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Original Article

Pharmacognostic Evaluation of Aerial Parts of *Euphorbia tirucalli*

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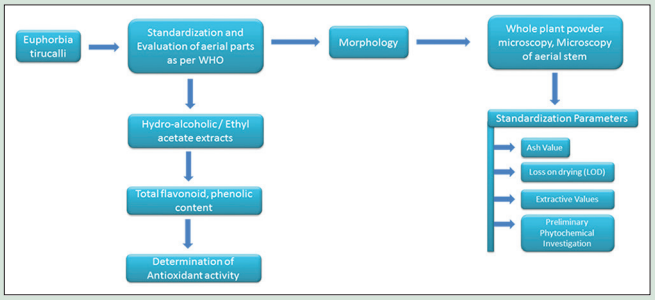
ABSTRACT

Objectives: This study aimed to establish the pharmacognostic profile of aerial parts of *Euphorbia tirucalli* (ET) as per World Health Organization guidelines for ensuring the quality and identification of adulteration. **Materials and Methods:** Standardization parameters such as macroscopic and microscopic characteristics of the study plant were evaluated. Hydroalcoholic and ethyl acetate extracts were prepared and subjected to preliminary phytochemical screening. Further, the extracts were used to analyze total phenol and flavonoid contents, and their antioxidant activities were estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide assay. **Results:** Shape, size, color, odor, surface characteristics, and microscopic images exhibited useful diagnostic characteristics of aerial parts of plants. Total ash, water-soluble, and acid-insoluble ash were found to be 16.65% ± 1.050%, 5.623% ± 1.11%, and 2.56% ± 0.706, respectively. The loss on drying was 9.6%, and water and alcoholic extractive values were 4.0% and 26%, respectively. Phytochemical screening revealed the presence of saponins, steroidal triterpenoids, phenols, and flavonoids. Total flavanoid and phenol content in hydroalcoholic and ethyl acetate extracts of ET was found to be 246 mg rut/g, 120 mg rut/g, 81.36 mg gallic acid equivalent (GAE)/g, and 279.58 mg GAE/g respectively. 2,2-diphenyl-1-picrylhydrazyl and nitric oxide scavenging assay revealed the IC_{50} values of hydroalcoholic and ethyl acetate extracts as 69.599 µg/ml, 20.454 µg/ml, 17.017 µg/ml, and 17.562, respectively. **Conclusion:** The findings obtained from the present study help to authenticate and establish the pharmacopeia standards for the ET plant.

Key words: 2,2-diphenyl-1-picrylhydrazyl, ethyl acetate extracts, *Euphorbia tirucalli,* flavonoid content, hydroalcoholic, nitric oxide assay, phenol

SUMMARY

People throughout the world have started focusing on herbs and herbal products in the health-care system. The alternative use of natural products has resulted in growth and interest in the traditional system of medicine. The growing focus on the importance of medicinal plants has driven our researchers to use one of the important herbs (*Euphorbia tirucalli* [ET]) in the traditional health-care system for combating diseases. The WHO has prescribed a set of specified guidelines for standardizing herbs and herbal products, and their authentication is necessary to maintain their quality and ensure their safe use. In the present work, the aerial parts ET were considered for standardization based on WHO guidelines and also to measure the antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide scavenging assay concerning the amount of flavonoid and phenol content present in ethyl acetate and hydroalcoholic extracts of ET. The macroscopical observation was in concordance with the available literature, and The transverse section of the stem exhibited small notches at some places due to sunken stomata. Distinct, conjoint, collateral, and open type of vascular bundles. Enlarged epidermis and cortex region with stone cells and cortex region showing fibers. Powder microscopy of the aerial parts of ET showed the presence of laticiferous cells with stomata, pitted reticulate vessels, latex, trichomes, and annular ring vessels. Acid-insoluble and water-soluble ash values were 2.56 ± 0.706 and 5.623 ± 1.1, respectively. Loss on drying was 9.6%, and the extractive value was 4.0% and 26% for water and alcohol, respectively. The %yield of the hydroalcoholic extract was found to be 8.076% and that of ethyl acetate extract was 4.73%. Phytochemical investigations showed the presence of flavonoids, phenols, carbohydrates, saponins, steroids, and triterpenoids. Total flavonoid and phenol contents were measured using hydroalcoholic and ethyl acetate extracts and the standards used were rutin and gallic acid. The hydroalcoholic and ethyl acetate extracts of flavonoid and phenol content were found to be 246.6 mg rut/g, 120 mg rut/g, 81.36 mg GAE/g, and 279.58 mg GAE/g, respectively. An antioxidant activity using DPPH and nitric oxide scavenging assay was carried out and ascorbic acid was used as standard. IC_{50} values of DPPH of hydroalcoholic and ethyl acetate extracts was 69.599 and 20.455 µg/mL, respectively, and that of nitric oxide scavenging assay was estimated as 17.017 and 17.562 µg/mL, respectively. The whole standardized parameters outlined above approach the assurance to herbal quality, safety, unadulterated, and also global acceptance of herbal products as remedies for various diseases and ailments. It could also serve as a basis for proper identification of the plant material and helps the investigator to distinguish the plant from other members of the same genera. Further, the presence of flavonoid and phenol content in the extracts and its antioxidant activity suggests that the ET can be used as natural antioxidant sources. It is also designed to take up the study further to determine the antioxidant activity by *in vivo* methods and also the anticancer potential of study plants by adopting *in vitro* and *in vivo* models of cancers because it evidenced the presence of flavonoid, phenol, and antioxidant activity.



Abbreviations Used: ET: *Euphorbia tirucalli*; GAE: Gallic acid equivalent; Rut: Rutin; WHO: World Health Organization; DW: Dry weight; DPPH: 2,2-diphenyl-1-picrylhydrazyl

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INTRODUCTION

For the treatment of a range of diseases, herbal drugs have been used since ancient times. Abundantly available herbs, which are comparatively cost-effective with fewer side effects, have been used as herbal medicines to treat many human ailments.[1] The raw materials used to make herbal medicines show seasonal variations such as ecotypic, genotypic, chemotypic, drying, and storage conditions.[2] The World Health Organization (WHO) has set specific guidelines for the assurance of safety, rigid quality control profiles and also specifies the parameters for standardization of herbs and herbal products and other health care.[3,4] Standardization is a tool in the quality control process with proper techniques and knowledge. Methods of standardization should take into consideration that contribute to the quality of the herbal drugs, namely the correct identity of the sample, organoleptic evaluation, pharmacognostic evaluation, quantitative evaluation (ash value, extractive values), and phytochemical test. In the present study, an attempt was made to encompass all possible information about the chemical constituents present in the aerial parts of the study plant.

The aerial parts of *Euphorbia tirucalli* (ET) have been most extensively investigated for their chemical composition and biological activities,[5] with the prevalent constituent being inganen. ET has been widely used in traditional medicine system against syphilis; has been used as an antimicrobial agent; has been used for its proteolytic activity, molluscidal activity, and larvicidal activity; is used as an laxative agent; has been used in the treatment of asthma, cough, rheumatism, cancer, epithelioma, sarcoma, and skin tumors; and its most important components have been isolated such as alcohol, eufol, alfaeuforbol, and taraxa sterol e-tirucallol.[6] Based on these evidence, its hydroalcoholic and ethyl acetate extracts were prepared and the same were subjected for qualitative preliminary phytochemical tests for identification of the category of constituents present. Further, an attempt was made to standardize these extracts for total phenol and flavonoid content. In addition to this, reports have been indicating the antioxidant potential of the herb, which is indicative of its anticancer potential,[7] therefore the antioxidant potential was measured by DPPH and nitric oxide scavenging assays. Based on the above facts and evidence, this plant has been adopted in the present study to investigate and establish the reproducible quality parameter before using the plant material for manufacturing herbal medicines.

MATERIALS AND METHODS

Aerial parts of ET were collected from Honnaiahnaroppa village, Challakere Taluk, from Chitradurga, and the same were identified, confirmed, and authenticated by Dr. V Rama Rao, Regional Ayurveda Research Institute for Metabolic Disorders.

Macroscopic examinations

The aerial parts of ET were subjected to morphological identification such as color, odor, and texture. Color, shape, and surface characters were noted by examining the plant material under diffuse daylight.

Microscopic evaluation

The thinnest possible sections of aerial parts of ET were obtained along the radial plane of a cylindrical portion of the stem; selected fine sections were placed in safranin for 3 min and then washed with 50% of alcohol; and further placed in 70%, 80%, and 90% of alcohol successively for 5 min each. They were then mounted and observed under a trinocular microscope and photographed (camera model-Magnus-Magcam-magnis Opto systems India Pvt. Ltd, Noida, Uttar Pradesh, India) with ×10 and ×40 magnifications.[8]

Powdered microscopy

The aerial parts of ET were dried and pulverized. A small quantity of the powder was soaked in 20% nitric acid overnight and washed with distilled water. The sample was mounted on a glass slide, stained with safranin, and observed under a microscope.[8]

Total ash value

The air-dried aerial parts of ET were accurately weighed (2 g) in a previously ignited tared crucible of silica. Using a maffle furnace, the dried material was ignited at 600°C until it becomes white, cooled in a desiccator, and the weight was noted. The percentage of ash with reference to the air-dried drug was calculated.

Water-soluble ash and acid-insoluble ash

Water and hydrochloric acid (25 mL) were added to the crucible containing the total ash and boiled for 5 min. The insoluble matter was collected on an ashless filter paper and washed with hot water and ignited at a temperature not exceeding 450°C. The percentage of water-soluble and acid-insoluble ash with reference to the air-dried drug was calculated.[8,9]

Loss on drying

Accurately weighed 10 g of the plant material was taken into a China dish, kept in a hot air oven at 105°C, and its weight was measured every hour until a constant weight was attained.[10] The total moisture content of crude drug was noted.

$$\text{Loss on drying } (\%)\ (\text{LOD}) = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Alcohol and water-soluble extractive value

Coarsely powdered crude drug of around 5 g was weighed and macerated in 100 mL of an iodine flask containing 70% V/V alcohol and water for the duration of 24 h with frequent shaking for 6 h and finally allowed to stand for 18 h. The solution was filtered rapidly, and the filtered solution was evaporated to dryness at 105°C in a tarred flat-bottomed Petri dish. The percentage of the alcohol-soluble extract was determined with reference to the shade-dried drug.[11]

Preparation of hydroalcoholic and ethyl acetate extracts

The instrument (FOSS scino (Suzhou) Co. State:- Suzhou country: China Sl no: 204500017) was used for extract preparation (hydroalcoholic and ethyl acetate) according to the procedure in the manual provided. Chemical tests such as Molish, Tollens, Fehling's, Barfords, Mayer's, Dragendorff's, Wagner's, Hager's, foam test, test for sterol, Salkowski's, Libermann–Burchard, ferric chloride, gelatin, chlorogenic, Shinoda, ferric chloride, mineral acid, lead-acetate, and sodium hydroxide tests wwereas performed to screen phytochemical constituents present in the study plant.[12-14]

Total flavonoid content

The total flavonoid content in the extracts was determined by aluminum chloride assay by colorimetry method.[15] The stock solution of rutin 100 μg/ml was prepared and used as standard. 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the above standard stock solution was pipetted into 10-mL volumetric flasks. Extracts of ET (hydroalcoholic and ethyl acetate) 1 mg/mL were dissolved separately and 0.5 mL in triplicate was pipetted into 10-mL volumetric flasks. To the volumetric flask of both test and standard rutin, 0.3 mL of sodium nitrite (5% $NaNO_2$ w/v) was added

and allowed to stand for 6 min followed by 0.3 mL of aluminum trichloride (10% $AlCl_3$) and incubated for 6 min at room temperature and finally, 4 mL sodium hydroxide (NaOH, 4% w/v) was added and the volume was made up to 10 mL with distilled water for standard and test extracts. All the samples were incubated at room temperature in dark for 15 min. After 15 min, the mixture turned pink whose absorbance was measured at 510 nm using an ultraviolet (UV) spectrophotometer. Methanol was used as a blank solution. The calibration curve was constructed for standard rutin and based on the test extracts' absorbance, flavonoid content was expressed as mg rutin/g dry weight (DW) (mg rutin/g DW).

$T = CV/M$ is the formula used to determine the concentration of total flavonoid compounds in the extract.[16]

Where T = Total flavonoid content in mg rutin/g DW (mg rutin/g DW) of the plant extract

C = Concentration of rutin obtained from the calibration curve

V = Volume of the extract in mL

M = Weight of the plant extract taken.

Total phenol content

The total phenolic content was estimated by Folin–Ciocalteu's method using gallic acid as standard.[17] Folin–Ciocalteu's reagent was prepared by diluting 1 volume of ready-to-use Folin–Ciocalteu reagent with 2 volumes of distilled water. Gallic acid 100 µg/mL was prepared, and 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the stock solution was pipetted into 25-mL volumetric flasks. Hydroalcoholic and ethyl acetate extracts of ET were prepared and dissolved separately to get 1 mg/mL. A volume of 0.5 mL of the above test stock extract was pipetted into 25 mL of volumetric flasks in triplicate. Methanol was prepared and considered as blank. Folin–Ciocalteu reagent (1.5 mL) was added to the volumetric flasks of test, standard, and blank and allowed to stand for 5 min. A volume of 4 mL of 20% sodium bicarbonate solution was added to the volumetric flasks and the volume was made up to 25 mL with distilled water of the test extract, standard, and blank. All the samples were incubated for 45 min at room temperature. The absorbance was measured at 765 nm using the UV spectrophotometer. The calibration curve was constructed for standard gallic acid, and the concentration of phenolics from extracts was calculated (mg/mL) from the calibration curve. The content of phenolics in the extracts was expressed in terms of GAE (mg of GA/g of extract).

The concentration of total phenolic compounds in the extract was determined by using the following formula:

$T = CV/M$

Where T = Total phenolic content in mg/g in terms of GAE

C = Concentration of gallic acid obtained from the calibration curve

V = Volume of the extract in mL

M = Weight of the plant extract taken.

Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl radical scavenging method

The DPPH assay is based on the reduction of DPPH, a stable free radical.[18,19] 3.94 mg of the DPPH was dissolved in 100 mL of methanol to get 0.1 mM DPPH. Ascorbic acid is used as a standard. Different concentrations of 5, 10, 15, 20, 25, and 30 µg/mL were prepared in triplicate by diluting with methanol of standard and plant extracts. 1 mL of each of the standard/test samples was mixed with 3 mL of DPPH, and the test tubes were kept in dark place and covered with an aluminum foil for 30 min. Methanol was taken as blank and DDPH alone as control throughout the study. Standard, control, and test extracts' absorbance was measured at 517 nm using a UV-visible spectrophotometer. (Shimadzu Corporation country: Japan, Model no: UV-1800 240V, Serial no: A116355304950 CD 1800). The % inhibition was calculated by using the following formula and compared with the values of standard ascorbic acid.

% inhibition of DPPH = $(A_0 - A_1) \times 100$

Where A_0 is the absorbance of control

A_1 is the absorbance of extract/standard.

The IC_{50} value was determined and compared with the standard ascorbic acid.

Nitric oxide radical scavenging activity

Nitric oxide scavenging assay was performed using the Griess reagent method.[20] Different concentrations of the standard (ascorbic acid) and plant extracts of 10, 20, 30, 40, and 50 µg/mL were prepared in triplicate and made up the volume to 1 mL with methanol. Exactly 0.3 mL of 10 mM sodium nitroprusside was added to 1 mL of each of the standard/plant extract. A volume of 0.5 mL of Griess reagent was added to the test tubes previously incubated for 150 min at 25°C. Methanol was used as blank and absorbance was measured at 546 nm using UV-visible spectrophotometer (Shimadzu 1800).

The % inhibition was calculated using the following formula given below:

% inhibition of NO scavenging activity = $(A_0 - A_1) \times 100$

Where A_0 is the absorbance of control

A_1 is the absorbance of extract/standard.

The IC_{50} value was determined and compared with the standard ascorbic acid.

RESULTS

Macroscopic examinations

ET is a flowering shrub or succulent tree with high branches, which can grow up to 3–5 m tall; plants are unarmed, branched terrate, and spread. Leaves are few, small, linear-oblong, and early caducous. Stems are green, are cylindrical with 0.5–2.0 cm in diameter, and ooze out milky exudates on breaking. Dried stems are greenish-brown and their surface are longitudinally finely striated. These observations confirm with the available literature,[21,22] and the picture of ET is shown in Figure 1a.

Microscopical characteristics

The transverse section of the stem has circular outline exhibiting small notches at some places due to sunken stomata. Central vascular cylinder consists of phloem and xylem encircling empty pith. Vascular bundles are distinct, conjoint, collateral, and open. Enlarged epidermis and cortex region consist of stone cells and fibers. The findings are in accordance with available literature.[23,24] The identified diagnostic characteristics of aerial parts of ET are exhibited in Figure 1b-g.

Powder microscopy

The identified powder microscopical characteristics of aerial parts of ET showed the presence of trichomes and vessels, laticiferous cells with stomata associated with parenchymal cells, sharp pitted vessel, xylem fibers, annular ring vessels, thick-walled parenchymal cells, single fiber cell, coiled and bunched laticiferous cells, pitted and reticulate vessels, and latex-showing canals. Identified stomata in the present study were found to be paracytic and it was further confirmed with the available literature.[25] The powder microscopic observations are depicted in Figure 2a-n.

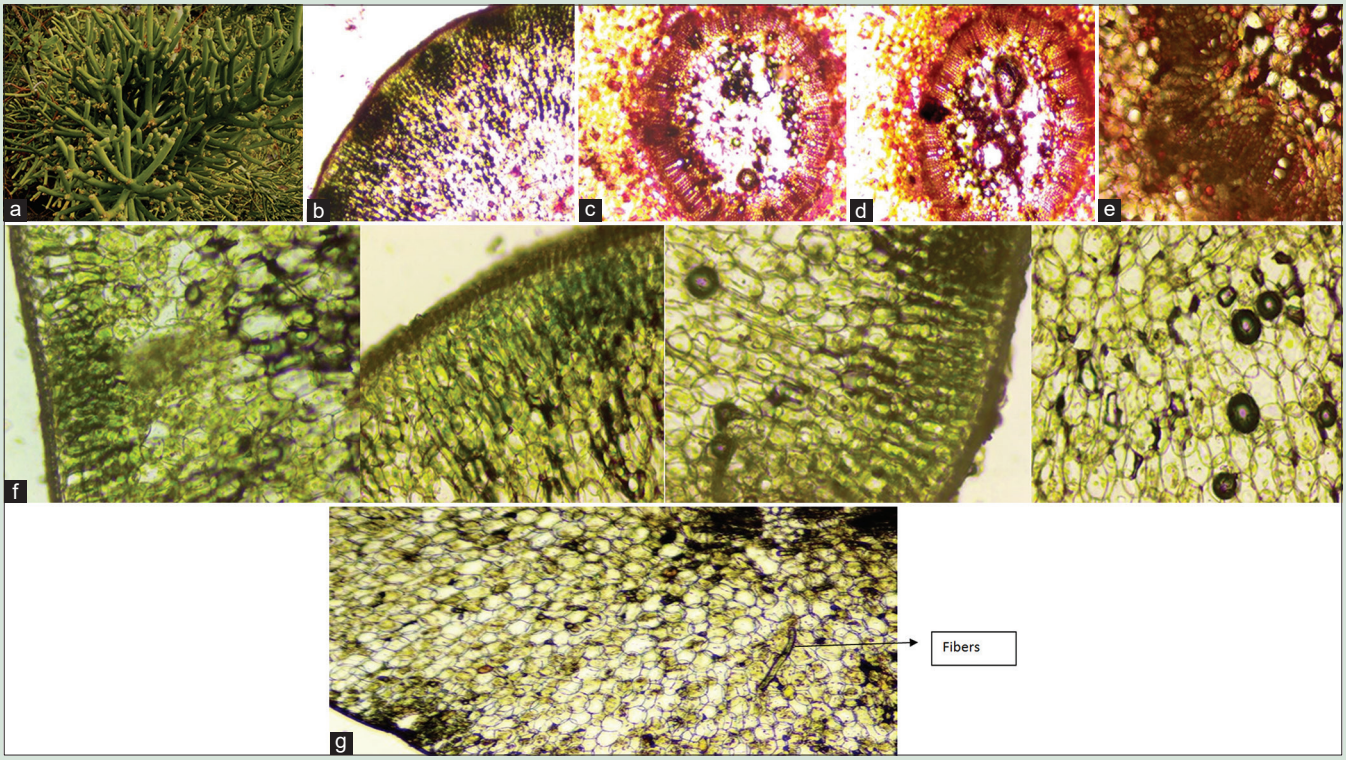

Figure 1: (a) Morphology of aerial parts of *Euphorbia trirucalli,* (b) epidermis with small notches, (c) phloem and pith region with latex canals, (d) vascular bundles, (e) enlarged vascular bundles, (f) enlarged epidermis, cortex region cells, and stone cells, (g) cortex region with fibers

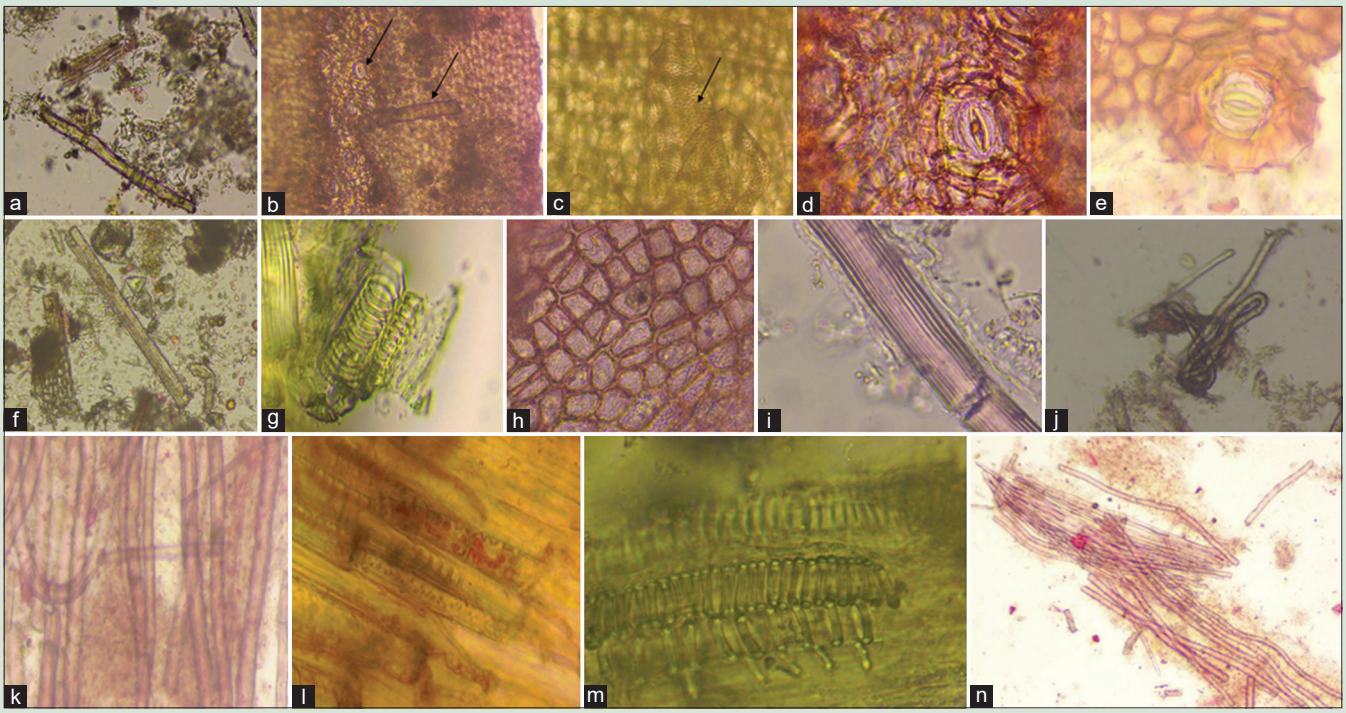

Figure 2: Powdered characteristics of aerial parts of *Euphorbia tirucalli:* (a) Vessels and trichome, (b) laticiferous cells associated with stomata, (c) pitted vessels, (d) stomata associated with parenchymal cells, (e) enlarged stomata, (f) xylem fibers, (g) annular ring vessels, (h) thick-walled parenchymal cells, (i) single fiber cell, (j) coiled laticiferrous cells, (k) bunch of laticiferous canals, (l) reticulate vessels and pitted vessels, (m) reticulate vessels, (n) latex-containing canals

Determination of ash value

The total ash and acid-insoluble ash values were found to be 16.65 ± 0.644 and 2.56 ± 0.706, respectively, however, total ash and water-soluble ash values were found to be 16.67 ± 1.050 and 5.623 ± 1.1, respectively.

Loss on drying and extractive values

The moisture content of dry aerial parts of ET was 9.6%. The water and alcoholic extractive values of ET were 4.0% and 26%, respectively.

Extraction of aerial parts of ET

The color of hydroalcoholic and ethyl acetate extracts of ET was found to be dark green and the extracts were semisolid in texture. The percentage yield of hydroalcoholic extract of ET was 8.076% and that of ethyl acetate extract was 4.73%.

All the standardization parameter results are tabulated in Table 1.

Phytochemical investigation

The extracts of hydroalcoholic and ethyl acetate were further subjected to phytochemical screening, which demonstrated the presence of flavonoids, phenols, carbohydrates, saponins, steroids, and triterpenoids. The results are tabulated in Table 2.

Table 1: Standardization parameter of *Euphorbia tirucalli*

Standardization parameters	Mean±SEM
Total ash and water-soluble ash (%)	16.67±1.050 5.623±1.11
Total ash and acid-insoluble ash (%)	16.65±0.644 2.56±0.706
Loss on drying (%)	Dry powder: 9.6
Extractive values (%)	Alcohol soluble: 26 Water soluble: 4.0
Percentage yield	Hydroalcoholic extract: 8.076. Ethyl acetate extract: 4.73.

SEM: Standard error of mean

Table 2: Results of preliminary phytochemical investigation of extracts

Chemical constituents	Test	Results	
		Hydroalcoholic	Ethyl acetate
Carbohydrates	Molish test	+	+
	Tollens test	-	-
	Fehlings A and B	-	-
	Barfords	-	-
Alkaloids	Mayer's test	-	-
	Dragendorff's test	-	-
	Wager's test	-	-
	Hager's test	-	-
Saponins test	Foam test	+	+
Steroidal test	Salkowski's test	+	+
	Libermann-burchard test	+	+
Triterpenoidal test	Salkwoski's test	-	-
	Libermann-burchard test	+	+
	Iscugajiu test	+	-
Phenol test	Ferric chloride test	+	+
	Gelatin test	-	+
	Chlorogenic test	+	+
Flavavanoids test	Shinoda test	-	-
	Ferric chloride test	+	+
	Mineral test	+	+
	Lead acetate test	+	+
	Sodium hydroxide test	+	+

+=present, - = absent

Total flavonoid content and total phenolic content

The total flavonoid content in hydroalcoholic and ethyl acetate extracts of ET was found to be 246.6 and 120 mg rut/g, respectively, and the total phenol content in hydroalcoholic and ethyl acetate extracts of ET was found to be 81.36 and 279.58 mg GAE/g, respectively. The results of flavonoid and phenol content are tabulated in Table 3, and the standard calibration curve is graphically depicted in Figure 3a and b.

Determination of the antioxidant activity of by 2,2-diphenyl-1-picrylhydrazyl and nitric oxide radical scavenging method

The antioxidant activity of aerial parts of ET was assessed by the DPPH and nitric oxide scavenging assay. Ascorbic acid was selected as the standard for both the activity, and IC_{50} value of DPPH and nitric oxide scavenging activity was found to be 15.893 and 29.759 µg/mL, respectively. DPPH assay of hydroalcoholic and ethyl acetate extracts of aerial parts of ET demonstrated IC_{50} value of 69.599 and 20.455 µg/mL, respectively, and nitric oxide scavenging assay was estimated as 17.017 and 17.562 µg/mL, respectively.

The results are presented in Table 4 and the graph is depicted in Figure 4a and b.

DISCUSSION

Chemotherapy for cancer is always associated with severe toxicity, and there is the possibility of relapse. There are several incidences where patients die of the treatment rather than diseases. Therefore, researchers worldwide are concentrating on evolving the efficient and safest treatment regimen for cancer. To achieve this, researchers are globally looking for natural sources, traditional systems of medicine, and native practitioners. As a result of this, several herbs and herbal products have been adopted in the treatment of various cancers. In continuation of this trend, ET a herb mentioned in the ancient system of medicine for treating cancer has been selected for the present study. In the first phase of the study, aerial parts of ET were collected and authenticated. The plant materials were shade dried, coarsely powdered, and used for further studies.

Morphologically, ET is a flowering shrub or succulent tree with high branches, which can grow up to 3–5 m tall; leaves are few, small, linear, oblong, and early caducous. Stems are green in color, are cylindrical with 0.5–2.0 cm in diameter, and they ooze out milky exudates. Dried stems are greenish-brown and their surface is longitudinally finely striated Figure 1a. The transverse section of ET showed the presence of sunken stomata, phloem, and xylem encircling empty pith. Conjoint, collateral, open vascular bundles, enlarged epidermis, and cortex region showing stone cells and fibers [Figure 1b-g]. The dried powdered ET was subjected to powder analysis, which demonstrated the presence of trichomes, pitted vessels, coiled, branched laticiferous cells, distinct stomata associated with thick-walled parenchymal cells, xylem fibers, annular ring vessels, and single fiber cell. The microscopic observations of the powder are depicted in Figure 2a-n. The identified laticifer cell is in concordance with the literature.[26,27,28] and the finding may be used as a diagnostic characteristic to authenticate and detect adulteration.

Total ash, acid-insoluble ash, and water-soluble ash experiments were performed to give an idea about the existence of carbonates, phosphates, silicates, silica, and other inorganic impurities along with the drug, and the values are tabulated in Table 1. These findings are useful for the identification, authenticity, purity, standardization, and quality standards as a part of the proximate analysis. Loss on drying is one the major factors in determining the deterioration and stability of the drugs and formulation. Considering these facts, moisture content was measured,

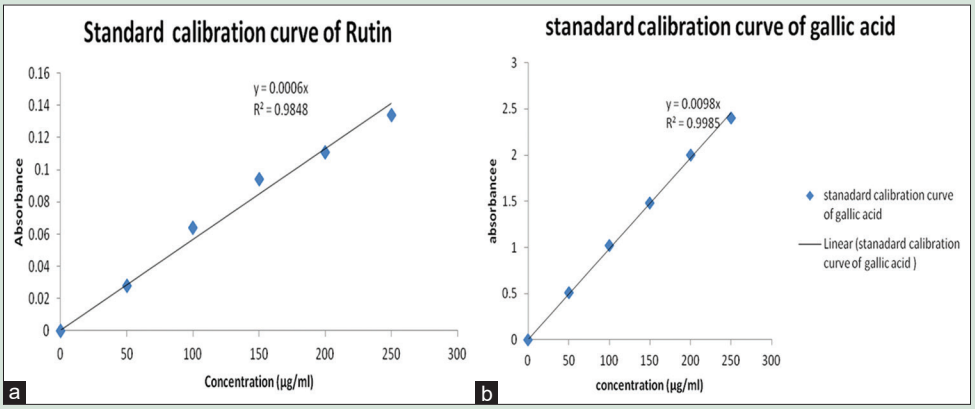

Figure 3: (a) Calibration curve of rutin, (b) standard calibration curve of gallic acid

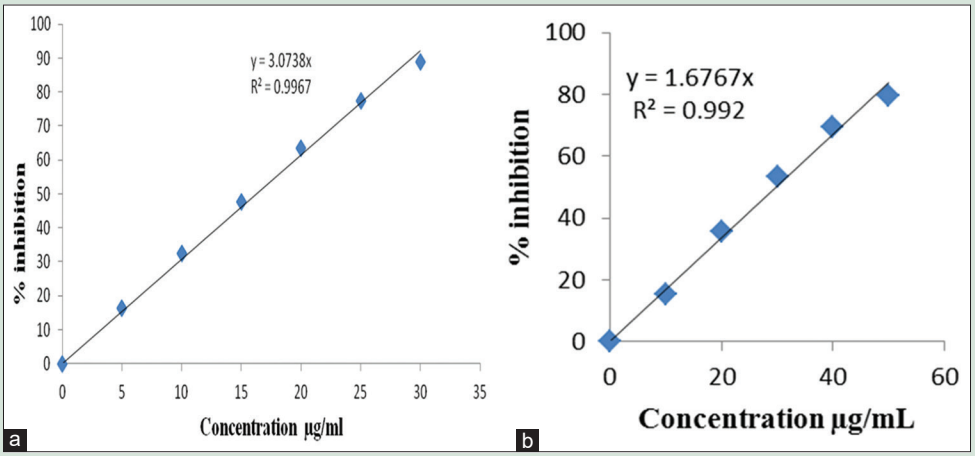

Figure 4: (a) Standard calibration curve of ascorbic acid by 2,2-diphenyl-1-picrylhydrazyl radical scavenging method, (b) standard calibration curve of ascorbic acid by nitric oxide radical scavenging method

Table 3: Total flavonoid and phenol content of hydroalcoholic and ethyl acetate extract of ET

Name of the assay	Concentration (µg/mL) Rutin	Absorbance	Name of the assay	Concentration (µg/mL) gallic acid	Absorbance
Flavonoid content	0	0	Phenol content	0	0
	50	0.028		50	0.513
	100	0.064		100	1.020
	150	0.096		150	1.480
	200	0.111		200	2.000
	250	0.134		250	2.400
Hydroalcoholic extract	1 mg/mL (0.5 mL)	0.074	Hydroalcoholic extract	1 mg/mL (0.5 mL)	0.398
Ethyl acetate extract	1 mg/mL (0.5 mL)	0.036	Ethylacetate extract	1 mg/mL (0.5 mL)	1.37

ET: *Euphorbia tirucalli*

which was found to be 9.6%, indicating the presence of moisture content, which is useful for establishing the storage stability of plant material.

The water and alcohol extractive values play a vital role in evaluating crude drugs and give an idea about the nature of chemical constituents present in them. The water-soluble extractive value was less than the alcohol-soluble extractive value, as indicated in Table 1, which might indicate that phytoconstituents of the plant material are more easily extracted and soluble in alcohol compared to water. Further, the reports suggest that anticancer principle of both the plants are better extracted in polar solvents like ethyl acetate, alcohol and water[29] hence, hydroalcoholic and ethyl acetate extract of the study plant was prepared and indicated the presence of flavonoids and phenols when subjected to preliminary phytochemical test [Table 2]. These hydroalcoholic and ethyl acetate extracts were standardized to know the total flavonoid and phenol content by using aluminum chloride assay by colorimetry and Folin–Ciocalteu's method, respectively.

The total flavonoid content of hydroalcoholic and ethyl acetate of ET was found to be 246.6 and 120 mg rut/g, respectively, and the total phenol content of the corresponding extracts of ET was found to be 81.36 and 279.58 mg GA/g, respectively. The findings of total flavonoid and phenol content in the study plant indicate important antioxidant components that are responsible for the deactivation of free radicals[30] and protective against several chronic diseases like cancer, cardiovascular disease, etc. Since antioxidant phytoconstituents (flavonoid and phenol) present in

Table 4: Antioxidant activity of standard ascorbic and hydroalcoholic and ethyl acetate extracts acid by DPPH and nitric oxide radical scavenging assay

Assay	Treatment	Average IC_{50} (µg/mL)
DPPH radical scavenging method	Standard ascorbic acid	15.893
	Hydroalcoholic extract	69.599
	Ethylacetate extract	20.454
Nitric oxide scavenging method	Standard ascorbic acid	29.759
	Hydroalcoholic extract	17.017
	Ethylacetate extract	17.562

DPPH: 2,2-diphenyl-1-picrylhydrazyl, IC_{50}: Inhibitory concentration

hydroalcoholic and ethyl acetate extracts of ET was proved by performing in terms of their free radical scavenging capacity by DPPH and nitric oxide method. The IC_{50} value of hydroalcoholic and ethyl acetate extracts of ET using DPPH method was 69.599 and 20.454 µg/mL, respectively, and that of nitric oxide scavenging assay was found to be 17.017 and 17.562 µg/mL, respectively. The relationship between total phenol and flavonoid content and scavenging activity by DPPH and nitric oxide assay indicates the presence of primary antioxidants, which are known to react with hydroxyl radicals and superoxide radicals, thereby inhibiting the growth of tumor cells with anti-inflammatory and antimicrobial properties.[31] These marked results of ET is further evident from the report that the methanol and aqueous extracts screened for phytochemical, antioxidant, and anticancer activities.[32] The study results indicate that extracts of study plants are found to be more potent or equipotent with that of the standard. However, there is a need to further confirm these findings.

The whole investigation could serve as a basis for proper identification of the plant material and helps the investigator to distinguish the plant from other members of the same genera. Even these parameters can be used as standardization parameters and also for the identification of adulterants.

CONCLUSION

The Indian herbal industry is growing at a tremendous rate, and several concerns regarding the safety and quality of herbal medicines have been observed. The various standardization parameters studied from the present study such as macroscopy, microscopy, and proximate analysis may be used as a rapid and specific tool in herbal research for the identification and adulteration of the study herb and also to set quality standards and specifications for therapeutic efficacy, safety, and shelf-life of herbal drugs. Antioxidant activitiy demonstrated by the study plant indicates anticancer activity. It is also designed to take up the study further to evaluate the anticancer potential of these plants by adopting *in vitro* and *in vivo* models of cancers.

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Conflicts of interest

There are no conflicts of interest.

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