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Phytochemical Profiling and Bioadsorbent Evaluation of Tinospora Cordifolia And Madhuca Longifolia : A Comprehensive Study

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ABSTRACT
The present experimental research was carried out to analyse the phytochemical profiling of Tinospora Cordifolia and Madhuca longifolia and to evaluate the efficiency of bioadsorbents developed from these plants for eco-friendly wastewater treatment. To perform the phytochemical profiling , the plant samples were immersed in 70% ethanol to obtain the plant extract. The plant extracts of Tinospora Cordifolia and Madhuca Longifolia were evaluate by using the standard phytochemical profiling to detect the occurrence of essential phytochemical constituents such as phenols or tannins, proteins, alkaloids, glycosides, flavonoids , terpenoids , acidity etc. The bioadsorbents were produced from the dried plant materials of Tinospora Cordifolia and Madhuca Longifolia and to examine the effectiveness of adsorption of wastewater treatment. The phytochemical profiling of both the plants i.e., Tinospora Cordifolia and Madhuca Longifolia revealed the presence of several phytochemical constituents demonstrating their pharmacological and therapeutic significance. The plant based bio adsorbents exhibits strong adsorption performance by revealing the efficiency of removing the contaminants and foreign materials from the water. Both the plants i.e., Tinospora Cordifolia and Madhuca Longifolia acts an important biological source for the production of environmental friendly , cost efficient and sustainable bioadsorbents. The research indicates the utilization of purification of water and offers a basis of scientific foundation for subsequent enhancement and eco friendly applications.

INTRODUCTION

Tinospora Cordifolia is a well developed non-hairy deciduous with woody climber that are categorized under the family Minespermaceae. Tinospora Cordifolia is extensively distributed across a wide geographical areas of tropical and subtropical regions like India,Sri Lanka, Africa and China, Thailand, Phillipines etc. In Hindu Mythology, the plant Tinospora Cordifolia is traditionaly known as Giloy which symbolizes the nectar of immorality and enduring youth. The stems of Tinospora Cordifolia is fleshy and possess thread like structures with air borne roots which develops from the branches. The bark is cream coloured to grey in colour with deeply cracked in helically arrangement. The plant bears tiny flowers that are pale yellow greenish in colour. [1] Singh, S. S., Pandey, S. C., Srivastava, S., Gupta, V. S., Patro, B., & Ghosh, A. C. (2003). Tinospora Cordifolia exhibits significant pharmacological and therapeutic affects for the treatment of variety of diseases and health related disorders such as fever ,diabetes, cardiovascular diseases, arthritis, allergy, joint pain, skin related diseases and immune related diseases. The term Guduchi is referred as amrita in Sanskrit due to its purifying and immune enhancing affects.[2] Tiwari, P., Nayak, P., Prusty, S. K., & Sahu, P. K. (2018). Tinospora Cordifolia is referred by numerous vernacular names throughout different parts of India. Some Vernacular names are- Tinospora in English, Giloy in Hindi, Gilo in Punjabi and Kashmiri and Guduchu in Telugu. Tinospora Cordifolia is a nutritious plants that possess a high amount of fibre of about 15.9% which plays a vital role in digestion, proteins of about 4.5 – 11.2% which promotes growth and tissue repair, and carbohydrates around 61.6% which serves as a energy source. Tinospora Cordifolia also possess essential minerals like potassium, Chromium, Iron and Calcium which facilitate in the regulating body blood sugar , transport oxygen in the blood, and also in heart function etc. [3] Choudhary, N., Siddiqui, M. B., Azmat, S., & Khatoon, S. (2013) Tinospora Cordifolia is a significant medicinal plant in Ayurveda and is referred as rasayna which signifies and enhance wellness, strength and durability. It has been traditionally used as rejuvenating health tonic and possess relieving inflammation, arthritis relieving and prevents from allergies, effective against malaria

and dengue and regulating blood sugar. [4] Chandrasekaran, C. V., Mathuram, L. N., Daivasigamani, P., & Bhatnagar, U. (2009). The leaves of Tinospora Cordifolia are simple, alternate and heart shaped with long petioles and glabrous surface. The size of the leaves are usually 6-15 cm long and 5-12cm wide. The fruits of Tinospora Cordifolia are tiny, fleshy like structure which turn red to orange red when ripe and grow in brunches. The flowers are unisexual and actinomorphic i.e., it is radially symmetrical. The flowers generally occurs in spring and Summers. The male flowers possess six sepals six petals that are arranged in two whorls. The female flowers are solitary and are fewer in number. It possess superior ovaries and possess short style. [5] Saeed, M., Naveed, M., Leskovec, J., Kakar, I., Ullah, K., Ahmad, F., ... & Chao, S. (2020). Tinospora Cordifolia is extensively explored in India as an immune strenghthening natural herbal treatment. Some experimental studies suggest it may stimulate the immune system, including stimulating immunoglobulin G (IgG) levels in in vivo animal models.[6] Kulkarni, A. V., Hanchanale, P., Prakash, V., Kalal, C., Sharma, M., Kumar, K., ... & Liver Research Club India. (2022).

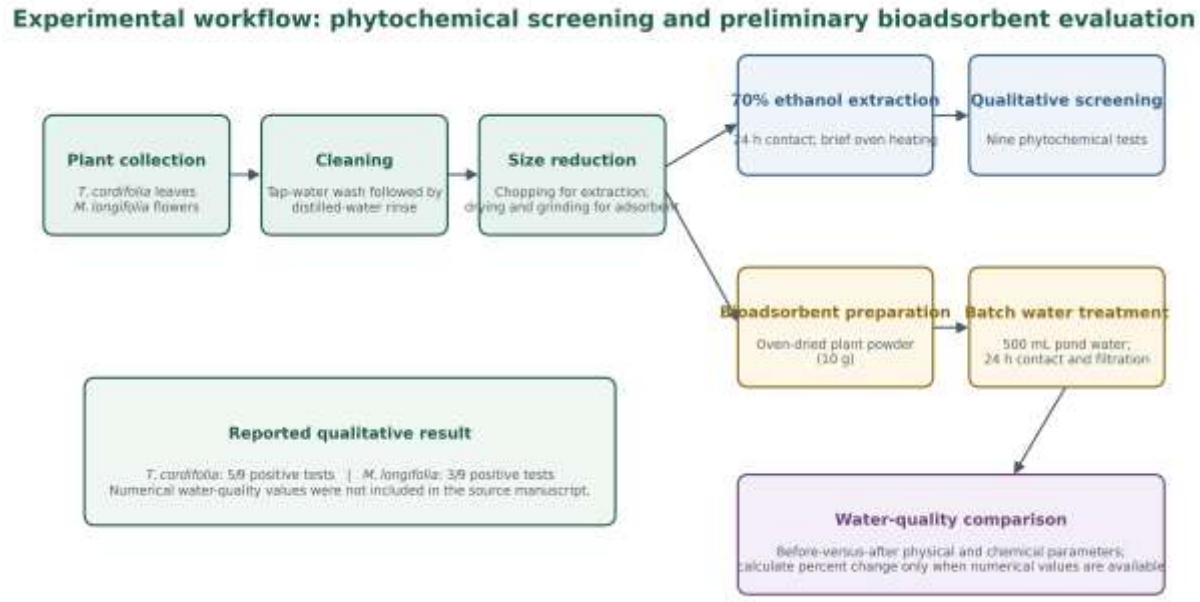

Fig.1 Tinospora Cordifolia

Madhuca longifolia, generally known as the Mahua or butter nut tree, is moderately large deciduous tree that are classified under the family Sapotaceae. Madhuca Longifolia is indigenous to the Indian subcontinent and is widely distributed across tropical and subtropical regions of South Asia. It is predominantly occurs in India, Nepal, and Sri Lanka**,** where it grows naturally in dry deciduous forests**,** mixed forest regions, and plains. In India, it is extensively distributed across states such as Madhya Pradesh, Chhattisgarh, Odisha, Maharashtra, Uttar Pradesh, Jharkhand, Bihar, Andhra Pradesh, Telangana, and parts of Karnataka and Tamil Nadu. The tree is highly adapted to hot and dry climatic conditions and can survive in nutrient-poor, rocky, and marginal soils. It is generally found in rural and tribal areas due to its high adaptability to environmental conditions and economic significance. [7] Yadav, P., Singh, D., Mallik, A., & Nayak, S. (2012). It is consists of a wide range of biologically active plant

constituents that plays a crucial role in therapeutic and pharmacological properties. These include terpenoids, proteins, starch, anthraquinone, phenolic compounds, mucilage, glycosides, tannins, and saponins. The bark is conventionally utilized in the treatment of diseases and health conditions such as skin related diseases, itching, swelling, bone fractures, snake bites, diarrhoea, chronic tonsillitis, leprosy, fever, and rheumatism etc. Madhuca Longifolia is observed that it possess some pharmacological properties such as inflammation reducing effects, anti -microbial and antioxidant, hypoglycemic effects and cancer inhibiting effects.[8] Khare, P., Kishore, K., & Sharma, D. K. (2018).

Madhuca Longifolia is an evergreen tree that grows up to approximately 70 feet in height. It attains maturity within 8-15 years and continues to produce fruit up to 60 years. The leaves of Madhuca longifolia are simple, alternate, and typically clustered at the terminal ends of branches. The leaves are elongated and are spear shaped of sixe about 10-15cm long with sharp and pointed apex. The lamina is thick and smooth with reticulate venation. The venation is pinnate with distinct lateral veins arising from the midrib. The fruits of Madhuca Longifolia are 2-4cm in length and are broadly ovoid in shape and fleshy and succulent and turns greenish to yellowish when it is fully matured or ripen. The flowers are abundant source of sugars, vitamin A, antioxidants, ascorbic acid, thiamine, riboflavin, anthocyanins and minerals such as calcium, phosphorus, iron, magnesium, and copper, flavonoids and saponins etc. The seeds of Madhuca Longifolia are brown, lignified seeds inside the fruit. They contain oil called mahua oil which is used as cooking oil in rural areas and is used to treat certain health related conditions like skin diseases and joint pain.[9] Devi, N., & Sangeetha, R. (2016). Madhuca Longifolia is taxonomically known by their several vernacular names across India and many other countries such as honey tree and butter tree in English, Mahua in Hindi, Mohua in Bengali, Madhukah in Sanskrit, Mahuda in Gujarati and Mahu in Marathi , Ippi in Telugu etc.[10] Dubey, I., Chhajed, M., Chourasiya, R., Chouhan, P. S., Walvekar, A., Bhandari, Y., & Verma, K. (2023).

Fig. 3

2. MATERIALS AND METHODS:

2.1. Materials:

2.1.1. Chemicals andReagents:

Distilled water, Ferric Chloride, Hydrochloric Acid, Sodium Hydroxide, Iodine, Potassium Iodide, Chloroform, Phenol, Phenolphthalein, methyl orange, EDTA, Nutrient agar media, pH 10 buffer Solution prepared from acid and base, EBT indicator, murexide, sulphuric acid, Potassium chromate. All the chemicals were procured from SRL Private Limited(Mumbai).

2.2.2 Glasswares and Laboratory Consumables:

Beakers, Conical Flasks, Volumetric Flasks, Measuring Cylinder ,Pipettes , Funnel, Burette, Test Tube, Test Tube stand, Micropipette, Pipette, wash bottle, mortar and pestle, petri dish, Microtips, glass rod, pH paper strips, spatula, scissors, Filter paper, Aluminium foil, Parafilm.

2.2.3 Equipments

Analytical Balance, Hot air oven, Refrigerator, Magnetic Stirrer, centrtifuge, autoclave, laminar air flow cabinet, incubator, spirit lamp.

2.2 Methods

2.2.1 Collection of Samples

The leaves of Tinospora Cordifolia and flowers of Madhuca Longifolia were collected from garden.

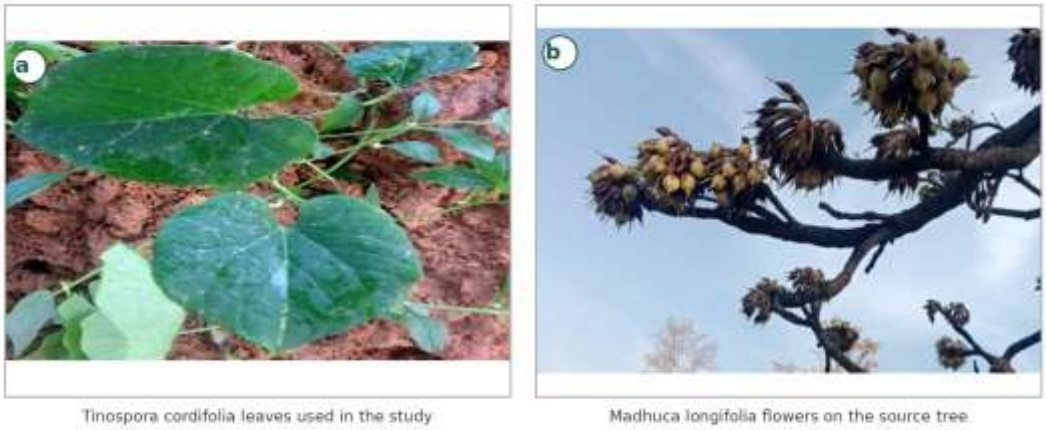

Fig. 4: Samples of Tinospora Cordifolia and Madhuca Longifolia

2.2.2 Extraction of Plant extracts

The collected samples were washed with tap water to remove the soil residues and foreign materials and then cleansed with distilled water to remove the remaining impurities and contaminants. The washed samples were then chopped using a sterile scissor. The chopped samples were then transferred into a beaker and 70% ethanol were poured into the beaker to fully submerged the plant material and were sealed with aluminium foil paper to minimise the evaporation of ethanol . The samples were kept for 24 hours to allow effective extraction of the samples . We later on heated in a hot air oven for 10 minutes for better efficacy and extraction of the samples.

Fig 4: Extract of Tinospora Cordifolia **Extract of Madhuca Longifolia**

2.2.3 Phytochemical Analysis of Plant Extracts

Table 1: Phytochemical Analysis tests

Table 1. Materials and equipment reported in the source manuscript.

Category	Reported items	Purpose in the study
Chemicals and reagents	Distilled water; ferric chloride; hydrochloric acid; sodium hydroxide; iodine; potassium iodide; chloroform; phenol; phenolphthalein; methyl orange; EDTA; pH 10 buffer; Eriochrome Black T; murexide; sulfuric acid; potassium chromate; nutrient agar	Qualitative screening and physicochemical water analysis
Glassware and consumables	Beakers; conical and volumetric flasks; measuring cylinders; pipettes and micropipettes; funnels; burettes; test tubes and racks; wash bottle; mortar and pestle; Petri dishes; microtips; glass rods; pH strips; spatula; scissors; filter paper; aluminium foil; parafilm	Sample preparation, extraction, filtration and titration

Category	Reported items	Purpose in the study
Equipment	Analytical balance; hot-air oven; refrigerator; magnetic stirrer; centrifuge; autoclave; laminar-flow cabinet; incubator; spirit lamp	Weighing, drying, extraction support and laboratory processing

S.No.	Phytochemical	Procedure
1.	Phenols	Add a few drops of ferric chloride solution to the extract. A blue – black or green colour may develop which indicates the presence of phenols.
2.	Flavonoids	Add sodium hydroxide to the extract, then add hydrochloric acid which results a yellow pale colour that become colourless which indicates the presence of flavonoids.
3.	Terpenoids	Add chloroform and then add HCL which results in the formation of a reddish – brown layer which indicates the presence of terpenoids
4.	Glycosides	Heat the extract with HCL and then neutralize with NaOH which results in the colour change which indicates the presence of glycosides.
5.	Saponins	Add distilled water to the extract and shake vigorously.presistent foam formation indicates saponins.
6.	Alkaloids	Add iodine- potassium iodide solution to the extract. A brown precipitate confirms alkaloids.
7.	Proteins	Heat the extract with NaOH solution . Formation of turbudity indicates the presence of proteins.
8.	Organic Acids	Add NaOH and phenolphthalein indicator in the extract which develops a pink colour which upon indicates the presence of organic acids.
9.	General Acids	Add methyl orange/BCG indicator to the extract which results in the colour change and indicates the presence of acid or basic nature.

2.2.4.1 Preparation of Adsorbent

The dried samples of 10g were accurately weighed by using analytical balance and then transferred into a 500ml of polluted water sample and are kept undisturbed for 24 hours.The sample of the mixture were subjected to filtration in order to eliminate the solid bioadsorbent from the solvent and the filtration is done in order to determine the physicochemical characteristics of adsorptive performance.

2.2.4.2 Analysis of Physicochemical parameters

The analysis of physicochemical parameters were conducted to determine the quality of water before and after the treatment with the use of bioadsorbents. The physical parameters include pH , odour, colour, TDS, TSS,TS. The Chemical parameters include pH , total hardness, alkalinity, chloride content, carbonate and bicarbonate alkalinity, Calcium Hardness etc. The difference in the initial and the final values determine the efficacy of the bioadsorbents in enhancing the wasterwater treatment.

3. RESULTS AND DISCUSSIONS:

3.1 Phytochemical Evaluation

Table 2: Phytochemical Tests for Tinospora Cordifolia and Madhuca Longifolia

S.No.	Phytochemicals	Leaves(Giloy)	Flowers(Mahua)
1.	Phenols	Present	Present
2.	Flavonoids	Absent	Absent

3.	Terpenoids	Present	Absent
4.	Glycosides	Absent	Absent
5.	Saponins	Present	Present
6.	Alkaloids	Absent	Absent
7.	Proteins	Present	Absent
8.	Organic Acids	Present	Absent
9.	General Acidity	Absent	Present

The qualitative analysis of phytochemical profiling represents that Tinospora Cordifolia (Giloy) possess the bioactive compounds like phenols, terpenoids, saponins, proteins, organic acids. It signifies the essential therapeutic and pharmacological properties. Flavonoids, Glycosides, Alkaloids and General Acidity are absent in Tinospora Cordifolia(Giloy).
Whereas, Madhuca Longifolia(Mahua) represents only three positive tests. It indicates the presence of Phenols, Saponins and General Acidity. Flavonoids, Terpenoids, Glycosides, alkaloids, proteins and Organic acids are absent in Madhuca Longifolia.This results that Tinospora Cordifolia has abundant source of therapeutic and pharmacological properties than Madhuca Longifolia.

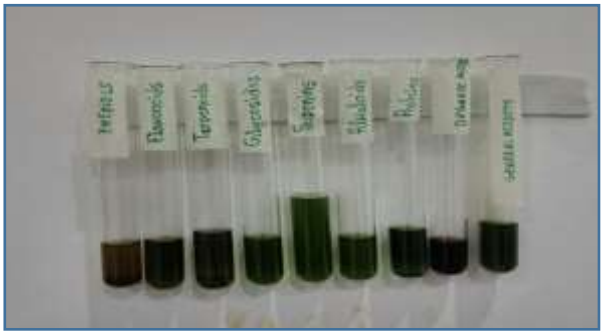

Fig.6: Phytochemical Extract of Tinospora Cordifolia

Fig.7: Phytochemical of Madhuca Longifolia

3.2 Study of Bioadsorbent

Table 3. Experimental conditions reported for the batch bioadsorbent evaluation and information needed for reproducibility.

Variable	Reported condition
Adsorbent materials	T. cordifolia leaf powder; M. longifolia flower powder
Adsorbent mass	10 g
Water volume	500 mL
Nominal dose	20 g L^{-1}
Contact time	24 h
Agitation	Not reported
Separation	Filtration
Replication	Not reported
Controls	Not reported

3 RESULTS AND DISCUSSION

3.1 Qualitative phytochemical profile

The two extracts produced distinct qualitative response patterns. T. cordifolia leaves were positive for phenols, terpenoids, saponins, proteins and organic acids. M. longifolia flowers were positive for phenols, saponins and general acidity. Both extracts were negative under the reported test conditions for flavonoids, glycosides and alkaloids.

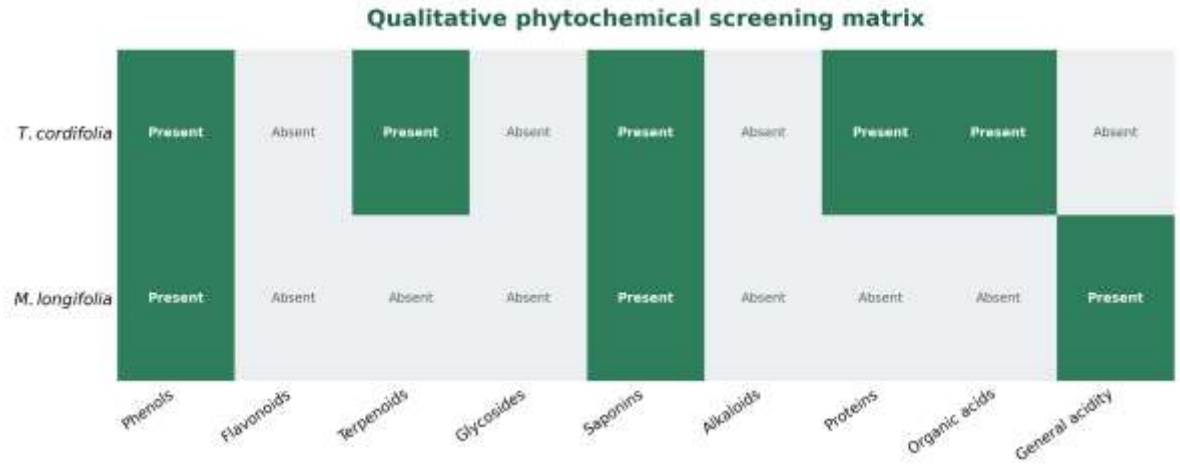

Figure 6. Qualitative phytochemical screening matrix. Green cells indicate a reported positive response; grey cells indicate a reported negative response.

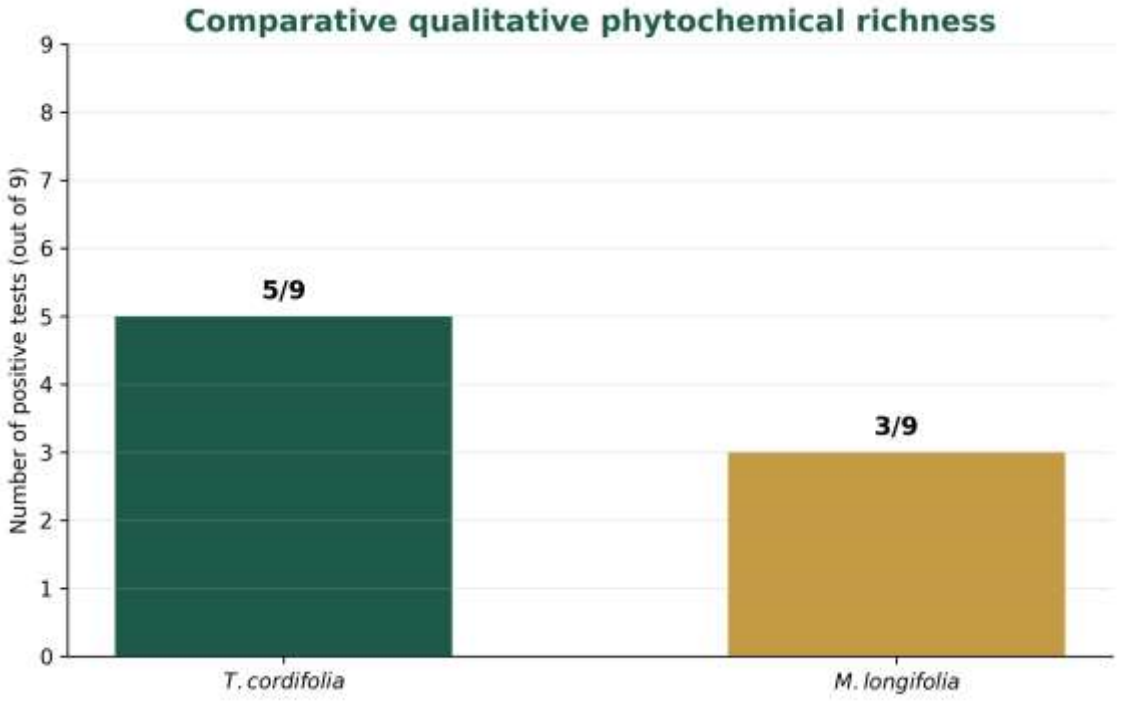

Figure 7. Number of positive qualitative tests: T. cordifolia leaves, 5/9; M. longifolia flowers, 3/9. Counts summarize test outcomes and do not quantify compound concentration.

Interpretation of the screening pattern

Under the specific extraction and test conditions used, the T. cordifolia leaf extract showed a broader positive profile than the M. longifolia flower extract. The difference should be interpreted cautiously. The comparison involves different plant organs, and preliminary tests are affected by extraction solvent, extract concentration, reagent quality, sample maturity and visual interpretation. A negative reaction therefore means "not detected by this procedure at the tested concentration," not necessarily complete chemical absence.

Published studies have reported variable profiles for both species. This variability is expected because different investigations use different organs, solvents and protocols [2,7-9,18]. The present observations should therefore be treated as a screening result specific to the tested samples rather than a definitive chemical inventory. Confirmatory analysis could use total phenolic and flavonoid assays, thin-layer chromatography, HPLC/UPLC, GC-MS where appropriate, and LC-MS or NMR for compound identification.

3.2 Preliminary bioadsorbent evaluation

The laboratory procedure demonstrates that both plant materials can be cleaned, dried, powdered, contacted with pond water and separated by filtration. However, the numerical before-and-after values in the source tables were blank. Consequently, absolute change, percentage removal, adsorption capacity, uncertainty and statistical significance cannot be calculated from the available record.

Table 4: Chemical parameters of Pond Water

Parameter	Unit	Before	After *T. cordifolia*	Change	After *M. longifolia*	Change
Colour	Qualitative / Pt-Co	NR	NR	NR	NR	NR
Odour	Qualitative	NR	NR	NR	NR	NR
pH	pH units	NR	NR	NR	NR	NR
Total suspended solids (TSS)	mg L^{-1}	NR	NR	NR	NR	NR

Parameter	Unit	Before	After *T. cordifolia*	Change	After *M. longifolia*	Change
Total dissolved solids (TDS)	mg L^{-1}	NR	NR	NR	NR	NR
Total solids (TS)	mg L^{-1}	NR	NR	NR	NR	NR

Table 6. Publication-ready reporting table for chemical water-quality parameters. NR = not reported in the source manuscript.

Parameter	Unit	Before	After *T. cordifolia*	Change	After *M. longifolia*	Change
Total hardness	mg L^{-1} as $CaCO_3$	NR	NR	NR	NR	NR
Calcium hardness	mg L^{-1} as $CaCO_3$	NR	NR	NR	NR	NR
Total alkalinity	mg L^{-1} as $CaCO_3$	NR	NR	NR	NR	NR
Phenolphthalein alkalinity	mg L^{-1} as $CaCO_3$	NR	NR	NR	NR	NR
Chloride	mg L^{-1}	NR	NR	NR	NR	NR
Residual chlorine	mg L^{-1}	NR	NR	NR	NR	NR
Acidity	mg L^{-1} as $CaCO_3$	NR	NR	NR	NR	NR
Carbonate alkalinity	mg L^{-1} as $CaCO_3$	NR	NR	NR	NR	NR
Bicarbonate alkalinity	mg L^{-1} as $CaCO_3$	NR	NR	NR	NR	NR

3.3 Conceptual basis for adsorption

Plant-derived powders are lignocellulosic matrices. Their surfaces may contain hydroxyl, carboxyl and phenolic groups associated with cellulose, hemicellulose, lignin and extractives. Depending on solution chemistry and pollutant identity, these sites may participate in several simultaneous interactions. The mechanism diagram below is therefore conceptual; it is not direct evidence that any particular mechanism occurred in the present experiment.

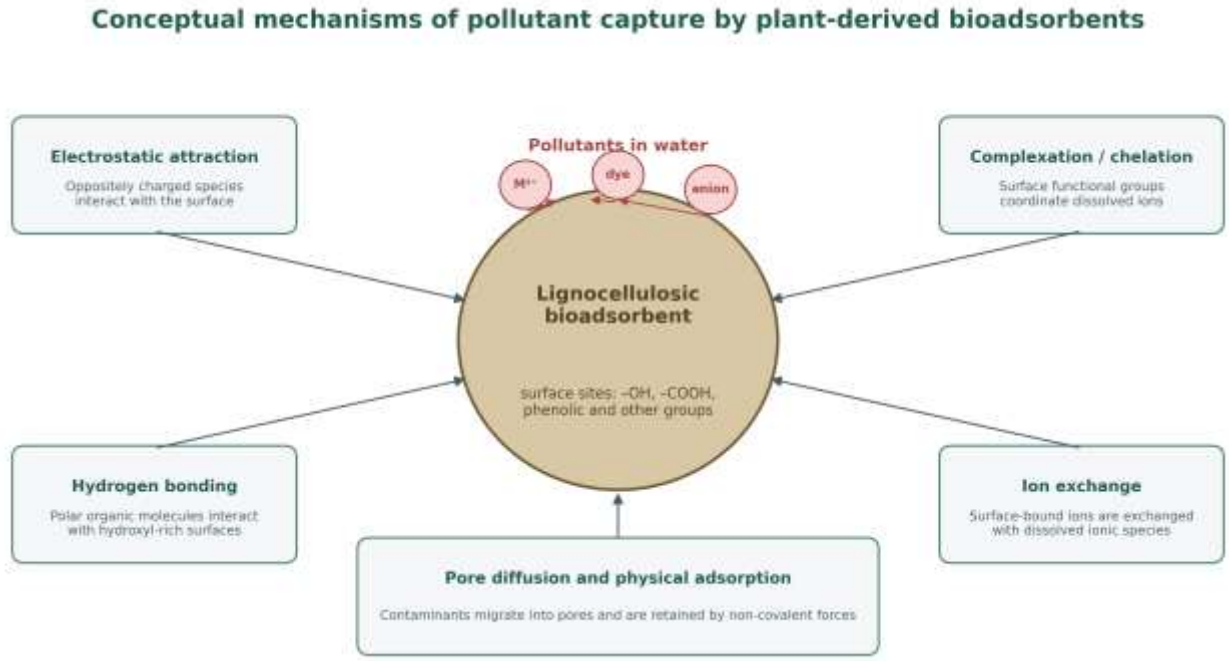

Figure 8. Conceptual mechanisms by which plant-derived lignocellulosic materials may retain dissolved pollutants. Verification would require adsorbent characterization and contaminant-specific experiments [13-16].

To identify the dominant mechanism, future work should characterize the material before and after adsorption using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy with elemental analysis (SEM-EDS), surface-area and pore-size analysis, point-of-zero-charge measurement and, where relevant, X-ray diffraction. Batch data should be collected across pH, dose, time and concentration ranges and evaluated using appropriate kinetic and equilibrium models. Model fitting should include uncertainty and goodness-of-fit diagnostics rather than relying only on the coefficient of determination.

4 LIMITATIONS AND QUALITY-IMPROVEMENT PLAN

The study provides a useful exploratory protocol and a documented qualitative screening pattern, but several reporting gaps prevent strong conclusions about bioadsorbent performance. Addressing these issues would substantially improve scientific validity and journal suitability.

Table 7. Principal limitations and recommended corrective actions.

Reporting or design gap	Why it matters	Recommended action
Plant identification not documented	Misidentification can invalidate biological interpretation.	Authenticate specimens and deposit voucher samples in a recognized herbarium.
Different organs compared	Leaf-versus-flower differences may reflect organ biology rather than species alone.	Compare matched organs where possible or clearly frame the study as an organ-specific comparison.
Extraction ratio and temperature missing	Concentration and heat exposure affect test sensitivity.	Report plant mass, solvent volume, temperature, filtration and storage conditions.
No positive/negative controls for qualitative tests	Colour and precipitate endpoints may be subjective or reagent-dependent.	Use reagent blanks, reference compounds and blinded replicate scoring.

Reporting or design gap	Why it matters	Recommended action
Drying temperature and particle size missing	Surface chemistry and available area affect adsorption.	Standardize drying, sieve to a defined size range and measure moisture content.
No untreated/process controls reported	Natural settling, filtration or container effects may be mistaken for adsorption.	Include untreated pond water, filtration blank and adsorbent leaching blank.
No numerical water-quality data	Removal efficiency and comparative performance cannot be calculated.	Report raw values, units, calibration details, mean ± SD and complete replicate-level data.
No pollutant-specific measurement	Aggregate parameters do not identify what was adsorbed.	Measure defined contaminants such as target metals, dyes, nutrients or organics.
No adsorbent characterization	Mechanisms and material differences remain speculative.	Use FTIR, SEM-EDS, BET, pH_{pzc} and proximate/compositional analysis.
No kinetics, isotherms or regeneration study	Process design and practical reuse cannot be evaluated.	Study contact-time profiles, equilibrium curves, desorption, reuse and safe disposal.

4. CONCLUSION

The phytochemical analysis of Tinospora Cordifolia and Madhuca Longifolia reveals the presence of various phytochemical constituents that plays a crucial role in pharmalogical and medicinal behaviour. The phytochemical profiling reveals the presence of some essential phytochemical constituents like phenols, alkaloids, proteins, General Acidity, Saponins in both the plants i.e., Tinospora Cordifolia and Madhuca Longifolia. The research revealed that Tinospora Cordifolia and Madhuca Longifolia serves as an efficient source of bioadsorbents. Both the plants plays a significant role to enhance the quality of water by optimization of impurities. Tinospora Cordifolia and Madhuca Longifolia acts as eco-friendly, economic viable, and sustainable bioadsorbents for the wastewater treatment.

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