Primary Assessment of the Biological Activity of Jojoba Hull Extracts

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Abstract: To our Knowledge no work up to the present moment has been reported in the literature on the phenolic extracts of jojoba hull. Thus the aim of the present work was to add value to this waste product by investigating the potentiality of different jojoba hull extracts as neutraceuticals. The efficiency of methanol, ethanol, acetone, isopropanol and ethyl acetate at concentrations of 100, 80, 70, 60, and 50% to extract phenolic compounds were investigated. Results revealed that 60% acetone extracted optimum phenolic compounds (13.9 mg/g hulls). Extraction at room temperature yielded more phenolic compounds than extraction at 45°C. On the other hand, 70% methanol extract of jojoba hulls exhibited the highest AOA (95.33%). The 70% methanol extract was added to a butter cake at 100 and 200 ppm as well as 200 ppm BHT. The cake was stored at room temperature and the butter analyzed every week for acid, iodine and peroxide values. Results proved that the addition of methanol extract delayed the oxidation of butter. The extracts of jojoba hulls exhibited different levels of antimicrobial activities on five food borne pathogenic bacteria. The 70% methanol extract of jojoba hulls showed potential as anti-carcinogenic agent on four different cell line carcinomas.
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1. Introduction

Due to the health awareness of the global population and the interest in a clean environment, the possibility of turning waste products into valuable nutraceuticals became desirable. Also substituting synthetic pharmaceuticals with neutraceuticals of plant origin became much advisable.

Seed hulls are among the food industry waste products that have proved to contain beneficial compounds such as phenolic compounds. Seed hulls reported to contain phenolic compounds include sunflower hulls (Mohamed and Taha, 2005), rice hulls (Asamarai *et al.,* 1996), buckwheat hulls (Watanabe, 1997), navy bean hulls (Onyenecho and Hettiarachchy, 1991), rapeseed hulls(Amarowicz *et al.,* 2000), peanut hulls (Duh and Yen, 1995), and sesame coat (Chang *et al.,* 2002).

Simmondsia chinensis (link) Schneider (syn. S. California nut, Buxus chinensis link) (Family simmondsiaceae) is a perennial dioeciously evergreen shrub, endemic to Sonoran desert of Arizona, California and New Mexico. It is now commercially cultivated in many countries all over the world. It is better known as jojoba (pronounced ho-ho-ba). Natives of California highly prized the fruit for food and the seed oil as medicine for cancer, kidney disorder, obesity, sore heart, warts and wounds **(**Leung and Foster, 1996**).**

Actually jojoba oil which is a wax ester and not oil is the most valued part of the seeds. Jojoba oil is used as a replacement for whale oil and its derivatives (Undersander *et al.,* 1990). Jojoba oil is used greatly in skin care (Gunstone, 1990). Jojoba oil is sought greatly by cosmetic manufacturers (Wisniak, 1994) Jojoba biodiesel has been explored as a cheap, sustainable fuel that can serve as a substitute for petroleum diesel (Bouaid *et al.,* 2007). The meal remaining after the removal of the oil from jojoba seeds represent a potential amendment for animals/ or humans. The jojoba meal contain toxic compounds simmondsin and its derivatives (kolodziejczyk *et al.,* 2000), which should be removed before being used as food or feed. No attention has been given to jojoba seed hull to our knowledge up to the present day.

Phenolic compounds exhibit a wide range of physiological properties, such as antioxidant, anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia, 1997; Samman *et al.,* 1998; Puupponen-Pimiwaw *et al.,* 2001; Manach, *et al.,* 2005). There are Several types of phenolics including simple phenolic compounds, such as the cinnamic acids (I) or aldehydes (II) and polyphenolics, such as the 'condensed' (III) and 'hydrolysable' tannins (IV) (Haslam, 1981) The main phenolic subclasses in oilseed products are phenolic acids (hydroxylated derivatives of benzoic and cinnamic acids), coumarin, flavonoid, tannins and lignin group of compounds (Shahidi and Naczk, 2003).

Extraction of phenolic compounds from plant material is influenced by extraction conditions, i.e. solvent polarity, particle size, concentration, temperature and time. The impact of the extraction of

phenolic compounds on the analysis has often been overlooked as substantial variations in the extraction procedures; and solvents are documented in the recent literature (Antolovich *et al.,* 2000). Several solvents such as methanol, ethanol, acetone, water, ethyl acetate; and to a lesser extent, propanol, dimethyl formamide, dimethyl sulfoxide and their combinations have been used for the extraction of different classes of phenolic compounds (Antolovich *et al.,* 2000; Naczk and Shahidi , 2004; Parejo *et al.,* 2004; Vrhovsek *et al.,* 2004).

The aim of the present investigation is to achieve the effective utilization of a wasted material from an industrial oil crop (jojoba seed). Synthetic pharmaceuticals are being replaced nowadays with phytochemicals from natural sources. Therefore the biological activity of the jojoba seed hull extracts will be studied. Several solvents as well as several solvent concentrations, followed by several extraction times will be investigated to determine optimum conditions for the extraction of phenolic compounds from jojoba hulls. The antioxidant activity of the phenolic extracts will be determined. The effect of the methanol extract of jojoba hull will be evaluated as an antioxidant in butter cake during storage. Then the antimicrobial effect of the different phenolic extracts and anticarcinogenic activity of the methanol extract will be examined. If the phenolic extracts of the jojoba hulls prove to have the previous biological activity, it will certainly be a great profit to the human health and the pharmaceutical industry.

2. Materials and Methods

Jojoba hulls: were obtained from the Egyptian Natural Oil Co., (NATOTL), Ismailia Branch and Farm Factory 10th of Ramadan City Egypt. It belonged to the crop of 2010. The hulls were ground using a Wiley mill to pass through 60 mesh screen. The ground hulls were defatted with n- hexane in a Soxhlet apparatus and saved for further work.

Wheat flour 72% extractions: were obtained from South Cairo Mills Co., Egyptian Ministry of Supply and Trade. Moreover, Fresh butter, vanilla and baking powder were purchased from the local market.

Microorganisms: were obtained from the Microbiological Resources Center (Cairo MIRCEN) Faculty of Agriculture, Ain Shams University: *E.coli* 0157:H7 ATCC 51659, *Staphylococcus aureus* ATCC 13565, *Bacillus cereus* EMCC 1080, *Listeria monocytogenes* EMCC 1875 and *Salmonella typhimurium* ATCC25566.

Cell line Carcinoma: Different cell line carcinomas included: Liver Carcinoma Cell Line (HEPG2), Colon Carcinoma Cell Line (HCT), Cervical Carcinoma Cell Line (HELA), and Breast Carcinoma Cell Line (MCF7) were supplied and used in The National Cancer Institute, Biology Department, Cairo, Egypt.

Analytical methods:

Moisture, ash, protein, oil and crude fibre were determined in jojoba hulls according to (AOAC, 2005). Dietary fibre fractions were determined as recommended by Goering and Van Soest (1970).

Analytical methods were carried out on different crude phenolic extracts of jojoba seed hulls. Total phenolic compounds were determined by the Folin Ciocalteu method according to Hung *et al.,* (2002) and measured as gallic acid equivalent. Antioxidant activity was determined by two methods: Free radical scavenging activity according to Kuda *et al*. (2005) where crude phenolic extracts were dissolved in methanol to obtain a concentration of 500 ppm. 0.2 ml of this solution was completed to 4 ml by MeOH and 1 ml of DPPH (6.09×10^{-5} mol/L) was then added. The second method used is the coupled oxidation of β- carotene/ linoleic acid method described by Al-Shaikhan *et al.,* (1995). Determination was done at a concentration of 500ppm of each phenolic extract and 200 ppm BHT. Acid value, iodine value and peroxide value were determined in butter according to AOAC, (2005). Anticarcinogenic activity was determined in the National Cancer Institute (Biology Department) on several cell lines by the measurement of potential cytotoxicity of the phenolic extracts which was carried out by the Sulfo-Rhodamine-B stain (SRB) assay, according to the method of Skehan *et al.,* (1990). Antimicrobial activity for different extracts was tested against five pathogenic bacterial strains using the disc diffusion method as described by Kotzekidou *et al.*, (2008).

Effect of solvent type, solvent concentration, temperature and time on the extraction of phenolic compounds:

A detailed study using extracting solvents with different polarities was carried out. These solvents included ethanol, methanol, acetone, isopropanol, and ethyl acetate at concentrations of 100%, 80%, 70%, 60%, and 50%. These experiments were carried out at room temperature. Two grams jojoba hulls were extracted with 100 ml of each solvent in a shaking water bath for 24 hours. The solution was then filtered and filtrate saved. The residue was re-extracted with 50 ml of same solvent under same previous condition. The first and second filtrates were combined, evaporated under vacuum using a Buchi-rotary evaporator, to dryness. The dried extract was used to determine phenolic content and antioxidant activity.

Same experiment was carried out at 45°C for 1, 3, 6, 9, and 12 hrs, and at a meal: solvent ratio 1:75 w/v in just one extraction. 70% ethanol, 70% methanol, and 60% acetone were chosen to be examined in this experiment.

Preparation of fortified butter cake:

Butter cake was fortified with jojoba hull extract (70% methanol) with the aim of testing the power of the hull extract to inhibit oxidation of the butter in the cake. The ingredients of butter cake are given in Table 1 according to Mizukoshi *et al.* (1979) with little modification.

The natural antioxidant from jojoba hull extract was added to the butter cake mixture at 100 and 200 ppm levels and compared with synthetic antioxidant (BHT) at level 200 ppm. The product was baked at 191°C ± 5°C for 25 min. in an electric oven and the cake was stored at room temperature for three weeks. The control sample was made by the same method but without any added antioxidant.

Table 1: Ingredients of butter cake

Ingredients	Weight/g
Flour	200
Sugar	250
Whole egg	150
Vanilla	1
Baking Powder	13
Water	40
Fresh butter	100

The cake was extracted every week by soaking in n-hexane at room temperature for 48 hrs. The extract was filtered and evaporated to dryness. Extracted butter was analysed for its acid value, iodine value and peroxide value.

Statistical analysis:

The results are presented as average ± standard deviation (St. Dev.). All results were evaluated statistically using one way analysis of variance according to McClave & Benson, (1991).

3. Results and Discussion

Chemical composition of jojoba hulls is represented in Table 2. Results in Table are self-explanatory. Verbiscar and Banigan, (1978) are the only available authors to analyse jojoba hull and reported 10.7% moisture, 7.0% protein, 0.7% oil, 4.4% ash and 15.6% crude fibre. Our values are within the same range.

Table 2: Chemical Composition of jojoba seed hulls

Composition (%)	Jojoba hull ± = St. Dev.
Moisture	7.92 ± 0.324
Protein	3.34 ± 0.535
Oil	1.76 ± 0.461
Ash	2.15 ± 0.436
Crude fiber	17.13 ±0.333
Nitrogen free extract	67.70 ± 0.228
Simmondsin	0.19 ±0.662
Dietary Fiber Fractions	
Cellulose	17.29 ± 0.516
Hemicellulose	1.34 ± 0.852
Lignin	35.29 ± 0.458
Neutral detergent fiber (NDF)	54.17 ±0.277
Acid detergent fiber(ADF)	52.83 ± 0.739
Acid detergent lignin (ADL)	35.54 ± 0.981

Optimization of the extraction of phenolic compounds from jojoba hulls.

Since the solubility of phenolic compounds (PC) in general is governed by their chemical nature which may vary from simple to very highly polymerized substances, and also because the solubility of PC is affected by the polarity of solvent(s) used, it was advisable to first examine the suitability of the type of solvent for optimum extraction of phenolic compounds from jojoba hulls. Results of the solvent extraction of jojoba hulls with methanol, ethanol, acetone, isopropanol and ethyl acetate at concentrations 100%, 80%, 70%, 60%, and 50% are represented in Table 2. Results reveal a significant difference ($p<0.05$) between all extracting solvents at all concentrations except for 60% methanol and 60% isopropanol that showed no significant difference between them. Highest phenolic extraction was achieved with acetone at 80, 70, 60, and 50% reaching 9.93, 12.48, 13.90, and12.74 mg PC/g hulls, respectively. As for methanol and ethanol highest amount extracted 10.09 and 10.70 mg PC/g hulls, respectively, was accomplished with 70% solvent concentration.

Acetone probably extracted more PC because it is more polar than the other solvents. Kim *et al.* (2007) developed a method of designing solvents for the optimal extraction of bioactive ingredients from mulberry leaves using an alcohol-water binary solvent. From their study they reported that the extraction efficiency of the bioactive ingredients was correlated with the solvent polarity. This finding is in agreement with our results. Taha *et al.* (2011) studied the optimization of phenolic compounds and chlorogenic acid extraction from sunflower meal. They found that 80% acetone extracted maximum phenolics and chlorogenic acid when applying conventional, microwave-assisted, and ultrasound-

assisted extractions. Sun *et al.* (2006) prepared oat groat phenolic extracts using acetone, methanol and hexane. The acetone extracted highest amount of phenolic compounds. Acetone extract exhibited highest radical scavenging activity and highest Trolox value. Sun *et al*., (2006) and Taha *et al* (2011) findings are in agreement with our results.

Table 3: Effect of different solvents at different concentrations on the yield of phenolic compounds (mg/g) extracted from jojoba hulls at room temperature

Extracting Solvent	Solvent Concentration				
	100%	80%	70%	60%	50%
Methanol	6.05 ^{a} ± 0.04	7.79 ^{b} ± 0.04	10.09 ^{c} ± 0.03	9.70 b ± 0.03	9.30 d ± 0.03
Ethanol	3.32 ^{b} ± 0.04	7.27 ^{c} ± 0.03	10.70 ^{b} ± 0.03	9.50 c ± 0.05	9.61 c ± 0.04
Acetone	1.30 ^{c} ± 0.40	9.93 ^{a} ± 0.04	12.48 ^{a} ± 0.05	13.9 a ± 0.04	12.74 a ± 0.04
Isopropanol	1.11 ^{d} ± 0.03	5.46 ^{d} ± 0.07	7.80 ^{d} ± 0.03	9.7 b ± 0.04	4.20 e ± 0.03
Ethyl acetate	1.01 ^{e} ± 0.08	3.84 ^{e} ± 0.04	6.30 ^{e} ± 0.03	4.09 d ± 0.05	9.71 b ± 0.04
LSD at 5%	0.087	0.081	0.062	0.073	0.059

Different letters in each column indicates significant differences between solvents at (P<0.05) for each extraction concentration.

Time and temperature are other parameters that can affect extraction of the PC. The previous experiment was carried out at room temperature. Table 4 show the effect of temperature (45°C) and time of extraction (1, 3, 6, 9, and 12 hrs) on the amount of extracted PC from Jojoba hulls, using the more efficient three solvents according to results in Table 3. It is clear that there is no significant difference between the PC extracts at 6, 9, and 12 hrs, when using 60% acetone, 70% methanol or 70%ethanol. This could be explained by Fick's second law of diffusion revealing that final equilibrium will be attained between the solution concentrations in the solid matrix and solvent after a particular duration (Pinelo *et al.,* 2006). At 1 and 3hrs less PC were extracted by the three solvents. This result indicated that at 45°C, extraction for 6 hours is suitable for maximum extraction. Comparing extraction at room temperature for 2 nights while shaking, with extraction at 45°C for 1-12 hrs, it is clear that more PC were extracted at room temperature. 60% acetone extracted 10.47mg PC/g hulls at 45°C for 6 hours and 13.90 mg PC/g hulls at room temperature for 2 nights, 70% methanol and 70% ethanol at 45°C for 6 hrs extracted 8.53 and 8.20 mg PC /g hulls, respectively; and at room temperature for 2 nights extracted 10.09 and10.70 mg PC/ g hulls, respectively. It is expected that increase in extraction temperature is directly proportional to increase PC yield. Al-Farsi and Chang (2007) reported that increased temperature could promote the phenolic extraction by increasing both diffusion coefficient and solubility of phenolic compounds in extraction solvent. Besides that, intense heat from solvent was also able to release the cell wall phenolics and bounded phenolics by breaking down of cellular constituents (Wang *et al.,* 2008).The findings of the previous authors are contrary to our results. On the other hand, (Mueller-Harvey, 2001) reported that some phenolic compounds decomposed rapidly under high temperature and thus causes a reduction of the antioxidant capacity of a plant extract. This statement is in agreement with our results. We believe that perhaps some of the phenolic compounds were degraded by heating, or 6h extraction time might not be enough to solubilize the entire PC From jojoba hulls.

Table 4: Effect of time on the yield of phenolic compounds extracted from jojoba hulls at 45°C

Time(h)	60% Acetone	70% Ethanol	70% Methanol
1	8.77 ^{c} ± 0.15	5.90 ^{c} ± 0.09	5.40 ^{c} ± 0.20
3	9.73 ^{b} ± 0.15	7.50 ^{b} ± 0.20	6.53 ^{b} ± 0.35
6	10.47 ^{a} ± 0.25	8.53 ^{a} ± 0.15	8.20 ^{a} ± 0.20
9	10.63 ^{a} ± 0.15	8.63 ^{a} ± 0.15	8.27 ^{a} ± 0.21
12	10.63 ^{a} ± 0.15	8.63 ^{a} ± 0.15	8.27 ^{a} ± 0.21
LSD at 5%	0.321	0.282	0.438

Different letters in each column indicates significant differences between time of extraction at (p<0.05) for each investigated solvent

Antioxidant activity of Phenolic compounds extracted from jojoba hulls.

Phenolic substances possess many biological effects which are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals (Bahman *et al.,* 2007). For examples, flavonols, cinnamic acids, coumarins and caffeic acids are well known polyphenolic compounds with strong antioxidant properties. Hence play an

important role in protecting food, cells and organs from oxidative damage (Osawa, 1999). These compounds (phenolic substances) all share the same chemical patterns, with one or more phenolic groups for hydrogen proton donors and neutralize free radicals (Pajero *et al.,* 2002; Milliauskas *et al.,* 2004; Atoui *et al.,* 2005; Galvez *et al.,* 2005).

Table 5 show the antioxidant activity of the different phenolic extracts resulting from extraction of jojoba hull with different solvents at room temperature. The antioxidant activity was determined by the DPPH scavenging method and the β- carotene/ linoleate method. In terms of antioxidant activity, the free radical scavenging activity (FRSA) is shown for three concentration levels of the phenolic extracts (25µl, 50µl, 100µl.). Statistical analysis of the FRSA made clear that there is no statistical difference (p<0.05) between the FRSA of the 70% methanol, 70% ethanol, and 60% isopropanol extracts of jojoba hulls and BHT at the three examined concentrations. A significant difference at (p<0.05) between 60% acetone jojoba hull extract and the other extracts and BHT at 25 and 50µl concentrations was detected, but no significant difference was found between all extracts and BHT at 100µl concentration, except 70% ethyl acetate which was significantly different at 100µl concentration. 60% acetone extract exhibited highest FRSA at 25 and 50µl concentration (59.93 and76.67 FRSA %, respectively), even higher than BHT (54.67 and73.35 FRSA %, respectively). At 100µl concentration the FRSA of the 70% methanol extract was the highest (81.19 FRSA %). In accordance the AOA as determined by the β-carotene/ linoleate method was also highest for 70% methanol extract of jojoba hulls (95.33%) compared to BHT (93.88%).These results led to the choice of the 70% methanol extract of jojoba hulls for further work. But in fact the methanol, ethanol and acetone extracts of the PC of jojoba hulls all possess very promising antioxidant properties. These results are in agreement with the results of other authors who reported on the efficiency of methanol extracts from seed hulls as antioxidants (Yen and Duh, 1995; Kyung *et al.,* 2006; Taha *et al.,* 2011). Seed coat plays an important role in protecting seeds from oxidative damage because the seed coat possesses large quantities of endogenous antioxidants such as phenolic compounds (Moure *et al.,* 2001).

Table 5: Antioxidant activity of jojoba hull phenolic extracts as determined by FRSA and β-carotene /linoleate methods

Phenolic extract	FRSA%		AOA%	
	25µl	50µl	100µl	
70% Methanol	52.66 ^{b} ± 2.83	66.44 ^{c} ± 1.90	81.19 ^{a} ± 4.06	95.33 ^{a} ± 1.53
70% Ethanol	54.23 ^{b} ± 2.95	67.03 ^{c} ± 3.25	80.74 ^{a} ± 4.11	89.66 ^{b} ± 1.53
60% Acetone	59.93 ^{a} ± 2.70	76.48 ^{a} ± 2.31	80.64 ^{a} ± 4.05	85.0 ^{c} ± 2.00
60% Isopropanol	53.28 ^{b} ± 3.00	69.67 ^{bc} ± 3.80	78.97 ^{a} ± 3.30	80.33 ^{d} ± 2.52
70% Ethyl acetate	45.38 ^{c} ± 2.36	48.84 ^{d} ± 3.61	63.97 ^{b} ±4.05	75.0 ^{e} ± 3.00
BHT	54.67 ^{b} ± 3.40	73.35 ^{ab} ± 3.71	76.87 ^{a} ± 2.77	93.88 ^{a} ± 3.01
LSD at 5%	5.148	5.663	6.69	3.8

Different letters in each column indicates significant differences between different solvents at (p<0.05) for FRSA at different concentrations, and AOA.

Effect of methanolic extract of jojoba hulls on the oxidation of butter-cake stored at room temperature for three weeks.

Seventy percent methanolic extract of jojoba hulls containing PC with AOA were added to a cake with butter as an essential ingredient in the cake. The methanolic extract was added at 100ppm and 200ppm to the cake to see its power of inhibition of butter oxidation during storage. BHT (200mg) was used for comparison. Acid value (AV), iodine value (IV) and peroxide value (PV) of the extracted butter were determined every week and taken as an indication for oxidation of butter. Results are represented in Table 6.

Statistical analysis revealed no significant difference (p<0.05) between AV, IV, and PV of the butter extracted from the cakes with 200ppm methanolic extract of jojoba hulls and 200ppm BHT, at zero time, 1, 2 and 3 weeks storage. Also no statistical difference between IV and PV of the butter with added 100and 200ppm methanolic extracts after one week storage period. All other treatments showed significant differences between all of them. AV, IV, and PV indicated that the 70% methanolic extract from jojoba hulls when added to the cake delayed oxidation of the butter, when compared to cake with no additions (control). 200 ppm methanolic extract showed more efficiency as antioxidant than 100ppm and was comparable to 200ppm BHT.

Table 6: Chemical characteristics of butter cake fortified with methanolic extract of jojoba hulls and BHT during three weeks of storage at room temperature.

Storage period (week)	Treatment	Acid Value (%)	Iodine Value (g/100g)	Peroxide Value (ml.equi.O_2/Kg)
Zero time	Control (no addition)	$0.71^{h} \pm 0.03$	$37.60^{bcd} \pm 1.39$	$2.7^{fg} \pm 0.20$
	Methanol 100ppm	$0.73^{h} \pm 0.04$	$39.83^{ab} \pm 1.40$	$2.36^{g} \pm 0.14$
	Methanol 200ppm	$0.75^{h} \pm 0.03$	$40.90^{a} \pm 1.75$	$2.27^{g} \pm 0.25$
	BHT 200ppm	$0.74^{h} \pm 0.03$	$41.20^{a} \pm 1.67$	$2.27^{g} \pm 0.25$
One week	Control (no addition)	$1.81^{c} \pm 0.04$	$30.37^{f} \pm 1.40$	$5.36^{dc} \pm 0.47$
	Methanol 100ppm	$1.16^{ef} \pm 0.04$	$35.33^{cd} \pm 1.81$	$4.07^{ef} \pm 0.45$
	Methanol 200ppm	$0.92^{gh} \pm 0.04$	$37.13^{bcd} \pm 1.75$	$2.7^{fg} \pm 0.04$
	BHT 200ppm	$0.85^{gh} \pm 0.04$	$38.47^{abc} \pm 1.75$	$2.53^{fg} \pm 0.40$
Two weeks	Control (no addition)	$2.51^{b} \pm 0.22$	$24.40^{g} \pm 1.18$	$10.73^{b} \pm 1.16$
	Methanol 100ppm	$1.53^{d} \pm 0.04$	$30.77^{f} \pm 2.55$	$7.53^{c} \pm 0.04$
	Methanol 200ppm	$1.19^{ef} \pm 0.04$	$34.57^{de} \pm 2.43$	$5.17^{de} \pm 1.16$
	BHT 200ppm	$0.99^{fg} \pm 0.10$	$35.90^{cd} \pm 2.33$	$4.93^{de} \pm 1.05$
Three weeks	Control (no addition)	$3.88^{a} \pm 0.35$	$19.63^{h} \pm 1.33$	$15.37^{a} \pm 1.33$
	Methanol 100ppm	$1.91^{c} \pm 0.25$	$26.53^{g} \pm 1.75$	$9.67^{b} \pm 1.84$
	Methanol 200ppm	$1.50^{d} \pm 0.13$	$30.33^{f} \pm 2.26$	$6.1^{cd} \pm 1.20$
	BHT 200ppm	$1.34^{de} \pm 0.07$	$31.36^{ef} \pm 2.66$	$5.83^{d} \pm 1.30$
LSD at 5%		0.22	3.158	1.614

Different letters in each column indicates significant differences between control butter and butter with additions and BHT at ($p<0.05$) for different storage times.

Acid value (AV), IV, and PV of the control butter at zero time were 0.71%, 37.60 g/100g , and 2.7 ml equiv O_2/ Kg., after three weeks of storage they changed to 3.88%, 19.63 g/100g, and 15.37ml. equiv. O_2/kg, respectively. Comparing AV of control (3.88%) after three weeks to 70% methanol extract 100 and 200ppm and BHT 200ppm it can be seen that the AV reached 1.19, 1.5, and 1.34%, respectively, IV reached 26.53, 30.33 and 31.36 g/100g, respectively compared to control 19.63 g/100g. While PV reached to 9.67, 6.1 and 5.83 ml equiv. O_2/Kg, respectively, in comparison to control (15.37ml equiv. O_2/kg). The increase in AV when compared to control at zero time is explained by the hydrolysis of the oil to free fatty acids which will lead to further formation of aldehydes and ketones (Kun, 1988). IV is the measure of unsaturation of any oil and the decline in iodine value is sometimes used to monitor the reduction of dienoic acids during the course of oxidation. Thus the decrease in IV during the whole storage period could be attributed to breaking of double bonds of unsaturated fatty acids of the lipid. Rehman (2006) added citrus peel extract to refined corn oil and stored at 25 and 45°C for 6 months to examine antioxidant capacity of the extract. He found that similar to our results a gradual increase in both the AV and PV, along with a decrease in IV. Last is the PV which is applicable for the early stages of lipid oxidation. It measures the peroxides which are the primary oxidation products. The PV of the butter increased during the three weeks but comparing PV of butter with methanolic extracts to control shows how the methanolic extract was a shelter that resulted in much less rancidity of the butter. On the other hand at end of the three weeks it became clear from the results that the addition of the 70% methanolic extract of jojoba hulls (100 and 200ppm) and 200ppm BHT delayed the rancidity of the butter.

Antimicrobial activity of phenolic compounds extracted from jojoba hulls.

There is considerable interest in the possible use of natural compounds as alternative food additives. They are used to prevent the growth of food borne pathogens or to delay the onset of food spoilage. Many naturally occurring compounds such as phenols (phenolic acids, polyphenols and tannins) have been considered in this context. Phenolics are being used in foods mainly for purposes such as antioxidants and other than antimicrobial agents (Nychas, 1995). Thus it seemed worthwhile to evaluate the phenolic extracts as antimicrobial agents.

The phenolic extracts of jojoba hulls using different extracting solvents were tested for their antimicrobial activity (AMA) against five bacterial strains using the disc diffusion method. The five bacteria were *Escherichia coli* 0157:H7 ATCC 51659, *Staphylococcus aureus* ATCC 13565, *Bacillus cereus* EMCC 1080, *Listeria monocytogenes* EMCC 1875 and *Salmonella typhimurium* ATCC25566. Comparing the effect of the different solvent hull extracts (80% methanol, ethanol, acetone, isopropanol and ethyl acetate) on the five bacteria strains, it is clear that the five extracts exhibited various degrees of inhibition against the 5 bacteria strains as presented in Table 7. Highest inhibition of *Bacillus cereus* was attained with ethyl acetate

jojoba hull extract, followed by isopropanol, methanol, acetone then ethanol with inhibition zone diameters 14.3, 10.8, 10.3, 8.3, and 8mm, respectively. *Staphylococcus aureus* show to be inhibited in decreasing order by methanol >isopropanol > acetone > ethanol > ethyl acetate extracts with inhibition zone diameter 15.5 >14 > 13.3 >13 >2.3mm, respectively. As with *Bacillus cereus* highest inhibition of Salmonella *typhimurium* was reached with ethyl acetate extract with 16.5mm inhibition zone diameter followed by ethanol extract with 11.6 mm inhibition zone diameter. Other extracts resulted in less zone inhibition between 9.7-5mm. For *Listeria monocytogenes highest* zone inhibition was with ethanol extract 16mm and lowest zone inhibition resulted from isopropanol extract10mm. *Escherichia coli* were inhibited by four extracts only and their inhibition zone diameters were in the following order 21.3 > 16.3 > 15.6 > 2.5mm for acetone, isopropanol, methanol and ethyl acetate, respectively. Ethanol extract exhibited no effects on *E. coli.*

The overall results indicated that different bacteria species exhibit different sensitivities towards phenolics. In the present work Gram-positive and Gram-negative microorganisms were affected by the phenolic extracts of jojoba hulls. Estevinho *et al.,* (2008) reported that the susceptibility of bacteria to phenolic compound and Gram reaction appears to have influence on growth inhibition. The inhibitory effect of phenols could be explained by interactions with the cell membrane of microrganisms and is often correlated with the hydrophobicity of the compounds (Sikkema et al., 1995; Weber and De Bont, 1996). Phenolic compounds could have an activating or inhibiting effect on microbial growth according to their constitution and concentration (Rauha *et al*., 2000; Reguant *et al*., 2000; Alberto *et al*., 2001; Estevinho *et al*., 2008; Rodríguez Vaquero *et al*., 2010).

Anticarcinogenic activity of a methanolic extract from jojoba hulls

This evaluation was carried out in the National Cancer Institute, Biology Department, Cairo. The experiment was done by the Sulfo-Rhodamine-B stain (SRB) assay. Jojoba hull extract (70% metabolic) has been evaluated as a chemopreventive agent. This was established by testing the jojoba hull methanolic extract for any cytotoxic activity against the following human tumour cell lines: Liver Carcinoma Cell Line (HEPG2); Colon Carcinoma Cell Line (HCT); Cervical Carcinoma Cell Line (HELA); Breast Carcinoma Cell Line (MCF7).

Figure 1 represents the effect of jojoba hull methanolic extract on the human carcinoma cell lines tested and the results are indicated by the IC50, which is the dose of the compound (jojoba hull phenolic extract) which kills surviving cells up to 50%. The smaller the concentration or dose the more effective is the compound. Looking at Figure 1, the following could be observed:

Methanolic extract of jojoba hulls was most effective on HELA cell line carcinoma with an IC 50 = 9.75µg / ml

IC 50 for HEPG was attained with 15.8 µg/ml of methanolic extract.

Following is IC 50 = 16µg/ml for MCF7 cell line.

Least affected was HCT cell line with IC 50 =19.8µg/ml.

These results indicate the potentiality of the 70% methanolic extract of jojoba hulls as an anticarcinogenic agent. This effect is probably due to the phenolic content of the jojoba hulls. The anticarcinogenic activity of phenolic compounds has been documented (Owen *et al.,* 2000; Cai *et al.,* 2004; Kerr and Sarangarajan, 2007). The Biology Department –National Cancer Institute-Cairo recommended that further pharmacological investigations *in vitro* and *in vivo* are required to confirm the activity of the tested 70% methanolic extract of jojoba hulls.

Conclusion

It can be concluded from this study that jojoba hull extracts are a very promising source of bioactive compounds with antioxidant, antimicrobial and anticancer properties. This neglected waste product from an industrial oilseed crop should be given more attention to get optimum benefits for the pharmaceutical (nutraceutical) industry.

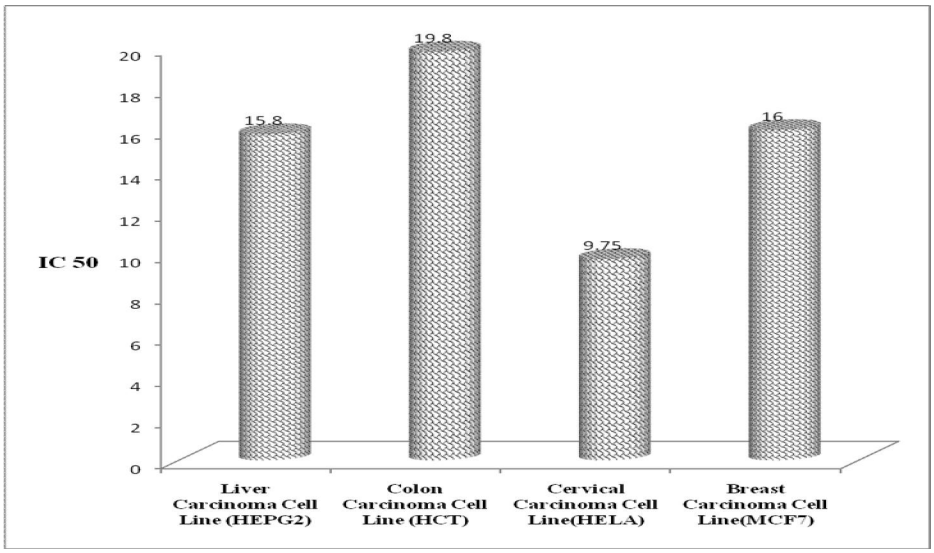

Figure 1: Anticarcinogenic effect of 70% methanolic extract of jojoba hulls.

Table 7: The effect of different jojoba hull extracts on the inhibition of some food borne pathogenic bacteria.

Jojoba hull extract	Strain/Inhibition zone diameter (mm)				
	B.c	St	Sa	Lis	E.coli
80% Methanol	10.3	15.5	9	11.6	15.6
80% Ethanol	8	13	11.8	16	-
80% Acetone	8.3	13.3	5	10.5	21.3
80% Isopropanol	10.8	14	9.7	10	16.3
80% Ethyl acetate	14.3	2.3	16.5	12.6	2.5

B.c= (*Bacillus cereus* EMCC1080), St.= *Staphylococcus aureus* ATCC 13565, Sa= (*Salmonella* typhymurium ATCC 25566), Lis = *Listeria monocytogenes* EMCC 1875, E. Coli = *Eschirechia.coli* 0157:H7 ATCC 51659

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References

1. Al Farsi, M.A. and Chang, Y., 2007. Optimization of phenolics and dietary fibre extraction from date seeds . Food Chemistry, 108: 977- 985.
2. Alberto, M.R. ,. Farias, M.E and. Manca, M.C da Nadra , M.C., 2001. Effect of gallic acid and catechin on *Lactobacillus hilgardii* 5w growth and metabolism of organic compounds. Journal of Agricultural and Food Chemistry. 49 : 4359- 4363.
3. Al-Shaikhan et al. Al-Shaikhan M.S., Howard, L.R. and Miller , J.C. Jr., 2005. Antioxidant activity and total phenolics in different genotypes of potato. Journal Food Science, 60: 341-343.
4. Amarowicz, R.,.Naczk, M. and. Shahidi, F., 2000. Antioxidant activity of crude tannin of canola and rapeseed hulls. Journal of the American Oil Chemist's Society,. 77: 956-961.
5. Antolovich, M., Prenzler, P.,.Robards, K. and. Ryan., D., 2000. Sample preparation in the determination of phenolic compounds in fruits. Analyst, 125: 989–1009.
6. AOAC, 2005 Association of Official Analytical chemists, "*Official methods of Analysis,* 15th, ed. Washington DC..
7. Asamarai, A.M., Addis, B.P., Epley, R.J. and Krick, T.P. 1996. Wild rice hull antioxidants.. Journal of Agricultural and Food Chemistry, 44: 126-130.
8. Atoui, A.K. , Mansouri, A., Boskou, G. and Kefalas, P. , 2005. Tea and herbal infusions: Their antioxidant activity and phenolic profile. Food Chemistry, 89: 37–36.
9. Bahman, N, Mohammed, K .and Hamidreza, I., 2007. In vitro free radical scavenging activity of five salvia species. Pakistan Journal Pharmacological Science, 20: 291–294.
10. Benavente-Garcia, J, Benavente-Garcia, O.,. Castillo, F. R. , Marin, A. Ortuno, and.Del Rio, J. A., 1997. Uses and properties of citrus flavonoids., Journal of Agricultural and Food Chemistry, 45: 4505–4515.
11. Bouaid , A., Bajo, L.,. Martinez, M. and Aracil, J., 2007 Optimization of biodiesel production from jojoba oil. Process Safety and Environmental Protection, 85: 378-382.

12. Cai , Y.,.Luo, Q ., Sun, M. and Corke,H., 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Science, 74: 2157-2184.
13. Chang, L.W., Yen, W.J., Huang, S.C. and Duh, P.D. , 2002. Antioxidant activity of sesame coat. Food Chemistry, 78: 347- 354.
14. Duh, P.D and Yen, G.C., 1995. Changes in antioxidant activity of and components of methanolic extract of peanut hulls irradiated with ultraviolet light. Food Chemistry, 54:127-131.
15. Estevinho, L., Pereira, A.P., Moreira, L., Dias, L.G and Pereira, E. , 2008. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chemistry and Toxicology, 46: 3774- 3779.
16. Galvez,M., Martin-Cordero, C., Hourghton, P.H. and Ayuso, M.J., 2005. Antioxidant activity of methanol extracts obtained from Plantago species. Journal of Agricultural and Food Chemistry, 53:1927–1933.
17. Gunstone , F.D., 1990. Jojoba oil. Endeavour, 14: 40-43.
18. Goering, H.K and Van Soest, P.V , 1970. In Forage Fiber Analysis ANKOM Technology 08|05, USDA Agricultural research Service, Handbook no.379 (1970) Washington, DC, USA.
19. Haslam, E.,1981. Vegetable tannins. In E. E Conn editor., The Biochemistry Of Plants. vol. 7, pp. 527-556. Academic Press, New York.
20. Hung Y.,.Sava, V.M, . Makan, S.Y. ,Chen, T.H.J., *et al.,* 2002. Antioxidant activity of melanins derived from tea: comparison of different oxidative states. Food Chemistry, 78:233-240.
21. Kerr, S.G. and Sarangarajan, R., 2007. Effects of phenolic antioxidants on four model cancer cell lines. The Journal of the Federation of American Societies for Experimental Biology, 21:882.10.
22. Kim, J.M., Chang, S.M.,.Kim, I.H., Kim, Y.E. 2007. Design of optimal solvent for extraction of bio-active ingredients from mulberry leaves. Biochemical Engineering Journal, 37: 271-278.
23. Kolodziejczyk, P.P.,.Lu,W., Ayerza,R. and Larminat, M.A., 2000. Capillary electrophoresis. A novel tool for Simmondsin analysis and its application to jojoba breeding. Industrial Crop Production, 12:193-202.
24. Kotzekidou, P.P., Giannakidis, P. and Boulamatsis, A., 2008. Antimicrobial activity of some plant extracts and essential oils against food born pathogens *in vitro* and on the fate of inoculated pathogens in chocolate. Swiss Society of Food Science and Technology, 41: 119-127.
25. Kuda, T.M., kawa, H.T.,Goto, H. and Araki R., 2005. Antioxidant property of four edible algae harvested in Noto peninsula Japan. Journal of Food Composition and Analysis, 18: 625-633.
26. Kun, T. Y, 1988. Improvements in frying qualities of lipid vegetable oil blending . Palm Oil Developments, 8: 1-4.
27. Kyung, I.J., Eunhu, P.,. Hae, R.P., You, J.H. , Seon, H.C and Seung, G.L., 2006. Antioxidant activity of far- infrared radiation of rice hull extracts on relative oxygen species scavenging and oxidative DNA damage in human lymphoctes Journal of Medical Foods. 9: 42-48.
28. Leung, A.Y and Foster, S., 1996. Encyclopaedia of common Natural Ingredients .2 nd ed. John Wiley and Sons. INC, New York.
29. Manach, C.,Mazur,A. and Scalbert, A., 2005. Polyphenols and prevention of cardiovascular diseases. Current Opinions in Lipidology, 16: 77–84.
30. McClave, J. T. and Benson., P. G., eds., 1991. In: Statistics For Business And Economics. Maxwell Macmillan International editions, pp. 272-295.Dellen Publishing Co. USA.,
31. Milliauskas, G., Milliauskas, P.R., Venskutonis, T.A. and Van-Beek, 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry,85:231–237.
32. Mizukosh, M., kawada, T. and Matsui, N., 1979. Model studies of cake baking .I: continuous observations of starch gelatinization and protein coagulation during baking. Cereal Chemistry, 56:305-310 .
33. Mohamed, G.F. and Taha, F. S., 2005. Extraction of sunflower hulls: their antioxidant activity on lipids of cooked mackerel fish. Alexandria Journal of Food Science and Technology, 2: 11-23.
34. Moure, A., Cruz, H.M., Franco, D., Dominguez , H.,. Sineiro , J., *et al.,* 2001. Natural antioxidants from residual sources. Food Chemistry, 72: 145-171.
35. Mueller-Harvey, I., 2001. Analysis of hydrolysable tannins. Animal Feed Science and Technology, 91:3-20.
36. Naczk, M. and Shahidi, F., 2004. A Review: Extraction and analysis of phenolics in food. Journal of Chromatography A., 1054: 95-111.
37. Nychas, G.J.E., 1995. Natural antimicrobials from plants. In G. W.Gould editor "New Methods of Food Preservation" Chapter 4, p.58, An Aspen Publication.
38. Onyenecho, S.N. and Hettiarachchy, S.N. , 1991. Effect of navy bean hull extract on the oxidative stability of soy and sunflower oils. Journal of Agricultural and Food Chemistry, 39:1701-1704.
39. Osawa, T. ,1999. Protective role of dietary polyphenols in oxidative stress. Mechanisms of Ageing Development, 111:133–139
40. Owen, R.W.,. Giacosa, A.,. Hull, W.E., Haubner, R., *et al.,* 2000 . The antioxidant/anticancer potential of phenolic compounds isolated from olive. European Journal of Cancer, 36: 1235-1247.

41. Parejo, O. Jauregui, F. Sanchez-Rabaneda, F. Viladomat, J. Bastida and C. Codina, 2004. Separation and characterization of phenolic compounds in fennel (Foeniculum vulgare) using liquid chromatography–negative electrospray ionization tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 52: 3679–3687
42. Parejo, I, Valadomat, F., Bastida, J., Rossa-Remero, A. *et al.,* 2002 .Comparison between the radical scavenging activities and antioxidant activity of six distilled and non-distilled Mediterranean herbs and aromatic plants. Journal of Agricultural and Food Chemistry, 50: 6882–6890.
43. Pinelo, M., Sineiro, J. and Núñe, M.J., 2006. Mass transfer during continuous solid–liquid extraction of antioxidants from grape by-products. Journal of Food Engineering, 77, 57-63.
44. Puupponen-Pimia, R, Nohynek, L., Meier , C., Ka¨hko¨nen, M.., 2001. Antimicrobial properties of phenolic compounds from berries. Journal of Applied Microbiology, 90: 494–507.
45. Rauha, J.P., Remes, S. Heinonen, M., Hopia A., and M. ,2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology, 56: 3-12.
46. Reguant C., Bordons, A., Arola, L. and Rozes, N., 2000. Influence of phenolic compounds on the physiology of *Oenococcus oeni,*. Journal Applied Microbiology, 88:1065-1071.
47. Rehman- Zia-ur, 2006. Citrus peel extract-A natural source of antioxidant. Food Chemistry, 99: 450-454.
48. Rodriguez-Vaquero, L.R., Serravalle, T., Manca de Nadra M.C. and. Strasser de Saad , A.M., 2010. Antioxidant capacity and antibacterial activity of phenolic compounds from argentinean herbs infusions. Food Control, 21: 779-785.
49. Samman, S., Lyons Wall, P. M. and Cook, N. C., 1998. Flavonoids and coronary heart disease: Dietary perspectives. In "Flavonoids in health and Disease" Rice-Evans C.A and Packer, L., editors pp. 469-481.
50. Shahidi, F. and Naczk M., 2003. Phenolic compounds in major oilseeds and plant oils .In FShahidi and Naczk editors, "*Phenolics in Food and Neutraceuticals*" Chapter 3, CRC Press.
51. Sikkema, J., De Bont, J. and Poolman, B., 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol Reviews, 59: 201–222.
52. Skehan, E.,.Storeng, R., Scudiero, D., Monks, A.,, 1990. New calorimetric cytotoxicity assay for anticancer drug screening. Journal National Cancer Institute, 82*:* 1107–1112.
53. Sun, T., Xu, Z., Godber, S. and Prinyawiwatkul, W., 2006. Capabilities of oat extracts in inhibiting cholesterol and long chain fatty acid oxidation during heating. *Cereal Chemistry,* 84, 451-454.
54. Taha, F.S., Mohamed., G.F., Mohamed, S.H.,Mohamed, S.S. and Kamil, M.M., 2011. Optimization of the Extraction of Total Phenolic Compounds from Sunflower Meal, and Evaluation of the Bioactivities of Chosen Extracts. American Journal of Food Technology, (published on line October 2011).
55. Undersander, D.J., Oelke., E.A, Kaminski E.A., Doll, J.D, Putnam, S.M. *et al.,* 1990. *"Jojoba". Alternative Field Crops Manual.*
56. Verbiscar, A.J. and Banigan, T.F, 1978. Composition of jojoba seed hulls and foliage. Journal of Agricultural and Food Chemistry, 26: 1456-1459.
57. Vrhovsek, U., Rigo, A., Tonon D. and.Mattivi, F., 2004. Quantitation of polyphenols in different apple varieties. Journal of Agricultural and Food Chemistry, 52: 6532–6538 .
58. Wang, J., Sun, B., Cao, Y., Tian, Y. and. Li, X., 2008. Optimization of ultrasound assisted extraction of phenolic compounds from wheat bran. Food Chemistry, 106: 804-810.
59. Watanabe, M ., Ohshita, Y. and Tsushida, T., 1997. Antioxidant compounds from buckwheat hulls. Journal of Agricultural and Food Chemistry, 45:1039-1044
60. Weber, F.J and De Bont, J.A.M., 1996. Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. Biochimi Biophys Acta, 1286: 225–245.
61. Wisniak, J., 1994. Potential uses of jojoba oil and meal- a review. Industrial Crops and Products, 3: 34-68
62. Yen and Duh, G-C. Yen and P-D. Duh, 1995. Antioxidant activity of methanolic extracts of peanut hulls from various cultivars. Journal American Oil Chemist's Society, 72: 1065-1067.

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