IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Evaluation of Antioxidant Properties and Phytochemical Analysis of extracts from *Euphorbia hirta* L.

Sadanand V. Aithal[1], Vishwamber A. Tidke[2], Vithal K. Jadhav[2]

[1] *Department of Botany, V.D.M.D. College, Degloor, Nanded-431717 Maharashtra*

[2] *Departments of Chemistry, V.D.M.D. College, Degloor, Nanded-431717 Maharashtra*

Abstract

This study aimed to evaluate the antioxidant properties of various *Euphorbia hirta* (*E. hirta*) plant parts and to identify new, safe, and cost-effective antioxidant sources. Methods: The research involved analyzing the total phenolic and flavonoid contents, as well as the in vitro antioxidant capacity, of *E. hirta* leaves, stems, flowers, and roots. Antioxidant activity was assessed using the diphenyl-1-picrylhydrazyl (DPPH) scavenging method, and the reducing power was determined. Results: Among the tested samples, the leaf extract showed the highest DPPH radical scavenging activity (60.96±0.44) %, surpassing the activities of the flower, root, and stem extracts, which displayed scavenging activities of (54.65±0.52) %, (50.52±0.97) %, and (42.46±0.74) % respectively. The activity of the standard antioxidant, ascorbic acid was (68.25±0.52) %. The IC_{50} values for the leaf, flower, root, and stem extracts were 0.723, 0.602, 0.459, and 1.118, mg/mL, respectively. The total phenolic content was highest in the leaf extract [(195.16±1.25) mg GAE/g], followed by the extracts from flowers, roots, and stems, which had values of (102.08±2.10) mg GAE/g, and (69.50±1.22) mg GAE/g, respectively. Similarly, the total flavonoid content was highest in the leaf extract [(33.970±0.521) mg CEQ/g], flower, root, and stem extracts showing lower values of (34.200±1.102) mg CEQ/g, (28.220±1.106) mg CEQ/g, and (19.180±0.004) mg CEQ/g, respectively. HPTLC analysis identified phenolic compounds, and phytochemical screening of the *E. hirta* leaf extract confirmed the presence of various bioactive compounds including reducing sugars, terpenoids, alkaloids, steroids, flavonoids, and phenolic compounds. Conclusion: The study underscores the potent antioxidant capacity of *E. hirta*, especially its leaves, highlighting its potential as a source of natural antioxidants for various applications.

Keywords- *E. hirta*, DPPH, GAE, Phenolic, Flavonoids compounds**.**

Introduction

Nowadays, there is a significant interest in antioxidants, particularly those capable of mitigating the harmful effects of free radicals in the human body and preventing the spoilage of fats and other food components. This interest is driven by the role free radicals play in causing various chronic diseases, such as cancer, diabetes, aging, atherosclerosis, hypertension, heart attacks, and other degenerative conditions [1]. Free radicals are produced during bodily metabolism, and the exogenous intake of antioxidants can aid the body in effectively scavenging these radicals. The preference leans towards natural sources of antioxidants over synthetic ones due to concerns associated with the latter. Currently, most antioxidants are synthesized chemically; however, synthetic antioxidants have been criticized for their potential side effects in vivo [2]. Studies have shown that synthetic antioxidants like butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) can accumulate in the body, leading to liver damage and carcinogenesis [3]. Consequently, strict regulatory standards on food safety have prompted the search for safer, natural alternatives for use as food preservatives.

Euphorbia hirta (*E. hirta*) L., from the Euphorbiaceae family, is a tropical, annual herb that can grow up to 90 cm tall, with a slender, hairy, and extensively branched stem. Its leaves are opposite, elliptical to oblong-lanceolate, with serrated edges and a darker top surface. The plant produces small, densely clustered flowers in the upper leaf axils and secretes a white sap when cut. *E. hirta* commonly thrives in disturbed areas like wastelands, watercourse banks, grasslands, and along roadsides and pathways [5,6,7].

E. hirta is highly valued in traditional medicine for its broad medicinal uses. Typically prepared as a decoction or infusion, it treats a wide range of conditions including gastrointestinal disorders, respiratory issues, urinary and reproductive system ailments, skin and mucosal infections, and provides pain relief for various complaints including headaches and rheumatism. It also offers antiseptic benefits and remedies for insect stings and bites [8]. This study focuses on exploring the phytochemical and antioxidant properties of *E. hirta* extracts, aiming to enhance our knowledge of its therapeutic potential.

Materials and methods

Plant collection

The fresh plant was collected from our campus V.D.M.D. College, Degloor, after washing; it was divided into leaves, flowers, stems, and roots, then shade-dried for ten days and oven-dried at 60 ℃ for 1-2 days. The dried parts were powdered using an electric blender and stored in labelled airtight bottles.

Preparation of the plant extract

Each part is powdered (100 grams) underwent maceration in 400 mL of methanol for 14 days with regular stirring. The resulting mixture was filtered through clean muslin cloth, then double-filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator under vacuum at 50 ℃, poured into glass Petri dishes, and dried in a 60 ℃ oven. The percentage yield of the crude extract was determined for each part, resulting in yields of 12.1%, 6.3%, 5.7%, and 4.8% for leaves, stems, flowers, and roots, respectively.

Diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay was employed to evaluate the free radical scavenging activity of *E. hirta* L, following the protocol outlined by Basma et al [9]. Plant extract solutions ranging from 0.031 to 2 mg/mL were mixed with 5 millilitres of 0.004% DPPH radical solution and incubated at room temperature in the dark for 30 minutes. The optical density (OD) was measured at 517 nm using a UV/Vis spectrophotometer. Methanol served as the blank, while a mixture of methanol and DPPH solution represented the baseline control (A_0), with Ascorbic acid used as the positive control. The scavenging effect (%) = (A_0 - A_1) x 100%/ A_0, where Ao is the absorbance of the control reaction and A_1 is the absorbance in the presence of the tested extracts. The IC_{50} (concentration providing 50% inhibition) was determined graphically from a calibration curve plotting extract concentration against scavenging effect within the linear range.

Determination of total phenolic content

The total phenolic content of *E. hirta* extracts was assessed using the Folin-Ciocalteu reagent, following the procedure [12]. In test tubes, 200 µL of plant extract (three replicates of 1.0 mg/mL) were combined with 500 µL of 10% Folin-Ciocalteu reagent, 500 µL of distilled water, and 800 µL of 7.5% saturated aqueous sodium carbonate (Na_2CO_3). After thorough mixing, the tubes were incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 755 nm using a spectrophotometer with distilled water serving as the blank. A standard calibration curve was generated using gallic acid (0-250 mg/L). The total phenolic content was expressed as Gallic acid equivalent per gram of dry weight (mg GAE/g) of extracts.

High performance thin layer chromatography (HPTLC) study of phenol and DPPH: -HPTLC was conducted using a silica gel aluminium sheet (20 cm × 20 cm, Silica gel 60 F254, Merck, Germany). The leaf extract, dissolved in methanol (10 mg/mL), was applied to a 3 cm × 7 cm section of the sheet. After drawing a baseline, the plant extract was spotted onto the baseline and allowed to air dry. The TLC plates were developed using an ethyl acetate/hexane (1:3) solvent mixture, and the solvent front was drawn. The developed plates were examined under a UV/Vis lamp, and spots were circled. Additionally, the plates were stained with Folin-Ciocalteu's reagent to visualize spots with phenolic content, appearing as blue spots against a yellow background.

For antioxidant activity determination, the developed silica gel sheets were dried and sprayed with a 0.04% solution of DPPH in 80% methanol. The spots indicating antioxidant activity were observed and analyzed. The findings revealed the highest phenolic content in leaves, with a value of (206.17±1.95) mg GAE/g, followed by flowers, roots, and stems extracts, which measured (117.08±3.10) mg GAE/g, (83.15±1.19) mg GAE/g, and (65.70±1.72) mg GAE/g, respectively. Furthermore, the leaves exhibited the highest flavonoid content, with (37.970±0.003) mg CEQ/g, followed by flowers, roots, and stems extracts, which measured (35.200±0.002) mg CEQ/g, (24.350±0.006) mg CEQ/g, and (24.120±0.004) mg CEQ/g, respectively [13].

HPTLC analysis of phenolic and antioxidant substance

In the HPTLC bioautography analysis, seven spots were detected under visible and ultraviolet lights, with retention factors (Rf values) of 0.1, 0.2, 0.4, 0.70, 0.85, 0.9, and 0.92, respectively. Upon examination for DPPH active spots, pale-yellow colored spots against a purple background indicated the presence of antioxidant substances. The first three spots near the baseline (Rf values: 0.1, 0.2, 0.4) appeared as a yellow smear rather than distinct spots. Additionally, three faint yellow spots were observed at the top (Rf = 0.75, 0.92, 0.96). In contrast, plates stained with Folin-Ciocalteu reagent displayed several blue-colored phenolic active spots against a yellow background. Notably, the spots that appeared blue with Folin-Ciocalteu reagent appeared yellow when sprayed with DPPH [13].

Phytochemical screening

The qualitative screening of phytochemical compounds [15-18]in *E. hirta* revealed the presence of reducing sugars, terpenoids, alkaloids, steroids, tannins, flavonoids, and phenolic compounds.

Results and Discussion

Recent years have witnessed resurgence in interest in plant antioxidants, possibly prompted by the emergence of adverse effects associated with certain commercial antioxidant products. Within the realm of medicinal plants, a vast array of bioactive compounds exhibit antioxidant properties, effectively terminating free radical chain reactions. In this study, we assess *E. hirta* as a novel antioxidant agent through various in vitro antioxidant tests.

The DPPH radical, a free radical, undergoes reduction by accepting an electron or hydrogen radical, resulting in the formation of a stable diamagnetic molecule. Antioxidants induce a decrease in the absorbance of DPPH at 517 nm, leading to its reduction to a pale-yellow colour through hydrogen atom abstraction from the antioxidant compound [21-28]. The extent of DPPH reduction correlates with the abundance of antioxidants in the extract, indicating higher scavenging activity in samples with greater antioxidant content. The IC_{50} value, representing the amount of antioxidant required to decrease the initial DPPH concentration by 50%, serves as a measure of antioxidant activity. A lower IC_{50} value indicates higher antioxidant activity. Notably, E. hirta leaf extract exhibited the lowest IC_{50} value, indicating the highest antioxidant activity. Additionally, the reducing power assay was employed to assess the antioxidant capacity of the extract. Appear as yellow spots on purple background.

DPPH radical scavenging activity (60.96±0.44) %, surpassing the activities of the flower, root, and stem extracts, which displayed scavenging activities of (54.65±0.52) %, (50.52±0.97) %, and (42.46±0.74) % respectively. The activity of the standard antioxidant, ascorbic acid was (68.25±0.52) %. The IC_{50} values for the leaf, flower, root, and stem extracts were 0.723, 0.602, 0.459, and 1.118, mg/mL, respectively. The total phenolic content was highest in the leaf extract [(195.16±1.25) mg GAE/g], followed by the extracts from flowers, roots, and stems, which had values of (102.08±2.10) mg GAE/g, and (69.50±1.22) mg GAE/g, respectively. Similarly, the total flavonoid content was highest in the leaf extract [(33.970±0.521) mg CEQ/g], flower, root, and stem extracts showing lower values of (34.200±1.102) mg CEQ/g, (28.220±1.106) mg CEQ/g, and (19.180±0.004) mg CEQ/g, respectively.

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