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Phytochemical screening and High-performance liquid chromatography (HPLC) profile of different extracts of *Euphorbia hirta* (Linn)

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Abstract

The present examination plans to phytochemical screening and evaluates the total flavonoid content in the different extracts of the whole plant of *Euphorbia hirta*. Acetone, Chloroform, Ethylacetate, Methanol, and Hydro-alcohol (70% Methanol) were used as extraction solvents and are employed for phytochemical screening as well as HPLC analysis. Phytochemical screening of all the concentrates demonstrated and revealed the presence of flavonoids in all the extracts. HPLC was completed utilizing a segment and acetonitrile and 0.1% phosphoric corrosive in water (7:3) dissolvable framework that is portable base and recognized by wavelength 254nm and 360 nm settled for flavonoids measure. Current work explores the flavonoid (rutin) richness in the whole plant of *E. hirta* and future work aimed to isolate the flavonoid in the plant.

Keywords: *Euphorbia hirta*, phytochemical screening, total flavonoid by HPLC.

1. Introduction

Medicinal plants are playing a very active role in traditional medicines for the treatment of various ailments [1]. *E. hirta*, traditionally known as *taua-taua*, is a slender-stemmed, annual hairy herb from the Family Euphorbiaceae. It can grow to a height of 40 cm traditionally, it is used for treating asthma, chronic bronchial disorders, acute nasal catarrh, skin problems, gastrointestinal disorders, warts, cuts and infections [2, 3]. The stem of the plant is slender, reddish in color, covered with yellowish bristly hairs. The leaves are about 5 cm long, lanceolate, oppositely arranged and are usually greenish or reddish in color. The stem and leaves produce white or milky latex on cutting. [4]. the flowers are unisexual, small, crowded together in dense cymes and green in color. The male flowers are sessile, linear bracteoles, fringed, lack perianth, and possesses one stamen, whereas the female flowers have short pedicel, the perianth is rimmed, with a superior ovary. Fruits are yellow in color which contains three brown, four-sided, angular, wrinkled whole plant of *E. hirta* L. is used in the treatment of many disease including bronchitis, antioxidant, antimicrobial, skin diseases, cough, hay asthma, bowel disease, worm infestation, kidney stones, bronchial disease, to decrease lactation as sedative, anxiolytic, analgesic, antipyretic, and as anti-inflammatory agent. [5] the whole plant of *E. hirta* also possesses various pharmacological actions including anti-inflammatory, antifungal, antibacterial, antidiarrhoeal, sedative, anxiolytic, analgesic, antipyretic, antioxidant, antiasthmatic, antitumor, antimalarial, larvicidal, diuretic etc. [6]. The use of the plant described herein ethnomedicine, as well as their potentials in herbal drug formulations, necessitates the standardization of their extracts. Thus, the present study aims to screen the five extracts of *E. hirta*, by the qualitative method, as well as quantification of flavonoid with HPLC profiling.

2. Materials and Methods

2.1. Reagents

The reagents and solvents used for the extraction and HPLC profiling were Merck and HPLC-grade respectively. All the chemicals were purchased from Esware Scientifics, Trichy. **2.2.**

Taxonomic classification:

Kingdom: Plantae,
Subkingdom: Viridiplantae,
Infra Kingdom: Straptophyta,
Division: Tracheophyta,
Subdivision: Spermatophyta,

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Infra vision: Angiosperms,
Class: Magnoliopsida,
Superorder: Rosanae,
Order: Malpighi ales,
Family: Euphorbiaceae,
Genus: Euphorbia,
Species: *Euphorbia hirta* (*Euphorbia pilulifera*)^[7].

2.3. Plant extraction

Plant material was washed with water and then allowed to dry in shade for about 3 to 4 weeks. Dried plant materials were ground by using the electronic grinder. The powder of the whole plants of *Euphorbia hirta* L. was extracted according to (Harborne and Baxter., 1995). Dried plants sample was powered and filed into the soxhlet apparatus and extraction was carry out by Chloroform and the procedure was repeated for Acetone, Ethylacetate, Methanol, and Hydro-alcohol (70% Methanol) solvents. The extracts were stored in a refrigerator for further use^[8].

2.4. Phytochemical screening

Phytochemical screenings were carried out for all the extracts of *Euphorbia hirta* Linn by following given standard methods.

2.4.1 Test for alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

- A. Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
- B. Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
- C. Dragendroff's Test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
- D. Hager's Test:** Filtrates were treated with Hager's reagent (Saturated picric acid solution). Presence of alkaloids confirmed by the formation of a yellow colored precipitate.

2.4.2 Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

- A. Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
- B. Benedict's Test:** Filtrates were treated with Benedict's reagent and heated gently. Orange-red precipitate indicates the presence of reducing sugars.
Prashant Tiwari, *et al.*: Phytochemical screening and Extraction: A Review
- C. Fehling's Test:** Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

2.4.3 Detection of glycosides: Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

- A. Modified Borntrager's Test:** Extracts were treated with Ferric Chloride solution and immersed in boiling water for

about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

2.4.4. Legal's Test: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

2.4.5. Detection of saponins

- A. Froth Test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
- B. Foam Test:** 0.5 gm of the extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

2. 4. 6. Detection of phytosterols

- A. Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicates the presence of triterpenes.
- B. Libermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of a brown ring at the junction indicates the presence of phytosterols.

2.4.7. Detection of phenols

- A. Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of a bluish black colour indicates the presence of phenols.

2.4.8. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

2.4.9. Detection of flavonoids

- A. Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of an intense yellow color, which becomes colorless on an addition of dilute acid, indicates the presence of flavonoids.
- B. Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

2.4.10. Detection of proteins and amino acids

- A. Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.
- B. Ninhydrin Test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of a blue colour indicates the presence of amino acid.

2.4.11. Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes^[9, 12].

2.5. HPLC Profile of extracts of *Euphorbia hirta*

The five Extracts flavonoids standards were subjected to High-Performance Liquid Chromatography using 600 series HPLC pump and 2487 dual wavelength UV detector-254 and 360 nm of biozymes, Bangalore having Reprobond C18 column-4.6x250mm and 7725 Rheodyne injectors. A sample volume of 20 microlitres each was injected in all cases and flow rate of 1.0 ml/min was maintained. The data analysis was done using Empower 2 software. The compounds were eluted by employing the following method. Gradient elution of two solvents such as acetonitrile (solvent A) and 0.1% phosphoric acid in water (solvent B) was used for the detection of rutin [13]. The total run time of the program was

30 minutes. The gradient program began with 85% B and was held at this concentration for the first 12 minutes. This was followed by 75% eluent B for the next 10 minutes with a linear gradient and after which its concentration was again increased to 85% B for the next 8 minutes.

3. Result and Discussions

3.1. Phytochemical screening

The result of phytochemical screening of all the extracts was given in the table-1 and it clearly explains the most of the phytocompounds are present in methanol and hydro-alcohol compare to all the extracts. It also resulted that all the extracts have the flavonoid contents rather than others.

Table 1: Phytochemical screening of all the extracts of *Euphorbia hirta*

Phytochemical Test	Extracts of <i>Euphorbia hirta</i>				
	Acetone	Chloroform	Ethyl acetate	Methanol	Hydroalcohol
Tannins	+	+	+	+	++
Saponins	-	-	+	+	+
Quinones	+	+	-	+	+
Terpenoids	+	++	+	+	+
Steroids	+	+	+	+	+
Flavonoids	+	-	++	++	++
Phenol	+	+	+	++	++
Alkaloids	+	+	-	-	-
Glycosides	-	-	-	+	+
Cardiacglycosids	+	-	+	+	+
Anthocyanin	-	+	-	-	-

3.2. Quantification of flavonoids by HPLC

The total flavonoid content in the acetone, chloroform, ethyl acetate, methanol, and hydro-alcohol extracts was determined and results were presented in Table 3. Standard used for the analysis was rutin and the profile details were displayed in table-2. The HPLC spectrum of standard (rutin), acetone, chloroform, ethyl acetate, methanol, and hydro-alcohol was given in figures 1, 2, 3, 4, 5, and 6 respectively. All the extracts show the characteristics peak of flavonoids thus, *E. hirta* contains the highest amount of rutin flavonoid and highest in the hydro-alcoholic extract (0.211). *E. hirta* crude extracts possess antioxidant activity [14, 15] and α -glucosidase inhibition activity [19] and the high flavonoid content of the hydro-alcohol extract could be correlated to the reported antioxidant activity of *E. hirta* in recent literature. Since hydro-alcohol extract has a maximum of flavonoid content it might be the reason for the activity [16, 19].

Table 2: HPLC and profile of the standard

Compound	Rutin
Classification	Flavonoid glycoside
RT(Sample)	30
Quantity(mg/ml)	10
Molecular formula	C ₂₇ H ₃₀ O ₁₆
Molecular weight (g/mol)	610.52

Table 3: Total Flavonoid Content of Five Extracts of *Euphorbia hirta*

S. No	Extracts	Quantity (mg/ml)	Total flavonoid content (mg/g)
1	Acetone	10	0.121
2	Chloroform	10	0.117
3	Ethyl acetate	10	0.139
4	Menthol	10	0.185
5	Hydro-alcohol	10	0.211

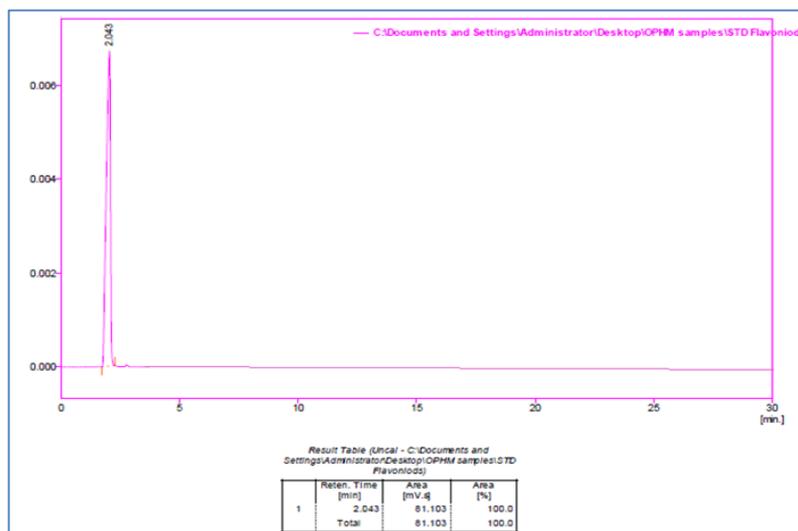


Fig 1: HPLC chromatogram standard of rutin

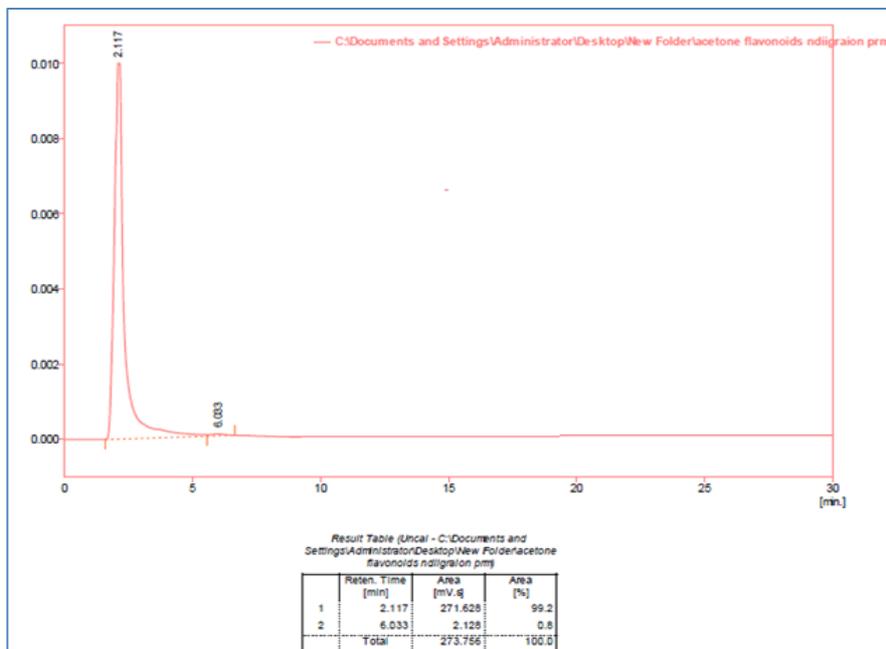


Fig 2: HPLC chromatogram of acetone extract of *Euphorbia hirta*

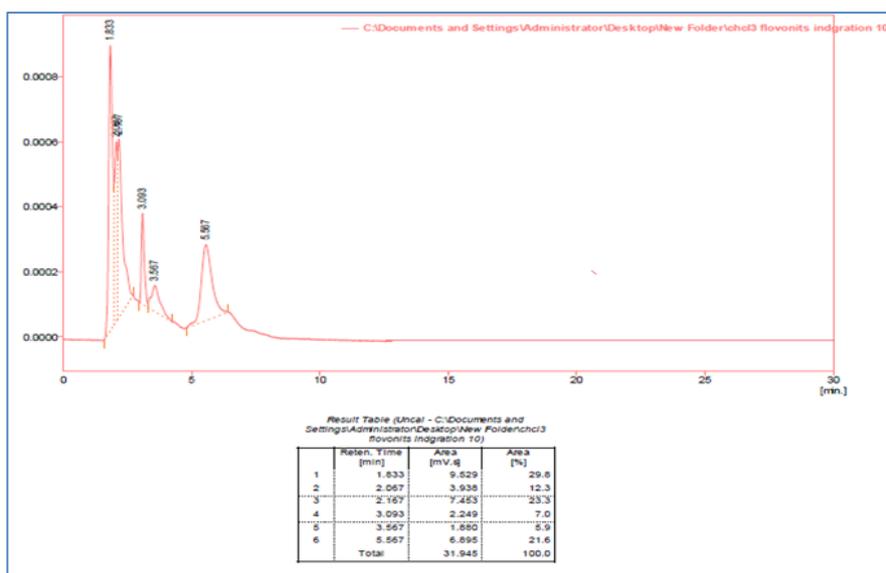


Fig 3: HPLC chromatogram of the chloroform extract of *Euphorbia hirta*

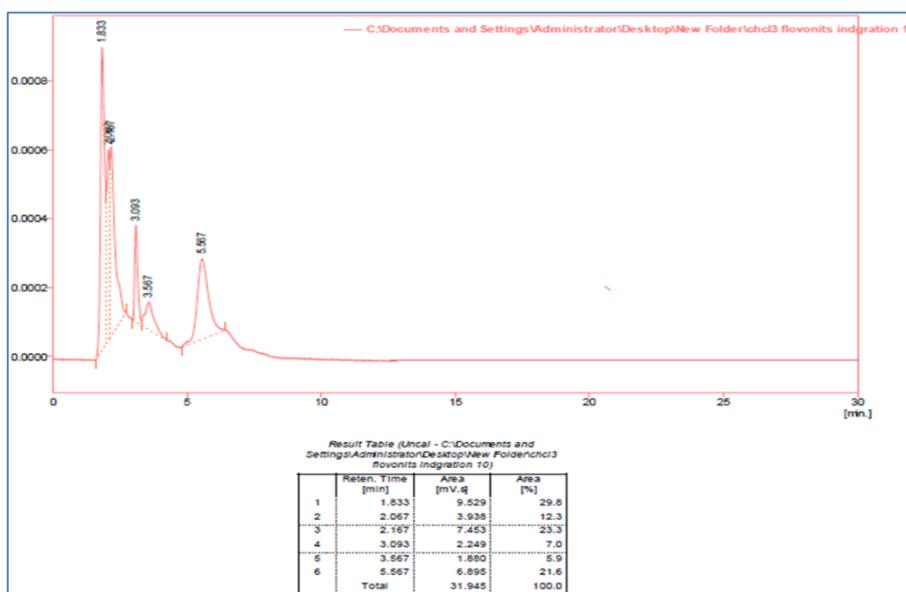


Fig 4: HPLC chromatogram of ethyl acetate extract of *Euphorbia hirta*

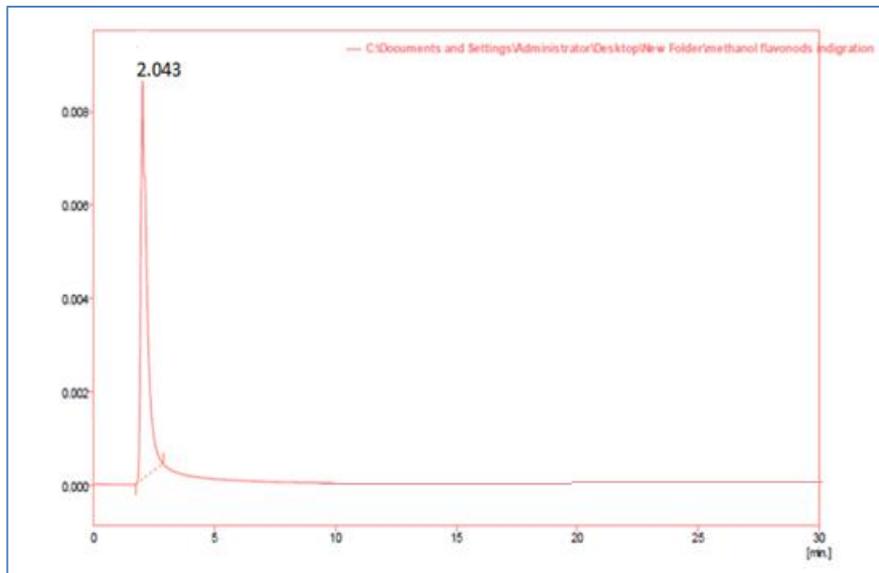


Fig 5: HPLC chromatogram of a methanol extract of *Euphorbia hirta*

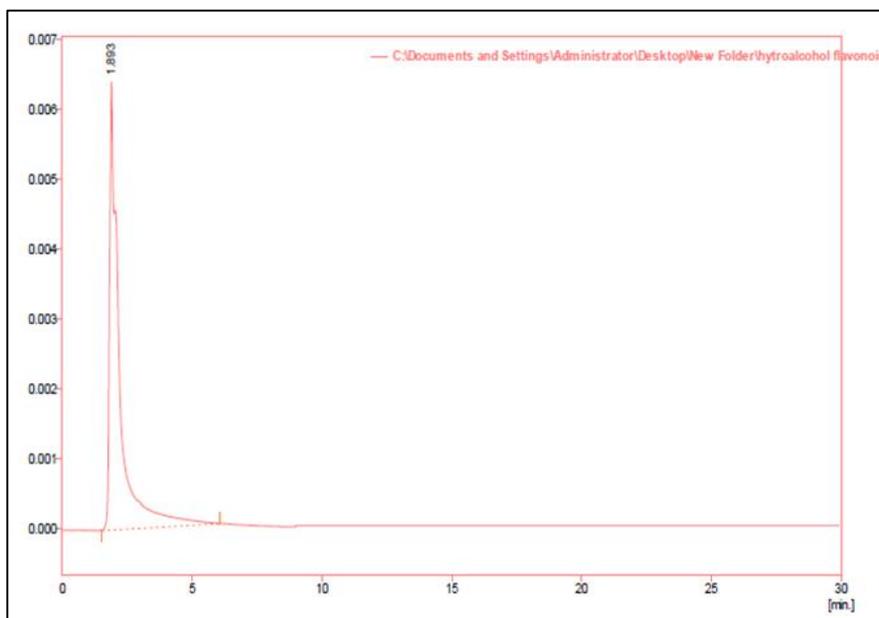


Fig 6: HPLC chromatogram of a hydro-alcohol extract of *Euphorbia hirta*

4. Conclusion

Natural flavonoid compounds play an important role in the medicinal field, especially in cancer prevention and treatment. They have a potential on antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects [20]. Rutin and its derivatives also showed numerous pharmacological activities such as antioxidant, apoptotic, antimicrobial [21] and hepatoprotective activities [22, 23]. The study evidencing the occurrence of flavonoid compounds in the medicinal plant *Euphorbia hirta* and the flavonoid rutin highly extracted in hydro-alcohol.

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6. References

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