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Analysis of biochemical components of the cultured copepod *Acanthocyclops trajani*, and evaluation of its extract as antimicrobial and anticancer activities

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Abstract

Aquatic environment is a vast reservoir of bioactive natural products, many of which exhibit structural and chemical properties not found in terrestrial natural products. However, studies on aquatic medicines have included a very small proportion of aquatic organisms. Copepoda is one of the most diverse groups on earth, yet there are no studies to test omnivores Copepod extract as an antimicrobial and anticancer agent. Therefore, this study aims to improve the sustainable mass production of the copepod *Acanthocyclops trojani* to evaluate its crude extract for antimicrobial and anticancer activity. GC-MS analysis of *A. trajani* extract detected 8 bioactive compounds known for their medicinal uses in several previous studies. The *A. trajani* crude extract showed activity against bacterial strain; *S. aureus*, *E. coli*, *E. faecali*, *K. pneumonia*, *S. lutea* and *S. typhi* with zones of inhibition of 14, 12, 8, 12, 17 and 12 mm, respectively. In addition, *A. trajani* crude extract showed moderate growth inhibitory activity against HepG2, A549, HCT and MCF7 cancer cell lines (IC50 = 63.064, 18.377, 46.905 and 21.736, respectively) compared to the activity of staurosporine (control). While the normal cells (THLE2, WI38, FHC and MCF10a) were more sensitive to staurosporine than the *A. trajani* extract. Based on the Cytotoxicity Selective Index (SI) values, the extract showed a selective effect on the lung and breast cancer cells (A549 and MCF7) (SI = 3.07 and 3.1, respectively). Therefore, *A. trajani* extract offers the potential for further exploitation in the discovery of a novel antibacterial agent and against lung and breast cancer.

Keywords: "Natural Bioactive Product, Aquatic Drugs, Copepoda, Antitumor, Antibacterial, Aquatic culture.

1. Introduction

Aquatic natural products are bioactive metabolites found in organisms including microbes, invertebrates, and plants **[1]**. While the majority of natural products used medicinally are derived from terrestrial organisms and not those that live in the sea, interest in aquatic organisms as a source for the production of bioactive compounds that can play an important role in the treatment of many has increased recently increased diseases such as cancer and bacterial infections. Yet the aquatic environment is a vast reservoir of bioactive natural products, many of which exhibit structural and chemical features not found in terrestrial natural products **[2]**. The marine ecosystem is the greatest source of discovery for useful therapeutics **[3]**. Although marine drug studies have covered a very small proportion of aquatic organisms, several thousand new compounds have been isolated, many of which are structurally unique and absent from terrestrial organisms **[4, 5]**. Cancer is a growing public problem whose estimated new incidence worldwide is a multistep process caused by accumulated errors in the genes that control cellular

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processes **[6, 7]**. It is one of the leading causes of death worldwide and responsible for almost 10 million deaths in 2020 **[8]**. Today, there are many treatment options for cancer patients, including radiotherapy, curative surg ery, and immunotherapy, however, all of these cancer treatment methods are ineffective at satisfactory rates. Furthermore, successful treatment with anticancer drugs remains challenged due to the non-selective cytotoxicity of available drugs. Therefore, research continues for unconventional drugs such as bioactive substances that could open new avenues for cancer treatment in the future **[9]**. In this regard, a large number of potent antitumor compounds have been isolated from aquatic invertebrates. The best-known examples include eleutherobin from Eleutherobia family of corals, sarcodictyin from Mediterranean Stolonigera corals, bryostatins from the bryozoan *Bugula neritina*, and dolastatins from the lump fish *Dolabell auriculate* **[10, 11].**

In addition, research on marine medicines has made great strides, and many of them have entered the global medicines market and clinical trials **[5]**. For example; marine peptides have been widely used in medicine for their health-promoting properties **[12]**, brentuximab vedotin and ziconotide isolated from cone snails have been approved by the FDA **[13]**. Moreover, the marine environment is indeed a source of useful compounds for pressing public health problems such as Resistance to antimicrobial agents. Efforts are ongoing to find not only new antimicrobial agents derived from marine animals, but also compounds derived from them that can block resistance mechanisms that can be used in combination with approved antimicrobial drugs **[14]**. Aquatic organisms, including marine invertebrates, show promise for the production of natural compounds as they face significant environmental challenges such as changes in temperature, pressure, nutrients and sunlight intensity. Therefore, these organisms create diverse natural products that allow them to protect themselves and adapt to these conditions. These products are biologically active against many diseases such as microbial diseases and others **[15]**. Among the aquatic invertebrates, copepods are widespread in all aquatic habitats. They are influenced by the surrounding water quality and emit chemical compounds. Copepods secrete bioactive compounds that mediate mate seeking or induce defensive traits in prey organisms. Some bioactive compounds extracted from copepods have been studied. For example, the content of copepods, *Temora longicornis*, was analyzed using high-resolution LC-MS and 87 compounds were found **[16]**. On the other hand, Calanus oil is a natural product obtained from copepods, Calanus finmarchicus inhabited the North Atlantic. Rich in waxy esters of polyunsaturated fatty acids, this oil is effective in the treatment of mild inflammation, insulin resistance and atherosclerosis caused by obesity **[17]**. However, no publications are available to test copepod extract for its anticancer and antimicrobial activity. Therefore, this study aims to improve the sustainable mass production of *Acanthocyclops trajani*, the most dominant copepod in the northern Egyptian lakes and fish farms **[18]**, to test its crude extract as anticancer and antimicrobial activity, as such trials may be open to new ones Horizons in finding a treatment for these serious diseases.

2. Materials and Methods

2.1. Culturing of copepods *Acanthocyclops trajani*

No informationis available on the culturing of *A. trajani*. Therefore, in order to improve the sustainable mass production of *A. trajani* for the evaluation of its extract as anticancer and antimicrobial assays, it is necessary to know and study the feeding behavior of this species and its ingestion **[19]**. Therefore, our study began by determining the grazing and ingestion (consumption rate) rates of A. trajani onvarious foods as follows:

2.1.1. Isolation of *A. trajani* copepods

Adults of *A. trajani* were collected from a fish pond at the Department of Freshwater and Aquaculture Branch of the National Institute of Oceanography and Fisheries (NIOF) Cairo, Egypt using a 350 m mesh size. The adult copepods were isolated under a tri-nuclear microscope using a wide-mouth pipette, which was immediately transferred to a 5-liter jar filled with groundwater, which continuously provided a mixture of food (algae, rotifers, and protozoans) for three days around the to get nauplii larvae.

2.1.2. Experiment on feeding behavior and feeding uptake

Experiment on feeding behavior and feed uptake rate of larval and adult stages of *A. trajani* was conducted before performing the mass culture. Feeding behavior of both larvae and adults was tested for 24 hours on several prey including protozoan *Paramecium* spp., rotifers and algae (*Chlorella vulgaris* and *Tetradesmus obliquus*), for 24 hs. The experiment was carried out in test tubes with 5 ml of ground water. A fixed number of *A. trajani* adults (3 individuals) were isolated under the microscope and then transferred to the test tubes. At the same time, the experiment was carried out on ten individuals of the nauplii larva. Each prey was offered separately with a fixed number to feed the adults and nauplii of *A. trajani* in triplicate for each prey, in addition to the control carried in triplicate without copepods. Each prey was counted as an individual ml-1 at the beginning of the experiment and after 24 hours. All experiments were performed at a water temperature of 241 °C and a pH of 8-8.5 with photoperiod (day/night). The consumption rate of copepods for each prey was calculated according to **[20]** equation:

$G = \frac{v}{n}\left(\frac{In\ Co - In\ C_t}{t} - A\right)$; $A = \frac{In\ Co - In\ C_t}{t}$; $I = F \sqrt{C_0\ Ct}$; *where:*

G =grazing rate (ml water purified by Copepods ind.$^{-1}$ day^{-1}); I =ingestion rate (individual number of prey consumed by copepods ind.$^{-1}$ day^{-1}); C0 = initial prey density ml^{-1}, Ct = final density, n =number of copepods in volume v (ml) water, t = experimental duration (day), A = correction coefficient for change density of prey in the control tubes to final density after the same time.

2.1.3. Preparation of food:

2.1.3.1. Protozoa represented by Paramecium spp. which flourished by adding 0.5 g cow manure and 0.5 g yeast l^{-1} on a tank with 100 L fish pond water, based on several preliminary tests. Then Protozoa were collected and washed from the groundwater in 20 m long plankton net before feeding the copepods. After washing and concentration, the average density of protozoan *Paramecium* spp. was 19450 individuals ml^{-1}. The feeding behavior experiment was started by adding 1 ml of Protozoa (19450 individuals) for each repetition.

2.1.3.2. Rotifers were collected from the outflow of the fish farm using a 55 µm plankton net. Then the collected samples were filtered on a 50 µm plankton net to remove organisms down to 150 µm. The sample was condensed and washed again with groundwater on a 55 µm plankton net and transferred to a five-liter jar. The feeding behavior experiment was started with 1840 rotifers ml^{-1} (520 individuals of *Brachionus calyciflorus*, 170 *B. caudatus*, 270 *Polyarthra vulgaris*, 800 *Collotheca* sp., and 80 *Keratella cochlearis*) in each repetition.

2.1.3.3. The green algae\ (*C. vulgaris* and *T. obliquus*) were cultured in the lab. BG-11 medium was used to pre-culturethe selected microalgae *C. vulgaris* and *T. obliquus*. The culture was supplied with continuous air bubbles under continuous illumination of approx. 3500 lux **[21]**. The culture was started with 10^3 cells ml^{-1} and 5x 10^2 cells ml^{-1}, respectively, for 7 days and then the microalgae were cultured in a new batch for 10-14 days, which reached 10^5cellsml^{-1}and 7x 10^3 cells ml^{-1}, respectively.

2.1.4. Sustainable mass production of *A. trajani*:

Our stock of collected *A. trajani* was kept in 10 vessels (5 liters ground water content) for six weeks as a preparatory culture. Based on the data from the feeding behavior experiment, we continuously offered an equivalent amount of feed with the number of *A. trajani*. The water quality was also maintained during the preparation period by a partial water change. Then all stages of *A. trajani* in the preparation vessels were transferred to a large ceramic pond (approx. 6000 L groundwater). The different stages of *A. trajani* were counted every two days. All dietary elements of protozoans, rotifers and algae were added in equivalent amounts to the counts of each *A. trajani* stage during the culture period. Approximately 500L of the pond was continuously filtered each week.

2.2. Preparation of the crude extract of *A. trajani*

The wet mass of the cultured species was dried at 50 °C for 24 hours. Approximately 500 mg of the dried sample was exhaustively extracted with 10 ml of

absolute methanol (HPLC grade, Merck, catalogue No. 107018) for 3 days in Checker at room temperature. The extraction was repeated three times until no color was obtained to ensure complete extraction. The combined extracts were filtered and concentrated through Whatman No. 1 filter paper and evaporated in a low-pressure vacuum at 40°C using a rotary evaporator (IKA Rotary Evaporator, IKA rv10) and stored at 20°C until use for GC/MS analysis, antimicrobial and cytotoxicity assays **[22, 23**].

2.3. Biochemical composition of A. trajani extract (GCMS) analysis

Compounds of *A. trajani* crude extract were separated using GC-MS at the National Research Centre, Cairo Egypt. The mass spectrum of the active isolates obtained was compared to standard reference spectra programmed on the instrument (Agilent 8860 GC with the Agilent 5977B GC/MSD). chemical compounds in the sample extract were identified as the closest matches with compounds recorded in the NIST MS Spectrum Library and Agilent's Retention Time Locked (RTL) database (agilent.com/en/product/gas-chromatography-mass-spectrometry-gc-ms/gc-ms-application-solutions/gc-ms-libraries).

2.4. Antimicrobial Activity

The antimicrobial activity of crude *A. trajani* extract against four gram-positive bacteria (*Bacillus subtilis (ATCC 6633)*, *B. cereus*, *Staphylococcus aureus (ATCC 29213),* and *Enterococcus fecalis(ATCC 29212*) and four gram-negative bacteria (*Escherichia coli (ATCC 25922),Sarcina lutea*, *Salmonella typhi (ATCC 14028)*, and *Klebsiella pneumonia(ATCC 10031)*) was tested by the Freshwater Hydrobiology Department (NIOF), Cairo, Egypt. The tested bacterial strains were cultivated overnight in tryptic soy broth medium (TSB, Difco Laboratories, Detroit, USA) at 37 °C and the cell density adjusted to 10^8 cells ml^{-1} with 0.5 McFarland standard **[24]**. Antibacterial activity was screened using the well-diffusion agar technique **[25]** at a test concentration of (26 mg ml^{-1}) *A. trajani* extracts. The commercial antibiotics (amikacin (30 µg/mL), ciprofloxacin (5µg/mL), and amoxicillin (25 µg/mL) were used as a positive control while 5% DMSO was used as a negative control.

2.1.5. Cytotoxicity assay

Cytotoxicity of crude *A. trajani* extract against cancer and normal cells was tested at Al-Azhar University Regional Center for Mycology and Biotechnology, Cairo, Egypt. The antiproliferative activity of the extract was measured using the MTT protocol **[26]**. Hep G-2 cells (human hepatocellular carcinoma), MCF-7 cells (human breast carcinoma), HCT cells (human colon carcinoma), and A-549 (human lung carcinoma) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). THLE2 (normal liver epithelial cells), WI38 (normal lung fibroblast cell), FHC (normal colon epithelial cells), and MCF10a (normal mammary epithelial cells) were obtained from Holding Company for Biological Products and Vaccines (VACSERA) Tissue Culture Unit. The relationship between the crude extract and the surviving cells was plotted to obtain the survival curve of each tumour and normal cell line after treatment. The 50% inhibitory concentration (IC_{50}) producing toxic effects in 50% of intact cells was estimated from dose-response curve plots for each concentration using GraphPad Prism software (San Diego, CA, USA) [26].

2.1.6. Data Analysis

A one-way analysis of variance (ANOVA) was performed to find the significant effect of the extract against cancerous and normal cells from the same organ using XLSTAT 2016. Also, ANOVA test was run to compare the activity of *A. trajani* extract with the commercial antibiotics against the pathogenic bacterial strains. In addition, the degree of selectivity of the extract expressed by the SI value obtained from the division IC_{50} of a normal cell/IC_{50} of a cancer cell of the same organ **[27]**. An SI value > 2 means the compound has selective toxicity to cancer cells, while a value below 2 means the compound is toxic to both cancer cells and normal cells.

3. Result and discussion

3.1. Feeding behavior and consumption rate of A. trajani on different foods

In order to promote and improve the sustainable mass production of zooplankton species for use in different applications, it is necessary to know and study the feeding behavior of these species and

their feeding rates. This information is very important and is needed for zooplankton culture**[19]**. Therefore, our study began by determining the grazing and uptake (consumption rate) rates of *A. trajani* on various foods including protozoa, rotifers, and algae. The results of feeding behavior and consumption rate of *A. trajani* on different foods are presented in Table 1. The results of grazing (G) and ingestion rate (I) showed that the adult stage of this species engaged in animal prey (protozoan *Paramecium* spp. and rotifers) and algal prey (*Chlorellavulgaris* and *Tetradesmus obliquus*). Nevertheless, the nauplii larvae only fed on algal prey; *C. vulgaris* and *T. obliquus*. Where adult G was 0.05, 0.9, 3.03 and 2.1 ml ind^{-1} of copepods x day, while I was 45.98, 43.09, 1082398 and 123792 $ind.^{-1}$ of copepods x day, on *Paramecium* spp., rotifers, *C. vulgaris* and *T. obliquus*, respectively. On the other hand, the results of feeding behavior and feeding rates of nauplii larvae differed from those of adults, with G and I being based on *C. vulgaris* and *Tetradesmus* (G = 0.06 ml ind^{-1} copepods x day; I = 7223.2 and 2374.3 $ind.^{-1}$ of the copepods x day, respectively) were clear, while G and I on *Paramecium* spp. and rotifers were not significant and mostly reached zero.

Based on our feeding behavior study, *A. trajani* is considered omnivorous because in its adult stages it fed on both algae and zooplankton (protozoa and rotifers), while its nauplii larvae only fed on algae. These results have been confirmed in other previous studies on other species of the same genus **[28-30]**. Where Brandl **[28]** found that the adults of *Acanthocyclops robustus* feed efficiently on zooplankton, they can also feed on algae. Garca-Chicote **[29]** mentioned that *A. robustus* is a predatory species that can feed on several species of protozoan, rotifers, cladocerans and copepods. Enriquez-Garcia **[30]** found that *A. americanus* only consumed algae in the larval stage and that in the adult stage it can also feed on algae in addition to animal prey. In addition, **[19]** found that *A. trajani* fed on *Chlorella vulgaris* but was not entirely dependent on the algal diet, and concluded with a recommendation for further studies on the feeding behavior of this species. In this regard, Brandl **[31]** mentioned that freshwater cyclopoids both can eat both plant and animal prey. Although predatory feeding is highly selective within this group, algae are heavily consumed by adults and later growth stages and are a necessary food source for younger stages.

Table 1

Grazing (G) and ingestion rates (I) of adult and nauplius larvae of *A. trajani* on various foods

Food item (prey)	Adult		Nauplius	
	G	I	G	I
• **Protozoa**				
Paramecium spp.	0.05	45.98	0.00	0.00
• **Rotifera**				
Brachionus calyciflorus	1.19	10.52	0.03	1.25
B. caudatus	0.81	4.09	0.00	0.00
Polyarthra vulgaris	1.10	5.79	0.00	0.00
*Collotheca*sp.	0.75	19.49	0.01	0.50
Keratella cochlearis	0.67	0.00	0.00	0.00
Total rotifers	0.90	43.09	0.00	0.00
• **Alga**				
Chlorella vulgaris	3.03	1082398.62	0.06	7223.18
Tetradesmus obliquus	2.10	123792.76	0.06	2374.34

3.2. Sustainable mass production of *A. trajani*

The mass culture was started by filling the ceramic pond with 8 ind. l^{-1} from adults, 11 ind. l^{-1} of copepodites and 17 $ind.l^{-1}$ from nauplii larvae. All copepod stages were encouraged to grow and their densities continued to increase over time. On day 20, adults reached the density of 360 ind. l^{-1}, while copepodite and nauplius larvae reached 480 and 3100 ind. l^{-1} (Fig.1). At this point, about 500 L of the culturing pond was harvested using 250 µm mesh plankton nets to collect the adults and copepodites, while the nauplius was returned to the pond. The pond was harvested at this point to maintain production continuity, although the density of cultivated species in the ponds may decrease due to their high density and/or due to changes in water quality. While the main problems limiting the success of copepod mass culture are its low productivity, long generation time, environmental changes and water quality changes **[32-34].**

The harvested biomass was concentrated as much as possible and washed many times with tap water through plankton nets of different mesh sizes to remove any components from the collective biomass. The harvested biomass was estimated to be 27.42 and 1.52 g for wet and dry weight, respectively (Fig. 2). The filtration and harvesting process was repeated weekly and continuously to

obtain amounts of *A.trajani* biomass for subsequent analysis.

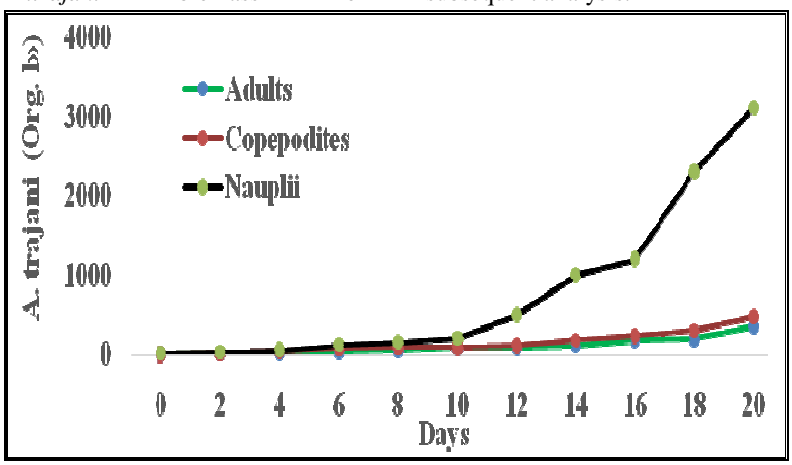


Figure 1. Growth densities of different stages of *A. trajani* during mass production

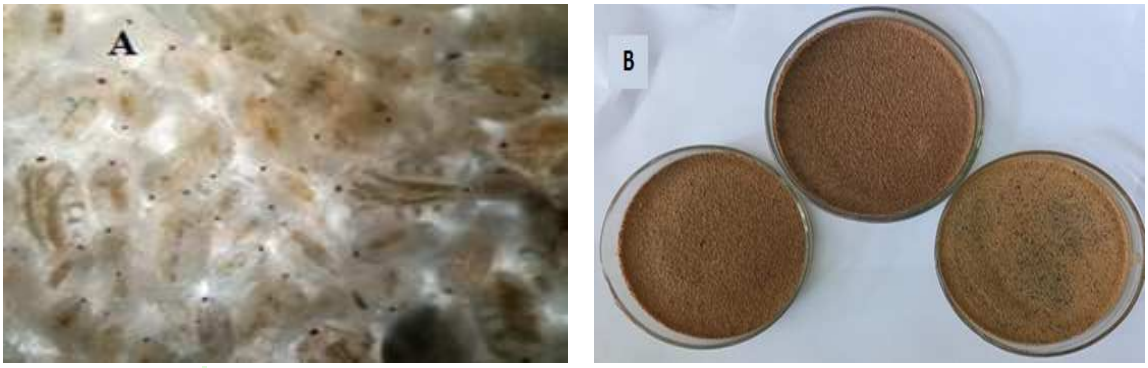


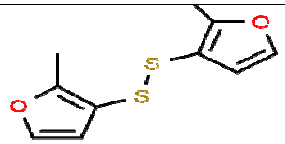
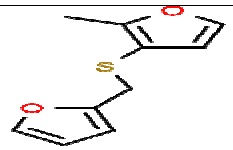
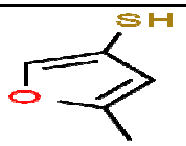
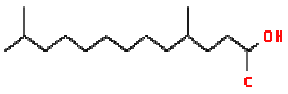
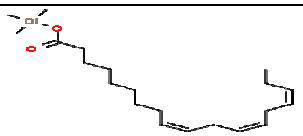
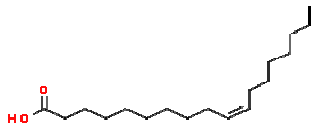
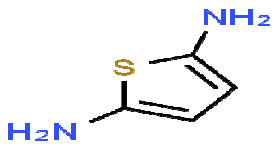
Figure 2. The collection mass of *A. trajani* after filtration and harvest; A: the biomass of *A. trajani* under a microscope, B: the dry biomass

3.3. Medicinal properties of biochemical compounds of *A. trajani* extract

GC-MS analysis of *A. trajani* extract, detected 8 bioactive compounds were shown in Table 2 and Fig. 3. These compounds are known for their medicinal use in several previous studies. Among the bioactive compounds of *A. trajani* extract, furan, 3,3-dithiobis [2-methyl- accounted for about 81.3% of the extract. This compound was found in marine-derived fungi and represents an interesting source of bioactive compounds, some of which exhibit antibacterial activity**[35, 36].** These studies showed that there is no clear difference in activity between volatiles produced by marine or terrestrial bacteria and showed that the antimicrobial activity of the volatiles against proliferative cells can be considered to be quite low, with the exception of furfuryl isovalerate and 2 ,2-[Thiobis(methylene)] bifuran compounds with surprising antimicrobial activity in several aspects. Also, several studies have shown that furan derivatives have moderate to excellent anti-inflammatory, antitumor and antimicrobial activity **[37-39]**. On the other hand, Huang **[40]** reported that several sulfur-containing heterocyclic compounds such as 2-formyl-2,3-dihydrothiophene showed high correlation with antioxidant activity and positive correlations with methylfurfuryl disulfide, which were significant correlated with both ferrous ion chelating activity and lipid peroxidation inhibitory activity, these are the main aroma compounds formed in the Maillard reaction and show antioxidant activity in lipid peroxidation systems. In addition to oleic acid, trans-oleic acid and linoleic acid, palmitic acid has high anticancer properties, antiviral, anti-inflammatory and strong antiproliferative activity **[41-45]**. A-linolenic acid has shown the largest investigations warranted to confirm its biological activities in vivo and to

investigate its possible candidacy for pharmaceutical and cosmetic applications **[46]**. Mohamed and Saber **[47]** strongly recommended the use of a species of marine algae found in Egyptian coastal waters as a promising and environmentally friendly source of bioactive compounds, particularly fatty acids such as stearic acid, oleic acid, linoleic acid, palmitic acid, which can be useful in the treatment or prevention of many human pathogenic fungal diseases and the management and control of fungal pathogens. Also **[48]** attributed the inhibitory effect of Pyrrosia piloselloides extract on human Hela cervical carcinoma to the presence of 2H-imidazole-2-thione, 1,3-dihydro-1-methyl

Table 2
The main bioactive compounds of *A. trajani* extract were detected by GC/MS

Chemical compound	Area Sum %	M. formula	M. wt.	Chemical structure
Furan, 3,3'-dithiobis[2-methyl-	81.3	$C_{10}H_{10}O_2S_2$	226.315	
Furfuryl sulfide	3.12	$C_{10}H_{10}O_2S$	194.250	
Thiophene, 2-formyl-2,3-dihydro-	1.75	$C_5H_6O_S$	114.166	
Palmitic acid	2.5	$C_{16}H_{32}O_2$	256.424	
Linoleic acid	1.25	$C_{21}H_{38}O_2Si$	350.6107	
Oleic Acid	6.01	$C_{18}H_{34}O_2$	282.461	
Stearic acid	1.71	$C_{18}H_{36}O_2$	284.477	
2H-Imidazole-2-thione, 1,3-dihydro-1-methyl-	0.92	$C_4H_6N_2S$	114.169	

Note: All names were checked using NIST Chemistry WebBook, SRD 69

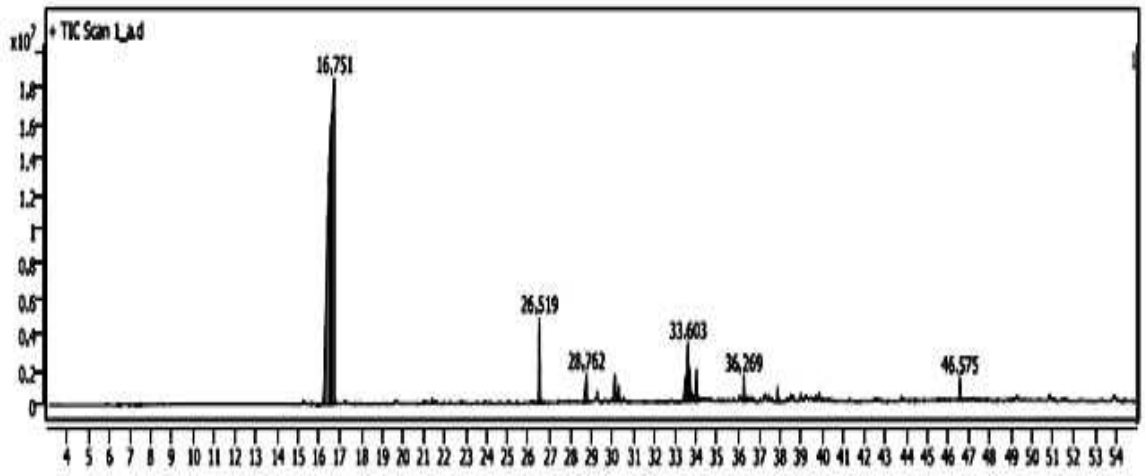


Figure 3. GC-MS analysis plots of active compoundsemitted from *A. trajani* extract

3.4. Antimicrobial activity of *A. trajani* extract

The *A. trajani* crude extract showed activity against bacterial strains; *S. aureus*, *E. coli*, *E. faecali*, *K. pneumonia*, *S. lutea* and *S. typhi* with zones of inhibition of 14, 12, 8, 12, 17 and 12 mm, respectively. While, the extract showed no activity against the other investigated bacterial strains. However, there were no significant differences in the activity of the extract and the antibiotic Amikacin against *E. coli*, *S. lutea,*and *S. typhi* (Table 3). The effectiveness of the extract against *S. aureus*, *E. coli*, *E. faecalis*, *K. pneumoniae*, *S. lutea* and *S. typhi* may be due to the presence of compounds such as furan, 3,3-dithiobis [2-methyl and fatty acid compounds (palmitic acid, oleic acid and linoleic acid) in the extract which, may have antimicrobial activity. Also, Toshkova-Yotova **[49]** reported that palmitic, oleic, and linoleic acids exhibited varying degrees of antibacterial and antifungal activity against gram-positive and gram-negative pathogens. Therefore, *A. trajani* crude extract shows promise for use as a source of alternative bioactive molecules that can be used as alternative antibiotics.

Table 3.
The activity of *A. trajani* extract and three commercial antibiotics against 8 bacterial strains. (Inhibition zones are expressed in mm). N/Z – non inhibition zone. Data are presented as mean ± SD, n = 3

Extract	*B. cereus*	*S. aureus*	*B. subtilis*	*E. coli*	*E. fecalis*	*K. pneumonia*	*S. lutea*	*S. typhi.*
***A. trajani* extract**	**N/Z**	**14± 0.3^{d}**	**N/Z**	**12± 0.7^{b}**	**8±0.6^{d}**	**12± 0.3^{c}**	**17±1.1^{b}**	**12± 0.5^{c}**
Amikacin	**25±1.2^{a}**	**20± 0.9^{a}**	**20± 1.7^{b}**	**13± 0.6^{b}**	**12±0.3^{c}**	**18± 1.6^{b}**	**20± 1.7^{b}**	**12± 2.1^{c}**
Ciprofloxacin	**35± 1.9^{b}**	**18± 0.9^{b}**	**25± 1.7^{a}**	**23± 1.2^{a}**	**24±1.2^{b}**	**30± 1.7^{a}**	**27± 0.8^{a}**	**24± 1.2^{b}**
Amoxicillin	**45± 1.9^{c}**	**8± 0.3^{c}**	**N/Z**	**N/Z**	**28±2^{a}**	**N/Z**	**35± 2.2^{c}**	**28± 1.2^{a}**
Negative control	**N/Z**	**N/Z**	**N/Z**	**N/Z**	**N/Z**	**N/Z**	**N/Z**	**N/Z**
ANOVA (p-value)								
***A. trajani Vs.* Amikacin**	--	< 0.04	--	< 1.0	**<0.01**	**<0.04**	**<0.06**	**<0.5**
***A. trajani Vs.* Ciprofloxacin**	--	< 0.04	--	< 0.001	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
***A. trajani Vs.* Amoxicillin**	--	< 0.004	**--**	< 0.001	**< 0.0001**	**--**	**< 0.001**	**< 0.0001**

[a-d]: Different letters in the same column indicate statistically significant differences groups (Tukey's test, $p < 0.05$)

3.5. Effect of *A. trajani* crude extract on in vitro cytotoxicity assay

The cytotoxic activity of *A. trajani* crude extract was screened against different histological types of human cancer cell lines (liver, lung, colon, and breast) cell lines (HepG2, A549, HCT, MCF7) as shown in Table 4. *A. trajani* crude extract showed a moderate anti-growth activity against the cancer cells (IC_{50} = 63.064, 18.377, 46.905 and 21.736, respectively) compared to the activity of staurosporine (control). Whereas staurosporine is a biological compound that has a potent effect on cancer cells by inhibiting protein enzymes by blocking the binding of ATP to kinases **[50]**. While the normal cells were more sensitive to staurosporine than the *A. trajani* extract, this means that the *A. trajani* extract is safer for the normal cells. However, the selectivity of the cytotoxic activity of the extract, determined by comparing the activity (IC50) of the extract against cancerous and normal cells of the same organ **[27]** based on SI values, showed a selective effect on the lung cancer cell (A549) breast cancer cell (MCF7) (SI = 3.07 and 3.1, respectively). In this regard, several studies have relied on the selectivity index (SI) evaluation of compounds as anticancer cells.

If the SI value of the compound is more than 2, it means that the compound has selective toxicity towards cancer cells **[51-53].** Also, the results of the ANOVA showed a significant effect of the extract ($P<0.005$) between A549 and WI38; and between MCF7 and MCF10a. Therefore, the crude extract of *A. trajani* may offer the potential for further exploitation in the discovery of new anti-cancer drugs, especially lung and breast cancer drugs. On the other hand, the moderate cytotoxic effect of *A. trajani* extract on cancer cells associated with staurosporine, hence the somewhat low SI values, can be explained by the fact that the extract contains several compounds, while staurosporine is thus a single compound it more focused and active. Therefore, it is necessary to complete this study with future detailed studies, in which the compounds should be separated, to test the activity of each compound separately, and to know a compound that can open new horizons in the search for the treatment of cancerous tumors. Among the expected compounds to be detected in this extract are compounds such as furan, 3, 3-dithiopis [2-methyl-, palmitic, oleic, oleic and linoleic acids, which have been found to have anti-cancer effects in previous studies **[37-39, 42, 43].**

Table 4

The IC_{50} values of the cytotoxic activity of *A. trajani* and staurosporine against cancerous and normal cells, selectivity index (SI) values and P values (ANOVA test). Data are presented as mean ± SD, n = 3

Organ	Liver		Lung		Colon		Breast	
Cell line	**HepG2**	**THLE2**	**A549**	**WI38**	**HCT**	**FHC**	**MCF7**	**MCF10a**
IC50 (ug ml^{-1}) of *A. trajani* extract	63.064 ± 2.8	83.072 ± 4.05	18.377 ± 1.3	56.564 ± 2.76	46.905 ± 2.08	42.275 ± 2.06	21.736 ± 0.96	68.021 ± 3.31
IC50 (ug ml^{-1}) of staurosporine (control)	7.787 ± 0.35	31.497 ± 1.53	6.246 ± 0.28	27.137 ± 1.32	8.822 ± 0.39	24.855 ± 1.21	6.296 ± 0.28	21.708 ± 1.06
SI	1.3		3.07		0.9		3.1	
P-value	0.51		0.005		0.99		0.005	
Significant	No		Yes		No		Yes	

4. Conclusion

A. trajani is an omnivorous species that feeds on animal and algal prey. *A. trajani* crude extract shows promise for use as a source of alternative bioactive molecules that can be used as alternative antibiotics. In addition, the crude extract of *A. trajani* offers the potential for further exploitation in the discovery of new anti-cancer drugs, particularly lung and breast cancer drugs. *A.trajani* crude extract has several therapeutic compounds. However, this preliminary study on the bioactive compounds of *A. Trajani* should be completed to determine and separate the therapeutic substance in the detected copepod species as antibacterial and anticancer drugs.

5. Conflict of Interests

Authors do not have any conflict of interest.

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