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Phytochemical investigations, *in-vitro* antioxidant, antimicrobial potential, and *in-silico* computational docking analysis of *Euphorbia milii* Des Moul

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ABSTRACT

Euphorbia milii Des Moul is a deciduous bush indigenous to Madagascar. The present study aims to investigate the presence of the phytochemical, *in-vitro* antioxidant and antimicrobial potency, and *in-silico* computational analysis of ethanolic and aqueous preparations of *E. milii* leaves and flowers. The ethanolic and aqueous extracts were tested for *in-vitro* antioxidant activity by DPPH, H_2O_2, TAC, and FRAP assay. In addition, antimicrobial potentials were assayed by agar well diffusion technique against *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *and Candida albicans* for various clinical isolates. The qualitative phytochemical analysis results confirmed the existence of alkaloids, flavonoids, phenolics, and tannins. The quantitative analysis elicits the availability of a magnificent number of alkaloids, flavonoids, phenolics, flavonols, and tannins. Among all the extracts, aqueous extracts of leaves exhibited potent antioxidant activity in DPPH, FRAP, and H_2O_2 assay with the IC_{50} value of 30.70, 60.05, and 82.92µg/mL, respectively. In agar well diffusion assay, all extracts displayed zone of inhibition varies from 2-24mm at different concentrations ranging from 10-320 mg/mL, whereas no activity was observed against *Candida albicans.* Furthermore, docking-based computational analysis has revealed that beta-sitosterol and taraxerol are the plant's active

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constituents responsible for their antimicrobial and antioxidant activities. Research findings suggest that the *E. milii* plant has an excellent prospect for further study for its extended antioxidative and antimicrobial potential. It could be a natural source of various ailments and can be utilized to develop new drugs.

1 Introduction

The instability that occurs at the cellular level between the generation and buildup of reactive oxygen species (ROS) and the capacity of organisms to neutralize them is what causes oxidative stress. ROS can seriously damage body tissues and cells (Aman et al. 2022, 2023). ROS is crucial in the aetiology of many common physiological conditions. In addition to cellular damage, cancer, hepatic, ageing, neurological, cardiovascular, and renal illnesses are strongly influenced by reactive oxygen species (Migdal and Serres 2011). Endogenous antioxidants might not be sufficient to face the overwhelming oxidative stress to ensure regular cellular activity, and to overcome this problem, dietary antioxidants are required (Aryal et al. 2019).

In addition, the development of microbial resistance to conventional antibiotics has occurred, which poses a significant risk to the efficiency of the antimicrobial treatments that are currently in use. Therefore, identifying new antimicrobial drugs to combat antibiotic resistance is a major medical issue of the 21st century (Chauhan et al. 2022). Since there is no effective therapy for cancer, tumours, and antimicrobial resistance in modern medicine, synthetic or semi-synthetic drugs have more severe adverse effects and are cytotoxic to humans, scientists are looking for a potent bioactive drug with non–cytotoxic qualities (Shriwastav et al. 2023). A significant foundation of possible antioxidants and antimicrobials has been found in natural sources, which may defend against the toxicity of ROS by preventing ROS generation and interfering with bacteria by inhibiting or destroying their cell walls. Antioxidants have a potent antimicrobial impact because they create a scavenging environment that inhibits bacteria. In addition, the antioxidant activity of the constituents minimizes the number of free radicals, which also boosts antibacterial activity (Singh and Sharma 2020). These activities have demonstrated a correlation between antioxidant and antimicrobial characteristics (Sagayaraj et al. 2020). The present emphasis on discovering and characterizing bioactive components responsible for radical scavenging and the ethnopharmacological effects of these components may open up possibilities for focused drug development (Habu and Ibeh 2015). Plants have an impressive potential for developing new therapeutics of immense benefit to humanity. Several epidemiological studies have demonstrated that antioxidant-rich plants benefit health and disease prevention, and their intake reduces the danger of cancer, heart disease, and hypertension (Rehni et al. 2008; Muanda et al. 2011)

Euphorbia milii is a deciduous shrub widely distributed in India and China and native to Madagascar, belonging to the Euphorbiaceae family (Figure 1)(Sreenika et al. 2015). Leaves, flowers, stems, roots, and thorns of *E.milii* were traditionally used in Chinese and Indian medicinal systems for various therapeutic purposes. In the conventional medicine approach, *E. milii* was used

Figure 1 The aerial part of *Euphorbia milii* Des Moul

to treat warts, cancer, and hepatitis in southern Brazil and China (Aleksandrov et al. 2019). On top of that, the seeds serve as a laxative for kids, the petals are applied to cure dermatitis and snake bites, and the entire shrub paste is applied to treat broken animal bones (Ajanaku et al. 2017). According to recent studies, more than 5% of the Euphorbia species are mainly used therapeutically (Sagar and Bisht 2021).

Phytochemical investigations of *E. milii* revealed the existence of subordinate metabolites like alkaloids, polyphenols, glycosides, and saponins (Narendra et al. 2015; Haleshappa et al. 2019). Furthermore, in this regard, several extracts of the *E. milii* aerial part were reported for their antioxidant (Gapuz and Besagas 2018; Mutalib et al. 2020) and antimicrobial potentials (Rauf et al. 2014; Pradyutha et al. 2015; Narendra et al. 2015). Also, methanol & chloroform extracts correspondingly testified for antiviral and anticancer activities (Chaman et al. 2019). Therefore, present research work has been deliberate in evaluating the antioxidant and antimicrobial potentials of ethanol and aqueous extract of leaves and flowers of *E. milii*, followed by the docking-based computational screening of various chemical constituents of the concerned plant to find the most active constituent in the plant accountable for its antimicrobial as well as antioxidant potential and to predict the most probable mechanism involved in it.

2 Materials and Methods

2.1 Collection and identification of the plant

Plants of *E.milii* were gathered from the botanical garden of Maharishi Markandeshwar (Deemed to be) University, Ambala, Haryana, India, in January 2022. The collected plant samples were identified by experienced plant taxonomist (IAAT: 337), Dr. K. Madhava Chetty and a voucher specimen (Number–0978) has been deposited in the herbarium of the Department of Botany, Sri Venkateswara University Tirupati, India (Rao et al. 2003).

2.2 Preparation of plant extracts

Fresh leaves and flowers were shade dried for 21 days and crushed to a coarse powder (Dobriyal et al. 2021). Based on their polarity, the powdered materials were weighed and extracted by Soxhlation with different solvents (ethanol and distilled water). The temperature was set based on the boiling point of the particular solvents. A rotary flash evaporator operating at decreased pressure concentrated the solvent extract separately. After that, each solvent extract was measured before being stored at a temperature of between 4 and 5 degrees Celsius in an airtight container. The percentage yield of the extracts was determined by using the formula given by Moges et al. (2021):

$$(\%\ \text{Yield}) = \frac{\text{amount of preparation}}{\text{amount of powdered drug}} \times 100$$

2.3 Phytochemical screening

Qualitative & quantitative phytochemical investigation of leaves and flowers of *E.milii* was carried out according to the established protocol and quantified the secondary phytoconstituents as designated by Sofowara (1993), Kokate et al. (2003), Trease and Evans (2009) and Harborne (2020).

2.3.1 Total alkaloids content (TAC)

Total alkaloids were quantified using the Harborne (2020) protocol with some modifications. First, 5 g of the sample plant extracts were poured into a beaker after being measured out, and a mixture of 200 mL of 10% acetic acid in ethanol was added. After that, the beaker was covered with a lid and set aside for four hours to rest. Next, the above mixture was drained, and the extracts were condensed to 1/4 of their original volume in a water bath. Next, the preparation was treated with drops of concentrated NH_4OH till the residue was obtained. When the mixture was settled, it was washed by the weak NH_4OH and filtered. Finally, the filtrate is desiccated and weighed to determine the total alkaloid (Abifarin et al. 2019).

2.3.2 Total phenol content (TPC)

The Folin-Ciocalteu method was used to determine the phenolic content of plant extracts (Jahan et al. 2012). First, 1 mL of the plant extract (1 mg/mL) and 1 mL of standard gallic acid consisting of a wide range of concentrations, starting from 10-2560 µg/mL, were added with 2.5 ml of Folin-Ciocalteu reagent. After 30 minutes, 2.0 mL of Na_2CO_3 (7.5%) was incorporated into the blend solutions, which was then nurtured (Al-zoreky and Al-Taher 2015) for 2.5 hours at 25°C with periodic stirring. The spectrum was then checked at 765 nm compared to a blank without extract (Gülçin 2005). Three copies of each test sample extract were run. The Absorbance against concentration data was used to plot the gallic acid calibration curve. The total phenol content was determined by mg GAE/g fw (Rajesh and Perumal 2014).

2.3.3 Total flavonoid content (TFC)

The Dowd assay was employed to check the presence of flavonoids in various solvents of *E. milii* (Arvouet-Grand et al. 1994). A mixture of 0.6mL of sterile water, 0.2mL of 10%(w/v) ethanolic $AlCl_3$ solution, and 1mL of potassium acetate was used to treat a portion of 1mL of sample solution (1Mg/mL) and 1mL of standard quercetin (10–320 g/mL). UV-spectrophotometer was utilized to determine the Absorbance at 415nm after the above mixture had been placed at 25°C for 30 min. The outcomes were given in mg QE/g (Godara 2022).

2.3.4 Total tannin content (TTC)

Total tannin content was identified using vanillin and p-dimethyl amino cinnamaldehyde method (Stavrou et al. 2018). The resulting

combination comprised 3mL of 50% sodium acetate solution, 2mL of ethanolic $AlCl_3$, and 2mL of plant preparation. The above blend was placed at 20°C for 2.5 hours before the wavelength at 440 nm was determined. Then, total flavonol was calculated using the calibration curve produced at different concentrations (10–1249 g/mL).

2.4 *In-vitro* antioxidant test

In-vitro evaluations of the antioxidant potential of ethanolic and aqueous extracts of flowers and leaves were carried out using the standard procedure to determine the antioxidant potentials of crude plant extracts at various concentrations (10-320 µg/mL).

2.4.1 Total Antioxidant Capacity (TAC)

The TAC was investigated using the phosphomolybdate method with some alterations (Prieto et al. 1999). First, a 0.3 mL plant sample (1 mg/mL) was mixed with 3 mL of phosphomolybdate and placed for 10 minutes at 95 °C. The Absorbance was then determined at 695 nm. Then, using the ascorbic acid calibration curve (10–320 g/mL), the TAC was defined as mg AAE/g raw weight of the sample.

2.4.2 DPPH assay

The radical scavenging activity (RSA) of isolated compounds has been utilized to determine antioxidant capacity as per the DPPH method with minor changes (Yan-Hwa et al. 2000). In this study, 0.1 mm DPPH solution and 2 mL of extracts (10–320 g/mL) in ethanol were mixed. The mixes were kept in an incubator at 25° C for half an hour, and the wavelength was obtained at 517 nm against a blank consisting of an equal quantity of DPPH and ethanol (Yao et al. 2022). The percentage was calculated by the formula given below.

$$\text{Percentage radical scavenging activity (\%)} = [\frac{(A_0 - A_1)}{A_0}] \times 100 \quad (1)$$

Where A_0 = Absorbance of the control and A_1 = Absorbance of the samples.

2.4.3 Hydrogen peroxide (H_2O_2) assay

The measurement of the H_2O_2 scavenging activity was carried out by employing Ruch et al. (1989) method with certain modifications. In this experiment, 1 mL extracts (10–320 µg/mL), 2.4 mL phosphate buffer (0.1M, pH 7.4), and 0.6 mL H_2O_2 (40 mM) were energetically mixed and placed at 27° C for 10 minutes. The wavelength was evaluated at 230 nm. To determine the percentage of H_2O_2 that was scavenged was estimated by the following equation:

$$\text{Percentage scavenging activity (\%)} = [1 - \frac{(A_1 - A_2)}{A_0}] \times 100 \quad (2)$$

Where A_0 = Absorbance of the control, A_1 = Absorbance of the samples, and A_2 = Absorbance of the sample only (phosphate buffer with sample)(Yan et al. 2018).

2.4.4 FRAP assay

The modified Fe^{3+} to Fe^{2+} reduction assay was utilized to determine the reducing power of the crude extracts (Oyaizu 1986). First, the response was carried out in a blend with 2.5 mL of sample (10–320 µg/mL) added with 2.5 mL of 0.1M sodium phosphate buffer (pH: 6.6) and 2.5mL of potassium ferrocyanide and kept at 50 °C for 20 min. Then, 2.5 mL of trichloroacetic acid (10% w/v) was put into the mixture. After that, 5 ml of the above mixture was diluted with 0.5 ml of fresh $FeCl_3$ (0.1%), and the intensity of the blue colour was measured at 700 nm against a blank. Finally, the percentage of radical scavenging activity was calculated using the following formula (1).

2.5 *In-vitro* antimicrobial activity

There has been rising attention to investigating and developing novel antibacterial substances derived from various natural resources to combat microbial resistance. This research focused on the *in-vitro* antimicrobial investigation of leaves and flower extracts of *E.milii* as a potential antimicrobial agent. The agar well diffusion method was used to test the antibacterial activity of the ethanol and aqueous extracts of *E. milii* on several different clinical isolates (Bonev et al. 2008; Sheela and Rajkumar 2013).

2.5.1 Collection and identification of microbial isolates

Clinical isolates of microbial strains (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, and Candida albicans*) were collected from M. M. Institute of Medical Science and Research (MMIMSR), India and Central Research Laboratory of Microbiology; Maharishi Markandeshwar University was used to perform the experiments. These microbes were isolated from clinical specimens after being cultured on blood and MacConkey agar. Microbial colonies were then exposed to a biochemical test to determine their phenotype, and the Vitek–2 automated system validated further bacterial identification (Pincus 2010). *S. aureus* and *E. coli* were used as the taxonomic key for bacterial identification.

2.5.2 Preparation and maintenance of bacterial Suspension

A pure microbial inoculum was taken, and 1–3 colonies were moved to a sterile tube having 500µl of standard saline solution and carefully mixed to generate a microbial suspension with turbidity equivalent to 0.5 McFarland's standard solution ($1.5x10^8$ CFU/mL) using a McFarland optic densitometer. A sterile Mueller-Hinton Agar (MHA) medium was inoculated with a microbial solution using

Table 1 Percentage extractive yields of E. milii leaves and flowers with various solvents

Parts of plants	Solvents	Wt. of dry powder	% Yields of dry extracts	Total % yields of dry extracts
Leaves	Ethanol	39.060 gm	35.671%	69.642%
	Aqueous		33.970%	
Flowers	Ethanol	18.621 gm	43.311%	64.702%
	Aqueous		21.391%	

a sterilized cotton swab. All microbial strains were grown by incubating MHA plates at 37°C for 24 hours.

2.5.3 Agar well diffusion assay

The microbial suspension (0.5 McFarland's) was engaged, and with the help of a sterile cotton swab, lawn culture was prepared on MHA. A6-8mm well was punched aseptically using a sterilized cork borer. The extract solution volume (10–200 µL) at the required concentration (10-320 Mg/mL) was put into the well to analyze its antimicrobial properties by agar well diffusion technique (Balouiri et al. 2016). The examined bacteria are cultured on the agar plates for 18–24 hrs at 35°C. Following incubation, the mean ±SD of the clear zone's diameter around the hole was measured to determine the levels of the antimicrobial activity of the plant extract against the organism being tested (Usman et al. 2009). All the experimental tests were accomplished in triplicates.

2.6 *In-silico* computational docking analysis

Antibacterial, antifungal, and antioxidant drug targets were selected based on their physiological involvement in the pathogenic life cycle and the oxidative metabolic processes inside the human body. The structural model of each pharmacological target utilized and considered for this investigation was retrieved from the protein databank (Berman et al. 2000). By employing the Chimera apparatus, the complex reference ligand was dissociated from the macromolecular complex associated with each target receptor. The separated receptor for all the macromolecular targets were prepared by adding hydrogens and Gasteiger charge, followed by its equal distribution among the macromolecular residues for docking analysis (Pradhan et al. 2015; Kaur et al. 2016). Covering the receptor's active binding site and creating an artificial grid box was the first step in the preparation process. Then the docking protocol for each macromolecular target is validated by redocking the reference ligand from the crystallized complex. In the next step, docking-based screening of the plant chemical components of the *E.milii* plant against different antimicrobial and antioxidant drug targets (Agrawal et al. 2021; Mujwar et al. 2022; Fidan et al. 2022; Shinu et al. 2022; Mujwar and Tripathi 2022).

3 Result and Discussion

3.1 Percentage extractive yields

The extraction of dry powdered leaves and flowers yielded an excellent quantity of crude dry extract. The percentage extractive yields of leaves and flowers of *E. milii* crude fractions were 69.64% and 64.70%, respectively. Furthermore, Table 1 illustrates the extraction percentage yield of dry fractions of leaves and flowers with various solvents based on their polarity (Et and Aq). The ethanolic fraction of flowers and leaves showed the highest extraction yield (43.312% and 35.671%, respectively), followed by aqueous fractions of leaves and flowers, which demonstrated relatively lower yields than Chohan et al. (2020).

3.2 Phytochemical investigation

The phytochemical analysis is essential to identify a new source of therapeutically valuable compounds. Medicinal plants have lately piqued the interest of researchers due to their immense therapeutic properties with no or minimal side effects. These medicinal plants contain various secondary metabolites, including alkaloids, flavonoids, flavonols, phenol, coumarins, terpenoids, essential oils, and tannins. Although they are utilized as defensive chemicals, they are also a vital source of phytomedicines due to their vast potential to battle bacterial, fungal, protozoal, and viral infections (Kabir et al. 2016). As a result, they represent a significant source of physiologically active chemicals, which are responsible for a vast number of medications employed in modern treatments. Thus far, natural products have played a critical role in ensuring human wellness and have been the medications of choice because of their safety and efficacy (Rauf et al. 2014). Phytochemical investigation of leaves and flowers reveals the existence of alkaloids, flavonoids, tannins, saponins, phenolics, glycosides, etc. (Table 2) (Pradyutha et al. 2015; Ogah et al. 2020).

According to the comprehensive literature review conducted for this study, plant phenolics are one of the essential classes of chemicals that operate as principal antioxidants (Roghini and Vijayalakshmi 2018). These compounds have various biochemical functions and scavenging capabilities for scavenging both active oxygen species and electrophiles. In addition, they have been correlated to multiple biological belongings with health-promoting qualities (Lelono et al. 2009), such as anticancer, antimicrobial,

Table 2 Qualitative phytochemical screening of E. milii leaves and flowers extracts

S. No	Phytochemical constituents	Leaves		Flowers	
		Ethanol	Aqueous	Ethanol	Aqueous
1	Alkaloids	+	+	+	+
2	Flavonoids	+	+	+	+
3	Carbohydrates	–	+	–	+
4	Reducing sugar	–	+	–	+
5	Cardiac glycosides	+	+	+	+
6	Protein & amino acids	–	+	–	+
7	Glycosides	+	–	+	–
8	Phenolic compounds	+	+	+	+
9	Tannins	–	+	–	+
10	Phlobatannins	+	+	+	+
11	Saponins	+	+	+	+
12	Coumarin	+	+	–	+
13	Anthocyanins	+	+	–	+
14	Quinones	+	+	+	+
15	Phytosterols	+	–	+	–
16	Triterpenoids	+	–	+	–
17	Steroids	+	–	+	–

Key: Positive result (+), Negative result (-)

Table 3 Quantitative phytochemical tests of leaves and flowers extracts of *E. milii* (mg/g)

Parts of plant	Extract	TAC	TFC	TPC	TTC
Leaf	Ethanol	5.58±0.43	38± 0.25	89.81± 0.43	38.25± 0.33
	Aqueous	11.24±0.31	133.94±0.38	252.41±0.56	14.42± 0.31
Flower	Ethanol	3.84±0.37	30.03± 0.73	76.29±0.42	21.46± 0.32
	Aqueous	8.39±0.47	62.93± 0.38	155.56±0.42	7.75± 0.32

TAC- Total alkaloid content, TFC- Total flavonoid content, TPC- Total phenol content, TTC- Total tannins content

anti-inflammation, and antiviral properties. Current investigations showed that the quantitative phytochemical analysis indicates sufficient alkaloids, flavonoids, phenolics, and tannins content in each extract (Table 3). Leaf aqueous and flower are aqueous showed potentially higher amounts of alkaloids, flavonoids, and phenolic contents, while the maximum amount of tannins was confirmed in leaf ethanol and flower ethanol extracts (Ghagane et al. 2017). Overall, aqueous extracts of leaves and flowers exhibited the highest amount of total alkaloid (11.24±0.31 and 8.39±0.47mg/g), total flavonoid (133.94±0.38 and 62.93±0.38mg/g) and total phenol content (252.41±0.56 and 155.46±0.42 mg/g) respectively, compared to the ethanolic extracts except total tannins. For tannins, ethanolic extracts of leaves and flowers exhibited the highest total tannin content, 38.25±0.33 and 21.46±0.32mg/g, respectively, compared to the aqueous extracts of leaves and flowers. According to the findings of this study, tested plant extracts can be employed as antioxidants and antimicrobial agents (Saleem et al. 2019).

3.3 Antioxidant assay

The antioxidant properties of polyphenols, including flavonoids, are primarily due to their redox characteristics, which can contribute to the absorption and neutralization of reactive oxygen species, quenching of singlet and triplet oxygen, and peroxide breakdown (Kabir et al. 2016). Antioxidants defend our bodies

from many illnesses by scavenging ROS or safeguarding the antioxidant defence systems (Jayathilakan et al. 2007). Natural antioxidants are found in medicinal plants with significant antioxidant properties. Antioxidant activity is present in many secondary metabolites classified as polyphenols, terpenes, and alkaloids (Lee et al. 2003). The major portion of plant antioxidant activity may be derived from phenolic molecules such as flavonoids, isoflavones, flavones, etc. Although their exact activities in this study were not studied, it has been reported that the most active components in these species include flavonoids, steroids, glycosides, and alkaloids (Ogah et al. 2020). Leaves aqueous extracts of *E. milii* showed the maximum inhibition on antioxidant capacity, DPPH, H_2O_2, and FRAP assay, as reflected in their IC_{50} values tested for antioxidant activity. Results of the antioxidant activity revealed that *E. milii* plants with relatively high flavonoid and phenolic content might be a substantial natural antioxidant source. Quantifying these active ingredients might be a foundation for this species to treat ROS-related conditions. The effectiveness of this species can be credited to the commonality and mix of subordinate metabolites found in each extract.

3.3.1 Total antioxidant capacity

All the extracts showed potential antioxidant capacity as compared to standard ascorbic acid. However, Aqueous extracts of leaves & flowers exhibited the highest total antioxidant capacity, 318.96±0.8 and 254.74±0.66mg/g, respectively, as compared to the ethanolic extracts of leaves and flowers. Table 4 demonstrates the results of the total antioxidant capacity in different *E. milii* leaves and flower extracts (Ogah et al. 2020).

3.3.2 DPPH assay

The results demonstrated a promising antioxidant effect in the DPPH experiment. The aqueous extracts of leaves and flowers showed maximum 90.31% and 87.22% inhibition with IC_{50} values of 30.70 and 36.34µg/ml, respectively, associated with the standard ascorbic acid of 27.9µg/ml with a maximum inhibition of 95.99±0.43% followed by the ethanolic leaves and flowers extracts (Chohan et al. 2020). Table 4 illustrates the results of various extracts of *E. milii* for the DPPH assay.

3.3.3 H_2O_2 assay

In the H_2O_2 scavenging assay, all the preparations demonstrated a dose-dependent scavenging activity. The aqueous leaves and flowers extracts showed the maximum inhibition of 91.79% and 84.62% with IC_{50} values of 82.92 and 89.16µg/ml, respectively, which is significant compared to the IC_{50} of standard ascorbic acid 71.73 µg/ml with a maximum inhibition of 86.92% (Chohan et al. 2020). Table 4 demonstrates the results of the H_2O_2 scavenging assay in various extracts of *E. milii.*

3.3.4 FRAP assay

The results revealed a proportionate increase in reduction power with increasing extract concentration. The reducing power results suggest the maximum reducing power of leaves aqueous and ethanolic extracts with EC_{50} values of 60.05 and 70.59µg/ml, respectively, compared to the EC_{50} of standard ascorbic acid 33.95µg/ml (Chohan et al. 2020). Table 4 demonstrates the results of the FRAP assay in various extracts of *E. milii.*

3.4 Antimicrobial assay

3.4.1 Agar well diffusion assay

Due to the emergence of microbial drug resistance, the indiscriminate and irrational use of antimicrobial medicines has posed an unprecedented threat to human civilization. The rise of multidrug-resistant pathogens has recently gained international attention and is now a significant concern for the future of humanity. Additionally, the adverse side effects of modern synthetic medications like antibiotics, fungicides, and antioxidants have driven researchers to develop new medicines without adverse effects on the human body. People are again looking to biodiversity for health care management, as natural resources have traditionally benefited people (Tollefson and Miller 2000). Since plants have a rich structural diversity of chemical compounds, utilizing this vast resource to develop novel medications to treat various infectious illnesses is crucial. As a result, there has been a renaissance in interest in investigating novel plants as a source of possible medication candidates in recent years. Isolated microbial

Table 4 Results of antioxidant assay of ethanol and aqueous extracts of E. milii

Parts of plants	Extracts	TAC (mg AA/g)	DPPH		H_2O_2		FRAP
			% RSA	IC_{50}	% RSA	IC_{50}	EC_{50}
Standard	Ascorbic A	-	95.99±0.43	27.95	86.92±0.36	71.73	33.95
Flowers	Ethanol	108.58±0.79	78.19±0.09	78.19	82.31±0.12	95.93	85.04
	Aqueous	254.74±0.66	87.22±0.26	36.34	84.62±0.29	89.16	75.56
Leaves	Ethanol	179.60±0.46	82.96±0.14	46.17	83.33±0.16	93.38	70.59
	Aqueous	318.96±0.8	90.31±0.21	30.70	91.79±0.27	82.92	60.05

strains were resistant to some modern medicines, so further investigation is required if an extract of *E. milii* could control these pathogens. The agar-well diffusion technique was used to examine the antibacterial activities. Zones of inhibition were seen in all isolates tested using the agar well diffusion technique ranging from 2-24mm at 10-320mg/mL concentrations (Tables 5 and 6). Both gram(+ve) *S. aureus* and gram(-ve) *K. pneumonia* bacterial isolates were discovered to be susceptible to flower ethanol extracts. Leaf ethanol and aqueous extracts showed broad-spectrum efficacy against gram -ve *E. coli*, and *P. aeruginosa* isolates. Except for *C. albicans*, all other species were shown to be effective against the tested isolates (Tables 5 and 6). This study's data on antifungal activity showed that *E. milii* was less effective in antifungal action. *E. milii* was shown to have a substantial inhibitory effect on both gram +ve and -ve bacteria tested. Several authors have well-documented the efficacy of *E. milii* as an antioxidant, antibacterial, and antifungal. The results of this experiment indicate that ethanolic extracts of the tested species were shown to be more effective when compared to aqueous extracts (Nayak et al. 2015).

3.5 Computational docking analysis

The structural model of the dihydrofolate reductase (DHFR) enzyme of *E. coli* (pdb id: 6cqa), DNA gyrase of *P. aeruginosa* (pdb id: 6m1j), DHFR of *S. aureus* (pdb id: 2w9s), acetolactate synthase of *K. pneumoniae* (pdb id: 5d6r), DHFR of *C. albicans* (pdb id: 1ai9), and cytochrome P450 reductase of *H. sapiens* (pdb id: 3qfs) was used in the current study (Berman et al. 2000). Prepared macromolecular receptors were saved in the default Auto dock format (*. Pdb qt). The imaginary grid-box was prepared for each target receptor by covering the extended conformations of the utilized ligands and the macromolecular binding residues for each target protein. Molecular docking analysis revealed that the taraxerol and beta-sitosterol were the active ingredients in the E. milii plant responsible for executing antimicrobial, antifungal, and antioxidant effects. The taraxerol is found to be interacting with the DHFR enzyme of *E. coli* and *C. albicans* for antimicrobial and antifungal effects. In contrast, the beta-sitosterol was found to be interacting with the acetolactate synthetase enzyme of *K. pneumoniae* for

Table 5 Zone of inhibition at various concentrations of *E. milii* extracts against several MDR isolates afterwards 24 hrs

Plant extracts	MDR isolates	Zone of inhibition (mm) at various concentrations (mg/ml)						Colistin (10µg)
		10mg	20mg	40mg	80mg	160mg	320mg	
Flower ethanol	*E. coli*	3.92±0.38	8.5 ±0.5	10.58±0.52	14.58±0.63	17.84±0.76	20.08±0.38	17.5 ±0.5
	P. aeruginosa	2.42 ±0.38	5.42 ±0.52	7.5 ±0.5	11.58 ±0.63	15.59 ±0.52	19 ±0.25	17.92 ±0.38
	K. pneumoniae	5.42 ±0.52	9.5 ±0.5	12.83 ±0.62	17.16 ±0.29	19.33 ±0.38	22.92 ±0.38	19.5 ±0.5
	S. aureus	3.5 ±0.5	7.75 ±0.25	14.25 ±0.25	17.25 ±0.25	19.75 ±0.25	24 ±0.25	19.83 ±0.29
	C. albicans	–	–	–	–	–	–	–
Flower aqueous	*E. coli*	2.33 ±0.58	5.25 ±0.66	7.5 ±0.5	10.73 ±0.75	14.58 ±0.52	18.08 ±0.38	17.08 ±38
	P. aeruginosa	2.25 ±0.5	5.08 ±0.35	8.42 ±0.52	10.92 ±0.38	14.42 ±0.38	18.58 ±0.52	17.41 ±0.38
	K. pneumoniae	2.25 ±0.25	6.58 ±0.62	10.42±0.38	16.5 ±0.5	18.33 ±0.38	20.25 ±0.25	18.42 ±0.38
	S. aureus	–	5.42 ±0.52	8.25 ±0.25	13.5 ±0.5	15.57 ±0.52	19.33 ±0.29	18.25 ±0.25
	C. albicans	–	–	–	–	–	–	–
Leaf ethanol	*E. coli*	5.33 ±0.57	7.5 ±0.5	10.75±0.66	13.5 ±0.5	18.08 ±0.38	20.75 ±0.25	17.58±0.52
	P. aeruginosa	3.75 ±0.25	7.83 ±1.04	12.75±1.08	15.58±0.8	19.42 ±0.62	21.58±0.52	19.16±0.29
	K. pneumoniae	2.25 ±0.25	7.08 ±0.15	11.5±0.5	14.5±0.5	17.25±0.43	20.92±0.38	18.92±0.38
	S. aureus	–	6.25±0.25	10.42±0.38	13.42±0.38	17.5±0.5	20.42±0.38	17.42±0.38
	C. albicans	–	–	–	–	–	–	–
Leaf aqueous	*E. coli*	-	1.33±0.38	3.5±0.5	7.48±0.5	12.75±0.66	18.42±0.52	18±0.25
	P. aeruginosa	2.5 ±0.5	7.42±0.94	10.08±0.88	12.58±0.8	18.17±0.76	23.58±0.36	17.5±0.5
	K. pneumoniae	–	3.33 ±0.38	6.67±0.62	10.5±0.5	16.5±0.66	19.58±0.52	17.58±0.63
	S. aureus	–	2.5±0.5	8.25±0.43	11.58±0.52	14.42±0.38	17.5±0.5	17.42±0.38
	C. albicans	–	–	–	–	–	–	–

Table 6 Analysis of the antimicrobial activity of *E. milii* extracts by well diffusion Method

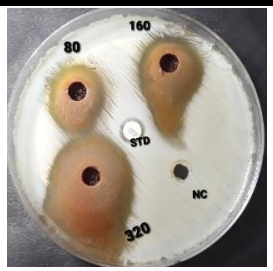
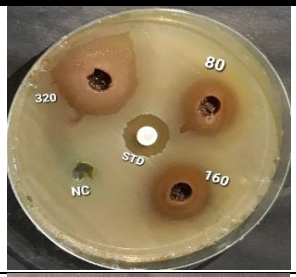
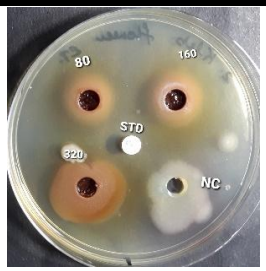
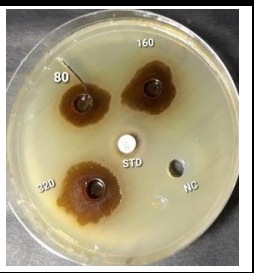
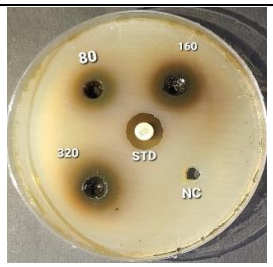
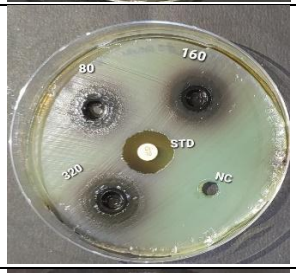
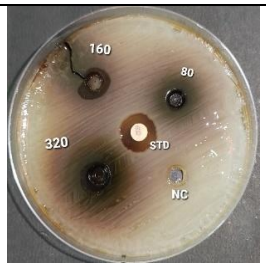
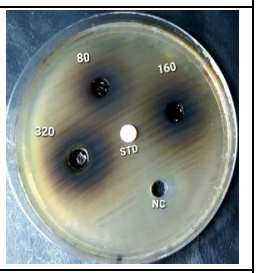
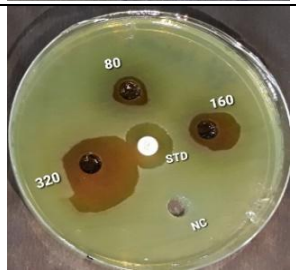
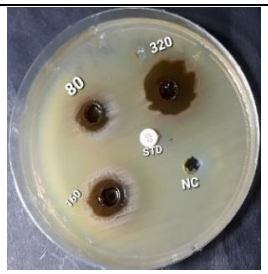
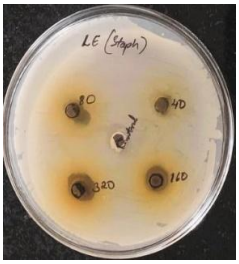
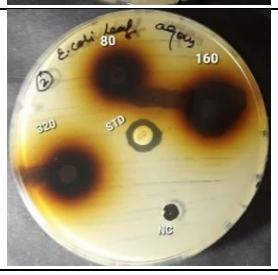
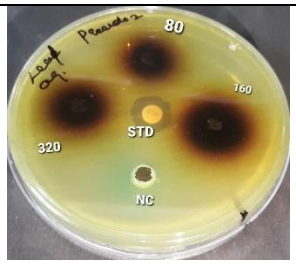
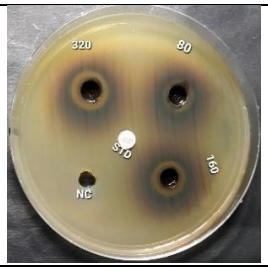
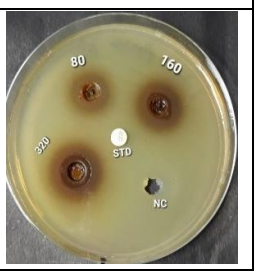
Plant extract	Escherichia coli	Pseudomonas aeruginosa	Klebsiella pneumoniae	Staphylococcus aureus
Flower ethanol				
Flower Aqueous				
Leaf ethanol				
Leaf aqueous				

Table 7 Coordinates are used to prepare a grid box for various macromolecular targets in the current computational paradigm

PDB Id	x-axis	y-axis	z-axis	Spacing (Å)	x centre	y centre	z centre
6cqa	40	40	40	0.386	14.844	26.998	6.265
6m1j	40	40	40	0.397	21.478	58.663	41.43
2w9s	40	40	40	0.369	5.984	-0.45	40.784
5d6r	40	40	40	0.375	-7.403	36.502	-12.131
1ai9	52	52	52	0.403	29.081	-6.919	11.8
3qfs	40	40	40	0.564	42.794	24.08	21.506

antimicrobial effect, with DHFR of *C. albicans* for antifungal effect and cytochrome P450 reductase enzyme for executing antioxidant effect. The grid parameters used in the current study were tabulated in Table 7. Docking results for the screened ligand, standard drug, and reference ligand were tabulated in Table 8. The three-dimensional binding mode of the taraxerol and beta-sitosterol against various macromolecular targets is shown in Figure 2.

Table 8 Binding energy obtained for chemical constituents from *E. milii* plant against different drug targets considered in the current study

S.N	Name	Antibacterial				Antifungal	Antioxidant
		E. coli DHFR (6cqa)	*P. aeruginosa* DNA Gyrase (6m1j)	*S. aureus* DHFR (2w9s)	*K. pneumoniae* AcetoA synthetase (5d6r)	*C. albicans* DHFR (1ai9)	*H. Sapiens* cP450 reductase (3qfs)
1	Taraxerol	-9.36	-7.95	-8.81	-8.66	-10.64	-8.48
2	2,8-hydroxyfriedelan-1,3-dione-29- oic acid						
3	Quercetin 3-O-(2"-O-galloyl)-a-Larabinofuranoside	-6.22	-5.52	-8.05	-6.06	-6.86	-6.15
4	7 7'- dihydroxy, 8, 6'-bicoumarin	-8.14	-7.45	-8.19	-7.61	-7.61	-8.36
5	9-acetyl-3'4'-dimethoxy dehydroconiferyl-3-alcohol	-7.49	-6.66	-6.24	-7.78	-7.13	-6.60
6	Beta-sitosterol	-9.00	-9.31	-9.42	-9.81	-8.95	-8.37
7	1-octacosanol	-3.35	-1.72	-4.82	-2.04	-4.13	-1.18
8	1-triacontanol	-2.31	-1.83	-4.49	-3.20	-2.34	-1.41
9	Cyclobarbital	-7.67	-6.40	-8.21	-7.06	-6.41	-5.96
10	Mephobarbital	-7.16	-5.78	-7.53	-5.89	-6.30	-6.46
11	N-methyl-N-acetyl-3,4 methylenedioxybenzylamine	-5.63		-5.95	-6.10	-6.27	-6.04
12	Palmitic acid	-4.90	-4.29	-4.45	-4.31	-4.87	-4.77
13	Butanoic acid	-2.97	-2.94	-3.14	-3.45	-4.51	-3.99
14	3-amino-1-phenylbutane acetyl derivative	-7.08	-6.53	-6.86	-7.39	-5.36	-5.93
15	Stearic acid	-5.11	-3.49	-4.63	-4.26	-5.12	-3.43
16	Standard	-5.78	-6.14	-6.08	-5.57	-5.85	-5.12

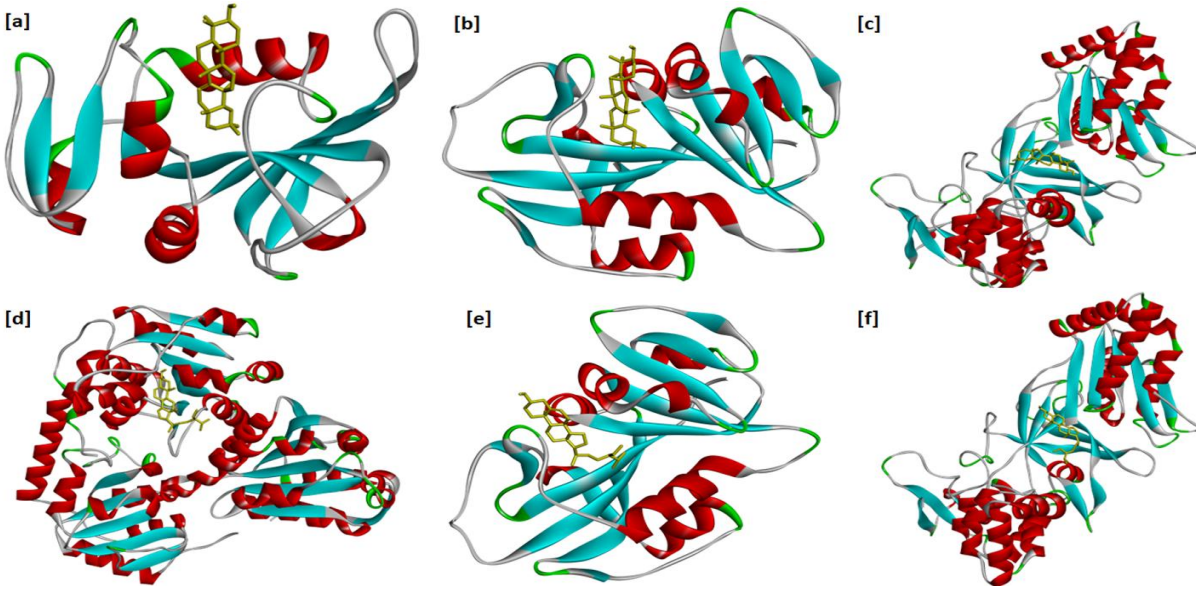


Figure 2 Three-dimensional binding conformation obtained after molecular docking for taraxerol against (a) DHFR enzyme of *E.coli*, (b) DHFR enzyme of *C. albicans*, (c) cytochrome P450 reductase enzyme of *H. sapiens*, (d) Beta-sitosterol against acetolactate synthetase enzyme of *K. pneumoniae*, (e) DHFR enzyme of *C. albicans*, (f) cytochrome P450 reductase enzyme of *H. sapiens*

Conclusions

In current findings, the quantitative phytochemical analysis indicates the existence of alkaloids, flavonoids, phenolics, and tannins content in the extract. Leaf and flower extracts exhibited antioxidant activity, likely due to their high flavonoid and phenolic content. Furthermore, *E. milii* showed impressive antibacterial potency against clinically isolated multidrug-resistant bacteria, while the extract does not exhibit antifungal activity against *Candida albicans*. Docking-based in-silico screening of the chemical constituents of the *E. milii* concerned has revealed that the taraxerol and beta-sitosterol are the active constituents that are supposed to be responsible for antimicrobial and antioxidant potential. Further research is required to clarify the mechanistic aspect of the antimicrobial & antioxidant traits of *E. milii*. This plant has the prospect of being a prodigious source of novel chemical compounds for appropriate new drug developments.

Abbreviations

E. milii: Euphorbia milii; EE: Ethanolic extract; AE: Aqueous extract; DPPH: α, α-Diphenyl-ρ-picryl hydrazyl; FRAP: Ferric reducing antioxidant potential; TAC: Total antioxidant capacity; IC_{50}: 50% Inhibitory concentration; TPC: Total polyphenol content; TFC: Total flavonoid content; TAC: Total alkaloid content; TTC: Total tannins content;GAE: Gallic acid equivalent; RE: Rutin equivalent; DHFR; Dihydrofolate reductase, cP450; Cytochrome P450 inhibitors

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Conflict of interest

All the authors declare no conflict of interest regarding this work.

Author contributions

All authors have combinedly contributed to complete the work. Therefore, we ensure and hereby declare that all authors have read and approved the final manuscript to submit to the selected journal.

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