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Evaluating Antioxidant and Antimicrobial Potential of *Albizia saman* Extract Against *Candida albicans*

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Abstract

Albizia saman is a tree of the Fabaceae family that has been used for ethnomedical purposes since olden times. Prior investigations have reported probable medicinal value against a wide array of ailments, which may be attributed to its varied phytochemical composition. Thence, there is a requirement for comprehensive studies on its efficacy against individual pathogens and their mechanisms. The present study is an effort to encompass a comprehensive description of the antimicrobial, anti-inflammatory, and antioxidant potential of A. saman extract with major emphasis against Candida albicans. Various microbial methods have been used for the determination of the antimicrobial potential of A. saman extract, including disc diffusion, well diffusion, streak plate, and various dilution techniques. The anti-inflammatory and antioxidant activities were assayed in vitro and in vivo by various models. A. saman extract exhibited significant antimicrobial activity against the tested pathogens, C. albicans. It also potent anti-inflammatory and anti-oxidant activity. Phytochemical screening for A. The phytochemical screening of the leaf extract of Saman revealed several important phytochemicals: tannins, alkaloids, carbohydrates, saponins, flavonoids, protein, phenol, and ninhydrin. In view of the antimicrobial, anti-inflammatory and antioxidant properties of the extracts of A. saman, it holds immense potential in the development of new therapeutic agents. Findings of the present study clearly show that Albizia saman could be utilized to reveal the traditional uses of this plant, and to discover new therapeutic uses.

Keywords: *Albizia Saman, Bioactive Compound, Candida Albicans, Efficacy and Safety, Health and Well-Being, Medicinal Properties, Phytochemical Composition, Therapeutic Potential.*

Introduction

Albizia saman is a deciduous tree that belongs to the Fabaceae family. Traditionally, it has been used for its medicinal properties. There are several studies documented that have reported its potential against many diseases and pathogens because of the variety of phytochemical composition within this plant. However, studies done have been patchy, so comprehensive research should be done to know its efficacies against specific pathogens and the exact mechanisms involved. Its potential antimicrobial, anti-inflammatory, and antioxidant properties give way to interest in

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the research. One of the significant areas of research would be into the antimicrobial activity of *A.saman* extract against a number of pathogens, like *Candida albicans* [1, 2]. In humans, *Candida albicans* is a common opportunistic fungus pathogen of many diverse infections, especially in immunocompromised patients. Several microbial methods have been utilized to evaluate the antimicrobial potential of *A. saman* extract, covering disc diffusion, well diffusion, streak plate, and dilution techniques [1]. All of these methods allow the determination of the minimum inhibitory concentration and inhibition zones, which act as indicators of potency and spectrum considerations of antimicrobial activity [2, 3]. The potential of β-sitosterol as a cytotoxic agent that causes oral cancer cells to undergo apoptosis presents encouraging opportunities for better treatment alternatives [4].

Other than antimicrobial properties, Anti-inflammatory and antioxidant activities of *A. saman* extract have also been investigated [1, 5]. Inflammation is one of the complex biological responses of an organism to various stimuli, including pathogens, damaged cells, or irritants. If not checked, then it may result in tissue damage. Antioxidants are the compounds capable of neutralizing free radicals and reactive oxygen species known to be awarding elements in various diseases such as cancer, cardiovascular disorders, and neurodegenerative diseases. Through its inhibition of HSC-3 oral squamous carcinoma cells' proliferation, migration, invasion, and aerobic glycolysis, calotropin has anti-cancer effects [6].

Extract of *A. saman* were evaluated for anti-inflammatory and antioxidant activities in various models, both in vitro and in vivo. These studies attempted to determine bioactive compounds such as phenols, tannins, and flavonoids, which may be responsible for their observed biological activities. It will therefore try to give a critical overview of the antimicrobial, anti-inflammatory, and antioxidant activity of the *A. saman* extract with a focus on *C. albicans*. This review will emphasize various microbial techniques that have been applied in evaluating the antimicrobial potential of these extracts, including their plausible mechanisms underlying their anti-inflammatory and antioxidant activities attributed to these plant extracts. It is expected that this review will improve the understanding of the therapeutic potential of *A. saman* and its applicability in developing new antimicrobial, anti-inflammatory, and antioxidant agents. Besides, it will give emphasis to new investigations directed toward the elucidation of bioactive specific compounds responsible for the biological activities observed and the evaluation of efficacy and safety then in clinical settings [1, 5]. H. pylori's significance in stomach disorders and reinfection, its prevalence in deep carious lesions, and the importance of eradicating active caries in children with H. pylori while advocating for more extensive study on eradication techniques [7]. Numerous genetic alterations in different grades of OSCC are found by NGS analysis, which helps with minimal intervention techniques and individualized treatment planning [8].

Materials And Method

Collection of Plant Materials

The fresh and healthy leave of the plant *Albizia saman* were collected from the area of Meenakshi ammal dental college and hospital Chennai, India. the plant was collected and preserved for previous used (Figure 1).

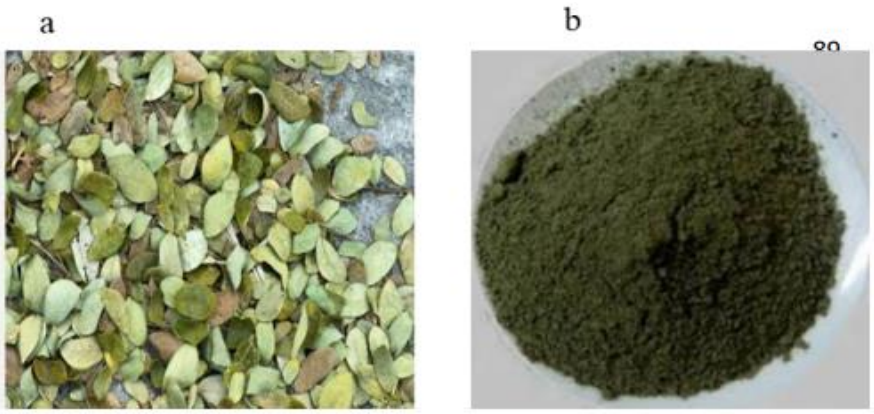

Figure 1. *Albizia saman* Plant Leave Dried at Shade (a). *Albizia saman* Powdered form (b)

Preparation of Plant Extract

Extraction

Extraction of the leaf extract of *Samanea saman* was modified at different steps. The leaves were dried in shade and powdered, then prepared an ethanol solution in the orbital shakers, and maintained for 48 h. This was allowed to shake for 3 h before incubating in a water bath at 60 °C for further 72 h under reflux avoiding increase in temperature above boiling point of solvent. This extract was filtered and evaporated in a rotary evaporator and dried out using a dessicator. The compound was obtained as dark greenish solid residue in 5.321 g (23.0% of the plant material). This process yielded dozens of additional extracts. Plants were then extracted and the sterilized bottles of plant samples fridge until analysed. The dry weight of the plant extracts was determined by solvent evaporation and used in calculating the concentration in mg/ml (Figure 2). These were preserved at a temperature of 2-4°C and were kept for further investigation of antimicrobial activity [9,10].

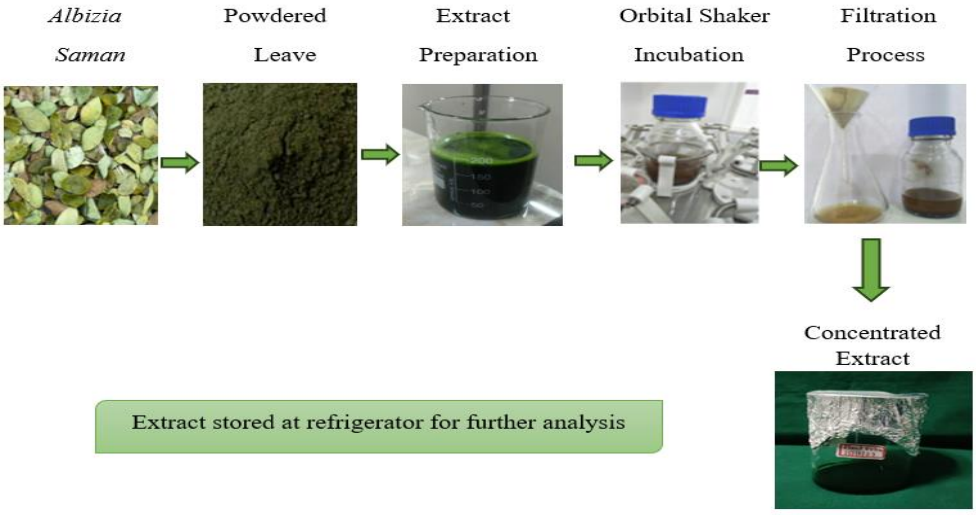

Figure 2. Extraction Method using Ethanolic Solvent System

Preliminary Phytochemical Screening

Phytochemical preliminary tests were carried out on the extracts to identify different chemical compounds. The powdered air-dried plant materials were screened for the presence of different chemical groups namely, saponins, tannins, alkaloids, flavonoids, terpenoids, steroids, cardiac glycosides, phenol, ninhydrin, carbohydrates, starch and protein as described in relevant [11, 12].

Phytochemical Screening

Phytochemical screening serves as a crucial initial step in understanding the bioactive compounds present in plant extracts. The diverse chemical constituents of *Albizia saman* may include alkaloids, flavonoids, tannins, saponins, and terpenoids, among others. These compounds have been previously associated with antimicrobial and antioxidative activities. The methodology involves the extraction of bioactive compounds from *Albizia saman* using suitable solvents, followed by qualitative analysis to identify the presence of various phytochemicals. The results of this screening will provide insights into the potential bioactive compounds responsible for the observed antimicrobial and antioxidative effects.

Test for Tannins

Boiled extract (0.1 g) in 2 ml of water /DMSO was filtered, the filtrate was made alkaline with a few drops of NaOH solution and then shaken with few drops of Plant Plant and 0.1% ferric chloride. You can find reddish with green or maybe, the coloring is typically blue and often black.

Test for Alkaloids

The extract (0.1 g) was dissolved in acidified alcohol (10 ml), heated in a steam bath for 10 min, and separated by filtration & the filtrate was equally distributed into two parts. Besides, 0.4 ml dilute ammonia (10% v/v) and 1 ml chloroform were added to one of the duplicate parts, this mixture was vortexed. This layer was extracted with 2 ml of acetic anhydride. In the second part of the filtrate, Dragendroff's reagents were used for detection. A cream color formation or reddish-brown with Mayer's or Dragendroff's reagents, respectively, was taken as alkaloids present.

Test for Glycoside

Test material (0.2 g) was extracted with 5 ml of each of 1% and 5% aqueous hydrochloric acid (boiling/warming), filtered, and neutralized with 5% aq. NaOH solution. Then, Fehling's A and B reagents were added as above. If a larger quantity of red precipitate was withdrawn from acid hydrolysate than that from the alkaline hydrolysate, glycosides may be present.

Test for Carbohydrates

The acidity adjusted to approximately neutral by including a measured amount of sodium hydroxide (1 N) from a 5 ml pipette after Molisch's reagent (alcoholic α-naphthol) is added to the extracts. The addition of 1 ml of concentrated sulfuric acid is then possible, carefully along the side of the test tube, a purple ring at the interface shows the presence of carbohydrates.

Saponins Test

Emulsification upon shaking vigorously of 3 drops of olive oil into the froth created by adding 0.1g of test extract into 1 ml of distilled water indicates the presence of saponins.

Flavonoids Test

The presence of flavonoids can be tested using two methods:

Alkaline Reagent Test

Add a few drops of sodium hydroxide solution to the test solution. If an intense yellow color develops and then turns colorless upon adding dilute acid, it indicates the presence of flavonoids.

Zinc Hydrochloride Test

After adding a mixture of zinc dust and concentrated hydrochloric acid to the test solution, the development of a red colour within a few minutes is considered positive for flavonoids.

Terpenoids Test (Salkowski’s test)

Add 0.4 ml of chloroform to 0.1 g of the test extract. Follow with concentrated sulfuric acid.

If a reddish-brown coloration appears at the interface, it indicates the presence of terpenoids.

Proteins Test

Add biuret reagent to the test solution. If a violet colour develops, it indicates the presence of proteins.

Phenol Test

Dissolve 50 mg of the test extract in 5 ml of distilled water. Add a few drops of neutral 5% ferric chloride solution. A dark green coloration after this addition is considered positive for phenolic compounds.

Antioxidant Activity

DPPH- Free Radical Scavenging Assay

In addition to antimicrobial properties, *Albizia saman* might show antioxidant effects, given the presence of various phytochemicals like flavonoids and phenolic constituents. Antioxidant assays will be performed such as the total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) to assess the antioxidant activity of the plant extracts. Understanding the antioxidant activities of *Albizia saman* extracts is crucial, since oxidative stress is relevant to many disease processes. The outcome of the antioxidant activity tests will give further evidence to support the rumor that the plants have potential in the fight against oxidative stress, and what their significances are, as well possible therapeutic uses of this plant [13].

Anti Inflammatory Activity

Bovine Serum Albumin (BSA) Denaturation Assay

To assess anti-inflammatory activity, *Albizia saman* extract (0.05 mL) was mixed with a 1% aqueous solution of bovine serum albumin (0.45 mL) at various concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, and 50 µg/ml). The solution's pH was adjusted to 6.3 using 1N hydrochloric acid. After pH adjustment, the samples were incubated for 20 minutes at room temperature and then placed in a water bath at 55°C for 30 minutes. Following cooling to room temperature, absorbance was measured at 660 nm using a spectrophotometer. Diclofenac sodium served as the standard drug for comparison, and DMSO (dimethyl sulfoxide) was the control in this experiment [14].

The percentage of protein denaturation was determined using the following equation:

$$Inhibition\ \% = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

Antimicrobial Assays

Albizia saman extracts will be assessed for their antimicrobial activity against *Candida albicans* using various microbiological methods. These include disc diffusion assays, broth microdilution assays, and determination of minimum inhibitory concentrations (MICs) to evaluate the efficacy of the plant extracts against these pathogens. All test were conducted with Nutrient agar incubated at 37^{o}c for 24hours, 48hours and 72 hours at different concentration like 150µg, 300µg, 450µg, 600µg, 750µg. The results from these assays will provide vital information on the potential utility of *Albizia saman* as a remedy or supplement to current antimicrobials. Knowledge of the antimicrobial potency and the concentration necessary to yield that activity could help to inform future research and development of plant-based therapeutics [15].

Results

Phytochemical Screening

The qualitative phytochemical screening of *Albizia saman* leaf extract revealed the presence of several important phytochemicals. The extract was found to contain Tannis, Alkaloid, Carbohydrate, Saponins, Flavonoids, Protein, Phenol, and Ninhydrin. However, the screening also showed the absence of

glycoside, terpenoide, and steroid in the extract showed in (Table-1). These findings provide valuable insights into the chemical composition of *Albizia saman* leaves, which could have implications for its potential medicinal and therapeutic applications. Further research may be warranted to explore the specific roles and properties of these identified phytochemicals in the plant.

Table 1. Phytochemical Screening of *Albizia saman* Leaf Extract Revealed the Presence of Several Important Phytochemicals

S.no	Phytochemical components	Test	Observation	Inference
1.	TANNINS	Galatin	White precipitate	+
2.	ALKALOID	Wagner's	Reddish brown precipitate	+
3.	GLYCOSIDE	Legals	Blood red colour	-
4.	CARBOHYDRATE	Benedicts	Orange red precipitate	+
5.	SAPONINS	Forth	Oil Emulsion	+
5.	FLAVANOIDS	Alkaline reagent	Yellow precipitate	+
6.	TERPENOIDS	Salkowski's	Violet colour	-
7.	PROTEIN	Xanthoproteic	Yellow colour	+
8.	PHENOL	Ferric chloride	Bluish black colour	+
9.	NINHYDRIN	Amino acid	Deep blue	+
10.	STEROID	Steroids	Violet colour	-

(+): - Present, (-):- Negative

DPPH- Free Radical Scavenging Assay

The anti-oxidant and anti-inflammatory assays on *Albizia saman* extract reveal that the extract's inhibitory effect changes depending on the concentration used. In anti-oxidant the sample concentration increased, the percentage inhibition also increased. Notably, the highest inhibition was observed at 100 µg concentration (74.15%) (Table-2). This indicates that *Albizia saman* extract has strong antioxidant properties, effectively neutralizing free radicals and oxidative stress (Figure 3).

Table 2. Percentage of Inhibition of DPPH

S.no	Sample Concentration	% Inhibition
1	20 µg	33
2	40 µg	39.8
3	60 µg	50.45
4	80 µg	58.7
5	100 µg	74.15

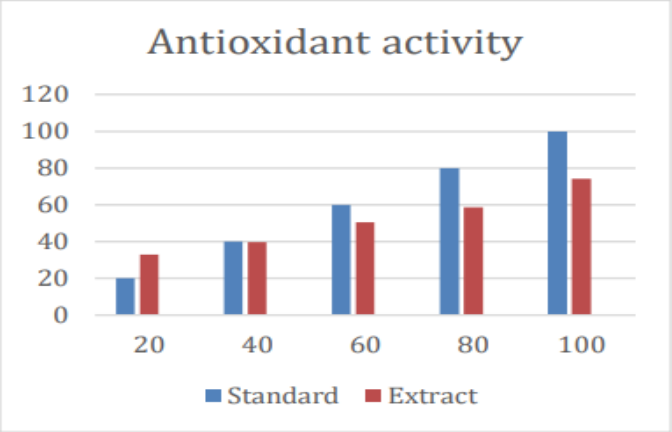

Figure 3. DPPH Radical Scavenging Activity

In anti-inflammatory assays at a concentration of 20µl, the extract showed a significant 30.5% inhibition of albumin denaturation, which increased as the concentration was raised. For instance, at 40µl, the inhibition rose to 36.7%, and at higher concentrations, such as 60µl, 80µl, and 100µl, the inhibition levels were 51.3%, 56.7%, and 73.45%, respectively (Table 3). These findings strongly suggest that *Albizia saman* extract possesses potent anti-inflammatory properties, with higher concentrations exhibiting stronger inhibitory effects. This concentration-dependent response highlights the potential of *Albizia saman* extract as a promising candidate for the development of anti-inflammatory therapies (Figure 4).

Table 3. Percentage of Inhibition of Albumin Denaturation

S.No.	Sample concentration	% Inhibition
1	20µg	30.5
2	40 µg	36.7
3	60 µg	51.3
4	80 µg	56.7
5	100 µg	73.45

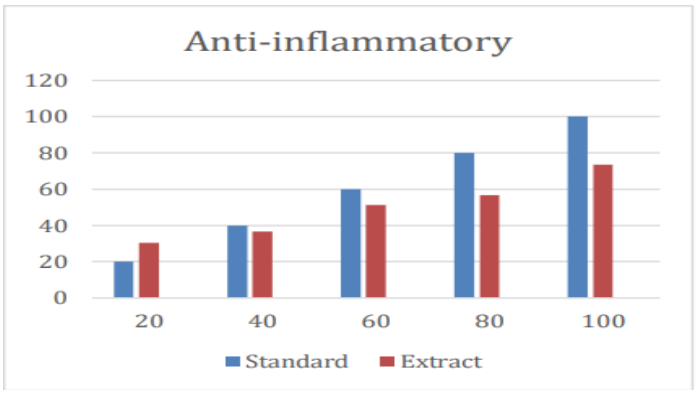

Figure 4. Protein Denaturation Inhibitory Activity

Antimicrobial Assay

Over a 72-hour period, the petri dishes displayed varying zones of inhibition around the central discs. Initially, after 24 hours, the zones indicated moderate antimicrobial activity. However, by 48 hours, the zones expanded significantly, suggesting increased effectiveness. Remarkably, after 72 hours, the plant extract exhibited even larger zones of inhibition, surpassing the standard substance. Inhibition Zone Increase with Volume: Across all incubation times, there is a general increase in the zone of inhibition up to a point with the volume of the substance at 700 µg. Incubation Time Effect: A longer incubation time shows a greater zone of inhibition, with 48 and 72 hours showing larger zones compared to the 24-hour incubation. It therefore means that the efficiency of the substance rises with an increase in time.

These observations could indicate that the substance has antibacterial properties, being most effective both with increasing volume and with longer incubation periods. These findings underscore the plant extract's potent antimicrobial properties, making it a promising candidate for further investigation and potential natural medicine applications (Figure 5).

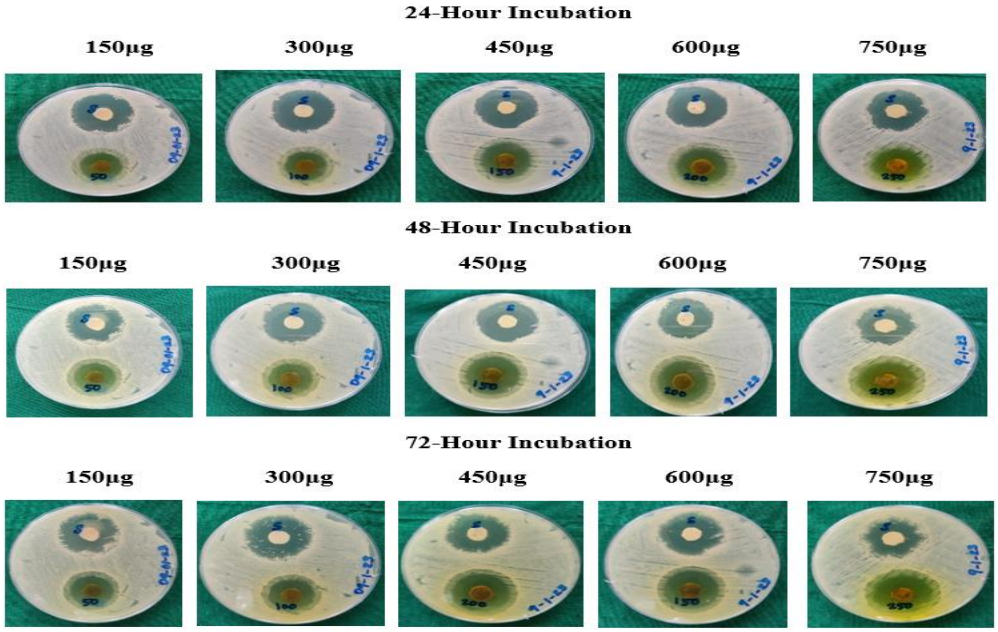

Figure 5. Showing Zone of Inhibition of Candida Albicans by the Plant A.Saman Extract

Discussion

The extracts derived from the *Albizia saman* (*A. saman*) tree have garnered significant attention due to their promising therapeutic properties, particularly their antimicrobial, anti-inflammatory, and antioxidant activities. These diverse biological activities make *A. saman* a compelling candidate for the development of new therapeutic agents. Antimicrobial Properties of *A.saman* Extracts The antimicrobial potential of *A. saman* extracts has been extensively studied, and the results have demonstrated their strong activity against a variety of pathogens [15]. Since elevated salivary MMP-9 levels are correlated with malignant transformation and OSCC severity, they may be used as a prognostic and early detection diagnostic [16].

The antimicrobial activity has been evaluated using several well-established methods, such as disc diffusion, well diffusion, streak plate, and dilution techniques. These diverse assays have helped to determine the strength and breadth of the antimicrobial effects exhibited by the *A. saman* extract. The disc diffusion and well diffusion methods, for

instance, have been employed to assess the zone of inhibition produced by the extracts against different microbial strains. These techniques provide a qualitative assessment of the antimicrobial potency, allowing researchers to compare the efficacy of the extracts against various pathogens. The results from these antimicrobial assays have consistently demonstrated the potent antimicrobial activities of the *A. saman* extract. The extracts have shown the ability to effectively inhibit the growth of a wide range of pathogenic microorganisms, including both bacteria and fungi. This broad-spectrum antimicrobial activity suggests that the *A. saman* extract may be a valuable resource in the development of new antimicrobial agents, potentially useful in the treatment of various infectious diseases [17]. Anti-inflammatory and Antioxidant Activities of *A.saman* extract. In addition to their antimicrobial properties, the *A.saman* extracts have also been extensively studied for their anti-inflammatory and antioxidant activities. The results of these investigations have confirmed the presence of both of these beneficial biological activities. The anti-inflammatory properties of the *A. saman* extract have been found to be concentration-dependent, indicating that the extracts can effectively inhibit inflammatory processes in a dose-responsive manner [18]. miRNAs exhibit promise as therapeutic targets and biomarkers for OPMD early detection and treatment [19]. This suggests that the *A.saman* extract may be useful in the management of various inflammatory conditions, as they have the potential to suppress the underlying inflammatory mechanisms. Furthermore, the antioxidant activity of the *A. saman* extract has been demonstrated to be significant. The extracts have shown the ability to neutralize reactive oxygen species and scavenge free radicals, which are known to contribute to oxidative stress and various disease states. This antioxidant capacity of the *A. saman* extract is an important attribute, as it suggests that they may be beneficial in the prevention and management of conditions associated with oxidative stress, such as chronic diseases and aging-related disorders. Phytochemical composition of *A. saman* extract. The phytochemical screening of the *A. saman* leaf extracts has revealed the presence of several important bioactive compounds, including flavonoids, tannins, alkaloids, carbohydrates, saponins, proteins, phenolics, and ninhydrin. The absence of glycosides, terpenoids, and steroids in these extracts suggests that these compounds may not play a significant role in the observed biological activities. The presence of these diverse phytochemicals, such as flavonoids, tannins, and alkaloids, is likely responsible for the antimicrobial, anti-inflammatory, and antioxidant properties of the A. saman extracts. Flavonoids, for instance, are known to possess potent antimicrobial, anti-inflammatory, and antioxidant properties, and their presence in the *A.saman* extract may contribute to the observed therapeutic activities. Similarly, tannins and alkaloids have also been associated with various pharmacological effects, including antimicrobial, anti-inflammatory, and antioxidant activities [20]. The synergistic or additive interactions between these different phytochemicals present in the A. saman extracts may further enhance their overall therapeutic potential [21]. Possible biomarkers to evaluate the risk of malignant transformation in individuals with leukoplakia, OSMF, and OSCC include circulating exosomal miRNAs miRNA 21, miRNA 184, and miRNA 145 [22]. The natural antimicrobial, anti-inflammatory, and antioxidant characteristics of the *A. saman* extract make them a promising candidate for the development of new therapeutic agents [23-25]. These properties could be particularly useful in the treatment of various infectious diseases, inflammatory conditions, and oxidative stress-related disorders. For instance, the antimicrobial activity of the A. saman extracts could be exploited in the development

of new antimicrobial drugs, potentially useful in the management of bacterial, fungal, or even viral infections. Similarly, the anti-inflammatory properties of the extracts may be beneficial in the treatment of chronic inflammatory conditions, such as arthritis, asthma, or inflammatory bowel diseases. Furthermore, the antioxidant activity of the *A. saman* extract could be leveraged in the development of novel therapeutic agents for the prevention and management of diseases associated with oxidative stress, such as cardiovascular diseases, neurodegenerative disorders, or certain types of cancer [26-28]. During the orthodontic treatment phases, salivary levels of IL-17A and 1-25dihydroxycholecalciferol correlate, indicating that vitamin D administration may hasten tooth movement with little tissue injury [29]. Further research is needed to fully understand the pharmacological activities of the bioactive compounds present in the *A. saman* extract and to determine their in vivo effects and potential toxicities in clinical trials. This additional research will be crucial in establishing the therapeutic potential of *A. saman* and paving the way for the development of new, effective, and safe treatments [27, 28, 30, 31].

Conclusion

Albizia saman leaf extract exhibits potent antimicrobial, anti-inflammatory, and antioxidative properties. Its rich phytochemical composition, including alkaloids, flavonoids, tannins, saponins, proteins, and phenols, contributes to these broad-spectrum biological effects. Further research is needed to identify specific bioactive compounds, understand their mechanisms, and validate their therapeutic potential through *in vivo* studies and clinical trials. To conclude, *Albizia saman* extract shows promise for developing novel treatments against infections, inflammation, and oxidative stress-related disorders.

Conflict of Interest

The author hereby declares that there is no conflict of interest.

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