Qualitative & quantitative phytochemicals evaluation of Euphorbia hirta linn. leaves extracts for antioxidant activity

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Abstract:

Euphorbia hirta Linn. Belongs to the family Euphorbiaceae and is widely known for its medicinal properties and extensively used globally. E.hirta leaves have been investigated for qualitative & quantitative evaluation such as microscopical, physical, and qualitative as well as quantitative evaluation of essential phytoconstituents inclusive of overall phenolic content, flavonoid content & antioxidant potential. This study aimed to screen the antioxidant potential of Hydro alcoholic, Ethyl acetate, and Acetone extracts of E.hirta Linn. Extracts (EH-HA, EH-EA, and EH-AC respectively). The results of 2, 2-diphenyl-1-pycrylhydrazyl (DPPH) radical scavenging assay and lipid per oxidation inhibition assay confirmed that EH-EA turned into the strongest antioxidant (IC50 = 95.05 ± zero.01µg/ml) compared to EH-HA (IC50 = 261.83 ± 0.01 µg/ml) & EH-AC (IC50 = 318.40 ± zero.01µg/mL) extracts. Our study results endorse that E.hirta Linn. Extracts possess tremendous antioxidant activity. Results show that this plant might be an awesome supply of natural antioxidants and a probable pharmaceutical complement. The various 3 analyzed extracts; EH-EA extract has the strongest activities and must be used to decide the phytochemicals and mechanisms of these activities.

Keywords:

E.hirta Linn., Antioxidant activity. Physio-chemical analysis, phytochemicals screening, hydro alcoholic extract, ethyl acetate extract, acetone extract.

1. Objective:

E.hirta contains several secondary metabolites, consisting of flavonoid, terpenoids, phenols, vital oil, and so forth. Many flavonoids had been diagnosed in E.hirta inclusive of quercetin, quercitrin, quercitol and derivatives. Some terpenoids in E.hirta had been isolated and identified successfully, including triterpenoids, α -amyrin, β -amyrin, friedelin, cycloartenol, 24-methylene-cycloartenol, triacetate β - sitosterol, campesterol, stigmasterol, etc. [14, 15]. The Ethyl acetate extract of E.hirta also showed greater outstanding antioxidant interest and a free radical scavenging capacity [14]. For the purpose of Qualitative & quantitative evaluation of leaves of E.hirta Linn., changed into completed to estimate the entire phenolic content, flavonoid content & Antioxidant activity of hydro alcoholic, ethyl acetate and acetone extracts of this herb.

2. Introduction:

Considering ancient instances, herbs were confirmed for their biological capability to relieve aches, prevent illnesses, and therapy illnesses. Nowadays, scientists around the world have developed a research machine for medicinal flora and their medicinal properties. Every plant species carries masses to heaps of different constituent chemical substances that interact in complicated ways within the bodies of animals and people1, 2, 3 in growing countries, in line with estimates through the sector health employer, approximately 80% of the population nevertheless relies upon folk medicines made from plants for prevention and remedy4. Conventional medication or folk medicine has been verified to be extra low-cost, clinically effective, and has quite fewer detrimental effects than contemporary drugs 5, 6; Plant-derived secondary metabolites which can be biosynthesized in plants are not best critical for plant survival but also are extraordinarily crucial for improvement, boom, reproduction, and plant safety. Secondary metabolites in plants encompass numerous businesses of molecules, inclusive of steroids, alkaloids, phenolics, lignans, carbohydrates, glycosides, etc., which own numerous biological activities. For instance, phenolic compounds are one large institution of secondary metabolites that can be popularly disbursed in plants and own a diversity of organic residences useful to people, consisting of their antiallergic, anticancer, antimicrobial, anti-inflammatory, antidiabetic, and antioxidant effects7, 8. Nowadays, the novel therapeutic agent is used against many diseases. Isolated bioactive molecules are utilized in the synthesis of new drugs. As a result, Extraction performs a critical step inside the itinerary of phytochemical processing for the discovery and

isolation of bioactive constituents from plant materials. Extracts organized by the usage of one-of-a-kind extraction techniques are stated to have versions in biological activities. Therefore, its miles vital to choose the perfect solvent and suitable extraction technique as an important step in the standardization of herbal products.

3. Plant profile[7, 8]:

3.1. Scientific name:

Euphorbia hirta. Linn

3.2. Family:

Euphorbiaceae

3.3. Vernacular name:

Dugdhika, mothi dudhi, dudhi

Figure. 1: Leaves of Euphorbia hirta. Linn

Figure. 2: Plant of Euphorbia hirta. Linn

3.4. Vernacular name:

Dugdhika, Mothi Dudhi, Dudhi

3.5. Description:

An erect herb up to 50 cm tall with greenish yellow OR white plant life, stem included with

yellowish hairs. Leaves are in opposite pairs.

3.6. Plant part used:

Leaves, complete plant.

3.7. Chemical constituents:

Alkaloids, Saponins, Tannins, Steroids, Flavonoids, and many others.

3.8. Conventional uses:

Used in the remedy of cough, allergies, diarrhoea, piles and semen debility, malicious program contamination, leprosy, pores and skin diseases, urinary contamination, and dis-urea. It is an aphrodisiac and enriches the blood and so on.

3.9. Description:

E.hirta. Linn. Is a small weed, erect or prostrate. Its miles hairy and greenish to reddish in colour. It exudes white latex as quickly as its far-cut. The plant has a deep root gadget. The short most important stem branches speedy deliver secondary creeping stems. These stems are abundantly furry and often tinged with purple. Leaves, with brief petioles, are arranged in pairs along the stem. Their area is serrated. They're furry in both aspects. The flora is grouped into balls, alternately arranged alongside the stem. They may be greenish and contained in a small cup with four glands of white edge. A globular fruit with 3 quarters emerges from the cut. The leaves are easy and distiches contrary, quick-stalked. The degree is as much as five cm lengthy and a couple of cm extensive. The bases of petioles are joined by using a skinny stipular collar with 2 to 4 filiform tines. Lamina is oval to elliptical, dark inexperienced in color. The bottom of the lamina is, especially uneven, vertex apex. Finely serrated margin. Higher and lower pubescent (denser hairs along the ribs on the underside, extra scattered on the top lamina).

4. Material & methods:

4.1. Chemicals:

All the chemical substances had been of analytical grade and were received from Merck India Ltd. and Loba Chemie Ltd., India

4.2. Authentication of plant:

The clean leaves of the E.hirta plant have been gathered from the local area of Nagpur region. The plant specimen turned dried and their herbarium sheet become organized .E.hirta became botanically identified & authenticated by Dr.Alka Chaturvedi, Professor & Head, Dept.of Botany, R.T.M. Nagpur University, Nagpur in July 2010.

Figure. 3: Herbarium of Euphorbia hirta. Linn

4.3. Plant material collection & preparation:

The leaves have been separated from the whole plant and gathered. The leaves had to begin with washed with distilled water to cast off particles and dust debris and dried on paper towels at room temperature (37±1) 0C. The leaves have been averted from exposure to daylight to save you the loss of active components. Dried leaves were packed in polyethylene bags. Inside the laboratory, after drying, the leaves had been subjected to size reduction in a mechanical grinder, accompanied by way of a blender, to get a coarse or fine powder. The coarse powder turned into then surpassed thru sieve no.40 mm to get the preferred particle size and stored in a properly - closed plastic box at room temperature until required to be used.

5. Determination of physio-chemical constant:[10, 11,12,13,15]

Physico-chemical values which include the ash values, extractive values, and percent of loss on drying had been finished as per the standard technique described in Indian Pharmacopoeia (1996).

5.1. Ash value:

Crude drug quality and purity became determined by using ash values. Sodium, potassium, magnesium, carbonates of calcium salts, phosphates, and silicates represent the full ash values. Powdered plant substances were subjected to total ash, acid-insoluble ash, and water-soluble ash.

5.2. Determination of total ash:

Coarsely powdered leaf material of about 2 to 3 grams turned into weighed and transferred to a pre-weighed silica crucible. In the crucible, the powdered drug changed into spread evenly as a nice layer and incinerated by progressively increasing the temperature not exceeding 4500 c until colourless, which suggests carbon-unfastened. After incineration final touch, the silica crucible became cooled in a desiccators and weighed. The same procedure becomes repeated to get a constant weight. The percentage of total ash was decided almost about the shade-dried plant material.

5.3. Acid-insoluble ash:

The ash received from the above approach changed into boiled for 5-10 min with 25 ml of dilute HCl. The insoluble ash became accumulated in an ash less filter paper or a Gooch crucible. The crucible or filter-out paper became washed with warm water. The acid-insoluble ash becomes transferred to a pre-weighed silica crucible. This became repeated till a regular weight turned into acquired. The acid insoluble ash percent was calculated close to the air-dried drug.

5.4. Water soluble ash:

The ash received in the dedication of total ash became boiled for about 5- 10 min in 25 ml of water. The insoluble depend turned into amassed in a silica crucible and washed with warm water. The insoluble ash so acquired turned into transferred to a pre-weighed silica crucible. The silica crucible became heated regularly to a temperature of 4500 c for 15 min to get a steady weight. The crucible was cooled and weighed. The proportion of water-soluble ash turned into calculated through subtracting the load of insoluble matters from the weight of total ash.

Table. 1: Ash value

Sr. No	***Ash Value***	***Observed***	***Max.Limit***
1	Total Ash value	8.4%	Not more 12 %
2	Water soluble Ash	1.06%	Not more than 10 %
3	Acid Insoluble Ash value	1.1%	Not more 07 %

5.5. Moisture content (loss on drying) (LOD):

2 gm. of powder drug appropriately weighed and brought in a porcelain dish. The dish turned into stored in a warm air oven maintained at a temperature of 1100C for 4 hours till a steady strong weight turned into recorded. The procedure became repeated. The dish was cooled in a desiccators at room temperature and weighed.

Table. 2: Moisture content

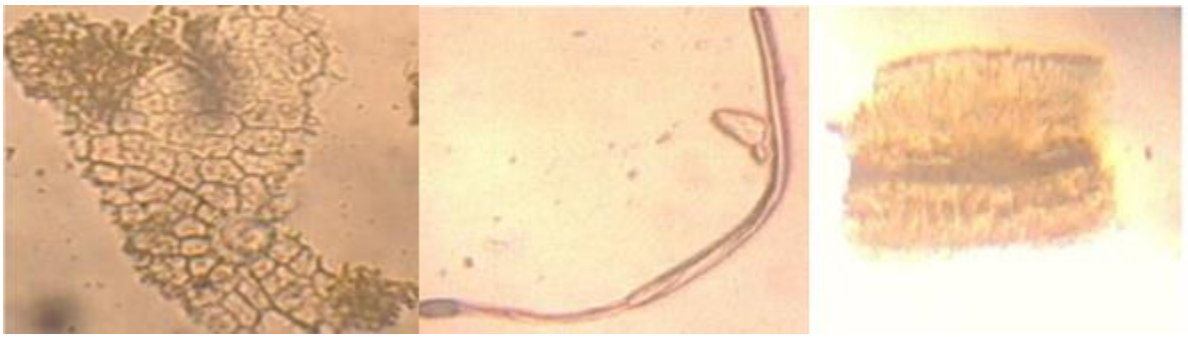
1	Moisture content:	7.85

6. Microscopy:[10,14,17,18]

6.1. Powder microscopy of *euphorbia hirta linn*. leaf:

Lignified Xylem Pericyclic fibre Anomocytic stomata

Figure. 4: Transverse section of Euphorbia hirta Linn. leaf



Epidermal cells with stomata | Fibre | Scalifarm vessel

Figure. 5: Powder microscopy of Euphorbia hirta. Linn

7. Extraction & fractionation:[13, 15]

7.1. Principle:

To prepare various extracts of E.hirta Linn., a successive solvent extraction method became accompanied. The plant substances have been subjected to successive extraction with precise solvents, starting from solvent of low polarity to high polarity.

7.2. Equipment:

Soxhlet equipment

7.3. Materials:

Dried leaves powder of E.hirta Linn.

7.4. Solvents:

Petroleum ether, hydro alcohol

7.5. Procedure:

About 200 gm of powdered drug of the selected medicinal plant subjected to extraction defatted by way of manner of the usage of Petroleum ether & then this defatted material turned into recharged with hydro alcoholic as a solvent in a Soxhlet apparatus. The extraction becomes persisted till the solvent inside the tumble turns clear or colourless. Then the heating emerge as stopped and the aggregate from the distillation flask was gathered and cooled. Then this mixture was filtered and concentrated through the usage of an evaporator at room temperature. The extract was changed into dried at room temperature and stored in an amber-colored glass container in a

refrigerator or desiccators for further experiment.

7.6. Fractionation:

The hydro-alcoholic extract is then fractionated through the usage of a separating funnel with ethyl acetate soluble extract & acetone soluble extract.

7.7. Determination of extractive value:

Extractive values are used to assess the character of phytochemicals present inside the crude drug. The extractive yield is a measure of the solvent's performance to extract precise components from the original material and it defined as the amount of extract recovered in mg. compared with the initial amount of the entire plant. Its miles are presented in percent and turned into decided for each technique examined.

Table: 3. Extractive value of E.hirta leaves extract

Sr.	***Extractive value***	***Extractive value found***	***Extractive value limit***
1	Water soluble extractive value	22.16%w/w	Not less than 10 % w/w
2	Alcohol soluble extractive value	14..08%w/w	Not less than 03 % w/w

8. Qualitative phytochemicals screening of euphorbia hirta linn. leaf extracts:[14, 15, 16]

The hydro alcoholic extract was acquired by way of successive solvent extraction techniques and ethyl acetate & acetone extract by using successive fractionation techniques. The above 3 focused extracts of the Euphorbia hirta Linn were subjected to numerous well-known phytochemicals assessment approaches to hit upon the presence or absence of numerous active phytoconstituents present inside the crude extracts

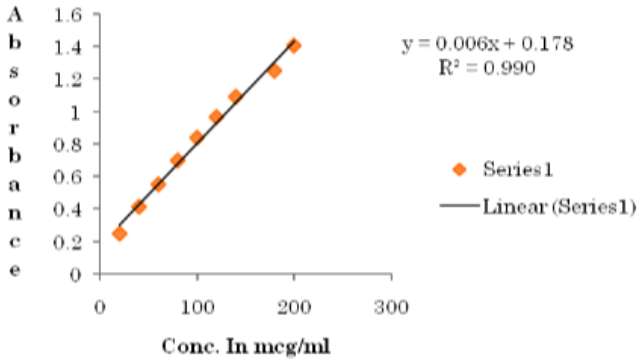
Table. 4: Qualitative Phytochemicals Sscreening of Euphorbia hirta Linn. Leaf Eextracts

Chemical Constituent	*Hydro alcoholic (HA)*	*Ethyl acetate soluble fraction of HA(EAS)*	*Acetone soluble fraction of HA(ACS)*	*Acetone insoluble residue of HA(ACIS)*
Proteins & amino acids	–	–	–	–
Carbohydrates	+	–	–	+
Steroids	+	+	–	–
Saponins	+	+	–	–
Tannins & Phenolics	+	+	+	–
Flavanoids	+	+	+	–
Alkaloids	–	–	–	–

+ Presence of compounds, - Absence of compounds

9. Determination of total phenolic content of the extracts from euphorbia hirta linn:

The total phenols content of the extract become determined through the Folin-Ciocalteu method. The conc. gradient of gallic acid prepared as a standard solution (0-50 µg/mL), and the calibration curve became installed the usage of gallic acid. The samples had been diluted with DMSO as sample solutions when they have been made up to 10 mL by means of methanol. The 6 mL of distilled water and 100 ?L of sample or widespread solution and 0.5 mL Folin-Ciocalteu reagent were combined for 5 min, followed by way of the addition of 1.5 mL of 20% sodium carbonate and 1.9 mL of distilled water. The mixture changed into located for 120 min at room temperature. Every test was accomplished in triplicate. The absorbance of the combination become measured at 758 nm the use of a UV spectrophotometer. The entire phenols content material of the samples was expressed as mg of gallic acid equivalents (GAE) per gram of dried extract. The experimental consequences are expressed as mean ± standard errors mean (SEM) of three replicates. [16,17,18]



Graph. 1: Absorbance vs. Conc. Total Phenolic Content of Euphorbia hirta Linn. leaf extracts

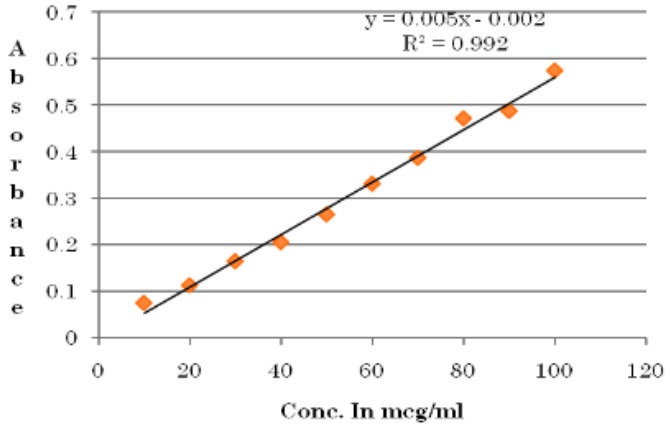
Table. 5: Total Phenolic Content of Euphorbia hirta Linn.leaf extracts

Plant part	Types of extracts /fractions	Total polyphenols (Gallic acid equivalent mg/g)				Avg. (mg/gm)
Leaves	Hydro alcoholic extract(HA)	0.1 mg/ml	0.3 mg/ml	0.5 mg/ml	1.0 mg/ml	119.60
		11.10	34.08	49.16	155.5	
	Ethyl acetate fraction(EAS)	12.58	37.83	64.16	183.50	140.89
	Acetone fraction(ACS)	9.83	32.66	44.83	140.00	109.17

10. Determination of total flavonoid content (tfc):

Total flavonoid content was determined by aluminium chloride colorimetric assay. The concentration gradient of quercetin prepared as a standard solution (0-80 µg/mL), and the calibration curve turn out to be mounted the usage of quercetin. The samples had been diluted with DMSO as sample solutions once they have been made as a lot as 50 mL via manner of methanol. The 4 mL of distilled water and 1 mL of sample or widespread answer, and 300 µL NaNO2 (5%) were delivered to a pitcher cuvette. The 300 µL of AlCl3 (10%) have been delivered after 5 min and 2 mL NaOH (1 M) and 2.4 mL distilled water were added after 6 min. The mixture was positioned for 10 min at room temperature. Every check was carried out in triplicate. The absorbance of the mixture changed into measured at 510 nm the usage of a UV spectrophotometer. The entire flavonoid content material of the samples grows to be expressed as mg of quercetin

equivalents (QE) per gram of dry extract. The experimental consequences are expressed as mean ± standard errors mean (SEM) of three replicates. [16,17,18]



Graph. 2: Absorbance vs. Conc.Total Flavonoids Content of Euphorbia hirta Linn. leaf extracts

Table. 6: Total Flavonoids Content of Euphorbia hirta Linn. leaf extracts

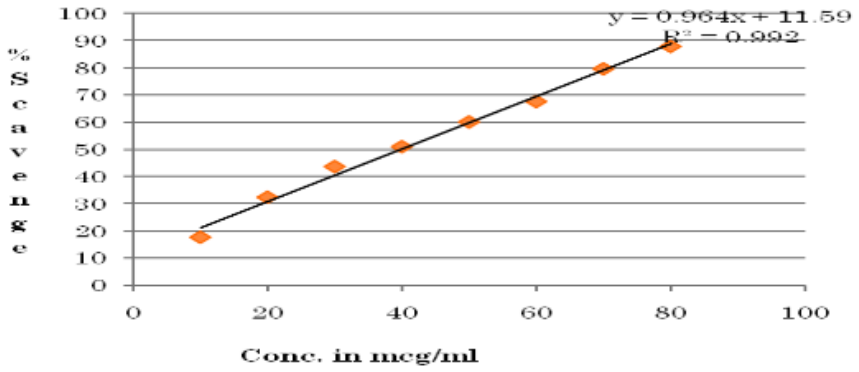
	Types of extracts /fractions	***Total Flavonoids content (Quercetin equivalent mg/g)***				***Avg. (mg/gm)***
Leaves	Hydro alcoholic Extract(HA)	0.1mg/ml	0.3mg/ml	0.5mg/ml	1.0mg/ml	mg./gm.
		11.2	30.00	49.5	107.4	104.57
	Ethyl acetate fraction of HA(EAS)	14.0	57.2	110.2	227.8	194.66
	Acetone fraction of HA(ACS)	7.5	24.2	41.00	83.2	80.19

11. Antioxidant activity:

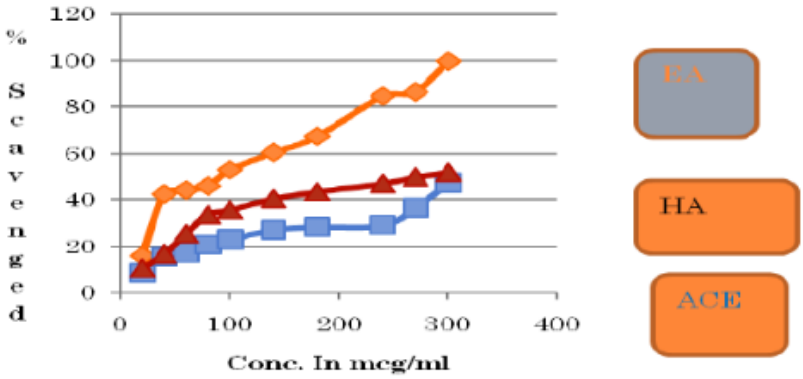
11.1. DPPH radical scavenging assay:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a strong free radical that has a most absorption of 515-517 nm (purple shade). While an antioxidant (as a hydrogen donor) reacts with DPPH, DPPH is reduced to DPPH-H, and therefore the solution turns from red to yellow. Consequently, the antioxidant activity of DPPH samples may be evaluated by the lower in absorption at 515-517 nm. The scavenging ability potential of the samples had been assessed using ascorbic acid (Merck, Germany) as positive control.Briefly, 0.5 ml. of diverse concentrations of each sample (10, 50,100,

250, 500, 750 and 1000 µg/mL) or ascorbic acid (0.5, 0.25, 0.1, 0.05, and 0.01 mM) became brought into a tube containing 0.5 ml of 0.6 mM DPPH solution dissolved in methanol and the volume became made uniformly to 4 ml by use of methanol. The solution became mixed and then allowed to stand in darkish at room temperature for 30 min. Absorbance became taken at 515 nm. The use of methanol as blank on UV-Visible spectrometer. Then, 0.5 mL of DPPH was brought to 3.5 mL of methanol and absorbance become taken for control reading. All analyses were run in triplicate. [19,20,21,22,23]



Graph. 3: % Scavenge vs. Conc. Antioxidant Content of Euphorbia hirta Linn. leaf extracts



Graph. 4: % Scavenge vs. Conc. Antioxidant Content of Euphorbia hirta Linn. leaf extracts

Table. 7: Antioxidant content of Euphorbia hirta Linn. leaf extracts

	Types of Extracts /fractions	***DPPH IC_{50} value (µg/ml)***
Standards	Ascorbic acid	38.99
E.hirta Leaves	HA Extract	261.83

	EAS Fraction	95.05
	ACS Fraction	318.40

12. HPTLC study: [24,25,26,27,28,29]

12.1. Comparative TLC images of HA, EAS and ACS Extracts At Visual, 254 and 366:

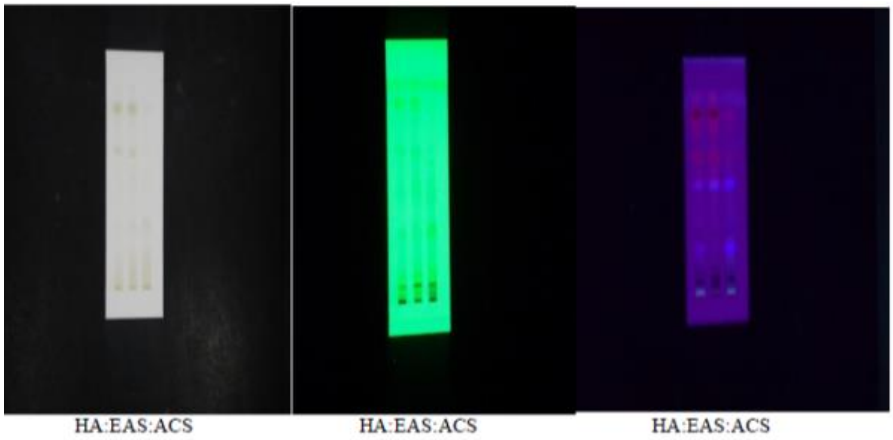


Figure.

Table. 8: HPTLC of Euphorbia hirta Linn.leaf extracts

Text extract	***Solvent system***	***Number of spots***
Hydro alcoholic extract (HA)	Toulene: Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)	09
Ethyl acetate fraction of HA (EAS)	Toulene: Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)	09
Acetone fraction of HA (ACS)	Toulene: Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)	08

12.2. E.hirta hydro alcoholic extract:

Scanning wavelength: 254nm

Stationary phase: HPTLC precoated, silica gel G 60 F25 (Merck, Germany)

Mobile phase: Toulene:Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)

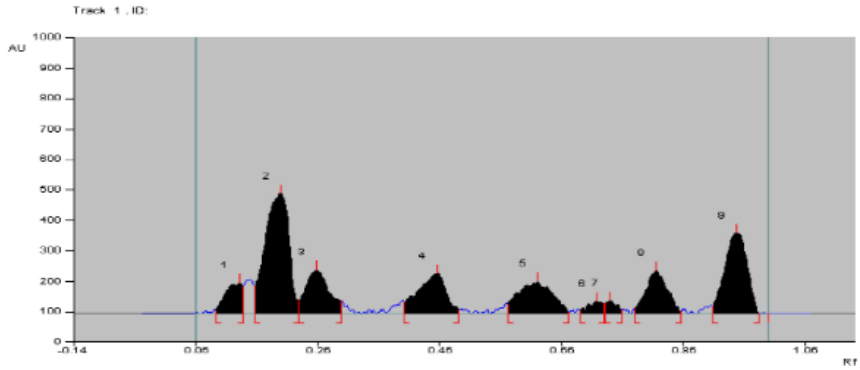


Figure. 6. 9: spots were found with the Rf values 0.13, 0.20, 0.26, 0.46, 0.62, 0.72, 0.74, 0.82, 0.95.

12.3. E.hirta Hydro alcoholic extract:

Scanning wavelength: 366nm

Stationary phase: HPTLC precoated, silica gel G 60 F25 (Merck, Germany)

Mobile phase: Toulene: Ethyl acetate: Methanol: Formic acid (6:3.5:0.5:0.1)

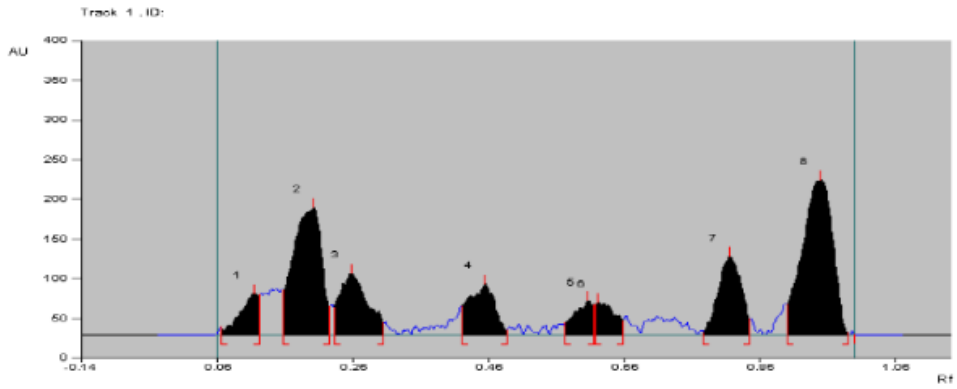


Figure. 7. 8: spots were found with the Rf values 0.11, 0.20, 0.26, 0.45, 0.61, 0.62, 0.82, 0.95

12.4. E.hirta ethyl acetate fraction:

Scanning wavelength: 254 nm

Stationary phase: HPTLC precoated, silica gel G 60 F25 (Merck, Germany)

Mobile phase: Toulene:Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)

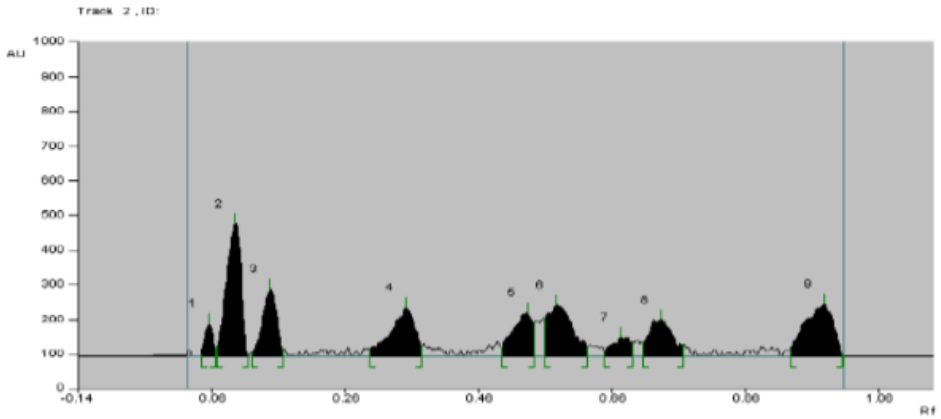


Figure. 8. 9: spots were found with the Rf values 0.06,0.09,0.15,0.35,0.53,0.58,0.67,0.73,0.98

12.5. E.hirta. ethyl acetate fraction:

Scanning wavelength: 366 nm

Stationary phase: HPTLC precoated, silica gel G 60 F25 (Merck, Germany)

Mobile phase: Toulene:Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)

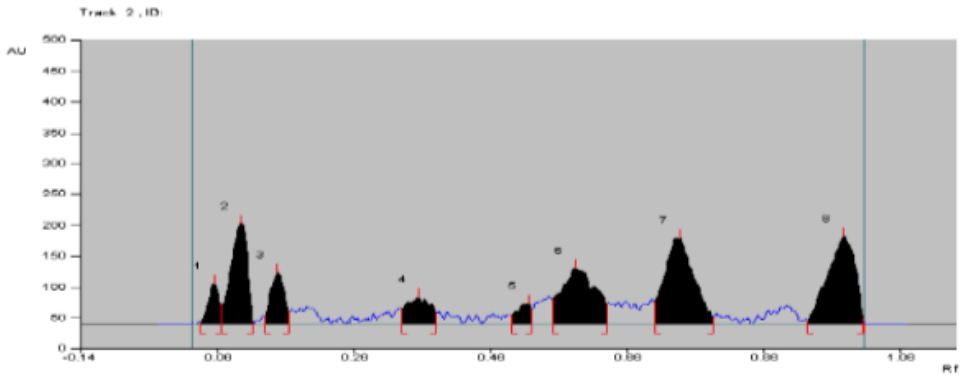


Figure. 20. 8: spots were found with the Rf values 0.05,0.09,0.15,0.35,0.52,0.58,0.74,0.98

12.6. E.hirta. Acetone fraction:

Scanning wavelength: 254 nm

Stationary phase: HPTLC precoated, silica gel G 60 F25 (Merck, Germany)

Mobile phase: Toulene:Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)

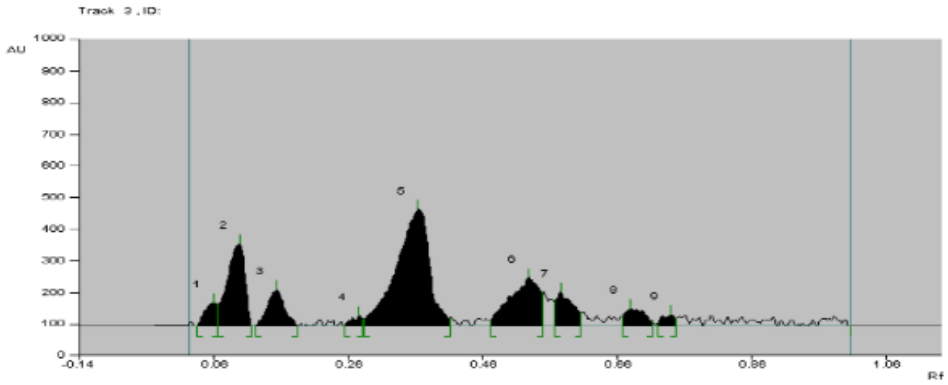


Figure. 9. 9: spots were found with the Rf values 0.06,0.10,0.15,0.28,0.36,0.53,0.58,0.68,0.74

12.7. E.hirta. Acetone fraction:

Scanning wavelength: 366 nm

Stationary phase: HPTLC precoated, silica gel G 60 F25 (Merck, Germany)

Mobile phase: Toulene:Ethyl acetate:Methanol:Formic acid (6:3.5:0.5:0.1)

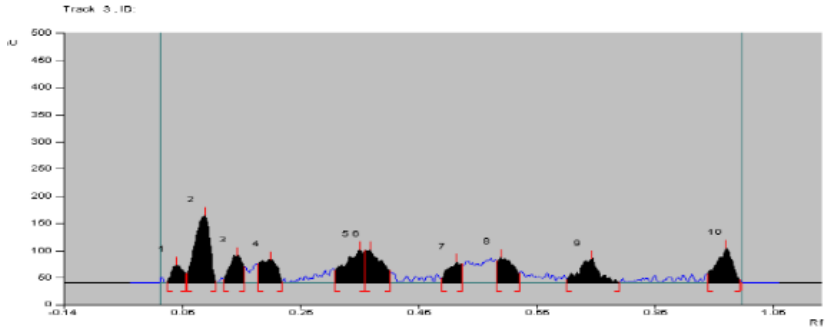


Figure. 10. 10: spots were found with the Rf values 0.05,0.10,0.15,0.21,0.36,0.38,0.52,0.60,0.75,0.98

13. Result and discussion:

In the ayurvedic device of drugs, E.hirta has long been in medical use. The plant is used in remedies for cough, asthma, diarrhoea, piles, semen debility, bug contamination, leprosy, skin illnesses, urinary infection, and dysuria. It's miles aphrodisiac and enriches blood and so on.

The existing research deals with the have a look at of pharmacognostic characteristics, extraction of dried leaves, preliminary phytochemicals screening, quantitative estimation of phenolic and flavonoid, strength of will of antioxidant ability, TLC and HPTLC studies.

Microscopic have a look at turned finished to decide the primary cellular composition of leaves and prove to be standards for the identity of plant species. Various specific bodily homes like ash

and extractive values were decided.

The dried leaves were defatted with petroleum ether and mark extracted with hydro alcohol. The crude extracts had been focused in a rotary vacuum dryer and the residue was further fractionated with ethyl acetate and acetone fractions.

Preliminary phytochemicals screening of extracts became finished to show the presence of different number primary and secondary metabolites. The hydro-alcoholic extract is located in the presence of steroids, tannins, Flavonoids, Saponins, and carbohydrates. The ethyl acetate soluble fraction of hydro alcoholic extract confirmed the presence of steroids, Saponins, flavonoids, tannins, and phenolics. Acetone soluble fraction showed the presence of flavonoids, saponins, carbohydrates, tannins, and phenolics. Acetone insoluble residue showed the presence of carbohydrates and was eliminated.

All extracts have been subjected to quantitative estimation of typical phenolic and flavonoid thru Folin-the Ciocalteu technique and the aluminium trichloride method respectively. The overall phenolic content of HA, EAS, and ACS became found to be 119.60, 140.89 and 109.17 respectively and flavonoid content become placed to be 104.57, 194.Ninety-six and 80.19 respectively.

All extracts had been subjected to assessment of antioxidant hobby by the manner of DPPH free radical scavenging technique. The conc. that inhibited 50% of free radicals have been discovered 261.83, 95.05, and 318.40 for HA, EAS, and ACS extracts respectively. The ethyl acetate fraction of HA extracts was determined to have a promising antioxidant effect. So it was shown to be the energetic extract and subjected to TLC and HPTLC research to estimate no. and sort of phytoconstituents are found in it. A number of solvent tools were tried, however suitable resolution become obtained inside the optimized solvent gadget. The presence of phytoconstituents changed confirmed by way of spraying TLC plates with suitable spraying reagents.

14. Conclusion:

In the present research work, all extracts have been subjected to qualitative & quantitative estimation of typical phenolics and flavonoids contents

Attempts were made to study the probable antioxidant activity of different extracts of leaves of Euphorbia hirta Linn.

Work has clearly proved that the ethyl acetate fraction of hydro alcoholic extract have considerable antioxidant activity.

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