Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3633

PHYTOCHEMICAL PROFILING, ELEMENTAL ANALYSIS, AND GC-MS INVESTIGATION OF *EUPHORBIA HETEROPHYLLA*: EXPLORING ITS POTENTIAL BIOLOGICAL ACTIVITIES

Azhar Abbas[1], Muhammad Sajid[2*], Hamed Ali Ismail[3], Shahid Ullah Khan[4], Safir Ullah Khan[5], Umber Zaman[6], Khalil ur Rehman[7], Muhammad Naveed[8*], Mah Noor Ayaz[9]

[1,2*]Department of Biochemistry, Hazara University, Mansehra, KP - Pakistan
[3]Department of Pharmaceutical Sciences, Irma Lerma Rangel College of Pharmacy|Texas A&M University, MS 131|1010 West Ave B | Kingsville, Texas 78363
[4]Department of Biochemistry, Women Medical and Dental College, Khyber Medical University KPK – Pakistan / [4]Integrative Science Centre of Germplasm Creation in Western China (CHONGQING) Science Cityand Southwest University, College of Agronomy and Biotechnology, Southwest University, Beibei,Chongqing 400715 - China.
[5]Affiliation; Hefei National Laboratory for Physical Sciences at the Microscale, School of Life Sciences, University of Science and Technology of China, Hefei 230027 - China
[6,7]Institute of Chemical Sciences, Gomal University, Dera Ismail Khan, KPK - Pakistan
[8*, 9]Department of Microbiology, Hazara University, Mansehra, KP – Pakistan

***Corresponding Author:** Muhammad Sajid,
Email Address: sajid931@hotmail.com

***Corresponding Author:** Muhammad Naveed,
Email Address: naveed.mic@hu.edu.pk

Abstract

Background: *Euphorbia heterophylla*, a popular medicinal plant from the Euphorbiaceae family, was investigated in this study to determine its phytochemical composition, Elementals profile, GC-MS analysis, as well as its antibacterial, antifungal and antioxidant activities.

Purpose: The antibacterial and antifungal activities were evaluated using the disc diffusion method, while antioxidant activity was assessed through the DPPH assay.

Study Design: Elemental analysis was conducted using atomic absorption spectroscopy. GC-MS analysis was carried on the chloroform extracts of *E. heterophylla*.

Method: Extracts were prepared using methanol, ethyl acetate, chloroform, n-hexane, and distilled water as solvents.

Results: The results revealed the presence of various phytochemicals, including alkaloids and carotenoids, across all five extracts. The most abundant Elements identified were K, Ca, and Na. Additionally, the prominent compound 2,4-Di-Tert-Butyle Phenole was identified through GC-MS analysis. Methanolic extracts exhibited the highest brine shrimp cytotoxicity, while the ethyl acetate extract displayed the largest zone of inhibition (24mm) against *Escherichia coli*. However, all five extracts of *E. heterophylla* exhibited relatively low antifungal activity, with methanolic extracts demonstrating the highest zone of inhibition (12mm) against *Aspergillus fumigatus*. Notably, the n-

hexane extract demonstrated the highest antioxidant activity, measuring 42.07 ± 0.76 µg/ml. Significant activities was observed.

Conclusion: These findings underscore the importance of *E. heterophylla* in pharmaceutical industries, as its abundance of essential phytochemicals and resistance against various pathogens make it a valuable candidate for further exploration.

Keywords: *Euphorbia heterophylla*; Plant-derived compounds; GC-MS study; Antibacterial infections; Antioxidant activity.

1. Introduction

Medicinal plants play a crucial role in pharmacological research, as they serve as essential sources of raw materials for the pharmaceutical industry [1]. Throughout history, nature has provided a vast array of therapeutic agents, with many modern medicines originating from natural sources, particularly plants, based on their traditional uses in folk medicine systems. The demand for herbal products has been exponentially increasing worldwide, prompting scientists to explore the pharmacological activities of plant extracts and isolate biologically active compounds for their potential medicinal value [2].

Euphorbia heterophylla, an herbaceous plant with an erect reddish-green stem and a smooth surface, varying in height from 20 to 200cm depending on the growth conditions, belongs to the Euphorbiaceae family [3,4]. Traditionally, this plant has been employed in various Asian regions for treating ailments such as cancer, tumors, respiratory tract disorders, skin infections, and viral warts. It is particularly renowned for its efficacy in addressing gonorrhea, migraines, and viral warts, while its latex finds application in the treatment of fish poison, asthma, bronchitis, constipation, purgative purposes, and as an insecticide [5,6].

Phytochemicals, the chemical compounds synthesized by plants, can be categorized into primary and secondary metabolites. Primary metabolites include purines, pyrimidines, amino acids, and other essential compounds [7]. Secondary metabolites, derived from primary metabolites, encompass biologically active compounds, many of which possess significant pharmaceutical potential [8]. About 20% of known plant species have been studied for their pharmaceutical properties, leading to positive impacts on healthcare by aiding in the treatment of cancer and various diseases. Plants have the capacity to produce a diverse array of bioactive compounds, and fruits and vegetables, in particular, can accumulate high concentrations of phytochemicals that protect against free radical damage. Such plants with beneficial phytochemicals can supplement the human body's needs by acting as natural antioxidants [9].

In light of these considerations, this study aims to investigate the phytochemical composition, Elemental content, and potential bioactivities of *E. heterophylla* through different solvent extracts. The evaluated bioactivities encompass antibacterial, antifungal and antioxidant properties. Furthermore, gas chromatography-mass spectrometry (GC-MS) analysis will be conducted to isolate and identify volatile and semi-volatile compounds. The findings from this research may establish E. *heterophylla* as a novel source of bioactive phytochemicals with potential applications in clinical and nutritional fields.

2. Material and methods

2.1. Sampling

The plant of *Euphorbia heterophylla* was collected from the hilly areas of Mansehra District, KP - Pakistan. The collected plants were initially washed with tap water, followed by distilled water, and subsequently dried at room temperature. Once dried, the plants were finely powdered using a mechanical grinder.

2.2. Extraction preparation

The plant powder (100g) was separately soaked in 500 ml analytical grade solvents (chloroform, ethyl acetate, n-hexane, methanol, and water) for fourteen days. All the extracts were dried and concentrated by a rotary evaporator. The extracts were condensed by vacuum evaporation in the rotary evaporator (Buchi, Switzerland) and dried at 45°C for the final crude extract in the vacuum oven (Yamato, Japan). Extractions were made in the four solvents (chloroform, ethyl acetate, n-hexane, methanol, and water).

2.3. The phytochemicals analysis

The screening of phytochemicals of plant extracts was analyzed with standard procedure for different phytochemicals, i.e., flavonoids, alkaloids, tannins, saponins, glycosides, protein, and carbohydrates [10].

2.3.1. Test for Alkaloids

For the investigation of alkaloids, Mayer's test was used. Briefly, 2ml of 1% HCl was mixed with 5mL of aqueous solution. Then 1-2 drops of Mayer and Wagner solutions were added to the mixture. The presence of the precipitate turbidity was evidence of the presence of alkaloids.

2.3.2. The flavonoids analysis

5 ml of the dilute ammonia mixture added to 1 ml of the aqueous filtrate of every plant extract and then by adding concentrated H2SO4 yellow color formation indicated the presence of flavonoids.

2.3.3. Test for Tannin

To analyze Tannins in the plant's extracts, 2 ml of D. water and 2 ml of the extract of every fraction were mixed. A few drops of $FeCl_3$ solutions were added. Green precipitate formation indicated tannin presence.

2.3.4. Test for the Saponins (Soap formation)

The 5ml of D. water was mixed with 2ml of aqueous extracts, then vigorously shaken and heated in test tubes. In the last, the soap formation indicated the presence of saponins in the plant extracts.

2.3.5. Tests for Glycosides (Legal test)

A part of the extract was hydrolyzed with HCl in a water bath for a few hours, and the hydrolysate was examined to detect the presence of various glycosides. About 1 mL of sodium nitroprusside was added to the hydrolysate. The change of color from pink to red indicated the presence of glycosides.

2.3.6. Elemental analysis

The contents of the Elements (sodium, copper, zinc, manganese, iron, nickel, cadmium, lead, cobalt, chromium, potassium, nickel, and magnesium) were analyzed by atomic absorption spectrophotometer. Briefly, 2 grams of sample powder was taken in the digestion flask, and solutions of HNO_3, H_2SO_4, and HCl (20:8:2 v/v) were added. The digestion flask was kept on the hot plate at the fume hood. The temperature of the hot plate was kept at 200ºC and slowly increased to three hundred eighty (380ºC) for one hour. The volume of the mixture was reduced to 3ml, and black crystals appeared. The flask was placed in a fume hood and allowed to cool, then 100 ml aqueous was added to the flask and poured into the blackish crystals. The solution was mixed thoroughly and was filtered using a filter paper 3-4 times till a pure white crystalline mixture was obtained. Distilled water was added up to 100 ml, which was used to analyze Elementals with atomic absorption spectrophotometer.

2.4. Gas Chromatography-Mass spectroscopy (GC-MS) analysis

For the GC-MS analysis, various extracts were selected to detect volatile and semi-volatile compounds. The column's initial temperature was set at 50ºC, with a temperature increase of 4ºC per minute. The final detection temperature of the instrument was maintained at 240ºC. Helium gas was employed as the carrier for compound isolation and detection. A 1 µl sample was injected into the instrument. Volatile and semi-volatile compounds were identified based on their electronic fragments (peaks). Each compound possessed its own retention time (R.T) and peak size. The x-axis of the chromatogram represented the retention time, while the y-axis depicted the strength and concentration of the compounds. The GC-MS chromatogram was compared to the reference search library program of the National Institute of Standards and Technology (NIST) for identification purposes.

2.5. Biological perspective

2.5.1. Antibacterial activity

The antibacterial potential was determined through well-diffusion methods. Bacterial cultures of *S. aureus, E. coli, K. pneumonia, B. subtilis,* and *S. pneumonia* were spread on nutrient agar plates. The antibiotics (Cefixime and Roxithromycin) were used as a control. The strains were cultured with the help of a cotton swab. The Wells were made with the help of a cork borer. The samples were loaded with the use of a micropipette. Different concentrations of samples were loaded into wells. Petri plates were incubated at 37 °C for 24 hours. The zones of inhibitions were measured using a Vernier caliper after the incubation periods. [11].

2.5.2. Antifungal activity

The suitable diffusion method determined each plant extract's antifungal activity. The *Aspergillus Niger* (FCBP-0198*), Aspergillus flavas* (FCBP-0064), and *Aspergillus fumigatus* (FCBP-66), (*Mucor species* (FCBP-0300) spores were cultured in distilled water. The swab was inoculated with each strain diluted in 100 µl deionized water and inoculated in Dextrose agar plates. The extracts (100mg/ml of DMSO), positive control (miconazole,4mg/ml), and negative control of DMSO were impregnated with sterile filter paper. All the substances were incubated for 24-48 hours at 28°C. The zone of inhibition was measured and recorded.

2.5.3. Antioxidant activity through DPPH Free radical scavenging assay

The free radical scavenging potential of the plant extracts against DPPH was determined following the method described by Bhaskar et al. in 2021.Sample solutions were prepared in methanol at a concentration of 1 mg/ml and further diluted to concentrations of 1000, 500, 250, 125, and 62.5 µg/ml. For the assay, 100 µl of each diluted sample was mixed with 3 ml of the DPPH solution in methanol. The absorbance of the samples, ascorbic acid, and blank solution was measured at a wavelength of 517 nm using a UV spectrophotometer. DPPH solution served as the negative control, while ascorbic acid was used as the positive control. The protocols for the DPPH assay were modified based on the method described by Bhaskar et al. in 2021. The percentage of inhibition was calculated using the following formula [12]: [Provide the formula for calculating the percentage of inhibition if available.]

$$\textbf{Inhibition percentage}(\%) = \frac{\textbf{Standard absorb} - \textbf{extract absorb}}{\textbf{Standard absorb}} \textbf{x100}$$

3. Results and discussion

3.1. Phytochemical analysis of *E. heterophylla*

The phytochemical analysis showed that alkaloids were highest in chloroform and methanolic extracts, followed by n-hexane, while partially present in the aqueous and ethyl acetate extracts. The flavonoid contents were partially present in chloroform and methanolic extracts and absent in other extracts. The tannin contents were present partially only in chloroform and Aqueous extracts. The

presence of terpenoid content was highest in chloroform and methanolic extracts, followed by the aqueous and ethyl acetate extracts, while in n-hexane terpenoids were absent. In ethyl acetate extracts, the glycoside contents were moderately and partially present in the aqueous and methanolic extracts, while it was not detected in the chloroform and n-hexane extracts. The volatile compounds were present moderately in the methanol chloroform and ethyl acetate and were absent in D. water and n-hexane.The results of the phytochemical screening of *E. heterophylla* are shown in Table 1. Similar results from Sajid *et al.* analyzed the phytochemicals in Alnus nitida. They found that the terpenoid content was highest in chloroform and methanolic extracts. The terpenoid was present in the Aqueous more than ethyl acetate in the same concentration. In ethyl acetate extracts, the glycoside is greater than aqueous methanolic extract [7].

Table: 1 Phytochemicals analysis of different extracts of *E. heterophylly.* {Highly present (+++), moderately present (++), partially present (+), nil (-)}

	Extract				
Phytochemicals	**Chloroform**	**n. hexane**	**Methanol**	**D. water**	**Ethyl Acetate**
Alkaloids	+++	++	+++	+	+
Flavonoids	+	-	+	-	-
Tannin	+	-	-	+	-
Terpenoids	++	-	++	+	+
Glycosides	-	-	+	+	++
Volatile	++	-	++	-	+

3.2. Elemental analysis of *E. heterophylla*

The analyzed Elements in *E. heterophylla* were cadmium, calcium, chromium, cobalt, copper, iron, lead, manganese, magnesium, nickel, potassium, sodium, and zinc. The detected value of sodium was 95.00±3.21mg/100g, the highest Element present in *E. heterophylla*. The copper level observed was 0.235±0.023 mg/100g. The calculated value of zinc was 0.125±0.015 mg/100g. The level of lead was 0.056± 0.005 mg/100g.

The chromium level observed in *E. heterophylla* was 0.030 ± 0.003 mg/100g. The analytical value of potassium and calcium was 60.00 ± 0.105 mg/100g and 50.050 ± 1.24 mg/100g, respectively. The magnesium was 4.041 ± 0.87 mg/100g. The lowest results were detected in the cobalt, i.e., 0.020 ± 0.004 mg/100g, as shown in Table 2.

Previous research indicates that the leaf samples of medicinal plants, such as *Alternanthera sessilis,* contain a high concentration of Mn, Fe, Na, and potassium. Copper and zinc were present moderately. In the current study, the similar results of *E. heterophylla* to the *Alternanthera sessilis.* The use of potassium and sodium is necessary for maintaining ionic equilibrium. These are also important for normal hypertension [13,14]. The strengthening of teeth, bones, and body rigidity is due to calcium. Calcium also helps in blood clotting. It is also helpful for food storage and involves a variety of cellular functions, nervous systems, hormone responses, cellular functions, and blood coagulation. It is also essential for healthy teeth and bones [15].

Table: 2 Elemental analysis of *E. heterophylla*

Elements	**(mg/100g)**
Copper	0.321 ± 0.031
Zinc	0.143 ± 0.013
Manganese	0.045 ± 0.045
Iron	2.321 ± 0.036
Nickel	6.345 ± 0.034
Cadmium	0.034 ± 0.034
Lead	0.067 ± 0.009
Chromium	0.050 ± 0.006

Cobalt	0.040 ± 0.007
Sodium	93.00 ± 4.21
Potassium	50.00 ± 0.106
Calcium	45.050 ± 1.24
Magnesium	5.041 ± 0.89

3.3. GC-MS Analysis of of chloroform extracts of *E. heterophylla*

Gas chromatography and mass spectroscopy(GC-MS) is a modern analogical technique for analysing volatile and semi-volatile compounds. The helium gas was used as a carrier. The chromatogram of the unknown component was compared with the spectrum of the component stored in the NIST library version (2005) software, Turbomas. The chemical compounds were analysed based on their retention time (RT), molecular mass (M. mass), and area concentration (%). A total of 16 compounds were identified by GC-MS analysis in the chloroform extract of *E. heterophylla.* The most abundant identified compounds were 2,4-Di-Tert-Butyl Phenol (Pentadecane, 2,6,10,14-tetramethyl- and 1-decanol, 2-hexyl. The results are shown in Table 3. The present study, which reveals the presence of components in *E. heterophylla,* suggests that the contribution of these compounds to pharmacological activity should be evaluated. The 2,4-Di-Tert-Butyle Phenol is one of the sixteen compounds in the recent study. Similarly, Jancy Rani *et al.* 2011 observed the presence of phytol in the plant of *Lantana Camara* [16]. Mangunwidjaja *et al*. (2006) reported the main components of 9, 12 octadecadienoic acid, Octadec-9-enoic acid and 9,12-actadecadienoic acid present in *Croton tiglium* seed. These compounds were found to have potential antioxidant and anticancer activities [17]. Elshamy et al.,2019 reported the GC-MS analysis of essential oils of *E. heterophylla* species collected from Egypt and they identified *β*-elemene camphor, endo-boneol, limonene, *α* pine, pentatriacontane and 1,8-Cineole major components [22].

Table: 3 GC-MS Analysis Results of chloroform extract of *E. heterophylla*

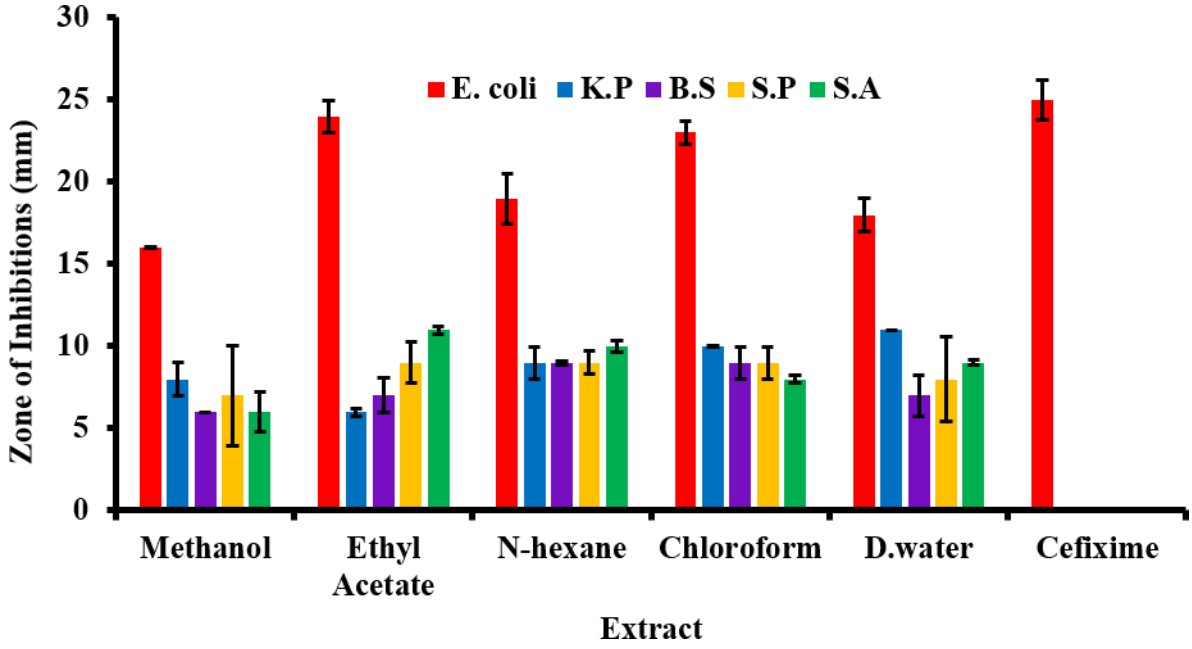
S.NO	Compounds Name	R-Time	Kovats RI of litrature	Calculated RI	Area	Area %
1	O-xylene	4.394	873	874	234502.063	3.367572
2	Nonane, 5-propyl-	8.846	1069	1053	214780.703	3.084363
3	1r,2c,3t,4t-tetramethyl-cyclohexane	9.436	1095	1075	102004.984	1.464845
4	Decane, 3,8-dimethyl-	11.697	1144	1156	212620.25	3.053338
5	4-chlorobutyric acid, 2-methylpentyl ester	12.938	1220	1200	236892.047	3.401894
6	Benzene, 1,3-bis(1,1-dimethyle	14.253	1245	1249	652893.75	9.375896
7	Undecan,3,6-dimethyl-	14.653	1249	1264	162358.609	2.331555
8	1-octanol, 2-butyl-	15.013	1277	1277	509548.125	7.317378
9	1-octacosanol, 2,4,6,8-tetramethyl-, (all-r)-	16.169	1285	1319	101763.117	1.461372
10	Disulfide, di-tert-dodecyl	16.284	1323	1323	175327.969	2.517801
12	Carbonic acid, eicosyl vinyl ester	18.41	1414	1404	280190.719	4.023685
13	2,6,10-trimethyltridecane	20.591	1465	1489	203463.234	2.921838
14	1-decanol, 2-hexyl-	20.721	1504	1495	745480.563	10.70549
15	2,4-di-tert-butylphenol	21.471	1519	1525	2285476	32.82063
16	Pentadecane, 2,6,10,14-tetramethyl-	26.503	1712	1830	846232.438	12.15234

4. Biological assessments of *E. heterophylla*

4.1. Antibacterial Activity

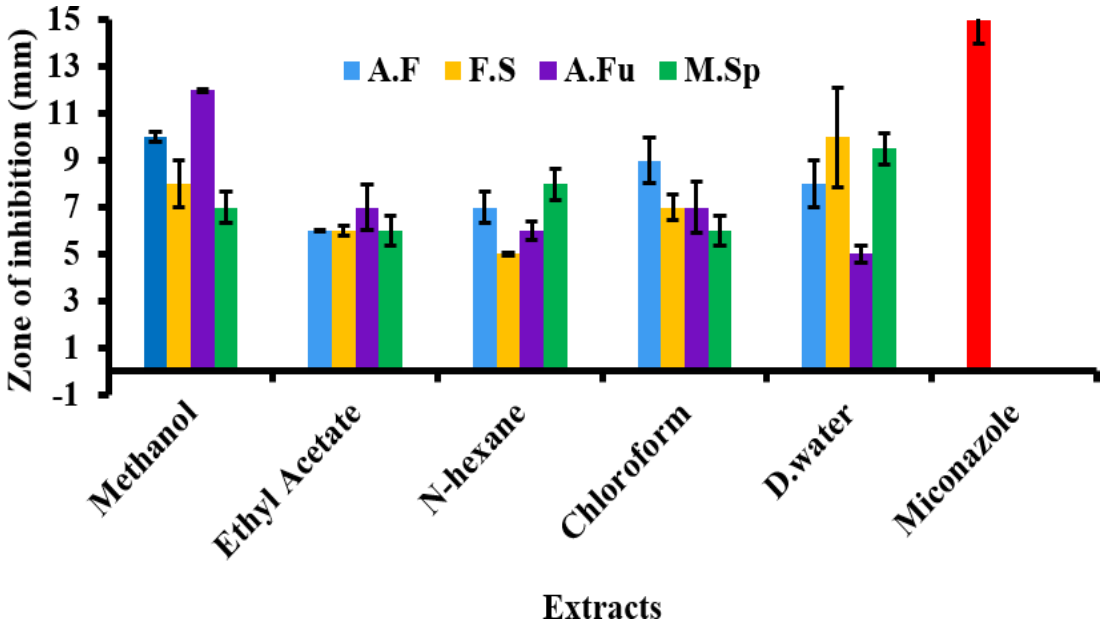
The various specific strains of bacteria showed resistance against different antibiotics such as penicillin G, macrolides, glycosamides, tetracycline, and gentamycin [18,19]. Due to these drastic conditions, the interest was more than in the last decades. Various extractions from *E. heterophylla* were checked for antibacterial activity against gram-positive and negative bacterial strains using the well diffusion method. In the present study, the five different bacterial strains (*Staphylococcus aureus, Escherichia coli, Bacillus subtitils, Salmonella typhi,* and *Klebsiella pneumonia*) were used for the antibacterial activity. A higher zone of inhibition against *E.*

coli and *K. pneumonia* species was measured. The *S. aureus* was more affected by ethyl acetate extract, forming zone inhibition of 11mm; similarly, extracts of ethyl acetate and Aqueous showed 24mm and 11mm zone of inhibition against *E. coli* and *K. pneumonia* species which were the highest zone of inhibitions as shown in Figure 3. The maximum zone of inhibition of *E. coli* was observed in the ethyl acetate, followed by chloroform, n-hexane, aqueous, and methanol. The second good antibacterial activity was shown against *S. aureus* by ethyl acetate followed by n-hexane distilled water, chloroform, and methanol extracts. In *K. pneumonia,* the highest zone of inhibition was recorded against the aqueous extracts. The highest zone of inhibitions was measured in methanolic and ethanolic extracts. of *Hopea parviflora beddome* [20]. Different gram-positive and gram-negative strains of bacteria were evaluated of ethanolic extracts for their antimicrobial activity of *brynopsis laciniosia.*



4.2. Antifungal Activity

In the recent study, the extracts of *E. heterophylla* were further evaluated for antifungal activity against four different fungal strains. i.e., *Fusarium saloni, Mucor* species, *Aspergillus flaves,* and *Aspergillus fumigatus.* The methanolic extracts of *E. heterophylla* showed the highest zone of inhibition (12mm) against *Aspergillus fumigatus* (*A. Fu*). Extracts of chloroform and ethyl acetate showed zone of inhibitions 9mm and 7mm against *Aspergillus flaves (A. F)* and *Aspergillus fumigatus*. The n-hexane extracts showed the maximum zone of inhibition (8mm) against *Mucor* species, followed by *Aspergillus flaves* (7mm), *Aspergillus fumigates* (6mm), and *Fusarium saloni* (5mm). The distilled water extracts have the zone of inhibitions of 10mm, 9.5mm, 8mm, and 5mm against *Fusarium saloni, Mucor* species, *Aspergillus flaves,* and *Aspergillus fumigatus,* respectively—the results as shown in Figure 4. A standard drug, miconazole was used reference control and it showed the highest antifungal activity. Different studies were conducted on different plants, such as *Sids corfoliaL, Tinospora cordifolia L, Winthania sonnifera L, Zizipus mauritiana L,* and *Acacia nilotica L.* All of these were investigated for the antifungal activities of *Aspergillus falves, Drreschleera turcica,* and *fusarium verticilloidis.* The highest antifungal effect was observed in the *Acacia nilotica* bark. The water and methanolic extracts showed a significant impact against *Xanthomonas spp.* The ethanolic extracts of the roots of *Psuedarthria viscida* have been studied for antimicrobial study [21].



4.3. Antioxidant activity by DPPH methods

The antioxidant activities of *E. heterophylla* were carried out by DPPH free radical scavenging assay. DPPH radical scavenging assay determined the antioxidant potential of the *E. heterophylla* extracts such as methanol, chloroform, ethyl acetate, n-hexane, and D. Water. The ascorbic acid was used as a positive control. In this research, the DPPH results showed the highest percentage of inhibitions at a maximum concentration (1000µg/ml) in methanol (84.62±0.47) followed by chloroform (83.00±0.51), n-hexane (80.99±1.02), ethyl acetate (78.37±0.23) and aqueous (75.24±1.28) extracts respectively. At minimum concentration (31.5µg/ml), the antioxidants activity through DPPH assay was the methanolic (49.05±1.62) extract followed by the chloroform (49.56±0.92) ethyl acetate (48.40±1.09), distilled water (45.09±1.13) and n-hexane (42.07±0.76) extracts. The percent inhibition of ascorbic acid was 85.96 ± 0.29 and 47.07 ± 0.98 at concentrations of 1000µg/ml and 31.5µg/ml, and all the results were compared with the standard as shown in Table 4.

Table: 4 Antioxidant activity of different extracts of *E. heterophylla* by DPPH assay

Extracts	1000 µg /mL	500 µg /Ml	250 µg /mL	125 µg /mL	62.5 µg/mL	31.5 µg mL
Methanol	84.62 ± 0.47	73.37 ± 0.48	68.68 ± 0.76	64.74 ± 1.15	57.31 ± 1.02	49.05 ± 1.62
Chloroform	83.00 ± 0.51	78.96 ± 1.22	73.70 ± 0.35	67.03 ± 1.04	56.21 ± 1.13	49.56 ± 0.92
Ethyl acetate	78.37 ± 0.23	72.25 ± 0.86	65.29 ± 1.03	59.06 ± 0.58	55.45 ± 1.03	48.40 ± 1.09
n-hexane	80.99 ± 1.02	74.71 ± 0.14	69.26 ± 0.13	62.66 ± 0.28	52.78 ± 1.37	42.07 ± 0.76
D. water	75.24 ± 1.28	68.36 ± 0.83	62.22 ± 0.16	56.03 ± 0.53	50.01 ± 0.65	45.09 ± 1.13
Ascorbic acid	85.96 ± 0.29	79.10 ± 0.57	73.48 ± 0.76	68.70 ± 0.47	53.19 ± 1.09	47.07 ± 0.98

4. Conclusions

The findings of this study demonstrate the diverse array of secondary metabolites present in different extracts of E. heterophylla, each possessing unique morphological properties. The comprehensive analysis of phytochemicals, Elements, GC-MS profiles, and biological perspectives sheds light on the presence of bioactive compounds with significant medicinal properties and therapeutic applications. The GC-MS analysis revealed the identification of 27 volatile and semi-volatile constituents, which contribute to various biological activities such as antimicrobial and antioxidant among others. The presence of these different compounds in various plant extracts likely plays a pivotal role in their observed therapeutic effects. These findings further underscore the

potential of *E. heterophylla* as a valuable source of bioactive compounds with promising medicinal implications.

Funding: Not applicable.
Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.
Data Availability Statement: Not applicable.
Conflicts of Interest: The Authors declare that there is no conflict of interest among them.

References

1. Verma, B., Karakoti, H., Kumar, R., Mahawer, S. K., Prakash, O., Srivastava, R. M., ... & Oliveira, M. S. D. (2023). Phytochemical Screening and Evaluation of Pesticidal Efficacy in the Oleoresins of Globba sessiliflora Sims and In Silico Study. *Evidence-Based Complementary and Alternative Medicine*, *2023*.
2. Hazra, S., Ray, A. S., Das, S., Das Gupta, A., & Rahaman, C. H. (2023). Phytochemical Profiling, Biological Activities, and In Silico Molecular Docking Studies of Causonis trifolia (L.) Mabb. & J. Wen Shoot. *Plants*, *12*(7), 1495.
3. Oso, B., & Ogunnusi, T. (2017). Antibacterial Activity of METHANOLIC EXTRACTS of Euphorbia heterophylla and Tithonia diversifolia against some microorganisms. *European Journal of Medicinal Plants*, *20*(3), 1-8.
4. Kouassi, G. F., Koné, G. A., Good, M., Assidjo, N. E., & Kouba, M. (2020). Effect of Hevea brasiliensis seed meal or Euphorbia heterophylla seed supplemented diets on performance, physicochemical and sensory properties of eggs, and egg yolk fatty acid profile in guinea fowl (Numida meleagris). *Poultry science*, *99*(1), 342-349.
5. Oloninefa, S. D., Abalaka, M. E., Daniyan, S. Y., & Mann, A. (2018). Phytochemical screening and antibacterial susceptibility of whole plant of Euphorbia heterophylla crude extracts against selected bacteria pathogens. *Bayero Journal of Pure and Applied Sciences*, *11*(1), 211-220.
6. Falodun, A., Agbakwuru, E. O. P., & Ukoh, G. C. (2003).? ANTIBACTERIAL ACTIVITY OF EUPHOR-BIA HETEROPHYLLA LINN (FAMILY-EUPHORBIACEAE). *Biological Sciences-PJSIR*, *46*(6), 471-472.
7. Sajid, M., Khan, M. R., Shah, N. A., Shah, S. A., Ismail, H., Younis, T., & Zahra, Z. (2016). Phytochemical, antioxidant and hepatoprotective effects of Alnus nitida bark in carbon tetrachloride challenged Sprague Dawley rats. *BMC complementary and alternative medicine*, *16*(1), 1-17.
8. Alqethami, A., & Aldhebiani, A. Y. (2021). Medicinal plants used in Jeddah, Saudi Arabia: phytochemical screening. *Saudi Journal of Biological Sciences*, *28*(1), 805-812.
9. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4), 42.
10. Siddiqui, S., Bhardwaj, S., Khan, S. S., & Meghvanshi, M. K. (2009). Allelopathic effect of different concentration of water extract of Prosopsis juliflora leaf on seed germination and radicle length of wheat (Triticum aestivum Var-Lok-1). *American-Eurasian Journal of Scientific Research*, *4*(2), 81-84.
11. Orchard, A., & van Vuuren, S. (2017). Commercial essential oils as potential antimicrobials to treat skin diseases. *Evidence-Based Complementary and Alternative Medicine*, *2017*.
12. Bhaskar, A., Nithya, V., & Vidhya, V. G. (2011). Phytochemical screening and in vitro antioxidant activities of the ethanolic extract of Hibiscus rosa sinensis L. *Annals of Biological Research*, *2*(5), 653-661.
13. Seal, T. (2011). Determination of nutritive value, Elemental contents and antioxidant activity of some wild edible plants from Meghalaya State, India. *Asian J. Appl. Sci*, *4*(3), 238-246.

14. Saupi, N., Zakaria, M. H., & Bujang, J. S. (2009). Analytic chemical composition and Elemental content of yellow velvetleaf (Limnocharis flava L. Buchenau)'s edible parts. *Journal of Applied Sciences*, *9*(16), 2969-2974.
15. Igwenyi, I. O., Agwor, A. S., Nwigboji, I. U., Agbafor, K. N., & Offor, C. E. (2014). Proximate analysis, Elemental and phytochemical composition of Euphorbia hyssopifolia. *Steroids*, *18*, 6-27.
16. Jancy Rani, P. M., Kannan, P., & Kumaravel, S. (2011). Screening of antioxidant activity, total phenolics and gas chromatograph and mass spectrometer (GC-MS) study of Delonix regia. *Afr J Biochem Res*, *2*, 341-7.
17. Mangunwidjaja, D., Raharja, S., Kardono, L., & Iswantini, D. (2006). Gas chromatography and gas chromatography-mass spectrometry analysis of Indonesian Croton tiglium seeds. *Journal of Applied Sciences*, *6*(7), 1576-1580.
18. Capasso, A. (2013). Antioxidant action and therapeutic efficacy of Allium sativum L. *Molecules*, *18*(1), 690-700.
19. Ahmad, R., Upadhyay, A., Ahmad, M., & Pieters, L. (2013). Antioxidant, antliglycation and antimicrobial activities of** Ziziphus oxyphylla** and** Cedrela serrata** eExtracts. *European journal of medicinal plants*, *3*(4), 520-529
20. Ehsan, B., Vital, A., & Bipinraj, N. (2009). Antimicrobial activity of the ethanolic extract of Bryonopsis laciniosa leaf, stem, fruit and seed. *African Journal of Biotechnology*, *8*(15).
21. Khan, W., Ali, M., Zaman, S. U., Nazir, N., Ullah, F., Ali, Z., & Nisar, M. In-vitro antioxidant and anti-microbial potential of the pollen extracts of Pinus roxburghii Sarg.
22. Elshamy, A. I., Abd-ElGawad, A. M., El Gendy, A. E. N. G., & Assaeed, A. M. (2019). Chemical characterization of Euphorbia heterophylla L. essential oils and their antioxidant activity and allelopathic potential on Cenchrus echinatus L. *Chemistry & biodiversity*, *16*(5), e1900051