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RESEARCH ARTICLE

Identification of bioactive metabolites in *Turnera ulmifolia*: Preliminary phytochemical screening and FTIR analysis

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Abstract

Turnera ulmifolia L., a member of the Passifloraceae family, is widely distributed across tropical and subtropical regions. Though frequently considered a weed, it has been commonly used in folk medicine to treat inflammation, infections, wounds and digestive ailments. Earlier studies have found alkaloids, flavonoids, tannins, terpenoids and polyphenols in species from the same genera that contribute to their therapeutic efficacy. Despite its ethnomedicinal value, the phytochemical profile and functional group characterization of *T. ulmifolia* are still unexplored. This study aimed to investigate the phytochemical composition of its leaf, stem and root extracts using different solvents (methanol, ethanol, hexane and acetone) and identify key functional groups through FTIR analysis. Phytochemical screening confirmed the presence of diverse secondary metabolites. FTIR analysis further revealed functional groups such as O=C=O, C=C and S=O, which are associated with therapeutic properties. Notably, alkaloids were abundant in leaf extracts, while sulfoxide groups, known for their herbicidal and medicinal effects, were detected in the stem. These findings reinforce the pharmacological potential of *T. ulmifolia* as a promising source of bioactive metabolites with medicinal and ecological applications. Its capacity to diversify in various habitats and create bioactive molecules under stress points to possible uses in medicine discovery, sustainable agriculture and environmental restoration. This study lays the groundwork for future research to validate its therapeutic potential and explore its integration into modern pharmaceutical and ecological solutions.

Keywords: bioactive; functional group analysis; *T. ulmifolia*; weed plant

Introduction

The sustainability of many valuable medicinal plant species is increasingly threatened by unsustainable harvesting, habitat deterioration and the impacts of climate change, along with the recent surge in their usage for COVID-19 prevention and remedies (1-3). Consequently, studies have also begun to focus on other wild plant species, such as weeds, as viable substitutes (4-6). *Turnera ulmifolia*, also known as Sage plant/ West Indian Holly, is a weed plant belonging to the family Passifloraceae (7). It is extensively dispersed throughout tropical and subtropical climates, especially in America, Africa and Southeast Asia (8). *T. ulmifolia* is an imported and naturalized species extensively grown as an ornamental plant in regions in India (9). These plants prefer warm temperatures and moderate to high humidity and thrive in various soil types but prefer well-drained, sandy, or loamy soils with enough aeration (10). This shrub shows multiple mutualistic interactions with pollinators and ants for seed dispersal by their nectar-producing flowers (11). It was also observed that extrafloral nectar produced by these plants throughout the fruit maturation stage attracts ants, allowing for efficient seed dissemination (12). A study found that enhanced blooming and nectar production of *T. ulmifolia* enhanced parasitism and decreased bagworm prevalence in oil palm farms (13). *T. ulmifolia* has a potential role in curing microbial infections and anti-helminthic and analgesic effects, with a promising role in drug discovery and medicinal research (14, 15). Researchers observed the hepatoprotective role of *T. ulmifolia* in treating chemical-treated rats (16). Another study found that *T. ulmifolia* can be an effective stabilizing and reducing agent in synthesizing nanoparticles of plant origin (17). In recognition of its relevance in medicine, methods for the micropropagation of *T. ulmifolia* were developed (18). Studies proved that microencapsulation improves the antiglycation and antioxidant potential of *T. ulmifolia* (19). Phytochemical profiling of the other species of the genus has been investigated thus far, but not for *T. ulmifolia* (20).

This study hypothesizes that using different solvents enables the differentiation of the various bioactive components in *T. ulmifolia*. The present study was carried out to examine the extraction capacity of solvents such as methanol, ethanol, hexane and acetone on sections of *T. ulmifolia* leaves, stem and roots to detect alkaloids, terpenoids, polyphenols, saponins steroids, flavonoids, coumarins, quinones and tannin and to determine the functional group present in various plant parts.

Material and Methods

Collection of plant materials

The flowering plants of *T. ulmifolia* were collected from Thiruvananthapuram, Kerala and the collected specimen was identified by Dr. N. Dhatchanamoorthy, Botanist, Plant Systematics and Nomenclature, Centre for Conservation of Natural Resources, Foundation of Revitalisation of local health traditions, Bangalore, Karnataka, India. (No. 127203). (Fig. 1). The leaves, stems and roots were properly cleansed individually twice or thrice under running water before being rinsed in distilled water. Once the plant parts were shade-dried to remove the excess moisture, they were ground into fine powder. The plant components were labelled and stored for later use (21).

Preparation of extract

Solvent extracts were prepared with a few modifications (22). The dried material weighed approximately 0.2 g and was placed in each test tube with 5 mL of solvents such as methanol (Mt), ethanol (Et), hexane (H) and acetone (A). After 48 hrs, the extract solution was filtered through Whatman No. 1 paper and the solvent was allowed to drain completely. It took 4 hrs for the solvent to evaporate entirely at 30°C . Extracts were re-suspended in individual extracting solvents and were further taken for the preliminary phytochemical analysis (Fig. 2).

Preliminary screening of the phytochemicals

Four different solvent extracts of *T. ulmifolia* were subjected to a preliminary qualitative phytochemical analysis to determine the pharmacologically active biochemical components like alkaloids, polyphenols, saponins, terpenoids, steroids, flavonoids, coumarins, quinones and tannin.

Test for alkaloid

Mayers' test detected The alkaloid in the samples (23). A mixture of dilute hydrochloric acid and alcohol was added to the crude extracts. This mixture was filtered to remove the impurities. The appearance of a creamy or white precipitate confirms the presence of alkaloids.

Test for polyphenols

Polyphenols were detected with a ferric chloride test (24). 1 mL of crude extract was mixed with 2 mL of 5 % ferric chloride ($FeCl_3$) solution. The mixture was then vortexed or gently swirled to ensure the extract and reagent were adequately mixed. The presence of polyphenols was indicated by a colour shift to blue-green or dark green, with the strength of the colour proportional to the concentration of polyphenols present.

Test for saponins

A forming test was used to perform the saponin test (25). 3 mL of plant extract was mixed with 3 mL of distilled water in a test tube. It was left to stand after aggressively agitating the mixture for a few minutes. The appearance of steady and persistent froth or foam on the surface suggested the presence of saponins in the extract.

Liebermann-Burchard test for terpenoids

The presence of triterpenes was detected using the Liebermann-Burchard test (26). 2 mL of chloroform was combined with 3 mL of concentrated sulphuric acid in a test tube containing 5 mL of plant extracts. The solution was carefully examined for colour changes. A reddish or purple colour indicated the presence of triterpenes.

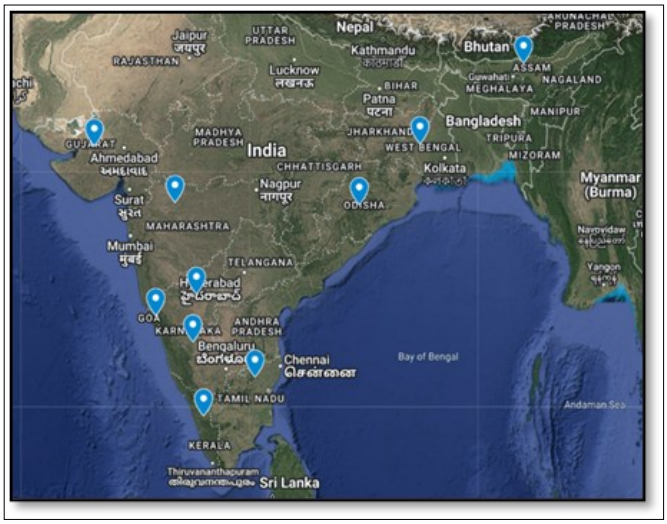
Test for steroids

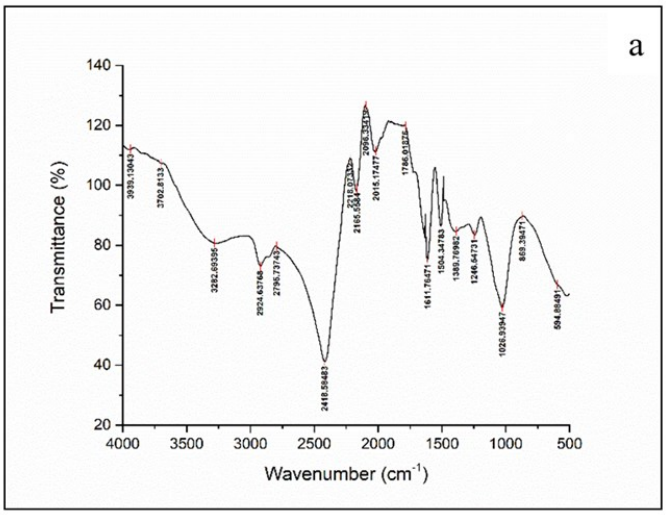
In a test tube, 1 mL of concentrated sulphuric acid was mixed with 5 mL chloroform and 2 mL of sample solution. The mixture was thoroughly blended and allowed to rest. A red or reddish-brown colouration at the interface showed the presence of sterols (27).

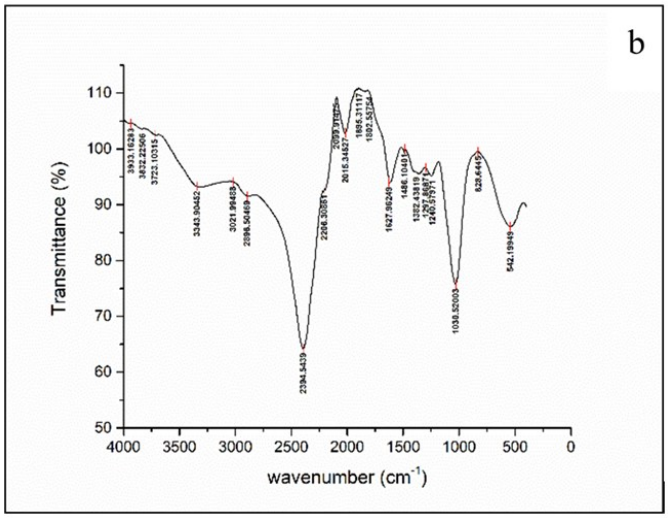
Test for flavonoids

To determine the presence of flavonoids, a test tube was filled with 1 mL of 10 % sodium hydroxide (NaOH) solution and 3 mL of plant extract. The appearance of a yellow colour suggested the presence of flavonoids. The colour change could be reversed by adding a few drops of dilute acid, confirming the flavonoid molecules' existence (28).

Fig. 1. Morphology of the plant with flowers of *T. ulmifolia* and its global distribution.







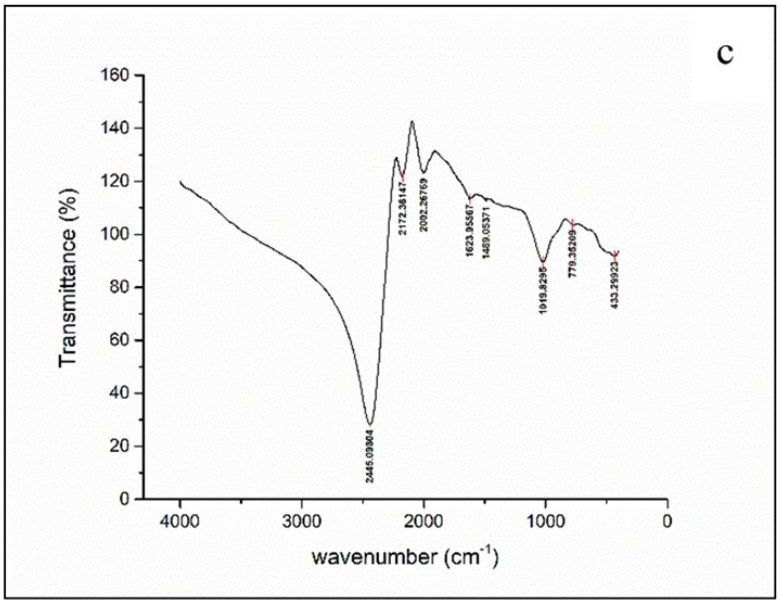


Fig. 2. FT-IR spectral graph of *T. ulmifolia* plant parts; a) Leaf b) stem c) root.

Table 1. Screening of *T. ulmifolia* for detecting bioactive compounds

Sl. No	Bioactive compound	Test name/Method	Reagents used	Positive observation
1	Alkaloids	Alkaloid Test	Alcohol, diluted HCl	White creamy precipitate
2	Polyphenols	Ferric Chloride Test	5 % Ferric chloride solution	Bluish-green colour
3	Saponins	Foaming Test	Distilled water	Stable and persistent foam
4	Terpenoids	Chloroform-Sulfuric Acid Test	Chloroform, sulfuric acid	Reddish-brown colour
5	Steroids	Salkowski Test	Sulfuric acid, chloroform	A reddish-brown ring at the interface
6	Flavonoids	Alkaline Reagent Test	10 % NaOH solution	Intense yellow colour
7	Coumarins	NaOH Test	10 % NaOH solution	Yellow color
8	Quinones	HCl Test	Concentrated HCl	Green appearance
9	Tannins	Gelatin Test	1 % gelatin, 10 % NaCl	White precipitate

Detection of coumarins

In a test tube, 2 mL of the crude extract was mixed with 3 mL of 10 % sodium hydroxide solution to detect flavonoids (29). The yellow colouration suggested the presence of flavonoids, which could be removed by adding a few drops of dilute acid, verifying their existence.

Detection of quinones

1 mL of plant extract was treated with a few drops of strong hydrochloric acid (HCl) in a test tube. The formation of a yellow, red, or orange colouration indicated the presence of quinones in the extract (30).

Test for tannins

A sample solution was mixed with a 1 % gelatin solution containing 10 % sodium chloride (NaCl) in a test tube to detect tannins. The production of a precipitate revealed the presence of tannins in the extract (31).

Study of FT-IR spectra

Fourier transform infrared spectroscopy (FTIR), an efficient technique, was used to characterize the chemical bonds and functional groups present in *T. ulmifolia*. The powdered sample of the plant was loaded in an FTIR spectrophotometer (Shimadzu, IR Affinity 1, Japan), with a scan range from 400 to 4000 per cm and a resolution of 4 per cm. The spectra were interpreted using the standard to identify the absorption peak and functional groups in *T. ulmifolia* leaf, stem and root samples.

Results and Discussion

Preliminary phytochemical analysis

The preliminary phytochemical analysis of different extracts (methanol, ethanol, hexane and acetone) of *T. ulmifolia* showed these results in Table 2. It was found that alkaloids, polyphenols, saponins, terpenoids, steroids, flavonoids, coumarins, quinones and tannins were present in the leaf and stem. Root contains alkaloids, polyphenols, saponins, terpenoids, steroids, flavonoids, coumarins and tannins, except for quinones. The extracting solvents, methanol, ethanol, hexane and acetone detected alkaloids, polyphenols, terpene, flavonoids, coumarins, quinones and tannins detected in the leaf, terpenes, flavonoids, coumarins and quinones in the stem and terpene, steroids and tannins were detected in the root. The polarity of the solvents plays a significant role in the extraction of bioactive compounds from plants (32). In this study, alkaloids were detectable in polar solvents such as methanol, ethanol and acetone and even in nonpolar solvents such as hexane. This describes the residual polarity of the alkaloids due to the nitrogen groups in them (33). Polyphenols were detected in all polar solvents of leaf fraction. The highly polar nature of the polyphenols made them insoluble in hexane in the stem and root fraction. A similar study showed hexane was not an effective solvent for extracting alkaloids, polyphenols, steroids and tannins compared to polar solvents (34). It was seen that methanol detected saponins in leaves, stems and root extracts. A study finding revealed that large molecules like saponins, which have intermediate polarity, would be recovered and further precipitated in a highly polar acetone (35). Researchers showed that hexane could extract saponins in all plant parts primarily due to the higher hydrophobic part that can dissolve in the nonpolar solvent (36). Polar and nonpolar solvent extraction detected terpenoids in the plant parts of *T. ulmifolia*. This is due to the polar functional groups in terpenoids with partial polarity, which may dissolve in the polar and nonpolar solvents depending on the balance of polar and nonpolar groups (37).

Methanolic extract detected steroids in the leaf and the root section of *T. ulmifolia*. This might be due to the higher concentrations of steroids in these areas, which have more accessible cellular structures or potentially more polar forms (38). Methanol did not extract these in the stem because of the rigid, lignified and tightly bound steroid. Using a highly nonpolar solvent or a combination of solvents may improve steroid extraction in the stem (39). However, the root sections were not dissolved in the polar solvents such as ethanol and methanol, which might be due to the presence of aglycons, that non-sugar form of glycosides, a type of flavonoid that has roles in stress response and inhibits the growth of competing plants (40). Coumarins found in plant parts were dissolved in polar and nonpolar solvents, which might be attributed to their lipophilic nature combined with specific polar properties (41). Hexane was the better solvent for the coumarins, possibly due to less polar derivatives or lipophilic coumarins in the root of *T. ulmifolia*. All four solvents of leaf and stem fractions of *T. ulmifolia* contained quinone.

Table 2. Preliminary phytochemical tests for *T. ulmifolia*

Sl. No	Bioactive compounds	leaf				stem				Root			
		Mt	Et	H	A	Mt	Et	H	A	Mt	Et	H	A
1.	Alkaloids	+	+	+	+	+	+	-	+	+	-	-	+
2.	Polyphenols	+	+	+	+	+	+	-	+	+	-	-	+
3.	Saponins	+	-	+	-	+	-	+	-	+	-	+	-
4.	Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+
5.	Steroids	+	+	-	+	-	+	-	+	+	+	+	+
6.	Flavonoids	+	+	+	+	+	+	+	+	-	-	+	-
7.	Coumarins	+	+	+	+	+	+	+	+	-	-	+	-
8.	Quinones	+	+	+	+	+	+	+	+	-	-	-	-
9.	Tannin	+	+	+	+	+	+	-	+	+	+	+	+

Methanol (Mt), ethanol (Et), hexane (H) and acetone (A); (+) =present; (−) =absent

Through preliminary screening, this study used four different extracting solvents to detect alkaloids, bioactive compounds potentially crucial in plant defence, growth and ecological interactions (42). They were also found to have a role in medicine for drug development, painkillers, anaesthetics and components of natural pesticides (43, 44). These secondary metabolites are essential in combating biotic and abiotic stress in plants (45, 46). The study detected plant steroids known for their potential against viral infections by acting as ligands (47). Flavonoids possess various bioactive properties such as anti-inflammatory, antioxidant and antimicrobial and are extracted using all the solvents in leaf and stem sections (48-50). Quinones are compounds that deter herbivores, cope with the stressful environment and defend against microbes (51). The functional group analysis further confirmed the presence of the bioactive compounds.

Functional group analysis by FTIR

The FTIR spectra of each plant part of *T. ulmifolia* are displayed in (Fig 2). The absorption range, intensity of the bond, type of vibration and functional group are shown in Table 3. FTIR spectra analysis detects the infrared spectra of organic molecules by measuring the absorption or transmittance of infrared radiation. It detects the chemical bonding and the nature of vibration. Applications of FTIR are

Table 3. FTIR spectral analysis of *T. ulmifolia* plant parts

Sl. No	Plant sample	Wavenumber range (cm^{-1})	Intensity of band	Chemical bond	Type of vibration	Functional group assignment
1.	Leaf	3702.81	medium	O–H	stretching	alcohol
		3282.69	medium	N–H	stretching	primary amine
		2924.63	medium	C=C	stretching	alkene
		2795.73	medium	C–H	stretching	aldehyde
		2418.58	strong	O=C=O	stretching	carbon-di oxide
		2218.07	weak	C-C	stretching	alkyne
		2165.55	strong	S – C-N	stretching	thiocyanate
		2096.33	strong	N=C=S	stretching	iso-thiocyanate
		2015.17	strong	N=C=S	stretching	isothiocyanate
		1786.01	strong	C=O	stretching	acid halide
		1611.76	strong	C=C	stretching	α,β– unsaturated ketone
		1504.17	strong	N–O	stretching	nitro compounds
		1389.76	medium	C–H	bending	aldehyde
		1246.54	medium	C–N	stretching	amine
		1026.93	strong	C–O	stretching	vinyl ether
		869.39	strong	CH	bending	1,3-disubstituted
		594.88	strong	C–Br	stretching	halo compound
2.	Stem	3723.10	medium	O–H	stretching	alcohol
		3343.90	medium	N–H	stretching	secondary amine
		3021.99	medium	C–H	stretching	alkene
		2896.50	medium	C–H	stretching	alkane
		2394.54	strong	O=C=O	stretching	carbon-di oxide
		2206.3	strong, broad	N=C=O	stretching	isocyanate
		2099	strong	N=C=S	stretching	thio isocyanate
		1802.55	strong	C=O	stretching	acid halide
		1627.96	strong	C=C	stretching	alkene
		1382.43	medium	O–H	bending	phenol
		1297.86	strong	C–O	stretching	aromatic ester
		1210.57	strong	C–O	stretching	ester
		1030.52	strong	S=O	stretching	sulfoxide
		823.6	medium	C=C	bending	alkene
		542.19	strong	C–Br	stretching	halo compound
3.	Root	2445.09	strong	O=C=O	stretching	carbon-di oxide
		2172.38	strong	N=N=N	stretching	azide
		2002.26	medium	C=C=C	stretching	allene
		1623.95	strong	C=C	stretching	α,β-unsaturated ketone
		1019.92	strong	C–O	stretching	vinyl ether
		779.36	strong	C–H	bending	1,3-undisubstituted

primarily in the mid-IR range, or roughly 4000 cm^{-1} to 400 cm^{-1}. The FTIR spectral data showed strong, intense peaks at 2418.58 cm^{-1} for the leaf, corresponding to the O=C=O stretching vibration of carbon dioxide bonds. Similar vibrations were found in the stem and the root samples at 2394.54 and 2445.09 cm^{-1}. A study showed that elevated levels of carbon dioxide in the atmosphere could improve the secondary metabolite concentration, which in turn has enhanced antimicrobial activity against pathogenic strains (52). A strong band was found at 1026.93 cm^{-1} due to the C-O stretching vibration of the vinyl ether bonds, primarily found in the phospholipids known as plasmalogens of plant tissue. These plasmalogens can scavenge the free radicals and act as antioxidant molecules. They could also be a sensitive method for detecting oxidative stress in biological systems. Similar bonds were present in the root of *T. ulmifolia* at 1019.92 cm^{-1}. Peaks at 1611.76 cm^{-1}in the leaf indicate the stretching vibration of C=C that makes α, β- unsaturated ketone. A study showed the importance of α, β- unsaturated as a compound with anticancer properties (53).

Previous studies revealed the role of terpene ketone in controlling insect pests, highlighting the insecticidal property of α, β-unsaturated ketone (54). The absorption of C–Br appearing at 594.88 cm^{-1} in the leaf and at 542.19 cm^{-1} in the stem indicated the presence of halo compounds. Organo-bromine compounds are not typically abundant in plants but appear only under specific contents. These compounds were known to have antimicrobial and fungicidal activities compared to their counterparts (55). Peaks at 1030.52 cm^{-1} in the stem of *T. ulmifolia*, which indicate S=O stretching, reveal the presence of a strong sulfoxide group. Researchers showed that therapeutic properties in plant-derived compounds are attributed to their sulphur (56, 57). The presence of the sulfoxide group in the plants was found to have herbicidal properties, which can be used to prepare the pesticidal formulation (58).

Conclusion

Phytochemical screening and FTIR analysis of *T. ulmifolia* yielded a wide range of bioactive chemicals with potential pharmaceutical and ecological uses. Methanol was the most effective solvent for extracting bioactive compounds, notably from the leaf. At the same time, ethanol and acetone were appropriate for the stem and a mixture of methanol, hexane and acetone could work well for root extractions. The presence of alkaloids, polyphenols, terpenoids, flavonoids, coumarins, quinones, tannins and steroids emphasizes the plants' medicinal value, notably for antibacterial, antioxidant and therapeutic purposes. FTIR spectrum revealed functional groups such as O=C=O, C=C and S=O, which are linked to antioxidant, antibacterial and anticancer properties. The presence of sulfoxide groups indicates potential herbicidal and pesticidal uses. The findings lend credibility to the concept that *T. ulmifolia* can be a renewable resource for medicinal and agricultural purposes. Furthermore, its capacity to create bioactive metabolites under stressful settings highlights its promise in sustainable medicine development and environmental restoration. These findings add to the growing body of evidence that supports the pharmacology of *T. ulmifolia*, laying the groundwork for future research into its medicinal, ecological and industrial applications, such as its role in combating antibiotic resistance and promoting sustainable biofuel production.

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Authors' contributions

MS conducted the preliminary phytochemical and functional group analysis and drafted the manuscript. MP has guided and edited the work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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