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Research Article

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Innovative Extraction Techniques for the Optimum Extraction of Phenolic Compounds from Peanut Meal and Evaluation of Their Biological Activities

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Abstract

There is a worldwide demand for phenolic compounds (PC) because they exhibit several biological activities. This work aimed at extracting phenolic compounds from peanut meal. The methods of extraction were mainly: conventional solvent extraction (traditional methods) and ultrasound assisted extraction (recent methods) and comparing their results. Peanut meal (PM) was prepared by defatting with n-hexane, and then extracted by the two previous methods. First, the conventional solvents used were 80% methanol, ethanol, acetone, isopropanol, and distilled water. Then studied Different parameters such as meal: water ratio, also the effect of temperature and the pH on the extraction process. Second, ultrasonic assisted extractions (USAE), the parameters investigated were temperature, time and speed of sonication. Finally, all the extracts were analyzed by HPLC for their phenolic contents. Results indicated that the highest extracted PC achieved by solvents was in distilled water where 1:100, Meal: Water ratio which extracted 40 mg PC / g PM at 30& 35°C. Highest extracted PC was achieved by alkaline medium at pH 12 more than acidic and neutral medium. While (USAE) at speed 8 ultrasonication and temperature 30°C, extracted 49.2mg PC /g PM. Sothe ultrasound assisted extraction exhibited great influence on the extraction of phenolic compounds from peanut meal. The ultrasonic peanut extract was examined for its antioxidant, antimicrobial and anticarcinogenic activities. The antioxidant activity of PM phenolic extract prepared by ultrasonic technique, was measured by, β-carotene, and DPPH methods, and reducing antioxidant power. Results revealed values: 84.57, 57.72 and 5960 respectively. The PM extract showed different levels of antimicrobial activity against the pathogenic bacteria used. As for the anticarcinogenic effect PM phenolic extract most effective on inhibiting colon carcinoma and lung carcinoma cell lines with IC_{50} = 20.7 and 20.8 µ/ml., respectively. This was followed by intestinal carcinoma and liver carcinoma cell lines with IC_{50}= 39.6 and 40.2µ/ml.

Keywords: Peanut Meal, Phenolic Compounds, Conventional Solvent Extraction, Ultrasound Assisted Extraction, HPLC, Antioxidant, Antimicrobial, Anticancer

Introduction

The value of phenolic compounds is continuously escalating worldwide. This derives from the reality that they exhibited many biological activities. Phenolic compounds possess a variety of physiological properties, for example antioxidant, anti-artherogenic, anti-allergenic, anti-microbial, anti-inflammatory, cardioprotective, anti-thrombotic and vasodilatory effects [1-5].

The pharmacological actions of phenolic antioxidants stem mainly due to free radical scavenging ability and metal chelating properties (to prevent pro-oxidative properties of metals), in addition to their influence on cell signaling pathways and on gene expression [6]. Phenolic antioxidants react with free lipidoxy or lipidperoxy free radicals, resulted in course of lipid oxidation thus preventing their further autoxidation [7,8]. The inhibition of lipid autoxidation is essential not just in foods throughout their storage or heating, but additionally to lessen the oxidation of lipids after ingestion and absorption within the intestinal wall [8]. Phenolic compounds are considered as functional food ingredients, nutraceuticals, and bioactive compounds.

Because of the high value of these products, several innovative methods of extraction were applied to maximize quantity of phenolic compounds extracted. In this work we shall examine and compare the results of two extraction techniques.

Conventional solvent extraction - Leaching: is the method in which inorganic, organic components are liberated from the solid phase in to the water phase under the influence dissolution, complexation, desorption processes as affected by dissolved organic matter, pH, redox, and (micro) biological activity. The method itself is universal, because material subjected to contact with water will leach constitutes from its surface based on the porosity of the material considered [9].

Ultrasonic–assisted extraction (UAE): Sound waves, that have frequencies above 20 kHz, are mechanical vibrations in a gas, liquid and solid. Recently many authors used successfully ultrasonic assisted extraction for the extraction of phenolic compounds and other bioactive compounds from plant sources Akl et al 2017, Zhong 2010 [10,11]. We et al., 2009 and Hoang et al., 2008, proved that peanut coat involved phenolic compounds which are considered a good source of antioxidants [12,13].

Materials and Methods

At the beginning of the work peanuts were bought from the Agriculture Research Centre - Giza, Egypt. Preparation of Peanut meal: the peanuts were shelled, then the kernels with the skins ground, the oil was extracted by subjecting to hot hydraulic pressing which removed a big portion of the oil. The remaining oil was removed by a soxhlet apparatus using n-hexane as extracting solvent, then the meal containing the skins was spread to dry at room temperature, then was ground to pass 60-80 mesh screen, then was saved. Peanut meals were analyzed for their chemical composition.

Experimental

Conventional Extraction of PC: The basic extraction of phenolic compounds was carried out as follows: Two grams of the defatted meal (PM) added to 200 ml different solvent (methanol, ethanol, acetone, isopropanol, and water and stirred by means of an electric stirrer for 30 min, then centrifuged at 3000 xg for 30 min. The supernatant (A) was kept, and the precipitate (ppt) was re-extracted with a fresh amount of solvent and centrifuged to give supernatant (B). The resulting ppt.was re-extracted for the third time with a fresh amount of solvent, centrifuge to give supernatant (C). The ppt. was discarded and supernatant A, B, C were collected to give (D). The phenolic compounds (PC) content in (D) were then determined.

Extraction of phenolic compounds (PC) at different pH values:

In this experiment 5 g (PM) was extracted with 5 ml water at pHs from 1-12. This was done by stirring on a magnetic stirrer and adjusting the pH to the desired value by using 6N – HCL or 1N-NaOH. The stirring was continued for 15 min at the fixed pH, and then centrifuged for 20 min at 4000 xg. The supernatant was taken for PC determination.

Extraction of PC by countercurrent technique

A schematic representation of the countercurrent extraction procedure is illustrated in [14].

Ultrasonic-Assistant Extraction of phenolic compounds (PC):

The basic ultrasonic extraction of PC comprises the following: 2g of (PM) was added to 200 ml distilled water (1:100, M: W ratio, as determined from conventional extraction)and the mixture extracted in an Ultrasonic water bath (crest ultrasonics NJ USA)for 30, 60,90, and 120 min., and at temperatures 30, 35 and 40^{o} C. The other variable was the speed of sonication which was 2, 4, 6, 8. First, the optimum temperature was determined at this optimum temperature the speed of sonication. At the fixed temperature and speed the time of extraction was then examined. The resulting extracts were centrifuged and the supernatants were examined for their PC content.

The ultrasonic extraction is extracted under the above conditions using the successive extraction technique.1g meal +100 ml distilled water were extracted in Ultrasonic water bath for 30 min, then centrifuged 20 min at 3000xg, and supernatant [1] kept. The ppt. [1] was re-extracted with 100 ml water, and then centrifuged; it gave supernatant [2]. The ppt. [2] was re-extracted again as described until we reached supernatant [4], the four supernatants were collected, evaporated by a rotary evaporator then lyophilized and PC determined.

Methods of Analysis

Moisture, protein, oil, ash, fiber was determined according to AOCS [15]. Standard methods of analysis. Nitrogen free extract was determined by calculation.

Determination of the Phenolic Compounds (PC) in the extracts

The phenolic content of the extracts were determined using Folin Ciacalteu reagent according to using Gallic acid as standard [16]. The absorbance was measured using a spectrophotometer (UV Vis spectrophotometer PG Instruments United Kingdom). The amount of total phenolic compounds in extract was determined as microgram of Gallic acid equivalent (GAE) using an equation that was obtained from a calibration curve of Gallic acid. The absorbance measured at 765nm.

Analysis of PC using HPLC Method:

Phenolic acids (PC) profile

Preparation of PC

Sample (1g) was placed in quick fit conical flask and 20 ml of 2M NaOH was added and the flasks were flushed with N2 and the stopper was replaced. The samples were shacked for 4 h at room temperature. The pH was adjusted to 2 with 6 M HCl. The samples were centrifuged at 5000 xg for 10 min and the supernatant was collected. Phenolic compounds were extracted twice with 50 ml ethyl ether and ethyl acetate 1:1. The organic phase was separated and evaporated at 45°C and the samples re-dissolved in 2ml methanol.

Analysis of Phenolic Compounds by HPLC

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 70 min and the gradient programmed was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards [17].

Antioxidant Determination of Phenolic Compounds (PC)

1. β-Carotene Coupled oxidation Method
   Determination of the AOA of the extracts was based on the coupled oxidation of β- carotene and linoleic acid according to the method of [18]. Absorbance of sample was measured against blank at 470nm.
2. Antioxidant activity by the (DPPH) free radical scavenging activity Method
   The scavenging activity of DPPH free radicals were measured according to [19]. DE colorization of the methanolic DPPH solution was determined by measuring the decrease in

absorbance at 517nm using a spectrophotometer model (UV VIS Spectrophotometer PG Instruments United Kingdom) and DPPH was calculated according to the following equation:
Scavenging rate = [1- (A1-A2)] × 100
Where A1 represents the absorption of the sample PC extract A2 represents the absorbance of control.

3. Measuring the reducing antioxidant power:
The reducing antioxidant power of the PC extracts was measured according to the method [20]. The absorbance was measured at 700nm a blank using a spectrophotometer model (UV VIS Spectrophotometer PG Instruments United Kingdom). Increased absorbance of the reaction mixture indicates increase in reducing power.

Determination of Antimicrobial Activity

The antimicrobial activity of PM or phenolic extracts of different concentration was determined by the agar well diffusion method [1]. The seven pathogenic indicator bacteria strains were obtained from the stock cultures of the Dairy Microbiological Lab, National Research Centre: Escherichia coli 0157: H7 ATCC 6933, Bacillus cereus ATCC 33018, Staphylococcus aureus ATCC 20231, Salmonella typhimurium ATCC 14028, Pseudomonas aeruginosa ATCC 9027, Listeria monocytogenes ATCC 7644 and Yersinia enterocolitica ATCC 9610. Each strain was activated in Tryptone soy broth by fermentation at 37 ºC for 24 h. One ml culture of the activated indicator strain (105 cells /ml) was inoculated into 20 ml of Mueller-Hinton agar (Becton Dickinson, USA) and poured in Petri dishes. After solidification of the agar, wells of 5 mm in diameter were cut from the agar with a sterile borer and 50µL of phenolic extracts delivered in each well. Dishes were incubated for 24 h at 37°C.

The zone diameter of wells cut in Mueller-Hinton agar was 5.0 mm and the diameter of inhibition zone (DIZ) of negative a control for each bacterium was also 5.0 mm. If the DIZ value is 5.0 mm (*), that means the sample has no inhibitory activity against that bacterium. The diameters of the inhibition zones were measured [21].

Cytotoxic Activity Test

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-4, 5-dimethylthiazol tetrazilium bromide) to purple formazan [22]. This cytotoxic activity test (*In vitro* bioassay on normal retina cell lines) test was conducted and determined by the bioassay cell culture laboratory, National Research Centre, El-Tahrir St., Dokki, Cairo, 12622, Egypt.

Determination of Anti-Carcinogenic Effect

Cell line Carcinomas: Liver Carcinoma Cell Line (HEPG2), Larynx Carcinoma Cell Line (HEP2), Colon Carcinoma Cell Line (HCT), Cervical Carcinoma Cell Line(HELA), Breast Carcinoma Cell Line(MCF7), Intestinal carcinoma cell line (CACO), Normal Melanocytes(HFB4) were supplied and used in The National Cancer Institute, Biology Department, Cairo, Egypt and The evaluation was done by the Sulfo-Rhodamine-B stain (SRB) assay, according to the method of [23].

Statistical Analysis

All determinations were carried out in triplicates and values expressed as mean ± standard deviation (SD). Significant statistical differences of investigated parameters were determined and analyzed using one way analysis of variance (ANOVA PC-STAT, 1985 VERSION IA copyright, university of Georgia). A confidence interval at 95% level and a probability (p) value less than 0.05 were considered statistically significant at 5% significance level ($p<0.05$).

Results and Discussion

The previous studies suggest that phenolic compounds can play a vital role in the maintenance of human health. So many researchers try to find out new methods and sources for extraction of phenolic compounds. In this work we have focused on two methods of extraction in order to reach the optimum amount of the phenolic compounds that can be extracted and to avoid the many losses of such a valuable material. The methods of extraction that will be studied include: conventional solvent extraction, ultrasonic-assisted extraction.

The chemical composition of peanut meal showed that it contained less than 1% oil and about 59% of protein. Results in table 1 are within the range reported [12,24].

Table 1: Chemical composition of Peanut meal

Composition	Peanut meal
Moisture %	6.67
Protein %	59.8
Oil %	0.8
Ash %	5.3
Crude Fiber %	5.91
Nitrogen free extract %	21.52

Conventional solvent extraction

Accordingly there are many factors that affected the extraction efficiency for example solvent type, temperature of extraction, contact time, particle size, solid to solvent ratio and solvent concentration [25].

Investigation of the type of solvent

The phenolic compounds solubility in different solvents was studied. The solvents investigated for the optimum extraction of phenolic compounds are 80% ethanol, acetone, methanol, isopropanol, and distilled water. Results, represented in Table 2, indicated that distilled water solubilized the highest amount of PC reaching 39.85mg PC / g PM. It is clear that the solubilization of PC was influenced by the polarity of the solvents [26,27]. Because of the safety and priceless of distilled water it was our choice for its utilization. Its storage stability is not long is the only drawback of the water extract thus it is stored in a freeze-dried form. This result is logical since water is more polar than the other investigated solvents (Wikipedia). Thus, water is our choice solvent for further work.

Table 2: Effect of different solvents on the extraction of phenolic content

Solvents	mg PC/g Peanut Meal
80% Methanol	15.91 ± 0.20^{c}
80% Ethanol	15.28 ± 0.20^{d}
80% Acetone	25.91 ± 0.02^{b}
80% Isopropanol	14.01 ± 0.10^{e}
Distilled water	39.85 ± 0.10^{a}
L.S.D 5%	0.344678

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

Investigation of Meal: Water ratio

Results are represented in Table 3. Results revealed a constant increase in the amount of PC extracted with the increase in M; W ratio. Tables 2&3 revealed that PM was rich in the PC. Probably this was due to the red skins of peanuts which are very rich in PC [28,29].

Table 3: Extraction of PC in water at different Meal: water ratios according to conditions of basic conventional extraction

Meal: Water ratio	mg PC/g Peanut Meal
1:10	27.74 ±.03^{i}
1:20	28.05 ±.04^{h}
1:30	29.35 ±.02^{g}
1:40	29.88±.04^{f}
1:50	30.54±.03^{e}
1:60	30.81±.05^{d}
1:70	31.99±.02^{c}
1:80	35.67±.09^{b}
1:90	35.87±.09^{b}
1:100	36.76±.11^{a}
L.S.D 5%	0.00958

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

Table 3: Results Indicates that a M: W ratio of 1:100 extracts the highest amount of PC, extracting 36.76 mg PC from PM. It is clear that with increase of the M: W ratio there is increase in PC extracted [10].

Effect of Temperature, And Time on the PC:

Tables 4 represented the influence of temp and time on PC. Results revealed that when comparing the effect of extraction time (30, 60, 90 min.) on phenolics extracted, a direct proportional effect on the amount of phenolic compounds extracted was noticed. This is in agreement with the results [30,31]. The quantity of PC extracted after 30, 60, and 90 min. extraction time at 35°C reached 36.94, 37.62 and 40.52 mg PC/ g PM, respectively. Results in the same tables showed that at 35° C maximum solubilization of the phenolic compounds was achieved, then with increase of temperature the solubilization decrease. This result indicates that with increases in temperature the solubility of phenolic compounds increase to a certain level then solubility starts declining. This is in agreement with the results [10,30,32].

Table 4: Effect of temperature on the solubility of phenolic compounds after 30, 60, 90 min of extraction

Temperature ° C	mg PC/g PM after 30 min	mgPC/g PM after 60 min	mgPC/g PM after 90 min
20	33.65±.04^{e}	35.26±.03^{c}	38.65±.01^{d}
25	34..36.02^{d}	36.65±.07^{b}	39.36±.06^{c}
30	34.57±.01^{c}	36.24±.07^{b}	40.24±.03^{b}
35	36.94±.02^{a}	37.62±.02^{a}	40.52±.02^{a}
40	35.94±.05^{b}	34.32±.02^{e}	36.79±.02^{f}
45	34.35.02^{d}	35.11±.02^{d}	36.94±.03^{e}
L.S.D 5%	0.01223	0.052211	0.025795

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

Table 4: Results indicates that temperature and time both have an effect on the quantity of PC extracted. Highest PC extraction was achieved at 35^{o} C at different time of extraction.

Finally, we needed to investigate the effect of pH on the solubility of phenolic compounds.

The Effect of pH on the Solubilization of PC

The pH had great influence on PC extraction. Results in Table 5 revealed that at very acidic pH the PC has the highest solubility which declines on increasing the pH towards the neutral then the solubility starts increasing again at pH 7 and continues increasing until reaching maximum solubility at pH 12. Wagdy and Taha reported the same solubility pattern for the PC in PM also, reported that phenolic compounds at the alkaline pH is more soluble than at neutral and acidic values while working with Jojoba meal [33,34]. The extraction of PC in alkaline medium also extract almost of the valuable protein, which is a big loss. Thus, the solubility pattern at different pH values cannot be used to prepare a phenolic extract.

Table 5: Effect of pH on the solubility of phenolic compounds

pH	mg PC/g Peanut Meal
1	46.1±.15^{c}
2	48±.10^{b}
3	28.5±.04^{g}
4	24±.02^{i}
5	16.5±.07^{j}
6	25.5±.01^{h}
7	34.5±.10^{f}
8	43.0±.04^{e}
9	45.1±.05^{d}
10	46.0±.15^{c}
11	48.0±.10^{b}
12	48.9±.05^{a}
L.S.D 5%	0.127623

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

It is clear from the results in Table 5 that the pH had a noticeable effect on the solubility of the phenolic compounds. The highest extraction was achieved at very acidic pH 1, 2, and very alkaline pH 11, 12. These results in the same line with Akl et al, the results showed that phenolic compounds extracted from flaxseed meal is more soluble in the alkaline medium than in neutral or acidic medium.

Countercurrent extraction technique

Using the countercurrent extraction technique was the last investigated method for solubilization of PC. Table 6 showed that

the first stage of extraction liberates and solubilized most of PC in the solvent then decrease in the followed stages. This procedure which is usually used to increase the yield such as when extracting plant protein with NaOH, unfortunately gave lower yield of PC. This low yield might be also due to the bonding of phenolic compounds to the protein or dietary fiber.

Table 6: Solubilized Phenolic Compounds by the countercurrent technique in PM

Stages	mg PC/g Peanut Meal
A	12.76
B	4.9
C	2.49
D	0.89
Total	20.77

Results in Table 6 prove that the countercurrent extraction procedure is not the suitable procedure to extract the highest quantity of PC from PM.

From this part of the study it was concluded that for the preparation of a PC recommended conditions would be extraction with distilled water as a solvent at 1:100, M: W ratio, temperature 30°C, 90 min extraction time, and pH 6-7.

Ultrasonic-Assisted Extraction

Ultrasonic-assisted extraction (USAE) is an alternative extraction process that can decrease extraction time and increase extraction yield in many plants [35]. Ultrasound-assisted extraction provides a mechanical effect, allowing greater solvent penetration into the sample matrix, increasing the contact surface area between the solid and liquid phase and, as a result the solute quickly diffuses from the solid phase to the solvent [36].

The effectiveness, simplicity and low cost are the main advantages of USAE (both instrument and operation cost) [37]. UAE is suitable for heat-sensitive compounds because it operates at moderate temperatures [38].

Tables 7and 8 clearly concludes that using USAE of phenolic compounds from PM was superior to the conventional extraction. USAE of Phenolic compounds from PM at 30°C, 120 min, and speed 8 yielded 49.2 mg PC / g PM, Conventional extraction yielded 40.52 mg PC /g PM. Akl et al., extracted PC from flaxseed meal by the aid of USAE. PC extracted 17.44 mg PC /g FM at 35°C, 120 min and speed 8, while extracted 12.65 mg/ g FM by conventional methods [10].

Wong et al. 2015, Wang et al. 2008, 2013 and wagdy et al., recommended the use of USAE for improving yield of phenolic compounds extraction from plant material [30,33,39,40].

Table 7: Effect of temperature on the phenolic compounds extracted by the aid of ultrasonic at 40°C, 35°C and 30°C

Speed	Time of extraction	Mg PC/ g Peanut meal AT 40°C	Mg PC /g Peanut meal at 35°C	Mg PC /g Peanut meal 30°C
8	30 min	23.5±0.02d	28.25 ±0.09d	28.15 ±0.05d
8	60 min	33.0 ±0.1c	37.58±0.01c	41.47 ±0.02c
8	90 min	37.5 ±0.03b	40.87±0.1b	45.20 ±0.1b
8	120 min	38.0 ±0.04a	45.50±0.04a	49.2 ±0.03a
L.S.D 5%		0.023645	0.12546	0.154376

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

In Tables 7 the speed of sonication was fixed at 8 and the temperatures examined were 30, 35, and 40°C. Results indicated that optimum extraction of PC from PM took place at 30°C, time of extraction 120 min and ultrasonic speed 8. The following experiments with PM were carried out at 30°C. Meanwhile the optimum temperature for extracting PC from FM was 35°C, 120 min, speed 8, the following experiment will be carried at 35°C, and however the speed was further tested.

Results in Table 8 show that with increasing the speed the more is the amount of PC extracted. The same with the of sonication the quantity of the PC extracted increase with increasing the duration of extraction, resulting in 49.2 mg PC/ g PM at speed 8 and 120 min. extraction time. The extraction temperature also affected the quantity of PC extracted. Best temperature was 30°C resulting in extraction of 49.2mg PC /g PM.

Table 8: Effect of speed of sonication 6, 4, 2 on the extracted PC by the aid of ultrasonic at 30°C

Time of extraction	Mg PC /g Peanut meal at speed 6	Mg PC /g Peanut meal at speed 4	Mg PC /g Peanut meal at speed 2
30 min	23.75 ± 0.04^{d}	25.46 ± 0.06^{d}	22.65 ± 0.10^{d}
60 min	33.60 ± 0.05^{c}	34.56 ± 0.03^{c}	32.22 ± 0.01^{b}
90 min	37.40 ± 0.1^{b}	36.84 ± 0.01^{b}	35.25 ± 0.1^{b}
120 min	39.40 ± 0.1^{a}	37.85 ± 0.03^{a}	37.01 ± 0.04^{a}
L.S.D 5%	0.27632	0.36962	0.245716

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

HPLC is an approved tool for analysis of PC as shown in figure 1: 8.

HPLC analysis of all phenolic extracts from PM with ethanol, acetone, methanol, isopropanol, distilled water, pH 4, pH 12 as well as ultrasound, assisted extractions were studied. Table 9 showed that p-hydroxybenzoic, extracted by all solvents and with USAE. Pyrogallol, genistinic, catachine.and p-coumaric extracted by all solvents except isopropanol, pH 4, distilled water, pH 4 respectively. pH 12 extracted also synergic, chlorogenic and Gallic. Methanol extracted different phenolic not extracted by other solvents such as cinnamic, quercitin, kaempherol and chyrsin. Finally, rosmarinic extracted only at Ph 4. The rest of data are shown in table 9.There is no fixed pattern for the extraction of phenolic compounds from peanut meal Akl et al., when using HPLC for the analysis of phenolic compounds from flaxseed meal found the same [10].

HPLC analysis of PC

Table 9: HPLC analysis of different extract resulting from different solvents and different methods of extractions (values in table are µg/ g Meal)

Phenolic Compound	Methanol	Ethanol	Acetone	Isopropa-nol	Dis.Water	PH 12	PH 4	USAE
Pyrogallol	1122.4	1055.51	48279.3	0	549.481	548.730	461.546	502.052
Gallic	0	0	0	0	0	21.315	0	0
Proto-catchuic	30.6	25.908	37.713	14.849	0	0	24.153	23.241
p-hydroxybenzoic	41.1	26.308	39.843	16.219	123.226	35.563	38.634	23.924
Genistinic	35.6	9.872	32.258	26.103	21.4206	287.233	0	34.350
Catachine	130.0	78.33	144.489	102.820	0	187.661	87.7401	108.777
Chlorogenic	0	0	0	0	17.625	20.669	0	0
Caffeic	0	0	0	0	0	0	0	0
Synergic	0	0	0	0	0	23.316	0	0
Vanillic	0	0	0	0	0	0	0	0
Ferulic	0	0	0	0	0	0	0	0
Sinapic	0	0	0	37.300	2.3229	0	0	0
Rutin	0	0	0	0	0	0	0	0
p-coumaric	27.9	7.9807	45.788	3.187	15.443	9.306	0	0
Naringeen	0	0	0	0	0	0	0	0
hisperdin	0	0	0	0	0	0	0	0
rosmarinic	0	0	0	0	0	0	6.796	0
quercitin	0	0	0	0	0	0	0	0
apeginin	0	0	0	0	0	0	0	0
cinnamic	12.5	0	0	0	0	0	0	0
quercitin	8.6	0	0	0	0	0	0	0
Apegnin	0	0	0	0	0	0	0	0
Kaempferol	6.8	0	0	0	0	0	0	0
chyrsin	24.8	0	0	0	0	0	0	0

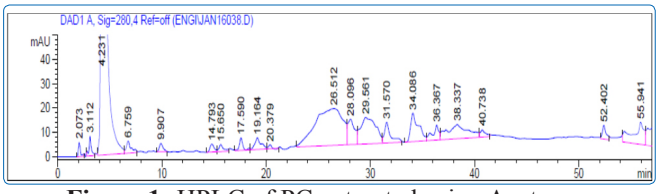

Figure 1: HPLC of PC extracted using Acetone

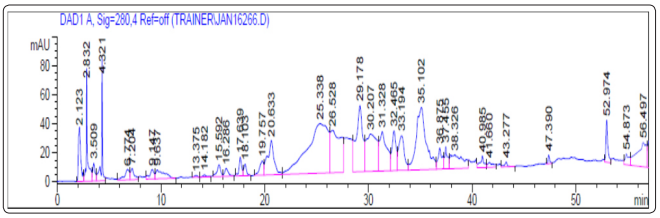

Figure 2: HPLC of PC extracted using Ethanol

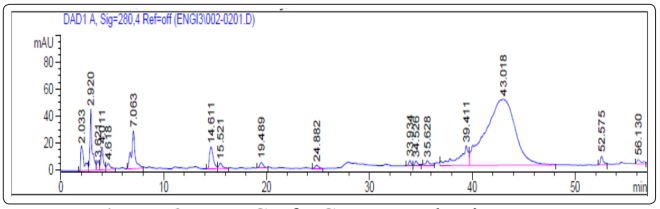

Figure 3: HPLC of PC extracted using H_2o

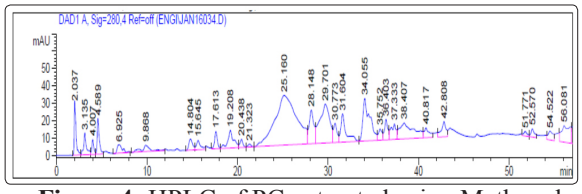

Figure 4: HPLC of PC extracted using Methanol

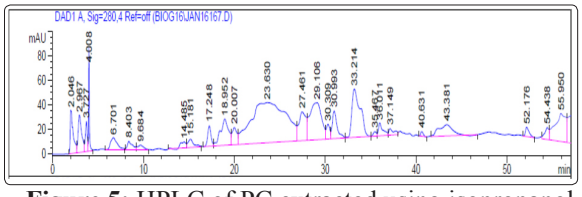

Figure 5: HPLC of PC extracted using isopropanol

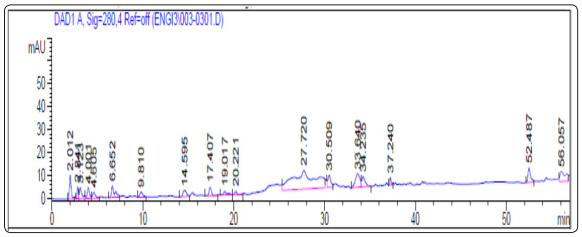

Figure 6: HPLC of PC extracted using pH 4

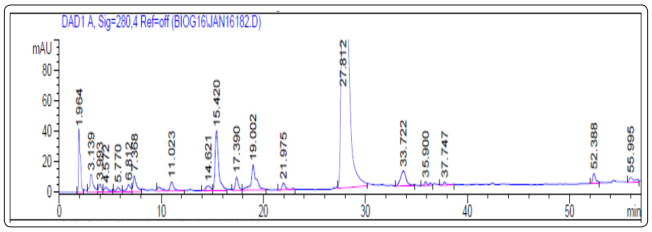

Figure 7: HPLC of PC extracted using pH12

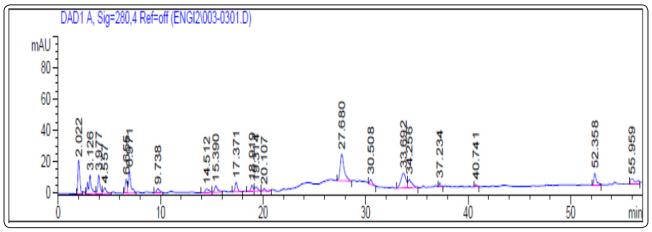

Figure 8: HPLC of PC extracted using Ultrasonic assisted extraction

Antioxidant activity of PC extract from PM

Table 10: Antioxidant activity as measured by three different methods

Antioxidant Activity			
PC extract	B-Carotene Method%	DPPH Method%	Reducing Antioxidant Power
Peanut meal	84.57	57.72	5960

Antioxidant activity

The antioxidant activity of PC extracts (Table 10) prepared by USAE from PM was determined. Results of the three experiments proved that PM phenolic extract possessed antioxidant power. β-carotene, DPPH method, and reducing antioxidant power were: 84.57, 57.72 and 5960 respectively.

Calomeni et al., revealed that the dried extracts from peanut skin exhibited remarkable antioxidant activity [41]. Taha et al., studied Antioxidant activity (AOA) as measured by β-carotene/ linoleate method and showed that AOA for roasted skin > unroasted skin > roasted hull > BHT > unroasted hull > unroasted defatted flour > roasted defatted flour, with values 89.13 > 86.65 > 80.33 > 76.33 > 75.27 > 39.34 > 30.37%, respectively [28]. Gaafar et al., working on peanut skin and peel [37]. Acetone extracts of peanut skin exhibited the highest DPPH• scavenging activity the highest DPPH• scavenging activity (IC_{50}=52.18 ± 0.47µg/ml) compared to peanut peel acetone extract (IC_{50}=70.51 ± 0.45µg/ml). Methanolic extract of peanut skin revealed the highest activity (86.10%) compared to peel extract (74.94%)when measured by Fe2+ chelating.

Toxicity Test

The major concerns for acceptability of such antioxidants are their activity and potential toxicity. The toxicity test proved that the extract from PM was nontoxic on normal retina cell line. Sample concentration range between 100 to0.78 µg / ml.

Table 11: Cytotoxic activity of PC extract from PM

Remarks	Sample Code
18.4% at 100 ppm	Peanut
5% at 100 ppm	DMSO

Phenolic antioxidants function as a free radical terminator. Phenolic compounds and some of their derivatives are very efficient in preventing autoxidation; however only a few phenolic compounds are currently permissible by law as food antioxidants.

Table 12: Antimicrobial activity of PC extract from PM

Species of pathogenic bacteria	Peanut extract mm
B.C, *Bacillus cereus* ATCC 33018,	15
List *Listeria monocytogenes* ATCC 7644	23
Staph *Staphylococcus aureus* ATCC 20231,	24
sal *Salmonella typhimurium* ATCC 14028,	19
E.C *Escherichia coli* 0157: H7 ATCC 6933,	17
Psed *Pseudomonas aeruginosa* ATCC 9027,	12
Yersinin *Yersinia enterocolitica* ATCC 9610	Nil

Antimicrobial activity of PC extracts from PM

The peanut extract exhibited antimicrobial effect on some pathogenic bacteria. The diameters of the inhibition zones were measured by mm. the clear zone is an indication to the inhibition effect of extract.

The phenolic extracts of PM showed various degrees of inhibition against the seven bacterial strains using the disc diffusion method as represented in Table 12. PM phenolic extract inhibited 6 strains out of 7. Growth inhibition of PM extract was highest for *Staphylococcus aureus* followed by *Listeria monocytogenes* > *Salmonella typhimurium* > *Escherichia coli* 0157: H7 > *Bacillus cereus* > *Pseudomonas aeruginosa* with inhibition zone diameter (mm) 24 > 23> 19 > 17 > 15 > 12. PM extract had no effect on *Yersinia enterocolitica*. Thus, PM phenolic extract can be considered to have as antimicrobial activity. Calomeni et al., revealed that the dried extracts from peanut skin showed bactericidal activity against Staphylococcus aureus and bacteriostatic activity against *Listeria monocytogenes* [41].

Determination of Anti-Carcinogenic Effect

Table 13: Anti-carcinogenic effect of PC from PM measured as µg / ml

IC50	
CELL LINE	PM
BHK	…….
CACO	39.6
MCF7	……
HCT	20.7
A549	20.8
HEPG2	40.2

Phenolic extract of PM exhibited highest anticancer effect on HCT and A594 cancer cell lines.PM phenolic extract have been evaluated as chemo preventive agents. Table 13 showed the influence of peanut extract on the six human tumer cell lines. Normal fibroblasts [BHK], Colon carcinoma cell line [HTC], Intestinal carcinoma cell line [CACO], Lung carcinoma sell line [A549], Breast carcinoma cell line [MFC7], Liver carcinoma cell line [HEPG2].

PM phenolic extract had no effect on the two cell lines: Normal fibroblasts, and breast carcinoma cell line. While PM extract was most effective on inhibiting colon carcinoma and lung carcinoma

cell lines with IC_{50} = 20.7 and 20.8 µ/ml., respectively. This was followed by intestinal carcinoma and liver carcinoma cell lines with IC_{50}= 39.6 and 40.2µ/ml.

Four identified phenolic compounds (resveratrol, ferulic acid, sinapinic acid and p-coumaric acid) in peanut test were studied for their histone deacetylase HDAC inhibitory and anticancer activities against colon cancer cell lines, Saenglee et al., [42]. In vitro study revealed that resveratrol possesses the greatest HDAC inhibitory activity. Resveratrol exhibited the most effective antiproliferative activity against both human colorectal carcinoma (HCT116) cells and human colon adenocarcinoma (HT29). Gaafari et al., concluded that peanut skin acetone extract; which contained antioxidant components; exhibited potential as an anticancer activity [37].

Conclusion

The ultrasonic assisted extraction improves the extraction yield of phenolic compounds when comparing with traditional methods. In addition, it is simple and save time needed for the extraction process. The optimum extraction of phenolic compounds achieved at speed 8 of ultrasonication and temperature 30 °C, which extracted 49.2 mg PC /g PM. The peanut extract showed potential as antioxidant. The PM extract showed different levels of antimicrobial activity against the pathogenic bacteria used. As for the anticarcinogenic effect PM phenolic extract most effective on inhibiting colon carcinoma and lung carcinoma cell lines.

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