Phytochemical analysis & Anti-microbial activity of *Jatropha gossypiifolia*

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ABSTRACT:

Jatropha gossypiifolia is a bushy, gorgeous shrub belonging to the Euphorbiaceous family, which grows practically everywhere in India. It has pesticidal, anti-cancer and anti-inflammatory properties. *Jatropha gossypiifolia* is commonly known as bellyache bush, and black physicnut. It is a medicinal plant used for multipurpose and largely used as folk medicine for the treatment of various diseases and the leaf decoction is used to treat wounds, sap from the stem stops bleeding and itching of cuts & scrapes, bark decoction is used as an emmenagogue, and leaves used as blood purifier. Regarding; to the phytochemical constituents that are present; alkaloids, lignans, phenols, saponins, tannins, were extracted from different part of the plant. In order to determine the potential effects of *Jatropha gossypiifolia* leaf and stem extract on pathogenic microorganisms, the current study was conducted and to detect the activity of compound it can assisted to do FT-IR spectral analysis and LC-MS to detect the chemical which are present in extract.

INTRODUCTION:

Many plants have Ayurvedic, significance and are used in a more primitive manner by indigenous communities in raw form. However, research is still being done to find powerful plants with fewer side effects that may be more effective than the medications already in use. The plants have been shown to be effective by this property in various conditions, including, cancer, memory deficit, Alzheimer's, atherosclerosis, diabetes, and other cardiovascular diseases. Traditional medicines have provided significant leads in the quest for new pharmaceuticals. For example, silymarin has been obtained from plants to protect the liver, artemisinin has been found to cure ''multidrug-resistant'' malaria, and vincristine and vinblastine have been found to treat specific types of cancer. The World Health Organization has been coordinating a network called the International Regulatory cooperation to improve medical products made from medicinal plants.

Infectious disease is the most serious health issue and the leading cause of death in society. The past ten years have seen a rise in the evidence of bacterial and fungal illnesses brought on by population growth, pollution, altered environmental conditions, and waste products from many sources, all of which might compromise foods with optimal nutritional value. Both humans and animals are less immunogenic as a result of this component. This feature, along with the fact that microbes have evolved resistance to allopathic agents and antibiotics, has led to increased toxicity in both humans and animals as a result of extended use of various antimicrobial drugs. These data encourage us to discover a novel herbal component that can cure opportunistic microbial infections by acting as an antibacterial biomolecule. Medicinal plants

continue to be an important therapeutic aid for alleviating the ailments of humankind[4]. The search for perpetual health and prolonged existence for remedies to mitigate pain and discomfort drove early man to explore his immediate natural surroundings and led to the use of many plants, animal products, and minerals, etc. and the development of an array of therapeutic agents. Today, there is a transformed interest in traditional medicine and an increasing demand for more drugs from the plant sources. This resurgence of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. Nature has bestowed upon us a very rich botanical affluence and a large number of diverse types of plants grow wild in different parts of our country. According to estimates from the World Health Organization (WHO), 4 billion people, or 80% of the global population, currently receive primary healthcare through herbal medicine. All indigenous peoples' traditional medicine heavily relies on herbal medicine, which is also a prevalent feature in naturopathic, homeopathic, ayurveda, traditional oriental, and Native American Indian medicine. According to the WHO, around 75% of the 119 pharmacological medications produced from plants are used in contemporary medicine in ways that are directly related to their original native cultural applications as plant medicines. The potential medical usefulness of plant materials collected from rain forests and other locations is currently the subject of intensive research by major pharmaceutical corporations.

Jatropha gossypiifolia

Jatropha gossypiifolia is a bushy, gorgeous shrub belonging to the Euphorbiaceae family, which grows practically everywhere in India. It has pesticidal, anti-cancer and anti-inflammatory properties. *Jatropha gossypiifolia* is commonly known as bellyache bush, and black physicnut. It is a medicinal plant used for multi-purpose and largely used as folk medicine for the treatment of various diseases and the leaf decoction is used to treat wounds, sap from the stem stops `cuts & scrapes, bark decoction is used as an emmenagogue, and leaves used as blood purifier. Regarding; to the phytochemical constituents that are present; alkaloids, lignans, phenols, saponins, tannins, were extracted from different part of the plant. In order to determine the potential effects of Jatropha gossypiifolia leaf and stem extract on pathogenic microorganisms, the current study was conducted.

Description of the plant:

A small deciduous shrub with succulent stem, 1-1.5 m tall. Leaves palmately 3-5 lobed, purple; petiole clothed with numerous stipitate glands. Flowers small, red in terminal corymbose cymes. Fruit a capsule, about 1.3 cm across.

Fig 1: ***Jatropha gossypiifolia***

Scientific classification:

Kingdom	**:**	Plantae
Subkingdom	**:**	Tracheobionta
Super division	**:**	Spermatophyta
Division	**:**	Magnoliophyta
Class	**:**	Magnoliopsida
Subclass	**:**	Rosidae
Order	**:**	Euphorbiales
Family	**:**	Euphorbiaceae
Genus	**:**	*Jatropha*
Species	**:**	*gossypiifolia*.

General Information:

Specific Name: Jatropha gossypiifolia

Vernacular names:

Telugu : Nela amudam

English : Bellyache bush, black physic nut

Hindi : Ratanjot

Synonym: Adenoropium gossypiifolum

PHYTOCHEMISTRY:

The entire jatropha gossypiifolia (Linn) plant is abundant in bioactive substances. Various phytochemical components are present in the plant parts. These substances are different solvent solution that are used to extract a components variety of phytochemicals including, **alkaloids, flavonoids, diterpenoids, tannins, steroids, saponins, and phenolic compounds. carotenoids,** are also abundant in this species. In phytochemical properties, particularly the interaction of compounds, will play a crucial role in biological activities.

Traditional uses:

- Jatropha gossypiifolia is also known as the castor oil plant, has many traditional uses, including
- The seeds can be used as anti-diarrheal agent.
- The leaves and bark have a purgative effect.
- Leaf of this plant shows anti-pyretic activity (reduces fever).
- It can help to use purification of blood.
- The plant has been traditionally used as an oral contraceptive and abortifacient.
- Microbial activity.
- Anti-inflammatory activity.
- Anti-cancer activity.
- Anti-diabetic activity.
- Wound healing activity

Objectives of study:

- From the literature survey done the plant, *Jatropha gossypiifolia,* was found to possess antimicrobial activity which was not yet reported.
- So, the aim of this study is to evaluate the antimicrobial activity of these plants
- Collection and authentication of the plant's aerial parts stem of *Jatropha gossypiifolia.*
- Extraction of plants by using methanol, hydro alcohol, and aqueous.
- Screening of the plant extracts for *in vitro* antimicrobial activity.
- Fractionation of selected potent extracts by column chromatography.
- Analytical characterization of isolated constituents.

Aerial parts stem of *jatropha gossypiifolia*, were collected and extracted with methanol, hydroalcohol, and aqueous the extracts were evaporated using rotary vacuum evaporator and the dried compound was evaluated for the in vitro antimicrobial activities and the potent extract were subjected to isolation. The isolated compounds were further characterized analytically by using IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopic techniques.

Plant Collection, Drying, & Pulverization

Plant collection & Authentication:

Leaves & stem of *jatropha gossypiifolia* were collected from Narasaraopet institute of pharmaceutical sciences college (Garden) Narasaraopet, Palandu, District in the month of November. They were authenticated by Dr. p. Satyanarayana Raju m.sc, M.Phil., Ph.D. department of botany and microbiology Acharya Nagarjuna university, Nagarjuna Nagar,522

510 Guntur Dist. (A.P).

Extraction:

To create aqueous, methanolic, and hydroalcoholic extracts of the stem of *Jatropha gossypiifolia,* the plant components were extracted using water, methanol, and hydro alcohol. Methanol, water, and hydroalcoholic at 60–70 C were used to reflux the ground plant material for an hour. This process was then done four times, each time with a different solvent. After the extract was fine-filtered, concentrated in a rotary water bath, and the solvent was totally eliminated, it was dried with a high vacuum or drier before being gathered in the form of thick paste or dry powder.

Codes of extracts were named as below and in the further discussion the extracts were mentioned as their codes.

1. Jg 01- *jatropha gossypiifolia* stem water extract
2. Jg 02- *jatropha gossypiifolia* stem hydroalcoholic extract
3. Jg 03- *jatropha gossypiifolia* stem methanolic extract

Table 1: Extraction of jatropha gossypiifolia leaves & stem

Extract	Amount Taken (g)	Extract obtained (g)	Solvent used
Jg- 01	100	11.77	Water
Jg- 02	100	12.68	Hydroalcohol
Jg- 03	100	7	Methanol

The obtained extracts of stem of *jatropha gossypiifolia* were tested for anti-microbial activity by using agar cup plate method. Amongst which the methanol extract of *jatropha gossypiifolia* stem extract was found to be potent so these were subjected to fractionation by column chromatography.

Soxhlet Extraction [18]

It is used when the compound is less soluble in the solvent and the impurities are soluble in the solvent. If the desired compound is highly soluble in the solvent the impurities can be removed by simple filtration. The

advantage is that the solvent is recycled in this method and hence there is less wastage of the solvent. Similar to the above method, thermolabile compounds cannot be extracted in this method.

The obtained extracts of stem of *jatropha gossypiifolia* were tested for phytochemical screening to fine the bioactive compounds which are present in jatropha gossypiifolia leaves, and stem.

Initial Phytochemical Examination Analysis of phytochemicals qualitatively

Qualitative Phytochemical Screening of *J. gossypiifolia*

Phytochemicals are chemicals of plant origin. Phytochemicals (from Greek *Phyto*, meaning "plant") are chemicals produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play a role in plant growth or defence against competitors, pathogens.

Phytochemical analysis is the process of identifying, characterizing, and quantifying the chemical compounds in plants. These chemical compounds, known as phytochemicals, can include a variety of bioactive substances such as alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds. The aim of this analysis is to understand the role of these compounds in plants and their potential benefits to human health, including their medicinal properties, antioxidant activities, and their role in preventing diseases. The chemicals that are produced by plants are called as phytochemicals.

S.No.	Test	Presence/Absence
1.	Terpenoid (Salkowski test)	+
2.	Steroids (Salkowski test)	+
2.	Saponin (Foam test)	+
3.	Flavonoid (Ammonia test)	+
4.	Triterpenoid (Liebermann-Burchard test)	+
5.	Tannin ($FeCl_3$ test)	+
6.	Cardiac glycosides (Keller-Killiani test)	+
7.	Reducing sugars (Fehling's test)	+
8.	Proteins (Xanthoproteic test)	+
9.	Starch (Iodine test)	+

+ indicates the presence of the constituent

ANTI-MICROBIAL ACTIVITY

Biological evaluation for Synthesized Compound by in vitro Studies.

Antimicrobial activity

Methods:

Determination of zone inhibition by cup plate method.

Principle:

Cultivation of microorganisms:

The method is based on the diffusion of an antimicrobial agent from a cup into an agar medium that has been Inoculated with a microbial culture. The antimicrobial agent diffuses radially (or) outwards, & the concentration decreases as distance from the well increases.

If the test substance is effective against the microorganism, it inhibits growth of clear zone (zone of inhibition) around the well. [19]

The following microorganisms were used to study the antimicrobial activity:

- Escherichia coli - Gram-negative bacteria
- Standard -> ciprofloxacin
- Solvent -> DSMO

Preparation of media:

Composition of nutrient agar medium:

- Beef extract - 10g (2g)
- Peptone - 10g (2g)
- Sodium chloride - 5g (1g)
- Agar - 20g (4g)
- Distilled water - 1000ml (100ml)
- pH = 7.2 ± 0.2

The medium was prepared by dissolving the specified quantity of dehydrated medium in purified water by heating on a water bath and dispensed in (100ml volume conical) flask. The conical flask was closed with cotton plugs and sterilized by autoclaving at 121°C (15 lbs/sq. in) for 15 minutes. The contents of the conical flask were poured aseptically into sterile Petri dishes and allowed to solidify. This sterilized media was tested to subculture the bacterial culture.

Procedure:

Each Petri dish was filled with a depth of 4-5mm with a nutrient agar medium that was previously inoculated with suitable inoculums of suitable test organisms and allowed to solidify. The Petri dishes were specially selected With a flat bottom and placed on a levelled surface so as to ensure that the layer of medium is in uniform thickness. The Petri dishes were sterilized at 160-170°C in a hot air oven for 30 minutes before use. A small sterile borer of uniform size was placed at approximately 1 cm height, having an internal diameter of approximately 6-8mm and made of aluminium or stainless steel. Each plate was divided into four portions equally along the diameter. To each portion, one cylindrical cavity was made in the medium with the help of a sterile borer. Three cavities for the test compound and one cavity for the standard. The Petri dishes were incubated at 37°C for 9 hours. The diameter of the zone of inhibition was measured, and the average diameter for each sample was calculated.

The diameter obtained by the test sample was compared with that produced by standard drug ciprofloxacin.

Fig 10 Anti-microbial activity

Identification and characterization: [32]

The compounds synthesized were identified and characterized by the following methods such as:

- Melting point determination
- Solubility
- Thin layer chromatography
- LC-MS
- IR- Spectroscopy
- Nuclear magnetic resonance spectroscopy (1HNMR and 13C NMR)

Melting point determination The melting point of an organic compound was determined by Thiele's melting point tube (capillary tube method). The determination of the melting point is the most important and easy way of differentiating this physical constant of one compound from another.

A pure substance melts sharply at definite temperature, while an impure substance will have a lower and indefinite melting point. The apparatus employed for the determination of the melting point of a given solid substance. The crystals are powdered and charged into a capillary tube sealed at one end. The capital tube should be 5 to 6cm.long and 1cm in diameter. The substance should be stand in capillary tube from bottom when thoroughly packed. This capillary remains striking to the thermometer by itself and is so adjusted that the solid in it stand. Just opposite of the idle of the mercury bulb. Thermometer is now lowered in beaker containing paraffin oil. The beaker is heated then the temperature at which solid completely melts is recorded as melting point of compound.

SOLUBILITY

When a mixture of a specified amount of a given solute and a specified amount of a given solvent forms a homogenous liquid, the former is said to be soluble in latter. Different solvents are methanol, DMSO, chloroform, acetone, water etc….

Thin layer chromatography

TLC is an important method for synthetic chemistry to infer the formation of the compound based on the Rr values since different compounds will have different Rr values. The mobile phase used for Scheme I and Scheme 2 was Ethyl Acetate; n-Hexane (2:8).

Liquid chromatography-Mass spectroscopy: [28]

Liquid Chromatography-Mass Spectrometry (LC-MS) is a powerful analytical technique that merges the separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. This technique is widely used in fields such as pharmaceuticals, environmental analysis, food safety, and biotechnology. Liquid chromatography separates the components of a mixture based on their interactions with a stationary phase and a mobile phase, while mass spectrometry measures the mass-to-charge ratio (m/z) of the ions to identify and quantify the compounds based on their unique mass spectra. The strengths of LC-MS include its high sensitivity, selectivity, and accuracy, allowing it to detect very low concentrations of analytes.

Spectral analysis: [27]

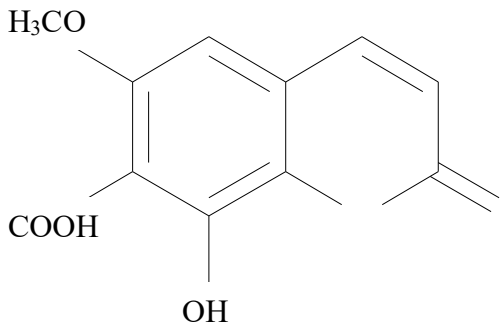
Spectrum analysis is a crucial technique for examining the frequency components of signals across various fields, including audio engineering, telecommunications, and physics. It involves breaking down a time-domain signal into its constituent frequencies using tools like the Fourier Transform, which provides a comprehensive view of the signal's frequency spectrum. The power spectrum, a key output of this analysis, shows the amplitude of different frequency components, allowing for detailed insights into the signal's structure. Spectrum analysers,

devices specifically designed for this purpose, visualize these frequency components, making it easier to interpret and apply the data in practical scenarios. Techniques like windowing are employed to reduce spectral leakage, ensuring more accurate results. Overall, spectrum analysis is a versatile and powerful method for signal examination, enabling advancements in technology and science by providing a deeper understanding of complex signals.

IR spectroscopy:

Infrared (IR) Spectroscopy is an analytical technique used to identify and study chemical substances by measuring their interaction with infrared radiation. When IR light passes through a sample, molecules absorb specific frequencies that correspond to the vibrations of their chemical bonds. The resulting IR spectrum, which is a graph of absorbance or transmittance at different wavelengths, can be analysed to identify functional groups and molecular structures.

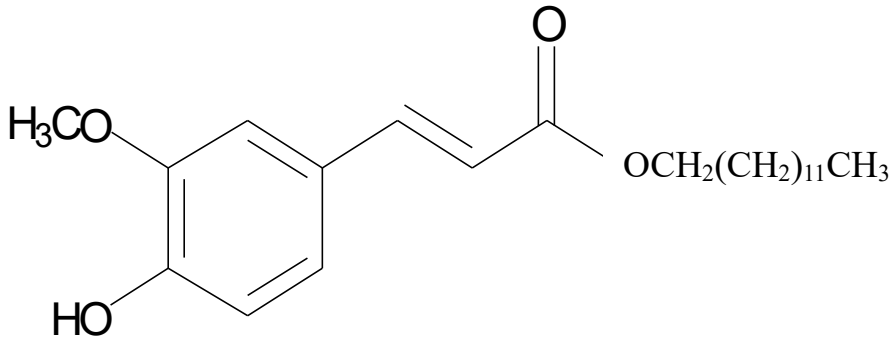
IR Spectrum analysis:



IR Spectrum analysis of fraxetin:

Property	Details
Molecular Formula	$C_{10}H_8O_5$
Molecular Weight	208.17 g/mol
IR Spectrum	-O-H Strech: 3200-3600 $^{cm-1}$
	- C= O Strech:- 1700 $^{cm-1}$
	-C-O Strech: 1000-1300 $^{cm-1}$
	-C=C Strech: -1600 $^{cm-1}$

IR Spectra of tetradecyl (E) ferrulate

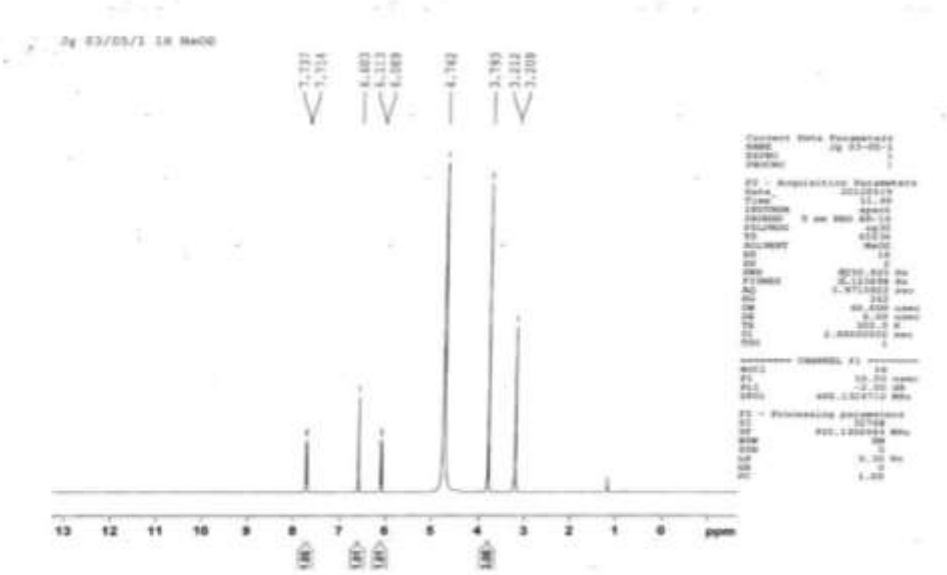


IR analysis of tetradecyl (E) ferrulate:

Property	Details
Molecular Formula	$C_{24}H_{28}$
Molecular Weight	391.57 g/mol
IR Spectrum	
-OH Strech	3427 cm-1
C=O Strech	1736 cm-1
C-H Strech (alkyl)	2924, 2853 cm-1
C=C Strech (aromatic)	1606, 1514 cm-1
C-O Strech	1267, 1171 cm-1
C-O-C Strech (ether)	1110, 1032 cm-1
C-H (aromatic bending)	831 cm-1

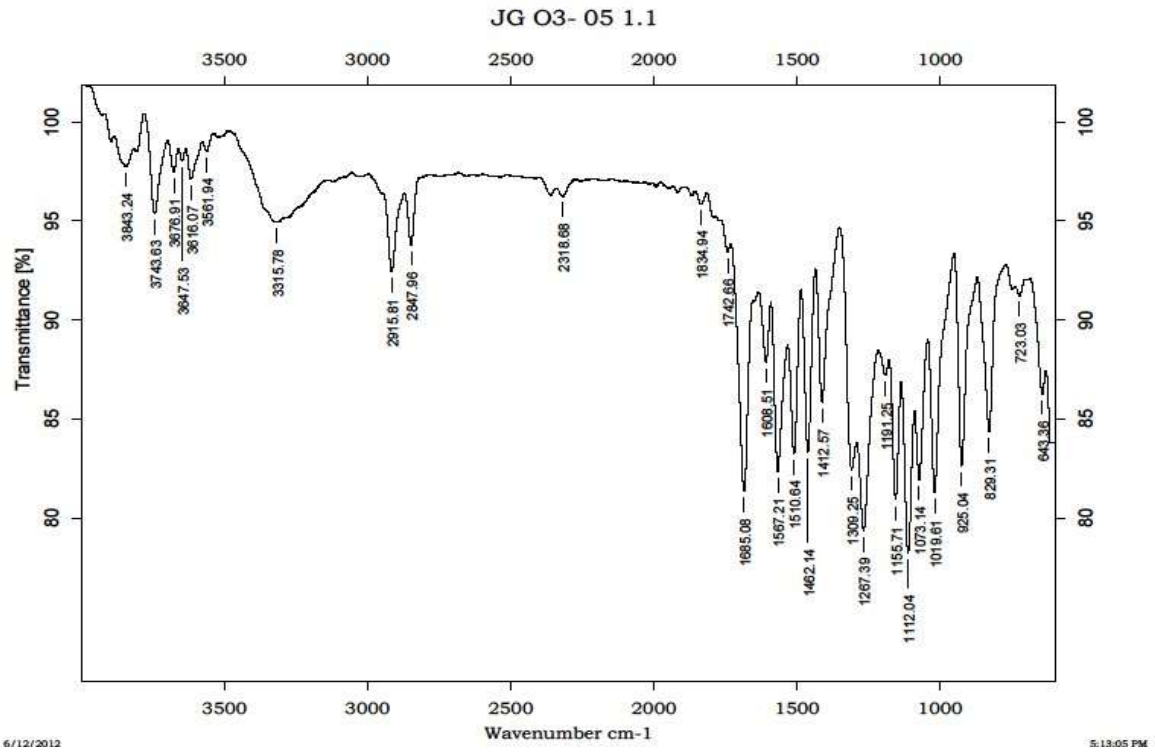
Liquid chromatography mass spectrum analysis of jatropha compounds:

Comments: m/z,413.5 $(M+Na)^+$ Corresponds to **390amu.**
Spectrum recorded in Positive ion mode.

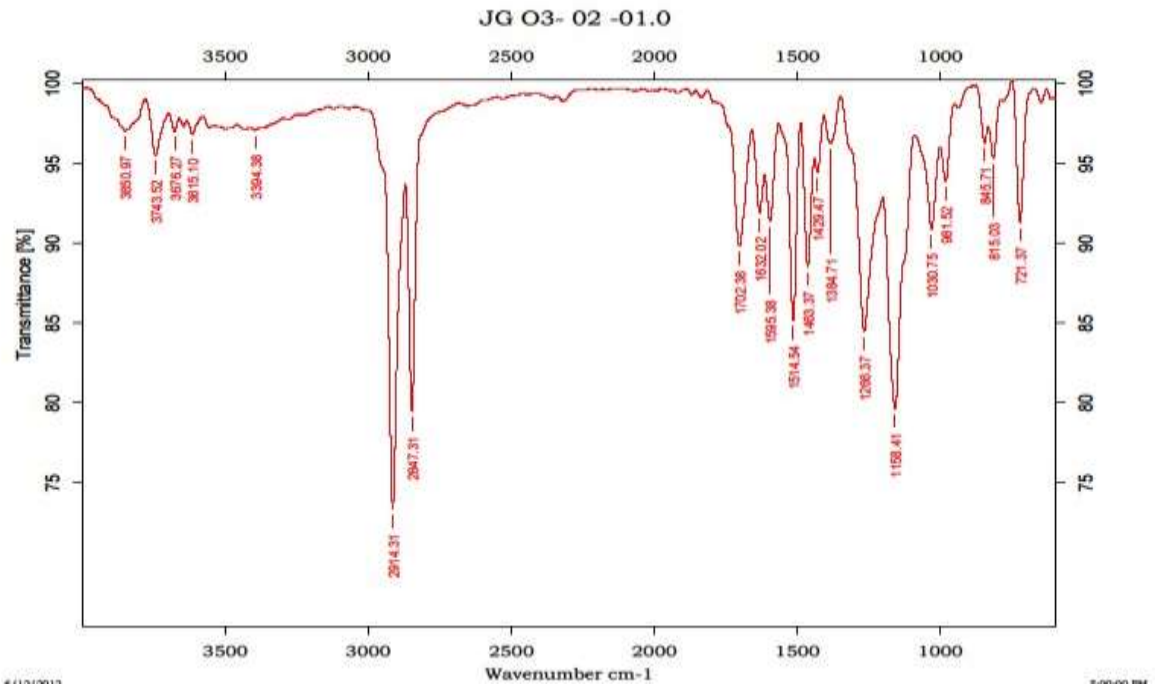


LC-MS of isolated compounds for 2 figures

IR spectrum analysis of fraxetin:



IR Spectrum analysis of tetradecyl (€) ferrulate



Conclusion:

The spectrum analysis of fraxetin, a coumarin compound found in Jatropha gossypiifolia, reveals significant insights into its chemical structure. Key absorption bands observed include a broad O-H stretch around 3200-3600 cm^{-1}, a sharp C=O stretch near 1700 cm^{-1}, C-O stretches between 1000-1300 cm^{-1}, and aromatic C=C stretches around 1600 cm^{-1}. These characteristic peaks confirm the presence of hydroxyl groups, carbonyl

groups, ether or ester linkages, and aromatic rings in fraxetin. This detailed analysis aids in identifying and confirming the structure of fraxetin, contributing to a better understanding of its chemical properties and potential biological activities. The IR spectral analysis of tetradecyl (E) ferulate reveals that the compound has a molecular formula of $C_{24}H_{38}$ and a molecular weight of 391.57 g/mol. The IR spectrum shows a significant peak at 3427 cm^{-1}, indicating the presence of hydroxyl (O-H) groups. The peak at 1736 cm^{-1} suggests the presence of carbonyl (C=O) groups, likely due to ester functionalities. The absorption bands at 2924–2853 cm^{-1} correspond to alkyl (C-H) stretches, confirming the existence of long alkyl chains in the molecule. The peaks at 1606 cm^{-1} and 1514 cm^{-1} indicate aromatic (C=C) stretching, supporting the presence of an aromatic ring. Additionally, the bands at 1267 cm^{-1} and 1171 cm^{-1} suggest C-O stretches, possibly from ester or ether linkages. The C-O-C stretches observed at 1110 cm^{-1} and 1032 cm^{-1} further support the presence of ether groups, while the peak at 831 cm^{-1} suggests aromatic C-H bending. Collectively, these spectral features confirm that tetradecyl (E) ferulate is a complex molecule containing aromatic rings, long alkyl chains, hydroxyl, and ester functionalities.

Isolation and Characterization of Fraxetin from Jatropha gossypiifolia

Considering the extractive value and bactericidal activity, the methanolic extract and hydroalcoholic extract of *Jatropha gossypiifolia* were subjected to fractionation and column chromatography for the isolation of phytoconstituents. From the methanol extract of *Jatropha gossypiifolia,* only the ethyl acetate fraction (Jg compound) was isolated and characterized by FT-IR and LC-MS. Based on the reported data, the isolated compound was identified as fraxetin, which demonstrated significant antibacterial activity upon analysis.

Additionally, the 20% ethyl acetate in hexane fraction from the methanol extract of *Jatropha gossypiifolia* was found to be active. This fraction was further subjected to sub-column chromatography using 80% chloroform in hexane and pure chloroform for isolation. The isolated compounds were characterized using FT-IR, LC-MS, the reported data, the isolated compound Jg/01, 02, 03 was confirmed as fraxetin, stigmasterol and sitosterol, tetradecyl €) ferrulate

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