



Phytochemical, Fluorescence and GC-MS Analysis of Methanolic Extract of *Sterculia foetida* L. Seeds

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Abstract— The present work phytoconstituents of the seed powder of *Sterculia foetida* L. extracted with 98% methanol. The extracted phytochemical compound subjected to qualitative analysis, quantitative analysis, fluorescence analysis and GC-MS analysis. The results of qualitative phytochemical screening confirm that the presence of tannins, phenols, steroids, cardiac glycoside and coumarin. The significant amount of carbohydrate, protein, lipid, tannin and total phenol estimated through quantitative analysis of phytochemicals. The seed powder with the picric acid exhibited fluorescent yellow during fluorescence analysis undertaken with short ultra violet light at 254 nm. There are 13 bioactive compounds were identified through GC-MS analysis of seed powder of *S. foetida* L. These various bioactive compounds possess a wide range of activities such as disease control, pest control and microbicidal effect.

Keywords— *Sterculia foetida*, seed extracts, phytochemical screening, fluorescence analysis, GC-MS analysis.

I. INTRODUCTION

The medicinal plants are widely used in traditional medicine to prevent and treat various diseases. The phytoconstituents present in the various part of the plant can be exhibit anti-cancer, anti-tumour, anti-diabetic, anti-spasmodic, anti-inflammatory, anti-oxidant and antibacterial activities²³. *Sterculia foetida* is commonly called as Wild Indian almond belonging to the family Sterculiaceae. The fruits, seeds and leaves of *S. foetida* have been conventionally known for its many therapeutic purpose⁵. A different variety of pharmacologically active compounds have been isolated from the leaves of *S. foetida* and these compounds from the leaves used as astringent, laxative, antifungal, anti-inflammatory and anti-ulcer medicines²⁶. Nanadagopalan *et al.*, (2015)²³ isolated twenty seven bioactive compounds from the methanolic extract of *S. urens* leaves. The important compounds present in the leaves extract were 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, sucrose, 2, 4-Dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one, 5(2H)-Oxazolone, 4-(phenylmethyl)-,

4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Megastigmatrienone and 2-Methoxy-4-vinylphenol etc and these compounds could contribute the medicinal quality of the *S. foetida* leaves. *S. foetida* seeds are not toxic and edible to humans and animals⁵. The bioactive compounds sterculinine-I, sterculinine-II, and soyacerebroside-I were isolated from the seeds of *S. lychnophora*³⁴. In view of that the present work aimed to identify the pharmacologically active biomolecules responsible for anti-cancer and antioxidant from the methanolic extract of *S. foetida* seed.

II. MATERIALS AND METHODS

2.1. Collection & processing of plant sample

Dried fruits of *Sterculia foetida* were collected from Kelambakkam-603103, Tamil Nadu, India. Seeds were identified and authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Science, Plant Anatomy Research Centre (PARC), Chennai-600045. The seeds

were collected from dried fruits were grinded and sieved to make fine powder for further study.

2.2. Preparation of extracts

Two fifty grams (250 g) from seed powder was extracted with 250 mL of 98% methanol in Soxhlet apparatus for 24 hours. After the extraction the crude extract was filtered through a Whatman number 1 filter paper. Later, the crude extract was subjected to evaporation in a rotary vacuum evaporator for dryness. After the evaporation process completed the concentrated extract was collected and stored at 4°C for further analysis^{9,31}.

2.3. Qualitative analysis of phytochemicals

Methanolic extract of *S. foetida* seed powder was subjected to qualitative phytochemical analysis. The phytochemical analysis such as Ferric chloride test and Lead acetate test for tannins²⁸, Ferric chloride test and Gelatin test for phenols²⁸, Libermann-Buchard test and Salkowski test for sterols²⁸, Keller Killiani test for glycoside²⁸, Coumarin test for Coumarin²⁷ and Phlobatanin test for Phlobatanin³ were performed.

2.4. Quantitative analysis of phytochemicals

The methanolic seed extract was subjected to quantitative analysis by spectrophotometer method. The extract was analyzed for Carbohydrate by Anthrone method¹², Protein by Lowry's method²⁰, Total Lipids³⁵, Total Phenol² and Total Tannin².

2.5. Fluorescence analysis

Fluorescence analysis is the most important parameter of pharmacognostical evaluation. This analysis was carried out as per the standard protocol^{16,18,29}. In the present work, the seed powder was treated with different solvents and chemicals. The seed powder was subjected to fluorescence analysis in visible/daylight and UV light (254 nm).

2.6. GC-MS analysis

Aim of this analysis is to identify the pharmacologically active biomolecule (anticancer & antioxidant) present in the seed extract. The methanolic extract of *S. foetida* seed powder was subjected to GC-MS analysis on the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m×0.25 mm I.D. The study conditions were 20 min. at 100°C, 235°C for column temperature at 3 minutes and 240°C for injector temperature, carrier gas was helium, and split ratio was 5:4. The 1 µl of the sample was evaporated in a split-less injector at 300°C and the run time was 22 min. The phytoconstituents of the extract was identified by Gas Chromatography coupled with Mass Spectrometry. The

GC-MS spectrum was analyzed using the NIST08 library which has more than 62,000 patterns^{4,25}.

III. RESULTS AND DISCUSSION

3.1. Qualitative analysis of phytochemicals

The essential information regarding the phytochemical constituents is generally determined through qualitative phytochemical analysis of plant extracts. The qualitative analysis of the methanolic seed extract showed the presence of secondary metabolites such as tannins, phenols, steroids, cardiac glycoside and coumarin and this analysis also confirmed that the absence of the phytochemical phlobatanin (Table 1). Tannin is an important secondary metabolite found in many plant species with remarkable amounts when compare to other secondary metabolites. Tannin can be present in various parts of plants such as roots, sap, stem, bark, leaves, fruits and seeds. Many researchers proved that the pharmacological properties of plant based secondary metabolites. Vieira Pereira *et al.*, 2015³³ reported that tannin is a pharmacologically active metabolites and it act as astringent and insecticidal agent. Phenol and phenolic compounds contain antimicrobial property, hence they are used to treat skin infection and wound²⁴. Phenol prevent the enzymes that cause antioxidant, inflammation, immune enhancers, hormone modulators and anti-clotting¹³. Sterols present in the plant possess anti-inflammatory effects and might enhance immune function⁷. Glycosides are considered as a useful drugs in therapeutics and it display antitumor activity and antiviral activity against rhinovirus²². Researchers, Agarwal, 2000; Goodman & Gilman's, 2006; Jain and Himanshu Joshi 2012^{1,8,15} proved that the anti-tumor, anti-cancer and anticoagulant activities of plant derived secondary metabolites coumarins. The above mentioned findings supported that the plant based secondary metabolites such as tannins, phenols, steroids, cardiac glycoside and coumarin have pharmacological properties.

Table 1: Phytochemicals present in the seed extracts of *Sterculia foetida*

S. No.	Secondary metabolites	Results
1.	Tannins	
	<i>Ferric chloride test</i>	+
	<i>Lead acetate test</i>	+
2.	Phenols	
	<i>Ferric chloride test</i>	+
	<i>Gelatin test</i>	+

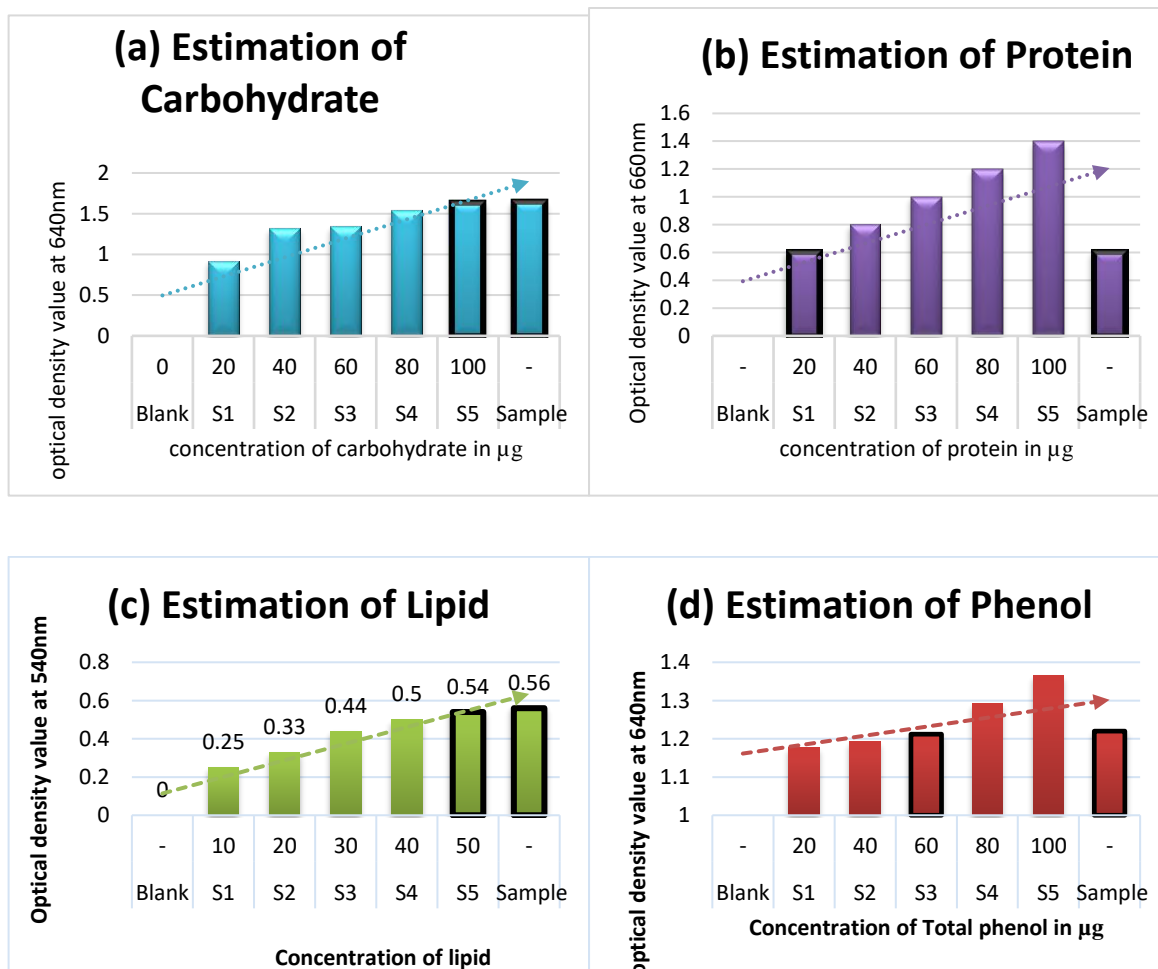
3.	Sterols	
	<i>Liebermann-Buchard test</i>	+
	<i>Salkowski test</i>	+
4.	Glycoside	
	<i>Keller Killiani test</i>	+
5.	Coumarin	
	<i>Coumarin test</i>	+
6.	Phlobatanin	
	<i>Phlobatanin test</i>	-

+ = Presence; - = Absence

3.2. Quantitative analysis of phytochemicals

The amount of Carbohydrate, Protein, Lipid, Phenol, and Tannin present in the seed extract was obtained by plotting the Optical Density value for the standard test tubes. The optical density value for sample tube is compared with one of the same optical density value of the standard tube. The optical density reading of the sample tube is plotted on the graph and the concentration of the component is determined. The amount of carbohydrate present in the

2ml of seed sample is estimated to be 100 μ g as shown in the Fig. 1a. The amount of protein present in the 2ml of seed sample is estimated to be 20 μ g as shown in the Fig. 1b. The amount of lipid present in the 2ml of seed sample is estimated to be 50 μ g as shown in the Fig. 1c. The amount of phenol present in the 2ml of seed sample is estimated to be 60 μ g as shown in the Fig. 1d. The amount of tannin present in the 2ml of seed sample is estimated to be 60 μ g as shown in the Fig. 1e. The secondary metabolites like carbohydrate, protein and lipid have antioxidant, antimicrobial and antiviral properties²¹. According to the literatures, phenolic compounds are known to exhibit antioxidants, anticancer, anti-inflammatory, antimicrobial, anti-allergic and antifertility activity^{14,30}. Tannin is one among the major active secondary metabolite found in wide variety of plants¹¹. Tannin has been reported to possessing antiviral, antioxidant, antibacterial and antitumor activity^{6,17}. In the present work confirm that there is a significant quantities of pharmacologically active secondary metabolites such as carbohydrate, protein, lipid, phenol, and tannin are present.



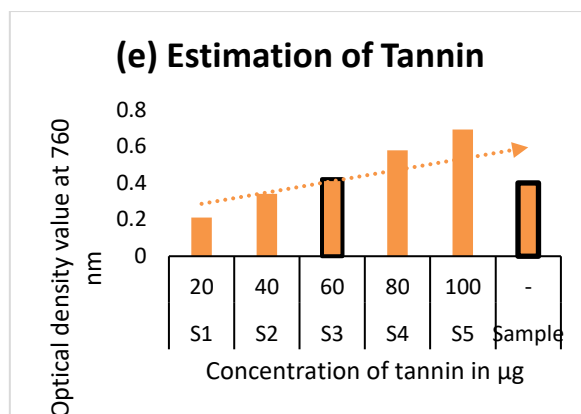


Fig 1 (a-e): The graphs showing results of quantitative analysis of methanolic extract of *Sterculia foetida* seed powder

3.3. Fluorescence analysis

The results of fluorescent analysis of dried seed powder of *S. foetida* with different chemical reagents are given in Table 2. The seed powder was treated with various solvents under visible and short UV light. Among the different solvents analyzed, picric acid showed characteristic coloration in both visible and UV light. Fluorescence analysis is a preliminary pharmacognostic parameter for determining various phytoconstituents present in the plant sample in short period of time¹⁹. The

crude powder of plants shows different coloration when subjected to different chemical reagents at varied wavelength¹⁰. The researcher, VidyaKamble & Nikhil Gaikwad (2019)³² reported that the secondary metabolites like coumarin, sapogenin and terpenoids show yellowish green fluorescence under short UV light. In the present work, the major bioactive metabolites present in the crude powder of *S. foetida* seed was found to be coumarin, phenols, tannins and sterols and this results could confirm that the pharmacognostic properties of *S. foetida* seed.

Table 2: Fluorescence analysis results of *S. foetida* seed powder



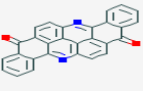

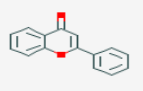

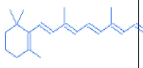
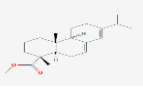

S. No	Treatment	Visible light	UV light (254 nm)
1.	Powder	Light brown	Blackish brown
2.	Powder+Water	Light brown	Blackish brown
3.	Powder+NaoH	Reddish brown	Dark reddish brown
4.	Powder+HCl	Brown	Dark brown
5.	Powder+Acetic acid	Light brown	Dark brown
6.	Powder+Picric acid	Yellow	Yellowish green
7.	Powder+Sulphuric acid	Reddish brown	Dark Reddish brown
8.	Powder+Nitric acid	Brown	Dark brown
9.	Powder+Iodine	Brown	Dark brown
10.	Powder+FeCl ₃	Green	Dark green
12.	Powder+KOH	Brown	Dark brown
13.	Powder+Ammonia	Light brown	Dark brown
14.	Powder+Ethanol	Brown	Dark brown
15.	Powder+Alc.NaOH	Dark brown	Black

3.4. GC-MS analysis

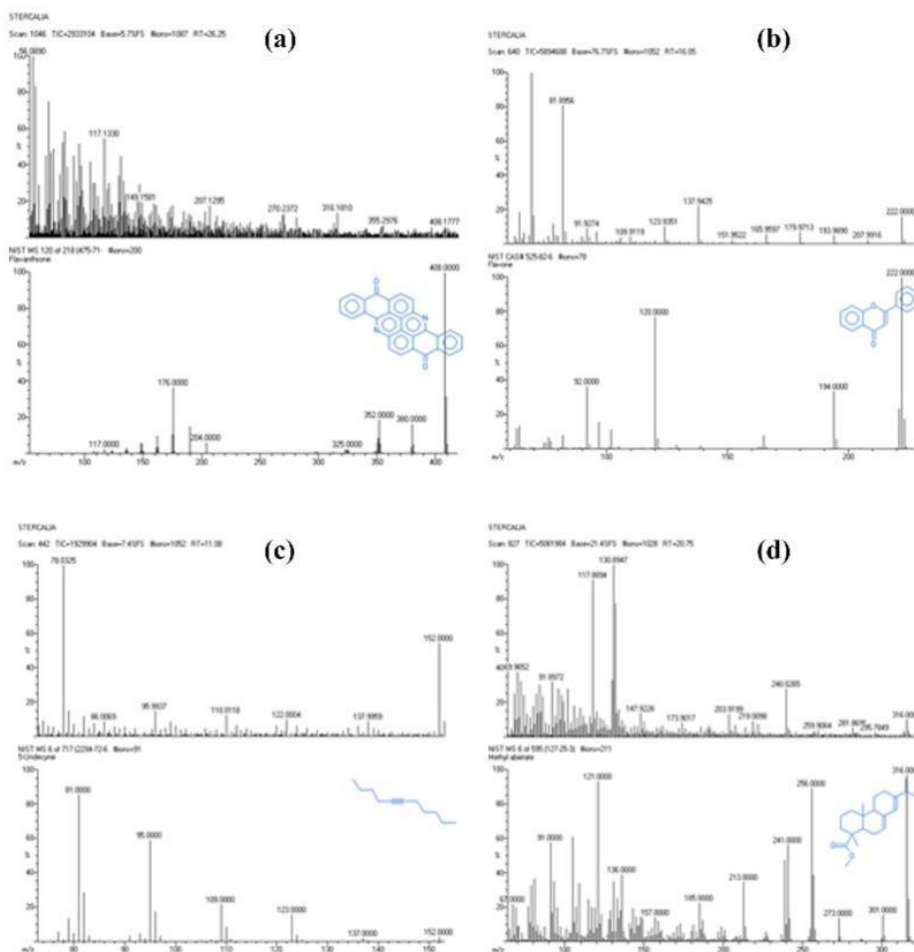
The GC-MS analysis of methanolic extract of *S. foetida* seed powder divulge the presence of thirteen phytochemical compounds that could possess the pharmacological and microbicidal activity. The identification of the biomolecule was confirmed based on the retention time and molecular formula. The biologically active compounds with their Retention time (RT), Molecular formula, Molecular weight, Molecular structure and their Biological activity are presented in Table 3. The pharmacologically active major compounds present in the seeds were Flavanthronone & Flavone (Anticancer activity), 5-Undecyne & Methyl abietate (Antioxidant), 2-cyclohexen-3-ol-1-one,2(11-phenylundecanoyl) (Antidiuretic), Androstan-6-0l-17-one,3-acetoxy-5A-

chloro (Vasodilator) and Testosterone Cypionate (Anti-inflammatory) apart from these compounds other major and minor compounds were also present. The GC-MS graph showing the compounds which showing anticancer (Figs. 2a & b) and antioxidant (Figs. 2c & d) activities are presented in Figs. 2 a-d. Similar to this study, twenty seven major phytochemical compounds were characterized through GC-MS analysis of the methanolic leaves extract of *S. urens* Roxb²³. Asif Jafri et al., 2019⁵ reported that there were thirty five bioactive compounds were characterized via GC-MS analysis of the ethanolic extract of *S. foetida* seed and they were confirmed further that among 35 bioactive compounds many of them possess pharmacological activity and these findings are similar to the present work.

Table 3: Phytoconstituents identified in the seed sample of *Sterculia foetida* using GC-MS

S. No	Retention time	Compound name	Molecular formula	Molecular weight	Structure	Activity
1	18.27	Oleic acid	C ₁₈ H ₃₄ O ₂	282.468g/mol		Herbicide, Insecticide & Fungicide
2	17.53	10-Octadecenoic acid, methyl ester	C ₁₈ H ₃₄ O ₂	282.468g/mol		Insecticide
3	26.25	Flavanthronone	C ₂₈ H ₁₂ N ₂ O ₂	408.416g/mol		Anticancer
4	11.08	5-Undecyne	C ₁₁ H ₂₀	152.281g/mol		Antioxidant
5	16.05	Flavone	C ₁₅ H ₁₀ O ₂	222.243g/mol		Anticancer
6	16.6	Palmitic acid	C ₁₆ H ₃₂ O ₂	256.43 g/mol		Herbicide
7	19.8	Retinal,9-cis	C ₂₀ H ₂₈ O	284.443g/mol		Cell differentiation & Embryonic development
8	20.75	Methyl abietate	C ₂₁ H ₃₂ O ₂	316.485g/mol		Antioxidant
9	21.5	Estra-1,3,5,(10)-trien-17a-ol,3-methoxy-17-(2-methylallyl)	C ₁₈ H ₂₂ O	254.373g/mol		Antibacterial

10	22.15	2-cyclohexen-3-ol-1-one,2(11-phenylundecanoyl)	C ₆ H ₁₀ O	98.145 g/mol		Antidiuretic
11	22.15	3,9-Methano-10 H – furo[3,2-d] azonine-10,11-dione , 9-[2]dimethyl amine]-3-methoxyphenyl] decahydro-2,6-dimethyl-[2 R ^x ,3R ^x ,3a5 ^x ,9R ^x ,10AR ^x]	C ₁₂ H ₉ NO	183.21g/mol		Antibacterial & Antidote
12	22.73	Androstan-6-0l-17-one,3-acetoxy-5A-chloro	C ₂₁ H ₃₂ N ₂ O	328.5 g/mol		Vasodilator & Antimicrobial
13	23.78	Testosterone Cypionate	C ₂₇ H ₄₀ O ₃	412.614g/mol		Anti-inflammatory



(a) Flavanthrone (b) Flavone (c) 5-Undecyne (d) Methyl abietate

Fig 2 (a-d): GC-MS graphs showing the compound possess anticancer and antioxidant activity

IV. CONCLUSION

On the basis of above findings, it can be concluded that the phytochemical screening and fluorescence analysis confirm that the methanolic extract of *Sterculia foetida* seed contains various pharmacologically active secondary metabolites. Furthermore, there are thirteen pharmacologically active biomolecules were identified through GC-MS study. Further studies are required to find out the efficacy of methanolic extract of *Sterculia foetida* seed as it may offer an effective alternative source against many diseases with less to no side effects.

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