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Evaluation of Antimicrobial, Cytotoxic and Larvicidal Activity of *Zygophyllum Coccineum* North Sinai, Egypt

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Abstract

The antimicrobial larvicidal and cytotoxic properties of *Zygophillum coccinium* collected from desert of north Sinai, Egypt. Antimicrobial activity of *Z. coccinium* extracts showed that ethyl acetate extract was the most potent antimicrobial effect on bacterial and fungal test organisms against *P. aeruginosa* with inhibition zone 20 mm and *F. moniliforme* with inhibition zone 22 mm. Cytotoxic effect of *Z. coccinium* extracts indicated that acetone extract showed maximum cytotoxic activity on HeLa cell line, whereas methanol extract showed maximum cytotoxic activity on MCF-7 cell line with cell line viability 13.36 and 35.19%, whereas the results of IC_{50} in HeLa cell line showed that the boiled water ethyl acetate extract had the most potent IC_{50} with 6.25 µg/ml. the results of IC_{50} in MCF-7 cell line the petroleum ether extract had the most potent IC_{50} with 18.75 µg/ml. Ethyl acetate extract of *Z. coccineum were the most potent medicinal plants as antibacterial effect with MIC 12.5* µg/ ml. Ethyl acetate with 200 ppm conc. of *Z. coccineum is the lowest conc. possess toxic effect* against 3rd instar larvae of *Culex pipiens* The results of column chromatography the antimicrobial activity of *Z. coccineum* against *K. pneumonia* showed that the highest fraction with antimicrobial activity was fraction No 3 with inhibition zone 22 mm. Spectroscopic characterization of antimicrobial agent. The chemical compound structure of of *Z. coccineum* was identified as was suggested as 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-6-methoxy-4-benzopyrone. e.

Keywords: Antimicrobial activity; Cytotoxic effect; *Larvicidal; Zygophyllum coccineum; North Sinai; Medicinal plants*

Introduction

Organized medicinal systems like most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc.,. It was suggested that several thousands of plants have been known with medicinal applications in various cultures [1].

Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early, cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components [2].

Bioactive principles isolated from plants appear to be one of the alternatives for the control of the antibiotic resistant plant and human pathogens. Though the literature is replete with information on the antimicrobial effect on human pathogenic bacteria, a similar investigation on plant pathogens has not been given an adequate attention. *Acacia nilotica* (Fabaceae) showed significant antibacterial activity [3].

Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [4]. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. These compounds are more complex and specific and are found in certain taxa such as family, genus and species, but heterogeneity of secondary compounds is found in wild species [5,6].

Experimental agents derived from natural products offer opportunities to evaluate not only totally new chemical classes of antitumor agents, but also novel and potentially relevant mechanisms of action [7]. Plants have been a main source of highly effective drugs for the treatment of many forms of tumor. In many instances, the actual compound isolated from the plant may not serve as the drug, but leads to the development of potential novel agents. The ability to attach agents to carrier molecules directed to specific tumors holds promise for the effective targeting of highly cytotoxic natural products to the tumors while avoiding their toxic side effects on normal healthy tissues [8].

Interest in ethnobotany has increased dramatically. Use of ethnobotanical information in medicinal plant research has gained considerable attention in segments of the scientific community [9]. Natural crude extracts and biologically active compounds isolated from plant species used in traditional medicine can be prolific resources for new drugs [10].

There are two main strategies for the selection of plants species

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in antitumor drug discovery. The First approach includes random screening and ethnomedical knowledge. The second approach includes plants used in organize traditional medical systems like herbalism and folklore [11].

Zygophyllin and quinovic acid exhibited anti-inflammatory activity, cortisone-like action, choleretic and antipyretic activities. The aqueous extract produced lowering in blood pressure, diuretic, antipyretic, local anesthetic and antihistaminic activities [12].

The aims of this study included investigating the antimicrobial larvicidal and cytotoxic properties of *Z. coccineum* collected from north Sinai, Egypt were evaluated.

Materials and Methods

Medicinal plants used in this study

Medicinal plant used in this study was collected from the North Sinai (Wadi El Maghara) *Zygophyllum coccineum*. Fresh areal parts leaves and stem of medicinal plants were stored in air tight container and maintained at 4°C until use to store and minimize the loss of volatile compounds.

Test organisms for Antimicrobial assay

Gram positive bacteria *Staphylococcus aureus ATCC 29213, Methicillin-resistant, Staphylococcus aureus (MRSA) clinical isolate Bacillus subtilis NRRL B 543, Alcaligenes faecalis ATCC 29217 and Enterococcus faecalis ATCC 29212.*

Gram negative bacteria were *Enterobacter cloacae* ATCC 13047, *Klebsiella pneumonia* ATCC 13883, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 10145.

Unicellular Fungi Candida albicans MTCC183, *Candida glabrata* clinical isolate and *Candida parapsilosis* clinical isolate.

Filamentous fungi such as *Aspergillus niger* NRRL 595, *Trichoderma viride* RCMB 017002, *Trichoderma longibrachiatum* RCMB 017015, *Fusarium moniliforme* RCMB 008002, *Rhizopus stolonifer* RCMB014001, *Curvularia clavata* RCMB 019003 and *Penicillium aurantiacum* RCMB 001025 (Figure 1).

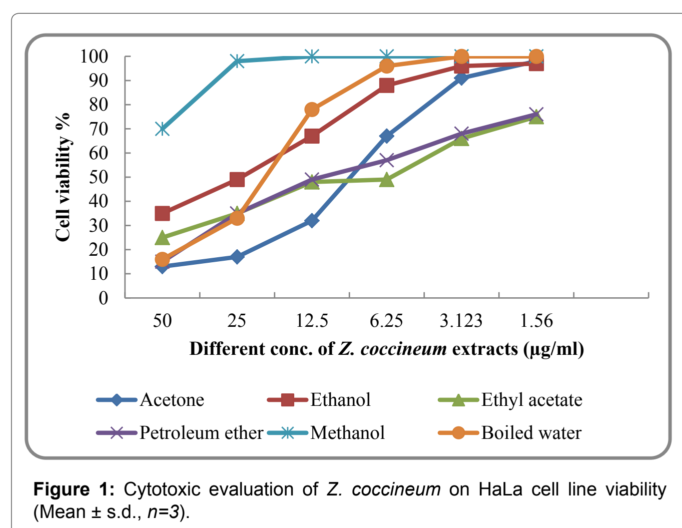


Figure 1: Cytotoxic evaluation of *Z. coccineum* on HaLa cell line viability (Mean ± s.d., *n=3*).

Mammalian cell lines

Mammalian cell lines: HeLa (cervical carcinoma cell line) and MCF-7 (human breast adeno-carcinoma cell line) and Vero (African green monkey kidney cells) were purchased from VACSERA, Egypt.

Plant Identification: Identification of plant species in a given stand has been tentatively recorded in the field and the authentication of their identification with the help of the documented floristic workers [13,14].

Preparation of plant extracts for *in vitro* testing: Different organic solvent were used for extraction of antimicrobial and cytotoxic substances from medicinal plants. Twenty gram of the aerial parts (leaves and stem) of all dried medicinal plants in this study was macerated in mortar dissolved in 100 ml of different organic solvents (methanol, ethyl alcohol (absolute), acetone, petroleum ether and ethyl acetate). Also, aqueous extract were prepared by boiled distilled water for 5 minutes left and then filtered using No.1 filter paper. Medicinal plants extracts were evaporated till dryness under reduced pressure using rotary vacuum evaporator at 40°C stored in sterile screw capped vials in the refrigerator until needed. Each concentrated crude extract was separately sterilized by filtration and further dilutions were made from the stock (Figures 2 and 3).

Determination of antimicrobial activity by Agar Well diffusion method

The Agar well diffusion technique has been widely used to assay plant extract for antimicrobial activity based on the observation of inhibition of microbial growth on microbial nutrient media. The previous mentioned human pathogenic bacteria Gram-positive and Gram-negative bacteria as well as unicellular and filamentous fungi were used in this test. For examination of antimicrobial activity nutrient agar for bacteria, Malt agar for fungi and Sabouraud agar medium for unicellular fungi were prepared and seeded with one of the tested organisms (100 µl of bacterial culture in 20 ml medium and 100 µl of fungal spore suspension in 25 ml broth medium). After solidification holes in media were made by cork-borer then medicinal plants extracts 100 µl were loaded into holes. Petri dishes were kept in refrigerator for 2 h, for diffusion of tested substances before incubation at 37°C for 24 h for bacteria, yeast and fungi 48 h at 28°C to allow complete diffusion of tested substances inside culture media after growth of test organisms. The detection of a clear zone around the well on the inoculated plates is an indication of antimicrobial activities of the medicinal plants extracts under study [15] (Tables 1 and 2).

Minimum inhibitory concentration (MIC) using dilution method

The series of double fold were performed in 96-well microtitre plates with U-shaped wells, using two-fold serial dilutions. One hundred µl of medicinal plants extracts were added to the first well and mixed. The series of douple-fold dilutions were done until the last well of the microwell plate and then 100 µl of bacterial suspension was added in respective wells and in control wells. The plates were sealed, placed in plastic bags and incubated at 37°C for 24 h. The MIC which is defined as the lowest concentration of extract that exhibited no growth by visual reading [16].

Cytopathic effect assay

The cytotoxicity medicinal plant extracts were measured by the cytopathic effect assay. The assay was carried out using 100 µl of cell suspension, containing 10,000 cells seeded in each well of a 96-well

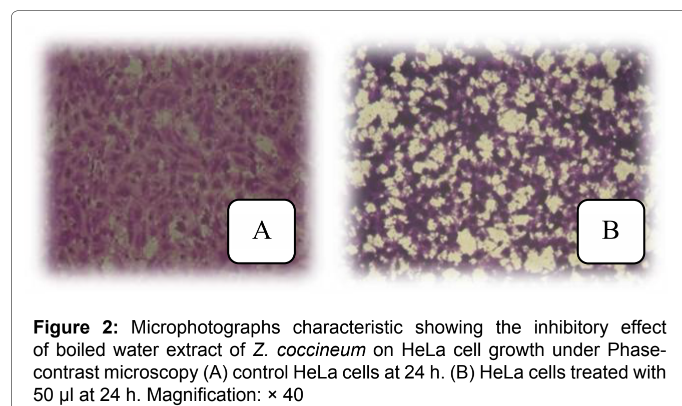


Figure 2: Microphotographs characteristic showing the inhibitory effect of boiled water extract of *Z. coccineum* on HeLa cell growth under Phase-contrast microscopy (A) control HeLa cells at 24 h. (B) HeLa cells treated with 50 µl at 24 h. Magnification: × 40

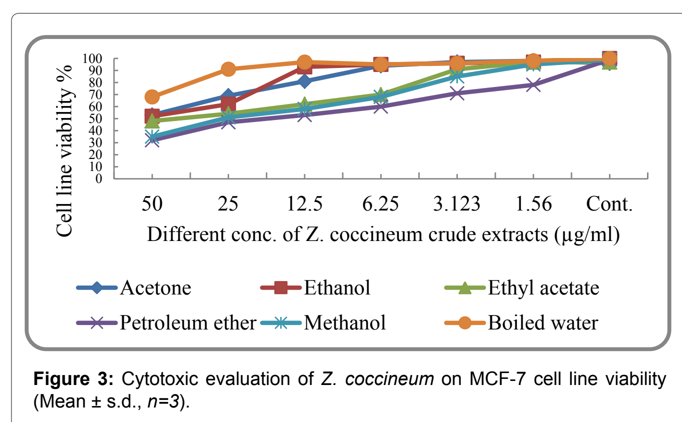


Figure 3: Cytotoxic evaluation of *Z. coccineum* on MCF-7 cell line viability (Mean ± s.d., *n=3*).

microtitre plate. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Then, serial two-fold dilutions of the medicinal plants extracts were added to confluent cell monolayer. The microtitre plates were incubated at 37°C in a humidified incubator with 5% CO2 for a period of 48 h. Six wells were used for each concentration of the test sample. Control cells were incubated without test sample. After the incubation period, media were aspirated and the cells were fixed with 10% formalin solution for at least 20 min. The fixed cells were rinsed with phosphate buffer solution (PBS) then stained with a 1% crystal violet for 1 hour. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. The cell cultures were examined for evidence of the cytopathic effect that observed microscopically as detectable alterations. The stained cells were lysed by using glacial acetic acid solution and then plates were read on ELISA reader, using a test wave length under 490 nm [17].

Evaluation of the larvicidal activity against the mosquito vector *Culex pipiens* L.

Mosquito culture: The mosquito *C. pipiens L.*was obtained from Medical Entomology Research Center, Doqqi, Giza. The sample was reared for several generations in the Department of Zoology, Faculty of science Al-Azhar University, Madenit Nasr, Cairo under controlled conditions (27 ± 2°C, RH 70 ±10% and 12 light-dark regime). Adult mosquitoes were kept in (30 × 30 × 30 cm) wooden cages and daily provided with sponge pieces soaked in 10% sucrose solution for a period of 3-4 days after emergence. After this period the females were allowed to take a blood meal from a pigeon host. Plastic cup oviposition (15 × 15 cm) containing tap water was placed in the cage. The resulting egg rafts picked up from the plastic dish and transferred into plastic

Plant extracts / Test microorganisms	Acetone	Ethanol	Ethyl acetate	Petroleum ether	Methanol	Boiled water
Methicillin-resistant, Staphylococcus aureus	NIZ	NIZ	17 ± 0.62	NIZ	NIZ	NIZ
Staphylococcus aureus	12 ± 0.63	NIZ	15 ± 0.47	NIZ	NIZ	11± 0.37
Bacillus subtilis	17 ± 1.2	11 ± 0.48	12 ± 0.52	12 ± 0.82	14 ± 1.2	13± 0.88
Enterococcus faecalis	NIZ	17 ± 0.71	13 ± 0.91	15 ± 0.39	NIZ	NIZ
Alcaligenes faecalis	11 ± 0.58	NIZ	14 ± 0.75	NIZ	NIZ	12 ± 0.35
Klebsiella pneumonia	12 ± 0.72	15 ± 0.83	17 ± 0.59	16 ± 0.44	12 ± 0.72	13 ± 0.32
Pseudomonas aeruginosa	NIZ	NIZ	20 ± 0.24	NIZ	NIZ	NIZ
Enterobacter cloacae	NIZ	NIZ	13 ± 0.59	NIZ	12 ± 0.78	NIZ
Escherichia coli	15 ± 0.80	12 ± 0.42	13 ± 0.58	13 ± 0.35	14 ± 0.38	13 ± 0.27

Table 1: Antibacterial evaluation of *Z. coccineum* extracts by using agar well diffusion method. The data are expressed as the mean in inhibition zone of diameter in mm ± standard deviation.

Plant extracts / Test microorganisms	Acetone	Ethanol	Ethyl acetate	Petroleum ether	Methanol	Boiled water
Candida albicans	18 ± 0.85	20 ± 0.27	13 ± 0.76	NIZ	11 ± 0.41	13 ± 0.58
Candida glabrata	12 ± 0.37	NIZ	15 ± 0.49	13 ± 0.44	14 ± 0.55	12 ± 0.27
Candida parapsilosis	NIZ	NIZ	13 ± 0.48	12 ± 0.53	12 ± 0.58	NIZ
Fusarium moniliforme	20 ± 0.46	NIZ	22 ± 0.36	15 ± 0.77	18 ± 0.75	NIZ
Trichoderma longibrachiatum	17 ± 0.85	18 ± 0.73	15 ± 0.34	NIZ	NIZ	NIZ
Trichoderma viride	NIZ	NIZ	12 ± 0.88	NIZ	NIZ	11 ± 0.61
Penicillium aurantiacum	NIZ	NIZ	15 ± 0.25	NIZ	NIZ	NIZ
Aspergillus niger	NIZ	NIZ	16 ± 0.36	NIZ	NIZ	NIZ
Rhizopus stolonifer	NIZ	NIZ	14 ± 0.64	NIZ	NIZ	NIZ
Curvularia clavata	11 ± 0.42	NIZ	17 ± 0.93	NIZ	13 ± 0.86	NIZ

Table 2: Antifungal evaluation of *Z. coccineum extracts* by using by using agar well diffusion method.

pans (25 × 30 × 15 cm) containing 3 liters of tap water left for 24 h. The hatching larvae were provided daily with fish food as a diet [18].

Larval treatment: In order to study the toxicity of the medicinal plant extracts, different concentrations of each extract were prepared in order to detect mortalities (200, 100, 50 and 25 ppm). All tested plant dried extracts were performed in 100 ml. of tap water contained in 200 ml plastic cups. Then, third instar larvae were put immediately into plastic cups contained different concentrations of extracts. At least three replicates were usually used for each tested concentration. All plastic cups were incubated under controlled conditions (27 ± 2°C, RH 70 ±10% and 12 light-dark regime). Control larvae not treated with any plants 100 ml water.

Mortality was recorded daily and the dead larvae. Abnormally formed pupae were removed daily and Placed in labeled glass vials containing 70% ethanol and one drop of glycerin for the photography under binocular microscope. Larval mortality was indicated by a failure to respond to mechanical stimulation [19].

Motility %=dead larva/ control × 100.

Separation and fractionation of the active compounds

Column chromatography: Prokaslo- England Column chromatography (20 × 2 cm). Ethyl acetate and acetone with different ratio were used as eluting solvent packed with Silica gel 60 GF 200 (MERK). Fractions were collected and assayed for their antimicrobial and cytotoxic activity. Finally the column eluted with methanol to wash and remove the remnant of biologically active compound (Figure 4).

Predicting the chemical structure: The spectroscopic data were employed to reach complete characterization of the chemical structure and molecular formula (Graph 1-3).

Statistical analysis: The data were expressed as mean ± S.D. The statistical significance of the difference mean values was determined by Student's unpaired *t*-test. Data were considered statistically significant at a significance level of $P<0.05$.

Results and Discussion

Medicinal plants of folk-origin are significant sources of synthetic and herbal drugs. In the commercial market, folk-medicinal plants were used as raw drugs, extracts or tinctures. Isolated active constituents are used for applied research for finding their bioactivity. For the last few decades, phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of antitumor compounds [3].

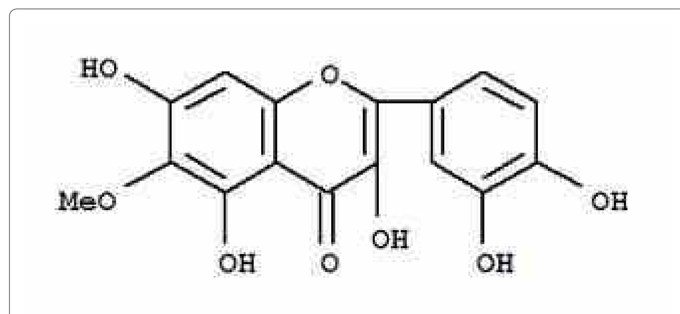
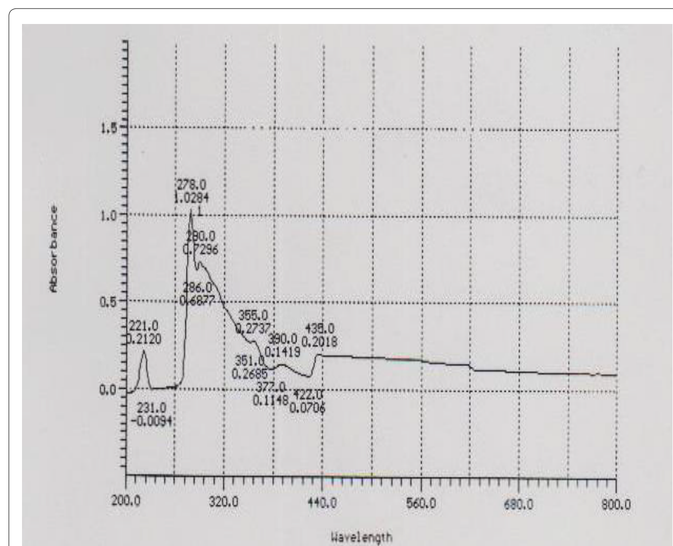
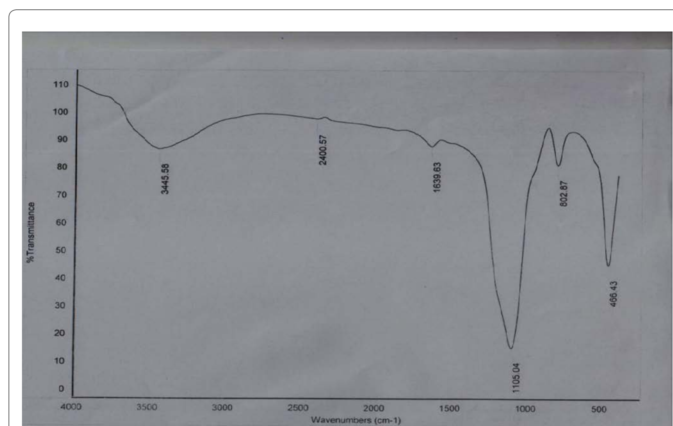


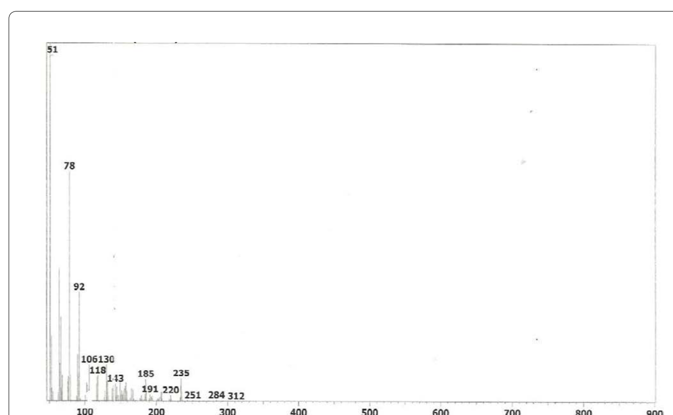
Figure 4: The suggested chemical for the purified compound of ethyl acetate extract *Z. coccineum* from column chromatography Fraction No. 3 at TLC violet band at R_f 0.62 was suggested as 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-6-methoxy-4-benzopyrone with molecular Formula $C_{16}H_{12}O_8$.



Graph 1: Ultra violet spectroscopy of the purified compound ethyl acetate extract *Z. coccineum* from column chromatography Fraction No.3 at TLC violet band at R_f 0.62.



Graph 2: Infra- red spectrum of the purified compound ethyl acetate extract *Z. coccineum* from column chromatography Fraction No.3 at TLC violet band at R_f 0.62.



Graph 3: Mass spectrum of the purified compound ethyl acetate extract *Z. coccineum* from column chromatography Fraction No.3 at TLC violet band at R_f 0.62.

Antimicrobial and cytotoxicity evaluation of *Zygophyllum coccineum*

Antimicrobial evaluation of *Z. coccineum* extracts showed that ethyl acetate extract was the most potent antimicrobial effect on bacterial and fungal test organisms. Antibacterial activities of *Z. coccineum* ethyl acetate extract against *P. aeruginosa* with inhibition zone 20 mm was the most sensitive test organism. Also ethyl acetate of *Z. coccineum* has antifungal activity against all fungal test organisms. Ethyl acetate extracts showed that *F. moniliforme* was the most sensitive organisms with inhibition zone 22 mm and 20 mm against *C. albicans* whereas boiled water extracts showed antifungal activity against *Curvularia clavata* with 20 mm inhibition zone.

Cytotoxicity evaluation of *Z. coccineum* on HeLa cell line viability indicated that acetone, petroleum ether and boiled water showed very strong cytotoxic activity with cell line viability 13.36%, 15.36% and 16.43% respectively. Cytotoxicity evaluation of *Z. coccineum* MCF7 cell line viability indicated that ethyl acetate, petroleum ether and methanol showed strong cytotoxic activity with cell line viability 48.15%, 67.59% and 35.19% respectively. Nevertheless, there is no scientific report on the *in vitro* and *in vivo* study the effect of *Z. coccineum* as anti-tumor. Thus, this work were studied to the antitumor effect of Z. *coccineum* also interesting to complete our study by the in vitro study.

Evaluation of the mortality effect of Z. *coccineum* extracts against the mosquito vector *Culex pipiens L*

The extracts of *Z. coccineum* were studied to evaluate the insecticidal activity with 200 ppm of each extract against 3rd instar larvae of *Culex pipiens* L. Mortality was recorded daily.

Ethyl acetate extract of *Z. coccineum* possess and induced a potent insecticidal and toxic effect against 3rd instar larvae of *Culex pipiens* L with 100% mortality after 24 h of treatment. So, the different concentrations of ethyl acetate of *Z. coccineum* were be studied to know the lowest conc. of ethyl acetate which possess toxic effect against 3rd instar larvae of *Culex pipiens* L with 100% mortality after 24 h of treatment. Also, ethanol extract of *Z. coccineum* possess and induced a potent insecticidal and toxic effect against 3rd instar larvae of *Culex pipiens* L with 58.07% mortality after 24 h of treatment and possess 100% mortality the 5th day of treatment as appeared in Table 3.

Different concentrations of ethyl acetate extract was selected to evaluate the lowest conc. used as insecticidal activity of *Z. coccineum* with 100, 50, and 25 ppm against 3rd instar larvae of *Culex pipiens* L for 7 days. Significant differences were observed among concentrations and exposure time on mortality in each treatment. At 100 ppm showed a moderate insecticidal effect with 41.46% mortality after 24 h of treatment (Table 4).

From the above results we observed that, ethyl acetate with 200 ppm conc. of *Z. coccineum is the lowest conc. possess toxic effect* against 3rd instar larvae of *Culex pipiens.*

Minimum inhibitory concentration (mic) of ethyl acetate extracts of the tested medicinal plants

To determine the smallest amount of antimicrobial agents necessary to inhibit growth of the tested organisms. MIC crude extract of ethyl acetate extracts of the tested medicinal plants were studied against *S .aureus, K. pneumonia, Enterobacter colaca* and *C. albicans.* The results obtained in Table 5 showed that *Z. coccineum were the most potent medicinal plants as antimicrobial effect with MIC 12.5* µg/ml whereas other medicinal plant extracts varied in its MIC activity. So, *Z. coccineum* were selected for purification and identification for their biological active compounds and also for more studies for their active compounds.

Plant extracts	1st day	2nd day	3rd day	5th day	7th day
	Mean of larval mortality % ± SD				
Acetone	20.82 ± 1.31	79.03 ± 1.67	100 ± 0.00	100 ± 0.00	100 ± 0.00
Ethanol	58.07 ± 2.01	60.98 ± 1.25	67.96 ± 1.70	93.74 ± 1.27	97.32 ± 1.49
Ethyl acetate	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
Pet. ether	22 ± 2.03	24.23 ± 4.93	24.67 ± 3.79	62.22 ± 5.82	62.89 ± 6.61
Methanol	42 ± 2.32	43 ± 3.61	44.53 ± 3.18	44.67 ± 4.62	44.89 ± 4.45
Boiled water	8.53 ± 0.27	23 ± 3.42	25.18 ± 3.39	25 ± 4.58	26.03± 5.26
Cont.	0	0	5.12 ± 1.18	14 ± 1.64	14 ± 3.53

Table 3: Evaluation of the mortality effect of *Z. coccineum* extracts against the mosquito vector *Culex pipiens* L.

Extract conc. (ppm)	1st day	2nd day	3rd day	5th day	7th day
	Mean of larval mortality % ± SD				
100	41.46 ± 2.96	39.24 ± 1.28	60 ± 2.58	61.58 ± 2.13	63 ± 3.16
50	22.25 ± 2.39	22.52 ± 1.84	26.74± 2.91	26.58 ± 1.68	29.16 ± 1.25
25	3.79 ± 1.53	24.30 ± 3.21	35.58 ± 2.93	34.92 ± 4.28	36.18 ± 3.37
Cont.	0	0	0	37.56 ± 0.31	37.79 ± 1.57

Table 4: Evaluation of the different concentration of ethyl acetate crude extracts of *Z. coccineum* against the mosquito vector *Culex pipiens* L.

Test Organisms / Medicinal plants	*S .aureus*	*K. Pneumonia*	*Enterobacter colaca*	*C. albicans*
Z. coccineum	12.5	12.5	12.5	12.5

Table 5: Minimum inhibitory concentration of ethyl acetate crude extracts of the tested medicinal plants. The data is expressed in µg/ml.

Separation, fractionation and identification of the active compounds

As the above results of antimicrobial activity in this study and the results of MIC of all medicinal plants showed that ethyl acetate extract *Z. coccineum were the most potent medicinal plants as antibacterial effect with MIC 12.5* µg/ml. *Z. coccineum* also showed significant antifungal activity so, purification and characterization of bioactive phytochemical compounds by column chromatography using different ratio of solvent system ethyl acetate and acetone and T.L.C. Toluene: Ethyl Acetate: Formic Acid (5:4:1) solvent system were used in this study.

From the results of column chromatography the antimicrobial activity of antimicrobial activity of *Z. coccineum* only fractions From 2 to 5 showed antimicrobial activity ranging from 13 to 22 mm of inhibition zone. The highest fraction with antimicrobial activity was fraction No 3 with inhibition zone 22 mm.

Minimum inhibitory concentration of the highest antimicrobial fractions were studied against *S .aureus, K. pneumonia, Enterobacter colaca* and *C. albicans* which showed that Fraction No 3 of *Z. coccineum antibacterial effect with MIC ≤ 12.5* µg/ml (Tables 6 and 7).

The chemical compound structure of ethyl acetate extract of *Z. coccineum* was identified as was suggested as 2-(3, 4-Dihydroxyphenyl)-3,5,7-trihydroxy-6-methoxy-4- benzopyrone (Table 8).

Fraction No	Mean of inhibition zone diameter (mm)	Fraction No	Mean of inhibition zone diameter (mm)
1	NIZ	11	NIZ
2	13	12	NIZ
3	22	13	NIZ
4	21	14	NIZ
5	18	15	NIZ
6	NIZ	16	NIZ
7	NIZ	17	NIZ
8	NIZ	18	NIZ
9	NIZ	19	NIZ
10	NIZ	20	NIZ

Table 6: Anti-bacterial evaluation of the fractions obtained after silica gel column fraction of ethyl acetate extract of *Z. coccineum* against *K. pneumonia.*

Active fractions	*MRSA*	*S .aureus*	*K. pneumoniae*	*Enterobacter cloacae*	*C. albicans*
Z. coccineum Fraction No 3	6.25	6.25	6.25	6.25	6.25

Table 7: Minimum inhibitory concentrations (MIC) of active fractions of Z. coccineum. The data are expressed in µg/ml.

Node	Shift	Base	Comment
CH	5.58	7.26	1- benzene
		-0.53	1 –O
		-0.11	1 –O-C
		-0.44	1 –O
		-0.07	1 –C- O
		-0.53	1 –O
CH	4.18	1.50	methine
		1.28	1 alpha – 1 C- C
		0.68	1alpha –C= C
		1.35	-O-C
CH	6.60	1.53	1- benzene
		7.26	–C= C
		0.04	1 –O
		0.17	1 –O
CH	6.69	7.26	1- benzene
		0.17	–C= C
		0.04	1 –O
		0.44	1 –O
OH	5.0	5.0	Aromatic C-OH
OH	5.0	5.0	Aromatic C-OH
OH	15.0	15.0	enol
OH	5.0	5.0	Aromatic C-OH
OH	5.0	5.0	Aromatic C-OH
CH	5.03	3.06	Tetrahydropyran
CH	3.37	1.60	Tetrahydropyran
OH	2	2	alcohol
OH	2	2	alcohol
CH_3	3.73	0.86	methyl

Table 8: Nuclear magnetic resonance spectroscopy (H1 NMR analysis).

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