PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE FLOWERS OF *CAESALPINIA MIMOSOIDES* Lam.

Project submitted

To

MAHATMA GANDHI UNIVERSITY

In partial fulfillment of the requirement in degree of

BACHELOR OF SCIENCE IN BOTANY

Submitted by

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CERTIFICATE

This is to certify that this project work entitled "**Preliminary Phytochemical Analysis of the Flowers of *Caesalpinia Mimosoides* Lam**.'' is a bonafide piece of project work done by **(SURYA BABU-210021022674) (**SREELAKSHMI VL-210021022661) (NIJOGEORGE-210021022657)in the Department of Botany, Bharata Mata College, Thrikkakara under my guidance and supervision for the award of Degree of Bachelor of Science in Botany during the academic year 2021-2024. This work has not previously formed the basis for the award at any other similar title of any other university or board.

Place: Thrikkakara

Date: 06/05/2024

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DECLARATION

I hereby declare that this project entitled "**Preliminary Phytochemical Analysis of the Flowers of *Caesalpinia Mimosoides* Lam**." is the result of work carried out by me under the guidance of **Dr. Abin Kurian,** Department of Botany, Bharata Mata College, Thrikkakara. This work has not formed on the basis for the award at any other similar title of any other university of board.

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INTRODUCTION

Medicinal plants are a natural boon bestowed upon people, aiding them in leading lives devoid of illness and in good health. India is renowned for its rich cultural and ethnic diversity, and the medicinal plant industry is a cherished tradition that continues to hold significance in the present day. There is substantial data indicating that the demand for medicinal plants is rising due to the global trend towards improved quality of life. India possesses a rich abundance of medicinal herbs. These plants are commonly used by many segments of society, either directly as traditional remedies or indirectly as pharmaceutical preparations for modern medical treatments (Raval *et al*., 2012).

India's rich heritage of traditional medicine, including Ayurveda, Siddha, and Unani, has flourished for centuries. The earliest records of Indian medicine are found in the Vedas, compiled in Sanskrit between 3000 and 1000 B.C. (Mukherjee, 2003). Growing awareness of chemical and synthetic medicine risks has increased herbal product use in both Eastern and Western worlds. In recent days, people are increasingly self-prescribing herbal products due to their perceived benefits (Basu and Pant, 2013).

The World Health Organisation defines a medicinal plant as a plant that includes chemicals in one or more of its organs that can be employed for therapeutic reasons or as precursors for chemo-pharmaceutical semi-synthesis. A plant with various components such as leaves, roots, rhizomes, stems, barks, flowers, fruits, grains, or seeds can be used to control or treat a disease. These components include medically active chemical substances. Phytochemicals or phytoconstituents are bioactive components found in plants that defend them from microbial infections or infestations by pests (Abo *et al*., 1991; Liu, 2004; Nweze *et al*., 2004; Doughari *et al*., 2009). Thus, the positive medicinal effects of plant materials often stem from the secondary products they contain. These effects are not usually attributed to a single compound but rather to a combination of metabolites. The medicinal properties of plants are specific to their species or group, supporting the idea that the combination of secondary products in a plant is taxonomically unique (Parekh and Chanda, 2005).

Plant's therapeutic properties are derived from their bioactive phytochemical ingredients, which have a variety of physiological effects on the human body. Phytochemical screening enables the identification of essential compounds that can serve as the foundation for developing new medications to treat a range of disorders (Sheikh *et al*., 2013). Medicinal plants contain phytochemicals such as flavonoids, alkaloids, glycosides and polyphenols,

which exhibit pharmacological activities. Screening these bioactive compounds has led to discovering new medicinal drugs with protective and treatment roles against various diseases (Govindappa *et al*., 2011). Plants extracts are one of the potentials that have rapidly garnered attention and expected that it will be active against synthetic medications resistant disease agents. Therefore, the search for plant based novel components with different biological activities are important (Kirtikar and Basu, 1993; Gaur, 1999; Joshi and Kumar, 2000; Manuchair, 2002).

The process of utilizing biologically active compounds from plant resources involves extraction, pharmacological screening, isolation, and characterization of the compound, followed by toxicological and clinical evaluations (Sasidharan *et al*., 2011). The different types of extraction methods separate the biologically active parts of plant tissue from inactive components using specific solvents and extraction technology. The quality of a plant extract relies on the plant material, the solvents chosen, and the extraction techniques employed. The solvents selected will penetrate the solid plant tissues and dissolve the phytoconstituents with the similar polarity (Das *et al*., 2011)

People from all social classes employ herbal drugs in various indigenous and modern pharmaceutical preparations or as direct folk remedies to treat common ailments. Although whole herbs, including roots, stems and leaves are usually preferred in medical procedures, flowers also have important therapeutic qualities that can effectively treat a variety of diseases (Retnam and Martin, 2006; Sundari *et al*., 2012). Various flowers have important medicinal properties. The flower decoction of *Hibiscus rosa-sinensis* treats urinary disorders and menorrhagia, while that of *Azadirachta indica* acts as a stimulant and tonic, curing gastric ulcers and killing intestinal worms. *Cassia auriculata* treats skin diseases and body odour, and *Calotropis gigantea* is used for bronchial asthma. *Clitoria ternatea* acts as a purgative and diuretic, and *Catharanthus roseus* provides chromium supplementation for certain leukaemia and circulatory disorders (Simon *et al*., 1984; Rao *et al*., 2005).

***Caesalpinia mimosoides* Lam.** [*Hultholia mimosoides* (Lam.) Gagnon & G. P. Lewis] belongs to the family Fabaceae. It is a spiny scandent shrub, sometimes erect; its branches covered with brown tomentum. Young parts are glandular and bristly, with erect and recurved prickles. Leaves are 30–60 cm long, with 18–20 pairs of pinnae. Leaflets are sensitive, opposite, subsessile, membranous, oblong and 10–20 pairs. Flowers are yellow in colour, 2– 2.5 cm long, in simple, axillary, and terminal racemes. Pods are 4–5 cm long, bristly, and

obliquely ovoid or oblong. Seeds are 1–4, mottled. *C. mimosoides* is native to Southeast Asia and found in India, southwest China, Bangladesh, Sri Lanka, Laos, Vietnam, Myanmar, and Thailand (Bhat *et al*., 2023).

In Northern and Northeastern Thailand, tribal communities traditionally consume tender shoots and immature leaves of the plant as a vegetable, appetizer, and for various health benefits (Chanwitheesuk *et al*., 2007). In southern states of India, different parts of the plant are used to treat various ailments. The *Mullu Kuruma* tribe in Kerala uses the plant leaves for epilepsy, while in Uttara Kannada district of Karnataka, the juice from tender shoots is used for skin disorders and as a blood purifier. Additionally, the paste of tender shoots is applied topically for boils, and in Udupi district, the root paste is used for wounds, ulcers, and arthritis (Bhat *et al*., 2016; Bhat *et al*., 2023)

More than 500 species belong to the genus *Caesalpinia,* with many of them still unexplored for potential pharmacological activity (Zanin *et al*., 2012). The genus *Caesalpinia* L. includes trees, shrubs, and prickly climbers distributed worldwide, with several species holding economic, medicinal, and horticultural significance. Many *Caesalpinia* species are utilized in ethnomedicine globally. They contain various phytochemical classes like flavonoids, diterpenes, and steroids. *Caesalpinia* species demonstrate diverse bioactivities such as antiulcer, anticancer, antidiabetic, anti-inflammatory, antimicrobial, and antirheumatic effects (Khatun and Rahman, 2006; Zanin *et al*., 2012)

The different phytochemical components are the basis for the different biological activities. Polyphenols found in the shoots and leaves of *C. mimosoides* show promise as valuable drug candidates for treating neurodegenerative diseases (Choi *et al*., 2012). Researchers have isolated several classes of chemical compounds, including flavonoids, diterpenes, and steroids, from *C. mimosoides.* Quercetin, a well-known potent antioxidant isolated from the plant, may scavenge radicals, chelate metal, inhibit enzymes, also induce the expression of protective enzymes (Lakhanpal and Rai, 2007; Sandhar *et al*., 2011). The different plant parts of *C. mimosoides viz*., root, stem, leaves and fruits has been extensively studied and shown to have antibacterial, anti-inflammatory, antioxidant, anticancer, and neuroprotective properties (Chanwitheesuk *et al*., 2007; Rattanata *et al*., 2016; Yodsaoue *et al*., 2010; Chanwitheesuk *et al*., 2005; Daduang *et al*., 2011; Palasap *et al*., 2014; Tangsaengvit *et al*., 2013).

The significance of the current study lies in the absence of detailed phytochemical studies on the analysis of the flowers of *C. mimosoides,* even though extensive studies were conducted on other plant parts. Hence, preliminary phytochemical analysis of the flowers of *C. mimosoides* were investigated in the current study to analyse its phytochemical potential.

OBJECTIVES OF THE STUDY

- Preparation of the different flower extracts of *Caesalpinia mimosoides* for phytochemical analysis
- Preliminary phytochemical analysis of the flower extracts of *Caesalpinia mimosoides*
- Standardization of the solvent systems for the chromatographic studies.
- Phytochemical investigation of the flower extracts of *Caesalpinia mimosoides* using TLC.

REVIEW OF LITERATURE

The beneficial effects of medicinal plants are often due to the combination of phytochemicals they contain. Phytochemicals are naturally occurring compounds found in fruits, vegetables, grains, nuts, tea, and seeds that promote health and prevent disease. These compounds, including flavonoids, alkaloids, sterols, terpenoids, phenolic acids, stilbenes, lignans, tannins, and saponins, contribute to the therapeutic effects of medicinal plants. Scientific evidence supports their antioxidant, antimicrobial, enzyme-modulating, immune-stimulating, antiplatelet, hormone-metabolism-modulating, and anticancer properties (Nyamai *et al*., 2016). Plants typically produce these phytochemicals in specialized cells during specific developmental stages, making their extraction and purification challenging. These various phytochemicals, acting as secondary metabolites, have been shown to have various physiological effects in mammals, including humans, earning them the name "active principles" of the plant (Shula *et al.,* 2009). The effectiveness of solvent extraction depends on several variables, including the choice of solvent, its concentration, extraction time, temperature, pH, number of extraction steps, liquid-to-solid ratio, and the particle size of the plant material (Cacace and Mazza, 2003).

Thin-layer chromatography (TLC) is a widely used and efficient technique for detecting, analysing, and separating phytoconstituents. It is estimated that 60% of analyses worldwide are conducted using TLC (Lade *et al*., 2014). TLC is valued for its simplicity, speed, and cost-effectiveness, providing researchers with quick insights into the components present in a mixture. It is also used to confirm the identity of compounds by comparing their Rf values with those of known compounds. Additional tests involve the use of phytochemical screening reagents, which induce colour changes corresponding to the phytochemicals present in a plant extract, or by examining the plate under UV light. This method is also employed to verify the purity and identity of isolated compounds (Sasidharan *et al*., 2011).

Caesalpinia mimosoides Lam., a prickly scandent shrub of the Fabaceae family native to Southeast Asia, is traditionally utilized by healers in India and Thailand for various medicinal purposes. These include treating epilepsy, boils, wounds, ulcers, and arthritis. The plant's immature leaves are also used as a vegetable and appetizer, as well as for their carminative properties and to alleviate giddiness. *C. mimosoides* has shown a wide range of phytochemical constituents and documented biological activities (Bhat *et al*., 2023).

2.1 Phytochemical analysis

He *et al*., (2017) isolated 17 compounds from the ethyl acetate extract of *C. mimosoides* twigs and determined their structures using 1H and 13C NMR spectroscopy. The identified compounds were mainly B, quercetin-3-O-glucoside, isoliquiritigenin, sulfuretin, bergenin, 8-methoxybonducellin, sitoindoside I, intricatinol, quercetin, eucomin, gallic acid, butein, bergenin 11-O-I-ferulate, coniferaldehyde, 10-methoxyisoprotosappanin B, 10-methoxyprotosappanin B, friedelin, and ethyl gallate (**Fig.1**).

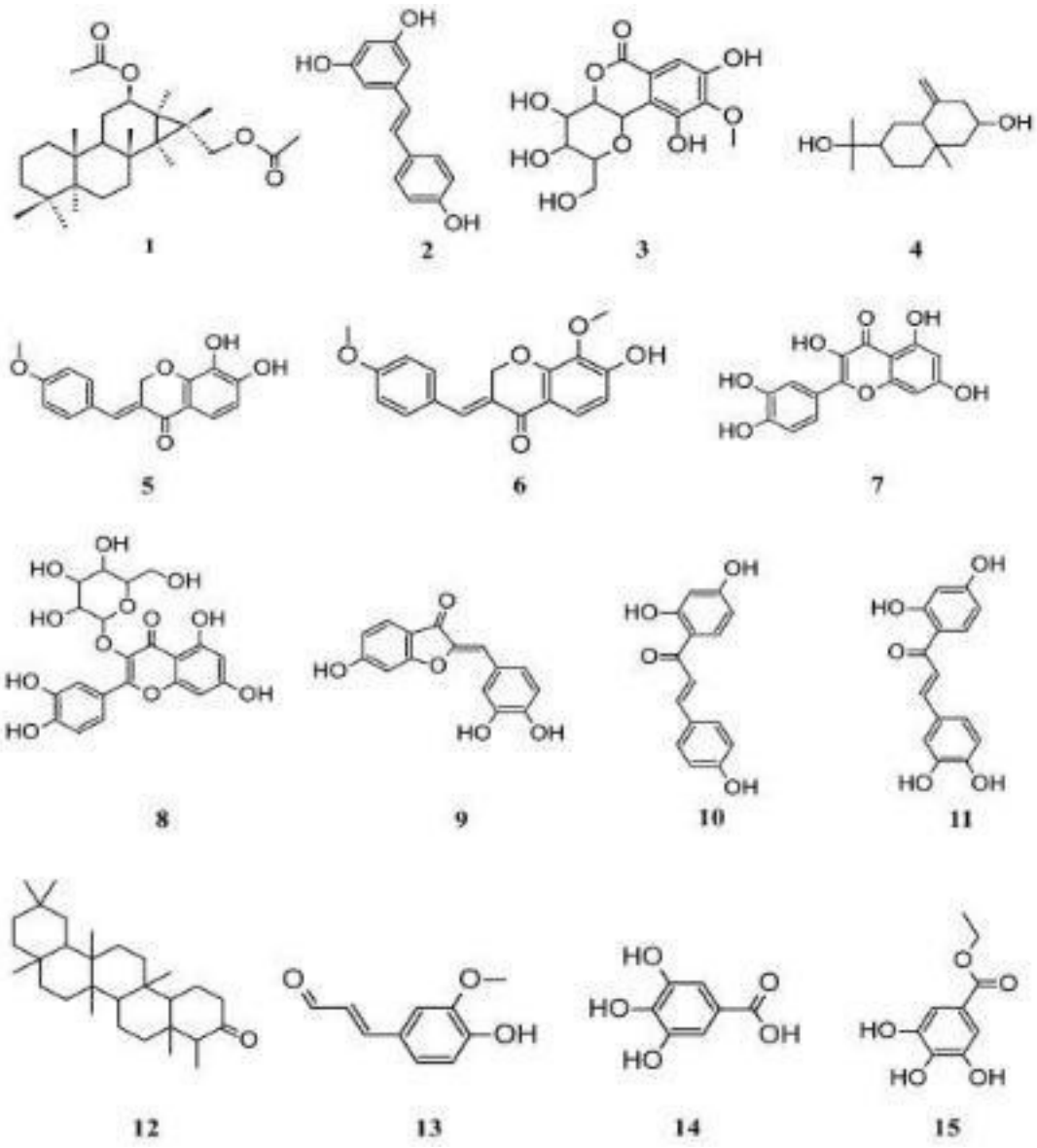

Fig.1: Structures of Mimosol-D (1), resveratrol (2), bergenin (3), pterocarpol (4), intricatinol (5), 8-Methoxybonducellin (6), quercetin (7),

Quercetin-3-O-glucoside (8), sulfuretin (9), isoliquiritigenin (10), butein (11), friedelin (12), coniferaldehyde (13), gallic acid (14), and ethyl gallate (15).

Preliminary phytochemical screening of the extracts from the stems, leaves, and tender shoots of *C. mimosoides* revealed the presence of phenolics, glycosides, flavonoids, tannins, resins, anthraquinones, saponins, phytosterols, steroids, alkaloids, proteins, reducing sugars, and ascorbic acid (Chanwitheesuk *et al*., 2007; Bhat *et al*., 2016; Viji and Wilson, 2017, Bhat *et al*., 2023). Tangsaengvit *et al*., (2013) isolated quercetin from the ethyl acetate extract of young shoots and leaves using the flash column chromatography technique. Analysis using GC–MS of the ethanol extract from tender shoots identified gallic acid and ethyl gallate as major compounds, with concentrations of 59.04 and 56.88 µg/mg, respectively (Bhat *et al*., 2016). Gallic acid was also isolated from shoots, leaves, and the whole plant by Chanwitheesuk *et al*., (2007) and Rattanata *et al*., (2016) using Sephadex LH-20 and silica gel column chromatography methods. Yodsaoue *et al*., (2010) isolated four diterpenes (mimosol A–D), a dimer (mimosol E), and two dibenzo [b,d] furans (mimosol F and G) through bioassay-guided fractionation and isolation of dichloromethane and acetone extracts of roots.

2.2 Pharmacological studies

The antimicrobial activity of hexane, chloroform, ethanol acetone and aqueous extracts of tender shoots of *C. mimosoides* was evaluated by Bhat *et al*., (2016) against bacterial and fungal skin pathogens using the serial tube dilution method. The ethanol extract exhibited the highest effectiveness against a range of bacteria, including *Staphylococcus aureus, Micrococcus flavus, Micrococcus luteus, Pseudomonas aeruginosa, Proteus vulgaris*, and *Salmonella typhimurium*, with concentrations ranging from 0.026 to 0.469 mg/mL. It also demonstrated significant antifungal activity against *Trichophyton mentagrophytes*, *Microsporum gypseum*, and *Malassezia furfur*, with concentrations ranging from 0.039 to 0.469 mg/mL, compared to standard drugs. They also studied the wound healing potential of ethanol and aqueous extracts and was evaluated using excision and incision wound parameters. Both extracts, at a 5% w/w dose, showed significantly higher percentages of excision wound closure compared to the positive control (Providine-AM).

The antioxidant activity of various parts of *C. mimosoides* was evaluated through different studies. Chanwitheesuk *et al*., (2005) reported that the methanol extract exhibited moderate antioxidant activity, while the study by Manasa *et al*., (2014) had the methanol extract of the root with most effective radical scavenging activity. The ethanol extract of the aerial parts demonstrated significant in vivo antioxidant activity. Additionally, the ethanol extract of tender shoots showed effective DPPH (19.25 µg/g) and nitric oxide scavenging (132.40 µg/g)

activities (Ranjith *et al*., 2014; Bhat *et al*., 2016). Rekha (2011), investigated an Ayurvedic herbal formulation called "Panchanga kwatha" derived from *C. mimosoides* for its antiarthritic and analgesic effects using a Wistar rat model. The study observed a noticeable reduction in paw volume in both the test and standard groups on the 17th, 19th, and 21st days. Additionally, the analgesic potential of the formulation was also tested, showing significantly higher responses compared to the standard group. Tangsaengvit *et al*., (2013) extracted quercetin from young shoots of *C. mimosoides* using ethyl acetate and studied its effects on neurite outgrowth in P19-derived neurons. They treated P19-derived neurons with quercetin (1 nM) for 24 hours and observed an increase in neurite number and length compared to untreated cells. Quercetin also protected the neurons from oxidative stress and cell death induced by serum deprivation. Pretreatment with quercetin (1 nM) significantly increased the survival rate of neuronal cells in cultures with and without serum supplementation.

MATERIALS AND METHODS

3.1 Collection and processing of plant material.

Flowers of *Caesalpinia mimosoides* Lam. [*Hultholia mimosoides* (Lam.) Gagnon & G.P.Lewis], was used for the study. The fresh flowers of *C. mimosoides* were collected from their natural localities of Kerala and were authenticated. The fresh flowers obtained were dried in shade for two weeks. After drying, the flowers were ground using a mechanical blender into fine powder and transferred into airtight containers at ambient temperature with proper labelling for further studies **(Plate: 1).**

3.2 Preparation of flower extracts

The flower extracts of *C. mimosoides* were prepared using different solvents such as Chloroform and Methanol. The cold extraction method was used to prepare the extracts. 5g of dried and powdered flower was taken in a conical flask (500ml) and 200 ml of chloroform was added for the preparation of the chloroform extract. Similarly, 5g of powdered flower was taken in a conical flask (500ml) and 200 ml of methanol was added for the preparation of the methanol extract. The two conical flasks were kept in a rotary shaker for 3 days at room temperature. The extracts thus obtained were filtered using Whatman No. 1 filter paper. The extracts were then transferred to air tight glass containers, labelled and stored in refrigerator for the analysis.

3.3 Qualitative preliminary phytochemical analysis.

To identify the presence of various classes of metabolites, different preliminary analysis was conducted.

3.3.1 Test for Alkaloids

3.3.1.1 Mayer's test: To a few ml of sample extract, two drops of Mayer's reagent are added along the sides of the tube. Appearance of white creamy precipitate indicates the presence of alkaloids (Tiwari *et al*., 2011).

3.3.1.2 Wagner's test: A few drops of Wagner's reagent are added to few ml of extract along the sides of the tube. A reddish-brown precipitate confirms the test as positive (Tiwari *et al*., 2011).

3.3.2 Test for Coumarins

Coumarins test: To two ml of sample extract, three ml 10% sodium hydroxide solution is added along the sides of the test tube. Appearance of yellow coloured solution indicates the presence of coumarins (Jayaprakash and Sangeetha, 2015).

3.3.3 Test for Flavonoids

3.3.3.1 Alkaline reagent test: 2 ml of 2% sodium hydroxide solution was mixed with plant crude extract, intensive yellow colour was formed, which turned colourless on addition of dilute acid (Jaradat *et al*., 2015).

3.3.3.2 Lead acetate test: To 2 ml of extract, few drops of lead acetate solution was mixed. Formation of yellow precipitate (Tiwari *et al*., 2011).

3.3.4 Test of Glycosides

Keller Killiani's test: To 2 ml extract glacial acetic acid is added along the sides of the test tubes and one drop of 5% ferric chloride solution added. Reddish brown colour appears at the junction of 2 layers and upper layer appears bluish green (Singh and Bag, 2013).

3.3.5 Test of Phenol

Ferric chloride test: To 2-3 ml of extract a few drops of 5% ferric chloride solution was added, presence of deep blue-black colour (Santhi and Sengottuvel, 2016).

3.3.6 Test for Quinones

Quinones test: To 2-3 ml of sample extract 3 ml hydrochloric acid is added. Appearance of yellow colour (Harborne, 1999).

3.3.7 Test for Saponins

Foam test: 2 ml sample is taken in a test tube to which 4 ml distilled water is added, mix well and vigorously. Indicates the formation of foam at the top of the sample (Hossain *et al*., 2013).

3.3.8 Test for Steroids

Salkowski's test: To 2 ml of extract 2ml chloroform is added. 2 ml concentrated sulphuric acid is added along the sides of the test tube. Chloroform layer appears red colour and acid layer shows greenish yellow fluorescence (Joseph *et al*., 2013).

3.3.9 Test for Tannins

Braymer's test: 2-3 ml extract is diluted by adding 2 ml of distilled water. To which 2-3 drops of 5% ferric chloride is added. Appearance of black green or bluish colour (Rishikesh *et al*., 2013).

3.3.10 Test for Terpenes

Copper acetate test: 2 ml extract is dissolved in distilled water, to which 3-4 drops copper acetate solution is added and mixed, results in the production of emerald green (Morsy, 2014).

3.3.11 Tests for Carbohydrates

Molish's test: To 2 ml of plant sample extract, two drops of alcoholic solution of α-naphthol was added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates (Banu & Cathrine, 2015).

3.3.12 Tests for Reducing sugars

3.3.12.1 Fehling's test: Fehling A and Fehling B reagents are mixed and few drops of extract was added and boiled. A brick red coloured precipitate of cuprous oxide forms, if reducing sugars present (Joseph *et al*., 2013).

3.3.12.2 Benedict's test: 0.5ml of aqueous extract of the plant material was taken in a test tube. 5ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. A red color precipitate of cuprous oxide was formed in the presence of a reducing sugar (Rishikesh *et al*., 2013).

3.4 Solvent system standardisation

The solvent systems for the chromatographic analysis of chloroform and methanol were standardised by comparing the trials of different solvent system combinations.

3.4.1 Standardisation of Chloroform

The solvent system for chloroform extract of flower was standardised by conducting different TLC trials by 10 different ratios of Toluene and Ethyl acetate combinations by increasing the ethyl acetate concentration.

Trials	Solvent system
T1	100 % Toluene
T2	Toluene : Ethyl acetate ; 9 : 1 ratio
T3	Toluene : Ethyl acetate ; 8 : 2 ratio
T4	Toluene : Ethyl acetate ; 7 : 3 ratio
T5	Toluene : Ethyl acetate ; 6 : 4 ratio
T6	Toluene : Ethyl acetate ; 5 : 5 ratio
T7	Toluene : Ethyl acetate ; 4 : 6 ratio
T8	Toluene : Ethyl acetate ; 3 : 7 ratio
T9	Toluene : Ethyl acetate ; 2 : 8 ratio
T10	Toluene : Ethyl acetate ; 1 : 9 ratio

3.4.2 Standardisation of methanol

The methanol extract solvent system was standardised by 8 different TLC trials of Toluene, Ethyl acetate and Methanol combination ratios by increasing the ethyl acetate concentration. The polarity of the solvent system was increased for the standardisation of methanol sample.

Trials	Solvent system
T1	Toluene : Ethyl acetate : Methanol ; 9:1:1 ratio
T2	Toluene : Ethyl acetate : Methanol ; 8:2:1 ratio
T3	Toluene : Ethyl acetate : Methanol ; 7:3:1 ratio
T4	Toluene : Ethyl acetate : Methanol ; 6:4:1 ratio
T5	Toluene : Ethyl acetate : Methanol ; 5:5:1 ratio
T6	Toluene : Ethyl acetate : Methanol ; 4:6:1 ratio
T7	Toluene : Ethyl acetate : Methanol ; 3:7:1 ratio
T8	Toluene : Ethyl acetate : Methanol ; 2:8:1 ratio

3.5 Thin Layer Chromatographic studies

TLC analysis was performed on Aluminium backed pre-coated Merck silica gel plate 60 F 254 plate (10 cm x 3 cm Merck, Germany). The origin was marked at 1cm from the bottom of the TLC plate. The TLC plates were activated and the test solutions was applied on to the TLC plate of uniform thickness of 0.2 mm in the form of bands with a width of 10 mm using a 2 mL capillary tube (Camag, Switzerland). The plates were then developed in their respective standardized solvent systems in a twin trough chamber previously saturated with solvent for 30 min to a distance of 9 cm. The plates were then treated at 100° C for 5 minutes before visualization.

3.5.1 Solvent system

Toluene: Ethyl acetate (8:2) was used as the solvent system for the chloroform extract.

Toluene: Ethyl acetate: Methanol (7:3:1) was used for the Methanol extract.

3.5.2 Visualization

Observed the plate under UV light at 254 nm and 365 nm in the UV visualization chamber (Remi, India). Plate was also visualised in the visible light.

3.6 Chemicals and reagents used in the study

Solvents such as Methanol, Chloroform, Toluene, Ethyl acetate was purchased from Merck and Nice was used. All other chemicals employed were of standard analytical grade from Merck, India.

Plate 1: A: Habit of *Caesalpinia mimosoides*. **B:** Fresh flower of *C. mimosoides* **C:** Dried and powdered flower of *C. mimosoides*

RESULT AND DISCUSSION

4.1 Preliminary phytochemical analysis

The preliminary phytochemical analysis of the flower extracts of *C. mimosoides* was done using different phytochemical tests. The potential phytoconstituents present in the samples were analysed and characteristic differences were observed regarding the chemical composition of different extracts.

4.1.1 Chloroform extract

The results of the phytochemical analysis of the Chloroform extract using different phytochemical tests showed the presence of some major classes of phytoconstituents **(Table 1).**

Table-1 Preliminary phytochemical tests for Chloroform extract

Sl. No.	Phytochemical constituents	Name of test	Inference
1	Alkaloids	Mayer's test Wagner's test	**+** **+++**
2	Coumarins	Coumarins test Alkaline reagent test	**+++**
3	Flavonoids	Lead acetate test	**+** **++**
4	Glycosides	Keller Killiani's test	**++**
5	Phenols	Ferric chloride test	**+**
6	Quinones	Quinones test	**+**
7	Saponins	Foam test	**+**
8	Steroids	Salkowski test	**-**
9	Tannins	Braymer's test	**-**
10	Terpenoids	Copper acetate test	**-**
11	Carbohydrates	Molish's test	**++**
12	Reducing sugar	Fehling's test Benedict's test	**++** **+**

(**+**) Present, (**-**) Absent

In the chloroform extract, phytochemical constituents such as alkaloids, coumarins, flavonoids, glycosides, phenols, quinones, saponins, carbohydrates and reducing sugars were present. Whereas, steroids, tannins and terpenoids were absent.

4.1.2 Methanol extract

The preliminary phytochemical analysis of the methanol extract to detect the presence of potential classes of phytoconstituents was done using different phytochemical tests **(Table 2).**

Table-2 Preliminary phytochemical tests for Methanol extract

Sl. No.	Phytochemical constituents	Name of test	Inference
1	Alkaloids	Mayer's test Wagner's test	**+** **++**
2	Coumarins	Coumarins test	**++**
3	Flavonoids	Alkaline reagent test Lead acetate test	**++** **++**
4	Glycosides	Keller Killiani's test	**+++**
5	Phenols	Ferric chloride test	**++**
6	Quinones	Quinones test	-
7	Saponins	Foam test	-
8	Steroids	Salkowski test	+
9	Tannins	Braymer's test	+
10	Terpenoids	Copper acetate test	+
11	Carbohydrates	Molish's test	**++**
12	Reducing sugar	Fehling's test Benedict's test	**-** -

(+) Present, (-) Absent

In methanol extract, phytochemical constituents such as alkaloids, coumarins, flavonoids, glycosides, phenols, carbohydrates, steroids, tannins and terpenoids are present. The phytoconstituents belonging to the classes of saponins, quinones and reducing sugar were not observed significantly in the phytochemical screening of the methanol extract.

4.1.3 Comparative preliminary phytochemical analysis

The preliminary phytochemical analysis of chloroform extract and methanol extract confirmed the presence of various classes of phytochemicals. Nine different phytoconstituents were observed in the preliminary phytochemical analysis of chloroform extract and methanol extract. Alkaloids, coumarins, flavonoids, glycosides, phenols and carbohydrates were found to be present in both chloroform and methanol extracts. They also had the highest intensities as compared to other compounds. Glycosides and flavonoids were also found to be reported as the major compounds in the preliminary phytochemical analysis of the flower extracts of *C. mimosoides* (Manasa *et al*., 2014). Alkaloids and coumarins in chloroform extract and glycosides in methanol extract had the best results and in turn, could be considered as their respective abundances. Saponins, quinones and reducing sugars present in the chloroform extract were not observed in the phytochemical screening of the methanol extract and similarly, steroids, tannins and terpenoids present in the methanol extract were absent in the chloroform extract.

The phytochemical screening of the leaf extracts of *C. mimosoides* using chloroform, ethyl acetate, petroleum ether and methanol indicated the presence of flavonoids, alkaloids, glycosides, phytosterols, reducing sugar, tannins, phenols, phytosterols, resins, saponins and anthraquinones. The presence of significant amounts of secondary metabolites such as phenols, flavonoids, and tannins may contribute to their potent antimicrobial and cytotoxic properties (Viji and Wilson, 2017). Manasa *et al*., (2014) carried out preliminary phytochemical analysis of methanol extracts of various parts of *C. mimosoides*, *viz*., leaf, root, flower and fruit. They identified a range of phytochemicals, such as steroids, flavonoids, glycosides, and tannins, in all the extracts. Similarly, the quantitative preliminary phytochemical screening of *C. mimosoides* was conducted by Chanwitheesuk *et al*., (2005) and determined the total phenolics and tannin contents.

Preliminary qualitative phytochemical analysis was conducted on the hexane, chloroform, acetone, ethanol and aqueous shoot extracts of *C. mimosoides* to determine their content of flavonoids, phenolic compounds, tannins, steroids, reducing sugars, proteins, glycosides, saponins, alkaloids and vitamins (Bhat *et al*., 2016). Ethanol and aqueous extracts encompassed all phytochemicals. Acetone extract contained reducing sugars, glycosides, flavonoids, alkaloids, tannins, phenolic compounds, and steroids, while the hexane and chloroform extracts contained only steroids. The total phenolic and flavonoid content of the ethanol extract was also estimated. Supriya *et al*., (2013) studied the preliminary phytochemical analysis of the methanol, ethyl acetate, chloroform and hexane stem extracts of *C. mimosoides*. Extensive studies were conducted in the phytochemical screening of the flower extracts of different species belonging to the genus *Caesalpinia*. The scientific data to support the phytochemical characterisation of *C. mimosoides* flower was in the pioneer stages.

4.2 Chromatographic studies

The chromatographic technique TLC was used for the analysis of the phytochemical composition of flower extracts of *C. mimosoides*. The TLC profile was developed for the chloroform and methanol extract. The solvent systems for the different extracts were standardized. Disparities were observed in terms of the number of bands and band intensity of the TLC profile developed for different extracts. These disparities in turn show the qualitative and quantitative deviations in chemical constituents.

4.2.1 Chloroform extract

TLC profile was developed for the flower extracts of *C. mimosoides.* The best solvent system for the separation of chloroform fraction was toluene: ethyl acetate mobile phase combination in the ratio 8:2. The developed plate was visualized at different wavelengths.

4.2.1.1 At 254 nm

A total of three compounds were found to be present in the chloroform extract at 254 nm. The bands were observed with an Rf value of 0.82, 0.87 and 0.91 respectively and all the three compounds were of relatively low polarity in response to their observed Rf values (**Table 3 and Plate 2).**

Table-3 TLC analysis of Chloroform flower extracts of *C. mimosoides* at 254 nm

Sl. No.	Rf	Band colour
1	0.82	Black
2	0.87	Black
3	0.91	Black

4.2.1.2 At 365 nm

The chloroform extract visualized at 365 nm had a total of six compounds. The compound with Rf value of 0.92 was found to be the prominent band with high intensity. The other major bands were observed with Rf values of 0.04, 0.78, 0.82 0.88 and 0.98. Four red coloured and 2 blue coloured UV active compounds were observed at 365 nm **(Table 4 and Plate 2).**

Table-4 TLC analysis of Chloroform flower extracts of *C. mimosoides* at 365 nm

Sl. No.	Rf	Band colour
1	0.04	Light red
2	0.78	Light blue
3	0.82	Light red
4	0.88	Light red
5	0.92	Red
6	0.98	Fluorescent blue

4.2.1.3 At visible light

TLC plate of chloroform extract at visible light had a total of 4 compounds. The bands were observed with a Rf values of 0.41, 0.86.0.92 and 0.98 **(Table 5 and Plate 2).**

Table-5 TLC analysis of Chloroform flower extracts of *C. mimosoides* at visible light

Sl. No.	Rf	Band colour
1	0.41	Faded yellow
2	0.86	Light yellow
3	0.92	Light grey
4	0.98	Light yellow

4.2.2 Methanol extract TLC profile was developed for the methanol flower extracts of *C. mimosoides.* The best solvent system for the separation of methanol fraction was standardised as toluene: ethyl acetate: methanol mobile phase combination in the ratio 7:3:1. The developed plate was visualized at different wavelengths and Rf values are calculated.

4.2.2.1 At 254 nm

A total of three compounds were found to be present in the methanol extract at 254 nm. The bands were observed with a calculated Rf value of 0.07, 0.59 and 0.98 respectively **(Table 6 and Plate 2).**

Table-6 TLC analysis of Methanol flower extracts of *C. mimosoides* at 254 nm

Sl. No.	Rf	Band colour
1	0.07	Black
2	0.59	Very light blue
3	0.98	Very light blue

4.2.2.2 At 365 nm

The methanol extract visualized at 365 nm had a total of seven compounds. The compounds with Rf values of 0.12 and 0.98 was found to be the prominent bands with high intensity. The compounds with Rf values of 0.6, 0.18, 0.35, 0.58 and 0.96 were also observed in the TLC analysis **(Table 7 and Plate 2).**

Table-7 TLC analysis of Methanol flower extracts of *C. mimosoides* at 365 nm

Sl. No.	Rf	Band colour
1	0.6	Grey
2	0.12	Dark blue
3	0.18	Light grey
4	0.35	Light red
5	0.58	Faded blue
6	0.96	Light grey
7	0.98	Red

4.2.1.3 At visible light

TLC plate of methanol extract at visible light had only 2 compounds. The bands were observed with a Rf values of 0.58 and 0.98 **(Table 8 and Plate 2).**

Table-8 TLC analysis of Methanol flower extracts of *C. mimosoides* at visible light

Sl. No.	Rf	Band colour
1	0.58	Faded yellow
2	0.98	Light yellow

4.2.3 Comparative TLC analysis of chloroform and methanol extracts.

The comparative analysis of the TLC profiles of chloroform and methanol extracts based on developed chromatographic plates showed that the Rf value of the bands which have the highest intensities in the developed chromatographic plates also possess a significant band width. The Rf values of the developed plates are manually calculated.

The comparative TLC analysis showed that a total of 25 distinct bands were observed among the two fractions. The maximum number of phytoconstituents was found to be present in the chloroform extract. A total of 13 different phytoconstituents were present in the chloroform extract. The methanol extract had a total of 12 compounds. Methanol flower extracts of *C. mimosoides* at 365 nm showed the highest number of bands (7) among all of the visualisation studies. Similarly, methanol flower extracts of *C. mimosoides* at visible light had the lowest number of bands (2). The comparative analysis showed that the phytoconstituents present in the flowers of *C. mimosoides* tends to be more on a medium polarity as the majority of the bands with high intensities and band width were observed in the chloroform extract.

The different chromatographic techniques have been adopted as a methodology for plant identification and quality evaluation (Farnsworth *et al*., 1985). Chanwitheesuk *et al*., (2007) extensively investigated different extracts of *C. mimosoides* such as distilled water, acetone, chloroform and ethanol using chromatographic and spectroscopic analysis for the presence of phytochemical components with biological activities and gallic acid was isolated found to have antimicrobial property. In that study, TLC analysis was conducted to the different fractions collected from column chromatography to ensure the presence of phytochemicals. Similar to the current study, the TLC analysis of four different leaf extracts of *C. mimosoides* consisting of methanol, ethyl acetate, chloroform and petroleum ether extracts were conducted by Viji and Wilson (2017). A total of 18 bands were visualised from the crude leaf extracts and methanol and ethyl acetate fractions showed better resolution. Ethyl acetate leaf extract of *C. mimosoides* showed maximum number of bands and petroleum ether extract had the least. These studies showed the presence of the potential phytochemicals in *C. mimosoides.* Extensive chromatographic and spectroscopic studies in *C. mimosoides.* were conducted by He *et al*., (2017) and 17 compounds were isolated from the twigs of *C. mimosoides* through column chromatography of the ethanol extract and their structures were elucidated. The compounds such as, eucomin, coniferaldehyde, intricatinol, ethyl gallate, sitoindoside and friedelin were reported for the first time from *C. mimosoides*.

The comparative investigation to validate the phytoconstituents present in *C. mimosoides* flowers was found to be deficient due to the limited research in the phytochemical characterisation of its flowers. However, thorough study has been undertaken on other *Caesalpinia* species. TLC studies of the ethyl acetate and acetone extracts of *Caesalpinia pulcherrima* flower were conducted by Phuse and Khan, (2018) using standards like gallic acid and quercetin. The quantitative estimation of phenolic and flavonoid content revealed that the flower extracts of *C. pulcherrima* were more potent than the leaf extracts. Similar findings were observed in the study conducted by Torre *et al*., (2017) on the different parts of *C. pulcherrima*. The *C. pulcherrima* flower extract had the most number of bands as compared to the leaf and seed extracts. The phytochemical investigation of the flowers of *C. mimosoides* could result in the identification and characterisation of the bioactive potential constituents that are beneficial for drug-based research.

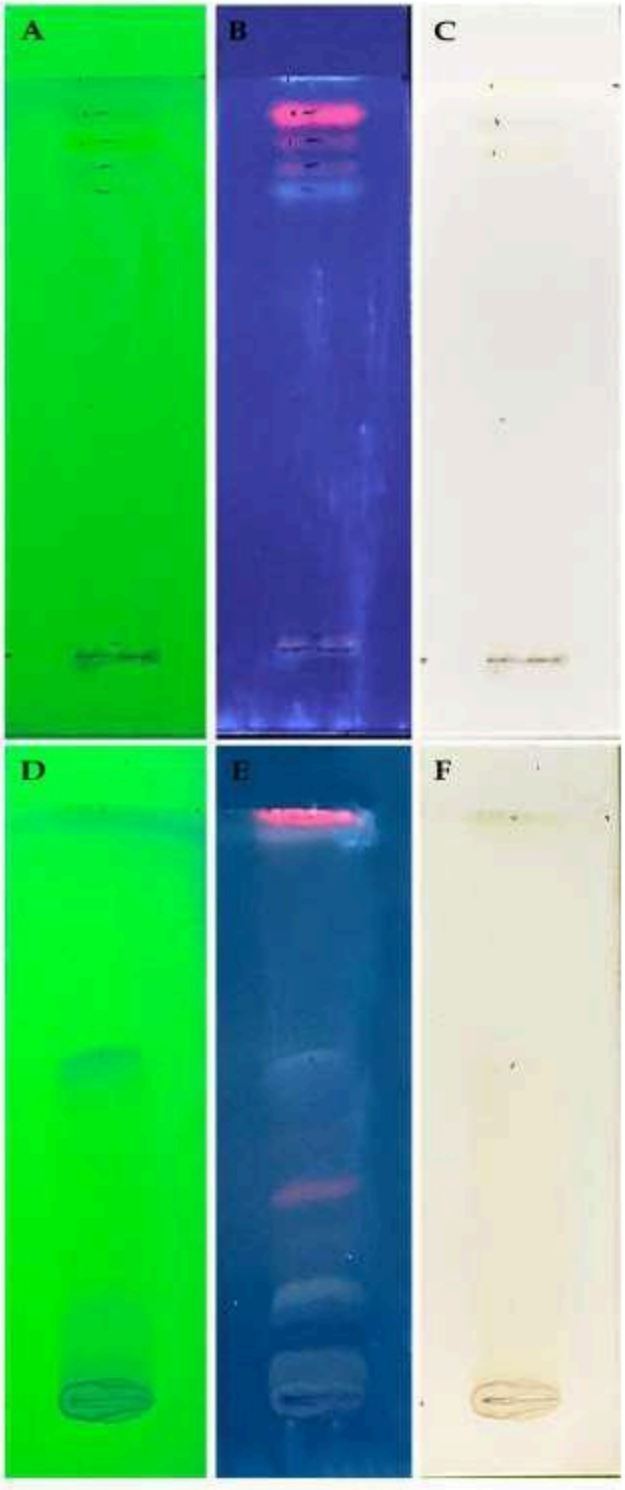

Plate 2: TLC profile of ***Caesalpinia mimosoides*** **flower. A,B & C:** TLC chromatogram of Chloroform extract under 254nm, 365nm and visible light respectively. **D,E &F:** TLC chromatogram of Methanol extract under 254nm, 365nm and visible light respectively

SUMMARY AND CONCLUSION

Medicinal plants have been utilized across cultures for their healing properties for thousands of years, serving as the basis for many modern drugs. Traditional medicine, largely plant-based, has provided leads for new pharmaceuticals and healthcare products. Numerous plant species are recognized for their therapeutic benefits, highlighting nature's role as a vital source of medicine.

The scientific validation of documented traditional knowledge regarding medicinal plants is a crucial step in the current scenario to meet the growing demand for herbal medicine. People generally favour using entire herbs, including leaves, stems, and roots, in medicinal practices. However, flowers also possess numerous medicinal properties that can treat various ailments. Therefore, there is a growing need to explore the medicinal potential of flowers to address a variety of health issues. The present work was undertaken to study the phytochemical constitution of the flowers of *Caesalpinia mimosoides*. *Caesalpinia mimosoides*, is an essential plant in traditional folk medicine, is utilized by healers in Uttara Kannada district of Karnataka, particularly for treating skin conditions and wounds. The *Mullu Kuruma* tribes of Kerala employ *C. mimosoides* in the treatment of epilepsy.

The preliminary phytochemical analysis of the flowers of *C. mimosoides* confirmed the presence of all classes of phytochemicals studied. The chloroform and methanol extracts contained alkaloids, coumarins, flavonoids, glycosides, phenols, and carbohydrates. However, phytochemical screening did not observe saponins, quinones, and reducing sugars in the methanol extract that were present in the chloroform extract. Similarly, the chloroform extract did not contain steroids, tannins, and terpenoids found in the methanol extract.

The chromatographic analysis of chloroform and methanol extracts of the flowers of *C. mimosoides*. The TLC analysis showed that a total of 25 distinct bands were observed among the two fractions studied. The maximum number of phytoconstituents (13) was found to be present in the chloroform extract and methanol extract had a total of 12 compounds. Methanol flower extracts of *C. mimosoides* at 365 nm showed the highest number of bands (7) among all of the visualisation studies. Similarly, methanol flower extracts of *C. mimosoides* at visible light had the lowest number of bands (2). The comparative analysis showed that the phytoconstituents present in the flowers of *C. mimosoides* tends to be more on a medium polarity as the majority of the bands with high intensities and band width were observed in the chloroform extract.

In conclusion, the present work was a phytochemical screening attempt to find out the presence of potential phytochemical constituents in the flowers of *C. mimosoides.* The detailed chromatographic and spectroscopic analysis of the flowers of *C. mimosoides* could result in the structural elucidation and identification of potential phytochemical constituents. Further pharmacological studies could, in turn, substantiate the biological activity of those phytoconstituents.

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