Phytochemical Profiling & Modern Standardization of Herbal Extract for Pharmacological Evaluation

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ABSTRACT

Chemical analysis was used to determine which phytoconstituents were present, and they were found mostly in the following extract: Carbohydrate, Glycoside, Tannin, Gum, and MucilageSugars, Glucosides, Proteins, Fatty Acids, Stable Oils, Tannins, Phytosterols, Gum, Mucilage, Alkaloids, Resin, Flavonoids, and Flavonoids.Sugars, lipids, proteins, peptides, phenolic compounds, tannins, phytes, sterols, alkaloids, resins, flavonoids, and phenolic acids The extract with a polarity somewhere in the middle -- chloroform, ethanolic, or aqueous -- was chosen for further pharmacological testing.Herbal product evaluation can benefit from quantitative chemical testing like acid value, saponification value, etc. Chromatography is one of the extensively utilised current methods of chemical standardisation.

Key Word-: Phytochemical, *Pongamiapinnata*(Linn.),Quantitative chemical,Aquous extract

1. INTRODUTION

Herbal medicinal products are defined as medicines that exclusively contain one or more active substances derived from herbs. These active substances can be herbal substances themselves or herbal preparations obtained from these substances through processes like extraction, distillation, fractionation, or fermentation. Herbal preparations include various forms such as powdered herbal substances, tinctures, extracts, essential oils, expressed juices, and processed exudates.

Pongamia pinnata, also known as Pongam or Indian Beech Tree, is a perennial legume plant with nitrogen-fixing capabilities and medicinal properties. Originally from the Indo-Malaysian region, it is now found in many countries. This medium-sized, glabrous, semi-evergreen tree can grow up to 18 meters tall and has a spreading crown and grayish-green or brown bark. Pongamia thrives in areas with annual rainfall ranging from 500 to 2500 mm and can withstand waterlogging, slight frost, and high salinity. It is often used for controlling soil erosion and binding dunes due to its dense network of lateral roots, and its drought tolerance is attributed to its long, thick taproot. The plant can be propagated successfully through seeds and cuttings.

The study of medicinal plants like Pongamia is crucial for various reasons. Traditional knowledge about these plants was largely passed down orally and has been lost over time due to cultural adaptations and invasions. Additionally, there was no standardized procedure for maintaining the inventory of these plants and their medicinal properties. Many contemporary and traditional medicinal systems use plants and plant-based products without written documentation or regulation. Hence, documenting and studying the uses of natural products is essential for systematic regulation and wider application of these valuable resources. In the future, nitrogen-fixing legumes, especially tree legumes like Pongamia, are expected to play a significant role in sustainable biofuel production. These plants can grow in marginal lands without competing with food crops, making them an attractive option for large-scale biofuel feedstock production.

2. REVIEW OF RELATED WORK

Author: Singh VK, Pandey DK, Shukla S.

Title: "Phytochemical analysis and comparative antimicrobial efficacy of Pongamia pinnata extracts against some pathogenic microorganisms."

Published: Journal of Pharmacy Research. 2019;13(6):824-829.

Summary: This study focused on the phytochemical screening of different extracts of Pongamia pinnata and evaluated their antimicrobial potential against various pathogenic microorganisms. The researchers identified various phytochemicals present in the plant and correlated them with the observed antimicrobial effects.

Author: Sahoo S, Ghosh G, Das D, Nayak S.
Title: "Preliminary phytochemical screening and in-vitro antioxidant activity of Pongamia pinnata Linn. leaves."
Published: International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(2):290-293.
Summary: This research aimed to identify the phytochemical constituents in Pongamia pinnata leaves and assess their antioxidant activity through in-vitro tests. The study highlighted the potential of the plant as a natural antioxidant source.
Author: Nayak S, Sahoo S, Acharya L.
Title: "Phytochemical screening and antibacterial activity of Pongamia pinnata Linn. seeds."
Published: International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(4):309-312.
Summary: This work focused on the phytochemical screening of Pongamia pinnata seeds and investigated their antibacterial properties against different bacterial strains. The study suggested that the seeds of Pongamia pinnata could be a potential source of natural antibacterial agents.
Author: Kumar S, Malhotra R, Kumar D.
Title: "Phytochemical and pharmacological profile of Pongamia pinnata: a review."
Published: Research Journal of Medicinal Plant. 2011;5(3):230-239.
Summary: This review article provides an in-depth analysis of the phytochemicals present in various parts of Pongamia pinnata and their pharmacological activities. The authors compiled information from multiple studies, highlighting the plant's potential in traditional medicine and modern drug development.
Author: Kumar VL, Rao NH.
Title: "Phytochemical investigation and anti-inflammatory activity of Pongamia pinnata leaves."
Published: Fitoterapia. 2001;72(6):698-701.
Summary: This study explored the phytochemical constituents of Pongamia pinnata leaves and evaluated their anti-inflammatory effects. The researchers found evidence of anti-inflammatory properties, supporting the traditional use of the plant in treating inflammatory conditions.
Author: Selvakkumar C, Jegadeeswari P.
Title: "Phytochemical screening and evaluation of antimicrobial activity of Pongamia pinnata."
Published: International Journal of Chemical Studies. 2014;2(3):38-42.
Summary: This study focused on the phytochemical screening of various parts of Pongamia pinnata and investigated their antimicrobial activity against pathogenic microorganisms. The researchers aimed to identify potential bioactive compounds responsible for the observed antimicrobial effects.
Author: Akhtar T, Waseem K, Sharma K, Shukla I, Ali M.
Title: "Phytochemical analysis and antimicrobial activities of Pongamia pinnata L."
Published: International Journal of Pharma Sciences and Research. 2015;6(2):82-89.
Summary: This research involved a comprehensive phytochemical analysis of Pongamia pinnata and evaluated its antimicrobial activities. The authors aimed to provide insights into the plant's potential as a source of natural antimicrobial agents.
Author: Sinha SN, Sharma BK, Singh RP, Sharma DK.
Title: "Phytochemical screening and anthelmintic activity of Pongamia pinnata (Linn.) Pierre."
Published: Journal of Applied Pharmaceutical Science. 2011;1(6):146-148.
Summary: This study explored the phytochemical content of Pongamia pinnata and investigated its anthelmintic (anti-parasitic) activity. The researchers aimed to determine if the plant possessed properties that could be useful in the treatment of parasitic infections.

Author: Rathod NR, Raghuveer I, Chitme HR, Chandra R.
Title: "Preliminary phytochemical screening and evaluation of anthelmintic activity of Pongamia pinnata in albino mice."
Published: Indian Journal of Natural Products and Resources. 2010;1(2):210-213.
Summary: This research involved a preliminary phytochemical screening of Pongamia pinnata and evaluated its anthelmintic activity in albino mice. The study aimed to validate the traditional use of the plant in folk medicine for treating parasitic infections.
Author: Reddy AC, Lokesh BR.
Title: "Studies on the antioxidant activities of Pongamia pinnata seeds."
Published: Journal of Food Science and Technology. 2010;47(2):223-227.
Summary: This study investigated the antioxidant activities of Pongamia pinnata seeds. The researchers focused on identifying the bioactive compounds responsible for the plant's antioxidant potential, which could have implications for various health benefits.

3. MATERIALS AND METHODS

Phytochemical screening

The isolation, purification, and identification of active components are all part of the chemical evaluation process. Herbal product evaluation can benefit from quantitative chemical testing like acid value, saponification value, etc. Chromatography is one of the most popular current methods of chemical standardisation. The groupings' qualitative characteristics were determined through a broad screening of plant extracts. To cite this entry: Basset,Jet al.,(1985).

Test for carbohydrates and glycosides

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to the following testes to detect the presence of Carbohydrate and glycosides.

(a) Molisch's test

The filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of concentrated H_2SO_4 was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

(b) Fehling's test

The filtrate was treated with 1 ml of Fehling's solution A and B and heated on the water bath. A reddish precipitate was obtained shows the presence of carbohydrate.

Test for fixed oils and fates

(a) Spot test

Small quantity of extract was pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

(b) Saponification test

Few drops of 0.5% alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on the water bath for 1-2 hours. Formation of soap pr partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for proteins and free amino acid

Small quantity of the extract was dissolved in few ml of distilled water and treated with following reagents.

(a) Millon's test – Appearance of red color shows the presence of proteins and free amino acids.

(b) Ninhydrin reagent –Appearance of purple color shows the presence of proteins and free amino acids.

(c) Biuret test – Equal volumes of 5% sodium hydroxide solution and 1% copper sulphatesolution were added, appearance of pink or purple color shows the presence of proteins and free amino acids.

Test for saponins

Foam test – The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

Test for phenolic compounds and tannins

Small quantity of the extract was taken in distilled water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

(a) Dilute ferric chloride solution (5% w/v) - Violet color.

(b)10% lead acetate solution-White precipitate.

Test for phytosterols

Small quantity of the extract was dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosteroles.

(a) Libermann-Burchard's test

The above preapared chloroform solution was treated with few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3 ml of acetic anhydride. A bluish green color appeared indicates the presence of phytosterols.

(b) Salkowski reaction

To 1 ml of the above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown color produced shows the presence of phytosterols.

Test for Alkaloids

Small quantity of the extract was treated with few drops of diluted hydrochloric acid and filtered. The filtrate was used for the following tests.

(a) Mayer's reagent – cream precipitate

(b) Dragendroff's reagent – Orange brown precipitate

(c) Hager's test – yellow precipitate

Test for flavonoids

(a) With aqueous NaOH solution

Small quantity of the extract was dissolved in aqueous sodium hydroxide. Appearance of yellow colour indicates the presence of flavonoids.

(b) With conc. sulphuric acid

To a small portion of extract, concentrated sulphuric acid was added. Yellow orange color was obtained shows the presence of flavonoids.

4. RESULT & DISCUSSION

In recent years, plant-derived natural products, such as flavonoids and phenolic compounds, have gained significant attention due to their potential health benefits and therapeutic properties. These compounds have been studied for their various biological activities and medicinal applications.

Phytochemical evaluation was performed on different plant extracts, and the phytoconstituents present in each extract were identified through chemical tests. The results are summarized as follows:

Hexane extract:

- Glycosides
- Carbohydrates
- Tannins
- Gum & mucilage

Chloroform extract:

- Carbohydrates
- Glycosides
- Proteins & amino acids
- Fixed oils & fats
- Phenolic compounds & tannins
- Phytosterols
- Gum & mucilage
- Alkaloids
- Resin
- Flavonoids

Ethanolic extract:

- Carbohydrates
- Glycosides
- Proteins & amino acids

- Fixed oils & fats
- Phenolic compounds & tannins
- Alkaloids
- Phytosterols
- Resin
- Flavonoids

Aqueous extract:

- Carbohydrates
- Glycosides
- Proteins & amino acids
- Saponins
- Phenolic compounds & tannins
- Phytosterols
- Alkaloids
- Resin
- Flavonoids

Based on the polarity of the extracts, the Chloroform, Ethanolic, and Aqueous extracts were selected for further pharmacological evaluation. These extracts offer a diverse range of phytochemicals, which may contribute to their potential therapeutic effects. Further research and testing can be conducted to explore the pharmacological properties and potential benefits of these extracts for various health conditions.

Table:1 Phytochemical Screening of different extract of dried seed of *Pongamia pinnata* Linn.:-

S. No.	Constituents	Tests	Hexane	Chloroform	50% Ethanolic	Aqu.
1.	Carbohydrate	Molish's test	+	+	+	+
		Anthrone test	+	+	+	_
		Fehling's test	_	_	+	+
2.	Glycoside	Legal's test	+	+	+	_
		Keller killanis test	_	_	+	+
3.	Fixed oil & fats	Spot test	_	+	–	+
		Saponification test	_	_	–	_
4.	Proteins & amino acids	Million's test	_	_	_	_
		Ninhydrin test	_	+	+	_
		Biuret test	_	+	_	_
5.	Saponins	Foam test	_	_	+	+
6.	Phenolic compounds & tannins	$FeCl_3$ test	+	_	+	+
		Leadacetatetest	+	+	+	_
7.	Phytosterol	Salkowiski test	_	+	+	+
		Libermann	_	_	_	+
8.	Alkaloids	Dragendroff's test	_	_	+	_
		Mayer's test	_	+	+	+
		Hager's test	_	_	–	_
9.	Gum& mucilage	Swelling test	+	_	–	_
10.	Resin	Resin	_	+	+	+
11.	Flavonoids	Aq. NaOH test	_	_	+	_
		Shinoda's test	_	+	+	_

Where:-(+)=Presence,(−)=Absence

Table No: 2 Quantitative Analysis of dried seeds of *Pongamia pinnata* Linn.:

Parameters	Range(%)	Mean ± SD(%)
Total sugar	0.560 -0.571	0.565 ± 0.007
Total Starch	1.237 -1.242	1.24 ± 0.003
Total Phenolics	0.189-0.186	0.187± 0.002
Total Alkaloid	0.33 -0.28	0.305 ± 0.035

5. CONCLUSION

Despite the ubiquity of modern medicine and the creation of synthetic pharmaceuticals, traditional medicaments, often known as herbal drugs in modern literature, continue to play a key part in therapy. The effectiveness, low cost, and lack of significant side effects of these herbal treatments have allowed them to maintain their popularity. Therefore, they are widely used and accepted even in developed nations. More and more people are becoming interested in the therapeutic potential of plants, and this has led to a rise in the popularity of goods made from plants. The phytochemical screening of the plant Pongamia pinnata has yielded valuable insights into its chemical composition. Through the analysis, we have identified several significant secondary metabolites present in the plant, including alkaloids, flavonoids, tannins, glycosides, and terpenoids. These compounds play crucial roles in the plant's defense mechanisms against environmental stresses, pathogens, and herbivores. The presence of alkaloids suggests potential bioactivity with possible medicinal applications. Flavonoids, known for their antioxidant and anti-inflammatory properties, may offer health benefits for humans. Tannins, with their astringent properties, can contribute to the plant's protection from external threats. Glycosides, on the other hand, have been associated with medicinal properties in traditional practices. Lastly, terpenoids, including essential oils and steroids, signify the plant's adaptability to its surroundings. While the phytochemical screening provides valuable information about the chemical constituents of Pongamia pinnata, further research is necessary to isolate and study individual compounds in depth. This comprehensive understanding will pave the way for potential applications in various fields, including medicine, agriculture, and industry. However, before any practical applications, it will be essential to assess the safety and efficacy of these compounds through extensive research and testing.

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