In Vitro Anti-Inflammatory Activity, Phytochemical and FT-IR Analysis of the Leaf Extract of Euphorbia Hirta

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Abstract
Researchers are paying close attention to medicinal plants in the present day because the majority of the pharmaceutical industry rely on them for the creation of therapeutic chemicals. Plants are the traditional source of therapeutic biochemicals, food colours, flavours, and scents in many nations, particularly India. Therefore, the study's primary goal was to identify bioactive chemicals from the leaves of Euphorbia hirta using phytochemical analysis and FT-IR analysis, and to assess its anti-inflammatory activity. The different phytocomponents present in the Euphorbia hirta extracts shows better anti-inflammatory activity.
Keywords: Anti-inflammatory, Euphorbia hirta, FT-IR, Phytochemical, Therapeutic.

Introduction

The usage of medicinal plants is spreading over the globe as an alternative treatment for a number of ailments. Various sciences, such as medical herbalism, have developed as a result of the abundance of plant species with therapeutic characteristics, enabling the widespread development of traditional medicine in some nations. Depending on where the plant is ingested and the social groups that use it, each medicinal plant has traditional applications [1]. Overuse of natural resources for food, medicine, and other uses is a major contributor to rapid population expansion, increased grazing of livestock, and aridity of the environment in many emerging nations [2]. The majority of people in nations that lack access to western medicine for the rural population or where it is prohibitively expensive and poorly accepted by the community rely on traditional herbal medicines. The added factor that most hospitals and health centres in these nations are dispersed far from the rural population, and the communication infrastructure is subpar, has contributed to the continued reliance on traditional herbal therapy. On the other hand, the community accepts and is able to afford herbal medications. Therefore, traditional medicine—in which traditional herbal medicine plays a very important role—is used by the vast majority of people as their primary healthcare systems [3-5].
With around 1600 species, Euphorbia is the biggest genus in the Euphorbiaceae family. It is distinguished by the presence of a more or less poisonous white milky latex. Rubber may be found in the latices of the eucalyptus species E. ingens, E. mey, E. tirucalli, and E. triangularis. Flavanoids, triterpenoids, alkanes, amino acids, and alkaloids are just a few of the unique substances that have been isolated from this category of plants [6]. There are numerous other Euphorbia species that are utilised in conventional medicine. When broken, all species of Euphorbia release a milky fluid that is somewhat deadly and is a component of arrow poisons. Euphorbia hirta has qualities that are antifungal, antibacterial, anthelmintic, anti-asthmatic, sedative, antispasmodic, antifertility, and anti-asthmatic [7-9]. Euphorbia hirta is a member of the plant genus Euphorbia and family Euphorbiaceae. It is a reddish or purplish, annual, hairy plant with a thin stem and several branches growing from the base to the top. The opposite, elliptic-oblong to oblong-lanceolate, acute or subacute, dark green above, pale below, 1-2 cm long, purple-blotched in the middle, and toothed at the edge leaves have these characteristics. The fruits are 1-2 mm in diameter, yellow, three-celled, hairy, keeled capsules with three brown, angular, four-sided, wrinkled seeds inside [6]. A vascularized living tissue's response to a local insult is inflammation. We started looking for novel anti-inflammatory medications from indigenous sources because of the terrible adverse effects of steroidal and non-steroidal anti-inflammatory drugs. The current study's objective is to assess the anti-inflammatory activity of leaf extracts from Euphorbia hirta and its phytochemical analysis.

Materials and methods

Sample Collection and Extraction

In December of the year 2021, fresh leaves of Euphorbia hirta were acquired from several locations in the Kanyakumari District. The leaves were cleaned, let to dry in the shade, and then used a home mixer grinder machine to grind them into a fine powder. For further processing and investigation, the resulting fine powder of the plant leaves was kept in an airtight container. Euphorbia hirta (leaves) that had been ground up were put into a flask with a circular bottom and covered with 95% ethanol. After being stopped, the flasks were permitted to stand for

doi.org/10.31838/hiv23.03.99 Received: 03.10.22, Revised: 15.11.22, Accepted: 10.12.22.

24 hours. Whatman No.1 filter paper was then used to filter the extract. The extraction solvent was completely evaporated at 80°C using moderate heat, concentrating the filtrate to a powdery state. Dried Euphorbia hirta powder weighing 50 gm was dissolved in ethanol over 24 hours. Excess solvent was decanted and saved for drying after incubation. The dried samples were preserved for Soxhlet extraction and wrapped in muslin cloth. The separated solute was put into a centrifuge tube and used for additional analysis.

Qualitative phytochemical Analysis

First, a tiny amount of the sample of the 95% ethanol-extracted ethanolic leaf extract of Euphorbia hirta was reconstituted in the solvent. The chemical makeup of the Euphorbia hirta extract was then examined using various qualitative chemical tests.

Detection of Alkaloids

A few ml of diluted hydrochloric acid are mixed with 50 mg of solvent-free extract before filtering. Mayer's reagent is used to analyse the filtrate. A drop or two of Mayer's reagent were applied along the test tube's sides to a few ml of filtrate. The test is considered positive when a precipitate of white or creamy colour forms.

Detection of carbohydrates

The 100 g of the extract are dissolved in 5 ml of water, then filtered. The Molish's test is performed on the filtrate. Formation of a violet ring indicates the presence of carbohydrates.

Detection of phenols

Using distilled water, the extract (50 mg) was dissolved in 5 ml. A few drops of a neutral 5% ferric chloride solution were then added to this. If a dark green colour develops, phenolic chemicals are present.

Detection of Tannins

The 20 ml of distilled water were used to dissolve the extract (1 g), which was then filtered. Two millilitres of the filtrate were mixed with three drops of a 10% FeCl3 solution. The presence of tannins was indicated by the emergence of a blackish-blue or blackish-green coloration. The production of a precipitate after adding 2 ml of the filtrate to 1 ml of bromine water was also considered to be a sign that tannins were present.

Detection of Flavonoids

The 1 ml of 5% lead acetate was added to 1 ml of plant extract, and the combination was then given some time to stand. The presence of precipitate in the sample suggests that flavonoids are present in the extract.

Detection of Saponins

The extract (50 mg) was prepared up to 20 ml by diluting it with distilled water. For 15 minutes, the suspension was shaken in a graded cylinder. Saponins are present when a 2 cm layer of foam forms on a surface.

In vitro Anti-inflammatory activity

Lymphocyte Culture Preparation

HPLs were grown in RPMI 1640 medium with 20% heat-inactivated FBS and antibiotics as supplements (Penicillin and Streptomycin). The stimulator for cell proliferation was PHA. The culture was filtered under strictly aseptic conditions using a Sartorius cellulose acetate filter with 0.2 m pore size. 1 x 106 cells/ml of fresh plasma were aseptically added to the culture. The 72 hours were subsequently spent incubating the culture. The culture was stimulated after 24 hours by adding 1 l of lipopolysaccharide (LPS). The incubation period was 24 hours. The Euphorbia hirta leaf extract was applied at a standard concentration of 500 g/ml and incubated for 24 hours. Isolation was accomplished by spinning at 6000 rpm for 10 minutes. The supernatant was removed, and 50 l of cell lysis buffer was added before centrifugation at 6000 rpm for 10 minutes. The supernatant was removed, and the anti-inflammatory assay was performed on the pellet suspended in a small amount of supernatant.

Cyclo-oxygenase Assay

The reagents used for the assay of cyclo-oxygenase are 100 Mm Tris HCl (pH 8), 5 mM GSH, 5 µM Haemoglobin, 200 µm arachidonic acid, 10% TCA in HCl, and 1% Thiobarbituric acid (TBA). The assay mixture contained Tris- HCl buffer, glutathione, haemoglobin and 50 µl of Lymphocyte culture. The reaction was started by the addition of arachidonic acid and terminated after 20 min incubation at 37°C by the addition of 0.2 ml of 10% TCA in 1N HCl, mixed and 0.2 ml of TBA was added. The contents were heated in a boiling water bath for 20 min, cooled and centrifuged at 1000rpm for 3 min. The absorbance of the supernatant was measured at 632 nm for COX activity.

Lipoxygenase Assay

The reagents used for the assay of lipoxygenase are 0.2 M sodium phosphate buffer (pH 6.1), 50 mM tris HCL buffer (pH 7.4), and Sodium linoleate 10 mM. The 70 mg of linoleic acid and equal weight of tween 20 was dissolved in 4 ml of oxygen free water and mixed back and forth with a pipette avoiding the formation of air bubbles. Sufficient amount of 0.5 N NaOH was added to yield a clear solution and then made up to 25 ml using oxygen free water. This was divided into 0.5 ml portions and flushed with nitrogen gas before closing and kept frozen until needed. The reaction was carried out in a quartz cuvette at 25°C with 1 cm light path. The assay mixture contained 2.75 ml tris buffer of pH 7.4, 0.2 ml of sodium linoleate and 50 µl of the Lymphocyte culture. The increase in OD was measured at 234 nm.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The term Fourier Transform Infrared Spectroscopy (FTIR) refers to a fairly recent development in the

manner in which the data is collected and converted from an interference pattern to a spectrum. Today's FTIR instruments are computerized which makes them faster and more sensitive than the older dispersive instruments. FTIR is perhaps the most powerful tool for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of extracts of plant materials was considered for instrumental analysis. For the FTIR study dried powder of different extracts 10 mg of plant material was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of plant specimens was treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). Scan range: from 400 to 4000 cm-1 with a resolution of 4 cm-1.

Results and Discussion

Qualitative phytochemical screening of Euphorbia hirta

Several qualitative phytochemical screening tests were carried out in the ethanolic leaf extracts of Euphorbia hirta. As reported in Table 1, the results confirmed the presence of phenols, alkaloids, tannins, and flavonoids while excluding carbohydrates and saponins.

The current study is consistent with the findings of Kala et al., 2011, who discovered that Euphorbia hirta extracts contain phytoconstituents such as alkaloids, saponins, tannins, phenols, coumarins, flavonoids, aminoacids, and sugars [10]. Mahmood (2009) discovered tannins, saponins, phenols, flavonoids, cardiac glycosides, anthroquinones, and alkaloids in Euphorbia hirta solvent extracts [11]. The findings of alkaloids, flavonoids, tannins, steroids, glycosides, and carbohydrates in various solvent extracts of Euphorbia hirta [12].

Table 1: Qualitative phytochemical screening of Euphorbia hirta extract

S. No	Parameters	Result
1	Carbohydrate	Absent
2	Phenol	Present
3	Alkaloid	Present
4	Tannin	Present
5	Flavonoid	Present
6	Saponin	Absent

Invitro anti-inflammatory activity using the Euphorbia hirta leaf extracts

It was performed by using cyclo-oxygenase and Lipo-oxygenase assay. From the results shown in the Tables 2 and Table 3 it can be observed that the leaf extracts Euphorbia hirta produced significant decrease in Cox activity (35 ± 1.23) and in Lox activity (29 ± 1.23). The rate of inhibition of inflammation is calculated.

Table 2: In vitro anti-inflammatory activity using the Euphorbia hirta leaf extracts (COX assay)

S. No	Samples	OD (632 nm)
1.	Control + LPS	0.632
2.	5 µl	0.407
3.	25 µl	0.514
4.	50 µl	0.520
5.	100 µl	0.604

Table 3: In vitro anti-inflammatory activity using the Euphorbia hirta leaf extracts (LOX assay)

S. No	Samples	OD (632 nm)
1.	Control + LPS	0.221
2.	5 µl	0.156
3.	25 µl	0.168
4.	50 µl	0.156
5.	100 µl	0.201

Due to Euphorbia hirta leaf extracts' potent anti-inflammatory properties, both assays showed a considerable decline. As a result, it is possible to hypothesise that the extraction and identification of a specific bioactive component from leaf extracts may result in the creation of very effective anti-inflammatory therapeutics. In order to use plants as natural anti-inflammatory medicines, it is therefore important to use a systematic strategy to determine their effectiveness against inflammation [13].

The inflammatory mediators' prostaglandins and leukotrienes, which by drawing polymorphonuclear leucocytes to the site of inflammation, would cause tissue damage most likely via the production of free radicals, are known to be produced by the enzyme phospholipase A2.

COX isoenzymes are the first step in the biosynthesis of prostaglandins (PGs). COX-1, a constitutively expressed enzyme in a variety of cell types, is thought to produce PGs primarily for physiological purposes. COX-2, an inducible isoform in inflammatory cells, produces PGs primarily for inflammation, fever, and pain [14].

FTIR Spectroscopy analysis

In Euphorbia hirta leaves, IR spectrum shows strong absorption peaks at 3452.34, 1643.24, 1481.23, 1429.15, 1170.71, 1031.85, 786.90, 721.33, 642.25 and 545.82 cm-1. In which 3452.34 cm-1 correspond to alcohol group; 1643.24 cm-1 corresponds to amide group; 1481.23 and 1429.15 cm-1 corresponds to aromatic group; 1170.71 cm-1 and 1031.85 cm-1 corresponds to ester group; 786.90, 721.33, 642.25 and 545.82 cm-1 corresponds to alkyl

halide group. FTIR is mainly done to find out the functional group present in the compounds. FTIR of Euphorbia hirta leaves contain different compounds with their functional group having different activity. As the present work is focused on anti-inflammatory activity, we are considering the compound which found to have anti-inflammatory activity. The presence of alcohol group in Euphorbia hirta reveals that the compound may be β-amyrin, β-sitosterol, 24-methylene cycloartenol, Euphorbin E, taraxerone, taraxerol, stigmasterol and campesterol. The presence of amide group in Euphorbia hirta reveals that the compound may be Euphorbin A, Euphorbin B, gallic acid, quericitrin, myricitrin and quericitol. The presence of ester group in Euphorbia hirta reveals that the compound may be 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucose, 2, 4, 6-tri-O-galloyl-D-glucose and β-amyrin acetate. The FTIR analysis is shown in the table 4 and Figure 1.

Table 4: FTIR Spectroscopy analysis of Euphorbia hirta leaf extracts

S.No	Peak	Characteristic absorptions (cm-1)	Intensity	Possible functional group
1.	3452.34	3200-3600	Strong, broad	O-H (Alcohol)
2.	1643.24	1640-1690	Strong	C=O (Amide)
3.	1481.23	1400-1600	Medium-weak, multiple bonds	C=C (Aromatic)
4.	1429.15	1400-1600	Medium-weak, multiple bonds	C=C (Aromatic)
5.	1170.71	1000-1300	Strong	C-O (Ester)
6.	1031.85	1000-1300	Two bands or more	C-O (Ester)
7.	786.90	600-800	Strong	C-Cl (Alkyl Halide)
8.	721.33	600-800	Strong	C-Cl (Alkyl Halide)
9.	642.25	600-800	Strong	C-Cl (Alkyl Halide)
10.	545.82	500-600	Strong	C-Br (Alkyl Halide)

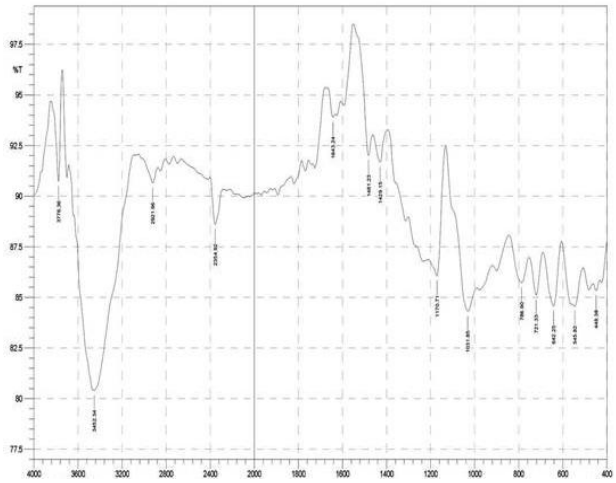

Figure 1: FTIR Spectrum of Euphorbia hirta Leaf Extracts

The current work is correlated with the findings of Shalini and Prema, 2012 showed the FTIR analysis in different plants. In Annona leaf, IR-spectrum shows strong absorption peaks at 3511, 2925, 2859, 2738 and 1075 cm-1, which corresponds to hydroxyl, methoxy, alkyl, carboxylic and glycosidic groups. In Annona seed, IR- Spectrum shows strong absorption peaks at 3417, 3409, 3398, 2925, 2862, 1749, 1646, 1452, 1318 and 1071 cm-1, which corresponds to alcohols, carboxylic acids, alkanes, C=O, alkenes, aromatics, alkanes, amide III compounds of proteins and aliphatic amine groups [15].

Conclusion

Through qualitative phytochemical screening procedures, secondary metabolites were checked for in Euphorbia hirta extracts. According to the screening results, phenols, alkaloids, tannins, and flavonoids were present. Using ethanol extracts of Euphorbia hirta leaves, the anti-inflammatory efficacy of the plant extracts was assessed in vitro using the Cyclooxygenase and Lipoxygenase test. 100µl and 50 µl of the studied extracts had the highest rates of inhibition in both experiments. In the Cox test and Lox assay, their maximum rate of inhibition was calculated to be 100 µl. The FTIR analysis of Euphorbia hirta leaves was done; IR spectrum shows strong absorption peaks at 3452.34, 1643.24, 1481.23, 1429.15, 1170.71, 1031.85, 786.90, 721.33, 642.25 and 545.82 cm-1. In which 3452.34 cm-1 correspond to alcohol group; 1643.24 cm-1 corresponds to amide group; 1481.23 and 1429.15 cm-1 corresponds to aromatic group; 1170.71 cm-1 and 1031.85 cm-1 corresponds to ester group; 786.90, 721.33, 642.25 and 545.82 cm-1 corresponds to alkyl halide group. In vitro experiments showed that Euphorbia hirta extracts had potent dose-dependent anti-inflammatory action that was comparable to that of the common medication indomethacin. Since serotonin, histamine, and prostaglandins are the main mediators of inflammation, Euphorbia hirta anti-inflammatory impact may be brought on by its ability to prevent either their formation or the creation of prostaglandins during the third stage of inflammation. Triterpenes and flavonoids may be responsible for some of their action. However, other substances may also have a role in the anti-inflammatory activity. To clarify the mechanism of action of the many components that give the extract its anti-inflammatory activity, more research can be done.

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