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PHYTOCHEMICAL SCREENING, QUANTITATIVE ESTIMATION OF TOTAL PHENOLICS AND FLAVONOIDS, AND ETHNOBOTANICAL PROFILE OF *EUPHORBIA HIRTA* L. FROM DURG

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ABSTRACT

Euphorbia hirta L. (Euphorbiaceae), commonly known as the asthma plant, has a rich history in global ethnomedicine for treating respiratory and gastrointestinal disorders. This study investigated the qualitative and quantitative phytochemical profile of *E. hirta* specimens collected from the natural habitats of the Durg district in Chhattisgarh, India. Using a sequential extraction method across four solvents of varying polarities: methanol, ethanol, distilled water, and n-hexane—preliminary qualitative chemical assays and quantitative spectrophotometric estimations were performed. Qualitative screening revealed an unequal distribution of primary and secondary metabolites, showing that polar organic solvents (methanol and ethanol) excel at dissolving vital bioactive compounds like phenols, flavonoids, tannins, and alkaloids, while lipophilic fixed oils and fats strictly localized in the non-polar hexane fraction. Quantitatively, the methanolic extract demonstrated the highest concentrations of both Total Phenolic Content (TPC: 265 ± 0.001) and Total Flavonoid Content (TFC: 254.8 ± 0.01), significantly outperforming the aqueous and hexane mediums. These findings validate that selecting alcohol-based organic solvent systems is crucial for effectively isolating antioxidant-rich therapeutic compounds from regional flora. The study highlights *E. hirta* as a potent source of secondary metabolites, supporting its traditional applications and prospective utilization in modern pharmaceuticals.

KEYWORDS: *Euphorbia hirta* L., Phytochemical screening, Solvent polarity, Secondary metabolites, Quantitative estimation.

1. INTRODUCTION

Herbal medicine has been an integral part of human history, with individuals across diverse cultures and geographic regions relying on plants to address a wide array of health issues since ancient times. Remarkably, approximately 80% of the global population is estimated to utilize herbal medicine in some form, either directly or indirectly, to manage or alleviate medical conditions (Izah *et al*., 2024).

Chhattisgarh, a state in India, is particularly abundant in herbal diversity. With its vast forests covering about 40% of the region, this area is a rich repository of medicinal plants. A recent study conducted in the bustling city of Bhilai, located in the Durg district of Chhattisgarh, identified an astounding 198 species of plants spanning 69 different families. Among these, there were 86 herbaceous plants, 44 shrubs, 61 trees, and 7 climbing varieties, showcasing the remarkable floristic diversity of the region. Notably, the Fabaceae family exhibited the highest species diversity. Bhilai City emerged as a significant hub for herbal medicinal plants, reflecting its rich botanical heritage (Sharma, 2016).

Known as the asthma plant or milkweed (Dudhi), *Euphorbia hirta* L., a member of the Euphorbiaceae family, is extensively spread in tropical and subtropical climates. In many parts of the world, it goes by different names. The plant's distinctive milky white latex makes it easy to identify. It has historically been used to treat skin infections, gastrointestinal issues, and respiratory diseases. A rich phytochemical profile comprising flavonoids, tannins, alkaloids, saponins, terpenoids, and phenolic chemicals is responsible for the medicinal qualities. Pharmaceutical development requires scientific proof of these qualities, despite their widespread use in ethnomedicine. Thus, the current investigation sought to assess the antibacterial and antioxidant properties of *E. hirta* extracts and perform phytochemical screening(Aithal and Tidke2025).

Taxonomic classification

Kingdom: Plantae,

Subkingdom: Viridaeplantae, Infrakingdom: Straptophyta,

Division: Tracheophyta,

Subdivision: Spermatophytina, Infradivision: Angiosperms,

Class: Magnoliopsida,

Superorder: Rosanae,

Order: Malpighiales,

Family: Euphorbiaceae,

Genus: Euphorbia,
Species: Euphorbia hirta (Euphorbia
pilulifera)

Typical Names: *Euphorbia hirta* L.is known by a number of common names in various languages and nations. It is known as Labeinah and Em elhaleeb in Arabic. Asthma Plant, Asthma Weed, Garden Spurge, Pill-bearing Spurge, and Snakeweed are some of the English names for this plant. It is frequently referred to as Dudhi in Hindi. It is known as Botobotonis in the Philippines and Daun BijiKacang in Indonesia. Names like Golondrina, Hierba de Boca, Lecherón Chico, Lecherita, Pichoga, and Yerba de Sapo are used in Spanish-speaking areas. It is referred to locally as Nam Nom Raatchasee in Thailand.

Distribution: *Euphorbia hirta* L.is found in many tropical and subtropical parts of the earth. In regions of North and South America, Africa, Asia, and Australasia, it is found naturally and in large quantities. The United States, Mexico, Brazil, India, Nepal, Sri Lanka, Thailand, Indonesia, Malaysia, and various African countries are among the places where the plant is frequently found. Because of its versatility, it thrives in wastelands, roadsides, farmed fields, and open grasslands.

Customary Applications: *Euphorbia hirta*L.has long been used to treat a variety of illnesses. It has been widely used to treat respiratory conditions such colds, hay fever, bronchitis, asthma, and cough. Additionally, the plant is used to treat gastrointestinal issues such as vomiting, peptic ulcers, intestinal worms, diarrhoea, dysentery, and stomach discomfort. It is used in traditional medicine to treat conjunctivitis, fungal infections, warts, wounds, and skin conditions. Menstrual problems, kidney stones, rheumatism, headaches, toothaches, and bites from snakes or scorpions have all been treated with it. The herb has long been used in India to treat inflammation, gonorrhoea, jaundice, and digestive problems. While the roots are utilised for sprains and inflammatory problems, the latex is frequently administered externally to wounds and skin infections.

Used Medicinal Parts:

The plant's leaves, stems, blossoms, roots, and latex are all significant medicinal components that are used in various traditional treatments.The southern Western Ghats of India and the northern east coast of Tamil Nadu are home to the medicinal rhizomatous herb Euphorbia hirta, which belongs to the Euphorbiaceae family. In both east and west Africa, the plant's

extracts are used to treat asthma and respiratory tract inflammations. Coughs, chronic bronchitis, and other lung ailments are also treated with it in Malagasy. The herb is also widely used in Angola to treat diarrhoea and dysentery, especially amebic dysentery. The plant's extracts or exudates are used as ear drops and to treat wounds, boils, and ulcers in Nigeria.(Singh Geeta and Kumar Padma,2013).Traditional herbal medicine practitioners frequently use Euphorbia hirta. Another name for Euphorbia hirta is "asthma herb and pill-bearing spurge" (M. Abu-Sayeed *et al*., 2005; H Anuradha *et al*., 2008). Particularly in the young portion of Euphorbia hirta, the steam is thin, reddish, and covered in yellowish, bristly hair. Euphorbia hirta has oppositely orientated leaves that are roughly 5 cm long and typically have reddish or greenish undersides.(M. Hussain *et al*.,2014).

2. MATERIALS AND METHODS

2.1 Study area: Durg district, situated in the central region of the Indian state of Chhattisgarh, serves as the designated study area for the present research (Figure 1). Geographically, the district lies between 20°54′ to 21°32′ North latitude and 81°10′ to 81°36′ East longitude, covering an area of approximately 2,238 square kilometers. It is bounded by Bemetara district to the north, Rajnandgaon to the west, Balod to the south, and Raipur and Dhamtari districts to the east. The region is part of the fertile plains of the Chhattisgarh Basin and is traversed by significant river systems, most notably the Shivnath River, a major tributary of the Mahanadi. The average elevation of the district is 317 meters above mean sea level, and it receives an annual average rainfall of around 1,052 mm, with the monsoon season contributing the majority of precipitation. Durg is characterized by a mixed land use pattern comprising urban, industrial, and agricultural zones. The district includes major urban centers such as Durg city, the administrative headquarters, and Bhilai, an industrial hub home to the Bhilai Steel Plant, one of India’s largest integrated steel manufacturing facilities. These urban centers form part of the Durg–Bhilai twin-city region, which is among the most densely populated and industrialized areas in Chhattisgarh. The district's strategic location along National Highway 6 (NH-6), the Howrah–Mumbai railway corridor, and the Raipur–Durg Expressway enhances its accessibility and economic significance. Administratively, the district is divided into several tehsils and development blocks, including Durg, Patan, and Dhamdha, encompassing a network of urban municipalities and rural gram panchayats. The region's socio-economic fabric is shaped by a blend of industrial development, agricultural activity, and a diverse demographic profile. Given its unique combination of physiographic

features, infrastructure, and development dynamics, Durg district offers a representative context for in-depth spatial, environmental, and socio-economicresearch.

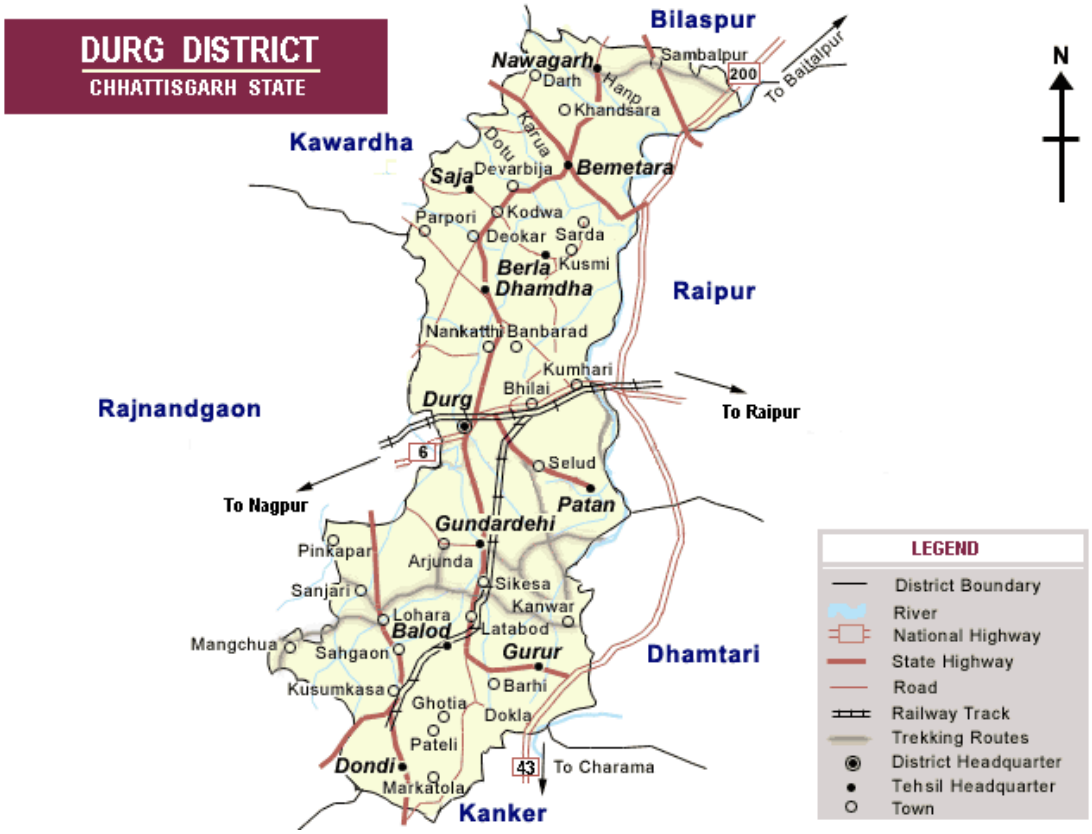

Figure 1: Map of Durg District.

2.2 Site selection: The selection of Durg district as the study site for research on medicinal plants is based on its rich ecological diversity, transitional landscape, and increasing ethnobotanical significance. The district encompasses a mosaic of urban, rural, and semi-forest areas, offering a varied habitat that supports a wide range of native flora. Particularly in its rural and forest-fringe regions, such as areas around Patan, Dhamdha, and parts of the western and southern blocks, local communities have traditionally depended on wild and cultivated medicinal plants for primary healthcare. These regions are characterized by red and lateritic soils, moderate rainfall (~1050 mm/year), and subtropical climate, which together support the growth of diverse herbal species. Additionally, Durg lies in proximity to forested belts and tribal settlements, where traditional knowledge systems related to medicinal plant use are still practiced and passed down through generations. The presence of natural water sources, uncultivated land patches, and home gardens further contributes to the distribution and conservation of medicinal plant species. Importantly, the area has not yet been extensively documented in academic literature for its medicinal flora, making it a promising

and underexplored region for systematic ethnobotanical and phytochemical research. Therefore, Durg district provides an ideal setting for investigating medicinal plant diversity, traditional usage patterns, and their potential for sustainable utilization and conservation.

2.3 Plant Collection and Identification

The plant samples (*Euphorbia hirta* L.) were collected from various natural habitats within Durg city and its adjacent areas (Chhattisgarh, India). The identity and botanical authenticity of the collected specimens were verified using standard regional floras, and a voucher specimen was deposited at the departmental herbarium for future reference. The collected plant parts were washed thoroughly with running tap water to remove soil particles, shade-dried at room temperature, and ground into a coarse powder using a mechanical grinder.

2.4 Preparation of Plant Extracts

The extraction process was carried out using the standard maceration/Soxhlet extraction method according to the procedures described by (Nikhal *et al.*,2010). Different solvents of varying polarities, including methanol, ethanol, distilled water (polar), and n-hexane (non-polar), were utilized for the extraction. The crude mixtures were filtered, and the filtrates were concentrated under reduced pressure using a rotary evaporator. The percentage yield for each solvent extract was recorded. The final concentrated dry extracts were preserved in amber-colored airtight containers and stored at 4°C for further phytochemical investigations.

2.5 Phytochemical Screening (Preliminary Analysis)

The prepared plant extracts were subjected to systematic preliminary qualitative chemical tests to detect the presence of various secondary metabolites and phytoconstituents using standard protocols.

2.5.1 Test for Alkaloids

- **Iodine Test:** The crude extract was treated with a few drops of iodine solution. The formation of a distinct brown or reddish-brown precipitate indicated the presence of alkaloids (Khandelwal, 2008).
- **Wagner's Test:** A small fraction of the extract was acidified with dilute hydrochloric acid (HCl) and treated with Wagner's reagent (iodine in potassium iodide). The appearance of a reddish-brown precipitate confirmed the presence of alkaloids (Kokate*et al*., 2001).

2.5.2 Test for Proteins

- **Biuret Test:** To 1–2 mL of the plant extract, an equal volume of 40% sodium hydroxide (NaOH) solution and a few drops of 1% copper sulfate solution were added. A characteristic violet or pink coloration indicated the presence of proteins (Harborne, 1998).

2.5.3 Test for Carbohydrates

- **Seliwanoff's Test:** The extract was treated with Seliwanoff's reagent (resorcinol in HCl) and heated gently in a water bath. The development of a cherry-red color indicated the presence of ketoses (Kokate*et al*., 2001).
- **Molisch's Test:** The extract was mixed with a few drops of Molisch's reagent (alpha-naphthol dissolved in ethanol), followed by the slow addition of concentrated sulfuric acid along the sides of the test tube. The formation of a purple or reddish-violet ring at the junction of the two liquids confirmed the presence of carbohydrates (Harborne, 1998).

2.5.4 Test for Fixed Oils and Fats

- **Saponification Test:** A small amount of the extract was boiled with an alcoholic potassium hydroxide (KOH) solution. The formation of soap or stable froth upon mixing indicated the saponification of fixed oils and fats (Kokate*et al*., 2001).

2.5.5 Test for Tannins

- **Ferric Chloride Test:** The crude extract was dissolved in water, heated, and filtered. To the filtrate, a few drops of 5% ferric chloride ($FeCl_3$) solution were added. The appearance of a dark green or blue-black coloration indicated the presence of tannins (Harborne, 1998; Kokate*et al*., 2001).
- **Lead Acetate Test:** The extract solution was treated with a few drops of a 10% lead acetate solution. The formation of a bulky white or yellowish precipitate confirmed the presence of tannins (Trease and Evans, 1985).

2.5.6 Test for Sterols

- **Liebermann-Burchard's Test:** The plant extract was dissolved in chloroform, followed by the addition of acetic anhydride and a few drops of concentrated sulfuric acid (H_2SO_4) from the side of the tube. A color transition from red to blue, and finally to emerald green, indicated the presence of sterols (Finisar, 1986).
- **Salkowski's Test:** The extract was treated with chloroform and an equal volume of concentrated sulfuric acid (H_2SO_4). A reddish-brown color in the upper chloroform layer and a yellow sulfuric acid layer with green fluorescence signified the presence of sterols (Kokate*et al*., 2001).

2.5.7 Test for Flavonoids

- **Pew's Test:** To the plant extract, zinc powder followed by a few drops of concentrated hydrochloric acid (HCl) were added. The appearance of a cherry-red or crimson color denoted the presence of flavonoids (Peach and Tracey, 1956).

- **Shinoda Test:** A small portion of the extract was treated with magnesium turnings, followed by the dropwise addition of concentrated hydrochloric acid (HCl).The development of a pink, crimson, or red color within minutes confirmed the presence of flavonoids (Kokate*et al*., 2001).
- **NaOH Test:** The extract was dissolved in dilute sodium hydroxide (NaOH) solution. The formation of an intense yellow color, which became colorless upon adding a few drops of dilute acid, indicated the presence of flavonoids (Khandelwal, 2008).

2.5.8 Test for Phenols

- **Ellagic Acid Test:** The extract was treated with a few drops of 5% glacial acetic acid followed by a 5% sodium nitrite ($NaNO_2$) solution. The appearance of a muddy-brown or red coloration indicated the presence of phenols (Gibbs, 1974; Hu, 2018).

2.6 Quantitative Phytochemical Analysis

2.6.1 Determination of Total Phenolic Content (TPC): The total phenolic content in different solvent extracts was determined spectrophotometrically using the Folin-Ciocalteu reagent method as described by Chandra and Gonzalez (2004). The absorbance of the reaction mixture was measured at a specific wavelength using a UV-Vis spectrophotometer against a blank. TPC was extrapolated from a standard calibration curve and expressed as mean ± standard deviation (SD).

2.6.2 Determination of Total Flavonoid Content (TFC): The total flavonoid content was quantified utilizing the aluminum chloride colorimetric assay following the protocol outlined by Woisky and Salatino (1998). The absorbance was measured spectrophotometrically. TFC values were computed from a standard curve and reported as mean ± SD.

3. RESULTS

3.1 Preliminary Qualitative Phytochemical Screening

The qualitative screening of the plant extracts across four distinct solvent systems revealed an unequal distribution of primary and secondary metabolites. Polar organic solvents (methanol and ethanol) showed highly positive profiles for secondary metabolites like phenols, flavonoids, tannins, and alkaloids. Fixed oils and fats were strictly localized in the non-polar hexane fraction.

Table 01: Preliminary Qualitative Phytochemical Screening.

S.No.	Phytochemical Groups	Specific Assays	Methanol	Ethanol	Distilled Water	Hexane
1	Alkaloids	Iodine / Wagner's	+++	++	+	-
2	Proteins	Biuret Test	++	+	++	-
3	Carbohydrates	Seliwanoff's / Molisch's	++	++	+++	-
4	Fixed Oils & Fats	Saponification	-	-	-	+++
5	Tannins	$FeCl_3$ / Lead Acetate	+++	++	+	-
6	Sterols	Liebermann-Burchard / Salkowski	+	++	-	+++
7	Flavonoids	Pew's / Shinoda / NaOH	+++	+++	+	-
8	Phenols	Ellagic Acid Test	+++	+++	++	-

Note: (+++) Strongly present; (++) Moderately present; (+) Weakly present; (-) Absent.

3.2 Quantitative Estimation of TPC and TFC

Quantitative analysis revealed significant variations ($p < 0.05$) among the different extraction mediums. The methanolic extract demonstrated the highest concentration of both phenols and flavonoids, whereas the aqueous and hexane systems yielded lower amounts.

Table 02: Quantitative Estimation of TPC and TFC.

S.No.	Extraction Solvent	Total Phenolic Content (TPC)	Total Flavonoid Content (TFC)
1	Methanol	265 ± 0.001^{d}	254.8 ± 0.01^{d}
2	Ethanol	215 ± 0.002^{c}	166.3 ± 0.001^{c}
3	Hexane	51 ± 0.0015^{b}	107.9 ± 0.007^{b}
4	Distilled Water	31.07 ± 0.007^{a}	76.9 ± 0.0011^{a}

Values are expressed as mean ± standard deviation (SD) of triplicate experiments. Different superscript letters (a, b, c, d) in the same column represent statistically significant differences ($p < 0.05$).

4. DISCUSSION

The extraction of bioactive compounds from plant matrices is highly dependent on the polarity of the solvents used, which directly influences both qualitative diversity and quantitative yield. In this study, plant samples collected from Durg and adjacent areas were extracted using four solvents spanning a wide polarity range: methanol, ethanol, distilled water, and n-hexane.

The preliminary qualitative screening (Table 1) confirmed that polar organic solvents, specifically methanol and ethanol, possess a higher capacity to dissolve crucial secondary metabolites. This aligns with the 'like dissolves like' principle. Phenolic compounds, flavonoids, and tannins carry hydroxyl groups (–OH) that readily establish hydrogen bonds with alcoholic solvent networks. Conversely, lipophilic components like fixed oils, fats, and sterols partitioned exclusively into the non-polar hexane phase, verifying the efficiency of using diverse polarities as suggested by (Nikhal *et al*.,2010).

Quantitatively, the Total Phenolic Content (TPC) peaked drastically in the methanol extract (265 ± 0.001^{d}), followed by ethanol (215 ± 0.002^{c}), while dropping significantly in hexane (51 ± 0.0015^{b}) and distilled water (31.07 ± 0.007^{a}). A identical trend was observed for the Total Flavonoid Content (TFC), where methanol achieved the maximum yield (254.8 ± 0.01^{d}) and distilled water exhibited the lowest (76.9 ± 0.0011^{a}).

The maximum recovery of TPC and TFC in the methanol extract can be attributed to its lower molecular weight and lower viscosity relative to water and ethanol. These physical properties enhance its ability to penetrate cell walls and optimize the solubility of intracellular polyphenols. Interestingly, despite water having the highest dielectric constant among the solvents, its quantitative yield for phenols was low. This phenomenon is often due to the activation of endogenous polyphenol oxidases in purely aqueous environments, which degrade free phenolic content during room-temperature processing. Organic alcohols effectively denature these enzymes, protecting the antioxidant yield.

Furthermore, hexane showed a higher value for TFC (107.9 ± 0.007^{b}) compared to its low TPC value. This suggests that certain highly lipophilic or polymethoxyflavones are present in the flora of the Durg region, causing them to separate more readily into non-polar systems. The clean statistical separation indicated by the alphabetic superscripts (a, b, c, d) confirms that selecting an alcohol-based organic solvent system is crucial for isolating therapeutic, antioxidant-rich compounds from these regional plant varieties.

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