

Qualitative Phytochemical Analysis of Methanolic Extracts of Indigenous Medicinal Plant *Cordia myxa*.

Abstract:

Medicinal plants have long been recognized as valuable sources of bioactive compounds with diverse therapeutic properties and continue to play an essential role in traditional and modern healthcare systems. The increasing demand for plant-derived medicines has encouraged researchers to investigate the phytochemical composition of medicinal plants to identify compounds that may contribute to their pharmacological activities. *Cordia myxa* is a medicinal plant traditionally used in the management of various ailments, including respiratory disorders, gastrointestinal diseases, inflammation, and infections. Despite its widespread traditional use, scientific evaluation of its phytochemical constituents remains important to validate its medicinal potential. Therefore, the present study was conducted to evaluate the phytochemical constituents present in the methanolic extract of *Cordia myxa* through qualitative phytochemical screening.

Fresh plant material of *Cordia myxa* was collected, cleaned, dried, and processed into powdered form before extraction with methanol using standard extraction procedures. The resulting methanolic extract was subjected to preliminary qualitative phytochemical screening using established laboratory methods. Different chemical tests were employed to identify the presence or absence of major classes of phytochemicals. Alkaloids were detected using Mayer's, Wagner's, Dragendorff's, and Hager's tests. Carbohydrates were analyzed by Molisch's, Benedict's, and Fehling's tests. Glycosides were identified using Modified Borntrager's and Legal's tests, while saponins were detected by the froth and foam tests. Phytosterols were evaluated using Salkowski's and Liebermann-Burchard's tests. Phenols, tannins, flavonoids, and proteins were detected using ferric chloride, gelatin, alkaline reagent, lead acetate, xanthoproteic, and ninhydrin tests, respectively. The observations obtained from these qualitative assays were recorded and interpreted according to standard phytochemical screening procedures.

The results of the phytochemical investigation revealed the presence of a wide range of bioactive secondary metabolites in the methanolic extract of *Cordia myxa*. All four alkaloid tests produced positive results, confirming the presence of alkaloids in the

extract. Carbohydrates were also positively identified by Molisch's, Benedict's, and Fehling's tests. Glycosides showed positive reactions in both Modified Borntrager's and Legal's tests, indicating their presence. Saponins were successfully detected by both froth and foam tests. Similarly, phytosterols were confirmed by positive reactions in Salkowski's and Liebermann-Burchard's tests. The ferric chloride test indicated the presence of phenolic compounds, while the gelatin test confirmed the occurrence of tannins. Flavonoids were detected through positive alkaline reagent and lead acetate tests. Protein analysis demonstrated a positive xanthoproteic reaction, whereas the ninhydrin test was negative, suggesting the presence of proteins containing aromatic amino acids but the absence of detectable free amino acids in the extract.

The detection of these phytochemical constituents suggests that *Cordia myxa* contains several biologically important compounds that may contribute to its traditional medicinal applications. Alkaloids, flavonoids, phenols, tannins, glycosides, saponins, and phytosterols are widely recognized for their antioxidant, antimicrobial, anti-inflammatory, analgesic, and other pharmacological activities. The simultaneous presence of these bioactive constituents indicates that the methanolic extract possesses considerable phytochemical richness and supports the ethnomedicinal importance of the plant. Although the present investigation was limited to qualitative phytochemical analysis, the findings provide valuable baseline information regarding the chemical composition of *Cordia myxa* and establish a scientific foundation for future studies.

In conclusion, the methanolic extract of *Cordia myxa* was found to contain numerous important phytochemicals, including alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, and proteins, whereas free amino acids were not detected. These findings support the traditional use of the plant and suggest that *Cordia myxa* represents a promising natural source of bioactive compounds. Further investigations involving quantitative phytochemical estimation, isolation and characterization of individual constituents, and pharmacological evaluations are recommended to identify the active principles responsible for the plant's therapeutic effects and to explore its potential for the development of novel herbal medicines.

Keywords: *Cordia myxa*; phytochemical screening; methanolic extract; medicinal plant; secondary metabolites; alkaloids; flavonoids; phenolic compounds; saponins; tannins.

65 1. Introduction

66 Medicinal plants have been the cornerstone of traditional medicine and continue to play a
67 crucial role in modern pharmacology due to their bioactive secondary metabolites
68 (Balasubramanian et al., 2020). *Cordia myxa* L., belonging to the family Boraginaceae, is
69 commonly known as assyrian plum, lasura, or gunda. The plant is native to South Asia,
70 the Middle East, and Southeast Asia, thriving in tropical and subtropical regions (Sharma
71 et al., 2020). *Cordia myxa* is a medium-sized deciduous tree belonging to the family
72 Boraginaceae. It is widely distributed in tropical and subtropical regions of Asia, Africa,
73 and the Middle East. The plant produces sticky, mucilaginous fruits that are traditionally
74 used for soothing throat ailments and respiratory issues. Various parts of the plant,
75 including its leaves, bark, and fruits, are known for their medicinal properties. *Cordia*
76 *myxa* contains bioactive compounds such as flavonoids, alkaloids, tannins, and phenolic
77 substances, which contribute to its antioxidant, anti-inflammatory, antimicrobial, and
78 demulcent activities.

79 *Cordia myxa* stands out as a medicinal plant of remarkable pharmaceutical and traditional
80 importance. With a rich phytochemical profile and a long history of therapeutic use, it
81 offers valuable benefits for respiratory, digestive, inflammatory, and microbial
82 conditions. Its natural mucilage, antioxidants, and diverse bioactive compounds make it a
83 promising candidate for modern herbal formulations and potential drug development.

84 The plant continues to play an important role in traditional medicine and is increasingly
85 recognized by pharmaceutical researchers for its therapeutic potential. As interest grows
86 in natural remedies and plant-based treatments, *Cordia myxa* remains a significant species
87 worthy of further scientific exploration and clinical validation. Traditionally, the fruits,
88 leaves, and bark of *C. myxa* are used to treat cough, asthma, diarrhea, dysentery,
89 inflammation, and skin disorders (Khan et al., 2019). Phytochemicals such as alkaloids,
90 flavonoids, tannins, saponins, glycosides, and phenolic compounds contribute to these
91 therapeutic effects by providing antioxidant, antimicrobial, anti-inflammatory, and
92 immunomodulatory properties (Pandey et al., 2020). Methanol is widely used for
93 extraction because it effectively dissolves both polar and non-polar plant metabolites
94 (Edeoga et al., 2005).

Phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds contribute to the therapeutic properties of the plant, providing antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory effects (Pandey et al., 2020). Methanol is often employed for extraction because of its efficacy in dissolving a wide range of phytoconstituents (Edeoga et al., 2005). This research work aims to perform a qualitative phytochemical analysis of the methanolic extract of *Cordia myxato* identify the presence of key secondary metabolites and assess its potential for further pharmacological development.

1.2. Aim & objective:

1.2.1. General Objective:

To study the phytochemical screening of *Cordia myxa*.

1.2.2. Specific Objective:

- Qualitative analysis of *Cordia myxa* for phytochemicals from methanolic extracts.
- Screening for alkaloids, carbohydrates, glycosides, phytosterols, phenol, saponins, tannins, flavonoids, proteins, and amino acids from *Cordia myxa*.

2.0. Plant profile of *Cordia myxas*:

2.1. Botanical Description of *Cordia myxa*:

Cordia myxa is a small to medium-sized deciduous tree, growing up to 10 meters in height. The plant exhibits simple, ovate to oblong leaves with a rough texture, white to yellowish flowers, and globular orange to brown fruits (Sharma et al., 2020). The fruits are edible and used both as food and medicine in traditional systems.

Ethnomedicinally, *C. myxa* is used as:

- A respiratory remedy for cough, asthma, and bronchitis
- A gastrointestinal aid for diarrhea, dysentery, and indigestion
- A skin treatment for wounds and infections
- A tonic for general health enhancement (Khan et al., 2019)



Figure 2.1: *Cordia myxa* plant



Figure 2.2: *Cordia myxa* fresh fruits



Figure 2.3: *Cordia myxa* dried fruits

2.2. Chemical Composition of *Cordia myxa*:

Phytochemical investigations of *C. myxa* reveal both primary and secondary metabolites:

Primary metabolites:

- Carbohydrates: glucose, fructose, sucrose
- Mucilage: polysaccharides contributing to demulcent activity
- Proteins and amino acids

Secondary metabolites:

- Alkaloids: exhibiting antimicrobial and analgesic activities
- Flavonoids: quercetin, kaempferol, luteolin – potent antioxidants (Sharma et al., 2020)
- Tannins: antimicrobial and anti-inflammatory properties
- Saponins: immunomodulatory, expectorant, and hemolytic effects
- Glycosides: cardioprotective and hepatoprotective activities
- Terpenoids: anti-inflammatory and cytotoxic properties

- Phenolic compounds: antioxidant and free radical scavenging properties (Pandey et al., 2020)

Different plant parts exhibit variations in phytochemical distribution. Fruits are particularly rich in sugars and mucilage, leaves have high flavonoid content, and bark is abundant in tannins (Khan et al., 2019).

2.3. Pharmacological Activity of *Cordia myxa*:

Bioactive compounds in *Cordia myxa* include flavonoids (which are potent antioxidants), phenolic acids, glycosides, sterols, alkaloids, and fatty acids (Rashed, *et al.*, 2024). Metabolic profiling has identified multiple phenolic glycosides, anthocyanins, lignans, and fatty acids in leaves and fruits, which are believed to contribute to observed pharmacological effects (Comparative metabolic profiling, 2025). These compounds exert antioxidant activity through radical scavenging and metal chelation, which can protect cells from oxidative stress—a central mechanism in many chronic diseases (Comparative metabolic profiling, 2025). The above pharmacological activities of *Cordia myxa* are largely consistent with its ethnomedicinal applications. The antioxidant activity may underpin many of its therapeutic effects, as oxidative stress contributes to inflammation, cancer, diabetes, and neurodegeneration. Anti-inflammatory and analgesic actions support traditional use for pain and swelling, and antimicrobial effects validate ethnobotanical claims for treating infections. Despite promising findings, many studies rely on in vitro assays or animal models, and clinical trials in humans remain limited. Isolation of specific bioactive compounds (e.g., triterpenoids, flavonoids) with defined mechanisms of action will help advance *C. myxa* toward pharmacological application. Moreover, standardized extraction methods and safety evaluations are needed to translate these effects into therapeutic formulas.

2.3.1. Antioxidant Activity of *Cordia myxa*:

Flavonoids and phenolic compounds in leaves and fruits exhibit strong free radical scavenging activity, reducing oxidative stress and preventing degenerative diseases (Sharma et al., 2020). Antioxidant capacity is one of the most extensively studied properties of *Cordia myxa*. Phenolic and flavonoid constituents are associated with radical scavenging and reduction of oxidative damage in biological systems (Abdel-

Aleem, *et al.*, 2019). In vitro assays, such as DPPH and ABTS, confirm significant antioxidant activity in leaf and fruit extracts. For instance, leaf extracts exhibited stronger radical scavenging effects compared to fruits, with high capacity in reducing DPPH and ABTS radicals (Comparative metabolic profiling, 2025). This antioxidant activity supports phytotherapeutic relevance, as oxidative stress plays a role in inflammation, diabetes, and cardiovascular disease.

2.3.2. Antimicrobial Activity of *Cordia myxa*:

Alkaloids, tannins, and saponins show inhibitory effects against bacterial and fungal pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Khan et al., 2019). Due to the emergence of antibiotic-resistant pathogens, plant extracts with antibacterial activity are of scientific interest. *Cordia myxa* extracts show inhibitory effects against various pathogenic bacteria. Ethanolic fruit extracts demonstrated antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* in agar diffusion tests (In vitro study, 2022). This antimicrobial action is likely linked to high phenolic content, which destabilizes bacterial cell walls and inhibits key metabolic pathways (In vitro study, 2022).

Chemical investigation has isolated compounds such as oleanolic acid, stigmasterol, and betulin that exhibited effective antimicrobial activity against organisms like *Vibrio cholerae*, *E. coli*, and *P. aeruginosa* (Chemical constituents, 2025). These compounds may disrupt membrane integrity or interfere with enzyme function.

In a study on *Klebsiella* isolates, alcoholic extracts of leaves showed significant inhibitory effects at higher concentrations, surpassing fruit and seed extracts in antibacterial effectiveness (Shamkhy& Abbas, 2021).

2.3.3. Anti-inflammatory and Analgesic Effects of *Cordia myxa*:

Methanolic extracts modulate inflammatory mediators and reduce pain, supporting traditional uses in asthma, arthritis, and skin disorders (Pandey et al., 2020). Inflammation is a physiological response to injury or infection, and *Cordia myxa* extracts demonstrate significant anti-inflammatory actions in experimental models (Abdel-Aleem, *et al.*, 2019).

The presence of flavonoids and phenolic compounds is believed to modulate eicosanoid synthesis by inhibiting cyclooxygenase and lipoxygenase pathways, thereby reducing pro-inflammatory mediators (Abdel-Aleem, *et al.*, 2019). Anti-inflammatory activity has been observed in carrageenan-induced edema models, where both ethanol extracts and fractions of leaves reduced inflammation comparable to standard non-steroidal anti-inflammatory drugs (NSAIDs) (Abdel-Aleem, *et al.*, 2019).

Analgesic effects have also been documented. Methanolic extracts of *Cordia myxa* bark showed significant pain relief in animal models, confirming traditional uses for reducing pain (IJSR, 2025). These effects are likely mediated by modulation of peripheral and central pain pathways, possibly via inhibition of pro-inflammatory enzymes and neurotransmitters involved in nociception.

2.3.4. Antipyretic and Antidiabetic Activities of *Cordia myxa*:

The antipyretic (fever-reducing) activity of *Cordia myxa* has been evaluated using yeast-induced pyrexia models. Total ethanol extracts and various solvent fractions showed significant reduction in body temperature comparable to acetylsalicylic acid, a known antipyretic agent (Abdel-Aleem, *et al.*, 2019). This suggests that compounds in *C. myxa* may act centrally to inhibit prostaglandin synthesis in the hypothalamus, which is central to fever development.

Antidiabetic potential has also been explored. Extracts and certain fractions significantly reduced blood glucose levels in streptozotocin-induced hyperglycemic models, supporting traditional uses for managing diabetes (Abdel-Aleem, *et al.*, 2019). The hypoglycemic mechanism is partly attributed to inhibition of carbohydrate-digesting enzymes like α -glucosidase, which reduces postprandial glucose absorption.

2.3.5. Anti-arthritic Activity of *Cordia myxa*:

Anti-arthritic properties of *Cordia myxa* have been supported by recent in vitro studies. Extracts of leaves demonstrated membrane-stabilizing activity in joint inflammation models, indicating potential to reduce synovial inflammation—a hallmark of arthritis (Tiwari *et al.*, 2025). This activity aligns with traditional uses of the plant for joint pain and swelling.

2.3.6. Hepatoprotective (Liver Protective) Effects of *Cordia myxa*:

Studies investigating liver protection have shown that formulations containing *Cordia myxa* extracts can mitigate chemically induced hepatic damage in rat models. Hepatoprotective effects were evident through reduction in liver enzymes (ALT, AST) and bilirubin levels, suggesting stabilization of cell membranes and enhanced detoxification mechanisms in the liver (Pharmacological evaluation, 2024). Bioactive compounds likely exert antioxidant effects that reduce oxidative stress in hepatic tissue.

2.3.7. Enzyme Inhibitory and Neuroprotective Activities of *Cordia myxa*:

Beyond classical effects, *Cordia myxa* extracts inhibit key enzymes implicated in metabolic and neurodegenerative diseases. Extracts demonstrated inhibitory activity against enzymes such as acetylcholinesterase and α -amylase, indicating potential neuroprotective and antidiabetic mechanisms (Comparative metabolic profiling, 2025). Acetylcholinesterase inhibition suggests possible relevance in conditions like Alzheimer's disease, where cholinergic dysfunction is implicated.

2.3.8. Cytotoxicity and Safety Profile of *Cordia myxa*:

Cytotoxicity assessments are critical in evaluating therapeutic safety. Ethanol extracts of *Cordia myxa* fruit exhibited negligible cytotoxic effects on fibroblast cell lines (L929), suggesting low toxicity at tested concentrations and potential safety for therapeutic development (In vitro study, 2022). Acute toxicity studies of leaf extracts also revealed no mortality up to high dose levels, supporting a favorable safety profile (Abdel-Aleem, *et al.*, 2019).

2.3.9. Emerging and Potential Anticancer Effects of *Cordia myxa*:

Emerging research has begun to explore the effects of secondary metabolites from *Cordia myxa* on cancer-related biomarkers. Flavonoids and other compounds isolated from the plant reduced tumor-associated enzyme levels like LOXL-2 and modulated oxidative stress markers in murine bladder cancer models, indicating potential anticancer activity (Rashid, Al-Sadoon & Mohammed, 2024). While preliminary, these findings underscore need for further mechanistic studies on anticancer potential.

2.3.10. Immunomodulatory Activity of *Cordia myxa*:

Saponins and flavonoids enhance immune response, potentially beneficial in immunodeficiency or infectious diseases (Sharma et al., 2020).

2.3.11. Nutritional and Demulcent Effects of *Cordia myxa*:

Mucilage and carbohydrates provide soothing effects on mucous membranes, particularly in the respiratory and gastrointestinal systems (Khan et al., 2019). *Cordia myxa* exhibits a range of pharmacological activities including antioxidant, anti-inflammatory, analgesic, antipyretic, antidiabetic, antimicrobial, hepatoprotective, enzyme inhibitory, and possible anticancer effects. These actions are supported by diverse phytochemicals such as flavonoids, phenolic acids, sterols, and triterpenoids. While research supports traditional medicine uses, further clinical investigations are necessary to establish efficacy and safety in humans.

3.0. Materials and Methods:

3.1. Plant materials:

Cordia myxa was selected as the experimental material.

3.2. Place and Period of study:

The study was carried out in the Biochemistry Laboratory of Faculty of Unani & Ayurvedic Medicine, Hamdard University Bangladesh during the period of August to November 2025.

3.3. Preparation of fine powder of *Cordia myxa*:

Cordia myxa was collected from the local market of Dhaka, Bangladesh. All the materials washed with clean water and sun-dry under shade then the dried fruits made into powder by means of mechanical blender and preserved in an air-tight container at room temperature until extraction.

3.4. Extracting materials:

277 Extraction of *Cordia myxadone* by polar (methanol) solvents. The following materials are
278 required while extracting them.

279 1. Dried *Cordia myxa*

280 2. Methanol

281 3. Distilled water

282 4. Beaker

283 5. Hot water bath

284 6. Drier

285 7. Weight machine

286 8. Glass rods

287 9. Tissue paper

288 10. Spatula

289 11. Titrating pipette

290 12. Foil papers

291 13. Vial.

292 14. Blender machine

293 15. Scissor

294 **3.5. Preparation of Extraction**

295 The grinded 100 gm powder taken into clean glass containers and was soaked in 500 ml
296 100% Methanol. Then the container was sealed and kept for 3 days with occasional
297 shaking and stirring. The whole mixture was filtered by a piece of clean, white cotton
298 material and then the filtrate was further filtered with Whitman filter paper No.1 filter
299 paper and then make the filtrates into one third of their obtaining volume and perform

phytochemicals experiments for qualitative analysis. Remaining filtrates placed on water-bath at 40°C to obtain crude extracts.

3.6. Reagents and chemicals

Reagents and chemicals such as Wagner's reagent (Iodine in Potassium Iodide), Hagger's reagent (saturated picric acid solution), Mayer's reagent (Potassium Mercurielodide), Draggendorf's reagent (Solution of Potassium Bismuth Iodide), Molisch's reagent (Alcoholic alpha-naphthol solution), Benedict's Reagent, Fehling's reagent, Salkowski reagent, concentrated sulphuric acid, 10% lead acetate, 0.1% ferric chloride, Fehling's solution, Dilute NaOH. Ammonia, Dilute HCl, Distilled water & Methanol were used in this study.

3.7. Phytochemical Analysis

The crude extract was subjected to different chemical tests to find out the qualitative presence of phytochemical which was identified by characterized color changes using standard procedure as described for alkaloids (Harborne, 1973), steroids (Trease & Evans, 1989). phenolics and flavonoids (Awe & Sodipo, 2001), saponins and cardiac glycosides (Sofowora, 1993), tannins (odebiyi & Sofowora, 1978), terpenoids and saponins (Obadonia & Ochuko, 2001).

3.7.1. Detection of alkaloids: Extract was dissolved in diluted Hydrochloric acid and filtered.

3.7.1.1. Mayer's Test: Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

3.7.1.2. Wagner's Test: Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

3.7.1.3. Dragendorff's Test: Filtrate was treated with Dragendorff's reagent (Solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

326 **3.7.1.4. Hager's Test:** Filtrate was treated with Hager's reagent (Saturated picric acid
327 solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

328 **3.7.2. Detection of carbohydrates:** Extract was dissolved in 5 ml distilled water and
329 filtered. The filtrate was used to test for the presence of carbohydrates.

330 **3.7.2.1. Molisch's Test:** Filtrate was treated with 2 drops of alcoholic α -naphthol solution
331 in a test tube. Formation of the violet ring at the junction indicates the presence of
332 carbohydrates.

333 **3.7.2.2. Benedict's Test:** Filtrate was treated with Benedict's reagent and heated gently.
334 Orange red precipitate indicates the presence of reducing sugars.

335 **3.7.2.3. Fehling's Test:** Filtrate was hydrolyzed with dil. HCl, neutralized with alkali and
336 heated with Fehling's A & B solutions. Formation of red precipitate indicates the
337 presence of reducing sugars.

338 **3.7.3. Detection of glycosides:** Extract was hydrolyzed with dil. HCl, and then subjected
339 to test for glycosides.

340 **3.7.3.1. Modified Borntrager's Test:** Extract was treated with Ferric Chloride solution
341 and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted
342 with equal volumes of benzene. The benzene layer was separated and treat with ammonia
343 solution. Formation of rose-pink color in the ammonical layer indicates the presence of
344 anthranol glycosides.

345 **3.7.4. Legal's Test:** Extract was treated with sodium nitropruside in pyridine and sodium
346 hydroxide. Formation of pink to blood red color indicates the presence of cardiac
347 glycosides.

348 **3.7.5. Detection of saponins**

349 **3.7.5.1. Froth Test:** Extract was diluted with distilled water to 20 ml, and this was shaken
350 in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the
351 presence of saponins.

352 **3.7.5.2. Foam Test:** 0.5 gm of extract was shaken with 2 ml of water. If foam produced
353 and persists for ten minutes, it indicates the presence of saponins.

354 **3.7.6. Detection of phytosterols**

355 **3.7.6.1. Salkowski's Test:** Extract was treated with chloroform and filtered. The filtrate
356 was treated with few drops of Concentrated Sulphuric acid, shaken and allowed to stand.
357 Appearance of golden yellow color indicates the presence of triterpenes.

358 **3.7.6.2. Libermann Burchard's test:** Extract was treated with chloroform and filtered.
359 The filtrate was treated with few drops of acetic anhydride, boiled, and cooled.
360 concentrated Sulphuric acid was added. Formation of brown ring at the junction indicates
361 the presence of phytosterols.

362 **3.7.7. Detection of phenols**

363 **3.7.7.1. Ferric Chloride Test:** Extract was treated with 3-4 drops of ferric chloride
364 solution. Formation of bluish black color indicates the presence of phenols.

365 **3.7.8. Detection of tannins**

366 **3.7.8.1. Gelatin Test:** To the extract, 1% gelatin solution containing sodium chloride was
367 added. Formation of white precipitate indicates the presence of tannins.

368 **3.7.9. Detection of flavonoids**

369 **3.7.9.1. Alkaline Reagent Test:** Extract was treated with few drops of sodium hydroxide
370 solution. Formation of intense yellow color, which became colorless on addition of dilute
371 acid, indicates the presence of flavonoids.

372 **3.7.9.2. Lead acetate Test:** Extract was treated with few drops of lead acetate solution.
373 Formation of yellow color precipitate indicates the presence of flavonoids.

374 **3.7.10. Detection of proteins and amino acids**

375 **3.7.10.1. Xanthoproteic Test:** The extract was treated with few drops of concentrated
376 Nitric acid. Formation of yellow color indicates the presence of proteins.

3.7.10.2. Ninhydrin Test: To the extract, 0.25% ninhydrin reagent ($C_9H_6O_4$) was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

4.1. Results:

4.0. Results and Discussion

4.1. Results of Phytochemical Screening of *Cordia myxa*:

The qualitative phytochemical screening of the methanolic extract of *Cordia myxa* demonstrated the presence of a broad range of biologically active phytoconstituents. The results revealed positive tests for alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, and proteins, while free amino acids were not detected. These findings indicate that methanol is an effective solvent for extracting a diverse array of secondary metabolites from *Cordia myxa*, thereby supporting its use in phytochemical investigations.

The presence of alkaloids was confirmed by Mayer's, Wagner's, Dragendorff's, and Hager's tests, all of which produced positive results. The agreement among these four qualitative tests strengthens the evidence that the methanolic extract contains alkaloidal compounds. Alkaloids are among the most important classes of plant secondary metabolites because they exhibit numerous biological activities, including antimicrobial, antioxidant, analgesic, anti-inflammatory, and anticancer properties. Their occurrence may contribute significantly to the medicinal importance of *Cordia myxa*.

Carbohydrates were detected by Molisch's, Benedict's, and Fehling's tests, indicating the presence of reducing sugars and other carbohydrate constituents in the extract. Although carbohydrates are primarily recognized as energy sources, they also play important roles in plant metabolism and may contribute to the nutritional value of medicinal plants.

The Modified Borntrager's and Legal's tests confirmed the presence of glycosides. These compounds are well known for their diverse pharmacological activities, including antioxidant, cardioprotective, antimicrobial, and anti-inflammatory effects. The detection of glycosides further supports the therapeutic potential of the plant and justifies its traditional medicinal applications.

Saponins were identified through positive froth and foam tests. Saponins possess surfactant properties and are widely reported to exhibit antimicrobial, expectorant, immunomodulatory, cholesterol-lowering, and anti-inflammatory activities. Their presence suggests that the methanolic extract may possess multiple pharmacological properties deserving of further investigation.

Phytosterols were positively detected using Salkowski's and Liebermann-Burchard's tests. These naturally occurring sterol compounds are recognized for their ability to reduce cholesterol absorption and exhibit antioxidant and anti-inflammatory effects. The presence of phytosterols increases the medicinal significance of *Cordia myxa* and supports its potential role in promoting human health.

Phenolic compounds and tannins were confirmed by the ferric chloride and gelatin tests, respectively. These polyphenolic constituents are among the most important natural antioxidants because of their ability to neutralize free radicals and reduce oxidative stress. In addition, tannins possess antimicrobial, wound-healing, and astringent properties that may contribute to the therapeutic uses of the plant.

Flavonoids were detected by positive alkaline reagent and lead acetate tests. Flavonoids are extensively studied for their antioxidant, anti-inflammatory, antiviral, antimicrobial, and cardioprotective activities. Their presence suggests that the methanolic extract contains compounds capable of protecting biological systems against oxidative damage.

Protein analysis showed a positive xanthoproteic test but a negative ninhydrin test. This finding indicates the presence of proteinaceous compounds containing aromatic amino acids while suggesting the absence or very low concentration of free amino acids in the extract. Overall, the phytochemical profile obtained in the present study demonstrates that the methanolic extract of *Cordia myxa* is rich in biologically important secondary metabolites. These findings provide scientific support for the traditional medicinal use of the plant and establish a strong foundation for future studies involving quantitative phytochemical estimation, isolation of active constituents, and evaluation of their pharmacological activities.

Table 4.1. Results of alkaloids in methanolic extract of *Cordia myxa*:

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Alkaloids	Mayer's Test	Positive (+)
2		Wagner's Test	Positive (+)
3		Dragendorff's Test	Positive (+)
4		Hager's Test	Positive (+)



Figure 4.1:
Mayer's Test

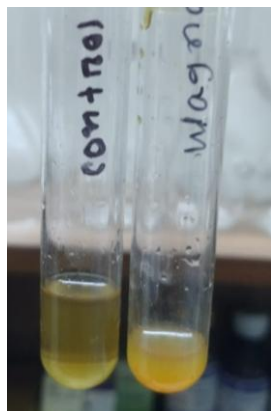


Figure 4.2:
Wagner's Test



Figure 4.3:
Dragendorff's Test

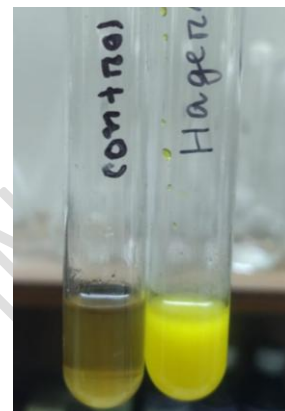
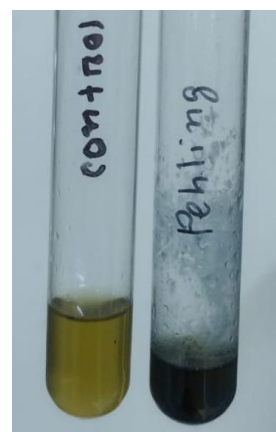
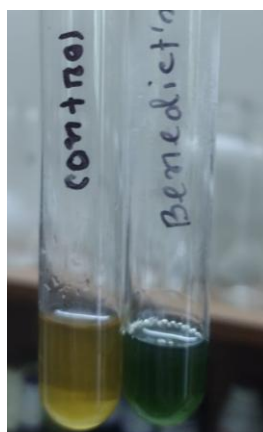
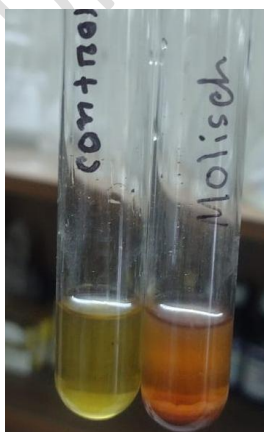


Figure 4.4:
Hager's Test

Table 4.2. Results of carbohydrates in methanolic extract of *Cordia myxa*:

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Carbohydrates	Molisch Test	Positive (+)
2		Benedict's Test	Positive (+)
3		Fehling Test	Positive (+)



**Figure 4.5.
Molisch Test**

**Figure 4.6.
Benedict's Test**

**Figure 4.7.
Fehling Test**

439 **Table 4.3. Results of glycoside in methanolic extract of *Cordia myxa*:**

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Glycoside	Modified Borntrager's Test	Positive (+)
2		Legal's Test	Positive (+)

440 **Table 4.4. Results of saponins in methanolic extract of *Cordia myxa*:**

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Saponins	Forth Test	Positive (+)
2		Foam Test	Positive (+)

441



**Figure 4.8.
Modified Borntrager's Test**



**Figure 4.9.
Legal's Test**



**Figure 4.10.
Forth Test**



**Figure 4.11.
Foam Test**

442 **Table 4.5. Results of phytosterol in methanolic extract of *Cordia myxa*:**

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Phytosterol	Salkowski's Test	Positive (+)
2		Liebermann Burchard's Test	Positive (+)

443 **Table 4.6. Results of phenols in methanolic extract of *Cordia myxa*:**

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Phenol	Ferric Chloride Test	Positive (+)

444 **Table 4.7. Results of tannins in methanolic extract of *Cordia myxa*:**

Sl.	Detection of Phytochemicals	Name of the test	Result
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1	Tannins	Gelatin test	Positive (+)
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Figure 4.12.
Salkowski's Test



Figure 4.13.
Libermann
Burchard's Test



Figure 4.14.
Ferric Chloride
Test



Figure 4.15.
Gelatin Test

Table 4.8. Results of flavonoids in methanolic extract of *Cordia myxa*:

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Flavonoids	Alkaline Reagent Test	Positive (+)
2		Lead acetate Test	Positive (+)

Table 4.9. Results of proteins and amino acids in methanolic extract of *Cordia myxa*:

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Proteins & Amino acids	Xanthoproteic Test	Positive (+)
2		Ninhydrin Test	Positive (+)

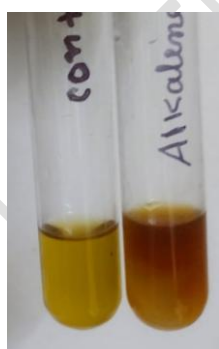


Figure 4.16.
Alkaline Reagent Test



Figure 4.17.
Lead acetate Test



Figure 4.18.
Xanthoproteic Test



Figure 4.19
Ninhydrin Test

4.2. Discussion

4.2. Discussion

The qualitative phytochemical screening of the methanolic extract of *Cordia myxa* demonstrated the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, and proteins, whereas free amino acids were not detected. The predominance of these secondary metabolites suggests that methanol efficiently extracted a broad spectrum of polar and moderately polar bioactive compounds. The present findings are highly consistent with previous phytochemical investigations of *C. myxa*, which reported similar classes of constituents in methanolic and ethanolic extracts of different plant parts (Al-Snafi, 2016; Ali et al., 2024; Fatima et al., 2025).

The detection of alkaloids by Mayer's, Wagner's, Dragendorff's, and Hager's tests indicates a significant presence of nitrogen-containing secondary metabolites. This result agrees with the findings of Ali et al. (2024), who identified measurable alkaloid content in *C. myxa* leaf extracts. Similarly, pharmacognostic studies on *Cordia* species have consistently reported alkaloids in methanolic extracts, emphasizing the effectiveness of polar solvents for alkaloid extraction (Chauke et al., 2022; Al-Snafi, 2016). Alkaloids are widely recognized for their antimicrobial, analgesic, anti-inflammatory, and antioxidant activities, which may partially explain the traditional medicinal applications of *C. myxa* (Al-Snafi, 2016).

Carbohydrates were confirmed by Molisch's, Benedict's, and Fehling's tests, indicating the presence of reducing sugars and other carbohydrate constituents. Comparable observations were reported by studies on *C. myxa* fruit and leaf extracts, where carbohydrates were consistently detected in methanolic and ethanolic extracts (ResearchGate phytochemical analysis of *C. myxa*; Al-Snafi, 2016). The presence of carbohydrates may contribute to the nutritional and demulcent properties traditionally associated with the fruits of *C. myxa*.

Glycosides were detected through positive Modified Borntrager's and Legal's tests. This finding is supported by several studies that reported glycosides as one of the major phytochemical groups in *C. myxa* extracts (Ali et al., 2024; Fatima et al., 2025; Al-Snafi, 2016). Glycosides possess diverse pharmacological properties, including antioxidant, antimicrobial, cardioprotective, and anti-inflammatory activities, suggesting that these compounds may contribute substantially to the therapeutic potential of the plant.

Saponins were positively identified by froth and foam tests. The presence of saponins agrees with the reports of pharmacognostic evaluations of *C. myxa* and related *Cordia* species, where methanolic extracts showed positive reactions for saponins (Pharmacognosy Journal; Al-Snafi, 2016). However, some fruit-based studies reported an absence of saponins, indicating that their occurrence may vary depending on the plant part, extraction solvent, geographical origin, and environmental conditions (Chemical Composition of *Cordia myxa* Fruit, 2017). Such variation is commonly observed in medicinal plants due to differences in secondary metabolite biosynthesis.

Phytosterols were detected by Salkowski's and Liebermann–Burchard's tests. The presence of sterols in the present study is consistent with previous reports identifying sterols, including β -sitosterol and stigmasterol, in *C. myxa* extracts (Chemical Composition of *Cordia myxa* Fruit, 2017; Al-Snafi, 2016). These compounds have been associated with cholesterol-lowering, antioxidant, and anti-inflammatory effects and may enhance the medicinal significance of the plant.

Phenolic compounds and tannins were strongly indicated by ferric chloride and gelatin tests. The detection of these polyphenolic constituents is one of the most important findings of the present study because phenolics and tannins are major contributors to antioxidant activity. Previous studies reported considerable phenolic and tannin contents in *C. myxa* leaves and fruits, with methanolic extracts generally exhibiting higher phenolic concentrations than less polar extracts (Ali et al., 2024; Fatima et al., 2025; Al-Snafi, 2016). Quantitative analyses have identified chlorogenic acid, caffeic acid, gallic acid, and other phenolic derivatives in *C. myxa*, supporting the antioxidant potential suggested by the qualitative results (Ali et al., 2024).

Flavonoids were confirmed by alkaline reagent and lead acetate tests. This observation closely agrees with numerous studies demonstrating abundant flavonoids in *C. myxa* extracts (Ali et al., 2024; Fatima et al., 2025; Al-Snafi, 2016). Identified flavonoids include rutin, naringin, hesperidin, and flavonoid glycosides, which are known for their antioxidant, anti-inflammatory, antiviral, and cardioprotective properties (Al-Snafi, 2016; Ali et al., 2024). The presence of flavonoids, together with phenolics and tannins, suggests a synergistic antioxidant defense system within the extract.

Protein analysis revealed a positive xanthoproteic test but a negative ninhydrin test, indicating the presence of proteins containing aromatic amino acids and the absence or very low concentration of free amino acids. Similar findings were reported in phytochemical studies where proteins were detected while free amino acids were either absent or present in negligible amounts (ResearchGate phytochemical analysis of *C. myxa*). The absence of detectable free amino acids may result from their low concentration, binding with other compounds, or degradation during extraction.

Overall, the present phytochemical profile is in close agreement with previously published studies on *C. myxa* and other *Cordia* species (Al-Snafi, 2016; Ali et al., 2024; Fatima et al., 2025; Chauke et al., 2022). The simultaneous presence of alkaloids, glycosides, saponins, phytosterols, phenolics, tannins, and flavonoids provides strong scientific support for the traditional medicinal use of *Cordia myxa* and highlights the methanolic extract as a promising source of bioactive compounds for future pharmacological and phytochemical investigations.

5.0. Conclusion and Recommendations:

5.1. Conclusion:

The present study successfully investigated the qualitative phytochemical composition of the methanolic extract of the indigenous medicinal plant *Cordia myxa*. The phytochemical screening revealed the presence of several important bioactive constituents, including alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenolic compounds, tannins, flavonoids, and proteins, while free amino acids were not detected. These findings demonstrate that methanol is an effective solvent for extracting a wide range of secondary metabolites from *C. myxa* and highlight the phytochemical richness of this medicinal plant. The detection of multiple classes of phytochemicals indicates that *Cordia myxa* possesses considerable therapeutic potential. The presence of phenolic compounds, flavonoids, and tannins suggests strong antioxidant properties, while alkaloids, glycosides, saponins, and phytosterols are known to exhibit antimicrobial, anti-inflammatory, cardioprotective, cholesterol-lowering, and other pharmacological activities. The qualitative phytochemical profile obtained in this study is consistent with several previously published reports on *C. myxa* and related *Cordia* species, thereby supporting the reliability of the present findings and providing scientific

evidence for the traditional medicinal use of the plant. The results suggest that the medicinal value of *Cordia myxa* may be attributed to the synergistic action of its diverse secondary metabolites. Although the present investigation was limited to qualitative phytochemical analysis, it provides an essential baseline for future research. Quantitative estimation of individual phytochemicals, chromatographic characterization of bioactive compounds, and evaluation of antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer activities are necessary to establish the pharmacological efficacy of the plant. *Cordia myxa* represents a promising source of natural bioactive compounds with significant medicinal potential. The findings of this study contribute to the growing body of scientific knowledge on indigenous medicinal plants and support the further exploration of *C. myxa* for the development of plant-based therapeutic agents and pharmaceutical formulations.

5.2. Recommendations:

Based on the findings of the present study, further research should focus on the quantitative estimation of the identified phytochemicals using advanced analytical techniques such as HPLC, GC-MS, LC-MS/MS, and FTIR spectroscopy. Isolation and structural characterization of individual bioactive compounds are recommended to identify the major therapeutic constituents of *Cordia myxa*. In addition, comprehensive pharmacological studies, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, and anticancer evaluations, should be conducted through in vitro and in vivo models. Toxicological and clinical investigations are also necessary to establish the safety, efficacy, dosage, and therapeutic applicability of *Cordia myxa*-based herbal formulations.

6. Conflicts of interests

The authors do not disclose any conflicting interests.

7. Acknowledgment

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