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Nano Capsulated Polyphenol Extracted from Oyster Mushroom (*Pleurotus ostreatus*), Characterization and Stability Evaluation

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Abstract: The development of serious diseases i.e. Breast, colon and prostate cancers in human population at large is affected by many factors i.e. food habits, chemical substances and stress. Polyphenols as secondary metabolites in specific plants could be extracted and used as antioxidant, anti-atherogenic, anti-diabetic, anti-cancer, anti-viral, and anti-inflammatory properties. These compounds could denote electron to free radical through different mechanism and thus affect oxidation and microbial growth.
The extraction kinetics of polyphenol Oyster mushroom (*pleurotus ostreatus*) by Supercritical Co_2 Fluid extraction at (100 °C and 400 bar) was evaluated. Total phenol extracts were determined using Folin-Ciocalteu reagents. SFE was found to produce higher phenol recoveries than traditional methods. The production of natural nanopolyphenols by using ultrasonic (mechanical process) aside with encapsulation could be used in treatment of some serial diseases such as cancer, (increase its capacity to induce apoptosis in cancerous cell by modulating cell signaling cascades).
Key words: Nanotechnology; SFE; characterization; stability; cell-line.

Introduction

The relationship between diet and health has a great effect to improve human consumer health. Therefore, efforts have been done to define some foods that have additional benefits to human health beyond the true one as functional foods.

The extraction of these functional foods and their use as antioxidant antiviral, antihypertensive contribute consumer's well-being. Supercritical fluid extraction, subcritical water extraction and pressurized liquids are the most used methods for extraction.

Functional foods nowadays have get a great interest as they provide many benefits in additional to nutritional and energetic i.e. physiological, antihypertensive, antioxidant or anti-inflammatory[1].

Antioxidant are the most important compounds with functional properties[2,3,4], they can play important role against lipid peroxidation, their losses during production and storage and thus increasing the interest of these products and their demand by consumers[5, 6].

Vegetal and plant kingdom have become the main source of antioxidant compounds. Among them the main families are phenolic compounds, carotenoids and tocopherols.

Polyphenolic compounds can be used as chemoperventive agents to inhibit cancer development and propagation[7].

Cancer nanotechnology products with their particular size and characteristics can onfiltrate tumors deeply with a high level of specificity[8, 9]. The nanoencapsulation is an important issue to enhance bioavailability of polyphenols[10, 12].

In this study, polyphenols supercritical fluid extraction was used as new technique to extract polyphenol compounds providing higher selectivity's, shorter extraction times and do not use toxic organic solvents comparing with traditional methods with low selectivity, low extraction yield, time consuming and laborious[13, 14].

Several reviews have been written about compressed fluid extraction of plants, microalgae and other stuff focused in the obtention and characterization of functional food[3, 6, 14].

The objective of this study was the extraction and characterization of new natural ingredients (phenolic nano particles) which can be used in functional foods and nutraceutical products through microencapsulation based delivery systems a side with their biological activity using new extraction method as well as their and characteristics.

Materials & Methods

Sample preparation

Oyster mushroom i.e *Pleurotus ostreatus* (Mycelia & Whole) obtained from the special unit of ″Production, Cultivation & Utilization of Mushroom″ at NRC was used.

Oyster mushroom (Mycelia & Whole) was treated with 1 % sodium metabisulfite for 3 minutes, sliced and homogenized to small particle size in a Sonicator (Ultrasonic processor) XL No. 2015-010 in dark place. The homogenized samples were placed in petri dishes and stored in a refrigerator at –80°C for 2 days until freeze. Then all mushroom samples were freeze-dried (LABCONCO, Kansas City, USA) at –50 °C & 0.014 mbar for 2 days to reach moisture content of 4 %. Mushroom powder was ground and stored at –80C° in brown glass bottle to prevent oxidative damage until extraction

Supercritical Extraction

Phenolic compounds were extracted from oyster mushroom (20 gm) at (100 °C) and (400 bar) pressure as described by Mendiola[15], until no significant amount of extracted phenolic could be collected. The extracted sample was stored in brown glass bottle in a refrigerator at –80°C until used for physical & chemical properties evaluation.

Physical & Chemical Properties

Physical Properties

Transmission Electron Microscopy

The morphology of the extracted phenolic compounds was examined by transmission electron microscopy (TEM) using JED 1230, JEOL Ltd., Tokyo, Japan).

FT-IR analysis

Extracted phenolic compounds were subjected to FT/IR - 600 Fourier Transform Infrared Spectroscopy (Jasco) and the structure of polysaccharides were measured[16].

HPLC

HPLC of phenolic compounds was performed in Mycotoxin Central Lab, NRC using a chromatograph. Supelcosil™ LC-18 column (15 cm, 2 µm) was used to separate individual lycopene at ambient temperature.

Samples (5µl) dissolved in methanol / dichloromethane (1:1 v/v) were injected into the HPLC system for phenolic compounds analysis. Methanol with 10 % (v/v) acetonitrile was used as mobile phase.

The mobile phase was delivered at a flow rate of 1ml / min under isocratic conditions. The separated ergotheonine, catechin, p-coumaric acid and quecertin, peaks were monitored by a UV detector at 200-400 nm. Standards were used for identification and quantification based on a calibration curve[17].

Chemical Properties:

Antioxidant activity measured by DPPH:

The hydron atoms or electrons donation ability of the corresponding extracts were measured from the bleaching of purple colored method solution of 1,1–Diphenyl-2-picrylhydrozyl (DPPH). The effect of mushroom methanolic extracts on DPPH radical was measured according to Hatano[18] as follow:

One ml of various concentrations (0.2-1.0 mg ml^{-1}) of the mushroom extracts in methanol and water was added to a 1 ml of DPPH radical solution in methanol (final concentration of DPPH was 0.2mM).

The mixture was shaked vigorously and allowed to stand for 30 min. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan).

Inhibition of the free radical DPPH in percent (1%) was calculated using Eq.1:

1% = 100 x (A control – A sample) / A control ……(1)

Where, A control is the absorbance of the control reaction (containing all reagent except the test compound), and A sample is the absorbance of the test compound. Butylated hydroxytoluene (BHT) was used as a control (All chemicals are from Sigma-Aldrich GmbH, Germany).

Total Antioxidant determined by conjugated diene methods

Antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide arising from linoleic acid[19].

Five mg of phenolic compounds - linoleic acid mixture were dissolved in 1 ml of chloroform (HPLC grade). Then, 25µl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml of oxygenated distilled water was added with vigorously shaking. The reaction mixture (2.5 ml) was transferred to test tubes and 0.5 ml of various concentrations (0.4-2.0 mg ml^{-1}) of the extracts in methanol and water were added and the emulsion system was incubated for up to 2 h at 50 °C. The same procedure was repeated with the positive control BHT.

After this incubation period, absorbance was continued until color of phenolic compounds disappeared. The bleaching rate (R) of phenolic compounds were calculated according to Eq. 2:

R = In (a / b) / t ……..... (2)

Where, In = natural log, a = absorbance at time 0, b = absorbance at time t

(30, 60, 90 and 120 min)[20].

The antioxidant activity (AA) is calculated in terms of percent inhibition related to the control using Eq.3:

AA = [(R control – R sample) / R control] x 100 …….. (3)

Antioxidant activities of the extracts were compared with butylated hydroxytoluene (BHT) at 0.4 mg ml^{-1} and blank consisting of only 0.5 ml methanol and water.

Reducing Power:

The reducing power of mushroom extracts was determined according to the method of Oyaizu[21]. Mushroom extracts (0.2 – 1.0 mg ml^{-1}) in methanol and water (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide and incubated at 50 °C for 20 min. Then, 2.5 ml of 10 % Trichloroacetic acid were added, and the mixture was centrifuged at 200 g (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml deionized water and 0.5 ml of 1 % ferric chloride. Finally the absorbance was measured at 700 nm against a blank. Butylated hydroxytoluene (BHT) was used as a control.

Total phenolic Compounds:

Total phenolic compounds of the Supercritical extracts were determined according to the method described by Oviasogie[22], including Folin-Ciocalteu reagent and gallic acid as standard. 1ml of the extract solution containaing 2000µg extract was added to a volumetric flask. 45ml distilled water and 1 ml Folin-Ciocalteu reagent was added, then the flask was shaked vigorously. After 3 min, 3 ml of Na_2CO_3 (2 %) solution was added and the mixture was measured at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from the standard gallic acid graph using Eq.4:

A = 0.0021 gallic acid – (µg) – 0.0074 (R2: 0.9908) …….. (4)

Microencapsulation

Phynolic compounds preparation of sodium alginate beads, sodium alginate was dissolved in distilled water to produce alginate solutions with concentration of 6 % w/v, (*d*p = 3 mm) was done by standard ionotropic gelation through a syringe as described by Kubic[23].

Transmission Electron Microscope

The morphology of microencapsulated phenolic compounds nanoparticle was measured as described previously.

Antioxidant activity measured by DPPH

The antioxidant activity of encapsulated phenolic compounds was measured as was described previously.

Cell-Line Assay

Human cell lines i.e. Human prostate cancer cells (DU145) and breast cancer cell line (MCF-7) were treated with prepared nano antioxidants i.e. lycopene, oleoresin , carotenoids and polyphenolic compounds (1-5 µM) for 48 and 96 h. Cell viability was monitored using the method of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. The cell cycle was analyzed by flow cytometry, and apoptotic cells were identified by terminal deoxynucleotidyl transferase- mediated dUTP-biotin nick-end labeling (TUNEL) and by 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) [24]. All experiments were done in vitro bioassay human tumors cell line for drug discovery Lab. National Research Centre (NRC).

Results & Discussion

Spurcritical Extraction of Phenolic Compounds:

Results showed an increase in the yield of phenolic compounds extracted by supercritical after optimization all conditions. The yield reached to 4430 µg/gm which is much higher than that extracted by conventional methods 1700 µg/gm (about 3 times).

Physical & Chemical properties

Transmission Electron Microscopy

The morphology of the phenolic compounds nanoparticles after freeze drying was obtained by using Transmission electron microscopy.

Figure (1) shows the TEM of phenolic compounds nanoparticles with diameter range from 61- 145 nm.

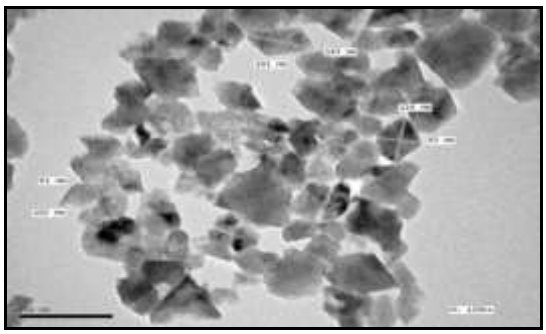

Figure (1): TEM of the phenolic compounds extracted by supercritical CO_2

FT-IR analysis

Figure (2) shows FTIR spectra of extracted phenolic compounds by supercritical fluid CO_2 with different absorption bands at wave numbers ranging from 4000-400 cm^{-1}. The (O-H stretching) appeared at 3425.92 cm^{-1} band, and is only identified in the high pressure extracts.

Bands at 2924.52 cm^{-1} and 2857.99 cm^{-1} referred to C-H stretching of methylene and methyl.

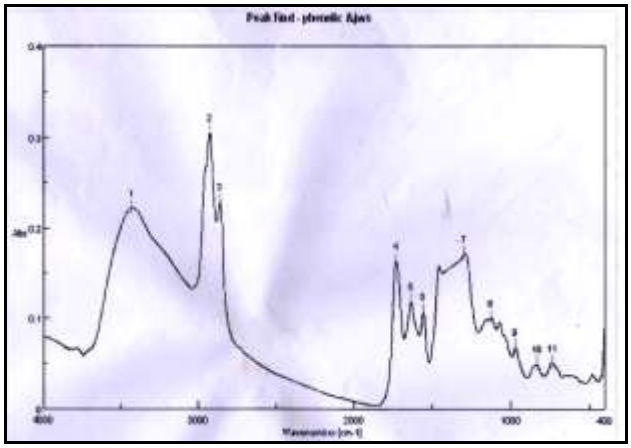

Fig. 2. FTIR analysis of extracted phenolic compounds by supercritical fluid CO_2

The individual bonds of functional groups (in fingerprint region) are shown at 1800-300 cm^{-1} bonds. While the C=OH stretching vibrations appeared as sharp bond at 1730.8 cm^{-1} and is due to the presence of high amounts of carboxylic acid in the extract. A small peaks at 1633.41 cm^{-1}, 1555.31 cm^{-1}, 1295.93 cm^{-1} and 1128.15 cm^{-1} were assigned to stretching C=C, s C-O and C-O respectively. Some peaks were observed out of plane at 970.983, 833.098 and 483.08 cm^{-1} referring to R-CH-CH-R, C-H and CH_2, respectively.

HPLC

Figure (3) show that the phenolic compounds of the nano phenolic capsules of all samples stored for 2,4and 6 months had several derived phenolics. The major peak for samples corresponds to the 2-Methyl4,6-DiNitro Phenol in comparison with standard used (retention time of 9.540). The compounds identified in comparison with standards used are : phenol, 4-nitro-phenol, 2,4 di nitro phenol, chloro-phenol, 2-nito phenol, 2-methyl 4,6- di nitro phenol 2,4-di methyl phenol, 4-chloro3-methyl-phenol, 2,4-di chloro phenol, 2,4,6-tri chloro-phenol and penta chloro phenol.

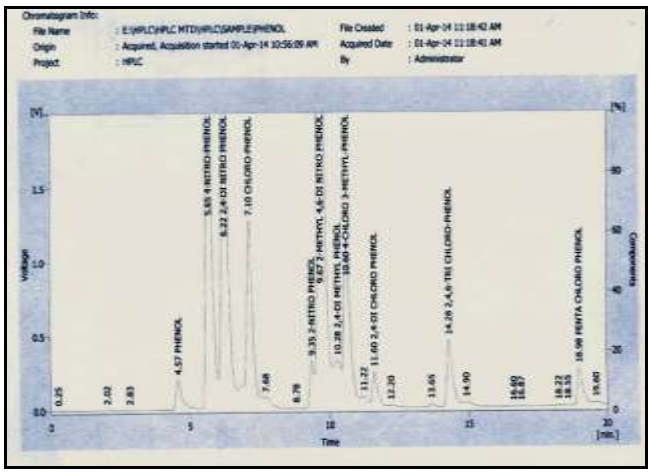

Fig.3. HPLC standard for phenolic compounds

Chemical Properties:

1-Antioxidant activity by DPPH:

Table (1) and Figure 4 showed the ability of polyphenolic compound extracted by supercritical from mushroom to decrease the absorbance of DPPH radical (discoloration from purple to yellow) which was due to the reaction of antioxidants present in mushroom extract and the radical.

This scavenging activity could exhibit IC_{50} value at the extract concentration of 97 µg/ml dry mushroom extract.

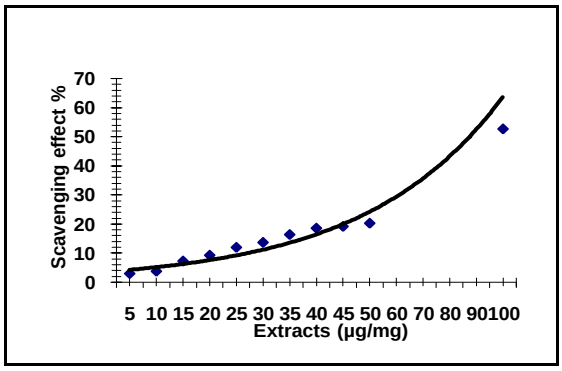

Fig.4. Scavenging effect % of various concentration extract (5 – 100 µg/ml) on DPPH

Table 1. Antioxidant activity as assessed by DPPH scavenging method:

Sample extract	DPPH (µg/mg)	Scavenging effect %
Ethyl Acetate	5.0	2.960
	10.0	3.763
	15.0	7.236
	20.0	9.201
	25.0	11.945
	30.0	13.697
	35.5	16.370
	40.0	18.500
	45.0	19.167
	50.0	20.232
	100.0	52.607

$$\text{Scavenging effect \%} = \frac{A_{515nm} \text{ of sample} - A_{515nm} \text{ of blank}}{A_{515nm} \text{ of blank}} \times 100$$

Conjugated diene methods

Table 2 and Figure 5 showed that oyster mushroom polyphenol had excellent antioxidant activity profile at 2.0 mg/ml comparing to control synthetic BHT which showed strong activity against linoleic acid oxidation.

Table 2. Antioxidant activity of polyphenol from oyster mushroom

Sample	Antioxidant activity % for Sample concentration (mg/ml)								
	0.4	**0.6**	**0.8**	**1.0**	**1.2**	**1.4**	**1.6**	**1.8**	**2.0**
Methanol soluble extract	63.78	67.48	72.00	78.66	79.32	80.16	81.08	81.78	82.05
BHT	94.67	95.77	95.82	96.01	96.57	97.60	97.73	98.14	98.54

$$\text{Antioxidant activity \%} = \frac{R_{control} - R_{sample}}{R_{control}} \times 100$$

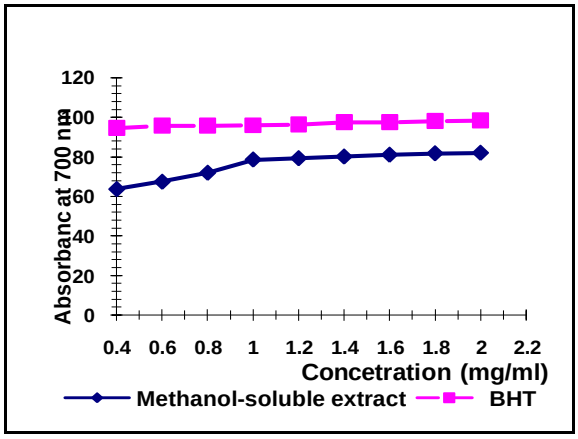

Fig.5. Antioxidant activity of essential oils of oyster mushroom

Reducing power

Table 3 and Figure 6 showed the comparison in the reducing power between the synthetic antioxidant BHT and the supercritical extracts of oyster mushroom. Results indicated that at concentration of 0.2 mg/ml the absorbance of BHT was 1.293 while that of oyster mushroom extract was 0.179, suggesting the ability of this extract to act as free radical as it reduce iron (III) and did so in a linear relationship.

Table 3. Reducing power of different extracts concentration

Sample	Sample concentration (mg/ml)				
	0.2	**0.4**	**0.6**	**0.8**	**1.0**
Methanol-soluble extract	0.179	0.244	0.391	0.513	0.602
BHT	1.293	1.650	2.416	3.434	3.561

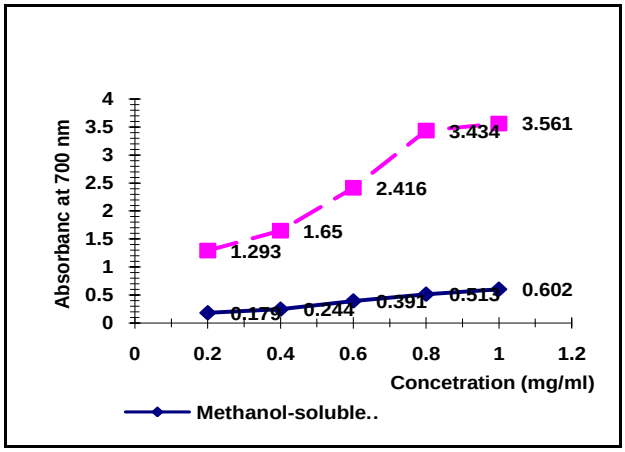

Fig.6. Reducing power of different extracts concentration

Total Phenol

Data obtained by total phenolic assay showed that total phenolic in mushroom extract was very high in methanol soluble extract being 30.248 µg GAE_s/mg indicating the positive relationship between antioxidant activity and amount of phenolic compounds in the extracts.

Transmission Electron Microscope

The morphology of the nano encapsulated phenolic compounds nanoparticles (entrapped in alginate) was measured by using Transmission electron microscopy.

Figure (7a and b) shows the TEM of the encapsulated phenolic compounds nanoparticles which showed the core and the wall of the entrapped phenolic compounds with diameter range from 131 to 669 nm.

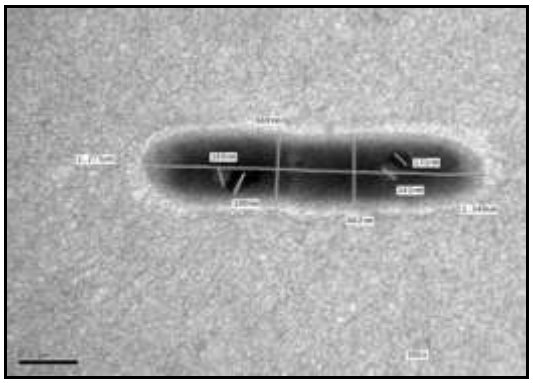

Fig.7a. TEM of nano encapsulated phenolic Compounds

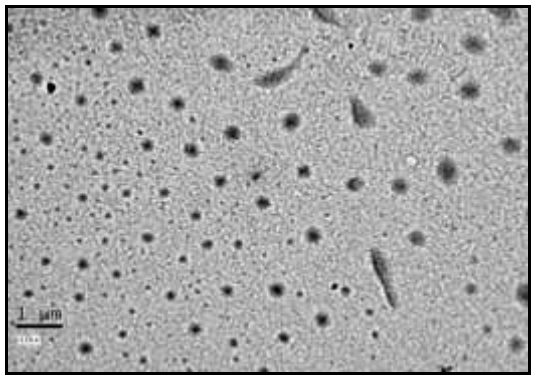

Fig.7b. TEM of nano encapsulated phenolic compounds

Antioxidant activity of nano phenolic by DPPH

The antioxidant activity of the nano phenolic compounds was determined after encapsulation (Table 4 and Figure 8). Results indicate that the microencapsulation process increased the ability of phenolic compounds to scavenge DPPH free radicals. Figure (8) revealed that the Ec_{50} of the encapsulated nano phenolic compounds was 80 µg/mg lower, than 97 µg/mg for nano extract, which indicates that the lower the Ec_{50} the greater the antioxidant activity[25].

Table 4. Antioxidant activity of nano phenolic compounds encapsulation by sodium alginate beads:

Sample extract	DPPH (µg/mg)	Scavenging effect %
Ethyl Acetate	5.0	2.362
	10.0	4.116
	15.0	8.038
	20.0	11.238
	25.0	14.592
	30.0	15.380
	35.0	18.098
	40.0	21.674
	45.0	24.234
	50.0	26.462
	60.0	29.851
	70.0	38.033
	80.0	46.392
	90.0	51.191
	100.0	57.856

$$\text{Scavenging effect \%} = \frac{A_{515nm} \text{ of sample} - A_{515nm} \text{ of blank}}{A_{515nm} \text{ of blank}} \times 100$$

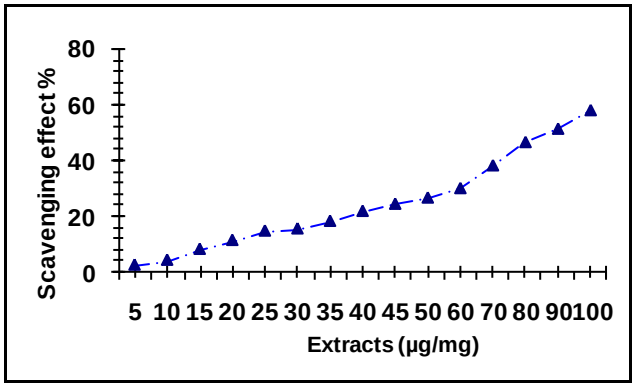

Fig.8. Scavenging effect % of various nano extract concentration (5–100 µg/ml) on DPPH after encapsulation by alginate beads.

Cell-Line

The effect of nano antioxidants on cell cycle was measured after treated for 48 and 96 hs (Table 5 and 6). Table 5 showed that MCF-7 cells treated with (3-5 µM) nano antioxidants for 48h resulted in G_0/G_1 arrest (by the accumulation of cells) aside with concomitant decrease in the G_2/M phase. While for DU145 cells, results showed minor changes in the profile distribution of cell cycle accompanied with an increase for cells in G_0/G_1 phase an decrease in G_2/M phase but only with the higher dose of nano antioxidant (5 µM).

Table 6 showed that the changes in cancer treated cells after 96h with nano antioxidant were significant as for MCF-7 cells, the same results were obtained after 95h like for 48h. While for DU145, there was an increase in cells in G_2/M phase, followed by a decrease in G_0/G_1 phase after treated with nano antioxidants (3 and 5 µM).

Table (5): Effect of NanoAntioxidants (1-5 μM) on cell cycle progression in different human cancer cell lines after 48 hours

Cell line	Cell Cycle Phases	CT	1ug	3ug	5ug
MCF-7	G_O/G_1	65.05	65.35	65.65	65.30
S		20.00	20.50	18.25	20.35
G_2/M		32.81	30.81	11.08	17.56
DU145	G_O/G_1	59.30	58.65	76.53	76.35
S		16.52	17.68	18.47	20.87
G_2/M		48.33	47.15	66.75	63.82

Table (6): Effect of NanoAntioxidants (1-5 μM) on cell cycle progression in different human cancer cell lines after 96 hours

Cell line	Cell Cycle Phases	CT	1ug	3ug	5ug
MCF-7	GO/G1	46.05	59.30	54.30	53.10
S		20.10	24.90	22.80	25.20
G2/M		69.45	70.85	63.60	59.07
DU145	GO/G1	46.89	56.28	42.27	36.27
S		15.19	19.36	11.02	11.57
G2/M		35.92	42.78	73.13	73.32

Apoptosis

Results in Table 6 showed an increase in apoptosis (which can be a useful indication of cancer cell kinetics) in the two cell lines (MCF-7 and DU145) after treated with nano antioxidant (3μM) for 96 h. While the cancer cell line (DU145) showed an increase number of cells and apoptosis G2/M phase by 3.5 and 4 fold, respectively for the same treatment. Nano antioxidants increased also by 2-fold apoptosis in MCF-7.

So, our results proved that there was a significant reduction in the viable cells number in the two cancer cells line after 48h treatment with nano antioxidant with the promotion of cell cycle arrest followed by decreased cell vialability in the majority of cell lines after 96h accompanied with an increase in the apoptosis. These results are in agreement with the results obtained in lycopene[26].

Conclusions

The rising need to extract either functional compounds or nutraceuticals from natural sources continuea for economically and ecologically feasible extraction technologies.

Using larger amount of solvent in the traditional extraction methods increase the operating costs and cause additional environmental problems. Therefore, compressed fluids have become an interesting alternative to obtain antioxidants from different natural sources.

Phenolic compounds extracted from oyster mushroom after treated with ultrasonication gave nanoparticles with high antioxidant activity. These treatment also improved the capacity of nano antioxidants to inhibit cell proliferation, arrest cell cycle in different phases and increase apoptosis in breast and prostate lines after 96h.

Microencapsulation process increased the ability of phenolic compounds to scavenge DPPH free radicals.

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References

1. Goldberg, L. (1996). Functional Foods, Designer Foods, Pharma Food Nutraceuticals. Chapman and Hall, London.

2. Halliwell, B. (1996). Oxidative stress, nutrition and health. Experimental strategies for optimization of nutitional antioxidant intake in humans. Free Radic. Res., 25: 57-74.
3. Herrero, M.; Cifuentes, A.; and Ibáñez, E. (2006). Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae Food Chemistry, 98 (1): 136–148.
4. Fogliano, V. and Vitaglione, P. (2005). Functional foods: planning and development Molecular Nutrition & Food Research, 49: 256–262.
5. Senorans, F.J.; Ibanez, E. and Cifuentes, A. (2003). New trends in food processing. Crit. Rev. Food Sci. Nutr., 43: 507-526.
6. Plaza, M.; Cifuentes, A. and Ibanez, E. (2008). In the search of new functional food ingredients from algae. Trend Food Sci. Tech., 19: 31-39.
7. Santos, I.S.; Ponte, B.M.; Boone, P.; Silva, A.M. and Souto, E.B. (2012). Nanocapsulation of polyphenols for protective effect against colon- rectal cancer. Biotechnology Advances, JBA-06603; No of pages 10.
8. Siddiqui, I.A.; Adhami, V.M.; Christopher, J.C. and Mukhtar, H. (2012). Impact of nanotechnology in cancer; emphasis on nanochemoprention. Int. J. Nanomedicine 7: 591-605.
9. Kloesch, B.; Becker, T.; Dietersdorfer, E.; Kiener, H.; and Steiner, G. (2013). Anti-inflammatory and apopotic effects of the polyphenol curcumin on human fibroblast-like synoviocytes. Int. Immunopharmacd, 15(2): 400-5.
10. Nair, H.B.; Sung, B.; Yadav, V.R.; Kannappan, R.; Chaturvedi, M.M. and Aggarwad, B. (2010). Delivery of anti-inflammatory nutraceuticals by nanoparticles for the prevention and treatment of cancer. Biochem. Pharmacol., 80: 1833-1843.
11. Sanna, V. and Sechi, M. (2012). Nanoparticle therapeutics for prostate cancer treatment. Maturitas http://dx.doi.org/10.1015/j.maturitas.201201.016.
12. Quiroz-Reyes, C.N.; Ronquillo-de Jesús, E.; Duran-Caballero, N.E. and Aguilar-Méndez, N.A. (2014). Development and characterization of gelatin nanoparticles loaded with a cocoa-derived polyphenolic extract. Fruits, 69(6): 481-489.
13. King, M. and Bott, T. (1993). Extraction of Natural Products Using Near-Critical Solvents, Chapman & Hall.
14. Mendiola, J.A.; Herrero, M.; Cifuentes, A. and Ibañez, E. (2007). Use of compressed fluids for sample preparation: food applications. J. Chromatogr A. 2007 Jun 8: 1152(1-2): 234-46.
15. Mendiola, J.A.; Rodriguez-Meizoso, I.; Señoráns, F.J.; Reglero, G. and Cifuentes, I. (2008). Antioxidants in Plant Foods and Microalgae extracted using Compressed Fluids, EJEAFChe 7:3301-3309.
16. Adt, I.; Toubas D.; and Pinon, J.M. (2006). FTIR spectroscopy as a potential tool to analyse structural modifications during morphogenesis of Candida albicans. Arch Microbiology, 185: 277-285.
17. Xu, F.; Yuan, Q.P. and Dong, H.R. (2006). Determination of lycopene and β-carotene by highperformance liquid chromatography using sudan I as internal standard. Journal of Chromatography B. 838: 44-49.
18. Hatano, T.; Kagawa, H.; Yasuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in Licorice root: their relative astringency and radical scavenging effects. Chem. Pharmacol. Bull, 36: 1090-2097.
19. Dapkevicius, A.; Venskutonis, R.; Van-Beek, T.A. and Linssen, P.H. (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J. Sci. Food Agric., 77: 140-146.
20. Cheung, L.M.; Cheung, P.C.K. and Ooi, V.E.C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem., 81: 249-255.
21. Oyaizu, M. (1986). Studies products of browning reactions: Antioxidatative activities of browing reaction prepared from glucoxamine. Jpn. J. Nutr., 44: 307-415.
22. Oviasogie, P.O.; Okoro, D. and Ndiokwere, C.L. (2009). Determination of total phenolic amount of some edible fruits and vegetables. African J Biotech. 8: 2810-2819.
23. Kubic, C.; Sikora, B. and Bielecki, S. (2004). Immobilization of dextranase and its use with soluble dextranase for glucooligosaccharides synthesis. Enzyme and Technology, 34: 555-560.
24. Levy, J.; Bosin E.; Feldman, B.; Giat, Y.; Miinster, A.; Danilenko, M. and Sharoni, Y. (1995). Lycopene is a more potent inhibitor of human cancer cell proliferation than either alpha-carotene or beta-carotene. *Nutr Cancer***,** 24:3, 257–266.
25. Chen, F.A.; Wu, A.B.; Shieh, P.; Kuo, D.H. and Hsieh, C.Y. (2006). Evaluation of the antioxidant activity of Ruellia tuderosa. Food Chemistry, 94: 14-18.
26. Teodoro, A.J.; Oliveira, F.L.; Martins, N.B.; Maia Gde, A.; Martucci, R.B. and Borojevic, R. (2012). Effect of lycopene on cell viability and cell cycle progression in human cancer cell lines, Cancer Cell International, 12(1): 36-45.

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