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LEAP OF RELIEF: FROG BASED EXPLORATION OF HERBAL ANALGESIA THROUGH POLYALTHIA LONGIFOLIA

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Abstract:
The current study looks at the analgesic potential of Polyalthia longifolia bark extract and compares its efficacy to the standard medicine acetyl salicylic acid, utilising Rana tigrina (Indian bullfrog) as an experimental model. The bark extract of Polyalthaia. longifolia was produced using appropriate solvent extraction procedures and delivered in graduated quantities. Analgesic activity was determined by measuring the frog's response to nociceptive stimuli such as acetic acid (aspirin)-induced pain reflexes and thermal stimuli using established pharmacological techniques. The results showed that Polyalthia longifolia bark extract significantly reduced pain responses in a dose-dependent manner. At greater doses, the impact was comparable to aspirin, indicating the presence of analgesic-like bioactive chemicals. The results indicate that Polyalthia longifolia bark has promising analgesic potential.It could be used as a natural alternative or supplement to conventional nonsteroidal anti-inflammatory medicines (NSAIDs). More research into the separation and characterisation of active ingredients is needed to understand the underlying mechanism of action.

Keywords: Polyalthia longifolia, analgesic activity, aspirin,Rana tigrina, Bark extract, phytochemical screening.

FIGURES 1,2,3

INTRODUCTION:

Plant-derived medicines have been used since ancient times, and according to the World Health Organization, over 80% of the global population—particularly in developing countries—relies on traditional plant-based remedies for primary healthcare needs. Medicinal plants are considered nature's gift, playing a crucial role in maintaining health, and India remains one of the most culturally rich regions where such practices continue to be respected and widely used. Traditional medicine is an evolving body of knowledge, with communities constantly adapting and discovering new therapeutic techniques.

Despite technological progress, drug discovery still faces an innovation gap, making natural products and ethnopharmacology important sources for identifying new lead compounds. Polyalthia longifolia (Annonaceae), locally known as "Ashoka," is native to the drier parts of India and commonly cultivated across India, Pakistan, and Sri Lanka. Although it is mainly grown as an ornamental tree, its bark is often used as an adulterant for the true Ayurvedic Ashoka, Saraca asoca, due to its easy availability.

India's rich ethnomedical and Ayurvedic traditions utilize many valuable plants whose pharmacological actions are yet to be fully validated through modern scientific methods.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage pain, inflammation, and fever, but their gastrointestinal side effects demand safer alternatives. In this context, Polyalthia longifolia, traditionally used as an antipyretic agent, has attracted scientific interest; its stem contains a lactone with antibacterial activity, and various parts of the plant exhibit analgesic, anti-inflammatory, and vasodepressant properties. Although the plant is widely used in traditional medicine for fever, its antipyretic action has not been extensively studied. Considering the presence of active constituents such as alkaloids, clerodane diterpenes, quercetin, bulbocapnine, β-sitosterol, stigmasterol, campesterol, enihalimane diterpenes, and sesquiterpenoids, an effort has been made to investigate the analgesic, anti-inflammatory, and antipyretic potential of the ethanolic extract of Polyalthia longifolia bark

METHOD OF PREPARATION

To prepare Ashoka bark powder extract for animal testing, the general procedure is as follows:

1. Collection and Drying:

- Collect fresh Ashoka (polyalthia longifolia) bark. Wash thoroughly with distilled water to remove dirt.
- Shade-dry the bark at ambient temperature away from direct sunlight until completely dry.
- Once dried, grind the bark into a coarse or fine powder using a mechanical grinder or mortar and pestle.
- Sieve the powder through a mesh (e.g., #40) for uniform particle size.


Figure 4


Figure 5

2. Extraction:(Traditional method)

- For aqueous extract: Suspend the powdered bark in distilled water or 80% alcohol (spirit-water mix) at a suitable ratio (e.g., 1:5 w/v)
- Alternatively, perform Soxhlet extraction using solvents such as ethanol, methanol, or acetone for 24-36 hours at controlled temperatures (50-75°C)
- Filter the extract through Whatman no. 1 filter paper or equivalent to remove solid residues.


Figure 6

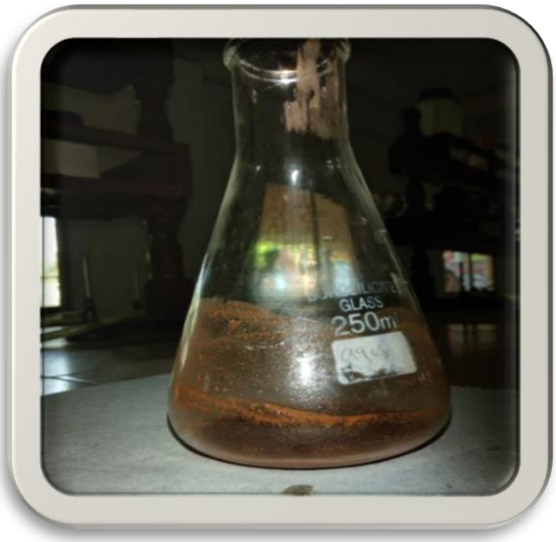


Figure 7

3. Concentration and Drying:

- Concentrate the filtrate under reduced pressure by gentle heating until it becomes thick at below 50°C to avoid loss of active constituents.
- Further dryness can be achieved by incubating the concentrated extract at 37°C or freeze drying
- - Store the dried extract in airtight containers at 4°C until use.

Figure 8

4. Preparation for Animal Testing:

- Dissolve or suspend the dried extract in a suitable vehicle such as distilled water, saline, or a minimal amount of DMSO for oral or subcutaneous administration. - Prepare fresh extract solutions at desired dosages (e.g., 50, 100, and 250 mg/kg body weight) right before administration

Figure 9

Figure 10

This method preserves phytochemical integrity (alkaloids, tannins, flavonoids) which are responsible for analgesic activity observed in experimental models. It is widely accepted for pharmacological testing in animals including frogs and rodents

This preparation allows standardized dosing and reproducible results for evaluating analgesic effects of Ashoka bark powder in animal tests

IDENTIFICATION TESTS FOR CHEMICAL CONSTITUENTS

FOR FLAVONOIDS

- **Alkaline reagent test**

Procedure: Add 1 mL 10% NaOH to extract, then a few drops HCl.
Positive: Yellow color with NaOH that turns colorless on acidification.

Figure 11

- **Lead acetate test**

Reagent: 10% lead acetate.
Positive: Brown precipitate.It indicates phenolic flavonoids

. Figure 12

FOR ALKALOIDS

- **Mayer's test**

Reagent: Mayer's reagent (K_2HgI_4 solution).
Procedure: To 2 mL extract (acidified with 2% HCl), add few drops Mayer's reagent.
Positive: Creamy/white precipitate

Figure 13

- **Dragendorff's test**

Reagent: Dragendorff's reagent (potassium bismuth iodide).
Procedure: Acidify extract, add Dragendorff's reagent.
Positive: Orange-red precipitate.

Figure 14

TANNINS

- Ferric chloride **test**

Reagent: 5% $FeCl_3$.
Procedure: Add to aqueous extract.
Positive: Blue-black (hydrolyzable tannins) or greenish black (condensed tannins).

Figure 15

- **Gelatin test (lead acetate optional)**

add a 1% gelatin solution (often containing 10% NaCl) to the plant extract; a positive result for flavonoids is the formation of a white precipitate.

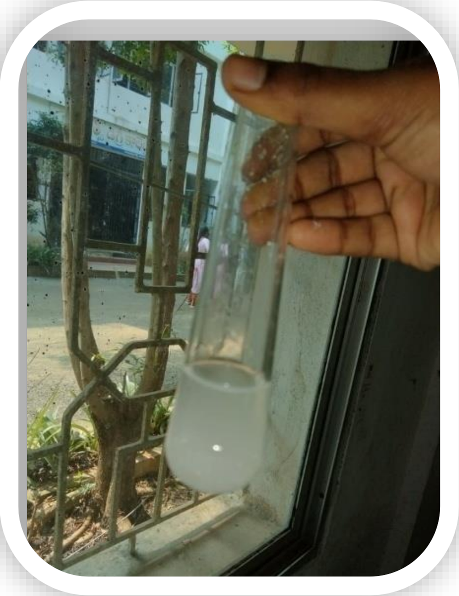


Figure 16

Procedure for Collection of Frogs

1. **Selection of Site:**
   - Frogs are usually collected from clean ponds, tanks, or paddy fields where they are abundant.
   - The area should be free from chemical pollution or sewage contamination.
2. **Time of Collection:**
   - Frogs are most easily collected in the evening or early morning, as they are more active during cool and humid conditions.
3. **Equipment Required:**
   - Hand nets or butterfly nets for catching.
   - Plastic or muslin bags or containers with small air holes for carrying frogs.
   - Gloves (for handling safely).
   - Identification chart (to ensure correct species, usually *Rana tigrina* / *Hoplobatrachus tigerinus* for laboratory use).

Figure 17

Collection Method:

- Approach the frog quietly and slowly to avoid alarming it.
- Quickly cover the frog with a net and lift it carefully.
- Transfer it gently into a container with moist grass or leaves to maintain humidity.

Transportation:

- Transport the frogs to the laboratory immediately after collection.
- Keep them in a cool, shaded area to prevent dehydration or heat stress.

Care After Collection:

- Place the frogs in large aquaria or glass troughs containing clean water.
- Provide sufficient aeration and keep the environment moist.
- Frogs should be used within a few days for experiments and handled humanely following ethical guidelines.

Ethical Considerations:

- Frog collection and use must follow Institutional Animal Ethics Committee (IAEC**)** and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) regulations.
- Ensure minimum pain and distress to the animals.
- Obtain necessary permission before collection and experimentation

Figure 18

PROCEDURE FOR TESTING

1. Selecting healthy frogs (e.g., Rana tigrina, 100-150 g).
2. Preparing Ashoka bark powder extract or suspension for administration.
3. Administering the test substance (Ashoka bark powder) either orally or via subcutaneous injection to the frog.
4. Using a behavioral observation setup such as a glass flask with a porous platform to house the frog during testing.
5. Inducing pain stimulus by injecting 4% NaCl subcutaneously in the lower abdominal area of the frog, which causes a measurable nociceptive response.
6. Measuring analgesic effect by counting the number of eye blinks and buccal oscillations before and after administration of Ashoka bark powder. A decrease in these responses indicates analgesic activity.
7. Alternatively, noxious thermal limb withdrawal tests or acetic acid tests (AAT) can Be used. The thermal test involves applying heat to the hind limb and measuring withdrawal latency, while AAT involves applying mild acetic acid drops to the skin and observing a wiping response.
8. Comparing responses in treated animals to controls (saline or untreated) to quantify analgesic efficacy.

Figure 19.

Figure 20

Design a frog nociception assay comparing bark powder aspirin

A frog nociception assay comparing bark powder, aspirin, can be designed based on the principles of chemical-induced nociceptive testing such as the acetic acid-induced writhing test or the formalin test adapted for amphibians. The following design reflects standard analgesic assay approaches with clear comparisons to aspirin (peripheral analgesic)

Experimental Design

1. Animals:
- Adult frogs (e.g., Rana pipiens or Rana tigrina), 100-150 g.
- Acclimate frogs in controlled environment.

2. Groups:
- Control group: vehicle-treated (saline or distilled water).
- Bark powder extract group: dose such as 100 or 250 mg/kg orally or subcutaneously.
- Aspirin group: standard dose, e.g., 50 mg/kg.
- Number of animals per group: ideally 6-8.

3. Preparation:
- Prepare bark powder extract by aqueous or ethanolic extraction.
- Dissolve aspirin in appropriate vehicles.

Figure 21

4. Nociceptive Stimulation:
- Use formalin test adapted for frogs:
- Subcutaneous injection of dilute formalin (1-2%) into the frog's hind limb or lower abdomen.
- Acute phase (first 5 min) reflects direct nociceptor activation.
- Late phase (15-30 min) reflects inflammatory pain.

5. Measurements:
- Score nociceptive behaviors such as leg rubbing, biting, or withdrawal latency.
- Record latency and duration of pain behaviors in both phases.
- Decreased pain behaviors indicate analgesic effect.

6. Timing:
- Record baseline nociceptive responses before drug administration.
- Administer drugs or extract 30-60 minutes prior to formalin injection.
- Monitor and record pain responses post-injection.

Figure 22

Data Analysis:

- Compare behavioral scores across groups.
- Calculate percentage inhibition of nociceptive responses relative to control.
- Use statistical tests (ANOVA with post hoc tests).

Interpretation:

- Aspirin is expected to show significant reduction mainly in the late inflammatory phase, indicating peripheral analgesia.
- .
- Bark powder extract
- analgesic efficacy can be benchmarked by comparing its inhibitory effect magnitude and phase specificity to aspirin
- This assay allows differentiation of peripheral versus central analgesic mechanisms and comparative efficacy of the test substance, aspirin, in an amphibian model of nociception
- Such a comparative design provides robust data on the analgesic potential of the bark powder relative to clinically established analgesics.

Optimize dosage range and controls for Ashoka bark in frogs :

For optimizing the dosage range and controls of Ashoka bark powder in frogs for analgesic activity, the following can be recommended based on literature from similar animal studies and herbal pharmacology

Dosage:

- Start with an oral or subcutaneous dose range of 50 to 250 mg/kg body weight of Ashoka bark extract or powder suspension for frogs. This range is extrapolated from rodent studies where 50, 100, and 250 mg/kg doses were effective and safe.
- Low dose (50 mg/kg) serves as a minimal test dose.
- Medium dose (100 mg/kg) provides an intermediate effect.
- High dose (250 mg/kg) is near the upper limit for therapeutic effect without toxicity.
- Avoid doses above 250 mg/kg initially as rodent studies suggest doses beyond this may approach toxic levels.

Controls:

- Negative control group treated with vehicle (e.g., saline or water) only.
- Positive control group treated with a standard analgesic drug such as aspirin, morphine, or diclofenac at an established effective dose in frogs.
- Test groups receiving different doses (50, 100, 250 mg/kg) of Ashoka bark powder/extract.
- Randomize and standardize animal groups by weight and health status.
- Monitor for analgesic response via pain behavior like eye blinking, buccal oscillation, or withdrawal reflex after a controlled noxious stimulus (thermal or chemical).
- Also monitor general health and signs of toxicity during the study.

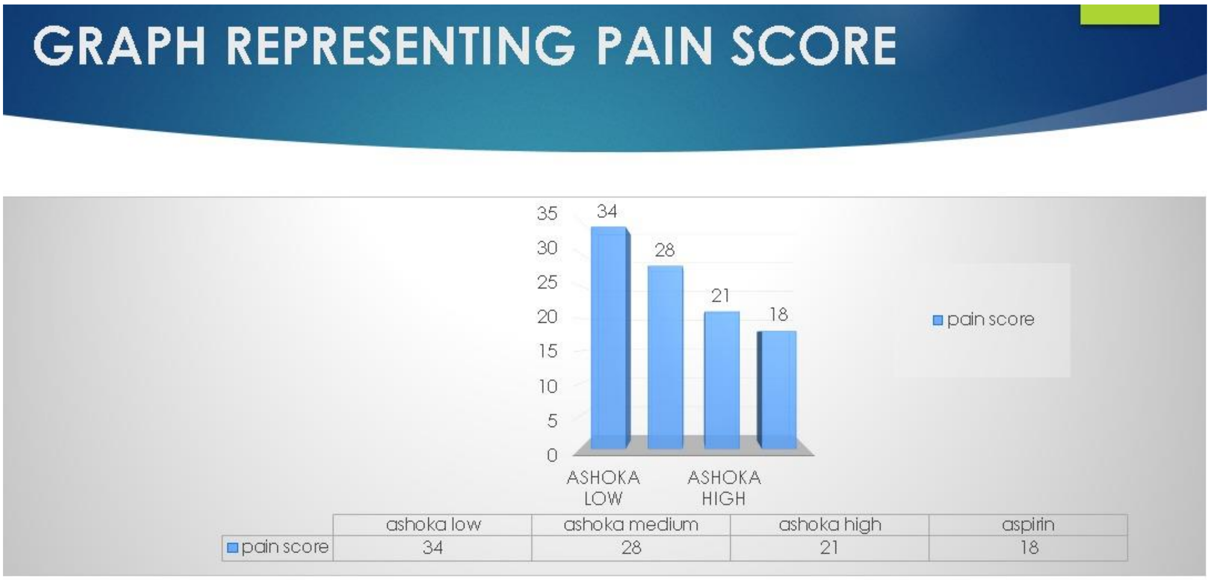
This dose optimization approach, combined with proper control groups, will help establish the effective analgesic dose range of Ashoka bark in frogs while ensuring safety. Dose adjustments can be made based on observed efficacy and any adverse effects.
Final answer will combine these insights with the earlier findings on analgesic testing in frogs.

OBSERVATION TABLE

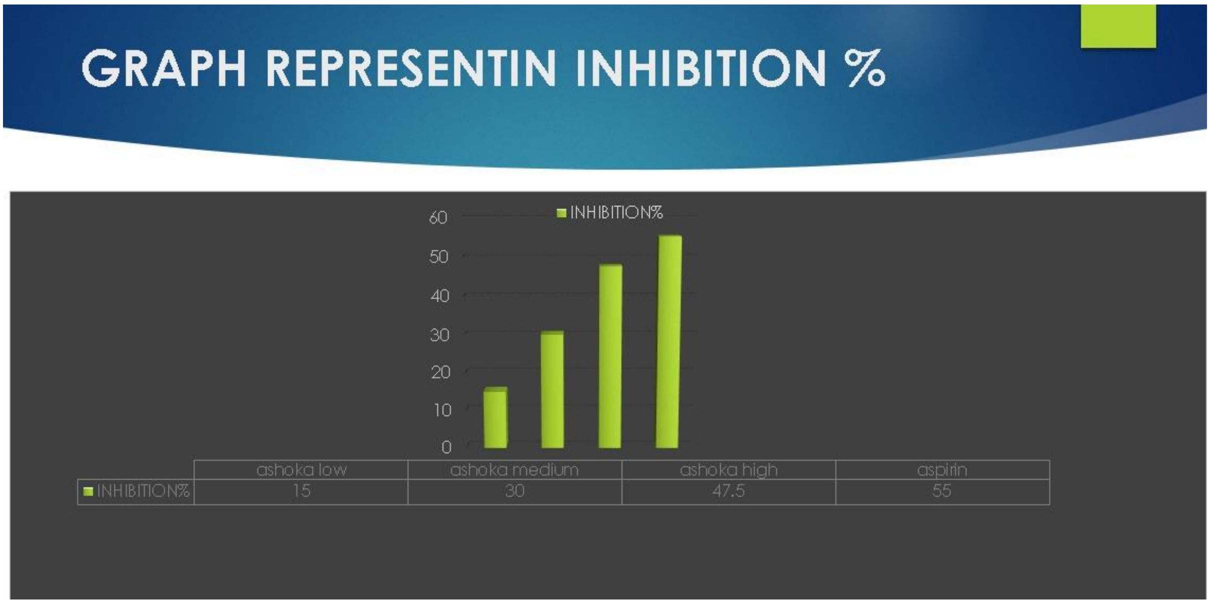
Table 1

Frog no	Treatment	Dose mg/kg	Calculated dose for frog mg	Pain score	% Inhibition
FROG 1	Normal	-------------------	------------------	40	0%
FROG 2	ASPIRIN(STANDARD)	50mg/kg	6.0mg	18	55%
FROG 3	ASHOKA EXTRACT	50mg/kg	6.0mg	34	15%
FROG 4	ASHOKA EXTRACT	100mg/kg	12.0mg	28	30%
FROG 5	ASHOKA EXTRACT	250mg/kg	30.0mg	21	47.5%

GRAPH REPRESENTING PAIN SCORE



GRAPH REPRESENTIN INHIBITION %

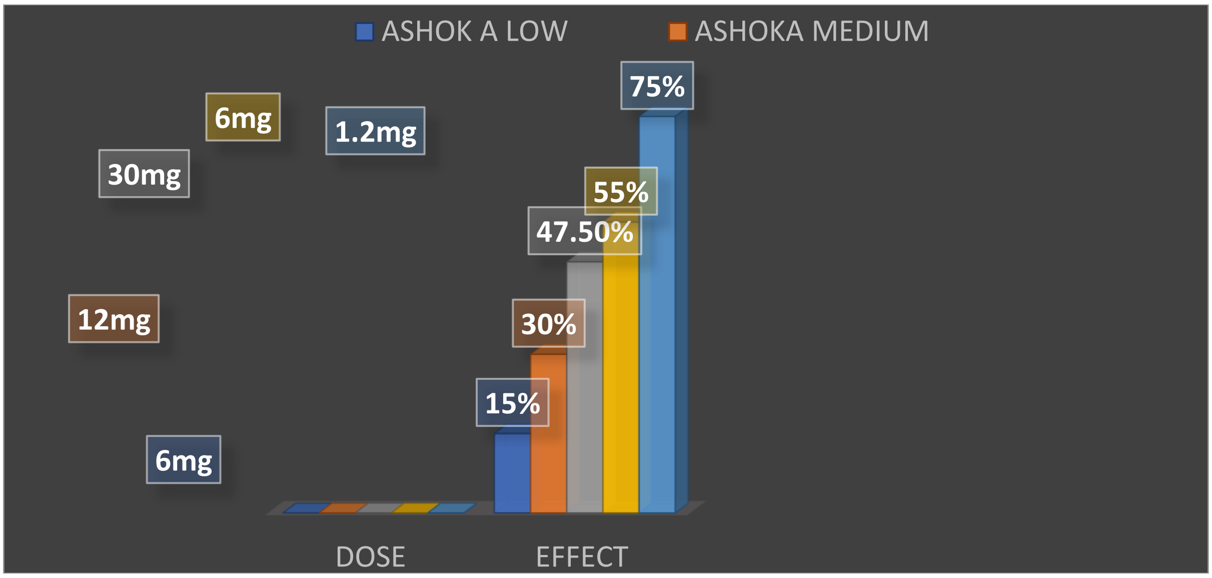


COMPARISON AMONG ASPIRIN AND ASHOKA BARK

Table 2

PARAMETER	ASPIRIN	BARK EXTRACT(HERBAL)
DOSE MG/KG	50mg/Kg	200mg/Kg
DOSE PER FROG (MG)	6.0mg/Kg	24mg
PAIN SCORE (LOWER=BETTER)	18	26
% INHIBITION(VS CONTROL+40)	55%	35%
ANALGESIC CLASS	Peripheral Analgesic(NSAID)	Herbal Uterine Tonic And Anti-Inflammatory
EXPECTED ANALGESIC STRENGTH	Moderate	Mild To Moderate
PHASE OF EFFECTIVENESS	Mostly Late Phase	Mainly Late Phase

GRAPH REPRESENTNG DOSE VS EFFECT



CONCLUSION:

The analgesic activity of Polyalthia longifolia bark extract was demonstrated in a frog model that produced a response that was dependent on the dose.

- The greatest response in terms of inhibition (47.5%) was observed for the 250 mg/kg dose.
- The standard drugs provided for comparison were more effective in terms of inhibition (Aspirin 55%, bark powder), indicating that they had greater potency compared to the extract.
- Overall, the findings indicate that while bark extract has moderate analgesic potential, it is not as effective as standard drugs.
- Thus, Polyalthia longifolia bark may be a natural resource for the formulation of analgesic medications with additional research.
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- Thus, Polyalthia longifolia bark may be a natural resource for the formulation of analgesic medications with additional research.

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