RESEARCH ARTICLE

Phytochemical Studies and Identification of Bioactive Compounds using High-performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry of Leaf Extract of *Jatropha curcas*

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ABSTRACT

Chronic, recurring, and remitting inflammatory disease with an unknown cause is inflammatory bowel disease (IBD). It is a condition that affects people throughout their lives and has many consequences because it is clinically common. Numerous risk factors are still unknown, and the pathophysiology of IBD is still not fully understood. There are a variety of side effects associated with conventional ulcerative colitis treatment. Globally, there is a rise in the use of herbal therapy for IBD. The medicinal plant *Jatropha curcas (L.)* belongs to the Euphorbiaceae family. It is a biofuel plant with a few pharmacological effects that have been researched. The study's purpose was to discover and investigate the bioactive components in an ethanolic extract of *J. curcas*. The leaves of L. *J. curcas* were gathered, shade dried, crushed up, and gradually extracted with ethanol using the soxhlet percolation method. By using fourier-transform infrared spectroscopy (FTIR) and high-performance liquid chromatography (HPLC), the phytoconstituents in the *Jatropha* leaf extract were confirmed. Along with the interpretation of mass spectra, the isolated chemicals were identified by contrasting their peak areas and retention times. Due to the discovery of possible, bioactive components as a result of the current inquiry, we are now able to investigate biological activity. After that, gas chromatography-mass spectrometry (GC-MS) was used to analyze the crude extracts. A large variety of secondary metabolites were detected in the extracts' profile, which was then spectroscopically described. The presence of phytoconstituents, including flavonoids, tannins, and triterpenoids significantly reduces internal inflammation and lessens damage to the intestinal mucosa. Herbal treatments work through a variety of processes, including immunological control and antioxidant activities.

Keywords: Intestinal Inflammation, Inflammatory bowel disease, *Jatropha curcas*, Mass spectroscopy, Murine model.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a term used to describe a set of conditions that cause the intestines to enlarge. Despite the fact that it is widely thought to be an autoimmune disorder, research suggests that continuous inflammation may not be the result of the immune system attacking the body. When the immune system attacks a harmless virus, bacterium, or food in the digestive tract, inflammation occurs, which leads to intestinal injury. The two most common IBD subtypes are ulcerative colitis (UC) and Crohn's disease (CD). Ulcerative colitis affects just the large intestine or colon. Crohn's disease, on the other hand, can affect the mouth and the anus as well as any other portion of the gastrointestinal tract. The interplay of environmental and genetic components that regulate immune responses promotes IBD.

Both CD and UC are chronic IBD disorders that cause gastrointestinal inflammation and dyspepsia. Weight loss, rectal bleeding, diarrhea, and stomach pain are some of the signs and markers of CD and UC. Their key characteristic is inflammation. Both disorders can affect men and women equally, as well as teenagers and adults.[1]

CD is an inflammatory disease that affects adults aged 15 to 35. IBDs were more difficult to treat than other inflammatory illnesses. The immune system is triggered, and a section of the gut is destroyed. Other symptoms and signs, include fever, diarrhea, and discomfort. In addition to the bottom region of the small intestine, CD can affect the large intestine, stomach, esophagus, and even the mouth.[2,3]

In severe cases, CD can produce bleeding, but UC causes blood in the stool, agonizing pain, and diarrhea. Rectal

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bleeding is more commonly connected with UC than CD, where it happens less frequently. More than half of UC patients are iron deficient, while more than half of CD patients are folate and vitamin D deficient.[4]

These conditions have an impact on a number of digestive tract components. CD, for example, has an effect on the ileum and a portion of the large intestine. Any gastrointestinal (GI) organ, including the mouth, esophagus, stomach, small intestine, rectum, and anus, could be affected. Unlike CD, which typically causes minor intestinal inflammation, UC is only present in the colon and is most commonly observed in the large intestine's rectum. In UC, the small intestine continues to operate properly while the large intestine becomes inflamed.[5]

Causes of the Disease

Even though the fundamental etiology of IBD is still unknown, numerous research in this domain emphasize the importance of genetic and environmental factors. Heymen *et al.* proposed the following two approaches to treating the underlying causes of IBD: When the mucous system is disturbed, the human microbiota's immune response rate increases.[6] The pathologic reaction in the healthy mucous system is caused by any change in gut flora or disturbance of epithelial function. On the contrast, Podolsky pointed out that a number of factors, including the patient's vulnerability, mucosal immunology, and gut flora, play a role.[7]

Despite numerous attempts, no knowledge of the bacteria that cause the onset of IBD has been achieved. Persons who are sick have different microbial flora than healthy people. Extraintestinal symptoms may include inflammatory reactions in the skin, joints, eyes, and hepatobiliary system. More recently documented involvements include thromboembolic events, osteopenia, and osteoporosis.[10]

The incidence of UC is calculated to be 1 to 25 per 1,00,000 per year and the disease affects people of all ages. The onset of disease in the first decade of life is unusual but there is a steep increase in incidence during puberty and the following adolescence and young adulthood. The etiology of the disease is yet to be revealed but the pathogenesis is believed to be multifactorial. Immunosuppressive medications, 5-amino salicylic acid, such corticosteroids and 6-mercaptopurine, and azathioprine are used to treat inflammatory bowel disease. Long-term glucocorticoid use is linked to unacceptably high rates of relapse and harmful effects.

However, azathioprine, a prodrug of 6-mercaptopurine, is effective in sustaining remission. There are fewer negative effects associated with biological drugs, including interferons, monoclonal antibodies, and other compounds produced by living creatures. Among the treatments for UC is infliximab. TNF-alpha is bound by the specific antibody in question (TNF-a). TNF-a is one of the immune cells' inflammatory protein messengers.

These medications do have adverse effects, though, and are pricey. As a result, there is a need for substitute agents that can be equally as effective or even more so, as well as being more affordable. These assertions and actions encouraged us to investigate the effectiveness of these herbs in protecting rodents from experimentally induced colitis.

Uses of Jatropha

Jatropha as an oil source *Jatropha curcas* has 35–40% of its seed oil content. The 21% of the oil's fatty acids are saturated, while 79% are unsaturated.[11] The seeds contain various chemical components that are lethal and toxic. This oil should not be consumed by anyone. Oil is a clean, renewable energy source. It is an effective substitute for furnace oil, diesel, kerosene, liquid petroleum gas (LPG), coal, and fuel wood. It is also used in the resin, polish, and candle industries[12] a supply of traditional medicine has utilized *Jatropha* to treat a number of conditions, such as arthritis, gout, and jaundice. pyorrhea, bleeding gums, and toothaches, human immunodeficiency virus (HIV), tumors, and dermato-mucosal conditions (wart), leprosy, leucoderma, scabies, smallpox, allergies, inflammation, hemostatic, burns, cuts, and wounds.[13,14]

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh mature, healthy leaves were collected from fully grown *J. curcas* Linn. Plant collected from nainarmandapam, Puducherry authenticated by French institute of Puducherry.

Preparation of Ethanolic Extract[15]

The air-dried leaves in the shade were pulverized into a fine powder using a mixer grinder. Using a soxhlet extraction equipment, 30 g of powdered material were extracted with 600 mL of ethanol and water (continuous hot percolation). Analytical grade ethanol was used for the extraction. The divided extracts were filtered through Whatman's no. 1 filter paper, and the aqueous and ethanol filtrates were separately concentrated to dryness with a rotary evaporator to remove the water and ethanol. The sticky, greenish-brown components were removed and chilled before use.[16]

Preliminary Phytochemical Screening[15,17]

Qualitatively, coumarins, flavonoids, phenolics, saponins, sterols, tannins, and/or triterpenes were discovered in powdered *J. curcas* leaves. These approaches allowed researchers to determine the presence or absence of phytochemicals depending on the color's strength or the precipitate's creation.

Fourier Transform Infrared Spectrometer (FTIR)

The existence of functional groups in the ethanolic leaf extract of *J. curcas* was confirmed by the results of FTIR spectra.

Gas chromatography/mass Spectrometry Analysis

The elite-5MS packed fused silica column (5% biphenyl, 95% dimethylpolysiloxane, 30 m, 0.25 mm ID, 250 m df) was analyzed using the Clarus 680 GC. Helium was employed as the carrier gas to separate the components, flowing at a constant rate of 1 mL/min. For the chromatographic run, the injector temperature was set to 260°C. The oven temperature was 1-L of sample extract was placed in the equipment: after sustaining a temperature of 60°C for two minutes, the temperature was

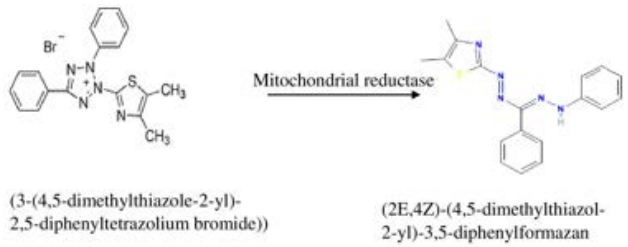


Figure 1: Mechanism of MTT assay

escalated to 300°C in six minutes at a rate of 10°C per minute. The mass detector was programmed to operate at 240°C for the ion source and transfer line, 70 eV for the electron impact in ionization mode, 0.2 seconds for each scan, and 0.1 seconds between scans, with bits ranging from 40 to 600 Da. The component spectrum was compared to a database of component spectrums from the GCMS-NIST library (2008).

Cytotoxicity Assay (Raw264.7 Cell line)[19]

To analyse the effect of various drugs on cell viability, the MTT test was utilised. After the CaCo-2 cells and murine RAW 264.7 macrophages had been seeded for 24 hours, fresh DMEM + 0.5% FBS was added to the culture. The cells were then exposed to substances at the measured maximum concentration for 24 hours in a 96-well microplate at 37°C (25, 50, or 100 M). Following the removal of the medium, 100 L of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide solution containing 0.5 mg/mL was applied to each well. The resulting liquid was then heated to 37°C to produce blue deposits. Dimethyl sulfoxide (DMSO) was then used to dissolve the coloured metabolite. Absorbance was determined at 490 nm using a Multiskan EX plate reader from Lab systems (Helsinki, Finland). Absolute absorbance measurements were used to express the findings; a decline in absorbance indicated a decline in cell viability Figure 1.[18]

The formula: % viability = Sample abs/Control abs x 100, was used to determine the percentage of viability.

RESULTS AND DISCUSSION

Phytochemical Screening

The *Jatropha* curcas leaf extract was subjected to preliminary chemical tests, it indicates the presence of some phytochemicals listed below.

As a result, it reveals the presence of alkaloids, triterpenoids, steroids, tannins, saponins, lipids, and oil components as shown in Table 1, which are responsible for its anti-inflammatory properties.

FTIR Analysis

FTIR analysis confirms that the presence of alkane, alkene, alcohol, and nitro, fluro and amine functional group present in the leaf extract of *J. curcas* as shown in Figure 2 and Table 2.

GC-MS

The chromatogram of ethanolic extract of leaves of *J. curcas* confirms the presence of various compounds with different retention times.

Table 1: Phytochemical screening

Sl.no	*Phytochemical test*	*Results*
1	Alkaloids	+
2	Glycosides	-
3	Proteins	-
4	Amino acid	-
5	Fats and oil	+
6	Triterpenoids	+
7	Steroids	+
8	Carbohydrates	-
9	Tannins	+
10	Saponins	+

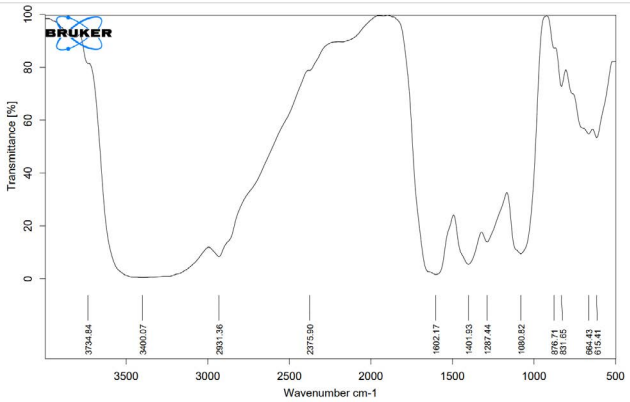


Figure 2: FTIR Reports of Ethanolic Extract of leaves of *J. curcas*.

The GC-MS chromatogram of ethanolic extract of leaves of *J. curcas* shows 7 peaks indicating 7 compounds in figure 3.In that major peak was 18.41.

Their retention time, molecular formula, area percentage are presented in Table 3. All compounds detected as 18.21 to 30.00 minutes. Eicosonaic acid observed at the retention time of 18.410 minutes, small peaks of the chromatogram was Tridecanoic acid,oleic acid,11-Tridecen-1-ol the retention time of 20.135, 20.340 (Figure 4).

Cytotoxicity Assay (Raw264.7 Cell Line).

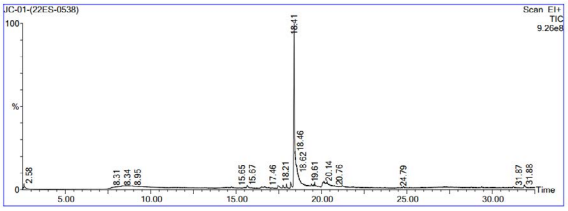
To ensure that the cells were healthy before the bioactivity studies, cell viability was measured after treatment with various extract concentrations (6.25, 12.5, 25, 50, 100 g). dosages of *J. curcas* leaf extract that resulted in cell viability The IC_{50} value of the extract was calculated to be 94.12 gas shown in Figure 5.

DISCUSSION

Due to advancements in efficacy and therapeutic value, as well as concerns about potential side effects from current medical therapy, complementary and alternative medicine, particularly with herbs and medicinal plants, is one of the best remedies for a wide range of ailments. The aroma and colour of a leaf extract from the *J. curcas* plant are remarkable. Preliminary phytochemical screening studies on *J. curcas* revealed several chemicals, including alkaloids, favonoids, saponins, fatty

Table 2: FTIR analysis for ethanolic extract of leaves of *J. curcas*

S.no	*Peak (wave no in cm^{-1})*	*Vibrational assignment*	*Functional group*
1	3734.84	OH Stretching	alcohol
2	2931.36	CH Stretching	alkane
3	2375.90	O=C=O stretching	CO_2
4	1602.17	N-O Stretching	Nitro compound
5	1401.93	C-F Stretching	Fluro compound
6	1287.44	C-N Stretching	aromatic amine
7	1080.82	C-O Stretching	primary amine
8	831.65	C=C bending	alkene
9	664.43	-C-Br Stretching	Halo compound



Y axis Area percentage; X axis Time

Figure 3: Gas Chromatography Mass Spectroscopy Chromatogram of Ethanolic extract of Leaves of *J. curcas*.

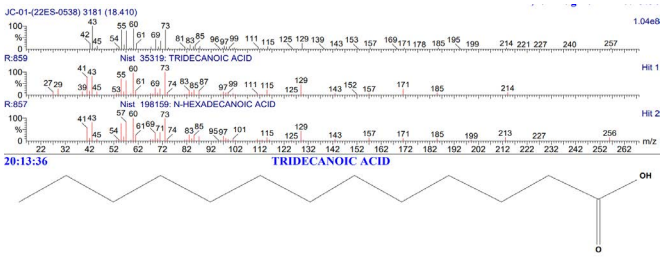


Figure 4A: Tridecanoic acid peak

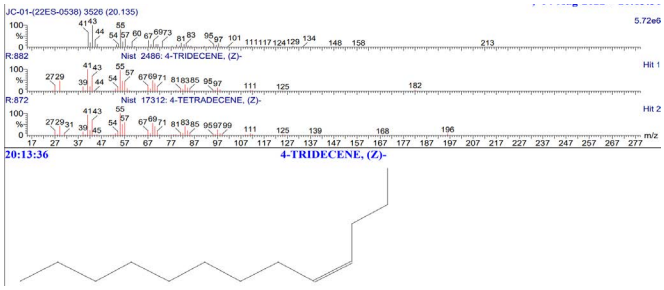


Figure 4B: Tridecene, (z) Peak

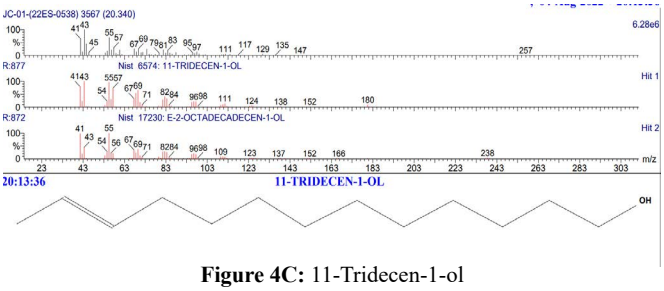
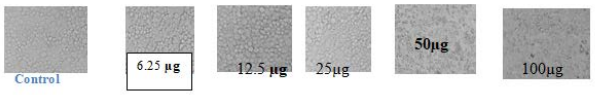
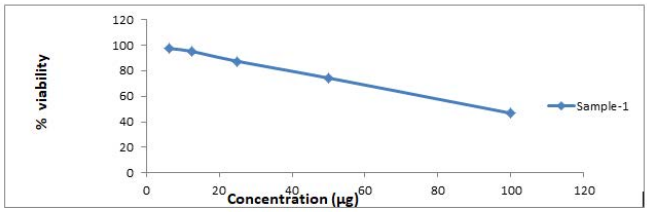


Figure 4C: 11-Tridecen-1-ol

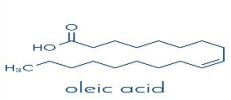




Sample 1	% of Viability
6.25	97.62290654
12.5	95.13776337
25	87.46623447
50	74.28417072
100	46.73149649

Figure 5: Cell viability assay

Table 3: Compounds identified in Ethanolic extract of Leaves of Jatropha curcas.

RT (min)	*Area %*	*Molecular formula*	*Name of compound*	*MW*	*Structure*
18.410	86.851	C7H6O5	Eicosonoic acid	312	
20.135	6.510	C15H14O6	Tridecanoic acid	290.27	
20.340	6.639	C27H30O16	Oleic acid	282 198	
		C13H26O	11-Tridecen -1-OL	198	

acids, tannins, and triterpenoids, the majority of which are thought to be useful in inflammation-related illnesses. *J. curcas* fared well in preliminary phytochemical screening tests for a range of phytochemicals such as flavonoids, saponins, and terpenoids, all of which have been demonstrated to have a significant impact in the treatment of a variety of ailments.[20]

In our investigation, ethanolic extract of *J. curcas* contained 3 distinct peaks identifying several chemical components. As shown in Figure. 3 and 4, the peaks for eicosonoic acid, tridecanoic acid, oleic acid, and 11-tidecan 1-ol were the most noticeable, with n-hexadecanoic acid and eicosonoic acid being the highest peaks. Numerous biological features can be found in the identified bioactive substances. Biological activity of identified chemicals at different levels. *J. curcas* has been researched so far for its ability to reduce inflammation by enhancing gut microbiota-mediated dysfunction of high levels of circulating branched-chain amino acids,[33,34] cytotoxicity against RAW 264.7 Cell Lines, oxidative stress,[37] and antimicrobials bioactivity reported for this extract.

The FTIR reports show 3734.84 – OH streching alcohol, 1602.17 N-O stretching nitro compound,1287.44 reprents C-N stretching aromatic amine (Table 2).

The free fatty acid group occupied the majority of the area, according to GC-MS data, with retention time 18.410 arachidonic acid having the biggest peak and area%. As a consequence of our research, we observed that this extract contains considerable bioactive components such as straight-chain saturated fatty acid, fatty acid, and furan. *J. curcas* ethanol extract had a significant effect on the RAW 264.7 cell line.[31] The IC_{50} value of the extract was found to be 94.12 µg. The phytoconstituents present in the extract exhibits a potent inhibitory activity against IL-6 *in-vitro* cell lines. RAW 246.7 cells can be used for immune-related investigations.

CONCLUSION

The results of this study's FTIR and GC/MS analysis indicate that *J. curcas* leaf extracts include a variety of phytochemical components and their functional groups, many of which are employed therapeutically in folkloric medicine. However, more research is required to identify the crude extract, define it, and determine the probable mechanism(s) by which it may be able to treat UC. Because of the significant potential of plant extract to treat a wide range of illnesses and disorders, pharmacokinetic activity has to be investigated. Additionally, the potential potentiation of action of identified drugs when docked with UC receptors might be investigated.

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