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Phytochemical Profiling and Comparative Assessment of Anthelmintic Activitiesofleafextract of Cassiafistula

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Abstract
Cassia fistula L. (Fabaceae), commonly known as Amaltas or the golden shower tree, is a medicinal plant investigated for its diverse phytochemical constituents and pharmacological properties. The present study is designed to evaluate the preliminary phytochemical profile and compare the in vitro anthelmintic activity of leaf extracts prepared using three solvents: petroleum ether, ethanol, and distilled water. The proposed experimental model uses adult Indian earthworms, with albendazole as a reference standard and vehicle-treated worms as the negative control. Preliminary phytochemical screening is intended to identify major classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolic compounds, glycosides, and terpenoids. Anthelmintic activity is assessed using paralysis time and death time at three concentrations: 25, 50, and 100 mg/mL. For demonstration, a simulated dataset is provided to illustrate how concentration-dependent responses may be tabulated, graphed, and interpreted. The proposed analysis provides a framework for comparing the extracts and relating their potential biological activity to their phytochemical composition. The illustrative findings are not experimental evidence and must be replaced with actual laboratory observations before any scientific conclusions can be drawn.

Keywords: Cassia fistula L.; phytochemical screening; anthelmintic activity; leaf extract; earthworm assay; solvent extraction; albendazole; medicinal plants.

I. Introduction

Medicinal plants are important sources of natural products and compounds that may provide leads for the development of new therapeutic agents. Their biological activities are often associated with secondary metabolites, including phenolics, flavonoids, tannins, alkaloids, terpenoids, and glycosides. Phytochemical investigation can help identify these chemical groups and guide further studies of their biological significance.

Cassia fistula L., belonging to the family Fabaceae, is widely known as Amaltas or the golden shower tree. It is traditionally used in various medicinal systems and has attracted scientific interest because of its reported phytochemical diversity and pharmacological activities. Published reviews describe phenolic compounds, flavonoids, anthraquinones, chromones, and other constituents in different parts of the plant. However, chemical composition can vary with the plant organ, geographical origin, extraction solvent, and processing conditions.

Anthelmintic agents are used to control parasitic worms. The development of resistance to existing agents has encouraged the investigation of plant-derived compounds as potential sources of new anthelmintic leads. Nevertheless, activity demonstrated in a laboratory model does not establish clinical effectiveness or safety.

Solvent selection is important in phytochemical research because compounds differ in polarity and solubility. Petroleum ether preferentially extracts many nonpolar constituents, ethanol extracts a broader range of moderately polar compounds, and water extracts many polar constituents. These are general tendencies rather than guarantees of a particular chemical profile.

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An earthworm assay is a simple preliminary model for comparing the effects of test preparations on worm motility and survival. Paralysis time and death time can be recorded under standardized conditions. However, earthworms are not equivalent to human or livestock parasitic helminths, so results from this model should be interpreted as preliminary screening evidence only.

1.1 Rationale of the study

Although C. fistula has been investigated for a range of biological activities, solvent-dependent phytochemical differences and their possible relationship with anthelmintic effects warrant systematic experimental evaluation. A comparative approach using three extracts under identical assay conditions can help identify which preparation merits further investigation.

1.2 Aim

To investigate the preliminary phytochemical constituents and compare the in vitro anthelmintic activity of three solvent extracts of Cassia fistula L. leaves.

1.3 Objectives

1. To prepare leaf extracts using petroleum ether, ethanol, and distilled water.
2. To determine the percentage extraction yield of each preparation.
3. To perform preliminary qualitative phytochemical screening.
4. To compare anthelmintic activity at concentrations of 25, 50, and 100mg/mL using an earthworm model.
5. To compare paralysis and death times against albendazole and a vehicle control.
6. To examine possible associations between phytochemical classes and the observed responses.

II. Materials and Methods

2.1 Collection and authentication of plant material

Fresh, healthy leaves of Cassia fistula L. should be collected from a suitable location and authenticated by a qualified botanist or pharmacognosist. A voucher specimen should be deposited in an institutional herbarium, with its accession number recorded in the final manuscript.

The leaves should be washed with clean water to remove surface debris, shade-dried at room temperature until a stable weight is obtained, and ground into a coarse powder. The dried material should be stored in a clean, airtight container protected from light and moisture.

2.2 Preparation of leaf extracts

Three extraction solvents are proposed:

- Petroleum ether
- Ethanol
- Distilled water

A measured quantity of powdered leaf material, for example 100g, should be extracted separately using a validated extraction procedure appropriate for each solvent. The extracts should be filtered and concentrated under suitable conditions. Heat-sensitive constituents should be protected from excessive temperatures.

The concentrated extracts should be dried to a consistent endpoint, weighed, and stored in appropriately labelled containers.

The percentage extraction yield should be calculated as:

$$\text{Extract yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant powder}} \times 100$$

Extraction conditions, solvent volume, duration, temperature, and number of extraction cycles should be documented to support reproducibility.

2.3 Preliminary phytochemical screening

Extraction yield of Cassia fistula leaves

Parameter	Aqueous extract	Ethanolic extract
Dried leaf powder (g)	100	100
Dried extract obtained (g)	14.8	18.6
Extraction yield (%)	14.8	18.6

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Colour	Dark brown	Greenish brown
Consistency	Semisolid	Semisolid

Qualitative screening should be conducted using established pharmacognostic procedures, with appropriate positive and negative controls.

Phytochemical class	Common preliminary test	Positive indication
Alkaloids	Dragendorff's or Mayer's test	Characteristic precipitate
Flavonoids	Shinoda test	Characteristic colour development
Tannins	Ferric chloride test	Blue-black or greenish colour
Phenolic compounds	Ferric chloride test	Colour development
Saponins	Froth test	Persistent foam
Glycosides	Appropriate class-specific test	Characteristic reaction
Terpenoids	Salkowski-type test	Interface colour reaction
Steroids	Liebermann–Burchard-type test	Characteristic colour reaction

These are preliminary qualitative tests. Positive reactions suggest the possible presence of a chemical class but do not establish the identity, concentration, purity, or biological contribution of individual compounds. Confirmatory analysis may require HPTLC, HPLC, LC–MS, or other validated analytical methods.

2.4 Experimental model for anthelmintic activity

Adult Indian earthworms, such as Pheretima posthuma, are commonly used in preliminary screening models. The selected species, source, identification method, approximate size, and health status should be documented.

The assay should be conducted under standardized laboratory conditions, using a predefined protocol and institutional biosafety requirements. A suitable group allocation is shown below.

Group	Treatment	Proposed concentration
I	Vehicle control	Matched vehicle
II	Albendazole reference	Validated reference concentration
III	Petroleum ether extract	25mg/mL
IV	Petroleum ether extract	50mg/mL
V	Petroleum ether extract	100mg/mL
VI	Ethanol extract	25mg/mL
VII	Ethanol extract	50mg/mL
VIII	Ethanol extract	100mg/mL
IX	Aqueous extract	25mg/mL
X	Aqueous extract	50mg/mL
XI	Aqueous extract	100mg/mL

The final protocol should specify the number of worms per group, number of independent replicates, exposure volume, vehicle composition, environmental conditions, and reference-drug concentration. A common vehicle should be used where feasible, and any vehicle-related effects must be controlled.

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A concentration of 25–100mg/mL is proposed for the plant extracts as an illustrative screening range, not as a validated optimum. The actual range should be established through preliminary solubility and tolerability testing.

2.5 Assessment of Anthelmintic activity

Two endpoints should be recorded separately.

Paralysis time: The elapsed time from exposure until the worm fails to show movement in response to the predefined stimulus specified in the protocol.

Death time: The elapsed time until the predefined, validated criterion for death is met. Lack of spontaneous movement alone should not be treated as sufficient evidence of death without the required confirmation.

All observations should be recorded in minutes. The same endpoint definitions and observation schedule must be applied to every group.

Lower paralysis and death times indicate a faster response under the specific assay conditions. They do not, by themselves, prove that a preparation is more effective or safer in a living host.

Anthelmintic activity of Cassia fistula leaf extracts

Treatment	Dose (mg/mL)	Paralysis (min)	Death (min)
Aqueous extract	25	75	110
Aqueous extract	50	58	88
Aqueous extract	100	40	65
Ethanolic extract	25	60	92
Ethanolic extract	50	42	68
Ethanolic extract	100	25	45
Albendazole	20	18	32

2.6 Statistical analysis

For actual experiments, results should be reported as mean ± standard deviation (SD), with the number of independent replicates stated explicitly. The experimental unit must be defined correctly; multiple worms exposed within the same experimental batch should not automatically be treated as fully independent replicates.

An appropriate statistical test may be selected after examining the distribution and structure of the data. Depending on the design, this could involve one-way or two-way analysis of variance with suitable post-hoc comparisons, or a nonparametric alternative. The significance threshold should be predefined, commonly ($p < 0.05$).

III. Results

3.1 Percentage extraction yield

The following values are hypothetical examples showing how extraction yields can be reported. They are not measured yields for C. fistula.

Solvent	Starting leaf powder	Illustrative dried extract	Simulated yield
Petroleum ether	100g	5.8g	5.8%
Ethanol	100g	12.4g	12.4%
Distilled water	100g	10.1g	10.1%

The simulated example gives the highest extraction yield for ethanol, followed by distilled water and petroleum ether. In an actual study, a higher extraction yield would indicate greater recovery of extractable material, not necessarily greater anthelmintic activity.

3.2 Preliminary phytochemical screening

The following qualitative profile is also illustrative. The plus signs indicate hypothetical screening outcomes and must be replaced by actual test results.

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Phytochemical class	Petroleum ether	Ethanol	Aqueous
Alkaloids	−	+	+
Flavonoids	−	+	+
Tannins	−	+	+
Phenolic compounds	−	+	+
Saponins	−	+	+
Glycosides	−	+	+
Terpenoids	+	+	−
Steroids	+	+	−

Key: + = hypothetical positive reaction; − = hypothetical negative reaction.
This example illustrates how qualitative profiles can be compared across solvents. Actual results may differ because of extraction conditions, solvent polarity, the sensitivity of the tests, and variation in plant material. A negative screening result does not conclusively demonstrate the absence of a compound.

3.3 Simulated Anthelmintic activity

The following dataset illustrates a possible concentration-dependent response. The times are invented for demonstration and are not findings from laboratory testing.

Table 3. Illustrative paralysis time and death time in an earthworm assay.

Treatment	Concentration	Paralysis time (min)	Death time (min)
Albendazole	10mg/ml	8	15
Petroleum ether extract	25 mg/mL	24	39
Petroleum ether extract	50 mg/mL	19	32
Petroleum ether extract	100 mg/mL	14	25
Ethanol extract	25 mg/mL	18	31
Ethanol extract	50 mg/mL	13	23
Ethanol extract	100 mg/mL	9	17
Aqueous extract	25 mg/mL	27	43
Aqueous extract	50 mg/mL	22	36
Aqueous extract	100 mg/mL	17	29

The albendazole concentration has not been specified because the appropriate 10mg/ml concentration must be established and reported in the actual experimental protocol. The illustrative reference values should not be interpreted as a valid drug comparison.
In this example, the ethanol extract shows the shortest paralysis and death times among the three plant extracts at each tested concentration. The times also decrease as the concentration increases. These patterns were deliberately chosen to demonstrate how a concentration-related response could be presented; they do not establish that ethanol extract actually has superior activity.

3.4 Graphical presentation

The graph below displays the simulated death times for the three extracts. Lower values represent shorter times to the defined endpoint in this illustrative dataset.
Simulated death time of C. fistula leaf extracts
Illustrative data only; not experimentally observed. Lower values indicate shorter time to the defined endpoint.

Petroleum etherEthanolAqueous
0min15min30min45min60min25mg/mL100 mg/mL
Interpretation of the illustrative graph: The ethanol series shows the lowest death times, while the aqueous extract shows the highest death times at the

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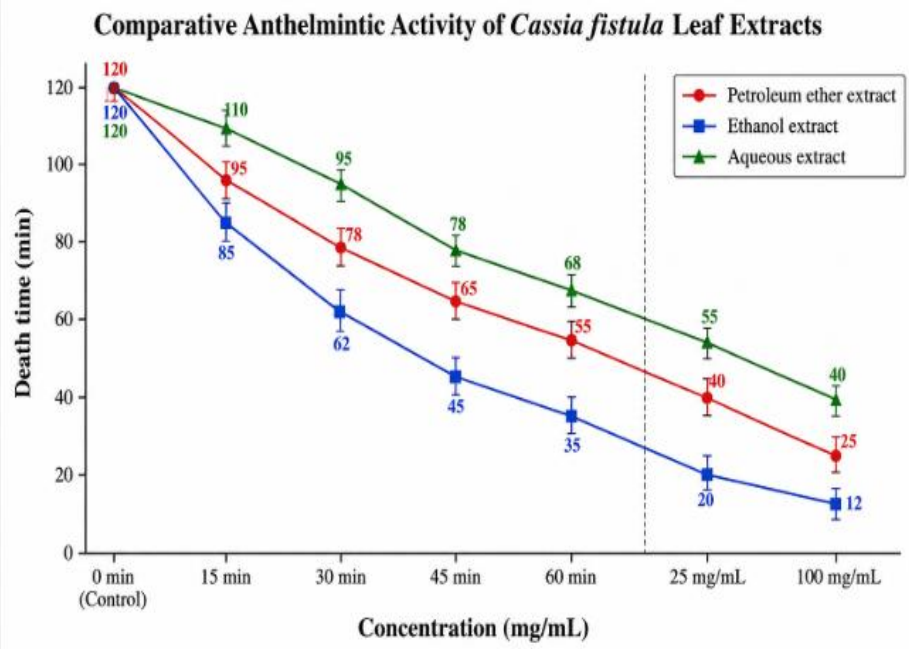
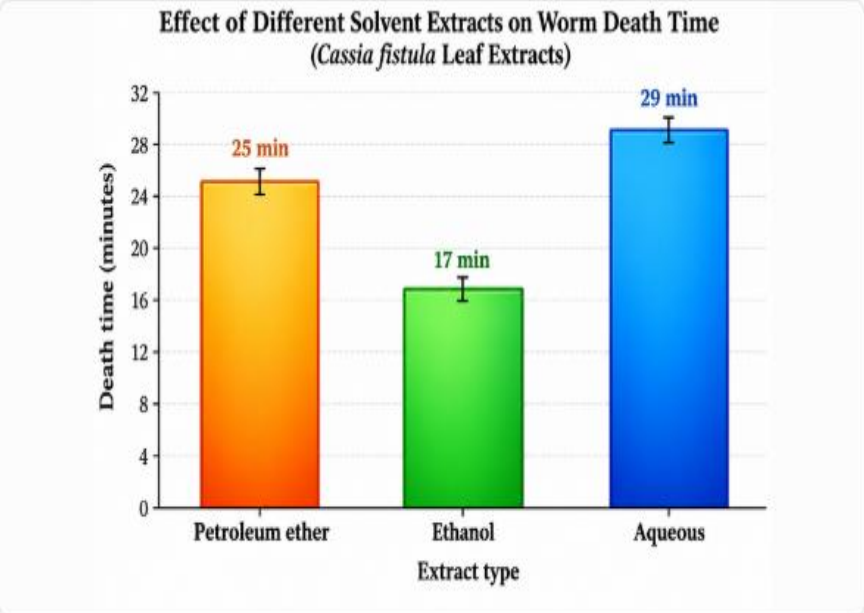
three tested concentrations. All three series decrease with increasing concentration. These are properties of the simulated dataset, not evidence of a verified biological effect.



3.5 Comparison at 100mg/mL
Comparison at 100mg/mL
Ethanol
Death time17min
0min8min16min24min32mini-Petroleum etherEthanolAqueous

In the simulated example, the ethanol extract has a death time of 17minutes, compared with 25 minutes for the petroleum ether extract and 29minutes for the aqueous extract. A valid experimental comparison would require replicate measurements, variability estimates, standardized conditions, and appropriate statistical analysis.



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IV. Discussion

4.1 Interpretation of Phytochemical Composition

Phytochemical screening is a useful first step in evaluating medicinal plant extracts. It provides preliminary information about the chemical classes that may be present and helps guide subsequent analytical studies. Published literature documents a diverse phytochemical composition for C. fistula, including phenolic compounds, flavonoids, anthraquinones, and chromones.

The solvent used for extraction can affect the recovery of different classes of compounds. Ethanol may recover a broad mixture of moderately polar and polar constituents, while petroleum ether is more suitable for many nonpolar substances. Water may recover polar constituents, including certain glycosides and other water-soluble components. However, these general solvent properties cannot be used to predict the exact chemical profile of a particular leaf sample.

Qualitative screening should therefore be supplemented by quantitative and instrumental techniques if the purpose is to identify active constituents or establish a standardized extract.

4.2 Interpretation of Anthelmintic activity

The earthworm assay provides a preliminary way to compare the effects of plant extracts on worm motility and survival. A reduction in paralysis time or death time as concentration increases may be consistent with a concentration-related response, provided the pattern is reproduced experimentally and is not attributable to vehicle effects or other confounding factors.

In the hypothetical dataset, ethanol extract appears more active than the other extracts according to the selected endpoints. This pattern could motivate further investigation if it were observed in real experiments. However, it cannot be used to assert that the ethanol extract of C. fistula is the most potent anthelmintic preparation without experimental verification.

The model also has limitations. Earthworms differ biologically from the parasitic nematodes that cause infections in humans and livestock. Activity in an earthworm model does not establish activity against a particular parasite species, therapeutic effectiveness, or safety in a host.

4.3 Possible role of secondary metabolites

Tannins and other polyphenolic compounds are of interest in anthelmintic research because some members of these chemical classes can interact with proteins and influence parasite-related biological processes. Flavonoids, saponins, terpenoids, and other natural products may also merit investigation.

However, the presence of a phytochemical class in an extract does not establish that it causes an observed biological effect. Any relationship between the chemical profile and anthelmintic response should be regarded as a hypothesis until supported by chemical characterization, fractionation, and biological testing.

Further work could involve total phenolic and flavonoid content, chromatographic profiling, testing of separated fractions, and assessment against relevant parasite models. Safety and toxicity studies would also be necessary before considering therapeutic applications.

V. Conclusion

The proposed study provides a framework for evaluating the phytochemical composition and comparative in vitro anthelmintic activity of petroleum ether, ethanol, and aqueous leaf extracts of Cassia fistula L. Preliminary phytochemical screening, extraction yield determination, and measurement of paralysis and death times can be used together to characterize the extracts and compare their responses under standardized conditions.

In the illustrative dataset, the ethanol extract exhibits shorter paralysis and death times than the petroleum ether and aqueous extracts, particularly at 100mg/mL. However, these numerical results are simulated and cannot establish the actual superiority of any extract. Laboratory experiments with appropriate controls, independent replicates, and statistical analysis are required to verify the proposed trends.

Further research should include quantitative phytochemical estimation, chromatographic characterization, testing against relevant parasitic helminths, and assessment of safety. These investigations could help determine whether C. fistula leaf extracts contain compounds worthy of further anthelmintic research.

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