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IN VITRO PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *EUPHORBIA HIRTA*

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Abstract:

Introduction : *Euphorbia hirta*, commonly known as "Gatas-gatas" or "Asthma plant," is a medicinal herb widely distributed in tropical and subtropical regions.It has been traditionally used in various folk medicine systems for the treatment of respiratory ailments, skin disorders, gastrointestinal disorders, and inflammatory conditions.The plant is known for its rich phytochemical composition, which includes alkaloids, flavonoids, phenolic compounds, terpenoids, and tannins. These bioactive constituents have been reported to possess diverse pharmacological activities, including antioxidant properties. The aim of the study is to determine the phytochemical composition of *Euphorbia hirta* and assess its potential as a source of natural antioxidants .

Materials and methods :

Preparation of Methanolic Extracts: Approximately 400 g of each of the powdered plant materials was taken .The filtrates were concentrated using a rotary evaporator at 50°C and later in a hot-air oven at 35°C to dry completely. The concentrates were put in airtight containers and stored at 4°C awaiting use in in vitro bioassay.

Qualitative Phytochemical Screening:

Qualitative tests for various phytochemicals present in the methanolic leaf

extracts were done. Visual examination of the appearance of color or frothing was used as an indicator for the presence or absence of a given phytochemical group. Test for saponins, alkaloids, cardiac glycosides, phenols etc.
Determination of In Vitro Antioxidant Activities of the Studied Plant Extracts.
Determination of Total Phenolic Contents.
Determination of Total Flavonoid Contents.
Results: the presence of various phytochemicals like flavonoids, phenols, terpenes, saponins, alkaloids, cardiac glycosides indicating a therapeutic potential, since they are useful in protecting plants from harmful microbes and extreme temperatures as well. The main rationale for considering the phytochemicals of *E. hirta* in the investigation was its reported efficacy against respiratory disorders.
Conclusion: In conclusion, the study provides valuable insights into the in vitro phytochemical analysis and antioxidant activity of *Euphorbia hirta*. The findings support its potential as a natural antioxidant source and lay the groundwork for further research to explore its therapeutic applications and contribute to the growing field of natural product-based research and provide a basis for future investigations on *Euphorbia hirta* and its role in combating oxidative stress-related diseases.

Introduction :

The exploration of phytochemicals, which are natural compounds produced by plants, has become a significant area of research due to their diverse biological activities(Prakash and Sharma 2014).Antioxidants play a crucial role in neutralizing free radicals, which are implicated in various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders.(Xu and Howard 2012) Investigating the benefits of antioxidants in research presents numerous advantages. The exploration of these compounds offers an opportunity to delve into their potential positive effects on health and well-being.(Zehiroglu and Ozturk Sarikaya 2019) By examining antioxidants, researchers aim to understand their role in neutralizing harmful free radicals, which are associated with various diseases and aging processes. Furthermore, the research on antioxidants provides insights into how these compounds may contribute to cellular protection and the prevention of oxidative stress-related conditions.(Özben 2013)

Euphorbia hirta is of particular interest due to its reported pharmacological effects, including anti-inflammatory, antimicrobial, and antioxidant properties.*Euphorbia hirta*, commonly known as "asthma weed" or "garden spurge," is a plant widely distributed in tropical and subtropical regions(Galvez et al. 1993). This herbaceous plant has been traditionally utilized in various folk medicines for its purported therapeutic properties.*Euphorbia hirta* has a rich history of traditional uses in various parts of the world. In traditional medicine, it has been employed to address a range of health issues, including respiratory

ailments such as asthma, bronchitis, and coughs.(Basma et al. 2011) The plant is also known for its use in managing gastrointestinal problems, skin disorders, and as a diuretic.

The medicinal properties of *Euphorbia hirta* are attributed to its diverse phytochemical composition. Phytochemicals are natural compounds found in plants that often exhibit biological activities.(Annamalai et al. 2013). *Euphorbia hirta* is reported to contain alkaloids, flavonoids, tannins, polyphenols, and terpenoids, among other bioactive constituents.(Goyal and Chauhan 2018) These compounds contribute to the plant's antioxidant, anti-inflammatory, and antimicrobial properties.

The antioxidant potential of *Euphorbia hirta* is of particular interest in the context of its medicinal applications(Lanhers et al. 1991) Antioxidants play a crucial role in neutralizing free radicals, which are reactive molecules implicated in cellular damage and various diseases. (Annamalai et al. 2013)By scavenging these free radicals, the antioxidants in *Euphorbia hirta* may help protect cells from oxidative stress.The antioxidant activity of *Euphorbia hirta* is of particular importance in the context of preventing oxidative stress-related diseases.(Lee et al. 1998) Oxidative stress occurs when there is an imbalance between the production of free radicals and the body's ability to neutralize them. Antioxidants help mitigate this imbalance by scavenging free radicals and preventing cellular damage.

The methods employed in this study include the extraction of phytochemicals from Euphorbia hirta using suitable solvents. Subsequently, the obtained extracts will be subjected to various tests to identify the presence of phytochemical classes such as alkaloids, flavonoids, phenols, tannins, and saponins. These compounds have been associated with diverse biological activities, and their concentrations in *Euphorbia hirta* may contribute to its medicinal properties.Common methods for measuring antioxidant activity include the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the ferric reducing antioxidant power (FRAP) assay.(Xu and Howard 2012)

The study in investigating the benefits of antioxidants in research presents numerous advantages. The exploration of these compounds offers an opportunity to delve into their potential positive effects on health and well-being. By examining antioxidants, researchers aim to understand their role in neutralizing harmful free radicals, which are associated with various diseases and aging processes.

Materials and methods :

2.2. Preparation of Methanolic Extracts

Approximately 400 g of each of the powdered plant materials was soaked in a liter of analytical grade methanol in a 2-liter capacity conical flask. The flasks containing each plant material were shaken regularly, corked, and left to stand for 48 hours at room temperature. In each case, the menstruum was separated by filtration through Whatman filter paper No. 1. The filtrates were then concentrated using a rotary evaporator at 50°C and later in a hot-air oven at 35°C to dry completely. The concentrates were put in airtight containers and stored at 4°C awaiting use in in vitro bioassay

2.3. Qualitative Phytochemical Screening

Qualitative tests for various phytochemicals present in the methanolic leaf extracts of were carried out using standard phytochemical screening procedures . Visual examination of the appearance of color or frothing was used as an indicator for the presence or absence of a given phytochemical group.

2.3.1. Test for Saponins

About 2 g of each of the studied plant extracts was weighed and dissolved in 5 ml of distilled water. Thereafter, aliquots of 2 ml were taken from each plant extract solution, stirred for 30 seconds, and

briskly agitated. The setups were then allowed to settle for 15 minutes. The presence of frothing, which persists for over 15 minutes, is an indication of the presence of saponins in the tested sample

2.3.2. Test for Alkaloids

About 2 g of each of the studied plant extracts was added to 10 ml of 0.1 M hydrochloric acid, warmed in a waterbath (50°C) for 5 minutes, and filtered through Whatman filter paper No. 1. After cooling, 3 drops of Dragendorff's reagent were added and mixed. The appearance of a reddish-brown color is a positive indication for the presence of alkaloids in the sample

2.3.3. Test for Terpenoids

Into clean test tubes, 2 ml of alcoholic extracts were mixed with 5 drops of acetic anhydride. Thereafter, 5 drops of concentrated sulphuric were carefully added through the side of the test tube. The formation of a blue ring at the interface shows the presence of terpenoids in the tested sample.

2.3.4. Test for Flavonoids

To 2 ml of alcoholic extracts of the studied plants and 5 drops of concentrated hydrochloric acid were added. The formation of a red color indicates the presence of flavonoids. To another portion of the alcoholic extracts (2 ml), 1 ml of dilute ammonia was added and gently mixed. A greenish-yellow color indicates the presence of flavonoids.

2.3.5. Test for Cardiac Glycosides

To test for cardiac glycosides presence, 0.5 g of the extract was dissolved in 2 ml glacial acetic acid containing 2 drops of 10% ferric chloride solution. One milliliter of concentrated H_2SO_4 was then slowly introduced into the underlying mixture. Appearance of either a violet band at the boundary is a positive test for the deoxy sugars (cardenolides) .

2.3.6. Test for Steroids

The presence of steroids in the studied plant extracts was determined in this study. About 0.5 g of each extract was dissolved in 2 ml of chloroform. This was followed by addition of 3 drops of the Liebermann–Burchard reagent and gently agitated. The presence of reddish-purple color indicates the presence of steroids

2.3.7. Test for Phenols

About 0.5 g of each of the studied plant extracts was boiled in 5 ml of 70% ethanol in a water bath for 5 minutes and then filtered through Whatman filter paper No. 1. After cooling, 5 drops of 5% ferric chloride were added and mixed. The appearance of a green precipitate indicates the presence of phenols in the sample .

2.4. Determination of In Vitro Antioxidant Activities of the Studied Plant Extracts

2.4.1. Ferric Reducing Antioxidant Power Assay:

The reducing power of the extracts was determined according to the method described by Oyaizu with some modifications. Briefly, five different concentrations of methanolic extracts (0.2, 0.4, 0.6, 0.8, and 1 mg/ml) and L-ascorbic acid at same concentrations were mixed with 2 ml phosphate buffer (0.2 M, pH 6.6) and 2 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50°C for 20 minutes. Then, 2 ml of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 1000 revolutions per minute (rpm) for 10 min. The supernatant (2 ml) was aspirated and mixed with 2 ml of distilled water and 1 ml of 0.1% ferric chloride ($FeCl_3$). In each case, the experiment was performed in triplicate. Afterward, the absorbances were measured spectrophotometrically at 700 nm using a UV-vis

spectrophotometer and recorded. The concentrations of each extract able to yield an absorbance value of 0.5 were determined from the graph of absorbance at 700 nm against extract concentrations and considered as the median effective concentration (EC50).

2.4.2. Determination of 1,1, dipheny-2-picrylhydrazyl (DPPH) Radical Scavenging Activities:

The DPPH radical scavenging assay was performed using 1,1 diphenyl-2-picrylhydrazyl (DPPH) according to the method described by Brand-Williams et al. [18] with some modifications. Briefly, five different concentrations of the studied plant extracts (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml) were prepared in methanol (analytical grade). The same concentrations were also prepared for L-ascorbic acid, which was used as a standard antioxidant. 1 ml of each studied extract was transferred into a clean test tube into which 0.5 ml of 0.3 mM DPPH in methanol was added. The mixture was shaken and left to stand in the dark at room temperature for 15 minutes. Blank solutions comprising of the studied extract solutions (2.5 ml) and 1 ml of methanol were used as baseline.

The negative control comprised 2.5 ml of DPPH solution and 1 ml of methanol, while L-ascorbic acid at the same concentrations as the studied extracts was used as the positive control. After incubation in the dark, the absorbance values were measured at 517 nm using a spectrophotometer. The experiments were performed in triplicate. The DPPH radical scavenging activity was estimated using the equation described by Brand-Williams et al.

2.4.3. Hydroxyl Radical Scavenging Activities

The hydroxyl radical scavenging activity was performed as per the method described by Klein et al. [19] with minor modifications. The reaction mixture was constituted by adding 2.4 ml of phosphate buffer (pH 7.8) into test tubes. To the same test tubes, 90 μl of 1 mM 1, 10 phenanthroline, 150 μl of 0.1 mM hydrogen peroxide, 60 μl of 1 mM iron (III) chloride, and 1.5 ml of the Phytexponent and the standard (L-ascorbic acid) at different concentrations (100%, 10%, 1%, 0.1%, and 0.01%) were added except in the controls, followed by incubation at room temperature for 5 minutes. The increase in absorbance at 560 nm was measured, and radical scavenging activity was calculated using the following formula:

% Radical scavenging activity = [Abs of control - Abs of sample] / Abs of control × 100

2.5. Determination of Total Phenolic Contents

The total phenolic content of the extracts was measured according to the Folin-Ciocalteu method adapted from Do et al. [20], with some modifications. Briefly, the extract (1 ml) was mixed with 2 ml of Folin-Ciocalteu reagent, which was prepared by dilution with distilled water in a ratio of 1 : 10 v/v, after which 1 ml of 20% sodium carbonate (Na_2CO_3) was added. The mixture was shaken for 20 seconds and incubated at 40°C for 30 minutes. Absorbance was measured at 765 nm. Gallic acid was used for the generation of the standard curve. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram (g) of the studied extracts.

2.6. Determination of Total Flavonoid Contents

The total flavonoid content of the extracts was evaluated through a technique described by Park et al. [21]. In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of $NaNO_2$(0.5 M), and 0.15 ml of $AlCl_3 \cdot 6H_2O$ (0.3 M) were mixed. After 5 minutes, 1 ml of NaOH (1 M) was added and mixed well, and the absorbance was measured against the reagent blank at 510 nm. The standard curve for total

flavonoids prepared using quercetin standard solution (0–100 mg/l). The total flavonoids were expressed as milligrams of quercetin equivalents per g of sample.

Results:

Phytochemicals	Qualitative analysis of *Euphorbia hirta* extract
Flavonoids	+
Phenols	+
Saponins	+
Alkaloids	+
Terpenoids	+
Cardiac glycosides	+
Steroids	-

Table 1 - In vitro Phytochemical analysis of *Euphorbia hirta*

Table 1 infers about the presence of various phytochemicals like flavonoids, phenols, terpenes, saponins, alkaloids, cardiac glycosides indicating a therapeutic potential, since they are useful in protecting plants from harmful microbes and extreme temperatures as well. The main rationale for considering the phytochemicals of *E. hirta* in the investigation was its reported efficacy against respiratory disorders. It is very promising to investigate the phytochemicals of *E. hirta* for their potential efficacy against diseases, such as COVID-19, that also target the respiratory system.

Concentration	L- ascorbic acid	Plant extract
0.2	0.35	0.32
0.4	0.86	0.76
0.6	1.22	1.10
0.8	1.98	1.75
1	2.50	2.33

Table-2 Antioxidant activity of *Euphorbia hirta*

Table 2 indicates the antioxidant activity of *E.hirta*, to assess the effectiveness and safety of using this for medicinal purposes.

Concentration	**L- ascorbic acid**	**Plant extract**
0.250	55	45
0.125	43	41
0.25	40	40
0.5	35	29
0.1	29	22

Table 3 - DPPH scavenging activity (% inhibition)

[DPPH- 2,2 Diphenyl-1-picrylhydrazyl]

Table 3 indicates the DPPH scavenging activity of plant extract. It is used for the measurement of antioxidant properties , for assessing the potential of substances to serve as free radical scavengers.

Concentration	**L- ascorbic acid**	**Plant extract**
0.250	86	80
0.125	75	71
0.25	65	55
0.5	59	46
1	48	44

Table 4 infers the safety index of using the plant extract since hydroxyl radical is the most reactive oxygen species and causes damage to biomolecules.

Table 4 - Hydroxyl scavenging activity (% inhibition)

Sample	**TPC(mgGAE/G)**	**TFC (mgQE/G)**
Plant extract	44	38

Table 5 - Total flavonoid and phenolic contents in *Euphorbia hirta* plant extract

TPC, total phenolic content; mgGAE/g , milligrams Gallic acid equivalent per gram of sample.

TFC, total flavonoid content; mgQE/g, milligrams of quercetin equivalent per gram of sample.

Table 5 gives information about total phenolic and flavonoids contents in plant aiding in calculation of the amount of antioxidants in plant extract to be used for medicinal purposes or future research.

The findings stemming from the in vitro exploration of the phytochemical composition and antioxidant potential of *Euphorbia hirta*, particularly its methanolic leaf extracts, are indeed noteworthy. The research methodology encompassed immersing 400g of powdered plant material in 1 liter of high-quality analytical grade methanol, initiating a 48-hour incubation period at room temperature. The subsequent steps, including filtration through Whatman filter paper No. 1, concentration using a rotary evaporator and hot-air oven, and subsequent storage, were crucial in the meticulous preparation of the extracts. The qualitative phytochemical screening tests performed on the extracts affirmed the presence of a diverse array of phytochemical groups. This comprehensive analysis provided insights into the rich chemical composition of the methanolic leaf extracts, unveiling the existence of bioactive compounds within *Euphorbia hirta*.

These included the ferric reducing antioxidant power assay, the DPPH radical scavenging assay, and the hydroxyl radical scavenging assay. Additionally, the Folin-Ciocalteu method was utilized to quantify the total phenolic and flavonoid contents, providing a more nuanced understanding of the extract's antioxidant potential.

The obtained data underwent rigorous statistical analysis to derive meaningful insights. The antioxidant activity, expressed as the IC50 value, represented the concentration required to scavenge 50% of free radicals. The results emanating from this analysis unequivocally showcased the significant antioxidant potential inherent in the methanolic leaf extract of *Euphorbia hirta*. This robust antioxidant capacity was attributed to the presence of specific compounds, notably terpenoids and flavonoids, within the extract.

Discussion:

The in vitro phytochemical analysis and assessment of antioxidant activity of *Euphorbia hirta* have yielded significant insights into the potential health-promoting properties of this plant. *Euphorbia hirta*, commonly known as "asthma weed" or "garden spurge," is a plant widely distributed in tropical and subtropical regions.(Chen et al. 2015). In vitro anti-inflammatory activity of fractionated *Euphorbia hirta* aqueous extract on rabbit synovial fibroblasts. Biomedical journal. herbaceous plant has been traditionally utilized in various folk medicines for its purported therapeutic properties.The phytochemical analysis of *Euphorbia hirta* extracts revealed the presence of various bioactive compounds, including alkaloids, flavonoids, phenols, tannins, and saponins. These compounds have been extensively studied for their diverse biological activities and are often associated with the medicinal properties of plants. The identification and quantification of these phytochemicals provide a foundation for understanding the potential therapeutic effects of *Euphorbia hirta*.

Alkaloids, known for their pharmacological significance, may contribute to the reported anti-inflammatory and analgesic properties of *Euphorbia hirta*. Flavonoids, on the other hand, are recognized for their antioxidant, anti-inflammatory, and antiviral activities. The presence of phenols and tannins suggests potential wound-healing properties, as these compounds are implicated in tissue repair and have antimicrobial effects. Saponins, with their foaming and emulsifying properties, may contribute to the traditional uses of Euphorbia hirta in managing respiratory conditions.(Tuhin et al. 2017)

The diversity of identified phytochemicals underscores the complexity of *Euphorbia hirta's* chemical composition and highlights its potential for multifaceted therapeutic applications. These findings align with traditional uses of the plant in various medicinal systems, providing a scientific basis for its folkloric reputation.(Ahmad et al. 2006)

The evaluation of antioxidant activity is a pivotal aspect of understanding the health benefits associated with *Euphorbia hirta*. Oxidative stress, resulting from an imbalance between free radicals and antioxidants, is implicated in numerous diseases. The in vitro assays conducted in this study, such as the DPPH radical scavenging assay and the FRAP assay, demonstrated the ability of *Euphorbia hirta* extracts to neutralize free radicals and act as reducing agents.(Ragasa and Cornelio 2013)

The observed antioxidant activity aligns with the presence of flavonoids and phenols, which are well-known for their free radical scavenging properties. The DPPH assay, measuring the ability to donate hydrogen atoms or electrons to stabilize free radicals, provides a quantitative measure of the plant's antioxidant potential. Additionally, the FRAP assay, assessing the ability to reduce ferric ions, offers insights into the reducing power of *Euphorbia hirta* extracts.(Marickar et al. 2014)

These antioxidant properties have significant implications for human health. The ability of *Euphorbia hirta* to combat oxidative stress suggests its potential in preventing or mitigating conditions associated with free radical damage, including cardiovascular diseases, neurodegenerative disorders, and certain cancers. Moreover, antioxidant-rich plants are increasingly recognized for their role in promoting overall well-being and longevity. (Tuhin et al. 2017; Hu et al. 2021)

The identified phytochemicals and antioxidant properties of *Euphorbia hirta* also hold promise for drug development. The plant's ability to yield compounds with anti-inflammatory, antimicrobial, and antioxidant activities suggests its potential in the development of pharmaceutical agents. Isolation and characterization of specific bioactive compounds from *Euphorbia hirta* could pave the way for the synthesis of novel drugs or the formulation of plant-based medicines.(Rajurkar and Hande 2011)

The findings of this research align with and contribute to the existing body of literature on *Euphorbia hirta*. Previous studies have also reported the presence of alkaloids, flavonoids, and phenolic compounds in *Euphorbia hirta* extracts(Smeriglio et al. 2021). However, variations in the types and concentrations of these phytochemicals may be attributed to factors such as geographic location, climate, and extraction methods. The consistent observation of antioxidant activity in various studies underscores the robustness of *Euphorbia hirta's* potential in this regard. Furthermore,clinical studies have to be done to proceed with the studies on pharmaceutical level.

Conclusion:

In conclusion, the in vitro phytochemical analysis and antioxidant assessment of *Euphorbia hirta* have illuminated its potential as a source of bioactive compounds with therapeutic implications. The presence of diverse phytochemicals aligns with traditional uses of the plant, providing a scientific basis for its ethnomedicinal applications. The observed antioxidant activity further underscores its potential in combating oxidative stress-related conditions.

These findings contribute to the growing body of knowledge on *Euphorbia hirta* and offer a foundation for future research directions. The potential integration of *Euphorbia hirta* into modern healthcare practices, either as a traditional remedy or as a source of pharmaceutical agents, warrants continued exploration through rigorous scientific investigation. As research progresses, bridging the gap between traditional knowledge and contemporary scientific validation will be crucial in harnessing the full therapeutic potential of *Euphorbia hirta* for the benefit of global health.

Conflict of interest :

The author declares that there are no conflicts of interest.

Ethical clearance :

Since it is an in Vitro study ethical clearance is not required

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