Research Article

Antioxidant Potential, Anthelmintic Activity and Quantification of Total Phenols, Tannins, Flavonoids of *E. milii* Leaf and Flower Extracts

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ABSTRACT

Plants possess a diverse biologically active compound that exhibits high antioxidant activity. The purpose of antioxidant studies of plants is to reveal the significance of the plants as a reservoir of new antioxidant substances and to entail them in treatment of diverse medical ailments. The current research investigates the antioxidant prospect of *E. milii* leaves and flowers. These leaves and flowers of *E. milii* were excerpted using various solvents of various polarities. The antioxidant action of the *E. milii* leaf and flower extracts was determined using the DPPH free radical scavenging assay. The results of antioxidant screening revealed that the aerial parts of the plant i.e., leaf and flower have prominent antioxidant properties. *In-vitro* anthelmintic activity results confirmed the potential bioactivity of *E. milii* leaf and flower extracts and could be a promising source for the development of effective anthelmintic drugs. The total flavonoid, tannin, and phenolic contents of the plant extracts were evaluated spectrophotometrically and found a positive relationship between these phytochemicals and the antioxidant activity. Ethyl alcohol extract of a leaf and acetone extract of flower contained the highest concentration of flavonoids. The maximum range of phenolics was observed in ethyl alcohol solvent extract of a leaf and methanol extract of the flower. The most elevated concentration of tannins was recorded in ethyl acetate extract of leaves and ethanol extract of flowers. The existence of phytochemical constituents and high antioxidant activity convinces the anthelmintic potential of *E. milii* aerial parts. The present study justifies the traditional use of *E. milii* to treat helminthic diseases.

Keywords: Antioxidant assay, Anthelmintic screening, DPPH, *E. milii*.

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INTRODUCTION

In the area of drug discovery, plant-based products have been proved to be a good source of medicinal compounds.[1] Despite the availability of many anti-bacterial drugs, there is still a significant demand for more powerful antibacterial agents. Most of the drugs available either have toxicity to the ecosystem or bacteria mold some sort of resistance towards them.[2] The majority of infections or illnesses are related to oxidative stress due to free radicals.[3] Free radicals are basic to any biochemical functions and express a vital part of aerobic life and metabolism. Antioxidant compounds are crucial in the prevention of human diseases and may act as free radical scavengers. Helminth infections are common to human beings and affect an immense proportion of the world's population.[4] Although commercial pharmaceuticals are available in the market, due to their prevailing ill effects, medicinal plants are considered an alternative source of anthelmintic medicines. A significant number of investigations have demonstrated the efficacy of various plants owning anthelmintic action.[5] Accordingly, researchers are increasingly shifting their focus on folk medicine, looking for natural leads to develop better drugs against bacterial, fungal,

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and helmintic diseases. However, despite great interest in the research of plants as new prospects for natural products, many plant sources and their components remain untouched.[6] In addition, the number of plant-derived secondary metabolites found to date is yet far from exhausted.

Euphorbia is one of the significant genera of therapeutic plants that belong to the family Euphorbiaceae.[7] Euphorbia milii, also known as the crown of thorns, with an extensive spread in tropical regions of the world. This plant has been traditionally used to treat different medicinal ailments like skin diseases, intestinal parasites, warts, and cancer in China and Brazil.[8] The current investigation is focused on the pursuit of the antioxidant and anthelmintic efficiency of *E. milii* aerial parts.

MATERIALS AND METHODS

Plant collection and solvent extraction of aerial parts

The plants of *E. milii* were collected from the Hyderabad region separated leaves and flowers, cleaned with water, and shade dried. These leaves and flowers were crushed in a hand-operated mill. The extraction of leaf and flower were taken out by soxhlet extraction procedure[9] by employing hexane, chloroform, ethyl acetate, acetone, ethyl alcohol, methanol, and aqueous solvents by increasing the order of polarity. All the extracts were dried in a vacuum with reduced pressure. The acquired crude extracts were stored in tightly packed vials for further studies.

Evaluation of Antioxidant activity

The antioxidant potential of the leaf and flower excerpts was screened using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging analysis.[10] The leaf and flower extracts in different concentrations viz., 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml were prepared in DMSO. 4 ml of 0.004% (w/v) solution of DPPH dissolved in methanol was added to 1 ml of each concentration. In dark conditions, this mixture was incubated for 30 min. Ascorbic acid was taken as a positive control to compare the antioxidant activity of the crude extracts of E.milii aerial parts. After recording the absorbance at 517 nm wavelength, % of antioxidant activity was measured by the following formula.

$$\% \text{ of DPPH scavenging activity} = [(Ao - As) / Ao] \times 100$$

Where, Ao -- absorbance of the control, As -- absorbance of the plant sample.

Screening of Anthelmintic activity

The present study aimed to investigate the invitro-anthelmintic activity of various solvent extracts of leaves and flowers of *E. milii* on Indian earthworm due to its anatomical and physiological resemblance with the intestinal roundworm (Ascaris lumbricoides), human parasites. Indian earthworms, *Pheretima posthuma* (Annelida), were gathered from the water-logged sites. The earthworms were cleaned with water to clear soil debris. The anthelmintic screening was done as per the procedure of[11] with minor changes. Anthelmintic activity was investigated at a 40 mg/ml concentration of various solvent extracts and results were represented in terms of time for paralysis and time for the death of earthworms. Albendazole (40mg/ml) was used as the standard drug for reference and tween 80 (0.1%) in saline as a control group. The mean paralysis time and mean lethal time for each sample were documented and each reading was taken in triplicate. The time taken by worms to become static except when the worms were shaken was recorded as paralysis time. Death time was recorded when the worms neither moved when shaken nor when dipped in warm water observed by withering out the body colour.

Determination of total phenolic content

The overall phenol content of different solvent extracts of E. milii aerial parts was estimated by the revised Folin Ciocalteu technique.[12] To plot the calibration curve, Gallic acid was taken as a standard reference. One ml of leaf and flower extract suspended in DMSO was infused with 1ml of Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (2% W/V). This mixture was incubated at 45°C temperature by shaking at 120 rpm for 15 min. After incubation, the absorbance of the respective samples were noted at 765 nm wavelength on a UV-Visible spectrophotometer. The content of total phenol compounds was represented as mg/g gallic acid equivalent (GAE) of dry extract.

Quantification of tannin content

The total tannin content of the plant crude extracts was estimated by the Folin-Denis method.[13] Tannic acid at a concentration of100 µg/ml was used as the standard reference. 1 ml of individual solvent extract of *E. milii* leaves and flowers was supplemented with 0.5ml of Folin-Denis reagent and 1 ml of saturated carbonate solution. Later, the mixture was shaken well, and read the absorbance at 760 nm against blank after half an hour. The total quantity of tannins was represented as mg of tannic acid equivalent/g of plant extract. The standard graph was conceived using tannic acid.

Estimation of total flavonoid content

The total amount of flavonoids was measured using the aluminum chloride technique.[14] 1 ml of the extracted sample mixed with 0.2 ml of 10% aluminum chloride, 3 ml of methanol, 0.2 ml of 1 M potassium acetate, and 5.6 ml of distilled water. The resulting sample mix was left at room temperature for 30 min. After incubation, the absorbance of the reaction mixture was assessed at 420 nm with a UV-Vis spectrophotometer. The total flavonoid content was recorded, from a standard graph which is plotted with catechin as a standard reference, and the concentration of total flavonoid content was presented as mg of catechin equivalent/g of plant extract.

RESULTS

The antioxidant potentiality of the leaf and flower of *E. milii* in various extracts were examined in search of novel biologically active compounds from natural resources. The results were compared with a reference standard, ascorbic acid. The *in-vitro* antioxidant activity of the *E. milii* clearly demonstrated the prominent antioxidant property of the aerial parts i.e., leaf and flower. All the leaf and flower extracts of *E. milii* exhibited DPPH free radical scavenging potency in a concentration-dependent manner (Figures 1 and 2). Leaf extracts showed relatively high radical scavenging activity than flower extracts. Polar solvent extracts of *E. milii* showed elevated inhibition which was not varied from ascorbic acid.

Antioxidant activity of various solvent extracts of *E. milii* leaves and flowers

The results disclosed that the *E. milii* leaf extracts possessed significant antioxidant activity. At 500 µg/ml concentration, high DPPH free radical scavenging activity was detected in methanol extract (91.08%)

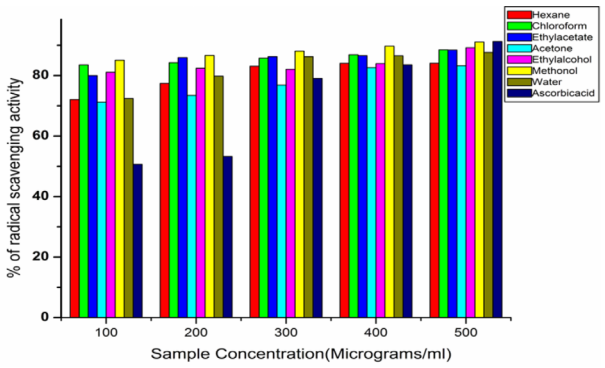

Figure 1: DPPH free radical scavenging activity of *E. milii* leaf extracts.

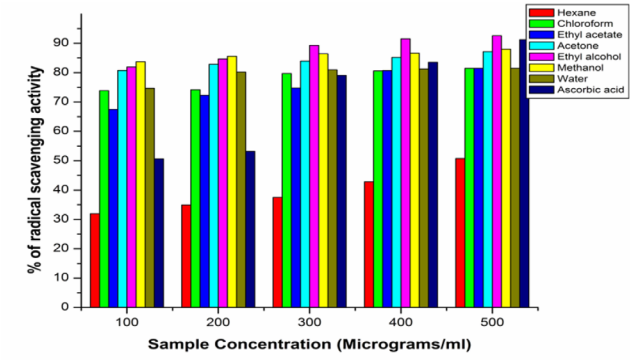

Figure 2: DPPH free radical scavenging activity of *E. milii* flower extracts.

subsequently followed by ethyl alcohol extract (89.22%), chloroform extract (88.50%), ethyl acetate extract (88.45%), aqueous extract (87.60%), hexane extract (84.05%) and acetone extract (83.20%). At the concentration of 100µg/ml and 200 µg/ml, all the solvent extracts displayed higher radical scavenging activity than ascorbic acid. At 300 µg/ml concentration, only acetone solvent extract showed relatively low antioxidant activity than standard ascorbic acid. At 400 µg/ml concentration, all the solvent extracts exhibited high free radical scavenger activity than standard ascorbic acid. However, only the methanol extract showed equal radical scavenging activity to that of ascorbic acid at 500 µg/ml concentration, whereas the antioxidant ability of other solvent extracts was less than ascorbic acid activity.

All screened extracts of the flower exhibited DPPH scavenging activity at various screened concentrations. It was observed that the ethanol and methanol solvent extracts of the *E. milii* flower displayed a prominent DPPH antioxidant assay percentage at all the tested concentrations. The elevated DPPH scavenging activity was recorded by methanol extract at 100 µg/ml and 200 µg/ml concentrations and ethyl alcohol at 300µg/ml, 400µg/ml, and 500µg/ml concentrations than all the tested solvent extracts including standard ascorbic acid. Tested solvent extracts, except the ethanol at 500µg/ml concentration, disclosed less antioxidant activity than ascorbic acid. A similar scavenging activity (81.48%) was noted with chloroform, ethyl acetate, and water extracts at 500µg/ml concentration. However, low to moderate DPPH scavenging activity was found with hexane extract (50.79%).

Anthelmintic activity

In-vitro anthelmintic study results revealed the adequate anthelmintic activity of ethyl acetate, methanol, and aqueous extracts of leaf and acetone, ethyl alcohol, and

methanol extracts of the flower of E. milii (Tables 1 and 2). It was assessed that the aqueous extract of leaf showed remarkable anthelmintic activity with 99.08 ±9.31 min of paralytic time and 129.3 ±16.04 minutes of death time when compared to all the screened extracts and standard of albendazole at the same concentration *i.e.*, 40 mg/ml (Figure 3,4 and 5). The time taken for paralysis at 40mg/ml of albendazole was 138.8 ±2.4 min., which is more or less similar to the paralysis time of acetone extract of flower i.e.,140.0 ±8.78 min. *Pheretima posthuma* exhibited resistance towards leaf and flower extracts of hexane and chloroform solvents of *E. milii*. Prominent anthelmintic activity was observed in the methanolic extract of leaves and flowers.

Quantification of phytochemical contents

The total flavonoid, tannin, and phenolic contents of the extracts of the plant were evaluated spectrophotometrically. The estimated total phenol, flavonoid, and tannin contents of *E. milii* leaf and flower extracts are shown in Table 3, 4, respectively.

Table 1: Anthelmintic activity of *Euphorbia milii* leaves.

Plant extract	At Concentration (40 mg/ml)	
	Time taken for paralysis(min)	Time taken for Death (min)
Albendazole	138.8±2.4	165.5±4.56
Hexane	------	----
Chloroform	------	-----
Ethyl acetate	163.1±12.4	244.6±22.06
Acetone	------	------
Ethyl alcohol	-------	------
Methanol	153±15.16	165.1±12.8
Aqueous extract	99.08±9.31	129.3±16.04

All values represent Mean ±SD; *n*= 6 in each group.

Table 2: Anthelmintic activity of *Euphorbia milii* Flower.

Plant extract	Concentration (40 mg/ml)	
	Time taken for paralysis(min)	Time taken for Death (min)
Albendazole	138.8±2.4	165.5±4.56
Hexane	------	-----
Chloroform	------	-----
Ethyl acetate	------	-----
Acetone	140±8.78	184.5±11.7
Ethyl alcohol	184.3±9.9	207.5±16.2
Methanol	170.5±11.9	256±17.2
Aqueous extract	-----	-----

All values represent Mean ±SD; *n*= 6 in each group.

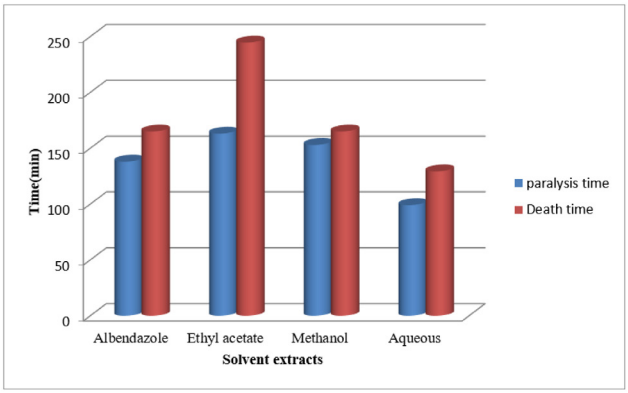

Figure 3: Anthelmintic activity of *Euphorbia milii* leaf extracts.

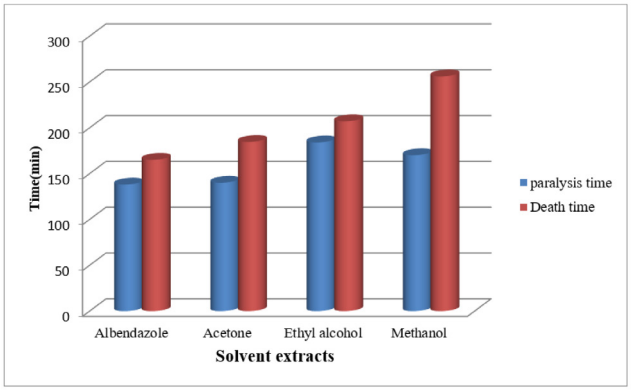

Figure 4: Anthelmintic activity of *Euphorbia milii* flower extracts.

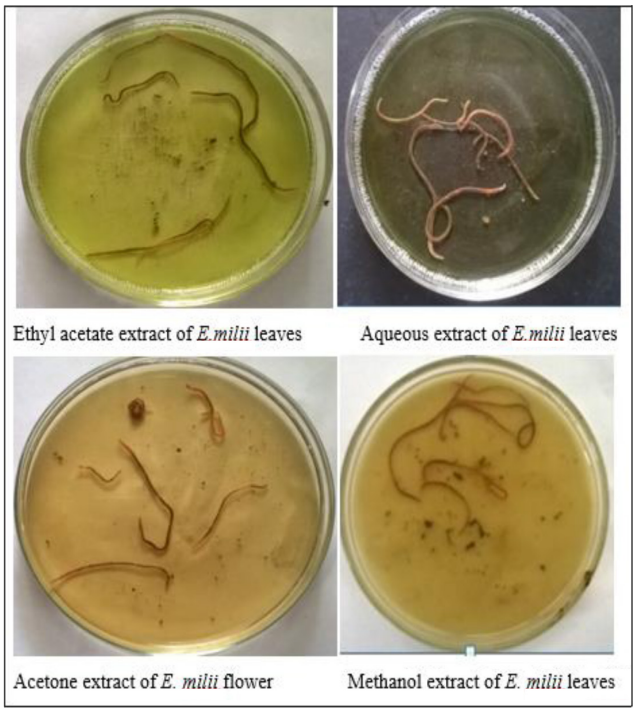

Figure 5: *In vitro* Anthelmintic activity of various solvent extracts of *E. milii* leaf and flower.

Table 3: Total Phenol, Flavonoid and Tannin content of *E. milii* Leaves extracts.

Solvent Extracts	Total Phenol content (mg of Gallic acid/g dry weight)	Total Flavonoid content (mg of catechol/g dry weight)	Total Tannin content (mg of tannic acid/g dry weight)
Hexane	28.0±1.5	36.2±0.1	35.6±1.5
Chloroform	46.3± 1.2	45.4±0.1	70.6±1.5
Ethyl acetate	57.6±0.5	59.5±0.0	81.8±1.0
Acetone	61.0±1.2	54.0±0.5	62.6±2.0
Ethyl alcohol	76.3±1.5	75.1±0.1	39.6±1.5
Methanol	22.1±0.8	68.4±0.6	41.6±1.5
Water	27.6±1.2	46.6±1.2	35.0±2.6

Table 4: Total Phenol, Flavonoid and Tannin content of *E. milii* Flower extracts.

Solvent extracts	Total Phenol content (mgof Gallic acid/g dry weight)	Total Flavonoid content (mg of catchecol /g dry weight)	Total Tannin content (mg of tannic acid/g dry weight)
Hexane	27.6±0.2	44.3±1.3	25.6±2.5
Chloroform	17.2±0.5	33.5±0.3	60.3±1.5
Ethyl acetate	41.5±0.8	24.5±0.6	41.3±1.5
Acetone	34.5±1.2	65.8±0.1	46.3±0.5
Ethyl alcohol	45.3±1.5	38.0±0.0	70.3±1.1
Methanol	63.0±0.1	25.5±0.3	30.3±1.1
Water	32.4±0.2	55.4±1.2	34.2±2.0

The estimated total phenolic content in the aerial parts of *E. milii* plant extracts was expressed in terms of gallic acid equivalent. The total phenolic content of *E. milii* leaves in hexane, chloroform, ethyl acetate, acetone, ethanol, methanol, and aqueous extracts were found to be 28.0±1.5, 46.3±1.2, 57.6±0.5, 61.0±1.2, 76.3±1.5, 22.1±0.8 and27.6±1.2mg of gallic acid/g dry weight, respectively. The maximum content of phenolic compounds was observed in ethanol solvent extract (76.3±1.5 mg GAE/g) followed by acetone (61.0±1.2mg GAE/g) and ethyl acetate (57.6±0.5mg GAE/g) extract, which are responsible for free radical scavenging activity. The lowest quantity of phenol was recorded in methanol extract.

The amount of phenolic content as dry weight recorded in flower extracts of *E. milii* was 27.6 ±0.2mg GAE/g (hexane extract),17.2±0.5 mg GAE/g (chloroform extract), 41.5±0.8 mg GAE/g (ethyl acetate extract), 34.5± 1.2 mg GAE/g (acetone extract), 45.3±1.5 mg GAE/g (ethanol extract), 63.0 mg GAE/g (methanol extract) and 32.4mg±0.2mg GAE/g (aqueous extracts). The highest quantity of phenolic compounds was observed in the methanol extract (63.0 mg GAE/g) of the flower and the lowest quantity of phenolic compounds was observed in chloroform extracts. The total phenolic content of the studied plant parts revealed the maximum in leaves (76.3±1.5 mg/g dry wt.) followed by flower (63.0±0.1mg GAE/gdry wt.). The phenolic content is a significant determinant in the study of antioxidant activity.[15] Phenolic compounds act as reducing agents, hydrogen donors, and are capable of scavenging free radicals.[16] Furthermore, phenolic compounds have been reported to be significant in the treatment of chronic inflammatory diseases associated with the over production of nitric oxide.[17]

The concentration of flavonoids in various solvent extracts of aerial parts of *E. milii* was determined using a spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of catechol equivalent mg of catechol/g dry weight of the extract. Ethyl alcohol extract of the leaf contains the highest flavonoid concentration as 75.1±0.1mg CE/g followed by methanol extract i.e., 68.4±0.6 mg of catechol /g dry weight. The lowest flavonoid concentration was measured in hexane extract. The amount of flavonoids recorded in flower extracts was in moderate concentrations. Flower of *E. milii* contained 65.8±0.1 mg CE/g of flavonoid content in acetone extract as highest concentration. All the other solvent extracts were found to have relatively lower concentration of flavonoids. It was found that the plant *E. milii* had high levels of flavonoids in ethyl alcohol extract of leaves and acetone extract of flower. Many research findings have demonstrated that flavonoids are essential in the fight against infections and can also act as antioxidants.[18]

The tannin content was examined in *E. milii* plant extracts using the Folin-Denis method and the results are expressed in terms of tannic acid equivalent. The values obtained for the concentration of tannin contents are expressed as mg of TAE/g of extract. Estimates of tannin levels varied greatly with various solvent extracts. The amount of the tannins varied from 35.0 to 81.8±1.0 mg TAE/g in leaves and 25.6± 2.5 to 70.3±1.1 mg in flower of *E. milii*. The highest concentration of tannins was measured as 81.8±1.0 mg TAE/g in ethylacetate extract of leaves and 70.3±1.1 mg TAE/ g in ethanol extract of flower. The lowest concentration of tannin content was observed in aqueous extract of leaves (35.0±2.6 mg TAE/g) and hexane extract of flower (25.6 ± 2.5mg TAE/g) of *E. milii*. Previous studies confirmed that tannins have

remarkable stringent properties. They are known to hasten the recovery of wounds and inflamed mucous membranes.[19]

DISCUSSION

The Euphorbiaceae plants are well known for their health effects and conventional use around the world.[20] Their biological activity is closely connected to the phytochemical profile. Antioxidants are essential substances that hold the strength to guard the body against harm caused by free radical-generated oxidative stress. Research inquisitiveness in plant antioxidants has emerged in contemporary days due to the building of unpleasant side effects of commercial antioxidants available in the market.[21,22] Many research findings confirmed that bioactive compounds from medicinal plants act as antioxidant agents and play a substantial role in ceasing free radical activity. Thus in this research work, we confirmed that *E. milii* leaf and flower extracts have potential antioxidant significance.

Results of antioxidant screening of various solvent extracts of leaf and flower were found to be concentration dependent and a similar trend was also reported previously by Muthoni.[23] Based on the results obtained both from leaf and flower extracts of *E. milii*, it was clear that the ethanol and methanol extracts, which are the highly polar solvents were found to have potent antioxidant activity when compared to the non-polar hexane extract. This result has been supported by various studies on other Euphorbia species such as *E. heterophylla*,[24] E. echinus,[25] *E. hirta*,[26] and *E. terracina*.[27] Additionally, the polar solvent extracts of *E. milii* produced the most noteworthy inhibition which was not significantly different from ascorbic acid. Thus the results suggest that polar solvents were significant for extracting compounds with increased antioxidant action. It was observed by these results that changes in solvent polarity alter its ability to dissolve antioxidant compounds. This is in agreement with the reports of Hertog and Qasim.[28,29] In their studies they confirmed that, polar solvent methanol is a widely used and effective solvent for effective solvent extraction of antioxidants and phenolic compounds. According to previous studies,[30,31] this activity was thought to be due to the high content of phenolics, flavonoids, and tannin components that act as significant antioxidants. The result of this work also furnishes further confirmation of the biological activity of the *Euphorbia milii* plant.

The *E. milii* leaf and flower extracts exhibited rich antihelmintic activity. Various research studies suggested the presence of diverse phytochemicals in plants contributes to the anthelmintic activity by acting on the metabolic routes of the worms.[31-33] Phenols and tannins selectively bind to free proteins present in the gastrointestinal tract and finally cause mortality.[34] The anthelmintic efficacy of the ethyl acetate, methanol, and aqueous leaf extracts and acetone, ethyl alcohol, and methanol flower extracts of *E. milii* may be due to a single compound or combined effect of the phytochemicals. Hence, the higher content of phenols, tannins, and flavonoids could be related to anthelmintic activity.

The total flavonoid, tannin, and phenolic content of the extracts of the plant were found to have a positive relationship between these phytochemicals and the antioxidant activity. This finding is well-matched with other published results.[35,36] Plant polyphenols serve as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups, so these polyphenols may be responsible for the experimental antioxidant activity screening.[37,38]

In this work, among the studied aerial parts (Leaf and Flower) of *E. milii* solvent extracts, leaf extracts exhibited the highest phenol (ethyl alcohol extract), flavonoid (ethyl alcohol), and tannin content (ethyl acetate) than the flower extracts of E. milii plant. This investigation results are unlike the investigation of Smeriglio *et al*.,[39] With their work they proved that, the flower extract of euphorbia species showed the highest Polyphenol profile and antioxidant activity followed by leaves. These fluctuations might be due to several factors including plant genotype, agronomic environment, postharvest conditions, and analytical methods.[40,41]

CONCLUSION

This investigation strengthened that the *E. milii* plant is an exciting source of interesting bioactive molecules that may be of future promise as an alternative resource for pharmaceutical leads. The antioxidant potency of the extracts may be a determining factor in the usage of plants in the control and therapy of various diseases. From the results of anthelmintic activity, it was concluded that the aqueous leaf extract of *E. milii* has a rich anthelmintic potency than the conventional anthelmintic drug, albendazole. Because of these properties, this plant might have been used in several traditional herbal medicines to treat helminth diseases. The present study makes an imperative contribution toward the understanding of the pharmacological importance and traditional medical use of the *E. milii* plant.

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CONFLICT OF INTEREST

The author declare that there is no conflict of interest.

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