liver-protecting) and possibly neuroprotective roles.

3. Hepatoprotective effects

- Several studies (in vitro & in vivo) show that *A. paniculata* protects the liver from toxic insult (e.g., CCl_4-induced liver damage).
- Mechanism: its antioxidant activity reduces oxidative stress in liver cells; andrographolide is considered a major contributor.

4. Immunomodulatory / Immunostimulant activity

- *paniculata* extracts stimulate both innate (e.g., macrophages) and adaptive immune responses.
- Andrographolide has been shown to modulate immune cell functions, possibly enhancing immune defences, but might also suppress over-activation in some contexts.

5. Antimicrobial (Antibacterial, Antiviral, Antiparasitic) activity

- Antibacterial: Extracts from *A. paniculata* leaves show activity against bacteria like *E. coli*, *Staphylococcus aureus*, *Salmonella*, etc
- Antiviral / Antiretroviral: The plant and andrographolide exhibit antiviral effects in some studies.
- Anti plasmodial / Antimalarial: There is reported activity against *Plasmodium* species; in one study, whole-plant extracts helped reverse drug resistance in malaria parasites.

6. Anticancer / Cytotoxic activity

- Andrographolide shows cytotoxicity against various cancer cell lines (colon, breast, glioblastoma, etc.).
- Mechanisms include induction of apoptosis, cell cycle arrest, inhibition of proliferation, and modulation of signalling pathways.
- There is also interest in structural derivatives of andrographolide to improve efficacy / reduce toxicity.

7. Antipyretic (Fever-reducing) and Analgesic (Pain-relieving) activity

- Extracts of A. paniculata (chloroform, methanol, aqueous) reduced fever in animal models.
- Analgesic effects have been demonstrated in experimental models (e.g., hot-plate, acetic-acid writhing, etc.)

8. Cardioprotective / Antithrombotic

- Some compounds from *A. paniculata* inhibit platelet aggregation. For example, 14- deoxy-11,12-didehydroandrographolide can inhibit thrombin-induced platelet aggregation.
- This suggests potential cardiovascular benefits, though more mechanistic and clinical research is needed.

9. Antidiabetic / Hypoglycaemic Activity

- Ethanol extracts of *A. paniculata* have been shown to reduce fasting blood glucose in diabetic animal models.
- It may also improve antioxidant status in diabetic subjects (reducing oxidative damage in liver & kidney) as part of its antidiabetic mechanism.

10. Neuroprotective Activity

- There is evidence for *A. paniculata* (and andrographolide) having neuroprotective effects: protecting against neurotoxicity, possibly via anti-inflammatory and antioxidant mechanisms.
- Some studies also suggest modulation of neurotrophic factors (e.g., TrkB) in neuronal injury

contexts.

11. Anti-venom / Antitoxic Effects

- Traditional uses include snake-bite treatment. Some pharmacological studies support antivenom or protective effects, though detailed mechanisms are less well-defined.
- Also, protective effects against other toxins (organ damage) are reported due to its antioxidant and anti-inflammatory actions.

12. Fertility / Reproductive Effects

- Some reports suggest *A. paniculata* or its constituents may have antifertility effects. b
- However, these effects are complex, and more research is needed to understand the safety and mechanism.

13. Nephroprotective (Kidney-protecting) Activity

- There is some evidence (from traditional and experimental studies) that *A. paniculata* can protect renal tissue, possibly via reducing oxidative stress and inflammation.

Extraction Process[16,17,18,19]

1. Plant material & pre-processing (standard)

- Harvest aerial parts (leaves ± flowering tops) at flowering stage, wash, shade-dry (or oven at ≤40°C) then grind to coarse powder (mesh #40–60). Record moisture content and voucher specimen. (Common practice in AP studies; yields and andrographolide content vary by plant part and harvest time).

2. Choice of solvent and general principles

- Andrographolide and related diterpenoids are moderately polar; common solvents: water, methanol, ethanol, ethyl acetate, dichloromethane. Methanol/ethanol (70–100%) and mixtures are widely used for high recovery of andrographolide. Non-polar solvents (hexane) for defatting prior to polar extraction.

3. Representative extraction methods

(a) Cold maceration (simple)

- Solid: solvent ratio: 1:10 to 1:20 (w/v). Macerate 24–72 hrs at room temp with agitation; filter and concentrate under reduced pressure. Suitable for screening and for thermolabile compounds. Example: dichloromethane: methanol 1:1 maceration used for andrographolide isolation.

(b) Soxhlet/ hot continuous extraction

1. Plant Preparation

- Shade-drying or oven-drying at 40–50°C
- Grinding to fine powder (40–60 mesh)

2. Extraction Setup

- 2–20 g powdered sample placed in a thimble
- Solvent volume: 100–300 mL depending on apparatus size
- Common solvents: ethanol, methanol, hydroalcoholic mixtures (50–70%).

3. Extraction Process

- Heating solvent to reflux
- Continuous siphoning cycles (6–10 hours in most studies)
- Hot extraction at solvent boiling point ensures efficient extraction of andrographolide

4. Post-Extraction

- Concentration using rotary evaporator
- Drying under reduced pressure
- Calculation of yield
- HPLC quantification of andrographolide.

(c) Ultrasonic-assisted extraction (UAE)

- Faster, non-thermal; commonly reported for andrographolide. Typical: 20–60 min, 20– 40 kHz bath or probe, solvent methanol/ethanol, solid: solvent 1:10–1:30, temp ≤50°C. Enhances yield and reduces time compared with maceration.

(d) Microwave-assisted extraction (MAE) / UAE-MAE hybrid

- Short times (minutes), good yields for andrographolide; optimize microwave power, time (30–180 s), solvent polarity. Useful for green extraction strategies.

(e) Supercritical CO_2 extraction (SFE) & pressurized liquid extraction (PLE)

- SFE is tunable and solvent-free (for nonpolar to moderately polar fractions with co- solvents). PLE (accelerated solvent extraction) delivers fast, reproducible extraction but requires instrumentation.

4. Fractionation & isolation

- Typical workflow: crude extract → liquid-liquid partition (n-hexane, chloroform/CH_2Cl_2, ethyl acetate, n-butanol, water) → column chromatography (silica gel; gradient hexane→EtOAc→MeOH) → preparative HPLC for pure andrographolide. Rapid isolation methods using DCM: MeOH 1:1 maceration + column/HPLC have been published.

5. Standardization / QC — quantification of andrographolide

- RP-HPLC (UV detection ~223 nm or 254 nm), HPTLC and LC-MS are routinely used. Example RP-HPLC mobile phases: water: acetonitrile (60:40) or chloroform: methanol mixes depending on method; andrographolide used as marker for standardization and reporting (mg/g extract). Report LOD/LOQ, linearity, recovery.

Medicinal Values[20]

1. Traditional Uses

- In Ayurvedic and other traditional systems, *A. paniculata* has been used for fever, diarrhoea, dyspepsia, dysentery, and as a liver tonic.
- Used for wounds, skin diseases, and insect/snake bites.
- Folk medicine also employs it for upper respiratory infections, loss of appetite, and general debility (e.g., convalescence after fevers).
- In some systems, it's used as a vermifuge (anti-helminthic)
- Also used traditionally for hypertension, diabetes, muscle pains, sinus issues, and skin sores.

2. Experimental uses

Based on modern research, *A. paniculata* has shown a variety of bioactivities. Below are some of the key ones:

Therapeutic Area	Evidence/ mechanism
Anti-microbial / Anti-infective	Exhibits antibacterial activity against *E. coli, B. subtilis, P. aeruginosa*, etc. Also, anti-protozoal (e.g., antimalarial) activity.
Anti-malarial	Extracts / andrographolide inhibit *Plasmodium* species; traditional use against malaria is backed by some experimental data.
Anti-inflammatory	Andrographolide and other compounds reduce inflammatory mediators (cytokines), showing anti-inflammatory effects.
Antioxidant	Extracts show antioxidant properties in vitro.
Immunomodulatory	Acts on immune system: stimulates immune responses, possibly via cytokine modulation.
Hepatoprotective (Liver-protective)	Several animal studies show liver protection; traditional use also includes liver stimulant / detox.
Antidiabetic / Hypoglycaemic	Some animal studies: e.g., ethanol leaf extract reduced blood glucose in diabetic models.

3. Safety, Dosage, and Clinical Use

- Clinical studies have used *A. paniculata* extract for respiratory infections (e.g., common cold) with some benefit.
- Doses in studies vary: for osteoarthritis, ~150–300 mg standardized extract (50% andrographolide) twice daily for 3 months was used.
- For ulcerative colitis: some trials have used 1200–1800 mg/day in divided doses.
- Safety concerns: possible side effects include gastrointestinal issues, and there is caution for use in pregnancy / lactation.
- In animal studies, very high doses affected male fertility by reducing sperm count and impacting hormones.
- Quality control: Andrographolide is often used as a chemical marker for standardization of herbal preparations.

4. Molecular / Mechanistic Insights

- Major active constituent: andrographolide, a diterpenoid lactone, is responsible for many pharmacological effects.
- The lactone ring in diterpenoids (like andrographolide) seems crucial for binding to target proteins.
- Mechanistically, it modulates inflammatory pathways (e.g., NF-κB), oxidative stress pathways, and immune signalling.
- Also studied for multi-target interactions (network pharmacology) and docking to various disease-relevant targets.

CONCLUSION

Andrographis paniculata (Burm. f.) Nees, widely known as the "king of bitters," is a medicinal plant with a rich phytochemical profile, predominantly comprising diterpenoid lactones such as andrographolide, neo andrographolide, and 14-deoxyandrographolide, along with flavonoids. Extensive pharmacological investigations reveal its potent anti-inflammatory, antioxidant, immunomodulatory, hepatoprotective, antimicrobial, anticancer, antidiabetic, neuroprotective, and cardiovascular-protective effects. Importantly, A. paniculata demonstrates significant potential in mitigating snake venom- induced toxicity. Its bioactive constituents can inhibit venom enzymes, including phospholipase A_2, hyaluronidase, acetylcholinesterase, and proteases, thereby reducing local tissue damage, systemic toxicity, oxidative stress, and inflammatory responses. Animal studies suggest that the plant extract can enhance survival, ameliorate venom-induced pathologies, and serve as an effective adjuvant to conventional antivenom therapy, potentially lowering the required dose of anti-snake venom serum and reducing adverse reactions. Phytochemical determination and detailed mechanistic studies highlight the strong correlation between specific bioactive compounds and their pharmacological activities, providing a scientific basis for traditional claims of snakebite management. While *in vitro and in vivo* studies are promising, further well-designed clinical trials are necessary to validate the safety, efficacy, and standardization of A. paniculata as a complementary or alternative therapy for snakebite envenomation. Overall, *Andrographis paniculata* emerges as a valuable natural therapeutic candidate with multifaceted pharmacological actions, offering a promising adjunct in the development of safer, plant-based antivenom strategies.

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