Original Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF ANTIOXIDANT, ANTI-INFLAMMATORY, ANTI-ALZHEIMER'S AND ANTICANCER ACTIVITIES OF *TAONIA ATOMARIA*

HANAN F. ALY[1], EMAN A. IBRAHIM[2], DOHA H. ABOU BAKER[3], KHALED MAHMOUD[4] AND FAROUK K. EL-BAZ*[2]

[1]Therapeutic Chemistry Department, National Research Centre (NRC), 33 El Bohouth st. (former El Tahrir st.), Dokki, Giza, Egypt, P.O.12622.
[2]Plant Biochemistry Department, National Research Centre (NRC), 33 El Bohouth st. (former El Tahrir st.), Dokki, Giza, Egypt, P.O.12622.
[3]Medicinal and Aromatic Plant Department, National Research Centre (NRC), 33 El Bohouth st. (former El Tahrir st.), Dokki, Giza, Egypt, P.O.12622.
[4]Pharmacognosy Department, National Research Centre (NRC), 33 El Bohouth st. (former El Tahrir st.), Dokki, Giza, Egypt, P.O.12622.

ABSTRACT

The present research aims to identify the phenolic and flavonoid composition as well as the biological effects of the *Taonia atomaria* different extracts as antioxidant, anti-inflammatory, anti-Alzheimer's and anticancer activities against different cancer cell lines including liver cancer cell line (HepG2), colon cancer cell line (HCT116), breast cancer cell line (MCF-7) and ovarian cancer cell line (A549). Beside, antioxidant scavenging activity of *T. atomaria* using DPPH and ABTS and total antioxidant capacity were also examined. The present results clearly indicated the presence of high contents of e-vanillic acid (4968.78µg/100g), benzoic acid (6286.57µg/100g) and pyrogallol (1531.04µg/100g). In addition, high contents of epicatechein (447.97µg/100g), caffeic (382.19µg/100g), ferulic (380.34 µg/100g) and saiycilic (653.93µg/100g) were found in phenolic composition of *T. atomaria*. The flavonoid composition of *T. atomaria* revealed the presence of high content of hisperdin and hespertin with contents 228.61 and 269.06µg/100g, respectively. Also, high content of narengin, rutin and luteolin were observed as they recorded 108.11, 109.19 and 104.01µg/100g, respectively. In addition to, rosmarinic, quercetin, kampferol, apegenin and 7-hydroxy flavone were identified. Moreover, the marine algae *T. atomaria* different fractions of ethanolic extract exhibited antioxidant, anti- inflammatory, anti-Alzheimer's and anticancer activities against various cancer cell lines in a dose dependent manner. Thus, it could be concluded that, *T. atomaria* different extracts demonstrated promising biological activities that need further *in vivo* studies and clinical extensive researches as, anti-inflammatory, anti- Alzheimer's as well as anticancer agents.

KEYWORDS:*Taonia atomaria*, **Phenolic and flavonoid, Antioxidant, Anti-Inflammatory, Anti-Alzheimer's, Anticancer**

FAROUK K. EL-BAZ
Plant Biochemistry Department, National Research Centre (NRC), 33 El Bohouth st. (former El Tahrir st.), Dokki, Giza, Egypt, P.O.12622..

INTRODUCTION

A wide variety of marine algae grows along the Egyptian Mediterranean coast, especially at Alexandria. The green, brown, and red algae are among the most abundant macroalgae on the Alexandria coast, particularly from spring to autumn. [1,2] Marine algae consider a rich source of pharmacologically active metabolites with antioxidant, antineoplastic, antimicrobial, antitumor, anti-inflammatory, anti-Alzheimers, neurotoxic and antiviral activities. [3-5] Marine algae contain a variety of major metabolites such as terpenoids, phlorotannins, polyphenols, anthocyanins, hydroxycinnamic acid derivatives, flavonoid, polysaccharides, lipids, proteins, carotenoids, vitamins, sterols, enzymes and antibiotics. [6] Among macroalgal are natural antioxidants. [7] The antioxidant capacity of algal compounds includes prevention of many diseases such as cancer, coronary heart diseases, inflammatory disorders, and neurological degeneration. [8,9] Methanol, ethyl acetate, and Hexane extracted from *Amphiroa fragilissimas* (macroalgae) have a strong antioxidant and antimicrobial activity except the methanol extract which showed inhibitory effect on lung cancer cell line. [9] This study aims to evaluate phenolic and flavonoid compounds of marine algae *T. atomaria*, characterize their chemical properties and assess anticancer, anti-Alzheimers, anti-inflammatory and antioxidants activities.

MATERIAL AND METHODS

(i) Material

(a) Collection of macroalgae sample

T. atomaria (brown algae) was collected from the Mediterranean Sea from (Abu-Qir near Alexandria) in August 2014. The algae were identified by Dr. Rauhaiya Abdul-Latif, Professor of Botany Department, Faculty of Science, Al-Azhar University, where voucher specimen was deposited (voucher No. 1848b:101).

(b) Preparation of macroalgae sample

Macroalgae was washed several times with tap water, air in shaded area. The dried of algal sample was grinded by electric mill into fine particle and then stored in glass containers at room temperature for further experiments.

(ii) Methods

a) Extraction and fractionation of phenolic and flavonoid compounds

Powdered alga (1kg) was extracted with ethanol (80%, 1L×5) by soaking at room temperature. The combined ethanol extracts were concentrated under reduced pressure at 45 °C (32g). The crude ethanolic extract was dissolved in hot water, left overnight, filtered and successively partitioned with peteleum ether (4.5g), chloroform (9g) and ethyl acetate (3.5g) which were further purified.

b) Identification of phenolic acid and flavonoid compounds

Phenolic compounds were determined by HPLC according to the method of Goupy et al. [10] Different fractions of *T. atomaria* ethanolic extract were filtrated through 0.20µm millipore membrane filter and set up to a known volume (10 ml). Three milliliters were collected in a vial for subsequent HPLC separation. HPLC instrument (Hewlett Packard, series 1050, country) equipped with column C18 hypersil BDS with particle size 5µm. Injection volume was 75 µl carried out with autosampling injector. Column temperature was maintained at 35°C. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate 1.0 ml/min. Elutes were monitored using UV detector set at 280 nm for phenolic acid and 330 nm for flavonoid. Chromatographic peaks were identified by comparing the retention times with the respective retention times of known standard reference material. Retention time and peak area were used for the calculation of phenolic acid and flavonoid compounds concentration by the data analysis of Hewlett Packard software. Phenolic acid and flavonoid compounds were expressed as µg/100g sample on dry weight basis.

c) Biological evaluation of different fractions of T. atomaria ethanolic extract

1. Antioxidant activity of different fractions of T. atomaria ethanolic extracts using

1.1. DPPH free radical scavenging assay

The ability of different fractions of *T. atomaria* ethanolic extract to scavenge DPPH free radical was determined according to Ye et al. [11] method. Briefly, a 0.1 mM of ethanolic DPPH solution was prepared, to give the initial absorbance value of 0.993 at 517 nm. The different concentration of samples (in 0.1 ml) of each sample (with appropriate dilution if necessary) was added to 3.0 ml of ethanolic DPPH solution. After incubation for 30 min in the dark, the absorbance was measured at 517nm. The percentage of DPPH scavenging activity which was scavenged was calculated using the following formula:

$$\text{Scavenging activity \%} = [1 - (A_{\text{sample}} - A_{\text{blank}}/A_{\text{control}})] \times 100$$

1.2. ABTS free radical scavenging assay

The ABTS free radical scavenging capacity assays were carried out according to the method of Arnao et al. [12]Potassium persulfate (2.6 mM) was added to 7.4 mM of ABTS and kept for 12-16 h at room temperature in dark. The ABTS solution (1ml) was diluted with 60ml methanol to an absorbance of 1.1 ± 0.02 at 734 nm before analysis. ABTS solution (2.80 ml) was added to sample fractions (0.150 ml, 50-150 µg/ml). The After incubation for 2h in the dark, the absorbance was measured at 734nm. Trolox was used as the positive control. The ABTS free radical-scavenging capacity (%) was calculated using the following equation:

$$\text{Scavenging activity \%} = [1 - (A_1 - A_2) / A_0] \times 100$$

Where, A_0 is the absorbance of the control (without sample), A_1 is the absorbance in the presence of the sample, and A_2 is the absorbance without ABTS).

1.3. Evaluation of total antioxidant capacity

One ml of different fractions of *T. atomaria* ethanolic extract (100 to 400 µg/ml) was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate, and 4 mM ammonium molybdate). The

tubes were capped and incubated at 95°C for 90 min. After cooling, the absorbance each samples was measured at 695 nm. Standard series concentrations of ascorbic acid were treated as the sample.

2. *In vitro* anti-inflammatory activity of different fractions of *T. atomaria* ethanolic extract using bovine albumin serum

Anti-inflammatory of different fractions of *T. atomaria* ethanolic extract were tested using the method of Rahman et al. [13] The different concentrations of *T. atomaria* extract or standard drug diclofenac sodium (50, 100, 150, 200 µg/ml) were mixed with 0.45ml bovine albumin serum. The sample extracts were incubated at 37°C for 20 min and then heated to 57°C for 3 min after cooling the samples was added 2.5ml phosphate buffer pH 6.4. The absorbance was measured using UV visible spectrophotometer at 255nm.

***3. Anti-Alzheimer's activity of different fractions of T. atomaria* ethanolic extract using cholinesterase inhibitory assay**

An assessment of cholinesterase inhibition was carried out in flat-bottom 96- well microtitre plates using the colorimetric method. A typical run consisted of 5µl of AChE solution, at final assay concentration of 0.08 U/ml; 200µl of 0.1 M phosphate buffer pH 8; 5µl of DTNB at a final concentration of 0.5mM prepared in 0.1 M phosphate buffer pH 7 containing 0.12 M of sodium bicarbonate; and 5µl of the test extract. The reactants were mixed and pre-incubated for 15 min at 30°C. The reaction was initiated by adding 5µl of ATChI at a final concentration of 0.5mM. As a control the inhibitor solution was replaced with buffer. Change in absorbance at 412 nm was measured on spectrophotometer. [14] Donepezil was used as standard.

4. Cytotoxic effect on human cell lines

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan. [15] Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium [(for HepG2- MCF-7 and HCT116 - DMEM for A549 and PC_3)], 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B) and 1% L-glutamine at 37 ºC under 5% CO_2.Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO_2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100-50-25-12.5-6.25-3.125-0.78 and 1.56µg/ml). After 48 h of incubation, medium was aspirated, 40µl MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200µl of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. A positive control which composed of 100µg/ml was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions. [16, 17]The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t- test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

[(Reading of extract / Reading of negative control) -1)] x 100

A probit analysis was carried for IC_{50} and IC_{90}determination using SPSS 11 program.

5. Statistical analysis

All results are expressed as mean value of three replicate. Data were statistically analyzed through analysis of variance (Anova) and Duncans test at P>0.01 using Co-State Statistics Software.

RESULTS

1. Phenolic compounds of T. atomaria

The phenolic composition of *T. atomaria* (Table 1) declared the presence of high contents of e-vanillic acid (4968.78 µg/100g), benzoic acid (6286.57 µg/100g) and pyrogallol (1531.04 µg/100g). In addition, high contents of epicatechein (447.97µg/100g), caffeic (382.19 µg/100g), ferulic (380.34%) and saiycilic (653.93µg/100g). Moreover, the analysis of phenolic compounds of *T atomaria* demonstrated the presence of moderate contents of protocatechuic, chlorogenic, catechol, catechein, caffeine, vanillic, iso-ferulic, ellagic, coumarin and p-coumaric. Also, low contents from gallic, 3-hydroxy tyrosol, 4-amino benzoic, P-hydroxy benzoic, reversetrol, α-coumaric, 3, 4, 5-methoxy cinnamic and cinnamic were detected.

2. Flavonoid compounds of T. atomaria

The flavonoid compositions of *T. atomaria* (Table 2) revealed the presence of high contents of hisperdin and hespertin (228.61 and 269.06µg/100g, respectively). Also, high contents of narengin, rutin and luteolin as they recorded 108.11, 109.19 and 104.01µg/100g, respectively were demonstrated. In addition to, rosmarinic, quercetin, kampferol, apegenin and 7-hydroxyflavone were detected in moderate concentrations.

3. Antioxidant activity of different fractions of T. atomaria ethanolic extract (DPPH scavenging activity and total antioxidant capacity):

a. DPPH and ABTS free radical scavenging activity

Data manipulated in Table (3) showed significant DPPH inhibitory activity of different fractions of *T. atomaria* ethanolic extract in a dose dependent manner, where ethanolic extract exhibited the highest inhibitory percent

at the dose 150µg/ml (63.6%) as compared to standard BHT followed by ethyl acetate (45.43%) at the same concentration of inhibitor. While, choloroform extract recorded the highest percentage of ABTS inhibitory activity at 150 µg/ml (85.12%), followed by petroleum ether extract (83.93%) as compared to standard Trolox.

b. Total antioxidant capacity (TAC)

Table (4) revealed the level of TAC in *T. atomaria* different extracts which recorded inhibition percentages in a dose dependent manner. Chloroform showed the highest percentage of inhibition in TAC at the dose 400 µg/ml (216.66%) as compared to standard (243.46%), at the same concentration of inhibitor. This is followed by total ethanol extract and petroleum ether as they recorded 212.72 and 194.04% inhibition respectively at the concentration of inhibitor 400 ug/ml.

4. Anti-inflammatory activity of different fractions of T. atomaria ethanolic extract

Results in Table (5) demonstrated the anti-inflammatory activity of different fractions of *T. atomaria* ethanolic extract. An insignificant change in anti-inflammatory activity of ethanol extract at the different concentrations (50-200) was obtained. Significant increase was found in an anti-inflammatory activity of petroleum ether, chloroform and ethyl acetate extracts of *T. atomaria* at the different concentrations i.e. dose dependant relationship (the activity is increase as the dose increase) as compared to the standard Diclofenac. Significant high percentages of an anti-inflammatory inhibition were recorded for chloroform extract at the different concentrations followed by petroleum ether and ethyl acetate extracts respectively as compared to standard drug, Diclofenac.

5. Anti-Alzheimer's activity (cholinesterase inhibitory activity) of different fractions of T. atomaria ethanolic extract

Results in Table (6) revealed significant increase in cholinesterase inhibitory activity with increase the concentrations of inhibitor (dose dependent relationship). Petroleum ether and choloroform extracts of *T. atomaria* showed more or less similar anticholinesterase activity at the different concentrations as compared to standard anticholinesterase drug (Donepezil). Choloroform extract of *T. atomaria* declared the highest inhibition percent of cholinesterase activity at 100 ug/ml (49.5%) followed by petroleum ether extract (46.4%) at the same concentration as compared to standard.

6. Anticancer activity of different fractions T. atomaria ethanolic extract against cancer cell lines (HepG2, HCT116, MCF-7 and A549)

Chloroform extract of *T. atomaria* (Table 7) exhibited the highest significant inhibition in hepatocelluar carcinoma cell line with percentage of inhibition 98.60 %, followed by petroleum ether extract which recorded inhibition percentage 87.60%. Moderate percentage of inhibitor was recorded for total ethanol extract (53.80%). While low percentage of inhibition was demonstrated for ethyl acetate extract of *T. atomaria* (17.50%). With respect to human colon cancer cell line, only ethanol extract of *T. atomaria* showed low inhibition percentage, while, no results were detected for petroleum ether, chloroform and ethyl acetate. Considering A549, insignificant high percentages of inhibition were recorded for both chloroform (100.00%) and ethanolic extract (92.70%), followed by petroleum ether extract (84.40%). Significant low percentage of inhibition was detected for ethyl acetate (11.30%) against A549. Also, chloroform extract of *T. atomaria* showed the significant high percentage of inhibition against breast cancer cell line which recorded 100.00% inhibition followed by ethanol extract (75.60%).In addition ,moderate significant percentage of inhibition was detected for petroleum ether (64.3%), while, significant low percentage of inhibition was detected for ethyl acetate extract of *T. atomaria* (9.3%).

IC_{50} and IC_{90} values of different fractions of T. atomaria ethanolic extract against cancer cell lines (HepG2, A549 and MCF-7)

Table (8) demonstrated the values of IC_{50} and IC_{90} of the different fractions of *T. atomaria* ethanolic extract. Results revealed that chloroform extract exhibited the lowest IC_{50} and IC_{90}, means the higher percentages of inhibition against A549 and MCF-7. More or less similar IC_{50} and IC_{90} were obtained for ethanol and petroleum ether extracts of *T. atomaria* against. A549 and MCF-7. While, no results were found for IC_{50} and IC_{90} against HepG2 and HCT 116 for all extracts.

Table 1
Phenolic compounds of T. atomaria

Phenolic compounds	Concentration (µg/100g DW)
Gallic	40.01
Pyrogallol	1531.04
3-Hydroxy tyrosol	54.53
4-amino benzoic	29.56
Protocatechuic	161.20
Chlorogenic	156.46
Catechol	208.66
Epicatechein	447.97
Catechein	208.66
Caffeine	267.39
***P*-hydroxy benzoic**	126.82
Caffeic	382.19
Vanillic	250.38
Ferulic	380.34
Iso-ferulic	240.97
e-vanillic	4968.78
Reversetrol	48.47
Ellagic	265.18

α- coumaric	59.36
Benzoic	6286.57
3,4,5-methoxy cinnamic	41.45
Coumarin	229.65
Saiycilic	653.93
***p*-coumaric**	194.44
Cinnamic	98.93

Table 2
Flavonoid compounds of T. atomaria

Flavonoid compounds	Flavonoid (µg/100g DW)
Luteolin	104.01
Narengin	108.11
Rutin	109.19
Hisperdin	228.61
Rosmarinic	47.778
Quercetrin	128.47
Quercetin	42.54
Kampferol	60.24
Hespertin	269.06
Apegenin	21.43
7-hydroxyflavone	35.32

Table 3
Antioxidant activity of T. atomaria different extracts DPPH and ABTS radical assay

		Scavenging activity%	
***T. atomaria* extracts**	**Concentrations**	**DPPH**	**ABTS**
Ethanol 80%	**50µg**	30.81±1.17^{ef}	12.86±0.87^{i}
	100µg	32.72±1.49^{ef}	54.27±12.75^{g}
	150µg	63.6±1.59^{d}	65.13±4.54^{ef}
Petroleum ether	**50µg**	32.53±0.26^{ef}	75.22±4.00^{cd}
	100µg	34.34 ±1.47^{ef}	80.17±0.46^{bc}
	150µg	37.88±1.21^{cdef}	83.93±0.49^{b}
Chloroform	**50µg**	32.81±0.37^{ef}	70.44±1.50^{de}
	100µg	34.4±0.79^{ef}	73.92±1.12^{cd}
	150 µg	37.76±5.28^{cdef}	85.12±1.06^{b}
Ethyl acetate	**50 µg**	32.81±0.37^{ef}	60.88±0.16^{fg}
	100µg	41.46±0. 87^{cde}	62.18±0.35^{efg}
	150 µg	45.43±1.85^{cd}	67.49±3.81^{def}
		BHT	**Trolox**
Control	**50 µg**	40.76^{h}±5.08	40.76±0.37^{h}
	100µg	81.53^{bc}±7.09	81.53±0.75^{bc}
	150 µg	107.51^{a}±13.11	122.3±1.13^{a}
LSD		**4.22**	**8.60**

Data represented as Mean±SD % of three replicates in each group, statistical significant is carried out using SPSS computer program and Co-state computer program, where unshared letter is significant at p ≤0.05.

Table 4
Total antioxidant capacity inhibition % of T. atomaria different extracts

	Concentrations (µg/ml)			
***T. atomaria* extracts**	**100µg/ml**	**200µg/ml**	**300µg/ml**	**400µg/ml**
Ethanol 80%	94.49 ±12.17^{k}	117.25±1.78^{ij}	171.52±10.0^{de}	212.72±6.52^{b}
Petroleum ether	97.61±5.95^{k}	125.95±3.04^{hi}	148.46±1.72^{f}	194.04±2.38^{c}
Chloroform	104.16±4.16^{jk}	142.85±2.38^{fgh}	174..97±2.38^{d}	216.66±3.57^{b}
Ethyl acetate	94.64±8.93^{k}	104.76±2.38^{jk}	145.83±9.88^{fg}	155.35±6.55^{ef}
Control (vit.c)	127.97±8.93^{ghi}	147.37±12.1^{f}	230.14±6.18^{ab}	243.46±3.56^{a}
LSD	8.93			

Data represented as Mean±SD % of three replicates in each group, statistical significant is carried out using SPSS computer program and Co- state computer program, where unshared letter is significant at p≤0.05.

Table 5
Anti-inflammatory activity of different fractions of T. atomaria ethanolic extract

***T. atomaria* extracts concentration (µg /ml)**	Inhibition %				
	Ethanol extract and partition fractions				Reference control
	Ethanol 80%	**Petroleum ether**	**Chloroform**	**Ethyl acetate**	**Diclofenattc Sodium**
50	50.72 ± 2.01^{j}	60.84 ±3.16^{gh}	63.95± 3.46^{g}	56.85± 4.20^{hi}	74.04 ± 0.37^{f}
100	53.00 ± 1.52^{ij}	80.05 ±1.54^{e}	78.23± 1.32^{ef}	76.78 ± 4.20^{ef}	81.74 ±0.63^{de}
150	53.39± 0.89^{ij}	95.33 ± 4.91^{b}	100.56± 0.2^{a}	88.61± 1.69^{c}	85.5 ± 0.21^{cd}
200	55.03±0.76`^{ij}	96.14±5.47^{ab}	100.54± 0.05^{a}	100.34± 0.3^{ab}	88.47 ± 0.30^{c}
LDS	5.17	3.22	2.98	5.99	7.12

Data represented as Mean±SD % of three replicates in each group, statistical significant is carried out using SPSS computer program and Co-state computer program, where unshared letter is significant at p ≤0.05.

Table 6

Anti-Alzheimer's activity % (cholinesterase inhibitory activity) of different fractions of T. atomaria ethanolic extract

	Extracts concentration (µg/ml)		
	25µg/ml	50µg/ml	100µg/ml
Ethanol 80%	34.8±0.14[a]	37.9±0.45[b]	42.0±0.8[b]
Petroleum ether	28.8±0.63[b]	40.4±0.9[a]	46.4±0.3[c]
Chloroform	28.2±0.7[b]	40.7±0.07[b]	49.5±1.2[bc]
Ethyl acetate	29.2±1.1[c]	31.1±1.06[a]	41.1±0.53[a]
Donepezil (drug)	49.0±0.9[b]	56.4±0.3[c]	59.5±1.2[bc]

Data represented as Mean ±SD of three replicates in each group, statistical significant is carried out using SPSS computer program and Co- state computer program, where unshared letter is significant at p ≤0.05.

Table 7

Anticancer activity of different fractions of T. atomaria ethanolic extract against cancer cell lines (HePG2, A549, HCT116 and MCF7)

T. atomaria extracts	Inhibition % Concentration (100ppm/ml)			
	HePG2	HCT116	A549	MCF7
Ethanol 80%	53.8±2.45[a]	2.5±0.05	92.7±3.78[e]	75.6±4.87[h]
Petroleum ether	87.6±5.89[b]	0	84.4±4.98[f]	64.3±7.98[i]
Chloroform	98.6±3.65[c]	0	100.00±3.87[e]	100.00±8.90[j]
Ethyl acetate	17.5±1.23[d]	0	11.3±1.10[g]	9.30±0.08[k]

Data represented as Mean±SD % of three replicates in each group, statistical significant is carried out using SPSS computer program and Co-state computer program, where unshared letter is significant at p≤0.05.

Table 8

IC_{50} and IC_{90} values of different fractions of T. atomaria ethanolic extract against cancer cell lines (HePG2, A549, MCF7 and HCT 116)

T. atomaria extracts	Cell lines					
	HePG2		A549		MCF7	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
Ethanol 80%	-	-	58.5	87.7	65.5	106.6
Petroleum ether	-	-	60.4	95.7	65.5	106.6
Chloroform	-	-	16.8	33.9	22.3	52.81
Ethyl acetate	-	-	-	-	-	-

DISCUSSION

The present results reveal the presences of vanillic acid (4968.78µg/100g), benzoic acid (6286.57 µg/100g) and pyrogallol (1531.04µg/100g) in *T. atomaria* phenolic composition. In addition, high contents of epicatechein (447.97µg/100g), caffeic (382.19 97µg/100g), ferulic (380.3497µg/100g) and saiycilic (653.9397µg/100g) as well as flavonoids were detected. While, the flavonoid composition of *T. atomaria* revealed the presence of high contents of hisperdin and hespertin (228.61 and 269.0697µg/100g, respectively). Also, high content of narengin, rutin and luteolin were found (108.11, 109.19 and 104.01µg/100g, respectively). In addition to, rosmarinic, quercetin, kampferol, apegenin and 7-hydroxyflavone were also detected. Flavonoids are known as nature's tender drug since it is having numerous biological and pharmacological activities. Recent findings regarding the activities of flavonoids such as antiviral, antifungal, antioxidant, anti-inflammatory, antithrombic, anticarcenogenic, hepatoprotective and cytotoxic have generated interest in researches of flavonoids. [18] The presence of various secondary metabolites in brown algae seaweeds is a clear indication of their pharmaceutical potential. [19]One of the confirmed properties of flavonoids is their ability to act as anti-oxidants. Flavonoids react directly with the free radicals lead to free radicals inactivity. Through, eliminating the free radicals, flavonoids decline LDL oxidation (low density lipids) *in vitro*. The second is the flavonoids interact with cell membranes, induced membrane changes that may affect the oxidation rate of the membrane lipid and/or protein. The separation of some flavonoids in the hydrofobic core of membranes may decrease the access of oxidants, protecting structure and function of membranes. [20] Moreover, a phenolic compound can be oxidized into a quinoid compound that is similar to vitamin K, participating in the redox reaction. By chemical and/or ezymatic oxidation, quercetin, which is the most representative flavonoid, is converted into ortho-quinone leading to quinone-methides by isomerisation, reactive intermediates which alkilate the DNA. In addition, the interference of flavonoids with NO-synthase activity is another way to remove ROS. [21]The anti-inflammatory effect of *T. atomaria* may be not reported and *may* be explained on the basis of the presence of phenolic and flavonoid in *T. atomaria* may have the potential to inhibit or minimize tissue injury and maintain homeostasis in the immune system in a dose dependent manner. It's well-known that, inflammation is the first response of the immune system to infection and plays a pivotal role in many diseases. [22] NO is considered as one of the factors leading to inflammation in macrophages. NO also, plays an important effect in body functions, but its over-production in macrophages, can cause an inflammation and autoimmune diseases. [23] COX-2 is involved in NO

generation causing inflammation. [24] So, the suppression of these inflammatory parameters has been shown to be critical in the inflammation treatment. According to the previous reports, pro-inflammatory cytokines, including TNF-α and IL-1β, induced iNOS which playsthe primer role of inflammation in macrophages. The pro-inflammatory activity of TNF-α can be mediated through its controlling several intracellular pathways and enhances IL-6 and IL-1β production, exerting inflammation, and cytotoxicity. [25] Thus, *T. ataomira* different extracts may inhibits NO production via the suppression of iNOS and COX-2 in a dose dependent manner and may reduce mRNA transcription of TNF-α, IL-1β, and IL-6 as well as may suppressed MAPKs activation or function which is the mechanical signals in inflammatory process.The present results declared anti-inflammatory activity of *T. atomaria* comparable to that standard. Methyl ester derivatives have been identified in the brown algae. The presence of these compounds in brown algae strongly suggested that these organisms possess an active LOX with ω-6. [26] The presence of the hydroxy fatty acids-HODTA and 13-HOTE, the likely products of 15-LOX catalyzed oxidation of linoleic or linolenic acids from *Laminaria saccharina*. [27] ATD-2 (stearidonic acid:SA), ATD-4 (EPA) and ATD-9 (AA) were identified from the edible brown seaweed *Undaria pinnatifida*. SA was anti-inflammation in mouse ear. EPA also exhibited anti-inflammatory activity against edema, erythema, and blood flow. Although AA declared anti-inflammatory activities at low concentration. [26] The brown alga is also known to have SA, which suppress leukotriene responsible for inflammation. [26] Yoon et al. [28] suggested that *S. micracanthum* brown algae blocking *NF*-kB signaling pathway due to inhibition of pro-inflammatory mediator. Also, the dichloromethane and ethanol extracts of *Sargassum fulvellum* and *Sargassum thunbergun* respectively inhibited an inflammatory symptom of edema due to the presence of identified anti-inflammatory compound6,6'-bieckol in ethanol extract. [26]The imbalances between the endogenous antioxidants and ROS cause serious disorders such as cancer, cardiovascular disease, hypertension, diabetes mellitus, inflammatory diseases, neurodegenerative diseases and ageing. [29] Among them, cancer and Alzeheimer diseases as they the leading threat for the world population; therefore, researchers are continually seeking a good source with potent antioxidant ability, cancer suppressing as well as cholinesterase inhibitory activities as an alternative for dietary supplements. Marine algae are considered as a rich source of natural antioxidants. [30] In fact, the most cytotoxic anticancer and amelioration of neurodegenerative agents including apoptosis characteristic cell changes, diminishing cancer cells and inhibition activity of cholinesterase. [29, 31] In this study, *T. atomaria* was identified as the apoptotic inducing potency against different cancer cells and cholinesterase inhibitory activity characterized neurodegenerative disorder. The present results confirmed the best anticancer activity as well as cholinesterase inhibitory activity of choloroform extract followed by petroleum ether and finally ethanol extract against the different cancer cell line under investigation and cholinesterase inhibitory activity. It has been suggested that, through their anti-oxidant action, flavonoids have a preventive role against stomach and colon cancers. The gastro-intestinal tract is continually exposed to ROS endogenously provided or resulting from food. Flavonoids also have the ability to inhibit deamination of nitrogenous bases of DNA structure by species derived from HNO_2, to up-regulate the metabolism of toxins or antioxidant enzymes in the gastro-intestinal tract. [21] ROS can change the DNA and leads to mutations. Previous works suggested that flavonoids target kinases phosphoring proteins in specific sites. Flavonoids interact with the signaling pathways of PI3-kinase (phosphoinositide 3-kinase), Akt/PKB (protein-kinase B), tyrosine kinase P1KC (protein-1 kinase C) and MAP (mitogen-activated protein) kinase. Stimulatory and suppressive actions of flavonoids on these pathways affect cell functions by altering the phosphorylation of the target molecules and by modulating gene expression. [32] Flavonoids may also alter growth signaling by inhibiting receptor phosphorylation or by blocking growth-factor receptor binding. In addition, flavonoids inhibit protein kinases Fyn and Lck, two representative members of SRC family of non-receptor kinases, involved in signaling in T cells transport. [21] So, the anticancer activity of flavonoids exert through blocking cells cycle. Treatment of prostate cancer by quercetin led to inhibition the rate of cell viability and proliferation. Beside, quercetin produce cancer cells apoptosis by controlling protein expression of Hsp90 (heat shock protein 90) which, in turn, suppressed the growth of cells and produce cancer cells death. [33] Furthermore, quercetin and kaempferola ctivating ERK (extracellular signal-regulated kinase) which suppressed A549cells proliferation derived from a lung carcinoma. [34] Quercetin also stimulating p21 and p53 protein expression which stopped cell cycle in HepG2 cells. [35] Moreover, quercetin (248 μM) controlling mutant p53-proteinexpression to undetectable levels in the cell breast cancer cell lines. From all previous explanation it can be said, flavonoids induce mechanisms that kill cancer cells and inhibit cell proliferation. Polyphenols also, can activate signals of cell-death that initiates apoptosis in maligne cells, inhibiting cancer development or progression. Malignant cell exposure to quercetin long time resulted in death of cancer cells and apoptosis through thymidylate synthase suppression. [36] Also, apigenin and ginestein elevated TRAIL-mediated cytotoxicity in HeLa cells, while this effect disappeared for kaempfeol and querectin. [37] Cytotoxicity was induced by luteoline causing suppressing of PI3K/Akt (phosphattidylinositol 3'-kinase), NF-kappa B (nuclear factor kappa B) and XIAP (X-linked inhibitor of apoptosis protein) and enhance pathways of apoptotsis especially p53 protein. [38] At high concentrations, most flavonoids inhibit AP-1 activity (activator protein 1) through MAPK (mitogen-activated protein kinase) pathway causing apoptosis. [21] In addition, the cytotoxicity effects showed by *T. atamaira* may be due to the presence of diterpenes common to brown algae, as they exhibited activity against tumor cells. [39] It was suggested that phenolic compounds inhibit telomerase activity in tumor cells. [40] On the other hand, all the tested extracts showed hydroxyl radical scavenging activity and total antioxidant capacity at the determined concentrations of inhibitors. The phenolic and flavonoid

contents of the extracts give the supportive facts that correlate with the determined antioxidant activity which improved neurodegenerative disorders including Alzeheimer disease. In addition, different kind of secondary metabolite could be involved for the antioxidant activity that correlated well with improving mental disorders [31].

CONCLUSION

T. atomaria different fractions declared antioxidant, anti-inflammatory, anti-Alzheimer's and anticancer activities against different cancer cell lines, HepG2, HCT116, MCF-7 and A549 that may be due to antioxidant scavenge activity of *T. atomaria* different fractions related to its phenolic and flavonoid composition. High contents of e-vanillic acid, benzoic acid, pyrogallol, epicatechein, caffeic, ferulic and saiycilic were detected in phenolic composition of *T. atomaria*. While, the flavonoid composition of *T. atomaria* revealed the presence of high contents of hisperdin, hespertin, narengin, rutin and luteolin, rosmarinic, quercetin, kampferol, apegenin and 7-hydroxyflavone. Thus, extensive further works must be done to elucidate *T. atomaria* biological activities in animal model and in clinical trial to use it as a supplement against different disorders.

CONFLICT OF INTEREST

Conflict of Interest declared none.

REFERENCES

1. Abou-El-Wafaa GSE, Shaaban KA, El-Naggar MEE, Shaaban M. Bioactive constituents and biochemical composition of the Egyptian brown alga *Sargssum subrepandum* (FORSK). Revista Latinoamericana de Química. 2011;39(1-2): 62-74.
2. Hanan KM, Shimaa EM. Seasonal variations in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt. Oceanologia. 2013;55 (2): 435-52.
3. Xu N, Fan X, Yan X, Tseng CK. Screening marine algae from China for their antitumor activities. Journal of Applied Phycology. 2004; 16: 451-6.
4. Ismail MM, Gheda SF, Pereira L. Variation in bioactive compounds in some seaweeds from Abo Qirbay, Alexandria, Egypt. Rendiconti Lincei. Scienze Fisiche E Naturali. 2015;1-11.
5. Rafiquzzaman SM, Kim YE, Lee MJ, Mohibbullah M, Alam BM, Moon SI, Kim JM, Kong IS. Anti-Alzheimers and anti-inflammatory activities of a glycoprotein purified from the edible brown alga *Undaria pinnatifida*. Food Research International. 2015;77:118-24.
6. Joshi NVM, Srisudha S. Biochemical characterization, haemagglutinating activity and cytotoxic activity of *Padina gymnospora* (Kutzing) Sonder. International Journal of Biological & Pharmaceutical Research. 2012;3(8): 956-61.
7. Vadlapudi V. Antioxidant activities of marine algae. In: "Medicinal plants as antioxidant agents: understanding their mechanism of action and therapeutic efficacy". ed: Anna Capasso. India: 2012;37/661(2): 189-203.
8. Ibañez E, Herrero M, Mendiola AJ, Castro-Puyana M. Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In: Marine bioactive compounds: Sources, Characterization and Application. Hayes M, editor. Springer Science+Business Media, LLC; 2012.p.55-98.
9. Viswanathan S, Ebciba C, Santhiya R, Nallamuthu T. Phytochemical screening and *in vitro* antibacterial, antioxidant and anticancer activity of *Amphiroa fragilissima* (Linneaus) J V Lamoroux. International Journal of Innovative Research in Science, Engineering and Technology. 2014;3(5): 12933-48.
10. Goupy P, Hugues M, Boivin P, Amiot MJ. Antioxidant composition and activity of barley (Hordeum vulgare) and malt extract and of isolated phenolic compounds. Journal of the Science of Food and Agriculture. 1999;79: 1625-34.
11. Ye H, Zhou C, Sun Y, Zhang X, Liu J, Hu Q, Zeng X. Antioxidant activities of ethanol extracts from brown seaweed *Sargassum pallidum*. European Food Research Technology. 2009;230(1): 101-9.
12. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry. 2001;73: 239-44.
13. Rahman H, Eswaraiah CM, Dutta AM. In-vitro anti-inflammatory and anti-arthritic activity of *Oryza sativa* Var. Joha rice (an aromatic indigenous rice of assam). American-Eurasian Journal of Agricultural & Environmental Sciences. 2015;15(1): 115-21.
14. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 1961;7(2): 88-95.
15. Mosmann T. Rapid colorimetric assays for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;65:55-63.
16. Thabrew MI, Hughes RD, McFarlane IG. Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. The Journal of Pharmacy and Pharmacology.1997;49(11):1132-35.
17. El-Menshawi BS, Fayad W, Mahmoud K, El-Hallouty SM, El-Manawaty M, Olofsson MH, Linder S. Screening of natural products for

therapeutic activity against solid tumors. Indian Journal of Experimental Biology. 2010;48:258-64.
18. Jiang H, Zhan WQ, Liu X, Jiang SX. Antioxidant activities of extracts and flavonoid compounds from *Oxytropis falcate* Bunge. Natural Product Research. 2008; 22(18):1650-56.
19. Melpha Y, Manchu N, James JE. Phytochemical evaluation of two brown seaweeds from Muttom and Rasthacaud coasts of Tamil Nadu, India. Journal of Chemical and Pharmaceutical Research. 2014;6(10):566-69.
20. Oteiza PI, Erlejman AC, Verstraeten SV, Keen CL, Fraga CG. Flavonoid-membrane interactions: A protective role of flavonoids at the membrane surface?. Clinical and Developmental Immunology. 2005;12(1):19-25.
21. Hertzog DI, Tica O. Molecular mechanisms underlying the anti-cancerous action of flavonoids. Current Health Sciences Journal. 2012;38(4): 145-49.
22. Kim KN, Heo SJ, Yoon WJ, Kang SM, Ahn G, Yi TH, Jeon YJ. Fucoxanthin inhibits the inflammatory response by suppressing the activation of NF-κB and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages. European Journal of Pharmacology. 2010;649:369-75.
23. Park HY, Han MH, Park C, Jin CY, Kim GY, Choi I.W, Kim ND, Nam TJ, Kwon TK, Choi YH. Anti-inflammatory effects of fucoidan through inhibition of NF-kB, MAPK and Akt activation in lipopolysaccharide-induced BV2 microglia cells. Food and Chemical Toxicology. 2011;49(8):1745-52.
24. Kim S, Kim E, Kang M, Lee J, Yang H, Lee J, Lim T, Jeon Y. Polyphenol-rich fraction from *Ecklonia cava* (a brown alga) processing by-product reduces LPS-induced inflammation *in vitro* and *in vivo* in a zebrafish model. Alage. 2014;29(2):165-74.
25. Ryu BM, Choi IW, Qian Z, Heo S, Kang D, Oh C, Jeon Y, Jang CH, Park WS, Kang K, Je J Kim S, Kim Y, Ko S, Kim G, Jun W. Anti-inflammatory effect of polyphenol-rich extract from the red alga *Callophyllis japonica* in lipopolysaccharide-induced RAW 264.7 macrophages. Algae. 2014;29(4):343-53.
26. Kathiravan V, Panneerselvam N, Palanikumar L. An untapped resource for natural anti-nflammatory compounds from marine macroalgae. International Journal of Pharm and Bio Sciences. 2015;6(2):579-95.
27. Rorrer GL, Modrell J, Zhi C, Yoo HD, Nagle DN, Gerwick WH. Bioreactor seaweed cell culture for production of bioactive oxylipins. Journal of Applied Phycology. 1995;7(2):187-98.
28. Yoon WJ, Ham YM, Kim SS, Yoo BS, Moon JY, Baik JS, Lee NH, Hyun CG. Suppression of pro-inflammatory cytokines, iNOS, and COX-2 expression by brown algae *Sargassum micracanthum* in RAW264.7 macrophages. EurAsian Journal of BioSciences. 2009;3(17): 130-43.
29. Valko M, Leibfritz D, Mancol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry and Cell Biology. 2007;39: 44-84.
30. Ngo D, Wijesekara I, Vo T, Ta QV, Kim S. Marine food-derived functional ingredients as potential antioxidants in the food industry: an overview. Food Research International. 2011;44: 523-9.
31. Lakmal HHC, Samarakoon KW, Lee WW, Lee J, Abeytunga DTU, Lee H, Jeon Y. Anticancer and antioxidant effects of selected Sri Lankan marine algae. Journal of the National Science Foundation of Sri Lanka. 2014;42(4):315-23.
32. Williams RJ, Spencer JPE, Rice-Evans C. Flavonoids: antioxidants or signalling molecules? Free Radical Biology and Medicine. 2004;36(7): 838-49.
33. Aalinkeel R, Bindukumar B, Reynolds JL, Sykes DE, Mahajan SD, Chada KC, Schwartz SA. The dietary bioflavonoid, quercetin, selectively induces apoptosis of prostate cancer cells by down-regulating the expression the expression of heat shock protein 90. Prostate. 2008;68(16):1773-89.
34. Hung H. Dietary quercetin inhibits proliferation of lung carcinoma cells. Forum of Nutrition. 2007;60: 146-57.
35. Mu C, Jia P, Yan Z, Lin X, Li X, Liu H. Qercetin induces cell-cycle G1 arrest through elevating Cdk inhibitors p21 and p27 in human hepatoma cell line (HepG2). Methods and Findings in Experimental and Clinical Pharmacology. 2007;29(3):179-183.
36. Haghiac M, Walle T. Quercetin induces necrosis and apoptosis in SCC-9 oral cancer cells. Nutrition and Cancer. 2005;53(2): 220-31.
37. Szliszka E, Czuba ZP, Jermas K, Krol W. Dietary flavonoids sensitize HeLa cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). International Journal of Molecular Sciences. 2008;9:56-64.
38. Lopez-Lazaro M. Distribution and biological activities of the flavonoid luteolin. Mini-Reviews in Medicinal Chemistry.2009; 9(1):31-59.
39. Gedara R, Zubía E, Ortega M, El-Sharkavy S, Salama O, Shier T, Halim A. Cytotoxic hydroazulenne diterpenes from the brown alga *Dictyota dichotoma.* Zeitschrift für Naturforschung C. 2003; 58:17-22.
40. Guedes ACÉ, da Silva TG, Aguiar JS, de Barros LD, Pinotti LM, Sant'Ana AEG. Cytotoxic activity of marine algae against cancerous cells. Revista Brasileira de Farmacognosia. 2013;23(4): 668-73.