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ORIGINAL ARTICLE

Comprehensive Chemical and Bioactivity Profiling of *Euphorbia milii* Des Moul. Flower Extract

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ABSTRACT
This study explores the identification of significant bioactive compounds and antimicrobial activity of the ethanol extract of Euphorbia milii flowers using Gas Chromatography-Mass Spectrometry (GC-MS) and agar well diffusion methods. GC-MS analysis was performed using Thermo GC-Trace Ultra Version 5.0 and Thermo MS DSQ II. The results of this analysis identified many significant bioactive compounds such as 1-methyl-4-(1-methyl ethylidene), 1,2-Benzenedicarboxylic acid, bis (2-methyl propyl) ester, Phthalic acid, decyl 2-methoxyethyl ester. The results of antibacterial studies indicate that the ethanol extract of a plant's flower exhibits potential inhibitory effects on Gram-positive and Gram-negative bacteria. A significant correlation between bioactive compounds and inhibition indices was confirmed by the statistical analysis, supporting the potential of the flower extract of Euphorbia milii for therapeutic applications in drug development.
Keywords*: Euphorbia milii, Flower extracts, GC-MS, Antimicrobial activity*

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INTRODUCTION

Plants have been used as medicinal resources across diverse cultures for centuries, serving as valuable sources of potent drugs due to their bioactive compounds, also known as phytochemicals or secondary metabolites. These compounds are crucial in treating various disorders through their individual, additive, or synergistic effects [1,2]. The pharmaceutical industry's phytochemicals are essential for developing new drugs and therapeutic agents. This process often begins with identifying active principles from natural sources and screening plant extracts for bioactive compounds [3, 4, 5]. Key phytochemicals, including flavonoids, tannins, saponins, alkaloids, and terpenoids, are known for their strong antioxidant activities and exhibit a broad range of biological effects, such as spasmolytic, antibacterial, antidiarrheal, antifungal, antiallergic, antimicrobial, antiviral, anthelmintic, insecticidal, and anti-constipation properties [6,7,8]. *Euphorbia milii*, commonly called the "Crown of Thorns," is a member of the Euphorbiaceae family and is distinguished by its upright structure with slender spines. The plant flourishes between spring and summer, blooming throughout the year. It is widely cultivated in regions such as China and Pakistan and is known for its characteristic milky latex. Traditionally, *E. milii* has been used in various medicinal practices; in Nepal, its latex is utilised to treat sprains and in China, it is employed for managing hepatitis and abdominal edema. The crude latex has also shown potent molluscicidal activity [9,10]. This study investigates the phytochemical profile of the flower of *Euphorbia milii* using polar solvent and evaluates its significant antibacterial activity, highlighting the plant's potential medicinal value.

MATERIAL AND METHODS

Preparation of Plant Extracts

The plant samples of *Euphorbia milii* were collected and identified by the Department of Botany, Osmania University, Hyderabad. Flowers were collected from different *Euphorbia milii* plants within the same natural habitat to account for intra-population variability. All samples were harvested during the same flowering season to maintain consistency in developmental and environmental factors influencing metabolite composition. The harvested flowers were dried in a sunshade and made into a fine powder using a pestle and mortar. Extraction was prepared using the Soxhlet extraction method with ethanol as the solvent, chosen for its efficiency in extracting polar bioactive compounds [11,12]. The crude extracts were concentrated using a rotary vacuum evaporator at a temperature of ≤ 50°C under reduced pressure to prevent degradation of the compounds. The colour and consistency of the concentrated extracts were documented, and the resulting crude extract was stored in a desiccator to maintain stability. These extracts were used for screening antimicrobial activity and GC-MS analysis.

GC-MS Profiling

GC-MS analysis of flower extract was performed at CDFD, Hyderabad, using an HP-5 MS fused silica capillary column (50 m × 0.25 mm). The front inlet and injector temperatures were set at 220°C and 240°C, respectively. High-purity helium was used as the carrier gas at a 1 ml/min flow rate. The oven temperature was programmed to increase from 50°C to 250°C at a rate of 10°C/min. Both the ion chamber and the GC interface were maintained at 250°C. A quadrupole mass analyzer and photomultiplier tube detector were employed, with a split ratio of 5:4 and a total run time of 22 minutes.

Screening of Antibacterial Activity

The agar well diffusion method is used to assess the antibacterial activity of the crude extracts [13]. Bacterial suspensions were prepared from 24-hour-old cultures. Nutrient agar medium served as the base for the screening. Sterile Petri plates were prepared by adding 1 ml of bacterial suspension to each plate, followed by molten nutrient agar, which was mixed thoroughly to ensure an even distribution of the microorganisms. After the agar solidified, wells were created using a sterile 6 mm cork borer. Each well was filled with 100 µl of the extract, which was prepared by dissolving 100 mg of the crude extract in 1 ml of DMSO (Dimethyl Sulfoxide). We used DMSO at a final concentration not exceeding 1**% v/v**, a concentration that is generally considered non-toxic and non-inhibitory to microbial growth, as supported by previous literature [14]. Streptomycin, a known antimicrobial drug, was used as a positive control. The plates were incubated for 24 hours at 37°C, and the inhibition zones were measured to assess the antibacterial efficacy.

RESULT AND DISCUSSION

Figure 1 shows the GC-MS chromatogram of the *Euphorbia milii* flower sample. The mass spectra of selected compounds are shown in Figure 2.The chemical compounds with their relative abundance and inhibition indices for four tested organisms are presented in Table 1. Extract of the plant's flower possesses eleven compounds, with 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester with the highest peak area (48.05 %). Prostaglandin methyl ester derivative is present in the least concentration with a peak area (0.03 %. Cyclohexene, 1-methyl-4-(1-methylethylidene)-, Imidazole, 2-acetoxy-, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, Phthalic acid, decyl 2-methoxyethyl ester are the significant major compounds in the flower extract [15-19].

Prost-13-en-1-oic acid and l-Gala-l-ido-octose, though present in minor concentrations, were included based on their reported pharmacological relevance, particularly anti-inflammatory and antimicrobial properties. Their consistent detection and known bioactivity support their potential role as bioactive agents in the extract [20, 21].

The ethanol extract of *Euphorbia milii* flowers exhibited significant antibacterial activity, with notable inhibitory effects on both Gram-positive and Gram-negative bacteria. Notably, *E. coli*, a Gram-negative bacterium, exhibited the largest zone of inhibition (16.1 mm), indicating that it was highly sensitive to the ethanol extract of the flower. The other tested organisms, such as *Staphylococcus epidermis, Streptococcus mutans* (a Gram-positive bacterium), and *Klebsiella pneumonia* (another Gram-negative bacterium) also showed notable inhibition zones than the positive control streptomycin, indicating the efficacy of the extracts against a range of bacterial types. (Figures 3, 4, 5)

This remarkable inhibitory activity is important due to the presence of bioactive compounds in the ethanol extract capable of penetrating and disrupting different bacterial cell structures. Gram-positive bacteria have a thick peptidoglycan layer in their cell walls, while Gram-negative bacteria have a more complex cell wall structure with an outer membrane that is generally more resistant to antibacterial

agents. The ability of the extract to inhibit both types of bacteria highlights its potential as an effective antibacterial agent [22].

The surpassing activity of *E. milli* flower extract over the positive control streptomycin may be due to its potent bioactive compounds. The interference of these compounds with essential bacterial processes, such as cell wall synthesis, protein synthesis, or membrane function, leads to bacterial death or inhibition of growth [25, 24, 25].

The identified compounds correlate with known biological activities, Notably, 1,2-Benzenedicarboxylic acid bis(2-methylpropyl) ester was found in higher concentrations in our research than the previous findings [26]. This may be attributed to environmental variations such as soil composition, climate, and geographic conditions. Literature supports the antimicrobial and antioxidant potential of these compounds. Therefore, they likely play a significant role in the observed bioactivity of the flower extract [27, 28].

The GC-MS analysis identified several bioactive compounds in the flower extract of *Euphorbia milii*, it is essential to mention the limitation of our study. The present identification was based on peak area normalization, which is based on semi-quantitative estimate of compound abundance. The exact quantification of individual compounds remains uncertain. Future studies should consider incorporating an internal standard-based quantification approach to enhance the accuracy and reliability of concentration estimates**.**

Statistical analysis

Two-way ANOVA was performed using IBM-SPSS version 26 to examine the combined effect of different chemical compounds and the four microorganisms on the inhibition index for the flower extract of *Euphorbia milii.*

Table 1: Chemical compounds and the inhibition index for each test organism for the *Euphorbia milii* flower sample

Chemical compound	**Peak area %**	**Molecular formula**	**Molecular weight**	**Inhibition index**			
				E.coli	***K. pneumonia***	***S. epidermidis***	***S. pneumonia***
Cyclohexene, 1-methyl-4-(1-methylethylidene)-	19.17	$C_{10}H_{16}$	136	0.85	0.70	0.39	0.67
Imidazole, 2-acetoxy-	13.26	$C_5H_6N_2O_2$	126.13	1.22	1.02	0.56	0.97
Diethyl Phthalate	3.47	$C_{12}H_{14}O_4$	222	4.67	3.89	2.13	3.72
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	48.05	$C_{16}H_{22}O_4$	278.34	0.38	0.28	0.15	0.27
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	4.08	$C_{16}H_{22}O_4$	**278.34**	3.97	3.31	1.81	3.16
Hexadecanoic acid, 3,7,11,15-tetramethyl-, methyl ester	3.09	$C_{21}H_{42}O_2$	326	5.24	4.37	2.39	4.17
Dibutyl phthalate	1.63	$C_{16}H_{22}O_4$	278	9.94	8.28	4.54	7.91
l-Gala-l-ido-octose	0.27	$C_8H_{17}NO_8$	255	60	50	27.40	47.78
1-Octadecanol, 18-bromo-	0.98	$C_{18}H_{37}BrO$	349	16.53	13.77	7.55	13.16
Phthalic acid, decyl 2-methoxyethyl ester	5.97	$C_{22}H_{34}O_5$	378	2.71	2.26	1.24	2.16
Prost-13-en-1-oic acid, 6-oxo-9,11,15-tris[(trimethylsilyl)oxy]-, methyl ester, (9.alpha., 11.alpha., 13E, 15S)-	0.03	$C_{33}H_{69}NO_6Si_4$	688	540	450	246.67	430

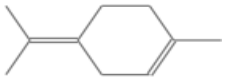
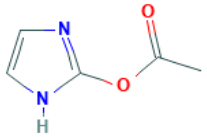
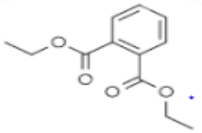
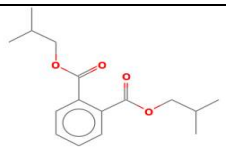
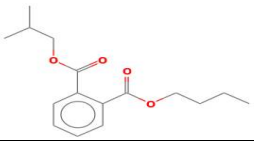
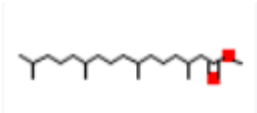
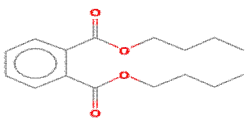
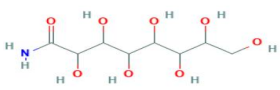
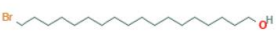
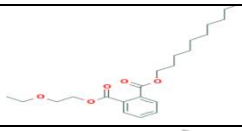
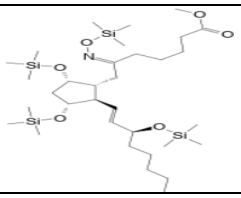
Table 2 shows structures and the Inhibition index's descriptive statistics (mean and standard deviation) for different chemical compounds and microorganism combinations. Each compound and microorganism combination has its mean inhibition index, and an overall mean is calculated for each combination.

From Table 2, a higher inhibition index indicates a more potent inhibitor. For instance, "Prost-13-en-1-oic acid, 6-oxo-9,11,15-tris[(trimethylsilyl)oxy]-, methyl ester, (9.alpha., 11.alpha., 13E, 15S)-" has an extremely high mean inhibition index of 416.6675, suggesting it is a very strong inhibitor.

Standard Deviation: The value of the standard deviation gives the steadiness of inhibition indices. The small value of the standard deviation indicates the values are very close to the mean, and the larger value

gives a wider spread. Compounds such as “Prost-13-en-1-oic acid..." and "l-Gala-l-ido-octose" exhibit significant effectiveness. Figure 6,7 shows a profile plot for the flower extract of *Euphorbia mili.* This line plot explains the interaction between chemical compounds and microorganisms on the inhibition index and is useful for understanding how different combinations affect the inhibition index.

Table 2: **Chemical compounds with their structures, mean and standard deviation of inhibition indices for the *E. milii* flower sample**

S.No	Chemical compound	Structure	Inhibition indices	
			Mean	Standard deviation
1	Cyclohexene, 1-methyl-4-(1-methylethylidene)-		0.6525	0.19190
2	**Imidazole, 2-acetoxy-**		0.9425	0.27693
3	Diethyl Phthalate		3.6025	1.06525
4	1,2-Benzenedicarboxylic acid, bis(2-methyl propyl) ester		0.2700	0.09416
5	1,2-Benzenedicarboxylic acid, butyl 2-methyl propyl ester		3.0625	0.90610
6	Hexadecanoic acid, 3,7,11,15-tetramethyl-, methyl ester		4.0425	1.19559
7	Dibutyl phthalate		7.6675	2.26418
8	l-Gala-l-ido-octose		46.2950	13.67212
9	1-Octadecanol, 18-bromo-		12.7525	3.76550
10	Phthalic acid, decyl 2-methoxyethyl ester		2.0925	0.61662
11	Prost-13-en-1-oic acid, 6-oxo-9,11,15-tris[(trimethylsilyl)oxy]-, methyl ester, (9.alpha., 11.alpha., 13E, 15S)-		416.6675	123.01608

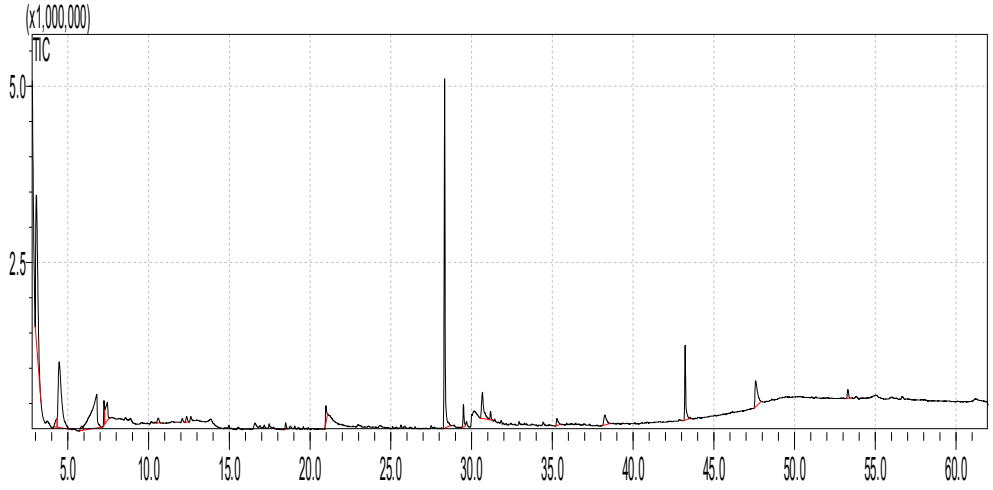


Fig. 1. GC-MS chromatogram of *Euphorbia milii* flower sample

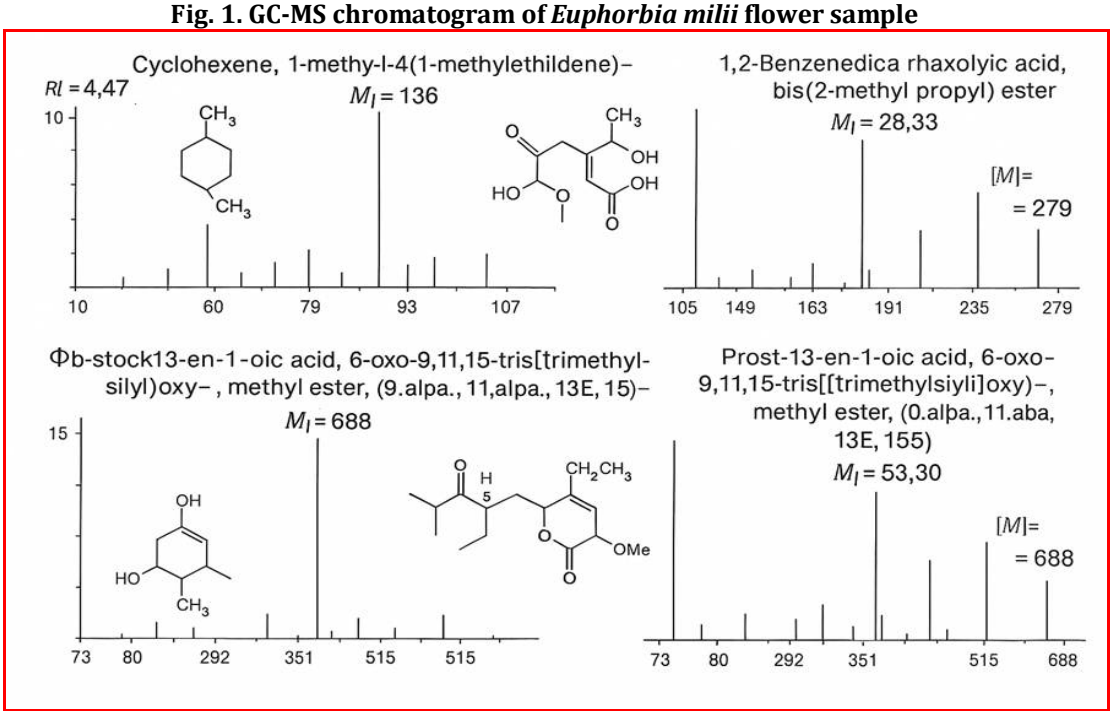


Fig. 2. Representative Mass Spectra of Selected Compounds from *Euphorbia milii* Flower Extract

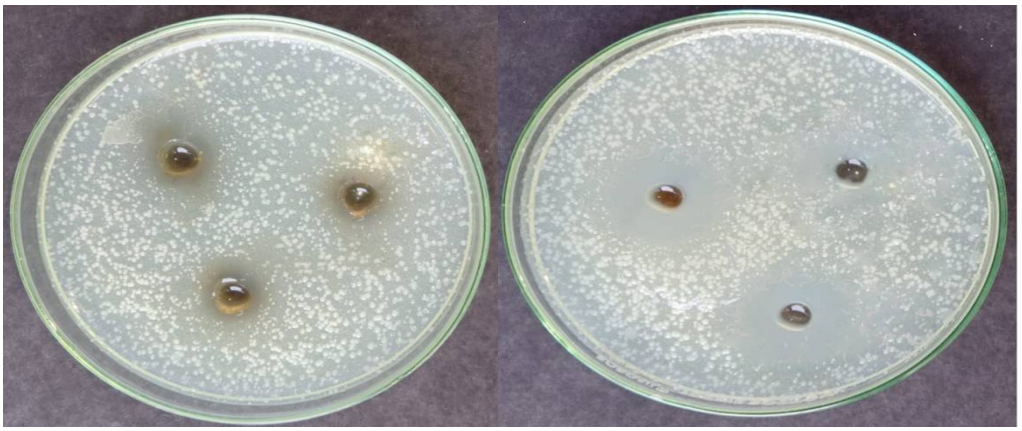


Fig.3.Ethanol extract of flower against *Staph.epidermis* **Fig.4. Ethanol extract of flower against *E.coli***

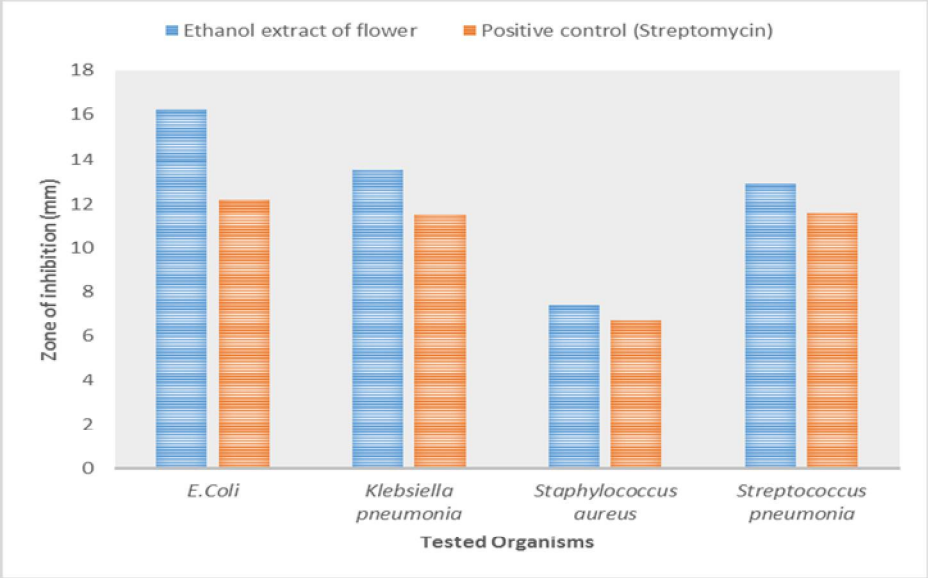


Fig.5: Biological activity of flower extract against G+& G- Bacteria

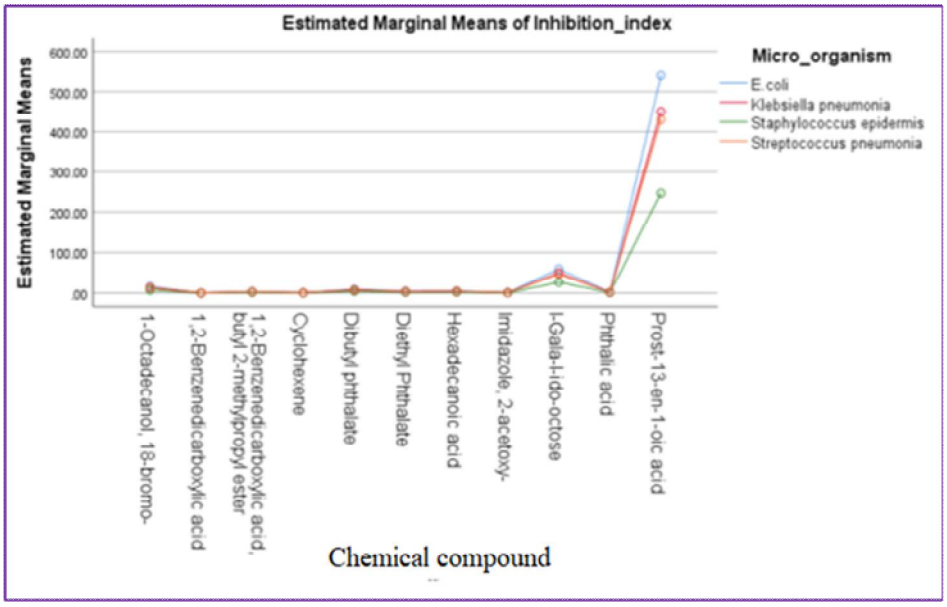


Fig.6: Interaction between chemical compounds and microorganisms on the inhibition index for flower extract

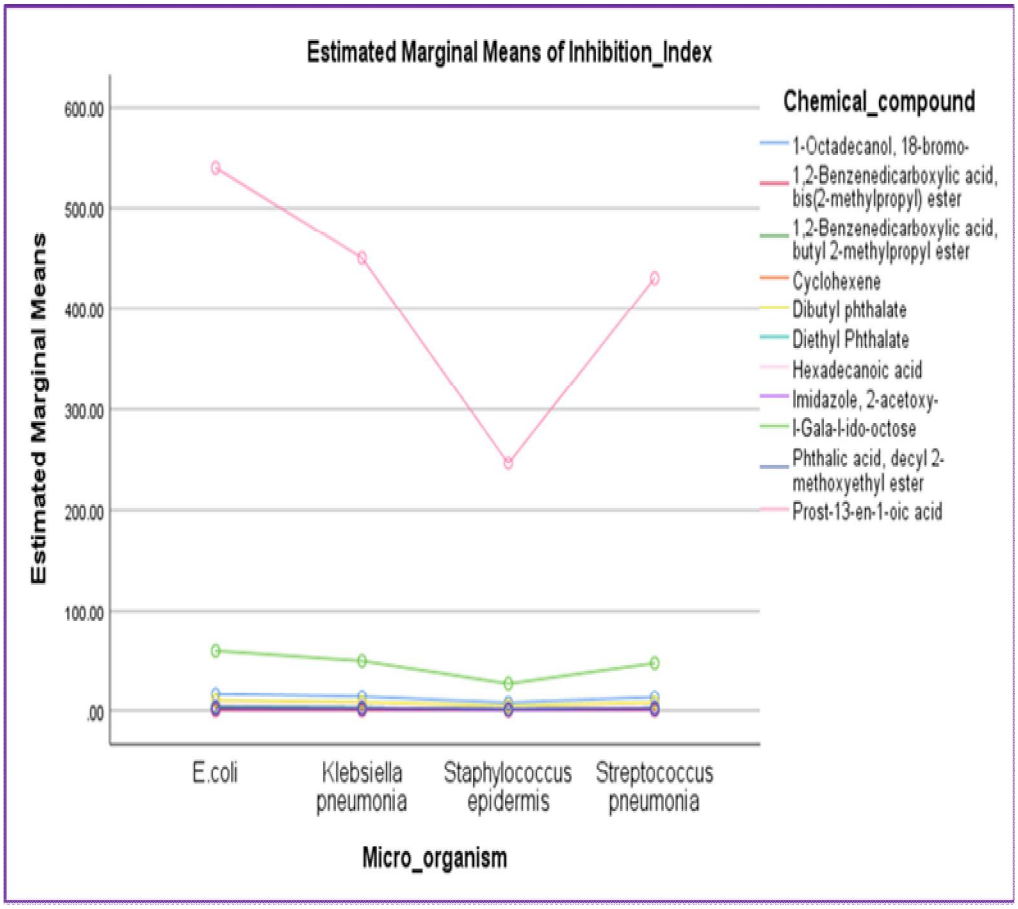


Fig.7: Inhibition indices of chemical compounds against the four microorganism for flower extract

CONCLUSION

The flowers of *Euphorbia milii* were collected and prepared for extracts. Significant bioactive compounds were identified with their relative abundance using GC-MS analysis. These results revealed that the flower extract consists of vital constituents such as 1,2-benzene dicarboxylic acid, cyclohexene, and imidazole. The observed potential antimicrobial activity surpassing the positive control streptomycin indicates the therapeutic properties of these compounds, supporting the plant's traditional medicinal applications. The presence of significant bioactive compounds and their potential antimicrobial activity indicates that *Euphorbia milii* could be a promising source for the development of new antimicrobial agents and therapeutic products. In future studies, we aim to isolate and characterize pure single compounds from the flowers to further evaluate their individual bioactivities and therapeutic potential.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human participants performed by any of the authors.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR'S CONTRIBUTIONS

A. Ch. Pradyutha: Investigation, drafted the manuscript; P. Sakuntala: Developed the manuscript by review, editing, and Statistical analyses.

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