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A Study on the Allelopathic Effects of *Samanea saman* (Jacq.) Merr. with Special Reference to Identification and Characterization of the Bioactive Principles

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Abstract: This study has been undertaken to investigate the allelopathic potential of *Samanea saman* (Jacq.) Merr. by identifying the bioactive compounds from the green leaves of the plant. Different concentrations (25, 50, 75, 100 %) of leaf extract were applied to the seeds of test plant *Vigna radiata* to investigate the allelopathic effect. From the experimental results percentage of inhibition increased with the increasing concentration of the extract. Phytochemical constituents of the leaf extract of *S. saman* were screened using standard qualitative analysis. Qualitative estimation revealed the presence of tannins, alkaloids, phenols and saponins. Thirty three bioactive compounds were identified from the methanolic extract of *S. saman* using GC-MS. The analysis revealed the presence of 2,4- Dimethoxyphenol, propanoic acid, coumaran, uric acid, and palmitic acid, methyl ester, having allelopathic potential to inhibit the growth of other plants or organisms. Thus, the presence of bioactive compounds in *S. saman* leaf extract has allelopathic potential and pharmacological properties.

Keywords- *Samanea saman*, Allelopathy, *Vigna radiata*, Bioactive compounds, GC-MS.

I.INTRODUCTION

Potential damage to human health and to the environment from herbicides is regarded today as a real problem. It has resulted in an increased interest in alternative strategies leading to the development of biodegradable compounds [1], [2]. Inhibition of growth of plants by other plants in their vicinity has been known for long time. The chemical interaction between plants, which can cause enhancement or inhibition of growth, has been termed 'allelopathy'[3].

The term allelopathy, was introduced by Molisch in 1937 [4], and was derived from the Greek words allelon 'of each other' and pathos 'to suffer' and mean the injurious effect of one plant upon other plants [5]. Allelopathy is the study of any direct or indirect, harmful or beneficial effect of plants, protists (e.g. microalgae, ciliates), bacteria, or viruses on another through production of chemical compounds that are released into the environment [6]. Chemicals released from plants and imposing allelopathic influences are termed allelochemicals or allelochemics. Most allelochemicals are classified as secondary metabolites and are produced as offshoots of the primary metabolic pathways of the plant. Often, their functioning in the plant is unknown, but some allelochemicals are known also to have structural functions (e.g. as intermediates of lignification) or to play a role in the general defence against herbivores and plant pathogens [3], [7], [8]. Allelochemicals are present in different parts of the plant including roots, rhizomes, leaves, stems, pollen, seeds and flowers. Allelochemicals are expelled into the environment by root exudation, leaching from aboveground plant parts, and volatilisation or by decomposition of plant material [6].

Samanea saman or monkey pod tree is a large canopied tree with symmetrical crown. Although globally distributed, it is of tropical American origin and belongs to the family Fabaceae. The tree is easily recognized by its characteristic umbrella shaped canopy [9]. Leaves are alternately arranged along with the twigs and have a prominent swelling at the petiole base. Glossy green on the top of the leaf and hairy beneath in bottom. The flowers of *S. saman* are tiny. 12-25 flowers per-head are massed with pinkish-red in color. Mature pods (monkey pods) are blackish- brown in color, quadrilateral and it is straight or slightly bowed. The bark of the mature tree is grey, rough fissured in long plates. The inner bark is a light color and bitter. On younger trees, the bark is smoother and pale gray to brownish in color [10].

Samanea saman shows several bioactive compounds which possess various properties such as antioxidants, antibacterial, anti-diabetic, analgesic, anti-ulcer, insecticidal, antifungal, and cytotoxic activities. The leaves were reported to constitute tanins, flavonoids, steroids, saponins, cardiac glycosides and terpenoids. Although the allelopathic potential of this plant were reported [11], the allelochemicals were not identified. The purpose of the present study was to elucidate the inhibitory effect of different

concentrations of leaf extracts of *S. saman* on seed germination of *Vigna radiata*. Thus, the present study on *S. saman* is focused on identification of bioactive compounds, its allelopathic potential and pharmacological activities.

II. RESEARCH METHODOLOGY

2.1 Plant material collection and Extraction:

The mature green leaves of the plant were collected from St. Albert's College Campus, Ernakulam, during the period of January to March, 2022. The Herbarium was prepared by standard method [23]. Voucher specimen (specimen accession no: 514) is deposited in the Herbarium SAC, of the college for future reference. The collected plant materials (mature green leaves) were cleaned under running water and dried under shade for one month and were then ground into powder, stored in air tight containers. Extracts were prepared from the powdered plant materials of mature green leaves of *S. saman* using Soxhlet extractor. For the extraction, 5g of plant material were taken in 250 ml distilled water. The extraction process was carried out for 6 to 8 hours.

2.2 Germination bioassay:

Vigna radiata were used in this experiment for biological assay, selected based on their growth pattern. Seeds of test plant were surface sterilized with 0.1% Mercuric chloride for 5-10 minutes before bioassay analysis. Autoclaved sterilized petri plates were lined with two layers of blotting paper and 20 seeds of *Vigna radiata* are placed on each petri plates. The petri plates were then flooded with sufficient amount of extract each with respective concentrations (25%, 50%, 75%, 100%), of the respective plant part (mature green leaves). Petri plates were labeled and kept in a glass hood at room temperature for 3 days.
Germination percentage was calculated using the formula;

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated} \times 100}{\text{Total number of seeds}}$$

2.3 Phytochemical Screening:

The phytochemical tests were carried out on the distilled water extracts of the powered leaves of *S. saman* using the standard methods to identify the phytochemical constituents [12].

Test for Tannins were done according to Obianime and Uche, 2008 [12]. The presence of tannins was indicated by the formation of greenish black colour. According to Adegoke et al., 2010, turbidity or precipitation was taken as evidence for the presence of Alkaloids by Dragendroff's test. In the Aqueous test, lather formation indicated the presence of Saponins [13]. Development of purple colour indicated the presence of Amino acids or proteins in Ninhydrin test [14]. Molisch's test indicated the appearance of reddish- violet ring at the junction of two liquids for the presence of Carbohydrates [14]. According to Nidal *et al.*, 2008, Sulphuric acid test indicates the appearance of reddish precipitate for Glycosides. Deep blue- black colour indicates the presence of Phenolic compounds [15].

2.4 Identification of Bioactive Substances using GC-MS:

The distilled water extract of *Samanea saman* were evaporated to dryness using temperature controlled water bath. The final residues were extracted using the solvent 2ml Methanol (HPLC Grade). 1ml of the supernatant was taken and 2µl were injected into the GC/MS/MS. The apparatus include micropipettes, syringe filter, methanol (HPLC Grade), helium gas as carrier gas (extra pure), and water bath. The samples were analysed by the Electron Impact (EI) GC/MS/MS technique, using a TRACE-1300 Gas Chromatograph and interfaced to a Thermo Scientific TSQ EVO-8000 MS/MS Mass Spectrometer. Injections are 2µl in size in the split less mode onto a 30m long 5% phenyl- 95% dimethylpolysiloxane low bleed capillary column (Thermo Scientific): TG-5ms 30m × 0.25mm, 0.25µm. Library used is NIST main library.

IV. RESULTS AND DISCUSSION

3.1 Germination percentage:

The results showed significant effect on the germination percentage of *Vigna radiata* seeds treated with different concentrations of the extract of green leaves of *Samanea saman*. The germination percentage was significantly lower in all treatments (25, 50, 75, 100%) than the control. The inhibitory effect gradually increased with progressive increase in concentration of the extracts. The degree of reduction increased as the extract concentration progressively increased from 25% to 100%. At the highest concentration of extract (100%), all aqueous extracts significantly reduced seed germination compared with distilled water control (Figure: 1). This finding is supported by Chung *et al.*, 1995 [16], who found that the degree of inhibition increased with increased concentration of the extract. Also, in the studies of Iqbal *et al.*, 2009 [17], reported that the extracts of *S. saman* caused inhibition in higher concentration than in lower concentration. Growth and germination significantly reduced could be due to the presence of inhibitory compounds. Inhibitory potential increased with increasing concentration of the extract suggests that the phytotoxicity may be proportional to the concentration of toxins in the environment [18]. From these observations, it can be concluded that the plant *S. saman* is having allelopathic potential to inhibit the growth of other plants or organisms.

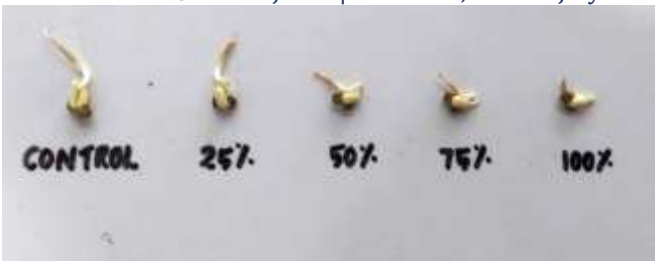
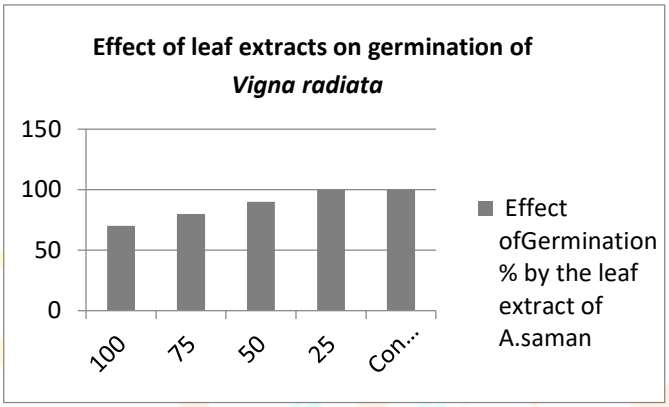


Figure: 1 Effect of leaf extracts on germination of *Vigna radiata*

Graph: 1 Germination Percentage of leaf extract of *Samanea saman*



3.2 Phytochemical screening:

The study revealed the presence of phytoconstituents in the extracts of *Samanea saman* leaves. The solvent extracts of green leaves of *S. saman* were done using distilled water. The phytochemicals screened for the analysis are tannins, saponins, alkaloids, phenols, amino acids, glycosides, and carbohydrates. Of the seven phytochemicals screened, tannins, alkaloids, saponins, and phenols were found present in the solvent extracts. Presence of phenol results in inhibition of germination. Phenols have been reported to interfere with the activities of respiratory enzymes in seed germination [19]. The presence of tannins, alkaloids and saponins also show the inhibitory effect on the germination of seeds. Thus these phytochemicals have allelopathic potential on other plants. Triterpinoids, saponins have been shown to inhibit the root and shoot growth of Alfalfa [20]. Saponins are a special class of glycosides having soapy characteristics. It has also been reported that saponins are active antifungal agents. Tannins are also known antimicrobial agents. Tannins are reported to have various physiological effects like anti-irritant, antimicrobial and antiparasitic effects [21]. Therefore, it could be concluded that the presence of allelopathic phytochemicals in *S. saman* leaf was responsible for the inhibitory effect on the seed germination and seedling growth of *Vigna radiata*. Hence, the phytochemical compounds from *S. saman* can be used as biodegradeable and environment friendly resource.

Table: 1. Result of phytochemical screening of leaf of *Samanea saman*

S. No.	Phytochemicals/ Solvent extracts	Distilled water
1.	Alkaloids	+
2.	Phenols	+
3.	Tannins	+
4.	Saponins	+
5.	Amino acid	-
6.	Carbohydrates	-
7.	Glycosides	-

+ = present, - = absent

2.3 Identification of Bioactive compounds using GC-MS:

The GC-MS analysis of leaves revealed the presence of thirty three compounds (phytochemical constituents) that could contribute the allelopathic quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area and retention time. The first compound identified with less retention time was Oxime-, methoxy- phenyl- (3.72 min) whereas Propanoic acid was the last compound which took longest retention time (24.09 min) to identify. Biological activities listed are based on Dr. Duke's Phytochemical and Ethnobotanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA [22]. From the GC chromatogram, with the reference to the Retention time, many useful constituents identified were found biologically active compounds. Each compound identified in the leaf extracts of *S. saman* has its own biological importance. It included alkaloids, phenolic compounds, and other organic compounds in the analysis. Most of them were found to possess antimicrobial, antibacterial, anti-inflamatory properties. The compounds possessing insecticidal, pesticidal, herbicidal inhibitory properties identified are coumaran (5.69), 2,4- Dimethoxyphenol (6.71), Imidazole, 4- methyl- 5- [2- methyl- 2- propenyl]- (6. 94), uric acid (11.33), and palmitic acid, methyl ester (14.57), propanoic acid (24.09) [22]. Presence of these

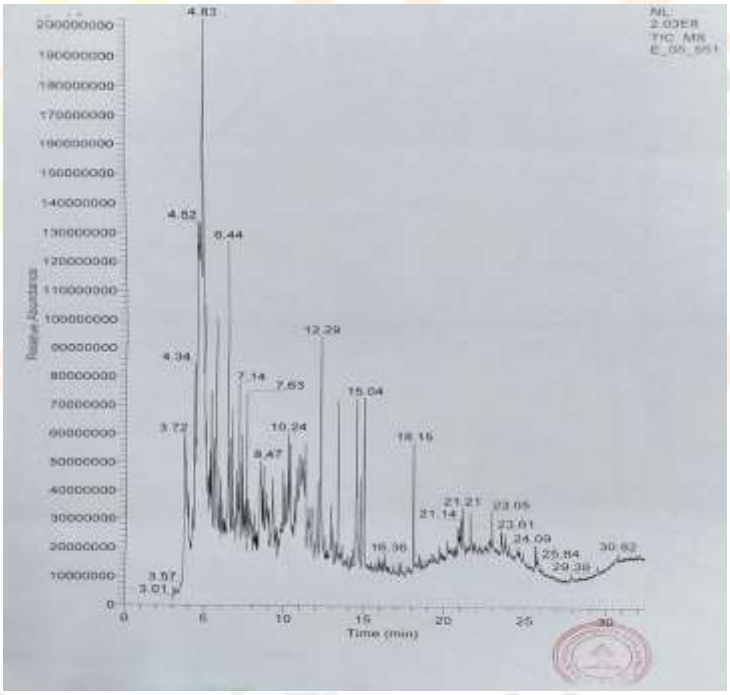
compounds may be the reason for the alleopathic potential. Thus, the plant *S. saman* is having the inhibitory property to suppress the growth of other plants or organisms.

Table: 2 Major phytochemical compounds identified in the distilled water extracts of *Samanea saman*

Peak	**Phytochemical compound**	**Retention time**	**Compound nature**	**Property**
1	Oxime- methoxy- phenyl-	3.72	Alkaloid	Antioxidant, antimicrobial activity
2	Glycine,N-[4-[(trimethylsilyl)oxy]benzoyl]- methyl ester	3.90	Amino acid compound	Antimicrobial
3	4- Hydroxy- N- methylpiperdine	4.34	Piperdine alkaloids	Antimicrobial
4	4- Hydroxypiperidine	4.51	Piperdine alkaloids	Antifungal, antimicrobial
5	4- Aminobutanoic acid	4.82	Organic compound	Regulates plant growth
6	6- Acetyl- a-d- mannose	5.04	Reducing sugar	Anti- arrhythmic effect
7	Dec- 9- en- 6- oxo- l -ylamide	5.25	Organic compound	Antifungal property
8	2- (2- vinyloxy- ethoxy)- cyclohexanol	5.39	Organic compound	Fragrant property
9	Coumaran	5.69	Acetyleholinesterase	Inhibitor, biopesticide
10	2- methoxy- 4- vinylphenol	6.44	Phenolic compound	Antioxidant, antimicrobial, and anti- inflammatory
11	2,4- Dimethoxyphenol	6.71	Phenolic compound	Antifungal, insecticide
12	Imidazole, 4- methyl- 5- [2- methyl- 2- propenyl]-	6.94	Organic compound	Antifungal, pharmaceutical, herbicide, precursor to a variety of agrichemicals
13	6- epi- shyobunol	7.06	Organic compound- elemane sesquiterpenoids	Antibacterial
14	Erucic acid	7.63	Mono unsaturated omega 3 fatty acid	Antibacterial
15	Melezitose	8.24	Non reducing trisaccharide sugar	Antioxidant
16	Deoxyspergualin	8.47	Organic compound	Anti- tumour activity
17	Uric acid	11.33	Purine alkaloids	Antioxidant, inhibitory property
18	Ingol 12- acetate	12.12	Terpene	Antibacterial, anti- inflammatory
19	6- Hydroxy- 4,4,7a- trimethyl- 5,6,7,7a tetrahydrobenzofuran-2 (4H)- one	12.30	Organic compound	Anti-inflammatory
20	Folic acid	13.20	Vitamins	Antioxidant
21	2-(4- Nitrobutyryl) cyclooctanone	13.39	Organic compound	Antitumor activity
22	Methyl phaseate	14.51	Ester	Antioxidant
23	Palmitic acid, methyl ester	14.57	Saturated fatty acid	Antioxidant, hypocholesterolemic nematicide, pesticide, lubricant, antiandrogenic, flavor,hemolytic,5-alphareductase

				inhibitor
24	5,10- Diethoxy- 2,3,7,8-tetrahydro- 1H, 6H-dipyrrrolo [1,2-a: 1^1, 2^1- d] pyrazine	15.04	Organic compound	Antibacterial
25	Aminoacetamide, N- methyl- N-[4-(1- pyrrolidinyl- 2- butynyl]-	16.27	Organic compound	Anti- diabetic property
26	Dasycrpidan-1- methanol, acetate (ester)	16.35	Ester	Anti-inflammatory
27	Methyl isostearate	18.16	Fatty acid methyl esters	Antioxidant, antibacterial
28	2- Myristynoyl pantetheine	18.65	Organic compound	Anti-inflammatory
29	a- D- Galactopyranoside, methyl 2,6- bis-o-(trimethyl silyl)-, cyclic methyl boronate	19.78	Unknown	New chemical compound
30	Cholestan- 3- one, cyclic 1,2-ethanediyl acetyl,(5a)-	21.72	Organic compound	Anti- inflammatory
31	Palmitin, 1,2-di-	23.05	Saturated fatty acid	Antioxidant
32	Methyl glycocholate,3 TMS derivative	23.81	Methyl ester	Anti-inflammatory, antioxidant, antimicrobial
33	Propanoic acid	24.09	Carboxylic acid	Antiseptic, insecticide

Graph: 1 GC-MS Chromatogram of the methanol extract of *Samanea saman*



IV CONCLUSION

From the present study it is evident that *Samanea saman* has allelopathatic potential. Phytochemicals found present in leaf extracts of *S. saman* indicates their potential as a source of allelopathy and pharmacological activity. Allelopathy can potentially be used as growth regulators, herbicides, insecticides and antimicrobial crop protection products. Insight of these concerns, allelopathy can be explored and used as alternative weed management over synthetic herbicides. Also the incorporation of allelopathic substances into agricultural management might reduce the development of pesticides and environmental deterioration.

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