PHYTOCHEMICAL SCREENING OF ACTIVE SECONDARY METABOLITES PRESENT IN THE LEAVES EXTRACTS OF *STERCULIA FOETIDA* LINN.

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ABSTRACT:

Sterculia foetida Linn is a soft handsome woody tree with various pharmacological properties and they are most prevalently found in India, Thailand, Indonesia, Ghana, and Australia. The biochemically active compounds present in the plant possess good medicinal properties which have been already reported in several research papers. The present study was designed to screen the phytochemicals present in the leaf of *Sterculia foetida*. The leaf powder was successively extracted with polar, non-polar solvents (hexane, chloroform, methanol, ethyl acetate and aqueous) by using various standard protocol for specific secondary metabolite. Secondary metabolites present in the cells of the plant are responsible for medicinal activity of plants. The phytochemical screening of the current study confirms the presence and absence of fifteen secondary metabolites (carbohydrates, tannins, saponin,flavonoids, alkaloids, quinones, terpenoids, glycosides, triterpenoids, phenols, coumarins, proteins, cardiac glycosides, steroids, phytosterols) in the five leaf extracts. The result of the phytochemical profilingfinally revealed that the methanol leaf extract of *Sterculia foetida* exhibit the presence of high content of important secondary metaboliteswhich possess various pharmacological actionwhen compared with other four leaf extracts. Further, the current research would be helpful for the scientific community to identify and design the new drug molecules with better medicinal activity.

Keywords: *Sterculia foetida* Linn, Leaves, Five solvents, Qualitative analysis, Secondary metabolites.

1. INTRODUCTION:

Medicinal plants possess numerous natural compounds which offer specific physiological function in the human body. Our earth has rich source of medicinal plants whose pharmacological importance has not yet been investigated till date [1], hence in the recent years, the scientists have turned their attention towards the plants which possess rich source of metabolites [2]. The plant metabolites serve as the raw material for herbal medicine and allopathic medicine in continents like Asia and Africa [3]. During the ancient period, people used plants for treating their disease without knowing their pharmacological properties [4]. Their knowledge was further transmitted to future generations and people in each generation started collecting information about the particular plant [5]. Then they started planting, collecting the plants and further used them as the medicine for treating various diseases even though they are not scientifically proven drugs [6]. In later years, the plant research increased numerically to identify its potential. This study revealed many chemical constituents present in the plants and these constituents can be further used as main drug target for treating various diseases, which would be later synthesized as new drug molecules [7].

The pharmaceutical and medicinal properties possessed by the plants are due to the presence of metabolites (chemical substance). They are abundantly seen in all parts of the plant – root, stem, leaves, fruits, flowers, and seeds. There are two types of metabolites; primary metabolites which are responsible for growth and development of plants, secondary metabolites which are responsible for defense mechanism of human [8, 9]. From 20th century onwards, several scientists concentrated their research in isolating secondary metabolites from different parts of the plants since bioactive compounds derived from secondary metabolites possess great therapeutic values and also due to its powerful defense mechanisms in humans [10, 11]. The bioactive compounds present in the secondary metabolites can be derived using phytochemical screening analysis [12].

Phytochemical screening was performed to extract the medicinally active substances. Qualitative analysis reveals the presence or absence of secondary metabolites present in the plantsand quantitative analysis reveals the amount of percentage of secondary metabolites present in the plants [13]. *Sterculia foetida* Linn is a tropical evergreen tree; it belongs to family Sterculiaceae, found in the western and southern part of India. It is commonly called as Java Olive, wild almond Poon tree and Pinari in tamil [14]. The tree is reported to have several useful aspects due its pharmacological properties present in the whole plant and especially in leaves. The phytochemicals present in the plant possess several biological properties like antifungal, antimicrobial, antiviral, antitumor activities15. In olden days the leaves of the plant were used as the medicine without the knowledge of their medical properties. Leaves were observed at the ends of the each branch, it was clumped with 7-9 leaflets with length of 10-17 cm with unpleasant smell [16].

The present study was designed to reveal the hidden potentials present in the leaves extract of medicinal plant *Sterculia foetida* Linn and to predict the presence of medicinally important secondary metabolites from various solvents like methanol, ethyl acetate, hexane etc. by using standard protocol for different bioactive molecules.

2. MATERIALS AND METHODS:

Collection and Authentication of the plant leaves:

The fresh leaves of medicinal plant *Sterculia foetida* Linn were collected in and around Pallavaram during the month of April 2014 and it were authenticated by Prof. P.Jayaraman, Ph.D. Director of Plant Anatomy Research Centre, West Tambaram and Retd Professor, Presidency College, Chennai - 5.

Preparation of plant leaves:

The 3 Kg of fresh and good leaves were collected. The leaves were further washed with the tap water to remove the dust particles and dried in shades for 10–15 days. The dried leaves were grinded into coarse powder using electric blender for the extraction of natural compounds present in the leaves.

Preparation of Extracts:

The 100 grams of powdered leaves were extracted using five different solvents (hexane, chloroform, methanol, ethyl acetate and aqueous) based on their increased polarity by continuous percolation process using soxhlet apparatus. After extraction from each solvent, the individual extracts were dried by reducing pressure and temperature in rotary vacuum evaporator. Then the five extracts were dried for calculating the percentage of yield present in each extract. Finally consistency and appearance of each extract were noted. Later individual extracts were collected and stored for further phytochemical and pharmacological studies.

Preliminary Phytochemical Screening:

The preliminary phytochemical screening were carried out using the standard specific protocols for the detection of bioactive chemical constituent such as carbohydrates, tannins, saponin,flavonoids, alkaloids, quinones, terpenoids,glycosides, triterpenoids, phenols, coumarins, proteins, cardiac glycosides, steroids,phytosterols [3, 6, 7, 17-24].

Various tests were performed for the detection of presence of qualitative biochemical:

1. **Detection of Carbohydrates:**

a) **Benedict's test**: To the 1ml of various extracts a few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) was added in the test tubes and later boiled in water bath for few minutes. Formation of reddish brown precipitate in the tube indicates the presence of carbohydrates in the extracts.

b) **Molish's Test:** To the 1ml of various extracts, 1ml of Molish's reagent and few drops of conc$H_2SO_4$was added. Formation of purple or red color in the tube indicates the presence of carbohydrates in the extracts.

2. **Detection of Tannins:**

a) To the 1ml of the various extract, 2ml of 5% ferric chloride was added in the tubes. Formation of greenish black or dark blue in the tube indicates the presence of tannins in the extract.

b) The 0.25 g of various extract was dissolved in 10 ml distilled water and then filtered. Further 1% aqueous Ferric chloride ($FeCl_3$) solution was added. Formation of intense green, purple, blue or black in the tube indicates the presence of tannins in the extracts.

3. **Detection of Saponin:**

To the 2 ml of various extracts, 2ml of distilled water was added and mixed well for 15 minutes by shaking lengthwise in a graduated cylinder. Formation of 1cm layer of foam indicates the presence of saponins in the extracts.

4. **Detection of Flavonoids:**

a) The 0.5 g of various extracts was mixed well by shaking with petroleum ether to remove the fatty materials (lipid layer). The defatted residue was dissolved in 20 ml of 80% ethanol and filtered. The filtrate was used for the following tests:

   1. 3 ml of the filtrate was mixed with 4 ml of 1% aluminium chloride in methanol in a test tube. Formation of yellow color indicates the presence of flavonols, flavones and chalcones in the extracts.
   2. 3 ml of the filtrate was mixed with 4 ml of 1% potassium hydroxide in a test tube. Formation of dark yellow color indicates the presence of Flavonoids in the extracts.
   3. 5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of conc H_2SO_4. Formation of yellow color indicates the presence of flavonoids in the extracts.

b) **Shinoda's Test:** The extracts were first dissolved in alcohol solution, and then portion of magnesium was mixed along with conc. HCl which was added dropwise. Formation of Magenta color indicates the presence of flavonoids in the extracts.

5. **Detection of Alkaloids:**

Mayer's test: To the 2ml of the leaves extract, 2ml of conc. HCl was added. Then a few drops of Mayer's reagent were added. Formation of green color or white precipitate indicates the presence of alkaloids in the extracts.

6. **Detection of Quinones:**

a) To the 1 ml of various extracts were treated with alcoholic potassium hydroxide solution. The color change from red to blue indicates the presence of quinines in the extracts.

b) To the 1ml of the various extract, 1ml of conc. H_2SO_4 was added. Formation of red color in the tube indicates the presence of quinones in the extracts.

7. **Detection of Terpenoids:**

Salkowski Test: 5 ml of various solvent extract was mixed in 2 ml of chloroform and 3 ml concentrated H_2SO_4 was added. A layer of the reddish brown color was formed at the interface of the tube that indicates the presence of terpenoids in the extracts.

8. **Detection of Glycosides:**

a) **Keller Killiani Test:**The various extract was treated with few drops of glacial acetic acid and Ferric chloride solution. The tube was mixed well by shaking vigorously and then conc. H_2SO_4 was added. The two layers can be observed in tube, the reddish brown layer in the lower part and acetic acid layer in the upper part. Finally if the solution turns bluish green color indicate a presence for glycosides in the extracts.

b) **Bromine water test:**The various extracts were mixed with a few drops bromine water. Formation of yellow precipitate in the test tube indicates the presence of glycosides in the extracts.

9. **Detectionof Triterpenoids:**

Liebermann Burchard test: The various extracts were added with few drops of acetic anhydride and then the mixture was boiled and cooled. The conc. H_2SO_4 was added along the sides of the test tube. Formation of a brown ring at the junction of two layers, deep red color in the lower part and green color in the upper part indicates the presence of triterpenoids respectively in the extracts.

10. **Detection of Phenols:**

a) **Ferric chloride test:** The 50 mg of extract was mixed well with distilled water and a few drops of neutral 5% FeCl solution was added. Formation of blue, green and violet color indicates the presence of phenols.

b) **Gelatin test:** A Small amount of extract was mixed well with distilled water and 2 mL of 1% solution of gelatin containing 10% NaCl was added and mixed well. Formation of white precipitate indicates the presence of phenols in the extracts.

11. **Detection of Coumarins:**

To the 1ml of the various extract, 1ml of 10% NaOHwas added. Formation of yellow color indicates presence of coumarins in the extracts.

12. **Detection of Proteins:**

Biuret Test: The various extracts were treated with a few drops of 10% NaOH solution and two drops of 0.1% copper sulphate solution in the tube and mixed well. Formation of violet or pink color indicates presence of protein in the extracts.

13. **Detection of Cardiac glycosides:**

To the 2 ml of various extracts a few drops of dilute Hydrochloric acid, 2 ml Sodium Nitropruside in pyridine and Sodium Hydroxide solution were added in the test tube. Formation of pink to blood red color indicates the presence of Cardiac glycosides in the extracts.

14. **Detection of steroids:**

To the 1ml of the various extracts, 1ml of chloroform and few drops of conc. H_2SO_4 were added. Formation of brown ring indicates the presence of steroids in the extracts.

15. **Detection of Phytosterols:**

To the 1ml of the various extracts, 1ml of chloroform and few drops of conc. H_2SO_4 were added. Formation of bluish ring indicates the presence of phytosteroids in the extracts.

3. RESULTS AND DISCUSSIONS:

Yield of leaf extracts obtained from *Sterculia foetida* using various solvents:

In the current study, the yield percentage of the five dried leaf extracts (hexane, chloroform, ethyl acetate, methanol and aqueous) was estimated by measuring its dryness per 100 grams of each extract. The yield percentage were found to be more in aqueous extract > methanol extract >ethyl acetate extract > chloroform extract >hexane extract. The consistency of the extract was found in the form of powder in chloroform and paste in other solvent extracts (table-1).

Table-1: Percentage yield of leaf extracts for *Sterculia foetida* and its consistency.

Plant name	Parts Used	Method of Extraction	Solvent	Yield (%) per 100 g	Consistency
Sterculia foetida Linn	Leaves	Continuous hot Percolation by Soxhlet Apparatus	Hexane	1.39	Paste
			Chloroform	2.41	Powder
			Ethyl acetate	3.87	Paste
			Methanol	7.25	Paste
			Aqueous	12.35	Paste

Screening for phytochemicals from the leaf extracts of *Sterculia foetida*:

The preliminary phytochemical screening was performed to confirm the presence of phytochemicals in the different leaf extracts i.e. hexane, chloroform, ethyl acetate, methanol and aqueous extract of *Sterculia foetida*. The screening tests aids in tracing the presences of active phytochemicals which elicit a major pharmacological attributes.

The result of phytochemical screening revealed that each solvent leaf extracts exhibit the presences of different phytochemicals in the leaf of *Sterculia foetida* (table-2). The occurrences of phytochemicals in the leaf extracts were discussed below:

The highly non polar hexane leaf extract exhibit the maximum presence of two phytochemicals, flavonoids, coumarins followed by the carbohydrates and trace of steroids, phytosterols. The phytochemicals like saponin, terpenoids, phenols, tannins, alkaloids, quinones, triterpenoids, glycosides, proteins, cardiac glycosides were not detected in the hexane leaf extract.

The chloroform leaf extract revealed the maximum occurrence of three phytochemicals like carbohydrates, tannins and quinones followed by triterpenoids and trace of phenol. The phytochemicals like saponin, flavonoids, alkaloids, terpenoids, glycosides, coumarins, acids, proteins, cardiac glycosides, steroids, phytosterols were absent in the chloroform leaf extract.

The Ethyl acetate leaf extract showed the maximum presence of two phytochemicals like tannins, quinones followed by flavonoids, carbohydrates, alkaloids, triterpenoids, coumarins and trace of saponin, terpenoids, phenols, steroids, phytosterols. The phytochemicals like glycosides, proteins, cardiac glycosides were not identified in the ethyl acetate leaf extract.

The methanol leaf extract were observed to exhibit the wide range of four important phytochemicals like carbohydrates, tannins, flavonoids, phenols, followed by saponin, alkaloids, steroids, quinones, phytosterols, coumarins and trace of proteins, terpenoids, triterpenoids. The phytochemicals like glycosides, cardiac glycosides were not detected in the methanol leaf extract.

Table-2: Qualitative phytochemical screening of solvent extracts obtained from the leaves of *Sterculia foetida* Linn.

S.No	Qualitative tests for phytochemicals	Leaf extract from solvents				
		Hexane Solvent	Chloroform Solvent	Ethyl acetate Solvent	Methanol Solvent	Aqueous solvent
1.	**Carbohydrates**					
	a)Benedict's test	++	+++	++	+++	+++
	b) Molish's Test	++	+++	++	+++	+++
2.	**Flavonoids**					
	Shinoda's Test	+++	-	++	+++	++
3.	**Alkaloids**					
	Mayer's test	-	-	++	++	+++
4.	**Terpenoids**					
	Salkowski Test	-	-	+	+	+++
5.	**Glycosides**					
	a)Keller Killiani Test	-	-	-	-	-
	b) Bromine water test	-	-	-	-	-
6.	**Triterpenoids**					
	a)Liebermann test	-	++	++	+	-
	b) Burchard test	-	++	++	+	-
7.	**Phenols**					
	a) Ferric chloride test	-	+	+	+++	+
	b) Gelatin test	-	+	+	+++	+
8.	**Proteins**					
	Biuret Test	-	-	-	+	-
9.	**Cardiac glycosides**	-	-	-	-	-
10.	**Tannins**	-	+++	+++	+++	-
11.	**Coumarins**	+++	-	++	++	++
12.	**Saponin**	-	-	+	++	+++
13.	**Quinones**	-	+++	+++	++	-
14.	**Steroids**	+	-	+	++	-
15.	**Phytosterols**	+	-	+	++	-

Note : +++ = Maximum; ++ = Average; + = Trace; - = Not detected

The higher polar aqueous leaf extract found to possess the maximum presence of four phytochemicals like carbohydrates, alkaloids, saponin, terpenoids which were followed by flavonoids, coumarins and trace amount of phenol. The phytochemicals like tannins, quinones, glycosides, triterpenoids, proteins, cardiac glycosides, steroids phytosterols were absent in the aqueous leaf extract.

The Preliminary screening of phytochemicals from the leaf extracts of *Sterculia foetida* revealed that the maximum number of phytochemicals was observed in the methanol leaf extract and aqueous leaf extracts. The current screening study determined that the methanol leaf extract showed the strong presence of four important phytochemicals flavonoids, phenols, carbohydrates, tannins in the leaf of *Sterculia foetida*, whereas aqueous leaf extract exhibit the presence of phytochemicals of carbohydrates, saponin, alkaloids, terpenoids. By comparing the result of phytochemical analysis, it was confirmed that the methanol leaf extract showed the presence of significant phytochemicals in the leaf of *Sterculia foetida* than other four solvent extracts.

The previous study on preliminary screening of ethanolic leaf extract of *Sterculia foetida* [14] confirmed the presence of metabolites of tannins, 2-deoxysugars, leucoanthocyanin and benzopyrone nucleus. The methanolic leaf extract of *Sterculia foetida* [26] showed the presence of five chemical constituents like alkaloids, proteins, glycosides, phytosterols and saponins whereas result of current study revealed strong presence of secondary metabolites like flavonoids, phenols, carbohydrates, tannins. The difference of the screening results was due to the reflection of geographical location of the plant *Sterculia foetida.*

4. CONCLUSION:

The present work depicts that the plant *Sterculia foetida* Linn possess rich source of the secondary metabolites. The secondary metabolites play a vital role by preventing and treating various diseases occurring in human. The above predicted bioactive compounds are enriched with various pharmacognostical properties like anti-cancer activity, anti-inflammatory activity, anti-microbial activity etc. Hence the

analysis of presence of phytochemicals in the plant play an important role in the identification of novel drug candidates which can be further tested for clinical trials. The above study provided a strong evidence that the methanol leaf extract of *Sterculia foetida* Linn contains high content of medicinally important secondary metabolites such as flavonoid, tannins, carbohydrates, phenols which has high potent in their pharmacological properties. The identification, purification and characterization of the bio active compounds present in those secondary metabolites which would be the major priority in our future studies.

5. CONFLICT OF INTEREST:

The authors declared no conflicts of interest.

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