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Research Article
Effect of Different Solvent Treatments on Peanut Meal Protein Fractions as 1 Bioactive Compounds

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Abstract

Background and Objective: Peanut is considered as one of the most abundant food legumes in the world. This study aimed at using organic solvents mixed with acid or base to optimizations of peanut by-products/waste with fast methods and low cost and investigating the protein content of soluble and insoluble fractions of peanut meal subjected to different treatments. It also aimed at estimating the bioactive compounds and its anti-oxidant activity in all extracts. **Materials and Methods:** The peanut meal was first defatted followed by grinding in coffee mill then subjected to the different concentration of single solvents, then extracted with acid and base. Another treatment was extraction of peanut meal protein with solvents mixed with acid and base. The soluble and the residue (insoluble) fractions of the defatted peanut meal were analyzed for their protein content by 8% native PAGE and 12.5% SDS-PAGE as a biochemical marker. **Results:** The gels showed bands at low molecular weight protein that were observed in area up to 25 kDa, these were extracted by acid and base treatments. There was a significant difference ($p<0.05 \pm SD$) between almost extracts. The soluble extracts showed high contents of phenolic compounds especially that extracted in the acidic, basic medium together with aqueous ethanol. It also contains appreciable amounts of saponins and flavonoids that exhibit anti-oxidant activities. The extracts of 60% ethanol and 100% ethanol were the most effective on Breast Carcinoma Cell Line (MCF7) and Liver Carcinoma Cell Line (HEPG2). **Conclusion:** The soluble extracts especially extracted by acid or base (which is rich with protein content) can be used in many food applications in the industries after its neutralization and for other purposes.

Key words: Peanut meal, protein, bioactive compounds, anti-oxidants, native, SDS-PAGE and anti-cancer

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Recently, scientists search for new alternative sources of animal proteins. Plant seeds are considered good sources of proteins. Peanut seeds have numerous uses in food products. It has more protein content in comparison with other nuts and legumes. The protein content in the peanut cake reaches[1] 50%. It contains also all types of amino acids with high level of protein and is reach in B-arginine[2]. The peanut proteins have good functional properties like emulsifying capacity stability, high solubility and foaming capacity[3]. Peanuts contain fiber and bioactive compounds in addition to protein which have numerous health benefits. Peanut meal contains a high quality protein reaches 50-55%, with high content of polyphenols in the skin, those support the use of peanut by products as natural antioxidants[3]. In addition peanut meal contains other important compounds such as flavonoids, fibres, phenolic compounds, lectin, resveratrol and piceid etc[4].

The peanuts and their skin have functional compounds like phenolic acids and flavonoids which are in all parts of peanut plants; it acts as protective against heart disease, it contains the same types of flavonoids that exist in green and black tea, apples red wine and soybeans[5]. It also contains p-coumaric acids. The role of phenolic compounds as anti-oxidants werestudied[6,7]. In addition to its nutritional compositions, it was also found saponins in peanut parts[8]. Resent researches proved thatpeanut coat is a good source of antioxidants and phytochemical compounds due to the presence of phenolic compounds[9].

Although, peanut proteins considered one of the important proteins in plants, it cannot be used reasonably, so scientists searched for new methods for separation of peanut proteins. There are several methods of separation of peanut protein including isoelectric precipitation, alcohol precipitation and alkali solution with isoelectric precipitation[10].

The functional properties of protein changed according to the methods and the extraction medium. The effect of heat, PH, ionic strength and water on the protein function properties was studied[11].

The aim of this work was a potential application of peanut meal (byproducts waste) with modern treatments and uses the soluble and insoluble extracts as food or feed supplements for human consumption and health promoting. The current study utilized different solvents and new treatments such as organic solvents with acid or base and investigated their effect on both protein quality and bioactive compounds by fast and low cost methods.

MATERIALS AND METHODS

Materials

Preparation of defatted peanut meal: The meal was already hydraulic pressed and then was subjected in the laboratory to complete defatting using a soxhlet apparatus and n-hexane as defatting solvent, then allowed to air-dry in a fume hood to remove residual hexane. The resulting defatted meal was ground in coffee mill to obtain a finely divided material suitable for extraction studies then saved for extraction studies. All chemicals were obtained from Sigma Chemical Co.

Methods: In this study, the bioactive compounds (phenolics, flavonoids and saponins) of defatted peanut meal were extracted by 10 different treatments, 100% organ solvents, aqueous organosolvents and mixture of organosolvents with acid or base.

Solvent extraction: One gram of dried peanut meal mixed with 45 mL of each solvents illustrated in Table 1 then leave in the ultrasonic bath for 1 h, soaking with solvents 24 h. The extracts centrifuged and the soluble phase were stored at -20°C until testing, while the residue allowed to air dry in a fume hood and saved in cool place.

Determination of protein extract: Protein was determined by using the Bradford[12] method. Hence, the assay was based on the colour change of coomassie brilliant blue dye G250 in response to protein concentrations. The absorbance of the mixture was measured after 5 min (and before 1 h) at 595 nm against blank (prepared from 0.1 mL of bidistilled water and 5 mL of dye reagent).

Determination of total phenolic extract: The content of phenolic compounds in the extracts was determined according to the method of McDonald *et al.*[13]. Absorbance of the solution was measured at 765 nm using a spectrophotometer (T80 UV-vis spectrophotometers).

Determination of Total Flavonoids Extract (TFE): The colorimetric determination of Total Flavonoids Extract (TFE) was performed according to Kanatt *et al.*[14].

Determination of Saponins Extract (TSE): The colorimetric determination of Saponins Extracts (TSE) was performed according to Hiai *et al.*[15].

Table 1: Effect of different solvents at different concentrations on the yield of protein phenolic, saponin and flavonoid compounds extracted and soluble from defatted peanut meal at room temperature

Treatment 1 g meal: 45 mL solvent, tow times, each time ultrasound for 1 h then soaking 24 h	Protein soluble (mg g^{-1} ±SD)	Phenolic extract (mg g^{-1} ±SD)	Saponin extract (mg g^{-1} ±SD)	Flavonoid extract (mg g^{-1} ±SD)
100% water	5.5±0.2[d]	10.1±0.19[d]	0.25±0.03[f]	0.001±0.0001[e]
100% methanol	2.6±0.1[f]	8.3±0.1[g]	0.83±0.02[a]	0.007±0.0002[b]
100% ethanol	2.4±0.09[g]	2.8±0.01[h]	0.32±0.04[e]	ND
Methanol: ethanol: H_2O 7: 7 : 6	11.6±0.2[c]	10.0±0.3[d]	0.41±0.06[b]	0.0063±0.0004[b]
Ethanol: H_2O (50 : 50)	5.4±0.029[d]	9.7±0.2[e]	0.36±0.05[d]	0.004±0.0005c
Ethanol: H_2O (60: 40)	5.5±0.049[d]	10.6±0.1[c]	0.43±0.03[b]	0.0063±0.0002[b]
Ethanol: H_2O (70: 30)	2.9±0.039[e]	10.2±0.2[d]	0.41±0.01[b]	0.007±0.001[b]
Ethanol: H_2O (80: 20)	3.2±0.021[e]	9.4±0.02[f]	0.39±0.02[c]	0.005±0.0002[b]
Ethanol: 1N HCl (80: 30)	38.1±0.29[b]	12.0±0.4[b]	0.46±0.03[b]	0.03±0.005[a]
Ethanol: 1N NaOH (80: 30)	99.8±0.39[a]	14.0±0.29[a]	0.44±0.04[b]	0.0023±0.002[d]
LSD at 5%	0.32023	0.03732	0.061173	2.7237

Different letter(s) in each column indicates significant differences at $p<0.05$, ±SD

Evaluation of anti-oxidant activity of peanut meal extracts (soluble phase): For each extract, three series of anti-oxidant capacity methods were applied, 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydrogen peroxide (H_2O_2) scavenging and total reducing-capability.

Determination of the free radical-scavenging assay (DPPH*): The DPPH radical has a strong absorbance at 517 nm due to its unpaired electron and giving the radical a purple color. But upon reduction with an anti-oxidant, its absorption decreases due to the formation of its non-radical form, DPPH-H[16] that was based on the method of De Ancos *et al.*[17] with some modification. Results were expressed as percentage inhibition of the DPPHC using the following equation:

$$\text{Inhibition of DPPH (\%)} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

where, Absorbance control is the absorbance of DPPHC solution without extract, Butylated Hydroxyl Toluene (BHT) was used as positive control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

Estimation of H_2O_2 scavenging activity: Hydrogen peroxide exhibits weak activity in initiating lipid peroxidation, however, its potential to produce highly ROS, such as hydroxyl radical through fenton reaction is very high. The H_2O_2 scavenging ability of each extract was determined according to Sfahlan *et al.*[18] with some modification.

Estimation of total reducing capability: For the measurements of the reducing ability, it investigated the Fe^{3+} to Fe^{2+} transformation in the presence of peanut meal extract. The reducing power of each extract was determined according to Zhao *et al.*[19] with some modifications.

Electrophoresis: A-Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE):

The SDS-PAGE was performed according to the method of Laemmli[20]. The residue of defatted peanut meal was dried. About 0.01 g of the defatted meal residue was extracted by adding 0.5 mL of trisHCl extraction buffer (0.125 M, pH 6.8) and was carried out (analyzed) in polyacrylamide gels vertical type using a Mini-Protein II Electrophoresis cell unit. About 1.0 mm thick, 10×10 cm notched glass plates. Using 12% separating gel and 5% stacking gel according to the methods prescribed by Laemmli[20] and Jensen and Lixue[21].

Gel documentation and analysis: Molecular weight of different bands was compared with (FISHER BIO reagents) a mixture of standard protein markers include range in size from 10-200 kDa when analyzed by SDS-PAGE and stained with coomassie blue.

B-native-electrophoresis

Sample preparation: About 0.01 g of the dried residue samples were mixed with 0.5 mL of sample buffer. Gel preparation and sample loading were similar as the running conditions, according to the methods prescribed by Laemmli[20] and Jensen and Lixue[21]. Except that SDS and β-mercapto-ethanol were not the part of sample and running buffers. The concentrate separating gel were prepared 8% (Fig. 1).

Anti-carcinogenic activity: Evaluation of anti-carcinogenic effect of peanut meal.

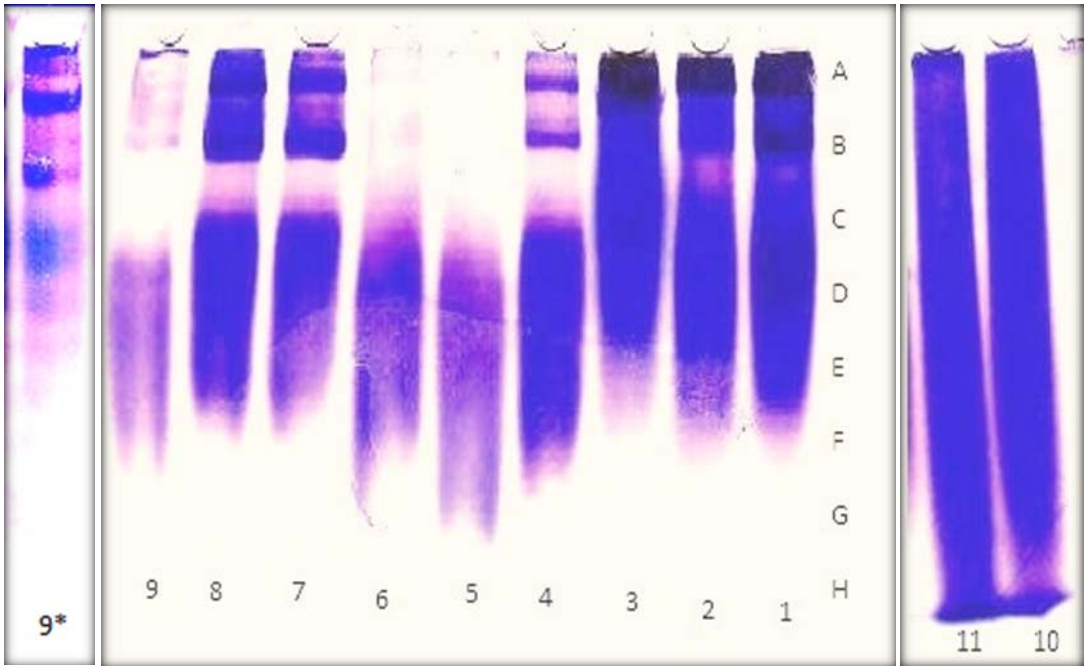

Fig. 1: 8% native-PAGE electrophoresis proteins of defatted peanut meal residue

Lane1: 100% Methanol, Lane 2: 100% Ethanol, Lane 3: Peanut meal, Lane 4: Methanol 7: Ethanol 7: H_2O_6), Lane 5: 50% Ethanol, Lane 6: 60% Ethanol, Lane 7: 70% Ethanol, Lane 8: 80% Ethanol), Lane 9*: 100% water, Lane 9: Extract from water then re ppt again, lane 10: 70 mL Ethanol: 30 mL 1 N Hcl) and Lane 11: 70 mL Ethanol: 30 mL 1 N NaOH

Measurements of potential cytotoxicity of the extracts were carried out by the SRB assay, according to the method of Skehan *et al.*[22]. This evaluation was done in the National Cancer Institute, Cairo, Egypt.

Statistical analysis: All results were carried out in triplicates and values were expressed as Means±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).

RESULTS AND DISCUSSION

The main aim of this study was to find out the best solvents for extraction of phytochemical compounds and to estimate its antioxidant activity of peanut meal with saving its contents of protein in good quality to be used in different applications. Ten extracts of peanut meal was obtained using solvents of different polarity. Their anti-oxidant activities were examined. This study will strongly increase the maximum utilization of peanut meal. The low molecular weight peptides were easily digested and were recommended as baby, sports people andgetriatic food. The soluble extracts studied the phenolic, flavonoids and saponins provide a natural source of bioactive compounds which is the goal of scientist in the whole world to shift naturally products for the cure or prevention of many diseases instead of synthetic medicine.

The results in Table 1 were an indication to the correlation between bioactive compounds and the soluble protein.

Total Proteins Extracted (TPsE): Ranged from 99.8-2.4 mg g^{-1} dried meal, the highest soluble extract yield were obtained by (Ethanol: 1 N NaOH) (Ethanol: 1 N HCl) and the least yield extract by 100% ethanol. The utilization of acid treatments with thermal hydrolysis increased the solubility of protein[23], this was because of the partial deamination and weak hydrolysis.

Total Phenolic Extracted (TPE): Ranged from 14-2.8 mg g^{-1} dried meal, the highest extract yield was obtained by (Ethanol: 1 N NaOH) 14 mg g^{-1} current results in the same line with Choi *et al.*[24], who noticed that bound phenolic were extracted in acidic hydrolysis from wheat bran. The phenolic compounds highly extracted in basic medium than in neutral or acidic medium[25]. The TPC extracted by 50% ethanol from defatted rice bran was found in appreciable amounts[26]. This result in the same line with present results in Table 1 where

maximum extraction of TPE and TPsE was accomplished with Ethanol: 1N NaOH. The highest anti-oxidant activity does not related to the highest in extraction yield[27]. Other studies noticed a direct correlation between antioxidant activity and phenolic content[14].

Total Extracted Soluble Flavonoids (TFE): Varied from 0.03-0.001 m g^{-1} in dried meal (Table 1), the highest extract yield was obtained by ethanol: 1 N HCl. The authors noticed that TFE values were higher in acidic hydrolysis than in neutral or basic hydrolysis. This agreed with Sani *et al*.[28] who observed that TFE were highest in the acidic medium than in basic medium. Ultrasonic assisted extraction to extract flavonoids with 65% ethanol from peanut hulls[29]. These results agreed with values in Table 1 where, methanol, ethanol: 1 N NaOH gave the best results for extraction of flavonoids.

Total Saponin soluble Extracts (TSE): Varied and ranged from 0.83-0.25 mg g^{-1} dried peanut meal, the highest extracted yield was obtained by Methanol 100% and with least extraction solvents H_2O which yield 0.25 m g^{-1}. Saponins have potential utilization in the industrial process and in pharmaceuticals. About 70% methanol in the extraction of saponins from saponaria seeds and this is in the same line with our results[30]. Peanut shell by products when soaked with methanol noticed low levels of total saponins[31].

Anti-oxidant activity of defatted peanut meal soluble extracts

DPPH· radical scavenging activity: The (ethanolic: 1N HCl) extracts in Table 2 showed a stronger DPPH radical scavenging activities than other solvents and aqueous extracts. Hydrogen peroxide-scavenging activity of peanut meal extracts showed that BHT>ethanol: 1 N HCl.

Reducing power of peanut meal extracts: The results showed that ethanol: 1 N HCl had the stronger reducing capability, in addition, soluble extract noticed higher reducing power than BHT.

Table 2 revealed that peanut meal soluble extracts exhibited an elevated anti-oxidant activity when measured by three methods. This was due to the high contents of phenolic compounds in the extracts correlated with the anti-oxidant activities[14,32]. The methanolic extracts exhibited high anti-oxidant activities from Xanthium strumarium L[33]. The germinated brown rice had anti-oxidant activity higher with acidic than basic hydrolysis[28]. Also the hydrogen peroxide is poorly reactive in aqueous solutions[34]. The previous studies showed that peanut meal extracts exhibited good anti-oxidant activities sometimes higher than that of BHT[14]. There was a strong anti-oxidant activity of water and ethanolic extracts of fennel seed when compared with BHT[35]. In this study the authors used defatted peanut with its skin because peanut skin contain anti-oxidant compounds such as phenolic compounds, flavonoids, procyanidin and others[10,36].

Electrophoresis: The electrophoresis of seed storage protein is a technique to investigate protein pattern and classification of plant varieties[37,38]. In the current study, the electrophoresis used to illustrate the effect of different treatments on the quality of peptide bands of defatted peanut meal protein after several treatments, also examined for the degree of aggregation and degradation.

The protein in the solid phase (residue) was subjected to native and SDS-PAGE analysis because modifications of protein structure effects on its functional properties when compared with the native proteins[39].

The result in Fig. 1 indicated that these large proteins were completely insolubilized in high concentration organic solvent, acid and base treatments (region A). In contrast, the smaller proteins (located at the gel from region C to H) were highly prone to degradation upon treatment, lanes 1-11 correspond to the same proteins treatments differently show higher mobility by comparing to peanut meal (lane 3 without treatment), this may be due to the presence of more charges than the starting protein at lane 3. The protein band (located at the gel from region B to C) seemed to be highly soluble with all pretreatment conditions tested except water extraction (lane 9*) and showed in Fig. 2, SDS-PAGE analysis of the protein in aqueous phase from (100-60 kDa). The peanut proteins classified as water soluble (albumins) and saline soluble (globulins)[40]. The globulins are made up of two major proteins, arachin and conarachin these showed in Fig. 1 in lane 9* (100% water) more intensive than lane 9.

SDS-PAGE analyses of the liquid and protein ppt Fig. 2 and 3a, b: This study had shown that SDS-PAGE method with a biochemical marker can be well used for illustration of different treatments. Figure 2 showed two visible bands of proteins from all treatments located from (100-60 kDa) after pretreatment referred to be highly soluble with all pretreatment conditions tested. In general, the SDS-PAGE patterns of proteins bands in these study look almost the same, except that the proteins of (25 kDa to the end of the gel) in lane 1 and 2 were more visible and intensive than others,

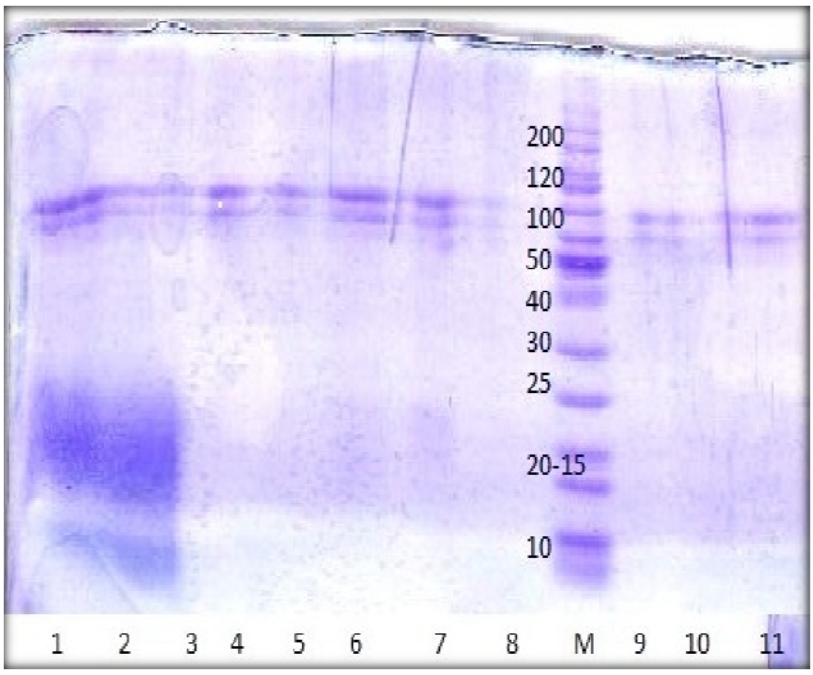

Fig. 2: About 12.5% SDS-PAGE electrophoresis proteins of defatted peanut soluble meal

Lane M: Marker, Lane 1: 70 mL Ethanol:30 mL 1 N NaOH), Lane 2: 70 mL Ethanol: 30 mL 1 N HCl), Lane 3: 80% Ethanol, Lane 4: 70% Ethanol, Lane 5: 60% Ethanol, Lane 6: 50% Ethanol, Lane 7: Methanol 7: Ethanol 7: H_2O_6, Lane 8: Buffer, Lane 9: 100% Ethanol, Lane 10: 100% Methanol and Lane 11: 100% Water)

Table 2: Anti-oxidant activity of defatted peanut meal soluble extracts at room temperature

Treatment 1 g meal: 45 mL solvent, tow times, each time ultrasound for 1 h then soaking 24 h	DPPH scavenging effect (%±SD)	Hydrogen peroxide scavenging effect (%±SD)	Total reductive capability ($m\ g^{-1}$)±SD
100% water	12.40±0.1^{h}	8.8±0.2^{d}	0.06±0.01^{i}
100% methanol	88.20±0.89^{c}	ND	4.90±0.02^{e}
100% ethanol	31.21±0.5^{g}	ND	1.22±0.01^{h}
Methanol: Ethanol: H_2O (7:7:6)	81.80±0.8^{d}	2.7±0.1^{f}	5.00±0.3^{d}
Ethanol: H_2O (50: 50)	77.00±0.7^{e}	6.4±0.3^{e}	2.10±0.02^{g}
Ethanol: H_2O (60: 40)	88.00±0.1^{c}	9.0±0.01^{c}	6.20±0.2^{b}
Ethanol: H_2O (70: 30)	77.00±0.5^{e}	ND	5.70±0.1^{c}
Ethanol: H_2O (80: 20)	89.40±0.4^{b}	ND	5.20±0.05^{d}
Ethanol: 1 N HCl (80: 30)	91.50±0.5^{a}	9.1±0.02^{c}	8.30±0.3^{a}
Ethanol: 1 N NaOH (80: 30)	90.90±0.9^{a}	11.4±0.3^{b}	4.40±0.04^{f}
BHT	51.50±0.3^{f}	23.7±0.2^{a}	1.10±0.1^{h}
LSD at 5%	1.030	0.3645	0.2691

Different letter(s) in each column indicates significant differences at $p<0.05$, ±SD

indicating to protein which bounded with bioactive compounds of peanut meal protein (used in this study). This may be due to the differentiation in solvent polarity that resulted in different extraction strengths and also different solvent systems[41,42].

SDS-PAGE: Totally invisible bands (absent) in the 5 lane (lane 2, 3, 4, 9 and 10), indication to different structure and the low molecular weight proteins (less than 10 kDa) were (lane 9 and 10) generated intense 4 bands (from 15-60 kDa) and highly prone to degradation upon pretreatment, leading to their complete hydrolyses and this was agreed with AOAC[43] which used acid thermal hydrolysis to obtain small peptides and amino acids. The examined samples showed new peptides of lower molecular weight $\leq$10 kDa and less intense bands from (200-80 kD). From the above results, most of treatments were suitable as potential food ingredients (Fig. 3a, b). According to the electrophoretic profile was classified into three regions with high, intermediate and low molecular weights related with typical bands corresponding to vicilin (7S fraction) and legumin (11S fraction) subunits and polypeptides[44]; which showed different susceptibilities to hydrolysis because of their different aggregation forms, happened by structural native conformation of globulins[44,45].

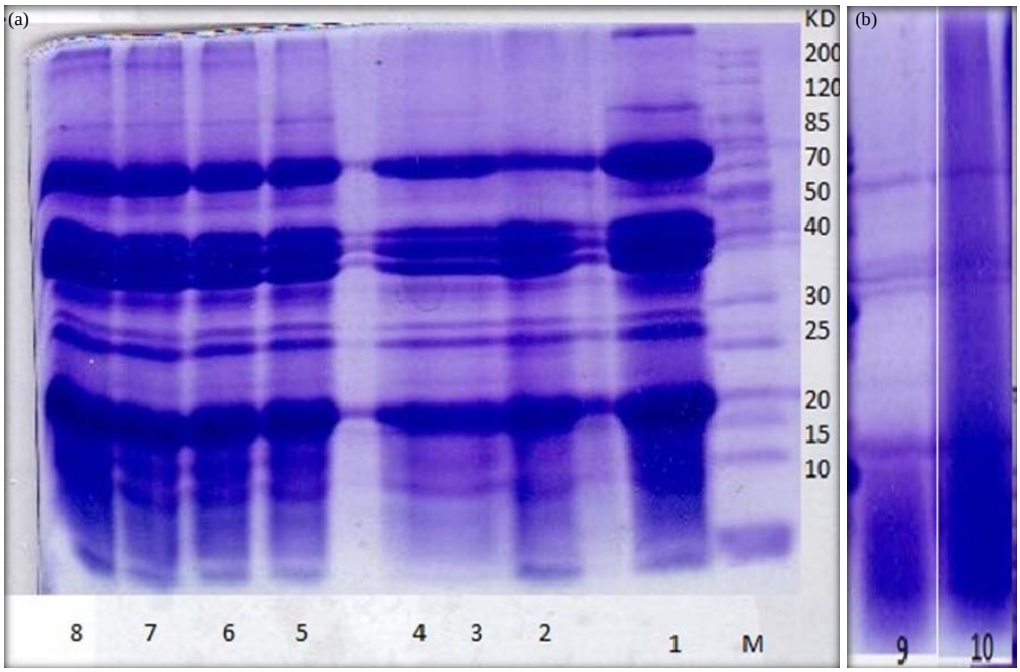

Fig. 3(a-b): About 12.5%SDS-PAGE electrophoresis proteins of defatted peanut meal residue

Lane M: Marker, Lane 1: meal, Lane 2: Extract from water then re ppt, Lane 3: 100% Methanol, Lane 4: 100% Ethanol, Lane 5: Methanol 7: Ethanol 7: H_2O_6, Lane 6: 50% Ethanol, Lane 7: 60% Ethanol, Lane 8: 70% Ethanol, Lane 9: 70 mL Ethanol: 30 mL 1 N Hcl, Lane 10: 70 mL Ethanol: 30 mL 1 N NaOH)

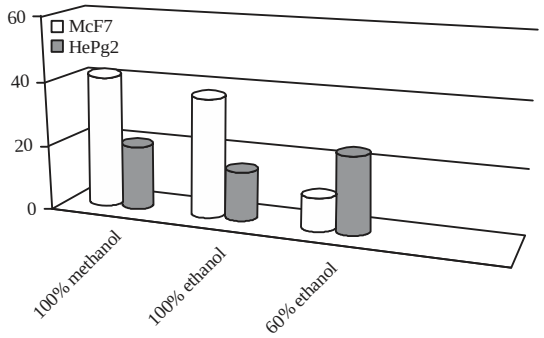

Fig. 4: Anti-carcinogenic effect of three different extracts of soluble peanut meal

IC_{50} is the percentage which kills surviving cancer cells up to 5

The hydrolyzed soy appeared large increases in the intensity of some bands for LH were perhaps correlated to the majority areas of the polypeptides exposed to hydrolysis[46].

Finally, SDS-PAGE and native electrophoresis were affected by different treatments to extract bioactive compounds and also showed good protein quality of the defatted peanut meal residue.

Anti-carcinogenic activity: In this work the bioactive compound extracts from peanut seed meal were studied for their anticancer activity. Figure 4 showed the effect of three phenolic extracts of different solvents (100% ethanol, 60% ethanol and100% methanol) from peanut meal on two human carcinoma cell line Liver Carcinoma Cell Line (HEPG2) and Breast Carcinoma Cell Line (MCF7). The results indicated that (60% ethanol was the most effective on (MCF7) and 100% ethanol were the most effective on (HEPG2). The IC_{50} is the percentage which kills surviving cancer cells up to 50%. Peanut testa contains various types of phenolic compounds such as resveratrol, ferulic acid p-coumaric acid and sinapic acid which possess anti-cancer and anti-oxidant activities[26,47]. Phenolic compounds exhibited anticancer activities against colon cancer cell line[48].

CONCLUSION

The favorable solvent for extraction of peanut meal protein was ethanol: 1N NaOH. It solubilized most protein and bioactive compounds in the meal sample. The analysis of soluble and insoluble of peanut meal proteins by native and SDS-PAGE showed peptide bands at low molecular weight in range(up to 25 kDa); which were extracted by acid and base treatments. These peptides were easily digested and were recommended as baby, sports people and geriatric food. The soluble extracts showed high contents of phenolic compounds especially that extracted by ethanol: 1N NaOH.

It also contains appreciable amounts of saponins and flavonoids that exhibited anti-oxidant activities especially DPPH scavenging activities 91% extracted by ethanol: 1N NaOH. The protein extracts of 60% ethanol were the most effective on (MCF7) and 100% ethanol were the most effective on (HEPG2).

SIGNIFICANCE STATEMENT

This study good described various treatments and recycling peanut by-products/waste that can be beneficial for people, who follow a plant diet, avoid dairy products for health reasons and children food. This study will help the researcher to uncover the critical areas of using organic solvents mixed with acid or base that many researchers were not able to explore. Thus a new theory on optimizations of peanut by-products/waste with fast methods and low cost may be arrived at. The future direction work II- studies more treatments as dietary supplement with and without nano to solve anemia problem and to compensate food deficiency.

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