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Research Article

Euphorbia Cyathophora Against Earias Vittella

Study on Phytochemical Profiling of *Euphorbia cyathophora* Leaf Extracts and Their Potential Antimicrobial, Larvicidal, and Pupicidal Properties Against *Earias vittella*

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Abstract: *Earias vittella* is a very serious polyphagous agricultural pest that can directly or indirectly damage crops and stored products worldwide. The use ofUsing synthetic chemicals in pest control has resulted in physiological resistance and adverse environmental effects. However, this alternative to plant-based pest control systems is ecologicalecologically friendly and biodegradable. *Moreover, Euphorbia cyathophora* is a medicinal plant used for many therapeutic activities. Therefore, the present study is aimed forat*E. cyathophora* leaf extract evaluation of the phytochemical, *in vitro* antimicrobial, larvicidal, and pupal activity of *E. vittella*. A preliminary phytochemical screening of *E.cyathophora* leaf various extracts revealed the presence of numerous bioactive components such as alkaloids, flavonoids, sterols, terpenoids, anthraquinones, carbohydrates, and essential oils. GC-MS analysis of *E.cyathophora* leaf different extracts revealed the presence of 29 phytochemicals. Among the extracts tested, the methanolic leaf extract showed the highest range of growth inhibition against gram-positive bacteria *Streptococcus iniae* (15 ± 0.26) and gram-negative bacteria *Aeromonas hydrophila* (13 ± 0.6), while antifungal activity against *Aspergillus flavus* (14 ± 0.2) and *Aspergillus niger* (12 ± 0.36) compared to other solvent extracts. In addition, the bioefficacy of *E.Cyathophora* leaf extract treated against IV instar larvae and pupae of *E. vittella*; however, the highest larval and pupal mortality was observed in the methanol extract at 750 ppm, followed by acetone and chloroform against *E. vittella*. Larvicidal activity of *E. cyathophora* leaf extracts showed LC_{50} and LC_{90} values: methanol 298.64 ppm (708.14); Acetone 362.61 ppm (848.56)), and chloroform 415.18 ppm (937.31), respectively. Similarly, thepupicidal effect of *E. Cyathophora* leaf extracts showed LC_{50} and LC_{90} values: methanol 470.25 ppm (958.01); acetone 527.36 ppm (996.35) and chloroform 545.55 ppm (997.88). This study strongly suggests that *E. cyathophora* leaf extract exhibited significant larval and pupal mortality and potential antimicrobial activity, which can be observed as the development of a new botanical preparation for economicaleconomic pest control.

Keywords: *Euphorbia cyathophora*, Phytochemical screening, GC-MS analysis, *Earias vittella,* antifungal, antibacterial

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1. INTRODUCTION

India is an agricultural country, and our economy is solely driven by agricultural productivity. Insects destroy 5 to 30% of the world's total agricultural production. Both agricultural production and stored agricultural products are under serious threat[1]. *Earias vittella* (Noctuidae: Lepidoptera) is a pest that affects a variety of economically significant crops and has a wide range of hosts, including cotton, okra, and peas.[2,3] *E. vittella* is generally an early and mid-season pest, attacking the delicate terminal shoots, sneaking into the stem, and feeding on flowers and green pods [4.] Larvae not only attack capsules and fruits, but also damage shoots, buds, and flowers [5]. However, this pest can cause up to 40% and 41.6% of cotton seed and okra losses, respectively. Conversely, cotton yield losses can reach 50% with a heavy infestation. The larvae cause 50-70% damage, including deformation and poor-quality fruit [6,7]. Infectious diseases remain a major public health problem, accounting for 41% of the global burden of disease measured in Disability Adjusted Life Years. Because of this problem of antibiotic resistance, attention is now turning to biologically active ingredients isolated from coexisting plant species used as herbal medicine as they could represent a potential new source of antibacterial and antifungal activity. Chemicals such as synthetic pesticides are indispensable to technologically advancing agricultural production processes. Using chemicals is the best strategy to prevent pre- and post-harvest crop losses from insect pests and diseases. However, synthetic insecticides have caused many unexpected problems at the time of their toxicity, causing acute and chronic poisoning of users, mold workers, consumers, fish, birds, and other wild animals, etc., with risks to humans, live animals, and the entire environment and ecosystem. Therefore, searching for plants or biopesticides can be an effective and advantageous substitute for commercial pesticides, resulting in an integrated approach to pest control due to specific and environmentally friendly targets, a small number of applications, higher acceptable properties, and an improvement in pest management insects and plant pests. Pathogenic microorganisms are currently an inevitable trend.[8,9]. Many Euphorbiaceae plants have been used in traditional, complementary, and alternative medicine.[10]. The annual herb *Euphorbia cyathophora* is used for many wound-healing, antimicrobial, anticancer, and Ayurvedic remedies, including dwarf star, fire tip, and painted leaf, found locally in North and South America and naturalized elsewhere. Medicinal plants are important because, according to traditional medicinal practices and modern scientific research, they are useful for medicinal purposes to alleviate diseases and improve human health. Many studies present medicinal plants as valid therapeutic aids for various ailments. Known as biopesticides, these natural plant products have long been used to control microorganisms that cause plant diseases. [11] These plants are considered a rich source of ingredients for drug synthesis and production.[12] In recent years, gas chromatography-mass spectrometry (GC-MS) has been widely used to identify various bioactive medicinal compounds in medicinal plants. GC-MS is one of the best, fastest, and most accurate techniques for detecting various compounds, including alcohols, alkaloids, nitro compounds, long-chain hydrocarbons, organic acids, steroids, esters, and amino acids, and requires a small number of plant extracts. Many of these benefits point to the potential role of phytochemicals in disease prevention and treatment and in treating human disease for hundreds of years. They may have a large and diverse collection of natural compounds that plant antioxidants trigger certain physiological movements in the human body. Plants produce a variety of secondary metabolites with significant differences in their structure and are useful to humans in various applications. Secondary metabolites are classified into glycosides, alkaloids, phenols, terpenoids, tannins, essential oils, saponins, resins, steroids, etc. Many of these secondary metabolites, likely true plant isolates, mixtures, and chemically modified derivatives, are of commercial importance. And are used in flavors, fragrances, and pharmaceutical preparations, biologically living compounds worldwide that can protect against diseases.[13,14,15] Today, several chemicals derived from plants are used as essential pills in several countries worldwide. [16.] Therefore, the present study investigated phytochemical screening and GCMS analysis of *E. cyathophora* leaf extract. In addition, this study evaluated the in vitro antimicrobial, larvicidal, and pupicidal -the effective activity of *E. cyathophora* leaf extract against *E. vittella*

2. MATERIALS AND METHODOLOGY

2.1. Collection and Identification of Plant Material

Fresh leaves of *Euphorbia cyathophora* were collected from in and around Namakkal District, Tamilnadu, India. The plant was taxonomically identified and authenticated by Dr. K. Raju , Associate Professor, PG and Research Department of Botany, KandaswamiKandar's College, Velur-Namakkal, Tamilnadu, Affiliated by Periyar University. The voucher specimen of the plant has been deposited in the college laboratory for further reference. Voucher Number: BSI/KKC/4/11/2017/Tech/212.

Fig 1 and 2: *Euphorbia cyathophora plant*

2.2. *Preparation of plant extract*

The leaves were washed in tap water and dried in the shade at room temperature (28 ± 2°C) for 5to10 to days. Because some compounds denature in the sun, they are dried in the shade to prevent decomposition. Therefore, 250 g of fresh and ripe leaves were rinsed with distilled water and dried in the shade. The dried leaves were placed in a Soxhlet apparatus (Borosil Glass Workers Ltd, Mumbai, India) and the extracts were prepared with chloroform, acetone, and methanol [(LobaChemiePvt. Ltd., Bombay, India. Purity 99%) (the concentration of chloroform, acetone, and methanol is 100%, the extraction time is 72 hours and the temperature is kept at 30-40°C)]. The extract yield was 100 g and evaporated to dryness in a vacuum rotary evaporator. The resulting dried residue was stored in airtight bottles in a refrigerator for later use.

2.3. *Preliminary Phytochemical screening*

Preliminary phytochemical testing of *E.cyathophora* leaf extracts was performed according to the standard qualitative methods of Harborne and Trease, and Evans. In addition, phytochemicals have been tested to distinguish the presence or absence of specific phytochemical groups.

2.4. *Gas chromatography-mass spectrum (GC-MS) analysis*

The Clarus 680 GC was used in the analysis using a fused silica column packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µmdf), and the Components were separated using helium as the flow medium constant gas. 1 mL/min The inlet temperature was set at 260°C during the chromatographic run. 1 µl of the extracted sample was injected into the device; the oven temperature was: 60°C (2 min); then from 300°C to 10°C min-1; and 300°C, holding for 6 min. Mass detector conditions were as follows: transfer line temperature of 240°C; ion source temperature of 240°C; electron collision in ionization mode at 70 eV, sample time 0. of 2 seconds, and a sampling interval of 0.1 seconds—fragments from 40 to 600 Da.

2.5. *Collection of bacterial and fungal culture*

Pathogenic bacteria were obtained from CMFRI Microbiology Laboratory, Cochin, India. The pathogenic fungus was obtained from TRI-Biotech, Trichy, India. The gram-positive bacterium Streptococcus iniae and the gram-negative bacterium *Aeromonashydrophila*, followed by the fungus *Aspergillus niger* and *Aspergillus flavus*, were used for antimicrobial screening.

2.6. *Antibacterial activity*

The antibacterial activity of *E. Cyathophora* leaf extracts was tested using the standard agar well diffusion method with negligible modifications. Pathogenic bacteria were grown on nutrient agar prepared by dissolving 28 g in 1000 ml distilled water and autoclave at 15 psi and 121°C for 15 min. To disinfect the Petri plate, the medium was poured and let to stand. After solidification, 70µl of the bacterial suspension of *A.hydrophila* and *S. iniae* were collected. Acorkscrew was used to make a well, and each sample(25µl,50µl,75µl)was poured in, and amoxyclav (10 mg) was used as a positive control. After placing the samples, the plates were incubated for 24hours at 37°C, and the zone of inhibition was measured in mm.

2.7. *Antifungal activity*

Potato dextrose agar medium was prepared by dissolving 40 g potato infusion, 4 g dextrose, and 3.5 g agar in 200 ml distilled water. The dissolved medium was autoclaved at 15 (lb) pounds at 121°C for 15 minutes. The autoclaved medium was mixed well and poured into 100 mm Petri dishes (25-30 ml/plate) while still molten. The antifungal in the sample was allowed to diffuse through the medium and act on the plate inoculated with the fungal cultures. To be tested. The resulting inhibition zones were uniformly circular because of a confluent growth pasture. Therefore, the diameter of the zone of inhibition was measured in millimeters. Petri dishes containing 20 ml of potato dextrose agar medium were inoculated with a 72-hourculture of a fungal strain (A. niger and A. flavus). Wells were cut, and different concentrations (25, 50, and 75 µl/ml) of samples were added. The plates were then incubated at 37°C for 48-72 hours. Antifungal activity was determined by measuring the diameter of the zone of inhibition formed around the wells. Amphotericin B

(100 units) was used as a positive control. The values were calculated with the Graph Pad Prism 6 software.

2.8. Collection and Mass Culture of Test Insects

Egg mass and larvae of *E. vittella* were collected from Bhendi fruit field in Namakkal, Tamil Nadu, India. The culture of *E. vittella* was carried out under laboratory conditions. Fresh Bhendis were fed larvae daily until pupation. The *E. vittella* was taxonomically identified and authenticated by Dr. K.Murugan, D.Sc., UGC-Emeritus Professor, Department of Zoology, Bharathiar University, Coimbatore, Tamilnadu.

2.9. Insect Mortality Bioassay

Sliced bhendi pods were treated with different concentrations *of E. cyathophora* leaf extract of 250, 500, 750, 1000, and 1250 ppm. Control leaves were treated with distilled water only. The leaves were allowed to dry for 10 min at room temperature and then placed on a damp filter paper disc in 10 cm diameter plastic Petri dishes. Experiments were performed with newly hatched and starved fourth-instar larvae for 3 hours using one larva per dish in five replicates (30larvae/concentration). After 4 days, the larvae were transferred to fresh, untreated, sliced bhendi pods and stored until they became adults or died. The total number of surviving normal adults was recorded. In addition, the larvae were observed for mortality and morphological changes associated with growth inhibitory effects. The data corrected for percent mortality were subjected to probit analysis to calculate the mean lethal concentration (LC50) (Finney, 1971). The results were modified for mortality in the control group using Abbott's (1925) formula.

$$P_t = (P_o - P_c / 100 - P_c) \times 100$$

Where,

P_t = Corrected percent mortality
P_o = Observed mortality
P_c = Observed mortality in the control

2.10. Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LC50, LC90, and other statistics at95 % fiducidal limits of upper fiducidal limit and lower fiducidal limit, and Chi-square values were calculated using the SPSS Statistical software package 16.0 version was used. Results with $P<0.05$ were considered to be statistically significant.

3. RESULTS

A preliminary phytochemical screening of various *E. cyathophora* leaf extracts revealed the presence of numerous bioactive chemical constituents such as alkaloids, flavonoids, sterols, terpenoids, anthraquinones, carbohydrates, and essential oils. The following anthocyanin phytonutrients, proteins, phenols, quinones, tannins, saponins, phytates, cardiac glycosides, glycosides, lignin, and coumarin are absent from all extracts tested. Alkaloids, flavonoids, and carbohydrates were present in all leaf extracts. Sterols were present in chloroform and methanol leaf extracts, except acetone.Terpenoids and anthraquinone are only found in acetone extract. In addition, essential oils were present in chloroform and acetone but not methanol extracts.*E. cyathophora* leaf extract, consisting of chloroform, acetone, and methanol, was analyzed by gas chromatography-mass spectrometry (GC-MS) to identify key phytochemicals. The results of GC-MS analysis of various solvent extracts from *E. cyathophora* leaves showed 29 chemical compounds. These compounds are known to have different therapeutic effects. Biologically active chemical components were characterized and identified based on retention time, molecular formula, and concentration (% peak area). GC-MS analysis of *E. cyathophora* leaf chloroform extract shows the presence of 10 bioactive compounds, including acidic acid, chloro, 5-dimethyl-2h-1,3-oxazine-3h-2,6-dione,1-propanol, 2-amino-, (s)-, 6h-purine-6-thione, 1,7-dihydro-7-methyl, dec-9-en-6-oxo-1-acrylamide, phenol, 4-[2-(methylamino) ethyl]-,4-amino-1-isobutyl piperidine, butylamine, 2,2-dinitro-n-methyl,1-aziridine-ethanol and pyrimidine, 1,4,5,6-tetrahydro-1,2-dimethyl.The GC-MS chromatogram of *E. cyathophora* acetoneextractshowed11 peaks, indicating the presence of 11 compounds shown in table 2 and figure2.hydroxyurea, pentane, 1,1'-oxy bis, 1-ethyl-2-phenylpyrazolium bromide, 2-azetidine, 3,3-diphenyl, 1-azabicyclo[2.2.2]oct-3-ylamine, urea, n-[2-[1-piperidyl]cyclohexyl]-, methyl 6,11-octadecadienoate, acetamide, n-[3-[3-(4-pyridinyl)ureido]phenyl]-, oxazole, trimethyl, cis,cis-1,6-dimethylspiro[4.5]decane and butylamine, 2,2-dinitro-n-methyl.Basedonthe GC-MS result,10 different bioactive compounds were confirmed in themethanolic extract of *E. cyathophora* leaves were confirmed. This was followed by5h-tetrazole-5-thione, 1-[2-(dimethylamino) ethyl]-1,2-dihydro, 4h-pyran-4-one, henethylamine, 3-methoxy-. alpha. -methyl-4,5-(methylenedioxy)-, 5-butyl-2-methyl-. Delta.1-pyrroline trimethadione, 7 azabicyclo[4.1.0] heptane,1-methyl-4-(1-methylethyl)-, 2-pyrrolidinone, 1-[4-(1-pyrrolidinyl)-2-butynyl]-, 3-azabutyl-1-ol, o-acetyl-4-cyclopropyl-n,n-dimethyl-, bromide, 9-thiabicyclo[3.3.1]nonan-3-one 9,9-dioxide and 4-amino-1-isobutylpiperidine respectively.

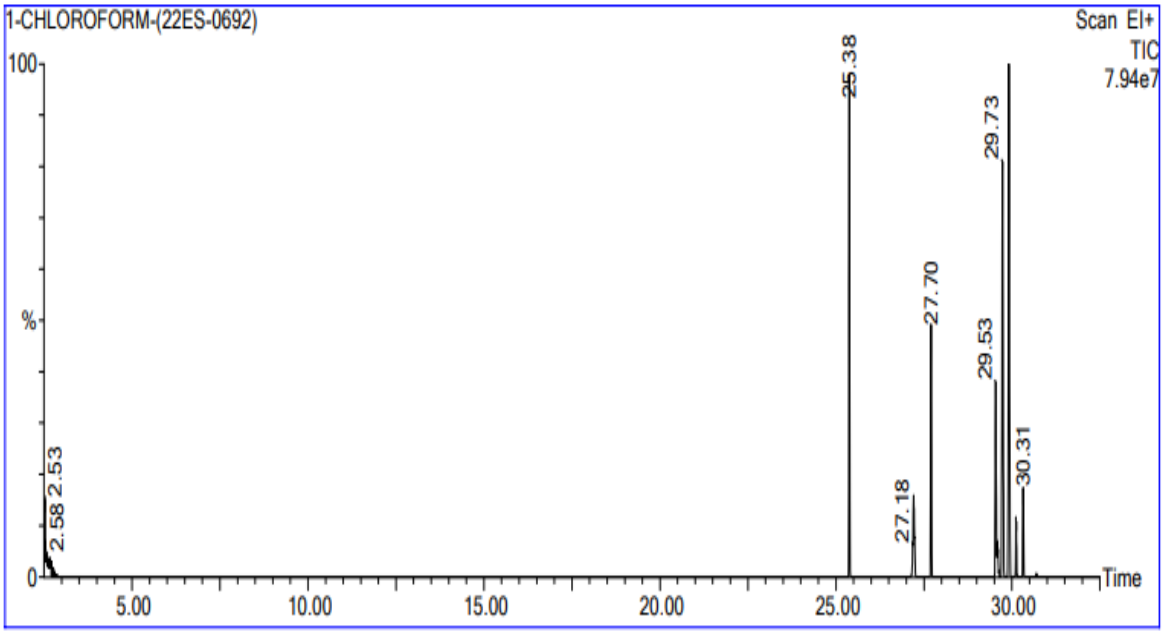


Fig 3: GC-MS chromatogram of *E. cyathophora* leaves chloroform extract

Table 1: GC-MS analysis of *E. Cyathophora*leaves chloroform extract

S. No.	RT	Name of the compounds	MF	MW	Area (%)	For	Rev
1	2.528	Acetic Acid, Chloro	$C_2H_3O_2Cl$	94	4.508	500	500
2	25.378	4,5-Dimethyl-2h-1,3-Oxazine-3h-2,6-Dione	$C_6H_7O_3N$	141	14.408	412	412
3	27.204	1-PROPANOL, 2-AMINO-, (S)-S	C_3H_9ON	75	6.150	656	656
4	27.699	6h-Purine-6-Thione, 1,7-Dihydro-7-Methyl	$C_6H_6N_4S$	166	9.907	525	525
5	29.535	Dec-9-En-6-Oxo-1-Ylamide	$C_{10}H_{17}O_2N$	183	9.064	429	429
6	29.590	Phenol, 4-[2-(Methylamino)Ethyl]-	$C_9H_{13}ON$	151	22.983	931	931
7	29.730	4-Amino-1-Isobutylpiperidine	$C_9H_{20}N_2$	156	22.983	496	593
8	29.910	Butanamine, 2,2-Dinitro-N-Methyl	$C_5H_{11}O_4N_3$	177	25.325	431	581
9	30.110	1-Aziridineethanol	C_4H_9ON	87	2.147	705	705
10	30.315	Pyrimidine, 1,4,5,6-Tetrahydro-1,2-Dimethyl	$C_6H_{12}N_2$	112	3.758	526	526

RT- Retention time; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse.

Gas chromatography-mass spectrometry (GC-MS) was used to evaluate the chloroform, acetone, and methanol-based leaf extract of *E. cythophora* to identify the major phytochemicals. It is shown in Table 1 and Figure 3. The phytochemicals in the various herbal solvent extracts were identified based on their retention time (RT), molecular formula, molecular weight, and percentage (area), Forward, and Reverse. Several solvent extracts of *E. cyathophora* leaves were subjected to GC-MS analysis, and the results revealed 29 chemical components. Several medicinal effects of these substances are well-known. Retention duration, molecular composition, and concentration (% peak area) were used to characterize and identify biologically active chemical components. There are 10 bioactive chemicals in the chloroform extract of *E. cyathophora* leaves, including acidic acid.

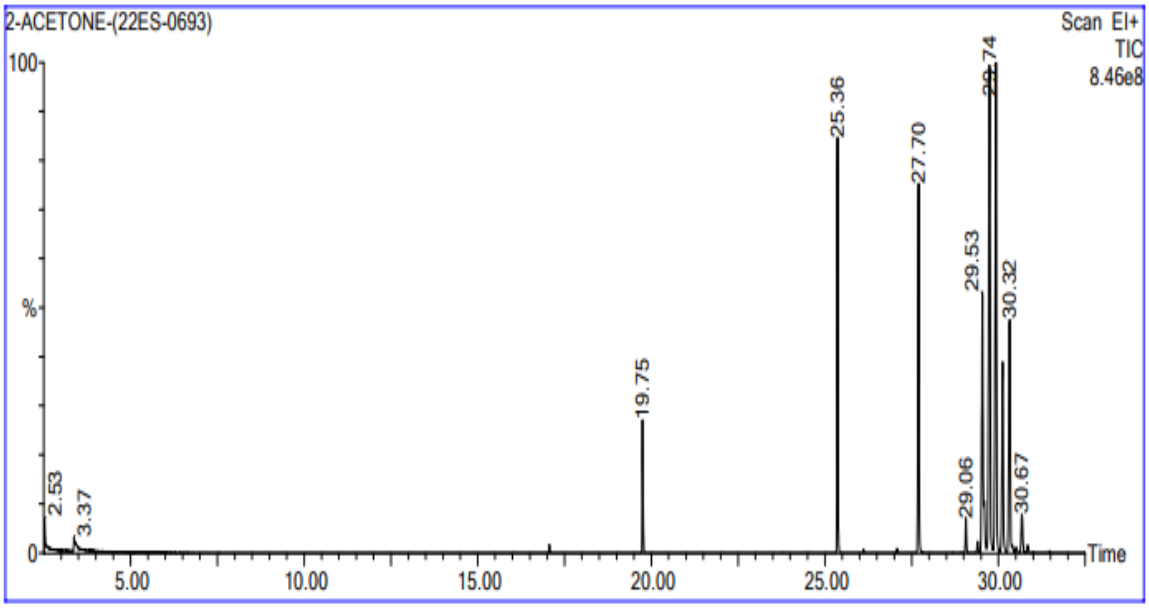


Fig 4: GC-MS chromatogram of *E. Cyathophora* leaves acetone extract

Table 2: GC-MS analysis of *E. cyathophora*leaves acetone extract

S. No.	RT	Name of the compounds	MF	MW	Area (%)	For	Rev
1	2.528	Hydroxyurea	$CH_4O_2N_2$	76	1.846	814	814
2	19.745	Pentane, 1,1'-Oxybis	$C_{10}H_{22}O$	158	2.902	626	752
3	25.363	1-Ethyl-2-Phenylpyrazolium Bromide	$C_7H_{13}N_2Br$	204	9.799	324	638
4	27.699	2-Azetidinone, 3,3-Diphenyl	$C_{15}H_{13}ON$	223	11.304	396	748s
5	29.059	1-Azabicyclo[2.2.2]Oct-3-Ylamine	$C_7H_{14}N_2$	126	0.997	506	527
6	29.534	Urea, N-[2-[1-Piperidyl]Cyclohexyl]-	$C_{12}H_{23}ON_3$	225	10.835	370	521
7	29.739	Methyl 6,11-Octadecadienoate	$C_{19}H_{34}O_2$	294	22.195	319	597
8	29.924	Acetamide, N-[3-[3-(4-Pyridinyl) Ureido] Phenyl]-	$C_{14}H_{14}O_2N_4$	270	20.472	300	576
9	30.120	Oxazole, Trimethyl	C_6H_9ON	111	7.662	346	346
10	30.320	Cis,Cis-1,6-Dimethylspiro[4.5]Decane	$C_{12}H_{22}$	166	10.134	315	703
11	30.670	Butanamine, 2,2-Dinitro-N-Methyl	$C_5H_{11}O_4N_3$	177	1.854	391	527

RT- Retention time; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse

Table 2 and figure:4 shows that 11 peaks were visible in the GC-MS chromatogram of the acetone extract of *E. cyathophora*, indicating the presence of the 11 chemicals such as.hydroxyurea, pentane, 1,1'-oxybis, 1,1'-dimethyl-1-ethyl-2-phenylpyrazolium bromide, 2-azetidinone, 3,3-diphenyl, and 1-azabicyclo [2.2.2] Octadecadienoate of methyl, urea, n-[2-[1-piperidyl]cyclohexyl]-, oct-3-ylamine, acetamide, and n-[3-[3-(4-pyridinyl)ureido]phenyl] -, trimethyloxazole, cis,cis-1,6-dimethylspiro[4.5]decane, and butanamine 2,2-dinitro-n-methyl.

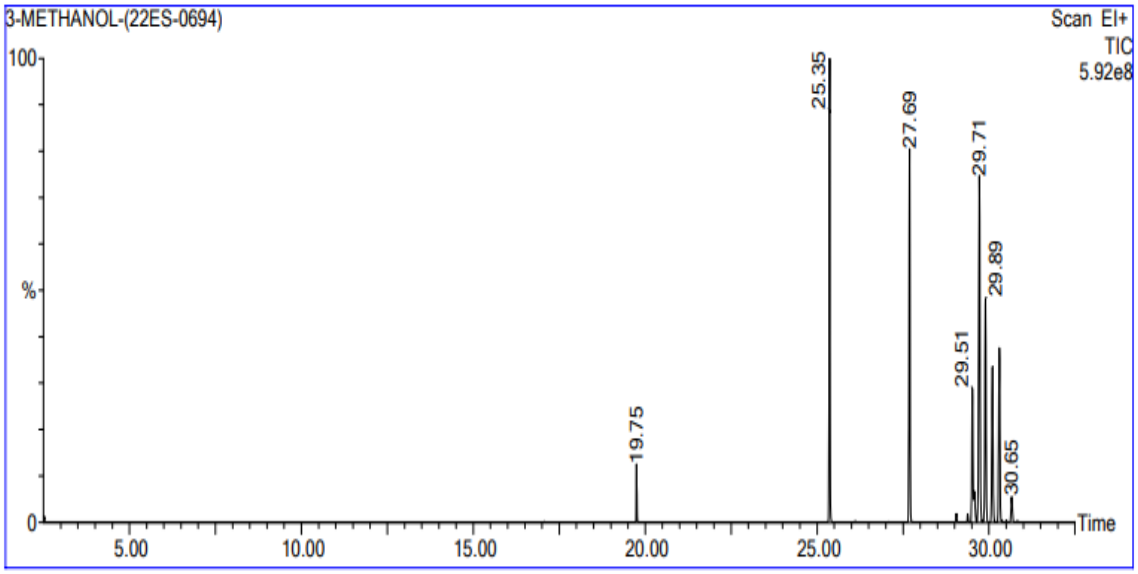


Fig 5: GC-MS chromatogram of *E. Cyathophora leaves* methanol extract

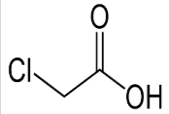
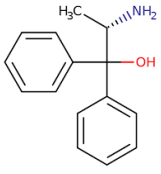
Table 3: GC-MS analysis of *E. cyathophora*leaves methanol extract

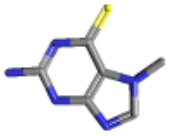
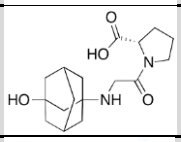
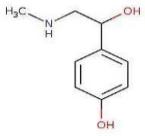
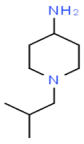
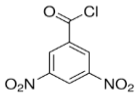
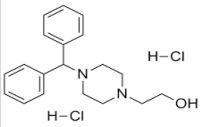
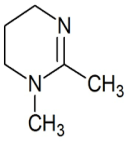
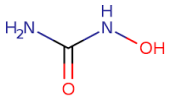
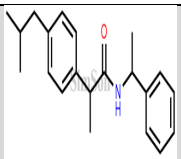
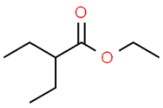
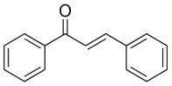
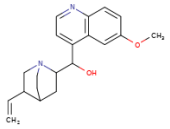
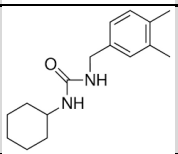
S.No.	RT	Name of the compounds	MF	MW	Area (%)	For	Rev
1	19.745	5h-Tetrazole-5-Thione, 1-[2-(Dimethylamino)Ethyl]-1,2-Dihydro	$C_5H_{11}N_5S$	173	1.782	654	664
2	25.363	4h-Pyran-4-One	$C_5H_4O_2$	96	17.047	460	672
3	27.689	Phenethylamine, 3-Methoxy-.Alpha.-Methyl-4,5-(Methylenedioxy)-	$C_{11}H_{15}O_3N$	209	17.232	478	729
4	29.514	5-Butyl-2-Methyl-.Delta.1-Pyrroline	$C_9H_{17}N$	139	6.960	393	491
5	29.569	Trimethadione	$C_6H_9O_3N$	143	1.810	606	628
6	29.714	7-Azabicyclo[4.1.0]Heptane, 1-Methyl-4-(1-methyl ethyl)-	$C_{10}H_{19}N$	153	21.920	320	559
7	29.895	2-Pyrrolidinone, 1-[4-(1-Pyrrolidinyl)-2-Butynyl]-	$C_{12}H_{18}ON_2$	206	12.750	347	486
8	30.100	3-Azabutyl-1-Ol, O-Acetyl-4-Cyclopropyl-N,N-Dimethyl-, Bromide	$C_{10}H_{20}O_2N$	186	8.540	305	470
9	30.295	9-Thiabicyclo[3.3.1]Nonan-3-One 9,9-Dioxide	$C_8H_{12}O_3S$	188	10.352	345	614
10	30.655	4-Amino-1-Isobutylpiperidine	$C_9H_{20}N_2$	156	1.609	537	537

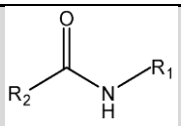
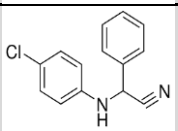
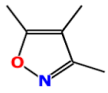
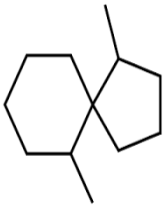
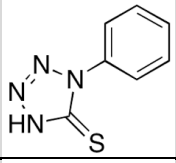
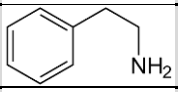
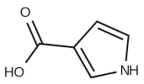
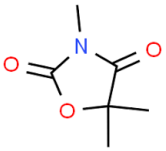
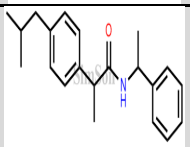
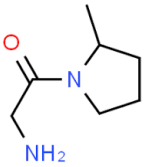
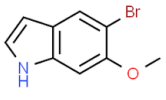
RT- Retention time; MF- Molecular formula; MW- Molecular weight; For- Forward; Rev- Reverse

Table 3 and figure: 5 exhibits GC-MS result in methanolic leaf extract of *E. cyathophora*, confirming thepresenceof10 different bioactive compounds, 5h-tetrazole-5-thione, 1-[2-(dimethylamino)ethyl]-1,2-dihydro, 4h-pyran-4-one, phenethylamine, 3-methoxy-.alpha.-methyl-4,5-(methylenedioxy)-, 5-butyl-2-methyl-.delta.1-pyrroline trimethadione, 7 azabicyclo[4.1.0]heptane,1-methyl-4-(1-methylethyl)-, 2-pyrrolidinone, 1-[4-(1-pyrrolidinyl)-2-butynyl]-, 3-azabutyl-1-ol, o-acetyl-4-cyclopropyl-n,n-dimethyl-, bromide, 9-thiabicyclo[3.3.1]nonan-3-one 9,9-dioxide and 4-amino-1-isobutylpiperidine.

Table 4: The GC-MS identified chemical compounds from *E. cyathophora* leaves extract and theirbiological properties

S.No.	Name of the compounds	Nature of the compound	Chemical structure	Biological Activity
1	Acetic Acid, Chloro	Chloroacetic acid		Anti-microbial activity,
2	4,5-Dimethyl-2h-1,3-Oxazine-3h-2,6-Dione	Alcohol		Anti-leukemia, Anti-malarial
3	1-Propanol, 2-Amino-, (S)-	Alcohol		Antiseptic and Disinfectants activity

4	6h-Purine-6-Thione, 1,7-Dihydro-7-Methyl	Ketone		Anti-bacterial, Anti-fungal
5	Dec-9-En-6-Oxo-1-Ylamide	Ketone/amide		Anti-parasitic, Anti-tumour, Cholesterol-lowering agent
6	Phenol, 4-[2-(Methylamino)Ethyl]-	Phenol		Anti-bacterial activity
7	4-Amino-1-Isobutylpiperidine	Heterocyclic compound		Natural repellents
8	Butanamine, 2,2-Dinitro-N-Methyl	Nitro/amine		Anti-microbial Insecticidal
9	1-Aziridineethanol	Alcohol		Anti-Bacterial activity
10	Pyrimidine, 1,4,5,6-Tetrahydro-1,2-Dimethyl	Heterocyclic compound		Anti-Bacterial activity
11	Hydroxyurea	Diamide		Cytotoxic and Anti-bacterial
12	Pentane, 1,1'-Oxybis	Ketone		Anti-microbial activity
13	1-Ethyl-2-Phenylpyrazolium Bromide	Heterocyclic compound		Anti-microbial activity
14	2-Azetidinone, 3,3-Diphenyl	Ketone		Anti-oxidant and Anti-bacterial activity
15	1-Azabicyclo[2.2.2]Oct-3-Ylamine	Bicyclic compound/amine		Anti-viral activity
16	Urea, N-[2-[1-Piperidyl]Cyclohexyl]-	Amide / Heterocyclic compound		Anti-microbial activity

17	Methyl 6,11-Octadecadienoate	Ester		Anti-bacterial activity
18	Acetamide, N-[3-[3-(4-Pyridinyl)Ureido]Phenyl]-	Amide / Heterocyclic compound		Anti-microbial activity
19	Oxazole, Trimethyl	Azo		Anti-Microbial,
20	Cis,Cis-1,6-Dimethylspiro[4.5]Decane	Spiro compound		Anti-microbial activity
21	5h-Tetrazole-5-Thione, 1-[2-(Dimethylamino)Ethyl]-1,2-Dihydro	Ketone		Anti-microbial, activity
22	4h-Pyran-4-One	Ketone		Anti-mycobacterium activity
23	Phenethylamine, 3-Methoxy-.Alpha.-Methyl-4,5-(Methylenedioxy)-	Amine		Anti-bacterial, Anti-fungal
24	5-Butyl-2-Methyl-.Delta.1-Pyrroline	Heterocyclic compound		Anti-microbial activity
25	Trimethadione	Ketone		Anti-microbial,
26	7-Azabicyclo[4.1.0]Heptane, 1-Methyl-4-(1-methyl ethyl)-	Heterocyclic compound		Anti-oxidant, Anti-bacterial
27	2-Pyrrolidinone, 1-[4-(1-Pyrrolidinyl)-2-Butynyl]-	Heterocyclic compound		Anti-microbial activity
28	3-Azabutyl-1-Ol, O-Acetyl-4-Cyclopropyl-N,N-Dimethyl-, Bromide	Alicyclic compound		Anti-microbial activity

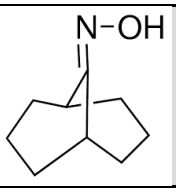
29	9-Thiabicyclo[3.3.1]Nonan-3-One 9,9-Dioxide	Bicyclic compound		Anti-bacterial activity

Table 4 indicates the results of GC-MS analysis of various solvent extracts from *E. cyathophora* leaves showed 29 chemical compounds. These compounds are known to have different therapeutic effects and potential biological properties.

3.1. Antimicrobial activity

The antibacterial and antifungal activity of various solvent extracts from *E. cyathophora* leaves were tested against gram-positive and gram-negative pathogenic bacteria and fungi at 25 µl, 50 µl, and 75 µl. The greatest antibacterial activity was observed for the methanol extract, followed by the chloroform extract with a zone of inhibition (ZOI) of 15 ± 0.26, 14 ± 0.17 at a concentration of 75 µL against *S.iniae.* The acetone extract showed the lowest inhibitory activity (7 ± 0.36) against S. iniae at a concentration of 50 µl. In contrast, at the concentration of 25 µl, no inhibitory activity was observed for the two bacterial strains tested, except the methanolic extract against *S. iniae*(8 ±0.45). Furthermore, the methanol extract showed the largest inhibition zone with a diameter of 13 ± 0.6 compared to *A. hydrophila* at a concentration of 75 µl. The three concentrations of *A.niger* and *A. flavus* were very sensitive to the methanolic leaf extract of *E. cyatophora*. The methanol extract showed the largest zone of inhibition of 14 ± 0.2 (*A.flavus*) and 12 ± 0.36 (A. niger) at a concentration of 75 µl. At the same time, minimal inhibitory antifungal activity was identified in an acetone extract of *E. cyathophora* leaves against *A. flavus* with a ZOI of5±0.34 at 50µl concentration.

Table 5: Antibacterial activity of *E. cyathophora* leaf extract

S. No	Solvent extracts	Concentration µl	Zone of inhibition (mm) Mean± SD	
			Gram-positive bacteria	Gram-negative bacteria
			S. iniae	*A. hydrophila*
1	Methanol	25µl	8±0.45**	-
		50µl	12±0.2**	9±0.1**
		75 µl	15±0.26**	13±0.6**
		Disc	20±0.43**	15±0.6**
2	Acetone	25µl	-	-
		50µl	7±0.36**	-
		75 µl	13±0.51**	8±0.7**
		Disc	17±0.62**	14±0.6**
3	Chloroform	25µl	-	-
		50µl	-	-
		75 µl	14±0.17**	11±0.6**
		Disc	12±0.55**	19±0.34**

(-Nil activity), SD – Standard Deviation; **Significance p< 0.01.

Table 5 shows the antifungal activity of *E.cyathophora* leaves treated with three different concentrations against *A.niger* and *A.flavus.* The methanol extract showed a wide zone of inhibition of 14 ± 0.2 (*A.flavus*) and 12 ± 0.36 (*A. niger*) at a concentration of 75 µl. At the same time, low inhibitory antifungal activity was identified in an acetone extract of *E. cyathophora* leaves against *A. flavus* with a Zone of Inhibition of5±0.34 at the concentration of 50µl.

Table 6: Antifungal activity of *E. cyathophora* leaf extract

S.No	Solvent extracts	Concentration µl	Zone of inhibition (mm) Mean± SD	
			Aspergillusniger	*Aspergillusflavus*
1	Methanol	25µl	9±0.36**	6±0.55**
		50µl	10±0.1**	8±0.7**
		75 µl	12±0.36**	14±0.2**
		Control	18±0.26	19±0.65
2	Acetone	25µl	6±0.45**	-
		50µl	7±0.17**	5±0.34**
		75 µl	9±0.62**	6±0.36**
		Control	16±0.81	13±0.51
3	Chloroform	25µl	7±0.55**	-
		50µl	8±0.43**	6±0.1**
		75 µl	11±0.17**	10±0.43**
		Control	17±0.75	15±0.4

(-Nil activity), SD – Standard Deviation; **Significance p< 0.01.

The results of Table 6 showed the antibacterial activity of *E . cyathophora* leaves against *S. iniae* and *A. hydrophila.* The greatest antibacterial activity was observed for the methanol extract, followed by the chloroform extract with a zone of inhibition (ZOI) of 15 ± 0.26, 14 ± 0.17 at a concentration of 75 µL against *S.iniae.* On the other hand, the acetone extract showed the lowest inhibitory activity (7 ± 0.36) against *S. iniae* at a concentration of 50 µl. In contrast, at the concentration of 25 µl, no inhibitory activity was observed for the two bacterial strains tested, except the methanolic extract against *S. iniae* (8 ±0.45). Furthermore, the methanol extract showed the largest inhibition zone with a diameter of 13 ± 0.6 compared to *A. hydrophila* at a concentration of 75 µl.

3.2. Larvicidal and Pupicidal bioassay

This study investigated the larvicidal bioefficacy of *E. cyathophora* leaves treated with different concentrations (150, 300, 450, 600, and 750 ppm) against stage IV larvae of *E. vittella* under laboratory conditions. The larvicidal activity of *E. cyathophora* leaf extract in chloroform, acetone, and methanol showed significant mortality at a higher concentration of 750 ppm than at the other concentrations. Of the leaf extracts tested against fourth-in star *E. vittella* larvae, methanol extracts showed the highest larval mortality at 94.71%, followed by acetone at 86.72% and chloroform at 83.37% at a concentration of 750 (ppm). Larval mortality was directly proportional to the concentration of leaf extracts.

Table 7: Larvicidal activity of *E.cyathophora* leaf extract against IV instar larvae of *E.vittella*

leaf extract	Mortality (%) (mean±SD) Concentration (ppm)					LC_{50} (LC_{90})	95% confidence LimitLC_{50}(LC_{90})		Regression equation	χ^2
	150	300	450	600	750		LCL	UCL		
Chloroform	28.62± 2.07	39.51 ±1.41	47.32 ±2.30	65.67 ±2.60	83.37 ±3.03	415.18 (937.31)	364.99 (835.15)	462.44 (1095.03)	y=-1.019 +0.002x	3.032*
Acetone	32.26± 2.38	43.48 ±2.07	52.63 ±1.92	71.82 ±2.28	86.72 ±2.54	362.61 (848.56)	311.31 (762.81)	407.23 (976.88)	y=-0.956 +0.003x	3.686*
Methanol	36.58± 2.23	47.25 ±1.92	63.62 ±2.07	82.48 ±1.81	94.71 ±2.22	298.64 (708.14)	248.91 (645.80)	339.69 (795.90)	y=-0.935 +0.003x	3.173*

Mortality rates are means ± SD of triplicate replicates. No mortality was observed in control. LC_{50} lethal concentration that kills 50 % of the exposed organisms, LC_{90} lethal concentration that kills 90 % of the exposed organisms, LCL lower confidence limit, Table7 shows thatLarvicidalactivityof *E. cyathophora* leaf extracts LC50 and LC90 values: methanol 415.18 (937.31); Acetone 362.61 (848.56) and chloroform 298.64 (708.14), respectively. *E. Cyathophora* leaf extract's potential activity against *E. vittella* larvae.

Table 8. Pupicidal activity of *E. cyathophora* leaf extract against *E.vittella*

leaf extract	Mortality (%) (mean±SD) Concentration (ppm)					LC_{50} (LC_{90})	95% confidence limitLC_{50}(LC_{90})		Regression equation	χ^2
	150	300	450	600	750		LCL	UCL		
Chloroform	13.54± 2.07	25.62± 1.41	37.78± 2.30	53.18± 2.60	74.42± 2.28	545.55 (997.88)	503.30 (899.80)	594.39 (1143.31)	Y=-1.546+ 0.003 x	0.879
Acetone	16.29± 2.38	27.76± 2.07	38.12± 1.92	56.31± 2.28	75.39± 2.28	527.36 (996.35)	484.25 (895.66)	576.22 (1146.99)	Y=-1.441+ 0.003 x	1.093*
Methanol	21.54± 2.38	32.62± 2.07	45.78± 1.92	61.18± 2.28	79.42± 2.28	470.25 (958.01)	425.66 (859.31)	516.55 (1106.74)	Y=-0.003+ -1.236 x	0.887

Mortality rates are means ± SD of triplicate replicates. No mortality was observed in control. LC_{50} lethal concentration that kills 50 % of the exposed organisms, LC_{90} lethal concentration that kills 90 % of the exposed organisms, and LCL lower confidence limit Table: 8 shows that *E. cyathophora* leaf extracts, the highest pupal activity was observed in the methanol extract, followed by acetone and chloroform. The LC_{50} and LC_{90} values were 545 methanol.55 (997,88); Aceton 527,36 (996,35) Chloroform 470,25 (958.01). To our knowledge, this is the first report of the use of *E. cyathophora* for the toxic effects of *E. vitella* pupae.

4. DISCUSSION

The current phytochemical screening study of *E. cyathophora* leaf extracts showed the presence of several bioactive chemical components. These components of plant origin, such as flavonoids, quinine, terpenoids, etc., have certain biological functions that enhance therapeutic effects, such as B. anti-cancer, anti-mutagenic, anti-inflammatory and antioxidant properties.[17]Phytochemical screening not only helps to identify the components of plant extracts and which of them are dominant, but is also helpful in finding bioactive substances that can be used in the synthesis of useful drugs.[18] The presence of classes of phytochemicals as such; Flavonoids, alkaloids, and tannins have shown cytotoxic activity.[19] Plants containing many flavonoids can be useful as antibacterial agents and reduce the risk caused by flavonoids, mainly in cardiovascular diseases and cancer. The flavonoids that provide color and flavor have been shown to have anti-cancer properties.[20] This study demonstrated the existence of bioactive compounds. These phytochemicals included alkaloids, tannins, flavones, flavonoids, terpenoids, and steroids, present in all solvent extracts, while cardiac glycosides and anthraquinone were absent from all solvent extracts. The qualitative phytochemical study of *P. marsupium* pearls showed that alkaloids, tannins, terpenoids, carbohydrates, flavonoids, glycosides, cardiac glycosides, proteins, and phenol were present in both the PAH extract and ethanol. At the same time,saponins were only present in the ethanol extract.Twenty-one reviewed preliminary phytochemical studies of MEEC showing the presence of saponins, terpenes, glycosides, alkaloids, and flavonoids.

Based on the phytochemical analysis, the extracts of petroleum ether, ethyl acetate, and ethanol (whole plant) from *Euphorbia cyathophora* show the presence of secondary metabolites such as flavonoids, alkaloids, glycosides, tannins, terpenoids, carbohydrates, proteins, saponins, and sterols. The phytochemical study found that Oleaafricana leaf extract contains alkaloids, flavones, flavonoids, steroids, tannins,terpenoid compounds, and potential antimicrobial activity. All extracts are rich in secondary metabolites; However, the activity depends not only on the presence of secondary metabolites in the plant extracts but also on their concentration and possible interaction with other components. [22] The presence of tannins, phenols, saponins, and alkaloids found in this study contradicts the report by other researchers who indicated that these components were not present in the ethanolic extract of C. dichogamous root. According to, the extraction of phytochemicals can depend on the polarity and molecular weight of the chosen solvent. [23], In addition, GC-MS analysis was to identify 64 chemical compounds from an ethanol extract of the aerial parts of Calendula suffruticosa. Ukwubile observed that GC-MS analysis of methanol extract from M. capitatum leaves showed the presence of six important bioactive compounds, namely 9,12-octadecadienoic acid, methyl ester (Z, Z), hexadecanoic acid, methyl ester, and methyl stearate. the most common are methyl tetra decanoate, -9-dodecanoic acid, methyl ester E, and methyl 18-methyl decanoate. The recent report shows that a GC-MS study of leaf and rhizome extracts of A. nilgiricum revealed the presence of 25 phytochemicals. However, some of the compounds identified were similar to those previously documented by Azwid. butanoic acid, 1,5-heptane, 3,3-dimethyl-(E) and 2-propanoic acid, 2-propanoic esters in leaves and squalene, 1 -Hexanol, 2-ethyl-2-propyl, 1,2-benzene dicarboxylic acid, hexanedioic acid, heptane, heptanoic acid and isooctanol from the bark of Moringaconcanensis. These identified organic compounds may be responsible for antimicrobial, anticancer, analgesic, hepatoprotective, and anti-inflammatory properties that support their widespread use as health aids by traditional medicine practitioners. Medicinal plant extracts that inhibit plant pathogens do not cause problems in the habitat, and the world is interested in ways to replace the chemicals. The antimicrobial properties of plants are related to their ability to produce multiple secondary metabolites with relatively complex structures that exhibit antimicrobial activity. Morus alba ethanolic extract has antibacterial activity against 15 species of bacteria and fungi. It was also confirmed by those who noted that the most active extracts of M. alba were observed against gram-positive and gram-negative bacteria. This activity may be due to the plant being rich in phytonutrients such as Tannins, phytosterols, sitosterols, saponins, anthraquinones, glycosides, and oleanoliccompounds. Previous studies reported that ethanol extracts have better antibacterial activity than aqueous or plant extracts. Previousstudies found a difference in the antimicrobial activity of different strains of E.tirucalli, which depend on biologically active phytochemicals.[24] Our results are consistent with several studies showing that olive leaf extracts have antimicrobial activity against multiple pathogenic organisms.[25] The antibacterial activity was measured in terms of the diameter of the zone of inhibition. Acetone and IPA extract (50 mg/ml) showed antibacterial activity against gram-positive bacteria, e.g., *Staphylococcus aureus* (8 mm zone of inhibition) and Bacillus cereus (8 mm zone of inhibition). Still, they showed no antibacterial activity against gram-negative bacteria, i. H. the ethanolic extract (50 mg/ml) showed no antibacterial activity. The zone of inhibition for the control (50 µg/ml ofloxacin) was 13 mm for S.aureus, 12 mm for B. cereus, 24 mm for E. coli and 8 mm for S. Typhi. Some plants,like O.africana extracts, showed antibacterial and antifungal activity; however, the highest antibacterial activity was observed for the ethanolic extract against the organisms tested. The lowest inhibitory activity was obtained for the aqueous extract.[26] The methanol extract showed the highest inhibitory activity against S. aureus (28 mM). The susceptibility of fungal pathogens to O. africana leaf extracts was compared to nystatin. The methanol extract showed the highest inhibitory activity against fungal pathogens, followed by the ethanol extract, and the hexane extract showed the lowest inhibitory activity. [27] found the good antibacterial properties of Abrusprecatorius, Terminaliaphanerophlebia, Indigoferaarecta, and Pentanisiaprunelloides confirm their traditional use in the treatment of respiratory diseases. A study on the methanolic extract of this plant showed significant antibacterial and antifungal activity against most pathogenic organisms: Bacillus subtilis, Proteus mirabilis, Staphylococcus aureus, Escherichia coli, Shigella dysentery, Proteus mirabilis, Salamonellatyphi, and two Candida albicans fungi, tineacapitis, particularly effective against dermatophytes (tineacapitis). Manandhar et al. (2019) evaluated the antibacterial potential of methanolic extracts of Oxalis corniculata, Artemisia vulgaris, Cinnamomumtamala, and Ageratinaadenophora tested against twelve pathogenic microorganisms and two reference bacterial strains were subjected to a single agar diffusion test.[28] The greatest potential was observed in the extract of O.corniculata against Escherichia coli, Salmonella Typhi, MDR Salmonella Typhi, Klebsiella pneumonia, and Citrobacterkoseri with a zone of inhibition (ZOI) of 17 mm, 13 mm, 16 mm, 11 mm, and 12 mm, respectively. Our survey also agrees with other studies that found that ethanol and methanol leaf extracts were equally effective against most strains tested. Our results were consistent with reports from Momordicacymbalaria. Phytochemicals extracted from plant parts using various solutions act as toxins, repellents, and insect growth regulators. [29,30,31] Previous authors have also described several important controls of *E. vitella*. Consistent with our findings, several plant extracts have been suggested as possible sources of bioinsecticides. Several reports describe many compounds isolated from different plants with different biological properties against different pests.The results of this study indicate that the plant-derived compound may be an effective alternative to traditional synthetic pesticides, with plant-derived toxins undoubtedly representing a variable source of potential insecticides. These insecticides and other natural products may be more important in future pest control programs. The results of these studies will significantly reduce the routine use of insecticides. A less expensive botanical insecticide that readily biodegrades to non-toxic products for use in a pest control program. The plant extracts showed insecticidal activity against Callosobruchusmaculatus.[32] These results are consistent with those of Hassam et al., 2022 who reported the larvicidal toxicity of a C. colocynthis seed extract with methanol, ethanol, hexane, water, and profenofos for "*E. Vitella* first stage. between colocyn this and methanol seed extract showed the highest mortality compared to the others.[33] The current results were similar to those of LeNT et al. (2021) on extracts from *E. tirucali*.[34]The extract from *E. cyathophora* leaves was revealed to have several possible bioactive chemicals, and this study also discovered that these

compounds have extremely effective antimicrobial activities and insecticidal effects.against*E. Vitella*.

5. CONCLUSION

The study results highlight the promising potential of *E. cyathophora* leaf extract as a convenient and environmentally friendly means of controlling agricultural pests and medically important microbial pathogens. In addition, research is needed to identify the structure of the biologically active compound and the biological properties of the leaf extract of *E. cyathophora*, which can be used for drug development and the pharmaceutical industry.

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7. AUTHORS CONTRIBUTION STATEMENTS

Mr. M. Kannan conceptualized the manuscript, conducted the experimental bioassay, and scrutinized the results. Dr.N.Kanagaraju contributed to the statistical data analysis.Dr.B.Chandramohan contributed to the literature collection and the definition of intellectual content and manuscript preparation and revised the carried-out corrections in the manuscript. Dr.P.Madhiyazhagan contributed to the drafted introduction and methods. Finally, all authors discussed the results and contributed to the final manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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