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In vitro Screening of Cytotoxic Activity of the Ink and Nidamental Gland Extracts from the Egyptian Cuttlefish *Sepia officinalis* (Cephalopoda: Sepioidea) on Cancer Cell Lines

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ABSTRACT

New cancer treatments are desperately needed since the available convetntional cancer drugs have numerous negative consequences. Therefore, the current work attempted to evaluate the cytotoxic properties of the ink extract (IE) and nidamental gland extract (NGE) from the Egyptian cuttlefish *Sepia officinalis* on four cancer cell lines: lung carcinoma (A-549), epidermoid carcinoma (A-431), colorectal carcinoma (HCT-116), and prostatic adenocarcinoma (PC-3). Both extracts were characterized through biochemical composition screening via gas chromatography-mass spectrometry (GC-MS) and investigating the levels of proximate composition, phytochemicals, and total antioxidant capacity (TAC). IE and NGE exhibited cytotoxic effects by decreasing viable cancer cells number with dose-dependent, with median inhibitory concentrations (IC_{50}) of 517.52 and 427.45µg/ ml against A-549; 511.03 & 262.83µg/ ml against A-431; 480.06 and 220.04µg/ ml against HCT-116 in addition to 372.21 & 242.22µg/ ml against PC-3, respectively. The NGE showed more toxicity toward all tested cancer cell lines than the IE due to higher concentrations of bioactive substances in NGE relative to IE. Moreover, all proximal compositions, phytochemicals, and TAC in NGE were higher than those detected in IE. Hence, the NGE of *S. officinalis* may be considered a promising cytotoxic agent against cancer cell lines, but more studies are required to explore the action's mechanism.

INTRODUCTION

Nowadays, cancer is regarded as one of the most common illnesses that endanger human survival in most countries, and there is no known cause for it **(Wilson *et al.*, 2004; Houghton *et al.*, 2007)**. Cancer is an illness marked by unregulated cellular division and the proliferation of aberrant cells into the surrounding tissues **(Mofeed *et al.*, 2018; Yousefi *et al.*, 2018)**. Currently, there are no safe cancer treatments due to their adverse effects, such as nausea, exhaustion, vomiting, and diarrhea. Moreover, the available cancer medications have exorbitant expenses. Cancer prevention and treatment studies have attracted worldwide interest in discovering more secure, affordable, and less toxic anticancer medicines from natural sources in recent years **(Fouad *et al.*, 2021)**. The major focus is on marine organisms due to their capacity for adaptation **(Afifi *et al.*, 2016;**

Padmanaban *et al.*, 2022). Marine molluscs contain several potent natural bioactive compounds with great pharmacological resources used to produce new drugs with various therapeutic applications **(Fahmy & Soliman, 2013; Senan, 2015; Mona *et al.*, 2021)**.

Among the marine molluscs, cuttlefish *Sepia officinalis* (Linnaeus, 1758), a marine invertebrate belonging to the class Cephalopoda, can secrete a dark ink, which is a secondary bioactive metabolite consisting of melanin granules suspended in a sticky colorless medium for their self-defense, escaping from predators and avoiding dangers **(Sheela *et al.*, 2014)**. The cuttlefish ink is manufactured by the mature cells in the ink gland and released into the ink sac at maturity **(Derby, 2014)**. *Sepia* ink can potentially contain beneficial bioactive chemicals, such as carbohydrates, proteins, lipids, melanin, glycosaminoglycan, dopamine, taurine, and epinephrine **(Zhong *et al.*, 2009; Derby, 2014)**. The ink has already been reported to exhibit various therapeutic activities **(Rajaganapathi *et al.*, 2000; Russo *et al.*, 2003; Fahmy & Soliman, 2013; Fahmy *et al.*, 2014; Soliman *et al.*, 2015; Ismail & Riad, 2018; Salem *et al.*, 2020)**. Despite that, it still has a poor market value in Egypt and is usually disposed of as waste that can lead to environmental pollution if improperly managed **(Riyad *et al.*, 2020)**.

The nidamental glands (NGs) are part of the female cephalopods' reproductive system. They are large, white, paired glandular structures in the mantle cavity intimately connected to the ink sac's ventral surface. NGs participate in forming egg sheaths and in the protection of eggs and embryos in cephalopods. Oocytes are encased in two layers of egg capsule proteins; the oviduct gland produces the inner layer, while the NGs secrete the outer layer, mainly consisting of mucopolysaccharides and glycoproteins **(Zatylny-Gaudin & Henry, 2018)**. The mucosubstance secretion from NGs is critical in defending against environmental threats during embryonic development **(Boletzky, 2003; Lee *et al.*, 2016)**. Although NGs of cuttlefish are eaten as food in various parts of the world, there are limited studies on their biological activity. **Ismail and Riad (2018)** confirmed that NG extract exhibits strong antimicrobial properties and might be useful for additional therapeutic uses. Hence, the goal of this work was to make a comparison between the cytotoxic properties of the ink and NG extracts prepared from the Egyptian cuttlefish *S. officinalis* on the cancer cell lines.

MATERIALS AND METHODS

1. Sample collection and identification

Fresh mature females of cuttlefish *S. officinalis* (n= 8) were obtained from the fishermen of Alexandria Mediterranean waters, Egypt. They were quickly brought into the laboratory, washed with sterile water to get rid of any impurities, and identified with the help of the taxonomical key given by **Riad (2020)**.

2. Preparation of ink extract and nidamental gland extract of *Sepia officinalis*

Mature females of *S. officinalis* were posteroventrally dissected. The ink sacs and NGs were separated aseptically, carefully removed, and frozen at –20°C till use (Fig. 1). The preparation of the cuttlefish ink extract (IE) was conducted following the method described by **Sheela *et al.* (2014)** and **Jeyasanta and Patterson (2020)**. The ink duct was cut with sterilized scissors, and the ink sac content was gently forced out. The ink was

dried using a hot air oven and then pulverized. The dried ink powder was mixed sufficiently with methanol in a 1:3 (*w/v*) ratio using a sterile glass rod for 30min. This mixture was incubated for 72h at 4°C and shaken for 8- 10h at an ambient temperature. The methanol extract was centrifuged for 15min at 10000- 16770×g and 5°C. For the preparation of nidamental gland extract (NGE), the NGs were cut into small parts, weighed, and kept with methanol (1:3 *w/v*) for 72h, and then homogenized. The methanolic extract of NGs was centrifuged for 20min at 16770×g and 4°C **(Ismail & Riad, 2018)**. The resulting supernatant of both extracts was collected, concentrated using a rotary vacuum evaporator, and then lyophilized. The extracts' residue was frozen at –80°C in brown bottles until use. The percent yield of IE and NGE was 6.21 and 14.68%, respectively.

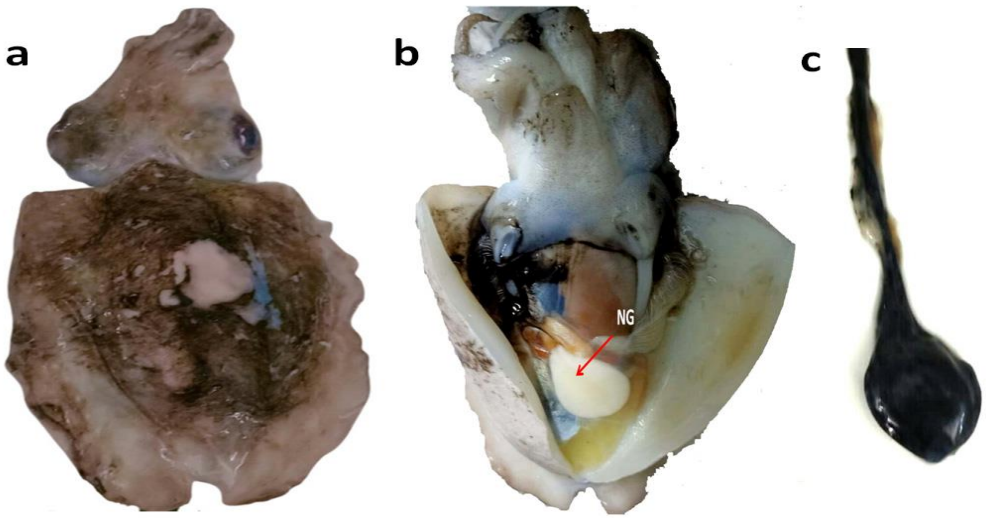


Fig. 1. Cuttlefish *Sepia officinalis*. **a:** External view, **b:** Dissected view showing nidamental gland (NG), and **c:** Ink sac

3. Characterization of ink extract and nidamental gland extract of *Sepia officinalis*

3.1. Chemical compounds identification using gas chromatography technique

The extract samples, IE and NGE, were examined using the gas chromatography-mass spectrometry (GC-MS) technique, followed in the study of **Ismail *et al.* (2019)** with minor modifications using GC-MS spectrometer (Perkin Elmer model: clarus 580/560 S) equipped with an Elite-5MS column (30m × 0.25mm ID, 0.25µm df). The instrument's temperature was first adjusted to 80°C for 8min before increasing it to 260°C. One microliter of samples was injected into GC-MS after keeping the temperature at 280°C for analysis. The mass spectra were set at 70eV ionization voltages and over the scan range of 40- 550Da in full scan mode. Helium was utilized as a carrier gas and pressurized to 2223psi with a constant flow rate of 122ml/ min. The chemical components were selected by matching their retention times and mass spectra with those in a mass spectral library database.

3.2. Proximate analysis

The protein estimation was calculated using the method described by **Bradford (1976)**. The quantification of carbohydrates and lipids was performed by simple colorimetric methods described in the studies of **Mishra *et al.* (2014)** and **Aziz (2015)**, respectively.

3.3. Measuring the total phenolic concentration

The total phenolic concentration in extract samples was estimated colorimetrically using the Folin-Ciocalteu reagent applying the method of described by **Singleton *et al.* (1999)** and **Wolfe *et al.* (2003)**. The absorbance of the outcome blue-colored complex was determined at 760nm by using a UV-spectrophotometer. Upon using the calibration curve, the phenolic concentration was calculated as mg gallic acid equivalent (GA eq.) per gram of dried extract.

3.4. Measuring the total flavonoid concentration

The aluminum chloride technique of **Kiranmai *et al*. (2011)** was used to assess the total flavonoid concentration. In order to measure the absorbance of the resulting yellow color, a UV-spectrophotometer was used at 420nm. The flavonoid concentration of the extract was quantified in milligrams of quercetin equivalent (quercetin eq.) per gram of dried extract by the use of the calibration curve.

3.5. Quantification of total alkaloid concentration

The extract's total alkaloid concentration was quantified using the method developed by **Li *et al.* (2015)**. The measurement of absorbance was performed with a UV–vis spectrophotometer at a specific wavelength of 418nm. The alkaloid concentration of the extract was determined by utilizing the calibration curve and reported as milligrams of berberine equivalent (berberine eq.) per gram of dried extract.

3.6. Evaluation of total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of extracts was estimated using the colorimetric method of **Prieto *et al.* (1999)**. This method relies on the reduction of Mo(VI) to Mo(V) by the extract/standard and the production of a green-colored compound of phosphate/Mo(V) at acidic conditions. The absorbances were recorded at 695nm using a spectrophotometer. Increasing the absorbance of the reaction mixture indicates a high level of TAC. The TAC was expressed as mM ascorbic acid equivalent (AA eq.) per gram of dried extract using the calibration curve.

4. *In vitro* cytotoxic activity of ink extract and nidamental gland extract of *Sepia officinalis* using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

Screening the cytotoxic activity of IE and NGE was conducted on four human cancer cell lines, namely lung carcinoma (A-549, ATCC: CCL-185, RRID: CVCL_0023), epidermoid carcinoma (A-431, ATCC: CRL-1555, RRID: CVCL_0037), colorectal carcinoma (HCT-116, ATCC: CCL-247, RRID: CVCL_0291), and prostatic adenocarcinoma (PC-3, ATCC: CRL-1435, RRID: CVCL_0035). All cell lines were acquired from VACSERA, Giza, Egypt. According to **Kalaba *et al.* (2022)**, all cancer cell lines at a concentration of 1×10^5 cells/ ml were maintained in 96-well tissue culture microtiter plates (100 µl/ well) and added to Roswell Park Memorial Institute (RPMI-1640) growth medium (Gibco BRL) containing 10% fetal bovine serum, 2mM L-glutamine, 1mM sodium pyruvate, 100 µg/ ml streptomycin, and 100IU/ ml penicillin for 24h at 5% CO_2 and 37°C to complete the formation of the monolayer sheet of cells. Then, the cells were supplied with 100µl of media and treated with 100µl of extract sample at levels of 1000, 500, 250, 125, 62.5, and 31.25µg/ ml (three replicates). Plates were incubated for 24h at 5% CO_2 and 37°C. Following this step, the media were removed, and plates were rinsed with phosphate-buffered saline (PBS) (pH 7.4, 0.137M), and the cells were incubated with 50µl/ well of 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (5 mg/ml PBS) solution for 4h to allow the formation of formazan after the reduction of MTT by the mitochondrial dehydrogenase activity in viable cells. Subsequently, 50µl of dimethyl sulfoxide (DMSO) solution was added to each well and left for 30min in a 5% CO_2 incubator at 37°C to dissolve the purple needle crystals of formazan. The optical density of each well was recorded at 560nm by an ELISA microplate reader. The cell viability percentage was calculated by the subsequent equation:

$$\text{Cell viability \%} = (\text{Absorbance of treated cells} / \text{Absorbance of negative control}) \times 100$$

$$\text{Cytotoxicity \%} = 100 - \text{Cell viability \%}$$

The median inhibitory concentration (IC_{50}) value of the extracted sample, the concentration needed to achieve a 50% inhibition of cell growth, was calculated by an online AAT Bioqest- IC_{50} calculator tool. Moreover, after 24h of treatment, the antiproliferative effects of various concentrations of IE and NGE of *S. officinalis* on the cancer cell lines were examined and photographed by an inverted phase contrast microscope (Helmut Hund GmbH, Wetzlar, Germany).

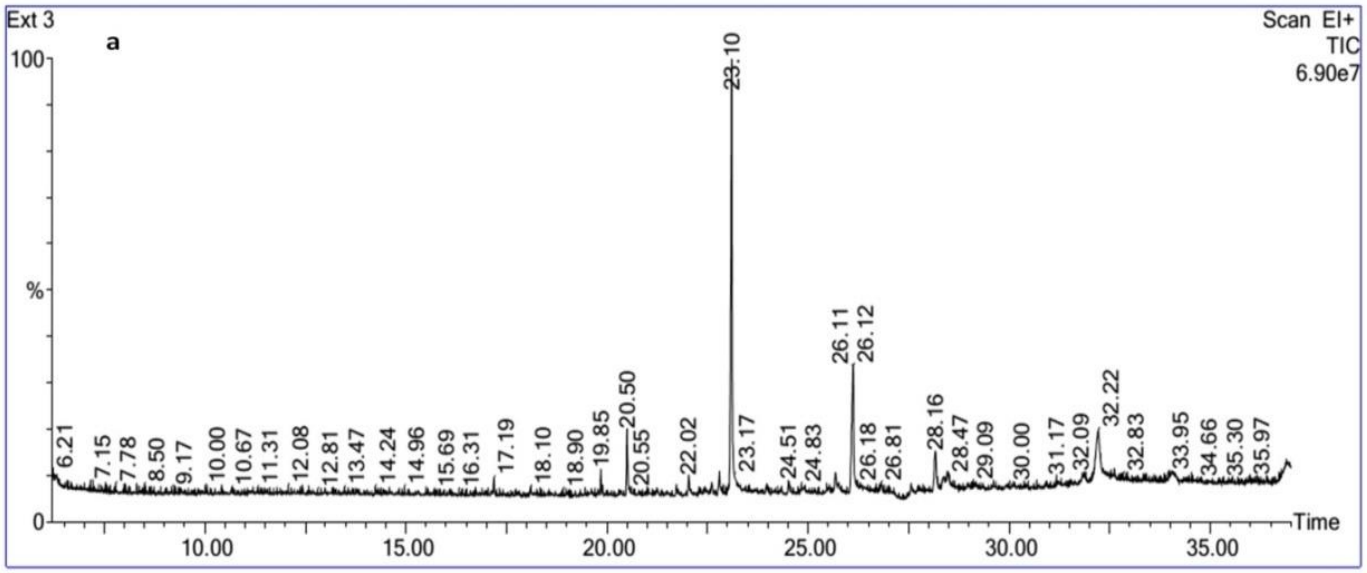
5. Statistical analysis of data

The Student's t-test was employed to analyze the results. All results were expressed as means ± standard deviations (SD) and performed in triplicate. Version 20.0 of IBM-SPSS software was used to conduct the statistical analyses. Distinctions were deemed to be significant when *P*- value was at 0.05 or less **(Kirkpatrick & Feeney, 2012)**.

RESULTS

1. Bioactive compounds identification of ink extract and nidamental gland extract of *Sepia officinalis* through using GC-MS technique

The resulting data from the GC-MS analysis of *S. officinalis* extracts, IE and NGE, present different compounds with different retention times, as shown in Fig. (2). GC-MS profile of NGE presents high peak height and area for saturated fatty acids (n-hexadecanoic acid, myristic acid, octadecanoic acid, dodecanoic acid, pentadecanoic acid, and heptadecanoic acid), hexadecanoic acid, methyl ester, unsaturated fatty acid (arachidonic acid), 1-hexadecanol, and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester in comparison with IE. Among all compounds extracted from both extracts, the n-hexadecanoic saturated fatty acid is the most abundant compound, with a peak area of 2941074.8 for IE and 28132282 for NGE (Tables 1, 2).



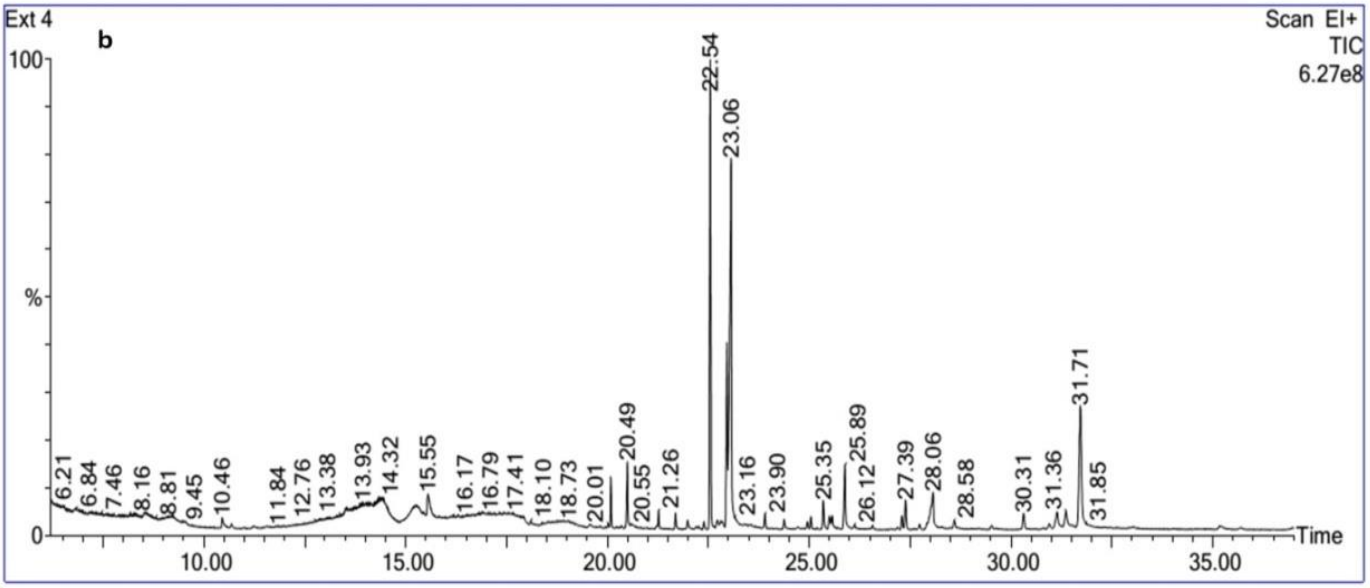


Fig. 2. GC-MS chromatograms of *Sepia officinalis* extracts with the identified compounds' retention times (RTs). **a:** Ink extract (IE) and **b:** Nidamental gland extract (NGE)

Table 1. Chemical constituents detected in ink extract (IE) of *Sepia officinalis* using GC-MS technique

Peak number	Compound name	Compound nature	Molecular formula	MW (g/mol)	RT (min)	Height	Area (IU)	Area %
1	Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	Monoterpene	$C_{10}H_{16}$	136.23	6.32	1164193	45869.5	0.24
2	Undecane, 2-methyl-	Alkane	$C_{12}H_{26}$	170.33	17.18	2638992	72138.6	0.38
3	Dodecanoic acid	Saturated fatty acid	$C_{12}H_{24}O_2$	200.32	18.10	1529166	62124.6	0.32
4	Heptacosane	Alkane	$C_{27}H_{56}$	380.70	19.84	4173200	108273.6	0.57
5	dl-Alanine	Non-essential alpha-amino acid	$C_3H_7NO_2$	89.09	19.89	1848520	38888.6	0.20
6	Myristic acid	Saturated fatty acid	$C_{14}H_{28}O_2$	228.37	20.49	10046847	360190.6	1.89
7	Pentadecanoic acid	Saturated fatty acid	$C_{15}H_{30}O_2$	242.40	21.73	1835321	66979.4	0.35
8	1-Hexadecanol	Fatty alcohol	$C_{16}H_{34}O$	242.44	22.03	3199968	140682.4	0.74
9	2-Bromotetradecane	Bromoalkane	$C_{14}H_{29}Br$	277.28	22.43	1096764	37066	0.19
10	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.45	22.60	1905919	80238	0.42
11	Linoleic acid	Polyunsaturated fatty acid	$C_{18}H_{32}O_2$	280.40	22.79	3623006	153229.4	0.80
12	n-Hexadecanoic acid	Saturated fatty acid	$C_{16}H_{32}O_2$	256.42	23.10	65041660	2941074.8	15.49
13	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	Amine alcohol	$C_{11}H_{16}O_3$	196.24	23.25	1779452	100321.6	0.52
14	Actinobolin	Polyketide	$C_{13}H_{20}N_2O_6$	300.31	23.31	1501024	50247.3	0.26
15	Oxirane, tetradecyl-	Alkyl oxirane	$C_{16}H_{32}O$	240.42	23.97	1611472	65938.4	0.34
16	Heptadecanoic acid	Saturated fatty acid	$C_{17}H_{34}O_2$	270.50	24.50	2179224	46222.3	0.24

17	3-Hydroxy-N-methylphenethylamine	Phenethylamine	$C_9H_{13}NO$	151.21	24.53	1899285	44209.7	0.23
18	Cyclopentolate -H_2O	Alkaloid	$C_{17}H_{25}NO_3$	291.39	24.82	1555161	54346.9	0.28
19	Behenic alcohol	Alcohol	$C_{22}H_{46}O$	326.60	24.90	1159951	48891	0.25
20	Undecanoic acid, 11-bromo-, methyl ester	Fatty acid methyl ester	$C_{12}H_{23}BrO_2$	279.21	25.53	1325175	37620.6	0.19
21	1-Eicosanol	Fatty alcohol	$C_{20}H_{42}O$	298.50	25.68	3001702	155888.7	0.82
22	Pterin-6-carboxylic acid	Pterin derivative	$C_7H_5N_5O_3$	207.15	25.76	1523140	81535.6	0.42
23	Octadecanoic acid	Saturated fatty acid	$C_{18}H_{36}O_2$	284.47	26.12	19332338	1182632.1	6.22
24	2,3-Dihydro-7-methyl-4-phenyl-1H-1,5-benzodiazepin-2-one	Benzodiazepine	$C_{16}H_{14}N_2O$	250.29	26.22	2163124	83663.7	0.44
25	dl-Alanyl-dl-valine	Dipeptide	$C_8H_{16}N_2O_3$	188.22	26.29	1776049	83009.9	0.43
26	Tetraacetyl-d-xylonic nitrile	Otro	$C_{14}H_{17}NO_9$	343.29	26.38	1257125	96949.3	0.51
27	1,2-Propanediamine	Aliphatic amine	$C_3H_{10}N_2$	74.12	26.48	1602796	48246.5	0.25
28	Octadecane, 1-(ethenyloxy)-	Alkane	$C_{20}H_{40}O$	296.53	26.81	2153214	90302.3	0.47
29	Quinomethionate	Quinoxaline	$C_8H_6N_2OS_2$	210.27	26.96	1616179	37594.4	0.19
30	Benzeneethanamine, 2,5-difluoro-á,3,4-trihydroxy-N-methyl-	Amine	$C_9H_{11}F_2NO_3$	219.18	27.00	1783791	43061	0.22
31	Cyclobutanol	Alcohol	C_4H_8O	72.11	27.04	1557296	64353.9	0.33
32	Dinoseb	Dinitrophenol	$C_{10}H_{12}N_2O_5$	240.21	27.55	2143604	118086	0.62
33	3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole	Triazole	$C_7H_9N_7O_2$	223.19	27.75	1505188	43079.1	0.22
34	Octanoic acid, 2-dimethylaminoethyl ester	Saturated fatty acid ester	$C_{12}H_{25}NO_2$	215.33	28.16	5886350	363081.3	1.91
35	15-Hydroxypentadecanoic acid	Omega-hydroxy-long-chain fatty acid	$C_{15}H_{30}O_3$	258.40	28.36	2013160	133338.5	0.70
36	Arachidonic acid	Unsaturated fatty acid	$C_{20}H_{32}O_2$	304.50	28.45	2828405	171006.2	0.90
37	Sinapic acid	Phenolic acid	$C_{11}H_{12}O_5$	224.21	29.60	1409394	42389.2	0.22

38	Pentanal	Saturated fatty aldehyde	$C_5H_{10}O$	86.13	29.91	911475	46393.8	0.24
39	Silane, methyl-	Organosilicon compound	CH_6Si	46.14	30.11	1375556	42735.1	0.22
40	Z-3-Octadecen-1-ol acetate	Fatty acid ester	$C_{20}H_{38}O_2$	310.50	30.22	911672	39105.1	0.20
41	Cyclopentolate	Carboxylic ester	$C_{17}H_{25}NO_3$	291.40	31.81	1790427	49076.4	0.25
42	Cyclotrisiloxane, hexamethyl-	Organosilicon compound	$C_6H_{18}O_3Si_3$	222.46	31.85	1978660	67917.2	0.35
43	1H-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-7-[2-[(1-methyl-2-phenylethyl)amino]ethyl]-	Xanthine derivative	$C_{18}H_{23}N_5O_2$	341.40	31.90	1952519	46053.2	0.24
44	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Amino compound	$C_{19}H_{38}O_4$	330.50	32.21	8165407	1136775.6	5.98
45	Benzoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-	Phenolic compound	$C_{17}H_{26}O_3$	278.40	32.40	1844137	84965.6	0.44
46	1-Monolinoleoylglycerol trimethylsilyl ether	Steroid	$C_{27}H_{54}O_4Si_2$	498.88	32.44	1682612	102151.1	0.53
47	3-Benzyloxy-1,2-dihydro-2-oxoquinoxaline	Quinoxaline derivative	$C_{15}H_{12}N_2O_2$	252.27	32.58	1401166	37076.7	0.19
48	Creatinine	Lactam	$C_4H_7N_3O$	113.11	32.60	2175382	90038.1	0.47
49	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	Alcohol	$C_{20}H_{40}O_2$	312.50	33.97	1744784	37867.6	0.19
50	Chlorothen	Aminopyridine (dialkylarylamine and tertiary amino compound)	$C_{14}H_{18}ClN_3S$	295.80	34.02	1946767	247145.9	1.30

MW: Molecular weight and **RT:** Retention time.

Table 2. Chemical constituents detected in nidamental gland extract (NGE) of *Sepia officinalis* using GC-MS technique

Peak number	Compound name	Compound nature	Molecular formula	MW (g/mol)	RT (min)	Height	Area (IU)	Area %
1	L-2-Aminobutyric acid	α-amino acid	$C_4H_9NO_2$	103.12	9.23	8411477	566276.9	0.23
2	1,2-Dimethyl-3-ethyldiaziridine	Diaziridine	$C_5H_{12}N_2$	100.16	10.45	14022866	794686.4	0.33
3	1,2-Propanediamine, N,N'-dimethyl-, (S)-	Primary aliphatic amine	$C_5H_{14}N_2$	102.18	12.48	7267193	1136147.2	0.47
4	3-Buten-1-amine, N,N-dimethyl-	Unsaturated primary aliphatic amine (Butenylamine)	$C_6H_{13}N$	99.17	12.65	8321576	1054123.6	0.44
5	1,2-Ethanediamine, N,N'-diethyl-	Primary aliphatic amine (Ethylenediamine derivative)	$C_6H_{16}N_2$	116.20	12.77	10972743	541381.1	0.22
6	N-Allyl-N,N-dimethylamine	Tertiary amine	$C_5H_{11}N$	85.14	12.90	13484808	1208485.2	0.50
7	Pseudoephedrine, (+)-	Alkaloid	$C_{10}H_{15}NO$	165	13.53	28435722	10521970	4.41
8	3-Dimethylamino-2,2-dimethylpropionaldehyde	Aldehyde and hydantoin	$C_7H_{15}NO$	129.20	13.93	35780436	17386356	7.29
9	N,N-Dimethylglycine	Amino acid derivative	$C_4H_9NO_2$	103.12	14.42	41646112	17176942	7.21
10	D-Pyroglutamic acid	Pyrrolidinemonocarboxylic acid (5-oxoproline)	$C_5H_7NO_3$	129.11	15.27	31462130	12817650	5.38
11	L-Proline, 5-oxo-, methyl ester	Amino acid methyl ester	$C_6H_9NO_3$	143.14	15.55	45585540	10351768	4.34
12	1-Propanol, 2-amino-	Amino alcohol	C_3H_9NO	75.11	16.30	18631584	5929704.5	2.48
13	Pyridine, 3-phenyl-	Phenylpyridine	$C_{11}H_9N$	155.20	16.90	22117442	11240693	4.71
14	2-Hexanamine, 4-methyl-	Primary amine	$C_7H_{17}N$	115.21	17.08	21813882	2586381.2	1.08
15	trans-2,3-Epoxyoctane	Epoxide	$C_8H_{16}O$	128.21	17.18	20693028	1521030.2	0.63
16	2-Butanamine, 3,3-dimethyl-	Primary amine	$C_6H_{15}N$	101.19	17.32	21606776	11894498	4.99
17	2,4'-Bipyridine	Pyridine	$C_{10}H_8N_2$	156.18	17.91	16774864	1729406	0.72

18	Dodecanoic acid	Saturated fatty acid	$C_{12}H_{24}O_2$	200.32	18.10	10187175	578811.6	0.24
19	[1,1'-Biphenyl]-3-amine	Aromatic amine	$C_{12}H_{11}N$	169.22	18.53	9483532	1262026.1	0.53
20	5-Pyrimidinecarboxaldehyde, 1,2,3,4-tetrahydro-2, 4-dioxo-	Pyrimidine derivative	$C_5H_4N_2O_3$	140.10	18.62	9302413	948705	0.39
21	l-Glutamic acid, monobenzyl ester	Amino acid ester derivative	$C_{12}H_{15}NO_4$	237.30	18.81	10616625	1203174.4	0.50
22	Tridecanedial	Aldehyde	$C_{13}H_{24}O_2$	212.33	18.90	10466953	797033.9	0.33
23	Benzenesulfonamide, 2-methyl-	Sulfonamide	$C_7H_9NO_2S$	171.21	19.01	10188645	2183259.2	0.91
24	1,1-Di(isopropyl)-1-silacyclobutane	Organosilicon compound	$C_9H_{20}Si$	156.34	19.19	6806219	535331.4	0.22
25	Benzenesulfonamide, 4-methyl-	Sulfonamide	$C_7H_9NO_2S$	171.21	19.56	5404372	622456.5	0.26
26	Myristic acid methyl ester	Fatty acid methyl ester	$C_{15}H_{30}O_2$	242.40	20.08	67220968	1679586.4	0.70
27	Myristic acid	Saturated fatty acid	$C_{14}H_{28}O_2$	228.37	20.49	88002208	2924462.5	1.22
28	Pentadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{16}H_{32}O_2$	256.42	21.26	24291418	691653.5	0.29
29	Pentadecanoic acid	Saturated fatty acid	$C_{15}H_{30}O_2$	242.40	21.68	21903296	815129	0.34
30	1-Hexadecanol	Fatty alcohol	$C_{16}H_{34}O$	242.44	21.98	12573477	591436.2	0.24
31	Hexadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.45	22.54	619746240	18959580	7.96
32	E-9-Tetradecenoic acid	Monounsaturated fatty acid	$C_{14}H_{26}O_2$	226.35	22.72	10529980	572828.8	0.24
33	Dibutyl phthalate	Phthalate ester	$C_{16}H_{22}O_4$	278.34	22.95	242129120	7389837.5	3.10
34	n-Hexadecanoic acid	Saturated fatty acid	$C_{16}H_{32}O_2$	256.42	23.06	485900064	28132282	11.81
35	Heptadecanoic acid, methyl ester	Saturated fatty acid methyl ester	$C_{18}H_{36}O_2$	284.50	23.90	19204178	710313	0.29
36	Heptadecanoic acid	Saturated fatty acid	$C_{17}H_{34}O_2$	270.50	24.37	13627907	509038.3	0.21
37	9-Octadecenoic acid (Z)-,	Unsaturated fatty acid	$C_{19}H_{36}O_2$	296.48	25.04	16602241	559483.6	0.23

	methyl ester	methyl ester						
38	Octadecanoic acid, methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.50	25.34	37647724	1444762.4	0.60
39	cis-Vaccenic acid	Omega-7 fatty acid	$C_{18}H_{34}O_2$	282.50	25.49	17272568	778289.6	0.32
40	Oleic acid	Unsaturated fatty acid	$C_{18}H_{34}O_2$	282.50	25.56	18312316	799590.2	0.33
41	Octadecanoic acid	Saturated fatty acid	$C_{18}H_{36}O_2$	284.47	25.88	84232872	3612593.8	1.51
42	Arachidonic acid	Unsaturated fatty acid	$C_{20}H_{32}O_2$	304.50	27.29	17582418	629119.6	0.26
43	cis-5,8,11,14,17-Eicosapentaenoic acid, methyl ester	Fatty acid methyl ester	$C_{21}H_{32}O_2$	316.50	27.38	38035664	1611347.9	0.67
44	Icosapent	Synthetic ethyl ester derivative of the omega-3 fatty acid eicosapentaenoic acid (EPA)	$C_{20}H_{30}O_2$	302.45	28.06	48851064	5039463	2.11
45	3-Trifluoroacetoxypentadecane	Fluro compound	$C_{17}H_{31}F_3O_2$	324.40	28.58	10209442	588580.4	0.24
46	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	Fatty acid methyl ester	$C_{23}H_{34}O_2$	342.50	30.30	21974012	1204908	0.50
47	Doconexent	Omega 3 fatty acid	$C_{22}H_{32}O_2$	328.48	31.13	20906494	1585622.6	0.66
48	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Palmitic acid glycerol ester	$C_{19}H_{38}O_4$	330.50	31.35	20645626	1523503.6	0.64
49	1,2-Benzenedicarboxylic acid, diisooctyl ester	Dialkyl phthalate	$C_{24}H_{38}O_4$	390.55	31.71	160682944	11743462	4.93
50	Pentadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Saturated fatty acid ester	$C_{18}H_{36}O_4$	316.50	35.15	4876874	591147	0.24

MW: Molecular weight and **RT:** Retention time.

2. Biochemical characterization of the ink extract and nidamental gland extract of *Sepia officinalis*

As shown in Table (3), all biochemicals detected in NGE are significantly higher than those detected in IE ($P< 0.05$), except for alkaloids ($P> 0.05$). The proximate compositions in both extracts contained a high amount of carbohydrates, followed by protein. However, the lowest contents were observed for lipids in both extracts. Total alkaloid concentration (5.60mg berberine eq./ g dried extract) is the highest phytochemicals in IE, while the total phenolic concentration (9.70mg GA eq./ g dried extract) is the highest in NGE over the other phytochemicals. Moreover, the TAC in NGE appears significantly higher ($P< 0.001$) than that recorded in IE.

Table 3. Biochemicals detected in ink extract (IE) and nidamental gland extract (NGE) of *Sepia officinalis*

Biochemical composition	Methanolic extract		*t*-value	Sig. (2-tailed)
	Ink extract (IE)	Nidamental gland extract (NGE)		
Proximate content				
Protein (mg/g dried extract)	3.50 ± 0.12	4.80 ± 0.15	-11.72-	0.00***
Lipid (mg/g dried extract)	2.30 ± 0.17	3.60 ± 0.23	-7.87-	0.001***
Carbohydrates (mg/g dried extract)	28.60 ± 1.60	57.30 ± 2.40	-17.23-	0.00***
Phytochemical				
Phenolics (mg GA eq./g dried extract)	5.40 ± 0.61	9.70 ± 0.54	-9.14-	0.001***
Flavonoids (mg quercetin eq./g dried extract)	3.70 ± 0.14	4.30 ± 0.33	-2.89-	0.04*
Alkaloids (mg berberine eq./g dried extract)	5.60 ± 0.25	5.90 ± 0.28	-1.38-	0.23
Total antioxidant				
Total antioxidant capacity (TAC) (mM AA eq./g dried extract)	0.49 ± 0.03	1.27 ± 0.08	-15.81-	0.00***

Values are presented as means of 3 replicates ± SD, * Statistically significant at a *P*-value of 0.05 or less, ** Statistically significant at a *P*-value of 0.01 or less, and *** Statistically significant at a *P*-value of 0.001 or less (Student's t-test).

3. Cytotoxic activity of the ink extract and nidamental gland extract of *Sepia officinalis*

The results in Figs. (3, 4) demonstrate that the cytotoxic activities of IE and NGE have dose-dependent viability and cytotoxicity on the four tested cancer cell lines at concentrations in a range of 31.25 - 1000µg/ ml. The results in Table (4) show that the wells treated with the IE presented dramatic changes in cell viability ranging from 99.90± 0.55, 99.77± 1.59, 100± 0.83, and 100± 1.60% (31.25µg/ ml) to 16.87± 2.39, 17.19 ± 2.18, 9.58± 1.29, and 12.21± 1.40% (1000µg/ ml) for A-549, A-431, HCT-116, and PC-3 cell lines, respectively. While the NGE showed percentages of cell viability varying from 99.72± 0.60, 99± 2.03, 99.74± 0.52, and 99.83± 1.77% (31.25µg/ ml) to 5.51± 1.59, 4.92±

1.66, 4.11± 0.75, and 3.52± 0.33% (1000µg/ ml) for A-549, A-431, HCT-116, and PC-3 cell lines, respectively.

NGE displayed potent cytotoxic effects against the four cell lines, A-549, A-431, HCT-116, and PC-3 compared to IE at most tested concentrations. The percentages of cell viability for all cell lines treated with NGE significantly decreased ($P< 0.01$), as compared with that recorded for IE, especially at concentrations of 1000, 500, and 250µg/ ml. IE extract exhibited a potent growth inhibition activity against PC-3 cells, followed by HCT-116, A-431, and A-549 with IC_{50} of 372.21, 480.06, 511.03, and 517.52µg/ ml, respectively. At the same time, NGE presented a potent growth inhibition activity against HCT-116 cells, followed by PC-3, A-431, and A-549 with IC_{50} of 220.04, 242.22, 262.83, and 427.45µg/ ml, respectively. The *in vitro* antiproliferative morphological impacts of different concentrations of IE and NGE of *S. officinalis* against these cell lines are presented in Fig. (5).

Table 4. The cytotoxic effects of *Sepia officinalis* ink and nidamental gland methanolic extracts following a 24-hour incubation period

Cell line	Concentration (µg/ml)	Ink extract (IE)			Nidamental gland extract (NGE)			*t*-value	Sig. (2-tailed)
		Viability %	Regression equation	IC_{50} (µg/ml)	Viability %	Regression equation	IC_{50} (µg/ml)		
Human lung carcinoma (A-549)	1000	16.87 ± 2.39	$Y= 0 + (101.6428 – 0)/[1+(X/517.5235)^{3.1295}]$	517.52	5.51 ± 1.59	$Y= 0 + (99.5573 – 0)/[1+(X/427.4579)^{3.4525}]$	427.45	6.83	0.002**
	500	49.15 ± 3.14			36.41 ± 0.96			6.70	0.003**
	250	98.99 ± 2.60			86.29 ± 1.47			7.33	0.002**
	125	99.31 ± 0.27			97.97 ± 1.10			2.02	0.11
	62.5	99.90 ± 1.04			99.26 ± 1.96			0.50	0.64
	31.25	99.90 ± 0.55			99.72 ± 0.60			0.38	0.71
Human epidermoid carcinoma (A-431)	1000	17.19 ± 2.18	$Y= 0 + (101.6652 – 0)/[1+(X/511.0379)^{3.1}]$	511.03	4.92 ± 1.66	$Y= 0 + (101.2986 – 0)/[1+(X/262.8308)^{2.9636}]$	262.83	7.73	0.002**
	500	47.82 ± 2.57			15.92 ± 2.17			16.38	0.000***
	250	98.56 ± 5.22			51.08 ± 3.19			13.41	0.000***
	125	99.67 ± 1.79			96.52 ± 3.23			1.47	0.21
	62.5	99.67 ± 5.06			98.56 ± 1.63			0.36	0.73
	31.25	99.77 ± 1.59			99.00 ± 2.03			0.51	0.63

Human colorectal carcinoma (HCT-116)	1000	9.58 ± 1.29	$Y= 0 + (100.8702 – 0)/[1+(X/480.0669)^{4.2465}]$	480.06	4.11 ± 0.75	$Y= 0 + (101.185 – 0)/[1+(X/220.0471)^{3.0974}]$	220.04	6.30	0.003**
	500	44.33 ± 3.08			10.19 ± 0.67			18.70	0.000***
	250	99.00 ± 2.05			38.76 ± 2.67			30.90	0.000***
	125	99.05 ± 2.05			88.25 ± 3.07			5.05	0.007**
	62.5	99.95 ± 0.67			99.20 ± 2.56			0.49	0.64
	31.25	100.00 ± 0.83			99.74 ± 0.52			0.43	0.68
Human prostate adenocarcinoma (PC-3)	1000	12.21 ± 1.40	$Y= 0 + (102.7231 – 0)/[1+(X/372.2196)^{2.1034}]$	372.21	3.52 ± 0.33	$Y= 0 + (102.0641 – 0)/[1+(X/242.2203)^{3.6787}]$	242.22	10.38	0.006**
	500	36.81 ± 5.80			11.99 ± 1.91			7.03	0.002**
	250	68.45 ± 1.34			45.21 ± 2.93			12.48	0.000***
	125	98.43 ± 2.00			98.99 ± 1.37			-0.39-	0.71
	62.5	99.72 ± 1.19			99.77 ± 1.40			--0.05-	0.96
	31.25	100.00 ± 1.60			99.83 ± 1.77			0.12	0.90

IC_{50}: Median inhibitory concentration. Values are presented as means of 3 replicates ± SD, * Statistically significant at a *p*-value of 0.05 or less, ** Statistically significant at a *p*-value of 0.01 or less, and *** Statistically significant at a *p*-value of 0.001 or less (Student's t-test).

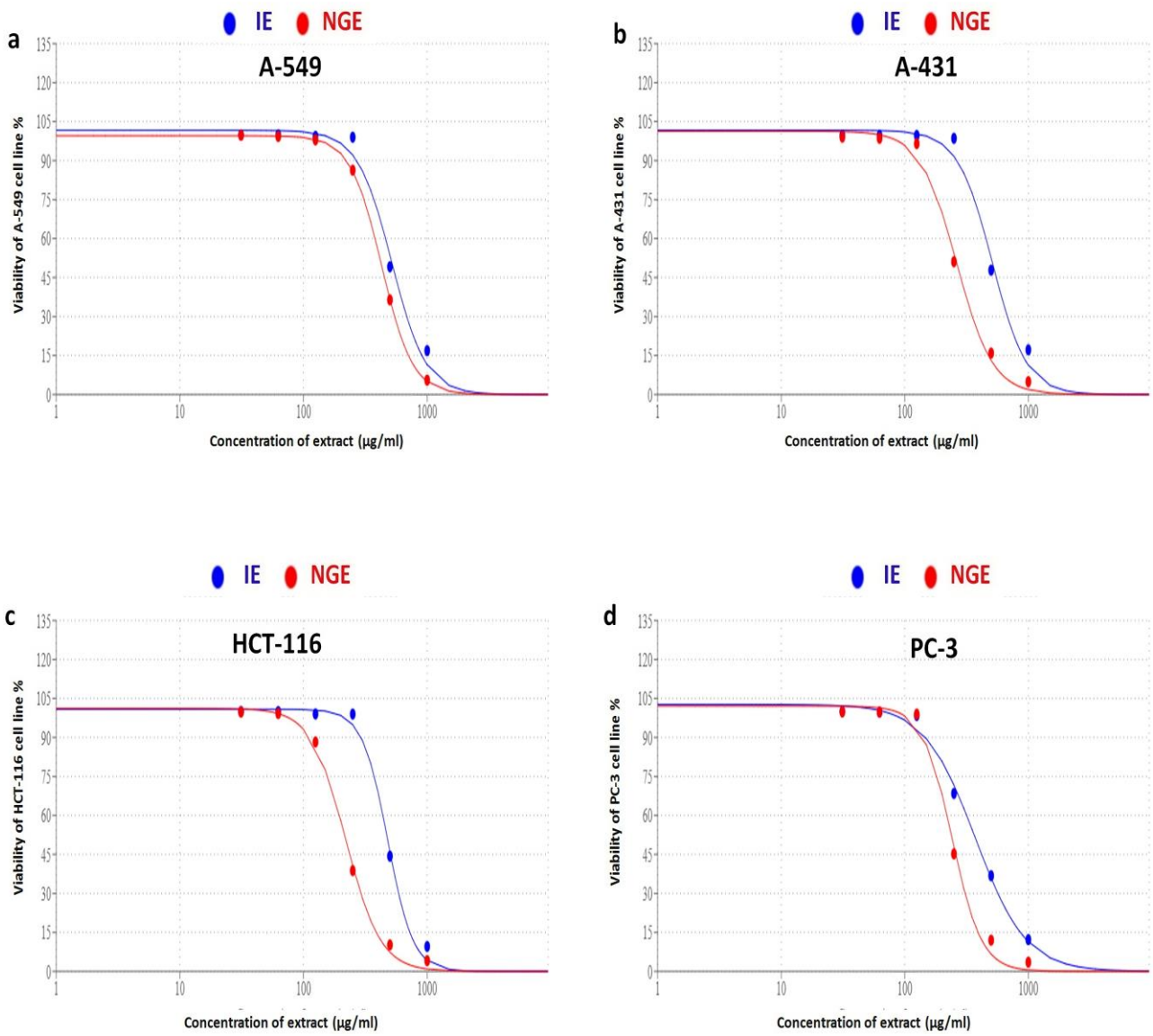


Fig. 3. Percentage of cell viability (%) of different human cell lines after 24 hour-incubation period with the methanolic ink extract (IE) and nidamental gland extract (NGE) of *Sepia officinalis*. **a:** Lung carcinoma (A-549), **b:** Epidermoid carcinoma (A-431), **c:** Colorectal carcinoma (HCT-116), and **d:** Prostate adenocarcinoma (PC-3)

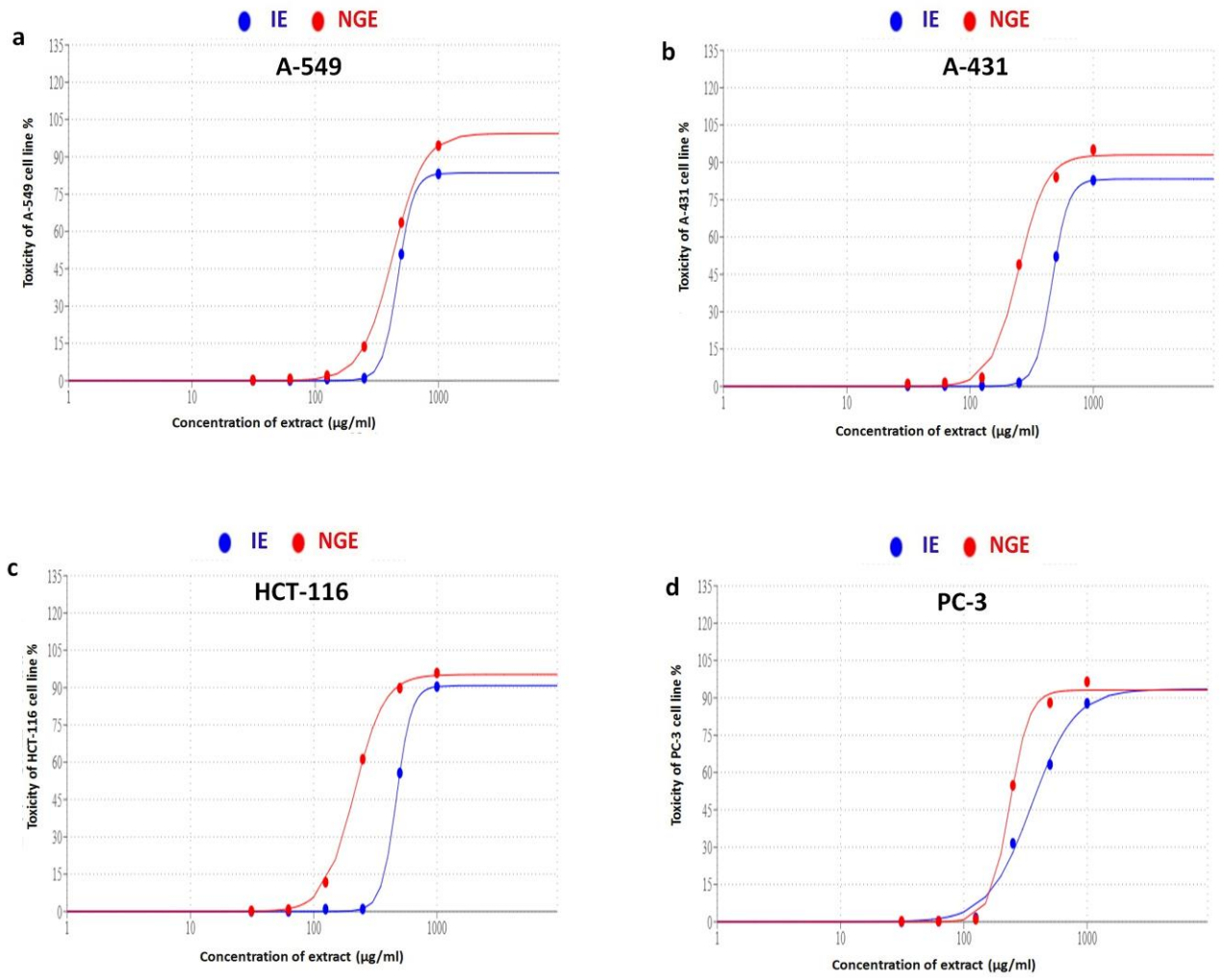


Fig. 4. Percentage of cytotoxicity (%) of different human cell lines after 24 hour-incubation period with the methanolic ink extract (IE) and nidamental gland extract (NGE) of *Sepia officinalis*. **a:** Lung carcinoma (A-549), **b:** Epidermoid carcinoma (A-431), **c:** Colorectal carcinoma (HCT-116), and **d:** Prostate adenocarcinoma (PC-3)

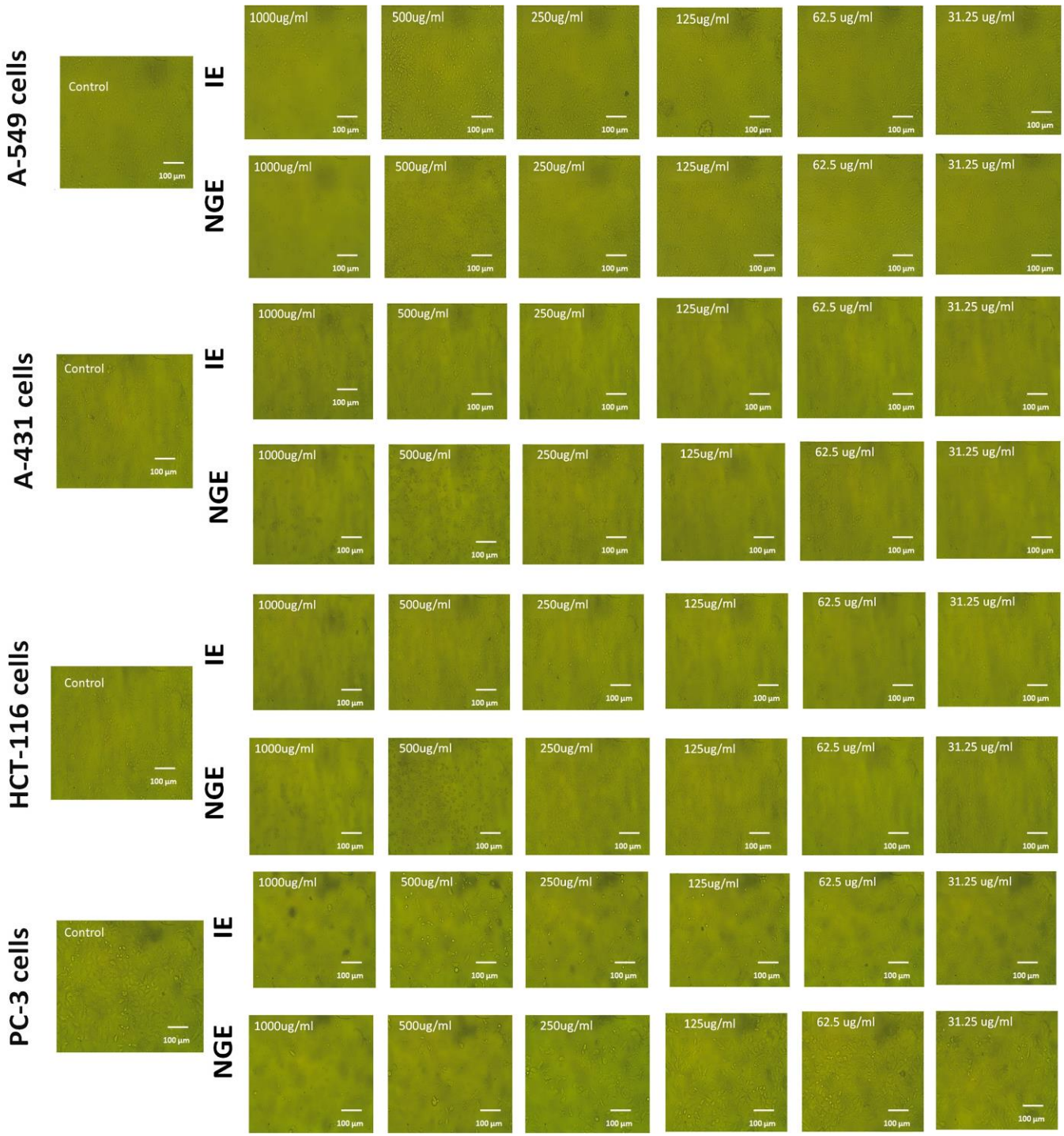


Fig. 5. *In vitro* antiproliferative morphological effect of different concentrations of the ink extract (IE) and nidamental gland extract (NGE) of *Sepia officinalis* against different human cell lines; lung carcinoma (A-549), epidermoid carcinoma (A-431), colorectal carcinoma (HCT-116), and prostate adenocarcinoma (PC-3) after 24 hour-incubation period, Scale bar = 100µm.

DISCUSSION

Cancer is still a major health issue worldwide. Numerous studies have been conducted on this field to find new sources of anticancer medications, particularly from marine invertebrates since they provide a wide variety of unique chemical structures for new bioactive chemical compounds **(Fahmy & Soliman, 2013; Senan, 2015)**. Among marine invertebrates, cephalopods are the most interesting source of extremely potent bioactive metabolites, with a great potential to develop new and much-needed drugs, primarily for cancer **(Khudair *et al.*, 2019; Salem *et al.*, 2020)**. They use these bioactive chemical compounds for self-defense and for preserving eggs and embryos. The ink secretion from cephalopods was considered one of the new sources of bioactive products **(Peruru *et al.*, 2012; Hossain *et al.*, 2019)**. The ink is a multifunctional marine bioactive agent that destroys cancer cells. It exhibited strong cytotoxicity on various cell lines through inhibiting cell growth **(Russo *et al.*, 2003; Fahmy & Soliman, 2013; Khudair *et al.*, 2019)**, initiation of apoptosis **(Derby, 2014; Salem *et al.*, 2020)**, and the reduction of viable tumor cell count **(Soliman *et al.*, 2015)**.

The present results demonstrated that the cytotoxic activities of IE and NGE from *S. officinalis* have dose-dependent viability and cytotoxicity on the four tested cancer cell lines. These results conform to the obtained results of **Diaz *et al.* (2015)**, **Riyad *et al.* (2020)** and **Salem *et al.* (2020)**, who indicated that there is a significant decrease in viability percentages of HepG2 liver cancer cells, A-549 human lung carcinoma cell line, and Ehrlich Ascites Carcinoma (EAC) cell line, respectively, as the concentration of extract increased.

The present results revealed that the cytotoxic activity of *S. officinalis* depends on the type of an extracted part from *S. officinalis* and the type of the cancer cell line. The NGE has a good cytotoxic activity on all treated cancer cell lines: A-549, A-431, HCT-116, and PC-3 compared to IE at most tested concentrations. Furthermore, the IE extract exhibited a potent growth inhibition activity against PC-3 cells, followed by HCT-116, A-431, and A-549 with IC_{50} of 372.21, 480.06, 511.03, and 517.52µg/ ml, respectively. At the same time, the NGE presented a potent growth inhibition activity against HCT-116 cells, followed by PC-3, A-431, and A-549 with IC_{50} of 220.04, 242.22, 262.83, and 427.45µg/ ml, respectively.

The present results are more or less similar to those obtained by the previous reports on the cytotoxic activity of the squid and cuttlefish extracts. **Diaz *et al.* (2014)** indicated that the crude and partially purified *Loligo duvauceli* squid inks exhibited a potent cytotoxic effect on the HepG2 liver cancer cell line with the IC_{50} value at 125µg/ ml concentration. **Diaz *et al.* (2015)** reported that the viability of HepG2 cells, after treatment with the methanolic extract of *L. duvauceli* and *Sepia pharaonis* bone powder, ranged from 63.161 (1000µg/ ml) to 73.366% (100µg/ ml) and 58.368 (1000µg/ ml) to 68.380% (100µg/ ml), respectively, with IC_{50} at a concentration of >1000µg/ ml. **Salem *et al.* (2020)** revealed that the viability of EAC cell line, treated with methanolic shell and ink extracts of *S. officinalis*, was 61% for 1000µg/ ml to 83% for 100µg/ ml and 61% for 100µg/ ml to 100% for 25µg/ ml, respectively. They recorded the IC_{50} values at a level of >1000µg/ ml for shell extract and >100µg/ ml for IE.

The cytotoxic activity of *S. officinalis* extracts on cancer cell lines is affiliated with the presence of a variety of bioactive chemical compounds **(Salem *et al.*, 2020)**. The present result of GC-MS analysis identified different bioactive chemical compounds in both extracts of *S. officinalis*, IE and NGE, having different therapeutic applications, such as saturated fatty acids (n-hexadecanoic acid, myristic acid, octadecanoic acid, dodecanoic acid, pentadecanoic acid, and heptadecanoic acid), hexadecanoic acid, methyl ester, unsaturated fatty acid (arachidonic acid), 1-hexadecanol, and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester. These bioactive compounds presented a high peak height and an area in NGE compared to IE. Most of these compounds have cytotoxic activities on cancer cell lines and antioxidant properties like n-hexadecanoic acid **(Harada *et al.*, 2002; Subavathy & Thilaga, 2016; Arora & Kumar, 2018)**, which is the most abundant compound in both extracts, with a peak area of 2,941,074.8 for IE and 28,132,282 for NGE among all compounds extracted. This fatty acid and its methyl ester have a role in human gastric cancer cells **(Yu *et al.*, 2005)** and inducing apoptosis in cervical cancer cell lines **(Paul & Kundu, 2017)**, respectively.

Other fatty acids like pentadecanoic acid **(Lin *et al.*, 2009)**, heptadecanoic acid **(Lalitharani *et al.*, 2010)**, octadecanoic acid **(Panigrahi *et al.*, 2014; Arora & Kumar, 2018)**, myristic acid **(Subavathy & Thilaga, 2016; Arora & Kumar, 2018)**, and arachidonic acid **(Muhammad *et al.*, 2020)** exhibited cytotoxic activities on cancer cell lines and antioxidant properties. Octadecanoic acid has the potential activity to prevent and treat breast cancer by inducing apoptosis and inhibiting the cell cycle of breast tumors **(Saadatian-Elahi *et al.*, 2004; Evans *et al.*, 2009a, b)** and has antiproliferative effects on prostatic cancer cells **(Hagen *et al.*, 2013)**.

Moreover, the present GC-MS results showed that IE of *S. officinalis* has bioactive compounds with a cytotoxic activity on cancer cell lines and antioxidant activities like 1-monolinoleoylglycerol trimethylsilyl ether **(Parthipan *et al.*, 2015; Singh & Patra, 2018)**, sinapic acid **(Chen, 2016)**, benzenemethanol, 2-(2-aminopropoxy)-3-methyl- **(Hussein *et al.*, 2016)**, and linoleic acid **(Raja *et al.*, 2016; Arora & Kumar, 2018)**. On the other side, NGE of *S. officinalis* presented some bioactive compounds that exhibited anticancer, antineoplastic, and antioxidant activities, such as 9-octadecenoic acid (Z)-, methyl ester **(Hema *et al.*, 2011)**, oleic acid **(Carrillo *et al.*, 2012; Wei *et al.*, 2016)**, , doconexent **(Babu *et al.*, 2014)**, and 3-trifluoroacetoxypentadecane **(Hadi & Hussein, 2016)**. In addition to the previous bioactive compounds, 1,2-benzenedicarboxylic acid, diisooctyl ester enhances protein phosphorylation in HeLa cells to high level through protein kinase C and casein kinase1 **(Lahousse *et al.*, 2006)**.

The present results indicated that both extracts, IE and NGE, have high amounts of carbohydrates, followed by protein and lipids. The low lipid concentration over all proximate compositions is consistent with those investigated by **Ganesan *et al.* (2017)**, **Jeyasanta and Patterson (2020)** and **Riyad *et al.* (2020)**. Polysaccharides are chemical components with multiple therapeutic benefits involving anti-virus, anti-inflammatory, and antitumor bioactivities **(Shi, 2016)**. **Luo and Liu (2013)** stated that marine bioactive squid ink polysaccharides have the antioxidant ability, with a potent scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals, as well as protecting deoxyribonucleic acid (DNA) from oxidative damage induced by free radicals.

The present result showed that the total phenolic concentration was 5.4 and 9.7mg GA eq./ g dried extract, total flavonoid concentration was 3.7 and 4.3mg quercetin eq./ g

dried extract, and total alkaloid concentration was 5.6 and 5.9mg berberine eq./ g dried extract, for IE and NGE, respectively. These results appear high when compared with those obtained in the study of **Nisha and Suja (2018)**, who found that the total phenol contents were 0.008 and 2.65mg GA eq./ g, and total flavonoid contents were 0.002 and 1.32mg quercetin eq./ g in the *L. duvauceli* methanol ink extract and its partially purified form, respectively.

The significantly high level of TAC in NGE can be returned to the presence of high levels of phenolic, flavonoid, and alkaloid concentrations in NGE, as compared with IE. The phenolic and flavonoid compounds are a major group of primary antioxidants and free radical scavengers due to their high redox potential activities, supporting their capacity to function as reducing agents **(Ozsoy *et al.*, 2009; Jeyasanta & Patterson, 2020; Makhlof *et al.*, 2023)**. They exhibited numerous biological activities, viz. anticancer, antioxidant, as well as anti-inflammatory activities **(Pourmorad *et al.*, 2006; Mateos *et al.*, 2020)**. Moreover, alkaloids are known to have antioxidant effects via scavenging or chelating free radicals and have numerous therapeutic applications as well **(Chen *et al.*, 2013; Zou *et al.*, 2016)**.

CONCLUSION

In conclusion, the current study showed that both *S. officinalis* extracts, IE and NGE, exhibited cytotoxic effects on cancer cell lines by decreasing the number of viable cancer cells. However, the NGE of *S. officinalis* showed a higher cytotoxic effect and had more toxicity toward all tested cancer cell lines than the IE due to the high level of all proximal compositions, phytochemicals, and TAC in NGE than IE. Hence, NGE can be the best toxic agent on cancer cells for developing anticancer therapy, but more research studies are needed to explore the mechanism of anticancer activity.

REFERENCES

Afifi, R.; Abdel-Nabi, I. M. and El-Shaikh, K. (2016). Antibacterial activity from soft corals of the Red Sea, Saudi Arabia. J. Taibah Univ. Sci., 10(6): 887-895. https://doi.org/10.1016/j.jtusci.2016.03.006

Arora, S. and Kumar, G. (2018). Phytochemical screening of root, stem and leaves of *Cenchrus biflorus* Roxb. J. Pharmacogn. Phytochem., 7(1): 1445-1450.

Aziz, M. A. (2015). Qualitative phytochemical screening and evaluation of anti-inflammatory, analgesic and antipyretic activities of *Microcos paniculata* barks and fruits. J. Integr. Med., 13(3): 173-184. https://doi.org/10.1016/S2095-4964(15)60179-0

Babu, M.; Raja, D. P.; Arockiaraj, A. A. and Vinnarasi, J. (2014). Chemical constituents and their biological activity of *Ulva lactuca* Linn. Int. J. Pharm. Drug Anal., 2(7): 595-600.

Boletzky, S. V. (2003). Advances in marine biology: biology of early life stages in cephalopod molluscs. 44: 143-203.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72(1-2): 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Carrillo, C.; Cavia, M. and Alonso-Torre, S. (2012). Antitumor effect of oleic acid; mechanisms of action: a review. Nutr. Hosp., 27(5): 1860-1865. http://dx.doi.org/10.3305%2Fnh.2012.27.6.6010

Chen, C. (2016). Sinapic acid and its derivatives as medicine in oxidative stress-induced diseases and aging. Oxid. Med. Cell. Longev., 2016: 3571614. https://doi.org/10.1155/2016/3571614

Chen, M.; Shao, C. L.; Fu, X. M.; Xu, R. F.; Zheng, J. J.; Zhao, D. L. and Wang, C. Y. (2013). Bioactive indole alkaloids and phenyl ether derivatives from a marine-derived *Aspergillus* sp. fungus. J. Nat. Prod., 76(4): 547-553. https://doi.org/10.1021/np300707x

Derby, C. D. (2014). Cephalopod ink: production, chemistry, functions and applications. Mar. Drugs, 12(5): 2700-2730. https://doi.org/10.3390/md12052700

Diaz, J. H. J.; Thilaga, R. D. and Sivakumar, V. (2014). Cytotoxic activity of crude and partially purified ink of *L. duvauceli* towards HepG2 cell line. Int. J. Pharm. Sci. Rev. Res., 3(6): 19-23.

Diaz, J. H. J.; Thilaga, R. D. and Sivakumar, V. (2015). *In-vitro* cytotoxic activity of squid and cuttlefish bone extract on HepG2 cell line. Int. J. Pharm. Sci. Res., 6(2): 778-782. https://doi.org/10.13040/IJPSR.0975-8232.6(2).778-82

Evans, L. M.; Cowey, S. L.; Siegal, G. P. and Hardy, R. W. (2009a). Stearate preferentially induces apoptosis in human breast cancer cells. Nutr. Cancer, 61(5): 746-753. https://doi.org/10.1080/01635580902825597

Evans, L. M.; Toline, E. C.; Desmond, R.; Siegal, G. P.; Hashim, A. I. and Hardy, R. W. (2009b). Dietary stearate reduces human breast cancer metastasis burden in athymic nude mice. Clin. Exp. Metastasis, 26: 415-424. https://doi.org/10.1007/s10585-009-9239-x

Fahmy, S. R. and Soliman, A. M. (2013). *In vitro* antioxidant, analgesic and cytotoxic activities of *Sepia officinalis* ink and *Coelatura aegyptiaca* extracts. Afr. J. Pharm. Pharmacol., 7(22): 1512-1522. https://doi.org/10.5897/AJPP2013.3564

Fahmy, S. R.; Ali, E. M. and Ahmed, N. S. (2014). Therapeutic effect of *Sepia* ink extract against invasive pulmonary aspergillosis in mice. J. Basic Appl. Zool., 67(5): 196-204. https://doi.org/10.1016/j.jobaz.2014.08.001

Fouad, M. A.; Orabi, M. A.; Abdelhamid, R. A. and Allian, A. (2021). Cytotoxicity and anti-leishmanial activity of the Red Sea soft coral *Sarcophyton spongiosum*. J. Adv. Biomed. & Pharm. Sci., 4(2): 107-110. https://doi.org/10.21608/jabps.2021.62964.1120

Ganesan, P. A. B. N.; Brita Nicy, A.; Kanaga, V. and Velayutham, P. (2017). Proximate analysis of cuttlefish ink procured from Thoothukudi coast: a comparative study. Int. J. Fish. Aquat. Stud., 5(3): 253-255.

Hadi, I. and Hussein, H. M. (2016). Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of Adiantum Capillus-Veneris using GC-MS and FT-IR spectroscopy. Int. J. Pharmacogn. Phytochem. Res., 8(3): 369-385.

Hagen, R. M.; Rhodes, A. and Ladomery, M. R. (2013). Conjugated linoleate reduces prostate cancer viability whereas the effects of oleate and stearate are cell line-dependent. Anticancer Res., 33(10): 4395-4400.

Harada, H.; Yamashita, U.; Kurihara, H.; Fukushi, E.; Kawabata, J. and Kamei, Y. (2002). Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res., 22(5): 2587-2590.

Hema, R.; Kumaravel, S. and Alagusundaram, K. (2011). GC/MS determination of bioactive components of *Murraya koenigii*. J. Am. sci., 7(1): 80-83.

Hossain, M. P.; Rabeta, M. S. and Husnul Azan, T. (2019). Medicinal and therapeutic properties of cephalopod ink: a short review. Food Res., 3(3): 188-198. https://doi.org/10.26656/fr.2017.3(3).201

Houghton, P.; Fang, R.; Techatanawat, I.; Steventon, G.; Hylands, P. J. and Lee, C. C. (2007). The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods, 42(4): 377-387. https://doi.org/10.1016/j.ymeth.2007.01.003

Hussein, H. J.; Hadi, M. Y. and Hameed, I. H. (2016). Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrophotometer and gas chromatography-mass spectrometry. J. Pharmacogn. Phytotherapy, 8(3): 60-89. https://doi.org/10.5897/JPP2015.0372

Ismail, G. A.; Gheda, S. F.; Abo-shady, A. M. and Abdel-karim, O. H. (2019). *In vitro* potential activity of some seaweeds as antioxidants and inhibitors of diabetic enzymes. Food Sci. Technol., 40(3): 681-691. https://doi.org/10.1590/fst.15619

Ismail, M. and Riad, R. (2018). Screening the antimicrobial activity of different *Sepia officinalis* (Cephalopoda: Sepioidea) parts collected from Alexandria Mediterranean Waters, Egypt against some human pathogens. Singap. J. Sci. Res., 8: 1-7.

Jeyasanta, I. and Patterson, J. (2020). Bioactive properties of ink gland extract from squid *Loligo duvauceli*. Ecol., 10(1): 9-19. https://doi.org/10.3923/ecologia.2020.9.19

Kalaba, M. H.; Sultan, M. H.; Elbahnasawy, M. A.; El-Didamony, S. E.; El Bakary, N. M. and Sharaf, M. H. (2022). First report on isolation of *Mucor bainieri* from honeybees, *Apis mellifera*: characterization and biological activities. Biotechnol. Rep., 36: e00770. https://doi.org/10.1016/j.btre.2022.e00770

Khudair, I. H.; Hanna, B. A. and Sugar, D. K. (2019). Cytotoxic effect of ink extracted from Cephalopoda on cancer cell line. Sci. J. Med. Res., 3(12): 139-145. https://doi.org/10.37623/SJMR.2019.31206

Kiranmai, M.; Kumar, C. M. and Mohammed, I. (2011). Comparison of total flavonoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction. Res. J. Pharm. Biol. Chem. Sci., 2(3): 254-261.

Kirkpatrick, L. A. and Feeney, B. C. (2012). A simple guide to IBM SPSS statistics for version 20.0, 12th ed. Wadsworth, Cengage Learning, Belmont, California, pp 128.

Lahousse, S. A.; Beall, S. A. and Johnson, K. J. (2006). Mono-(2-ethylhexyl) phthalate rapidly increases celsr2 protein phosphorylation in HeLa cells *via* protein kinase C and casein kinase 1. Toxicol. Sci., 91(1): 255-264. https://doi.org/10.1093/toxsci/kfj135

Lalitharani, S.; Mohan, V. R. and Regini, G. S. (2010). GC-MS analysis of ethanolic extract of *Zanthoxylum rhetsa* (roxb.) dc spines. J. Herb. Med. Toxicol., 4(1): 191-192.

Lee, M. F.; Lin, C. Y.; Chiao, C. C. and Lu, C. C. (2016). Reproductive behavior and embryonic development of the Pharaoh cuttlefish, *Sepia pharaonis* (Cephalopoda: Sepiidae). Zool. Stud., 55(41): 1-16. https://doi.org/10.6620/ZS.2016.55-41

Li, L.; Long, W.; Wan, X.; Ding, Q.; Zhang, F. and Wan, D. (2015). Studies on quantitative determination of total alkaloids and berberine in five origins of crude medicine "Sankezhen". J. Chromatogr. Sci., 53(2): 307-311. https://doi.org/10.1093/chromsci/bmu060

Lin, F. L.; Wu, S. J.; Lee, S. C. and Ng, L. T. (2009). Antioxidant, antioedema and analgesic activities of *Andrographis paniculata* extracts and their active constituent andrographolide. Phytother. Res., 23(7): 958-964. https://doi.org/10.1002/ptr.2701

Luo, P. and Liu, H. (2013). Antioxidant ability of squid ink polysaccharides as well as their protective effects on deoxyribonucleic acid DNA damage *in vitro*. Afr. J. Pharm. Pharmacol., 7(21): 1382-1388. https://doi.org/10.5897/AJPP12.1335

Makhlof, M. E.; Ghareeb, D. A. and El-Kenany, E. T. (2023). Assessment of anticancer, antibacterial, antifungal, and antioxidant activities of *Micractinium reisseri* (Chlorophyta, Trebouxiophyceae) methanolic extract. Rend. Lincei. Sci. Fis. Nat., 34(2): 483-489. https://doi.org/10.1007/s12210-023-01139-3

Mateos, R.; Pérez-Correa, J. R. and Domínguez, H. (2020). Bioactive properties of marine phenolics. Mar. Drugs, 18(10): 501. https://doi.org/10.3390/md18100501

Mishra, S. K.; Suh, W. I.; Farooq, W.; Moon, M.; Shrivastav, A.; Park, M. S. and Yang, J. W. (2014). Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. Bioresour. Technol., 155: 330-333. https://doi.org/10.1016/j.biortech.2013.12.077

Mofeed, J.; Deyab, M. A. and El-Halim, E. H. A. (2018). Anticancer activity of some filamentous cyanobacterial isolates against Hep-G2 and MCF-7 cancer cell lines. Int. J. Life Sci., 8(1): 10-17.

Mona, M. H.; El-Khodary, G. M.; Omran, N. E.; El-Aziz, K. K. A. and El-Saidy, S. A. (2021). Antimicrobial activity, cytotoxic effect and characterization of marine bivalve extracts *Cerastoderma glaucum*. Rend. Lincei. Sci. Fis. Nat., 32: 149-161. https://doi.org/10.1007/s12210-020-00964-0

Muhammad, Y.; Nur Fitriani, U. A.; Sri, I.; Rahmawati, S.; Mahyati, L. and Akhmad, R. (2020). Optimization ultrasonic assisted extraction (UAE) of bioactive compound and

antibacterial potential from sea urchin (*Diadema setosum*). Curr. Res. Nutr. Food Sci., 8(2): 556-569. https://dx.doi.org/10.12944/CRNFSJ.8.2.22

Nisha, N. and Suja, S. (2018). Phytochemical evaluation and antioxidant activity of methanol extract of *Loligo duvauceli* ink. J. Pharmacogn. Phytochem., 7(1): 1764-1767.

Ozsoy, N.; Candoken, E. and Akev, N. (2009). Implications for degenerative disorders: antioxidative activity, total phenols, flavonoids, ascorbic acid, β-Carotene and β-Tocopherol in *Aloe vera*. Oxid. Med. Cell. Longev., 2(2): 99-106. https://doi.org/10.4161/oxim.2.2.8493

Padmanaban, D.; Samuel, A.; Sahayanathan, G. J.; Raja, K. and Chinnasamy, A. (2022). Anticancer effect of marine bivalves derived polysaccharides against human cancer cells. Biocatal. Agric. Biotechnol., 39: 102240. https://doi.org/10.1016/j.bcab.2021.102240

Panigrahi, S.; Muthuraman, M. S.; Natesan, R. and Pemiah, B. (2014). Anticancer activity of ethanolic extract of *Solanum torvum* sw. Int. J. Pharm. Pharm. Sci., 6(1): 93-98.

Parthipan, B.; Suky, M. G. T. and Mohan, V. R. (2015). GC-MS analysis of phytocomponents in *Pleiospermium alatum* (Wall. ex Wight & Arn.) Swingle, (Rutaceae). J. Pharmacogn. Phytochem., 4(1): 216-222.

Paul, S. and Kundu, R. (2017). Induction of apoptosis by fatty acid rich fraction of *Solanum nigrum* on cervical cancer cell lines. Int. J. Pharm. Pharm. Sci., 9: 199-206. https://doi.org/10.22159/ijpps.2017v9i11.21628

Peruru, D.; Ahmed, R. S. N. and Sandeep, V. P. S. (2012). Isolation of eumelanin from *Sepia officinalis* and investigation of its antimicrobial activity by ointment formulation. Int. J. Pharm., 2(2): 67-72.

Pourmorad, F.; Hosseinimehr, S. J. and Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol., 5(11): 1142-1145.

Prieto, P.; Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem., 269(2): 337-341. https://doi.org/10.1006/abio.1999.4019

Raja, R.; Hemaiswarya, S.; Ganesan, V. and Carvalho, I. S. (2016). Recent developments in therapeutic applications of Cyanobacteria. Crit. Rev. Microbiol., 42(3): 394-405. https://doi.org/10.3109/1040841X.2014.957640

Rajaganapathi, J.; Thyagarajan, S. P. and Edward, J. K. (2000). Study on cephalopod's ink for anti-retroviral activity. Indian J. Exp. Biol., 38(5): 519-520.

Riad, R. (2020). Monograph of the Egyptian cuttlefishes Order: Sepioidea; Cephalopoda: Mollusca (Part I). Egypt. J. Aquat. Biol. Fish., 24(2): 555-590. https://doi.org/10.21608/EJABF.2020.86923

Riyad, Y. M.; Rizk, A. E. and Mohammed, N. S. (2020). Active components of squid ink and food applications. Egypt. J. Food Sci., 48(1): 123-133. https://doi.org/10.21608/ejfs.2020.27247.1049

Russo, G. L.; De Nisco, E.; Fiore, G.; Di Donato, P.; d'Ischia, M. and Palumbo, A. (2003). Toxicity of melanin-free ink of *Sepia officinalis* to transformed cell lines: identification of the active factor as tyrosinase. Biochem. Biophys. Res. Commun., 308(2): 293-299. https://doi.org/10.1016/S0006-291X(03)01379-2

Saadatian-Elahi, M.; Norat, T.; Goudable, J. and Riboli, E. (2004). Biomarkers of dietary fatty acid intake and the risk of breast cancer: a meta-analysis. Int. J. Cancer, 111(4): 584-591. https://doi.org/10.1002/ijc.20284

Salem, M. L.; Mona, M. H.; El-Gamal, M.; Mansour, M. A. and Ghonem, E. (2020). *In vitro* anticancer activities of ink and internal shell extracts of *Sepia officinalis* inhabiting Egyptian water. BFSZU., 2016(1): 160-174. https://doi.org/10.21608/bfszu.2016.31073

Senan, V. P. (2015). Antibacterial activity of methanolic extract of the ink of cuttlefish, *Sepia pharaonis* against pathogenic bacterial strains. Int. J. Pharm. Sci. Res., 6(4): 1705. https://doi.org/10.13040/IJPSR.0975-8232.6(4).1705-10

Sheela, J.; Dhanya, S. and Pugazhendhi, A. (2014). Antibacterial effect of ink gland extracts from Indian squid *Loligo duvauceli* and cuttlefish *Sepia officinalis* from North Chennai. Indian J. Appl. Microbiol., 17(1): 32-37.

Shi, L. (2016). Bioactivities, isolation and purification methods of polysaccharides from natural products: a review. Int. J. Biol. Macromol., 100(92): 37-48. https://doi.org/10.1016/j.ijbiomac.2016.06.100

Singh, S. K. and Patra, A. (2018). Evaluation of phenolic composition, antioxidant, anti-inflammatory and anticancer activities of *Polygonatum verticillatum* (L.). J. Integr. Med., 16(4): 273-282. https://doi.org/10.1016/j.joim.2018.04.005

Singleton, V. L.; Orthofer, R. and Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Meth. Enzymol., 299: 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Soliman, A. M.; Fahmy, S. R. and El-Abied, S. A. (2015). Anti-neoplastic activities of *Sepia officinalis* ink and *Coelatura aegyptiaca* extracts against Ehrlich ascites carcinoma in Swiss albino mice. Int. J. Clin. Exp. Pathol., 8(4): 3543-3555.

Subavathy, P. and Thilaga, R. D. (2016). GC-MS analysis of bioactive compounds from whole body tissue methanolic extract of *Cypraea arabica* (L. 1758). World J. Pharm. Res., 5(3): 800-80.

Wei, C. C.; Yen, P. L.; Chang, S. T.; Cheng, P. L.; Lo, Y. C. and Liao, V. H. C. (2016). Antioxidative activities of both oleic acid and *Camellia tenuifolia* seed oil are regulated by the transcription factor DAF-16/FOXO in *Caenorhabditis elegans*. PLOS ONE, 11(6): e0157195. https://doi.org/10.1371/journal.pone.0157195

Wilson, C. M.; Tobin, S. and Young, R. C. (2004). The exploding worldwide cancer burden: the impact of cancer on women. Int. J. Gynecol. Cancer, 14(1): 1-11. http://dx.doi.org/10.1136/ijgc-00009577-200401000-00001

Wolfe, K.; Wu, X. and Liu, R. H. (2003). Antioxidant activity of apple peels. J. Agric. Food Chem., 51(3): 609-614. https://doi.org/10.1021/jf020782a

Yousefi, M. K.; Hashtroudi, M. S.; Moradi, A. M. and Ghasempour, A. R. (2018). *In vitro* investigating of anticancer activity of focuxanthin from marine brown seaweed species. Glob. J. Environ. Sci. Manag., 4(1): 81-90. https://doi.org/10.22034/gjesm.2018.04.01.008

Yu, F. R.; Lian, X. Z.; Guo, H. Y.; McGuire, P. M.; Li, R. D.; Wang, R. and Yu, F. H. (2005). Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. J. Pharm. Pharm. Sci., 8(3): 528-535.

Zatylny-Gaudin, C. and Henry, J. (2018). Biological resources of water: egg-laying in the cuttlefish *Sepia officinalis*. InTech. https://doi.org/10.5772/intechopen.71915

Zhong, J. P.; Wang, G.; Shang, J. H.; Pan, J. Q.; Li, K.; Huang, Y. and Liu, H. Z. (2009). Protective effects of squid ink extract towards hemopoietic injuries induced by cyclophosphamine. Mar. Drugs, 7(1): 9-18. https://doi.org/10.3390/md7010009

Zou, Z.; Xi, W.; Hu, Y.; Nie, C. and Zhou, Z. (2016). Antioxidant activity of citrus fruits. Food Chem., 100(196): 885-896. https://doi.org/10.1016/j.foodchem.2015.09.072

ARABIC SUMMARY

الفحص المختبري للنشاط السام للخلايا لمستخلصات الحبر و الغدة العُشية من الحبار المصري *Sepia officinalis* (Cephalopoda: Sepioidea) على خطوط خلايا سرطانية

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هناك حاجة ماسة لعلاجات السرطان الجديدة و ذلك لأن أدوية السرطان التقليدية المتاحة لها العديد من العواقب السلبية. ولذلك، فإن العمل الحالي هو محاولة لتقييم الخصائص السامة للخلايا لمستخلص الحبر (IE) ومستخلص الغدة العُشية (NGE) من الحبار المصري ***Sepia officinalis*** على أربعة خطوط خلوية سرطانية: سرطان الرئة (A-549)، وسرطان البشرة (A-431)، وسرطان القولون والمستقيم (HCT-116)، وسرطان البروستاتا (PC-3). تم توصيف كلا المستخلصين من خلال فحص التركيب الكيميائي الحيوي عن طريق قياس الطيف اللوني للغاز (GC-MS) والتحقيق في مستويات التكوين التقريبي، والمواد الكيميائية النباتية، والقدرة الكلية لمضادات الأكسدة (TAC). أظهر الحبر ومستخلص الغدة العُشية تأثيرات سامة للخلايا السرطانية عن طريق تقليل عدد الخلايا السرطانية الحية بناءا على الجرعة ولها تركيزات مثبطة (IC_{50}) تبلغ 517.52 و427.45 ميكروجرام/مل ضد-A 549 و511.03 و262.83 ميكروجرام/مل ضد A-431 و480.06 و220.04 ميكروجرام/مل ضد HCT-116 و372.21 و242.22 ميكروجرام/مل ضد PC-3، على التوالي. أظهر مستخلص الغدة العُشية سمية أكبر تجاه جميع خطوط الخلايا السرطانية التي تم اختبارها مقارنة بمستخلص الحبر و ذلك بسبب التركيزات العالية من المواد النشطة بيولوجيًا في مستخلص الغدة العُشية مقارنة بمستخلص الحبر. وعلاوة على ذلك، كانت جميع التكوينات التقريبية، والمواد الكيميائية النباتية، والقدرة الكلية لمضادات الأكسدة في مستخلص الغدة العُشية أعلى من تلك المكتشفة في مستخلص الحبر. وبالتالي، يمكن اعتبار مستخلص الغدة العُشية ل ***Sepia officinalis*** عاملًا واعدًا ساما للخلايا السرطانية، ولكن هناك حاجة إلى مزيد من الدراسات لإستكشاف آلية العمل.