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Octopus vulgaris ink chemical profiling and validation of its potential as antioxidant, antimicrobial, anti-cancer as well as anti-Schistosomal drug *in vitro*

[Perfil químico da tinta de Octopus vulgaris e validação de seu potencial como antioxidante, antimicrobiano, anticancerígeno e antiesquistossomótico in vitro*]*

S.A.H. *Hamdi*[1], M.A.M. *El-Shazly*[2], M.F. *Fol*[1], H.S. *Mossalem*[3], M.A. *Ghareeb*[2], A.M. *Ibrahim*[3], A.S *Aloufi* [4], M.N. *Abd El-Ghany*[5*], S.M. *Korany*[6]

[1]Zoology Department, Faculty of Science, Cairo University, Egypt
[2]Medicinal Chemistry Department, Theodor Bilharz Research Institute, Egypt
[3]Medical Malacology Department, Theodor Bilharz Research Institute, Egypt
[4] Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia.
[5] Botany and Microbiology Department, Faculty of Science, Cairo University, Giza 12613, Egypt
[6]Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo 11795, Egypt

ABSTRACT

Among marine creatures including squids, cephalopods and *Octopus*, one of the most unique features is production of ink which is an extremely valuable source of pharmaceuticals. The current study investigates the ink produced from *Octopus vulgaris* chemically as well as its potential antioxidant, anticancer, antimicrobial, and anti-schistosomal activities *in vitro*. Twenty-one different compounds were identified by GC-MS analysis of *Octopus vulgaris* ink. Results revealed that *O. vulgaris* ink had antioxidant capability to cover DPPH free radical when compared to ascorbic acid. Moreover, *Octopus vulgaris* ink exhibited antibacterial activity against *Bacillus subtilis* followed by *Escherichia coli* and *Pseudomonas aeuroginosa* and exhibited a molluscicidal activity against adult *Biomphalaria alexandrina* snails and had a distinguished mortal effect on free larval phases of *Schistosoma mansoni*. Furthermore, its anticancer activity was confirmed, where IC_{50} value of breast cancer cell line MCF-7, human hepatocellular carcinoma (HCT) and human liver cancer cell line (HepG2) were 29.8, 38.29 and 30.38µg/mL, respectively. In conclusion, ink extracted from *O. vulgaris* may be considered as source of valuable compounds that can be used as molluscicidal, antioxidant, antimicrobial, anticancer and anti-schistosomal agents.

Keywords: *Octopus vulgaris*, antioxidant, antimicrobial, anticancer, anti-schistosomal

RESUMO

Entre as criaturas marinhas, incluindo lulas, cefalópodes e polvos, uma das características mais exclusivas é a produção de tinta, que é uma fonte extremamente valiosa de produtos farmacêuticos. O presente estudo investiga quimicamente a tinta produzida a partir do Octopus vulgaris, bem como suas possíveis atividades antioxidantes, anticancerígenas, antimicrobianas e antiesquistossômicas in vitro. Vinte e um compostos diferentes foram identificados pela análise GC-MS da tinta de Octopus vulgaris. Os resultados revelaram que a tinta de O. vulgaris tinha capacidade antioxidante para cobrir o radical livre DPPH quando comparada ao ácido ascórbico. Além disso, a tinta de Octopus vulgaris apresentou atividade antibacteriana contra Bacillus subtilis, seguida por Escherichia coli e Pseudomonas aeuroginosa, e apresentou atividade moluscicida contra caramujos adultos Biomphalaria alexandrina e teve um efeito mortal distinto nas fases larvais livres de Schistosoma mansoni. Além disso, sua atividade anticancerígena foi confirmada, onde o valor IC50 da linha celular de câncer de mama MCF-7, carcinoma hepatocelular humano (HCT) e linha celular de câncer de fígado humano (HepG2) foi de 29,8, 38,29 e 30,38µg/mL, respectivamente. Em conclusão, a tinta extraída de O. vulgaris pode ser considerada como fonte de compostos valiosos que podem ser usados como agentes moluscicidas, antioxidantes, antimicrobianos, anticancerígenos e antiesquistossômicos.

Palavras-chave: Octopus vulgaris, antioxidante, antimicrobiano; anticancer, antiesquistossomótico

*Corresponding author: dr.mohamed.naguib@cu.edu.eg
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INTRODUCTION

Recent years have seen a rise in the use of marine products as functional foods, nutraceuticals, supply materials for pharmaceutical research, and specific health foods (Suleria *et al*., 2015; Najmi *et al*., 2022). Because aquatic organisms, which live in an entirely dissimilar and diverse environment, produce highly potent protective compounds. Marine species are typically more varied and active than their analogues from terrestrial sources (Besednova *et al*., 2017). Cephalopods are common and valuable in fisheries in terms of both domestic consumption and commercial value (Rubaie *et al*., 2012). Natural products obtained from squid, cuttlefish, and octopus were commonly used in old traditional medicine and assisted in curing a wide range of diseases (Atta *et al*., 2021; Ibrahim *et al*., 2021; Amina M Ibrahim *et al*., 2022a). Cephalopods, without an outer shell, guard themselves in a many of ways, such as venom to kill enemies as well as preys and liquid ink to avoid predator attacks (Cooke, *et al*., 2015). Cephalopod ink is usually created at the termination of the maturation process in a viscous, colorless medium (Liu *et al*., 2011). The blue-black color of cephalopod ink is due to the presence of a high concentration of melanin pigment. It also contains a high concentration of proteins, lipids, minerals, and other nutrients (Ganesan *et al*., 2017). Scientists have paid attention to ink sacs scientifically, because of their intense black color (Derby, 2014). *Octopus vulgaris* ink by-products are a potent source of unique bioactive compounds with many important pharmaceutical compounds such as antimutagenic, cytoprotective, antiproliferative, antioxidant and proapoptotic effects in selected human cancer cell lines (Vate and Benjakul, 2013; Hernández-Zazueta *et al*., 2021a). Furthermore, Derby (2014) identified cephalopod ink not to contain toxins. The main profiles components of ink such as carbohydrate, protein, lipid, and moisture content are often necessary to ensure that they meet the requirements of food regulations and commercial specifications (Riyad *et al*., 2020). However, there is insufficient literature focused on the composition and pharmaceutical evaluation of cephalopod inks.

Therefore, the current study aimed to investigate the chemical composition of ink extracted from the marine cephalopod *Octopus vulgaris* and evaluate its antioxidant, antimicrobial, anticancer, and anti-schistosomal activities.

MATERIAL AND METHODS

Fresh cephalopod (*O. vulgaris*) was bought and rapidly transported to the laboratory where dissection took place, and then the ink solution was collected and diluted immediately with an equal volume of distilled water and mixed sufficiently. The solution was collected instantly, concentrated, and finally lyophilized to form a black residue using a lyophilizer (LABCONCO lyophilizer, shell freeze system, USA).

GC-MS investigation was carried out according to the confirmed techniques (Madkour *et al*., 2017; Ghareeb *et al*., 2022), using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1mm film thickness). For GC/MS measurment, an electron ionization system (70 eV) was used. The carrier gas (Helium) was used at a steady flow rate of 1ml/min. The temperature was fixed at 280°C at the injector and MS transfer line. The temperature of the oven was set up to be at initial temperature of 50°C for 2min and then raised up to be 150°C with an increasing rate of 7°C/min. then raised up to 270°C at an increasing rate of 5°C/min (hold 2 min) then to 310°C as a final temperature at an increasing rate of 3.5°C/min (hold 10min). The measurements of all the identified constituents were quantified by calculating a percent relative peak area. An identification of the compounds was done by comparing the irrelative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

Antioxidant activity was estimated via using 2,2'-diphenyl-1-picryl-hydrazyl (DPPH) test using the reported procedures by Ghareeb *et al*. (2016).
The antioxidant activity was estimated using phosphomolybdenum assay according to the reported procedures (Abdel-Motleb *et al*., 2022).

Antimicrobial activity of aqueous extracts of *O. vulgaris* ink was carried out using a modified disc diffusion method (Bauer, *et al*., 1966) against filamentous fungi as *Aspergillus flavus* at 25^{o}C for 48h; Gram (+) bacteria as *Streptococcus faecalis, Staphylococcus aureus,* and *Bacillus*

subtilis; Gram (-) bacteria as *Escherichia coli*, *Pseudomonas aeuroginosa* and *Neisseria gonorrhoeae*. They were incubated at 35-37°C for 24-48 h and yeast as *Candida albicans* incubated at 30°C for 24-48h and, then the diameters of the inhibition zones were measured in millimeters. Standard discs of Ampicillin (Antibacterial agent), Amphotericin B (Antifungal agent) served as positive controls for antimicrobial activity, but filter discs impregnated with 10µl of distilled water were used as a negative control. Four wells of 5 mm size each made into the agar plates with the help of sterile borer. The wells were loaded with appropriate test concentrations of ink extract. After incubation, the plates were observed for clear inhibition zone around the well, indicating the presence of antimicrobial activity of the tested samples. For the disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards (Methods…, 1997).

All set LC_{50} was determined using a range of concentrations from the ink's stock solution (200, 100, 50, and 25ppm) (Report.., 1983). Three replicates of *B. alexandrina* were utilized for each concentration, and the exposure period was followed by another 24 hours for recovery (150 snails). Thirty snails were utilized in a control group in a tri-replica set in dechlorinated water. To determine LC_{50}, fatality percentages were noted and examined using probit analysis (Molluscicide…, 1965).

The micro titre plate well was filled with fifty pairs of worms. Each well of the microtiter plates holding worms received a triplicate of 1 ml of various ink concentrations (50, 40, 30, 20, and 10ppm). Three pairs of dechlorinated water were used to sustain the control group of worms in a tri-replica. Following various experiment time intervals, treated and control worms were gathered and counted for the dead one (Pereira *et al*., 2019).

Five milliliters of the ink's LC_{50} concentration (88.3 ppm) were employed with one hundred miracidia or cercariae in five milliliters of water. In addition, 100 newly emerged cercariae or miracidia were kept in 10 milliliters of dechlorinated tap water as the control groups (Ritchie *et al*., 1974). Under a dissecting microscope, the movements of miracidia and cercariae were examined at intervals of 10, 20, 40, 80, 160, and 320 minutes. Static miracidia were deemed dead (Eissa *et al*., 2011).

Breast cancer cell line (MCF-7), Human hepatocellular carcinoma (HCT), and human liver cancer cell line (HepG2) from Michigan Cancer Foundation-7 were used to test the ink's antitumor activities in presence of normal Baby Hamster Kidney fibroblasts (BHK) cell lines. The RPMI-1640 media containing 10% inactivated fetal calf serum and 50g/mL gentamycin was used for cell cultivation. Subsequent subculture (2 to 3 times) was performed after week at 37°C in a humidified CO_2 incubator. This analysis was done in the micro-analytical center at Cairo University, Cairo, Egypt (Hamdi *et al*., 2022).

All analyses were carried out using spss 25 and sample analysis were in triplicate, and results are reported as mean ±SD.

RESULTS

GC-MS analysis of *Octopus vulgaris* ink includes 21 compounds (Figure 1). The total peak areas of the identified ingredients constitute 41.33%, the prediction of the chemical structures of the identified compounds are recorded in Table 1. The chief identified compounds are (E)-1,2,3,4-Tetra(4-phenylphenyl)-2-butene-1,4-dione (3.15 %), Lipo-3-episapelin A (2.68 %), and 5,10-Dihexyltetrabenzoporphyrin (2.50 %).

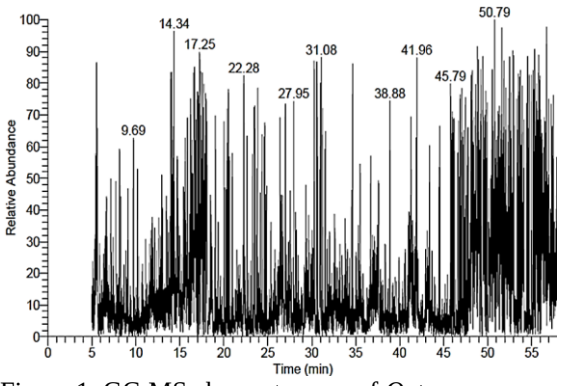

Figure 1. GC-MS chromatogram of *Octopus vulgaris* ink.

Table 1. Chemical composition of *Octopus vulgaris* ink

No.	R_t	Area %[a]	M. W.	M.F.	Identified compounds	Class/Category
1	8.16	2.14	612	$C_{40}H_{44}N_4O_2$	4,16-Di-t-butyl-10,11,22,23-tetramethyl-1,8,13,20-tetraazatet rabenzo[c,d:h:l,m:r]cyclooctadecahexadecaene-6,18-diol	Porphyrin derivative
2	14.05	1.42	694	$C_{48}H_{62}N_4$	2,7,12,17-Tetraethy-l3,5:8,10:13,15:18,20-tetrakis(2,2-di methylpropano)porphyrin	Porphyrin derivative
3	14.34	1.60	584	$C_{40}H_{56}O_3$	Deepoxy-Salmoxanthin	Carotene derivative
4	15.63	1.79	692	$C_{44}H_{44}N_4O_4$	N,N'-Dicyclohexyl-1,7-dipyrrolidinylperylene-3,4:9,10-tetracarboxylicacid bisimide	Pyrrolidine derivative
5	16.69	1.74	691	$C_{51}H_{33}NO_2$	2,6-Bis(2,3,5-triphenyl-4-oxocyclopentadienyl)pyridine	Pyridine derivative
6	16.87	2.03	657	$C_{32}H_{19}NO_3S_6$	11-phenyl-2,4,6,8-tetra(2-thienyl)-11-aza-5,13-dithiatetra cyclo[7.3.0.1(2,8).0(3,7)]trideca-3,6-diene-10,12,13-trione	Diene derivative
7	16.91	1.38	644	$C_{41}H_{40}O_7$	5''-(1,1-Dimethylethyl)-2,2',2'',2''',2'''' pentamethoxy[1,1':3' ,1'':3'',1''':3''',1''''-quinquephenyl]-3,3''''-dicarboxyaldehyde	Aldehyde derivative
8	17.87	3.15	692	$C_{52}H_{36}O_2$	(E)-1,2,3,4-Tetra(4-phenylphenyl)-2-butene-1,4-dione	Alkene derivative
9	18.02	2.32	248	$C_{15}H_{12}N_4$	3-Amino-2-(phenylazo)cinnamonitrile	Phenylazo derivative
10	22.28	2.18	544	$C_{26}H_{24}O_{13}$	Methyl-2,4',6'-tri-O-acetylneothamnolate	Hexacarboxylic acids derivatives
11	30.29	1.57	510	$C_{38}H_{26}N_2$	1,3,4,5,8-Pentaphenyl-2,7-naphthyridine	Naphthyridine derivative
12	30.58	1.81	675	$C_{39}H_{41}N_5O_6$	2-Methoxycarbonylvinyl-4(2-bromoethyl)deuteroporphyrin dimethyl ester	Porphyrin derivative
13	31.0	1.58	208	$C_{13}H_{20}O_2$	Trans-á-ionon-5,6-epoxide	Oxepanes
14	46.0	2.50	678	$C_{48}H_{46}N_4$	5,10-Dihexyltetrabenzoporphyrin	Porphyrin derivative
15	47.30	1.41	685	$C_{46}H_{63}N_5$	3(1),5(1)-cyclo-5(1)-(N-methylamino)-2,7,8,12,13,17,18-heptapropyl-3(1)-ethyl-21H,23H-porphrin	Porphyrin derivative
16	47.47	1.68	245	$C_{15}H_{19}NO_2$	(2E,4E)-N-Isopropyl-6-hydroxy-6-phenyl-2,4-hexadien amide	Hexadien amide derivative
17	48.26	2.51	658	$C_{44}H_{34}O_6$	(P,P,R)-Dimethyl-5,5'-dihydroxy-1,1',12,12'-tetramethyl [6,6']bi(benzo[c]phenanthrenyl)-8,8'-dicarboxylate	Phenanthrene derivative
18	49.79	1.55	679	$C_{47}H_{45}N_5$	5-[4-(Dipropylamino)phenyl]-10,20-diphenyl-15-propyl porphyrin	Porphyrin derivative
19	50.79	1.87	205	$C_{13}H_{19}NO$	4-[(4-methylphenyl)amino]-3,3-dimethyl-2-butanone	Beta-amino ketones
20	51.46	2.42	620	$C_{44}H_{28}O_4$	Bis(spirodienone)calix[4]naphthalene derivative	Naphthalene derivative
21	51.59	2.68	656	$C_{42}H_{72}O_5$	Lipo-3-episapelin A	Tirucallane-type triterpenoid derivative
		T% 41.33			Rt: Retention time; M.W.: Molecular weight; M.F.: Molecular formula.	

Ink of *O. vulgaris* was evaluated for its total antioxidant capacity (TAC) and free radical scavenging activity (DPPH). The present results showed that the total antioxidant capacity value of *Octopus vulgaris* ink was 159.33mg AAE/g dry extract, reflecting its reduction capability to convert Molybdenum (VI) to Molybdenum (V). Also, it had the ability to remove DPPH free radical with LC_{50} value of 56.24±2041µg/ml when comparing with ascorbic acid as reference compound with LC_{50} value of 7.46±1.35µg/ml (Table 2).

Table 2. Total antioxidant capacity (TAC) and free radical scavenging activity (DPPH) of *Octopus vulgaris* ink

Sample	Total antioxidant capacity (mg AAE/g dry extract)[1,2]	DPPH IC_{50} (µg/ml)[3]
Ink extract	159.33 ± 4.16	56.24 ± 2.41
Ascorbic acid	-	7.46 ± 1.35

[1]Results are (means ± SD) (n = 3).
[2]AAE (Ascorbic acid equivalent).
[3]IC_{50}: The amount of extract needed to scavenge 50% of DPPH radicals.

The results presented in Table 3 indicated that the maximum antimicrobial activity was found with *Bacillus subtilis* with zone inhibitor of 25 mm followed by *Escherichia coli* and *Pseudomonas aeuroginosa* which showed 23 mm zone inhibitor. However, the other strains showed zone inhibitor varied between 19 mm for *Aspergillus flavus* to 21 mm for *Streptococcus faecalis, Staphylococcus aureus, Neisseria gonorrhoeae* and *Candida albicans.*

The present study showed that ink of *O.vulgaris* has a molluscicidal activity at LC_{50} 88.3 mg/l against *B. alexandrina* snails. Also, it has a wormicidal effect against *S. mansoni* worms with LC_{50} 24.2mg/L after 24h of exposure. Furthermore, it has larvicidal activities against *S. mansoni* miracidiae and cercariae (Table 4, 5 and Fig. 2).

The present results showed that the cell viability percentages were decreased by the gradual increase of the concentration of the *Octopus* ink, where the LC_{50} value of MCF-7, HCT HepG2 tumor cell lines were 29.8µg/mL, 38.29µg/mL and 30.38µg/mL, respectively (Figure 3).

Table 3. Antimicrobial activity of *O. vulgaris* ink

Microorganisms	Ampicilin (Antibacterial agent)	Amphotercin B (Antifungal agent)	Control: DMSO	Extracted ink
	Inhibition zone diameter (mm/mg sample)			
Streptococcus faecalis	20± 0.6	-	Nil	21± 0.72
Staphylococcus aureus	21 ± 0.71	-	Nil	21 ± 0.71
Bacillus subtilis	22 ± 0.75	-	Nil	25 ± 0.81
Escherichia coli	21 ± 0.81	-	Nil	23 ± 0.84
Pseudomonas aeuroginosa	23 ± 0.83	-	Nil	23 ± 0.85
Neisseria gonorrhoeae	20 ± 0.56	-	Nil	21 ± 0.67
Aspergillus flavus	-	17 ± 0.54	Nil	19 ± 0.61
Candida albicans	-	21 ± 0.67	Nil	21 ± 0.66

Data represented as mean ± SD

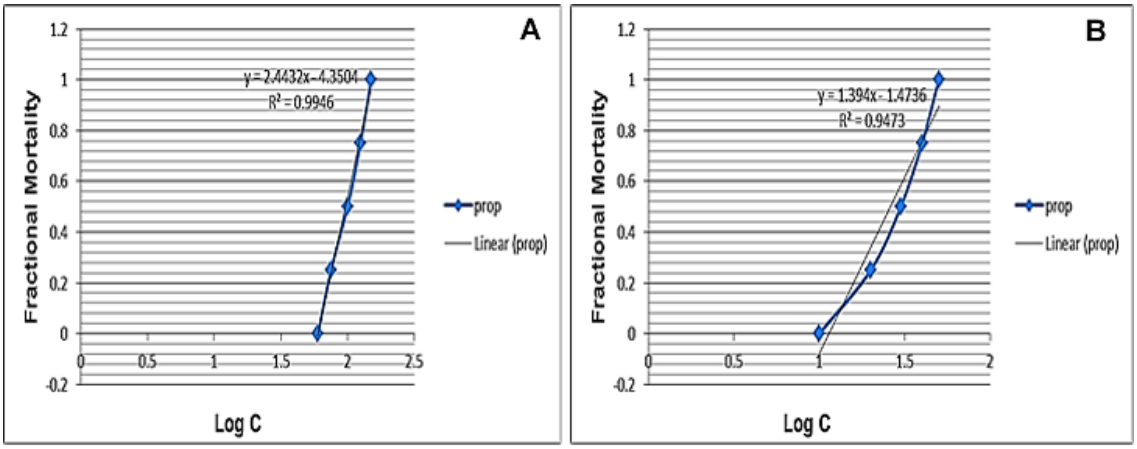

Figure 2. Fractional mortality of A) *Biomphalaria alexandrina* snails; B) *Schistosoma mansoni* worms

Table 4. Molluscicidal and wormicidal activities of *Octopus vulgaris* ink against *B. alexandrina* snails and *S. mansoni* worms after 24 h of exposure

Concentration (ppm)	LC_{10}	LC_{25}	LC_{50}	LC_{90}	Slope
B. alexandrina	44.07	65.03	88.3	132.5	1.1
Schistosoma mansoni worms	4.7	14.01	24.2	43.7	1.2

Table 5. Effect of *Octopus vulgaris* ink against *Schistosoma mansoni* miracidiae and cercariae

Concentration (ppm)		% cumulative mortality/time (min)					
		10	20	40	80	120	320
Miracidiae	Control	0	0	0	5	10	20
	Exposed	0	20	60	100		
Cercariae	Control	0	0	0	10	20	30
	Exposed	10	30	60	80	100	

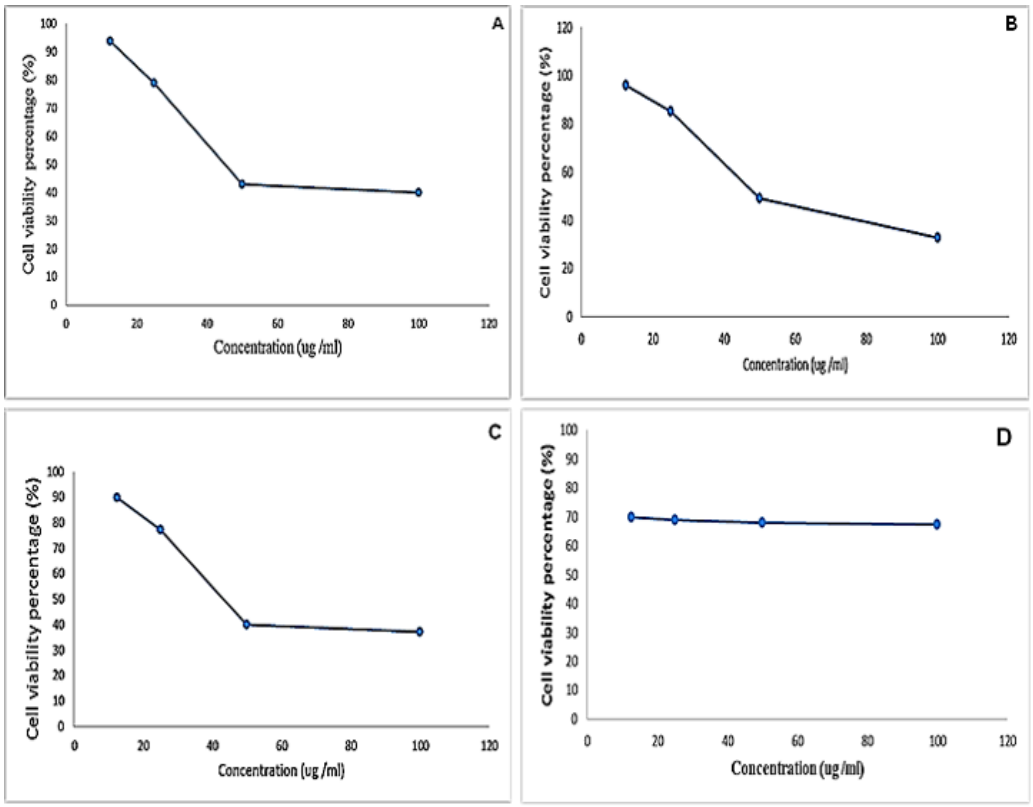

Figure 3. Anticancer activity of *Octopus vulgaris* ink against: A) breast cancer (MCF-7); B) human hepatocellular carcinoma (HCT); C) liver cancer cell line (HepG2) (HepG-2); D) Baby Hamster Kidney fibroblasts (BHK) cell lines.

DISCUSSION

The curiosity about bioactive compounds obtained from marine organisms is increasing every year. Cephalopoda as a members of the class mollusca are considered as promising candidates for the discovery of new BASs (Besednova *et al*., 2017). Cephalopod ink is a natural dark substance made up of melanin granules dispersed in a colorless medium that has been shown to be a multifunctional bioactive material with a wide range of therapeutic applications (Karim *et al*., 2016). Additionally, it has antioxidants (Liu *et al*., 2011), anti-radiation, anti-retrovirus, and anti-bacterial properties (Zhong *et al*., 2009; Nithya *et al*., 2011; Vennila *et al*., 2010). According to the current findings, GC-MS analysis of *Octopus vulgaris* ink

revealed 21 compounds. The identification was accomplished via computer search user-generated reference libraries, incorporating mass spectra (Madkour *et al*., 2017; Abdel-Wareth *et al*., 2019; Shawky *et al*., 2019; Khalaf *et al*., 2021). Chemical characterization of the dichloromethane extract of *O. vulgaris* ink collected from the coast of Hermosillo, Sonora, Mexico led to identification of cholesterol and [N-(2-ozoazepan-3-yl)-pyrrolidine-2-carboxamide (Hernández-Zazueta *et al*., 2021a). Moreover, hexadecanoic acid and 1-(15- methyl-1-oxohexadecyl)-pyrrolidine were detected in the dichloromethane extract of *O. vulgaris* ink collected from Mexico (Hernández-Zazueta *et al*., 2021b). Antioxidant activity is considered as one of the most important properties of biologically active compounds, as many diseases are complemented by oxidative stress (Besednova *et al*., 2017). *Octopus vulgaris* ink was evaluated for its total antioxidant capacity (TAC) and free radical scavenging activity (DPPH). The ink had the capability to disguise DPPH free radical in comparison with ascorbic acid. Moreover, it has antioxidant capacity where it can convert Molybdenum (VI) to Molybdenum (V). The anti-inflammatory, the antioxidant, antihypertensive and anticancer activities were confirmed in many marine species *Paphia malabarica* and *Villorita cyprinoides* (Joy *et al*., 2016). The free radical scavenging activities could protect the mollusks bodies from photoxidation Rockenbach *et al*., 2011). Similarly, Hernández-Zazueta *et al*. (2021a) correlated the antioxidant activity of *O. vulgaris* ink with the melanin that acts either as an antioxidant or reductive agent, stabilizing natural constituents from the ink and preventing its oxidation. Antioxidant characteristics have also been proven for polysaccharides in cephalopods. On the same line, Ramasamy *et al*. (2014) reported that chitosan from the cuttlefish *Sepia cobiensis* characterized as a potent natural antioxidant, as well as a supplement to food and a promising object for the pharmaceutical industry. Also, Luo and Liu (2013) showed that a polysaccharide from squid ink inhibits cyclophosphamide-induced oxidative stress in different organs of animals that received an immunosuppressive agent. Additionally, it caused reduction of Fe^{3+} dose dependently and effectively repressed DPPH and hydroxyl radicals.

There are many antimicrobial agents with diverse mechanisms. However, many of them have many side effects; therefore, finding new, effective, and harmless drugs remains an issue. Numerous studies have shown that cephalopods are an inexhaustible resource of biologically active substances with antimicrobial properties (Besednova, *et al*., 2017). The current study revealed that the ink extracted from *O. vulgaris* have antibacterial activities against gram-positive as *Streptococcus faecalis, Staphylococcus aureus,* and *Bacillus subtilis* and gram-negative bacteria; as *Escherichia coli, Pseudomonas aeuroginosa* and *Neisseria gonorrhoeae*. Also, it revealed antifungal activity against *Aspergillus flavus* and the yeast, *Candida albicans*. On the same line, Hossain *et al*. (2019) who reported that ink of *Octopus vulgaris* showed antibacterial effects on gram negative bacteria, *Vibrio cholera, Salmonella enterica, serovar Paratyphi, Shigella boydii, Shigella dysenteriae, K. pneumonia.* Also, Nirmale *et al*. (2002) suggested that ink of the Indian squid *Loligo duvauceli* has good antibacterial effects. Likewise, Giriji *et al*. (2011) reported on a novel antimicrobial protein, Lolduvin-s, which was isolated from the ink of Indian squid (*Loligo duvauceli*) and showed promising antibacterial and antifungal activities against different pathogens. Correspondingly, Girija *et al*. (2014) revealed that squid (*L. duvauceli*) ink extract has antibacterial potential against dental caries pathogens. It has also been reported that squid ink has good antibacterial properties against *E. coli* and *Klebsiella pneumonia* (Girija *et al*., 2012). Moreover, Nicomrat and Tharajak (2015) found that squid (*L. duvauceli*) and soft cuttlefish (*Sepioteuthis lessoniana*) ink have strong antimicrobial activity against biofilms causing microorganisms. Similarly, Vennila *et al*. (2010) reported that cuttlefish (*S. aculeate*) ink and squid (*L. duvauceli*) ink have antifungal effects against *Fusarium* spp. and *Aspergillus fumigates*. Also, Diaz and Thilaga (2016) revealed that crude and partially purified ink extracts of squid (*L. duvauceli*) and cuttlefish (*S. pharaonis*) have antibacterial effects against eight different bacterial strains. Moreover, Fahmy *et al*. (2014) confirmed the antifungal properties of the extract from the cuttlefish ink towards *A. fumigatus*. Additionally, Hossain *et al*. (2019) stated that in most of the studies, different kinds of ink samples showed prominent antimicrobial activities against most of the pathogenic bacteria

which made cephalopod ink a very good antimicrobial agent and thus it became an object of attraction among researchers.

Schistosomiasis is endemic in tropical regions, and it affects billions of people worldwide (Rees *et al*., 2019; Hussein *et al*., 2023). To control the spread of this disease, several strategies have been used through eliminating its intermediate host, *Biomphalaria alexandrina* snails or larval stages (Omobhude *et al*., 2017; Khalil *et al*., 2022) either by using biological materials or chemical synthetic materials (Morad *et al*., 2022; Ibrahim, 2018). Recent studies search for an ideal alternative instead of the chemical molluscicides as these chemicals pollute the water ecosystem and have high costs (Ibrahim and Ghoname, 2018). The present results verified molluscicide activity of the ink extracted from *O. vulgaris* against *B. alexandrina* snails also, it has a wormicidal and larvicidal effect on both *S. mansoni* worms, miracidae and cercariae. Consequently, it will decrease the transmission of schistosomiasis and can be used in the control strategies of this disease. Similarly, Hamdi *et al*. (2021) revealed that raw chitosan had molluscicide and larvicidal effects on both *Biomphalaria alexandrina snails* and *Schistosoma mansoni* larval stages. Similarly, miracicidicical and cercaricidal activities were reported after using *Nerium oleander* and *Tecoma stans* extract (Ibrahim *et al*., 2022b).

The vitality, motility, pairing, and oviposition of the worms must all be investigated to evaluate any new compound's potential as a schistosomicidal agent (Pereira *et al*., 2019). After being exposed to modest concentrations of edelfosine (5–10µM), the motility of *S. mansoni* worms was evaluated; a lack of motility is indicative of the parasite's mortality. Furthermore, a sign of worm mortality is the division of paired adult worms into single female and male worms. It would be a hopeful decision to lessen the spread of schistosomiasis because this split will result in a decrease in egg production (Yepes *et al*., 2014).

Following exposure of *S. mansoni* cercaria to varying doses of *Solanum nigrum*, cercarial abnormalities were detected. Whereas the cercariae displayed darkness, a swollen head, corrugation of the tail and shortness, partial separation of the tail or complete detachment of the head and tail with disintegration and loss of contents, the control, non-exposed *S. mansoni* cercaria had a bright appearance and smooth surface walls of the head and tail (Pereira *et al*., 2013).

Anticancer drugs are distinguished from other pharmaceuticals by their high level of aggressiveness and pronounced local irritating action. For this reason, scientists seek novel effective compounds with lowered side effects. In recent decades, anti-tumor properties of biologically active substances derived from cephalopods have been analyzed quite actively (Karthigayan *et al*., 2006; Senan *et al*., 2013). Furthermore, the present results showed that *O. vulgaris* ink has anticancer activity on the breast cancer cell line MCF-7, human hepatocellular carcinoma (HCT), and human liver cancer cell line (HepG2). These results were in a good accordance with Hernández-Zazueta *et al*. (2021a) who confirmed the antimutagenic and antioxidant capacity of *O. vulgaris* ink extracts on human cancer cell lines (22Rv1, HeLa, A549). Also, Hossain *et al*. (2019) confirmed the anticancer effects of cephalopods ink through its initiation of apoptosis. On the same line, Senan *et al*. (2013) reported the antitumor effect of an extract from the cuttlefish *Sepia pharaonis* towards chick embryo fibroblasts. Also, , Diaz and Thilaga (2014) demonstrated the cytotoxic effect of an extract from the cuttlefish *Sepia* (inner shell) on HepG2 human liver cancer cells.

CONCLUSIONS

In conclusion, natural bioactive substances, when compared to synthetic products, have the fewest side effects, and the marine environment can be a good source of natural bioactive compounds. Among different marine products, cephalopod ink is one of the best sources of bioactive products. Results showed that ink extracted from *O. vulgaris* provides benefit characteristics related to its antioxidant component content, antimicrobial and anticancer effect. Additionally, it had anti-schistosomal activities. Thus, it offers a chance for identifying new, prospective drugs. However, more experiments must be performed to study their effect on the shelf life of foods.

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