IJP (2025), Vol. 12, Issue 1 **(Research Article)**

Received on 14 January 2025; received in revised form, 28 January 2025; accepted, 30 January 2025; published 31 January 2025

EVALUATION OF PHYTOCHEMICAL COMPOSITION AND ANTI-MICROBIAL PROPERTIES OF HERBAL PLANT

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Keywords:

Phytochemical, Antimicrobial Activity, *Euphorbia hirta*, Flavonoids, Antibiotics

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ABSTRACT: This study explores the phytochemical composition and antimicrobial potential of *Euphorbia hirta*, a medicinal plant known for its therapeutic properties. Phytochemical screening of its aerial parts revealed the presence of bioactive compounds such as alkaloids, flavonoids, saponins, terpenoids, steroids, and sterols. Methanol extracts were evaluated for antimicrobial activity using the agar cup-plate diffusion method, demonstrating significant inhibitory effects against *Escherichia coli*. These findings highlight the plant's natural antimicrobial properties, suggesting its potential as a valuable resource for addressing bacterial infections. Our findings suggest that *Euphorbia hirta* holds promise as a natural antimicrobial agent and Isolated Flavonoid from *E. hirta* Extracts shows maximum antimicrobial activity as compared with the whole extract of *Euphorbia hirta*. The study underscores the importance of *Euphorbia hirta* in the development of herbal-based therapeutics. Future research will focus on isolating and characterizing the active compounds responsible for its antimicrobial effects. Understanding the mechanisms of action of these compounds could lead to the creation of novel, plant-derived antibacterial agents, offering an alternative to conventional antibiotics in combating resistant bacterial pathogens.

INTRODUCTION: Medicinal plants have been a vital part of traditional healthcare, especially in developing countries, for thousands of years. These plants contain bioactive compounds that have been used to create numerous drugs. According to the World Health Organization (WHO), over 80% of the global population relies on plant-based medicines for primary healthcare. Phytomedicines, derived from these plants, are valued for being safe, effective, and environmentally friendly, with their use dating back to pre-recorded history.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.IJP.12(1).22-26

Article can be accessed online on:
www.ijpjournal.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.IJP.12(1).22-26

Infectious diseases are still the leading cause of death worldwide, and the rise of antibiotic resistance has become a major concern. Many of the antibiotics we rely on are losing their effectiveness as pathogens evolve resistance. Herbal remedies, however, have long been used to treat these diseases, and recent research is shedding light on the potential of plant compounds to help fight infections. Secondary metabolites in plants, which were once not well understood, are now being studied for their medicinal properties.

These natural compounds, found in various parts of the plant, include flavonoids, alkaloids, and tannins, and have shown promise as antimicrobial agents. Phytomedicines have gained increasing popularity as a result of their broad range of therapeutic benefits. Plants naturally produce a variety of bioactive molecules, known as

phytochemicals, which help protect them from environmental stressors. These compounds vary in type and concentration depending on factors like climate, plant species, and age. Over the last two decades, there has been a surge of interest in exploring these phytochemicals as potential sources of new medicines, particularly in the pharmaceutical industry. As a result, many new plant-based drugs have been discovered and are now undergoing commercial screening.

One plant that has shown promise is *Euphorbia hirta*, a small herb found in tropical regions. Known for its traditional use in treating respiratory issues, this plant has also been studied for its antimicrobial properties. While research on its antifungal activity is more established, there is growing interest in exploring its effects against bacteria as well. **Euphorbia hirta** contains several bioactive compounds, including flavonoids, which may contribute to its ability to fight infections. Despite its potential, much more research is needed to fully understand its pharmacological effects and unlock its full therapeutic potential.

MATERIALS AND METHODS:

Materials:

TABLE 1: RAW MATERIAL

Sr. no.	Ingredients	Uses
1.	*Euphorbia hirta* Extract	Anti-microbial Agent
2.	Fractional Flavonoidal content of *Euphorbia hirta* Extract	Anti-microbial Agent

TABLE 2: CHEMICALS/ REAGENTS

Sr. no.	Chemicals/ Reagents
1.	Methanol
2.	Petroleum Ether
3.	Aluminum Chloride
4.	Sodium Hydroxide
5.	Ethyl Acetate
6.	Dil. Ammonia
7.	HCL
8.	Peptone
9.	Beef Extract
10.	Agar
11.	Sodium Chloride
12.	Dichloromethane
13.	Citric acid
14.	Citric Nitrite
15.	Chloroform
16.	Distilled Water
17.	Quercetin

TABLE 3: INSTRUMENTS/ ACCESSORIES

Sr. no.	Instruments/ Accessories
1.	Electronic Balance
2.	Soxhlet Apparatus
3.	Water Bath
4.	Sonicator
5.	Hot Air Oven
6.	Colorimeter
7.	Laminar Air Flow
8.	Incubator

Methods:

Collection of Plant Material: The plant of *Euphorbia hirta* were freshly collected from Aayurvan BKC Garden, Sawantwadi, Maharashtra, India in JULY &AUGUST 2024.

Authentication of Plant Material: The collected plant material was identified and authenticated by Department of Botany, S.P.K College, Sawantwadi, India.

Processing of Plant Material: The plant material was washed under running tap water and dried under shade. Dried plant material was coarsely powdered and stored in neatly labelled air tight container till the future use.

Determination of Extractive Value: Weighed accurately 4grams of sample was macerated with 100ml of alcohol in conical flask for 24 hour, with frequent shaking at an interval of 6 hours. It was then allowed to stand for 18 hours and filtered rapidly to prevent any loss during evaporation. 25ml of filtrate was evaporated until it dries in a porcelain dish and dried at 105°C to a constant weight. The percentage of alcohol soluble extract was calculated with reference to air-dried material.

Extraction Method: Coarsely powdered plant material was subjected to cold maceration with methanol with occasional shaking. Extract was filtered through Whatman filter paper and the filtrate was collected in clean glass container. Mare was dried and subjected to Soxhlet extraction using methanol (till the colored extract become colorless) at 40°C. Extract was mixed with filtrate of cold maceration. A total extract was concentrated by using water bath. Obtained concentrated extract was dried and stored in air tight container.

Fractionation of Flavonoid: Extract the sample (crude plant material) in suitable solvent. Evaporate it at 40°c to obtain residue. The obtaining residue is

dissolved in 2 diff. parts of citric acid & dichloromethane. X gm of sample dissolved in X ml of 5% citric acid. Add 25ml of dichloromethane keep it for 10 min for separation. Separate the dichloromethane & aqueous layer in separating funnel. Evaporate both liquid to dryness. Dichloromethane residue is dissolve in petroleum Ether & 90% methanol in the ratio of (1:1). In next step we get two layers of petroleum ether and methanol. In petroleum ether consist of lipids & waxes whereas methanol layer contains phenolics, terpenes & sterols. The obtained fractions are used for further phytochemical testing.

Phytochemical Screening:

Tests for Flavonoids:

Alkaline Reagent Test: Add 5 drops of dilute sodium hydroxide (NaOH) to 2 ml of plant extract, then add diluted hydrochloric acid (HCl). If the yellow solution turns colorless, flavonoids are present.

Ammonium Test: Heat 10 ml of ethyl acetate with the extraction in boiling water for 3 minutes, then filter. Mix the filtrate with 1 ml of dilute ammonia solution, then shake. If the ammonia layer is not yellow, flavonoids are present.

Shinoda Test: Add 2-3 ml of filtrate extraction with magnesium metal, then add HCL concentrate. If the color is magenta, flavonoids are present.

Determination of Total Flavonoid Content: The total flavonoid content (TFC) in plants can be determined using a colorimetric assay with aluminum chloride. Add 100 microliters of extract to 4 milliliters of distilled water. Add 0.3 milliliters of 5% sodium nitrite.

After 5 minutes, add 0.3 milliliters of 10% aluminum chloride. After 6 minutes, add 2 milliliters of 1 M sodium hydroxide. Immediately, add 3.3 milliliters of distilled water and mix thoroughly. Determine the absorbance at 510 nm versus a blank. Similarly, the standard solution of quercetin was prepared in range of (0, 0.2, 0.4, 0.6, 0.8, 1) mg/ml. Calculate the TFC using a calibration curve.

TABLE 4: FORMULAE

Sr. no.	Equation	Explanation
1	y=0.2671x+0.348	The equation obtained from the standard QE graph, where y is the absorbance of the sample and x is the concentration of quercetin.
2	A= (c x v)/m	The equation used to determine the total flavonoid content in the sample, where A is the total flavonoid content, c is the concentration of quercetin, v is the volume of extract, and m is the mass of the extract.

Microbial Assay:

Bacterial Culture: The human bacteria *Escherichia coli* was obtained from culture collection and were maintained in Nutrient agar at 4 ºC for experiment studies.

Preparation of Standard Culture Inoculums of Test Organism: The colonies of Escherichia coli bacteria were inoculated in the 10ml nutrient broth and incubated for 24- 72 hours.

Assay of Anti-bacterial Activity: Assay of anti-microbial activity of *Euphorbia hirta* extracts, isolated flavonoids of *Euphorbia hirta* extracts and standard solution of azithromycin tablet was done by Cup-plate method. In this method 100ml of sterilized Nutrient Agar was equally poured into 3 sterile petri plates, after solidification, 120µl of bacterial culture poured on the plates and the culture was spread on plates using spreader.

Then, the Whatman's filter paper discs (6mm in diameter) were kept over the 3 agar plates using sterile forceps at various concentrations. First prepare plate containing the standard azithromycin solution, second plate containing the *Euphorbia hirta* extracts, and third one containing the isolated flavonoids of *Euphorbia hirta* extracts. The anti-bacterial assay plates were kept incubator, where all the plates were incubated at 37ºC for 24 hours. The diameter of inhibition zone was noted down.

RESULTS AND DISCUSSION:

Determination of Extractive Value: The Extractive Value (Methanol as a solvent) of the *Euphorbia hirta* was found to be 7.2 % w/w. The Standard Extractive Value of the *Euphorbia hirta* is 9.71`% w/w.

Extraction and Fractionation of Plant Material: Methanolic extract of *Euphorbia hirta* Plant was

prepared and used for further studies. Extract was dark green in color. Obtained extract was subjected to fractionation for separation of Flavonoids. Methanolic fraction was used for study the anti-microbial activity.

Phytochemical Screening: Obtained result of phytochemical screening of Isolated Flavonoids from Euphorbia are as follow;

TABLE 5: REAULTS

Sr. no.	Phytochemical Test for Flavonoids	Observations	Conclusion
1.	Shinoda test	Magenta color is observed	Flavonoids are present
2.	Alkaline reagent test	Yellow solution turns colorless	Flavonoids are present
3.	Ammonium test	Ammonia layer is not yellow	Flavonoids are present

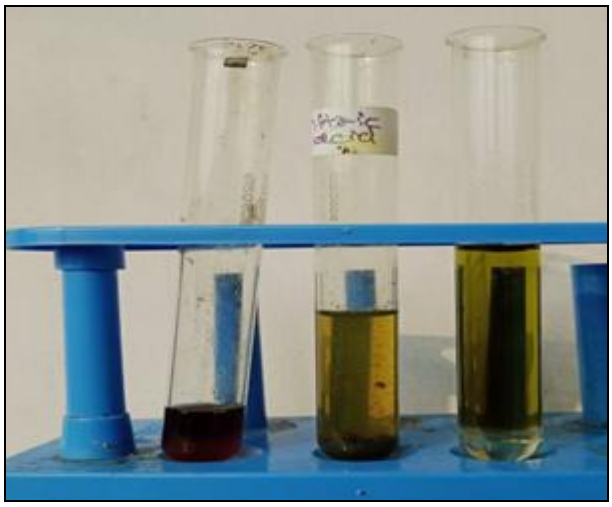

FIG. 1: PHYTOCHEMICAL TESTS

Determination of Total Flavonoid Content: The total flavonoid content of *Euphorbia hirta* was found to be 11.57 mg QE/g of sample by colorimetric assay method. The TFC of Quercetin can range from 87.53 ± 0.30 mg QE/g. Thus, the total flavonoid content (TFC) of *Euphorbia hirta* is lies between the TFC of Quercetin.

TABLE 6: OBSERVATION TABLE

Sr. no.	Concentration (mg/ml)	Absorbance
1.	0	0
2.	0.2	0.13
3.	0.4	0.14
4.	0.6	0.20
5.	0.8	0.25
6.	1	0.29
7.	Sample Extract	0.25

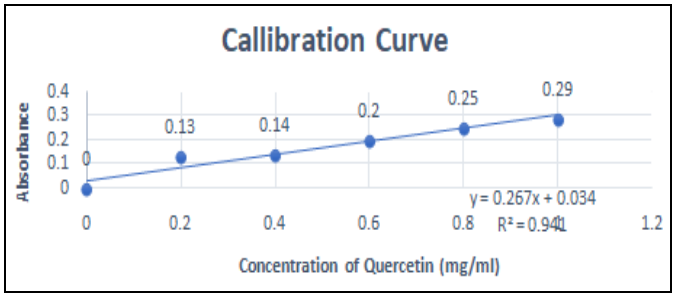

FIG. 2: CALLIBRATION CURVE

Anti-Microbial Activity: Antimicrobial activity refers to any action that destroys bacteria or inhibits their growth and reproduction. This can be achieved through heat, chemicals such as chlorine, and antibiotic drugs, all of which possess antimicrobial properties. Antimicrobial are commonly used to treat bacterial infections and are generally considered to have low toxicity in humans and other animals. However, they may have negative health effects in some cases. The Isolated flavonoid of *E. hirta* shows the highest inhibition zone (i.e. 20 mm) against the *Escherichia coli* and whole plant Extracts shows the lowest inhibition zone (i.e. 10 mm) against the *Escherichia coli*.

TABLE 7: ZONE OF INHIBITION

Sr. no.	Agar Plates containing;	Diameter of Zone of Inhibition
1.	Standard	40 mm
2.	*E. hirta* Extracts	10 mm
3.	Isolated Flavonoid from *E. hirta* Extracts	20 mm

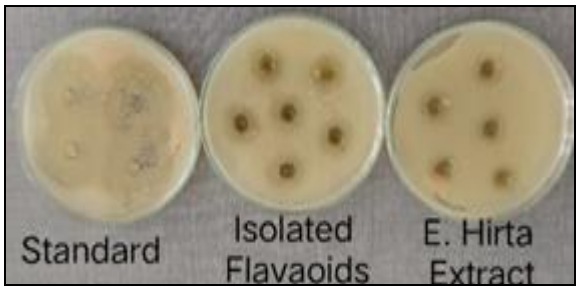

FIG. 3: ZONE OF INHIBITION

CONCLUSION: Our study revealed that the leaves of *Euphorbia hirta* contain significant amounts of alkaloids and flavonoids, along with smaller quantities of terpenoids, saponins, tannins, and carbohydrates. This indicates that these plant parts can be an important source of phytochemicals with antimicrobial potential. In terms of antimicrobial activity, the plant showed significant

antimicrobial effects against selected Gram-negative *E. coli* bacterial strains. Our findings suggest that *Euphorbia hirta* holds promise as a natural antimicrobial agent and Isolated Flavonoid from *E. hirta* Extracts shows maximum antimicrobial activity as compared with the whole extract of *Euphorbia hirta*.

ACKNOWLEDGEMENT: Authors are thankful to the Head of Department of Botany, University of Mumbaiand Yashwantrao Bhonsale College of Pharmacy, Sawantwadi for providing all necessary facilities for present work.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Thakur AL, Jagtap VA, Salunkhe OP, Shirodkar BS, Shetkar AV, Pednekar SN and Gawade SC: Evaluation of phytochemical composition and anti-microbial properties of herbal plant. Int J Pharmacognosy 2025; 12(1): 22-26. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.12(1).22-26.