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Study the Hemolytic and Cytotoxic Effects of Saponin Isolated from Soy Bean.

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Abstract: Saponin is an important class of natural products that can be found primarily in many plants, as well as in some marine animals. It has a diverse range of properties, which include pharmacological and medicinal properties, strong hemolytic properties, as well as antimicrobial, insecticidal, and anticancer activities. Our study aimed to isolate the saponin from different plant sources (legumes) and to evaluate its hemolytic and cytotoxic activity in higher plant source. In this study the saponin was extracted from legumes using choloroform and ethanol. The concentration of saponin was determined by colorimetric method. Soybean saponin source was loaded on silica gel column chromatography and was detected by thin layer chromatography (TLC), infrared spectroscopy (IR) and confirmed by high performance liquid chromatography (HPLC). In addition the hemolytic activity of soybean saponin was evaluated on human blood erythrocyte and the cytotoxic effect on human colon cancer cell line (Coca) was determined by using MTT assay. Our result showed the presence of blue violet spot on thin layer chromatography in soya been saponin and confirmed by IR spectrum by presence of C-H bond of at 2927cm^{-1}, C-O stretching at 1059 cm^{-1},C=C bond at 1628cm^{-1} and OH bond at 3400cm^{-1}. Hemolytic dose (HD50) that made hemolysis for 50% of erythrocyte was 0.412 mg /ml. MTT assay releaved that saponin extracted from soya bean had IC_{50} 43.4µg/ml against colon cancer cell line in 48hr treatment. **In conclusion**, Soya saponin inhibits the proliferation of colon cancer and thus it may have therapeutic application in future.

Key words: *soybean, saponin, hemolysis, colon cancer*.

Introduction:

For thousands of years natural products have played a very important role in health care and prevention of diseases **(Phillipson, 2001)**. Natural products, which are a rich source of compounds with enormous structural diversity, have been extensively explored in the field of drug discovery and have led to remarkable successes. This is particularly evident in the field of cancer therapeutics, where over 50% of the approved drugs discovered in the last two decades of the 20th century were of natural origin **(Newman et al., 2002).**

Legumes play an important role in the traditional diets of many regions throughout the world especially soybean because it is a concentrated source of isoflavones, phytosterols, phytates and saponins and thus it has received considerable attention for their

potential role in preventing and treating cancer **(Messina and Barnes, 1991).**

The name 'saponin' is derived from the Latin word sapo, which means 'soap', because saponin molecules form soap-like foams when shaken with water due to the combination of hydrophilic and lipophilic properties ***(Gepdiremen et al., 2005)***.

Saponins are one of the groups of secondary metabolites and it is generally composed of triterpenoid or steroid aglycone moiety with complex substituent of oligosaccharide ***(Adiukwu et al., 2013***).These saponins are heterogeneous or diverse group of glycosides which is produced mainly by medicinal plants or natural products and also by lower marine animals and some bacteria ***(Gepdiremen et al., 2005).*** The presence of both polar (sugar) and non-polar (steroid or triterpene) groups provide saponins with strong surface-active properties which then are responsible for many of its adverse and beneficial biological effects such as hemolytic and anticancer properities ***(Zhao et al., 2008)***. The haemolytic action of saponins is believed to be the result of the affinity of the aglycone moiety for membrane sterols, particularly cholesterol, with which they form insoluble complexes (***Man et al., 2010)***.

Differences in saponin structure which include the type, position, and number of sugar moieties attached by aglycosidic bond at different positions of the rings can characteristically influence biological responses, especially for the antitumor activity so the antitumor effect of saponin worked through various pathways, such as anti-cancer, anti-metastatic, immunostimulation and chemoprevention **(Bachran *et al.*, 2008)..** Hence this study is aimed to evaluate saponin from Egyptian plant sources for hemolytic and cytotoxic activity.

Material and methods:

1. Plant materials:

Legumes: bean (*Vicia faba*), soybean (*Glycine max*), cowpeas (*Vigna unguiculata)*, chichpea (*Cicer arietinum)*, kidney beans (*Phaseolus vulgaris*) and lupine (*Lupinus albus* L.), which were purchased from Tanta market, Egypt.

2. Chemicals:

Standard saponin was purchased from Fisher Scientific, UK. Silica gel (60-200 mesh size) was purchased from Loba chemie, Mumbai, India. DPPH (2,2 diphenyl-1-picrylhydrazyl), (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich, USA. All other chemicals used were of high grade.

3. Extraction and determination of saponin from legumes:

A modified method of **Tani *et al.* (1985)** was used**.** Samples were ground and defatted with chloroform in a soxhlet apparatus to remove the interfering pigments and lipids. 50 g of the defatted sample were re-extracted with 250ml of 95% ethanol (volume enough to make reflux) in a soxhlet extractor and the source of heating was water bath at 75^0c (the boiling point of ethanol)for 16hrs or till the color of the solvent in the thimble became colorless. The ethanolic extract was then concentrated by evaporation of the solvent using rotary evaporator under reduced pressure. The concentrated extract was then precipitated using excess volume of acetone. The resulting precipitate was filtered and dried to give crude saponin mixture then calculated the saponin mass.

(Yield) %SP= A/SM,

While A is the mass of saponin and SM is the sample mass (per gram).

4. Colorimetric method for quantification analysis of saponin:

The determination of total content of triterpenoid saponins was performed according to the method of **(Xiang et al., 2001)** and (**Yoko et al., 2000)** using standard saponin.

Ten mL of 2 M HCl was added to 10 mL ethanol solution of saponin and hydrolyzed for 3 h at 90°C, cooled, and extracted twice with 80 mL diethyl ether. The saponin fraction in the ether layer was isolated and the ether was removed under reduced pressure and the residue which contained sapogenin was dissolved with ethyl acetate and made up to 10 mL for spectrophotometry. 10µl of hydrolyzed saponin from each sample completed to 0.6ml and was determined by adding 0.2ml mixture of 5%vanillin-acetic acid (weight per volume) and 1.2ml of perchloric acid , the mixture was incubated at 70^0c for 15 min. After that the tubes were taken out and cooled in running water for 2min. then, ethyl acetate was added in order to make the total volume being 5ml. Blank solution of acetic acid was used as reference, the absorbence was measured at 550nm.

The standared curve was ranged from (0.1-1mg/ml)and the concentration of saponin extracted from different legumes was calculated according to the following equation:

$A=0.75C(R^2=0.9933)$

5. Chromatographic analysis:

5.1 Column chromatography:

A silica gel column (60-200 mesh) was used for soybean saponin purification. Concentrated crude saponin sample was applied to the column. The impurities were washed with n-hexane: ethanol (1:1)

(two bed volume) through a 12x6 cm bed of silica gel. The column was eluted with n-butanol/ ethanol/water (1:1:1). The flow rate was 4 ml/5min and the total bed volume was 144 ml. Fractions (4 ml) were collected. Aliquots from fractions were applied to a strip of TLC plate and the concentration of saponin was determined in each fraction **(Khalil and El-Adawy, 1994)**

5.2 Thin-layer chromatography (TLC):

TLC analysis was carried out on aluminium plates coated with silica gel 60 (Merk KGaA Darmstadt, Germany) according to the method of (**Kerem et al., 2005).** 10µl of the solution of the standard saponin, saponin enriched extracts from different legumes and soybean saponin after column chromatography were applied on TLC paper and the plate was developed in the solvent system n-butanol: water: acetic acid (84:14:7) to a distance of 15cm from the origin. The developed plate was dried in the air. TLC plate after development and drying was sprayed with freshly prepared solution of ethanol :sulphuric acid (90:10).After spraying the plate was heated at 110^0c for 10 minutes**.**

6. Fourier transform infrared (FTIR):

Positive fractions from column chromatography were pooled then the solvent was evaporated and the sample was dried. The sample and standard saponin were ground to quite powder with mesh sieve and mixed 1/1 with vacuum dried KBr powder to make compressed pellet with subsequent recording of infrared spectrum. A JNS-CO Spectrum System 4100 LE FTIR spectrometer (Japan) was used for the analysis in the range between 4000 cm−1 and 400 cm−1 at a resolution of 4 cm−1 **(Amini et al., 2014)**

7. High performance liquid chromatography (HPLC):

Saponin from soybean was confirmed and quantified after column chromatography by using YL9100 HPLC system, Japan (C18 column) according to the method of **(Snyder et al., 1997).** 20 milligrams of dried soy sample was dissolved in1ml of 80% HPLC grade aqueous methanol and used for HPLC analysis. The mobile phases were 0.05% trifluoroacetic acid in water (solvent A) and acetonitrile (solvent B). The column temperature was 30 °C. The injection volume was 50 μl. The flow rate was 1 mL/min. The UV absorbance was monitored at 205nm. The concentration of saponin in soybean was calculated by using standard saponin as reference. The results were expressed as milligrams of soyasaponin per gram of soy sample.

8. Determination of hemolytic activity of soybean saponin:

The hemolytic activity of soybean saponin was measured on the basis of method **of (Hassan et al., 2010).** Human blood was obtained and collected on heparin. Separation of human erythrocyte were performed by centrifugation at 4 000 rpm for 5 min. The obtained pellet was washed three times with PBS and 2% erythrocyte suspension was used for assessment hemolytic activity. The concentration of soybean saponin that were used for evaluation of hemolytic activity were prepared in PBS as follow(0.1, 0.3, 0.5, 0.7, 0.9, 1.1 and 1.3 µg/ml) which were incubated with 2ml of 2% erythrocyte suspension for 1hr at room temperature. Saline and distilled water were included as minimal and maximal hemolytic controls. The hemolytic percent developed by the saline control was subtracted from all groups. the samples were centrifuged at 4000rpm for 10min and the free hemoglobin in the supernatant was measured spectrophotometrically at 412nm.

9. Evaluation of cytotoxic effect of soybean saponin:

Cell line and culture

Human colon cancer cell (Coca) line was obtained from institution Serum and Vaccine, Cairo. Coca cells were maintained in DMEM (Dulbecco's Modified Eagle Medium) containing 10% fetal bovine serum (FBS) with 100 U/mL of penicillin and 100 lg/mL of streptomycin in 5% CO2 at 37^0 C. Cells were maintained at a concentration between 2 x 10^5 and 1 x10^6 cells/mL. Cells were subcultured every 2-3 days by total media replacement using trypsin –EDTA. Viable cells were assessed by 0.04% trypan blue exclusion dye using a hemocytometer.

MTT assay

The effect of soybean saponin on Coca cells viability was determined using the MTT assay .Coca cells were seeded in 96-well plate at final concentration 1x10^4. After incubation overnight, the cells were treated with or without different concentration of soyasaponin (10. 20, 40, 60, 80 and 100µg/ml). 48h later, 50 µl of MTT stock solution (2 mg/ml)was added and after incubation for 4 h, the supernatant from each well was removed and the formazen crystals formed by viable cells were dissolved with DMSO (150µl/well) with shaking (at highest speed) for 15min at room temperature. The absorbance was read with a microplate reader at 540nm.

The cytotoxicity was evaluated with reference to the IC50 value. The tests were performed 3 independent times (**Zhang and Popovich, 2008)**

Results

The obtained results in table (1) showed that the total concentrations of saponin in soybean, lupine, cowpea, chickpea, kidneybean and bean were 94.89, 20.77, 13.23, 10.59, 6.18 and 5.7 mg/g dry weight respectively.

In addition, TLC analysis showed the presence of blue violet spot in each extract with Rf 0.4 after the plate was sprayed with ethanol: sulfuric acid (90:10), **Fig. (1).** The soybean extract that contain the highest saponin concentration was partially purified using silica gel column chromatography and the fractions were confirmed of the presence of saponin by TLC then the positive fractions which appeared the presence of blue violet spots without impurities **Fig. (3)** were pooled and the concentration of saponin after pooling was determined compared to before the column, the data showed that the concentration of the pooled saponin was 1671.32 mg with yield percent 56.36% as shown in **Table (2)**.

The FTIR spectrum of purified soybean saponin releaved the presence of C-H bond at 2927cm-1, C-O-C bond at 1052 cm-1,C=C bond at 1628cm-1 and OH bond at 3400cm-1 were assigned in **Fig. (4)** as compared with standard saponin. Also, HPLC analysis releaved the presence of saponin in soybean with concentration 20.5mg/gm dry weight. **Fig. (5) and (6)** showed the chromatogram of standard saponin and soybean saponin with retention time 1.7 and 1.8 respectively. There were other peaks with different retention times but with lower concentrations.

The soybean saponin was screened for its hemolytic activity when the human blood erythrocytes were incubated with different concentrations of soybean saponin for 1h and the free hemoglobin was measured. The results releaved that soybean saponin had the ability to hemolyze the human blood erythrocyte with HD50 0.412mg/ml as in **Fig. (7).**

Fig. (8) showed the effect of standard and soybean saponin on Coca cell viability which releaved that the treatment of colon cancer cells with standard saponin and soybean saponin at a concentration 10µg/ml for each for 48h, the viability of cells were 75.8 and 85.5% respectively. When the concentrations of standard and soybean saponin increased, the viability of colon cancer cells significantly (<0.01) decreased with IC_{50} 28.7 and 43.4µg/ml for standard and soybean saponin.

Table 1. The concentration of saponin in different legumes

Legumes	Concentration of triterpenoid saponin (mg/ml)	Concentration of triterpenoid saponin (mg/g dry weight)	% Yield
Soybean	185.33	94.89	5.12%
Lupine	45.96	20.77	4.5%
Cowpea	82.71	13.23	1.6%
chickpea	57.6	10.59	1.84%
kidneybean	35.16	6.18	1.42%
Bean	55.4	5.7	1.04%

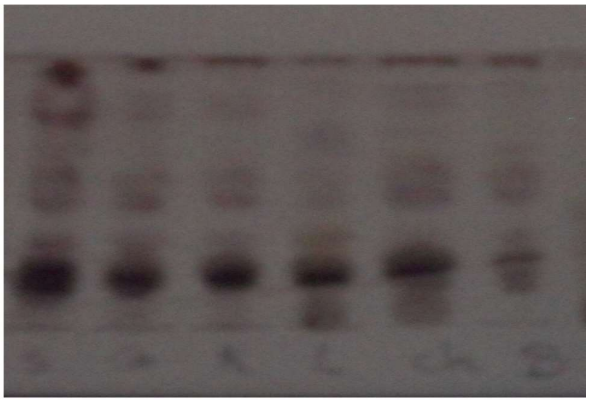

Fig (1): Thin layer chromatography of crude saponin extracted from different legumes

Table 2. Purification scheme of soyabean saponin

Step	Concentration of saponin (mg/ml)	Volume (ml)	Total concentration of saponin (mg)	%yield
1.Crude extract	185.33	16	2965.28	100%
. 2. Silica gel column chromatogr-aphy	34.82	48	1671.36	56.36%

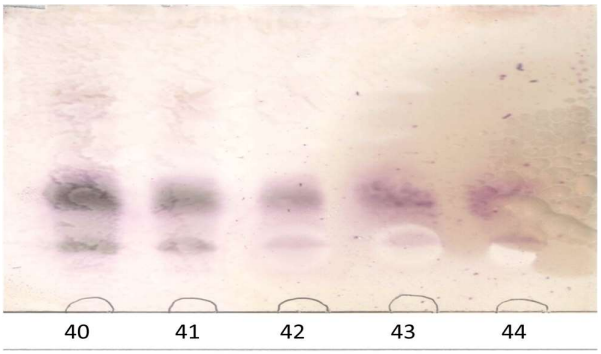

Fig. (2): Thin layer chromatography of soybean saponin after column chromatoraphy.

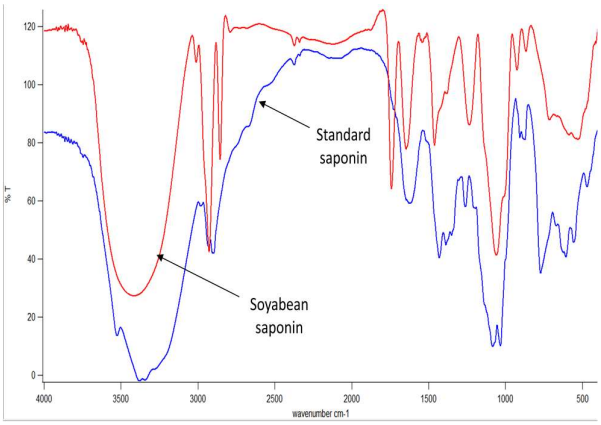

Fig. (3): FTIR spectrum of standard saponin and soybean saponin

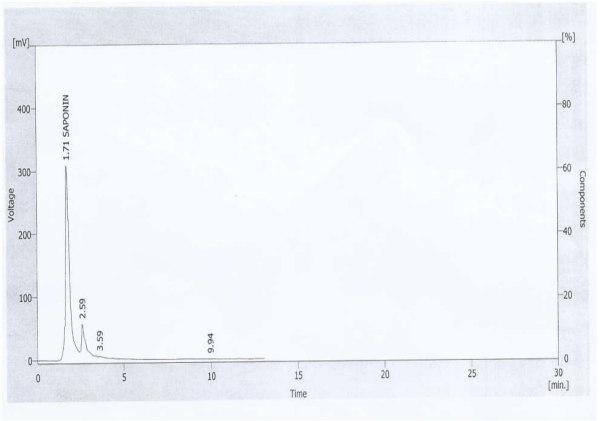

Fig. (4): HPLC chromatogram of standard saponin

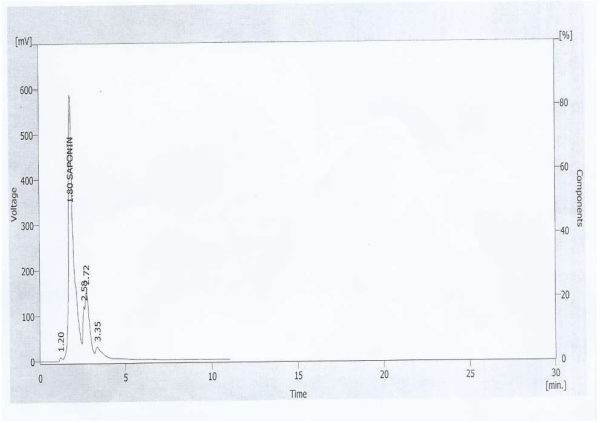

Fig. (5) : HPLC chromatogram of soybean saponin

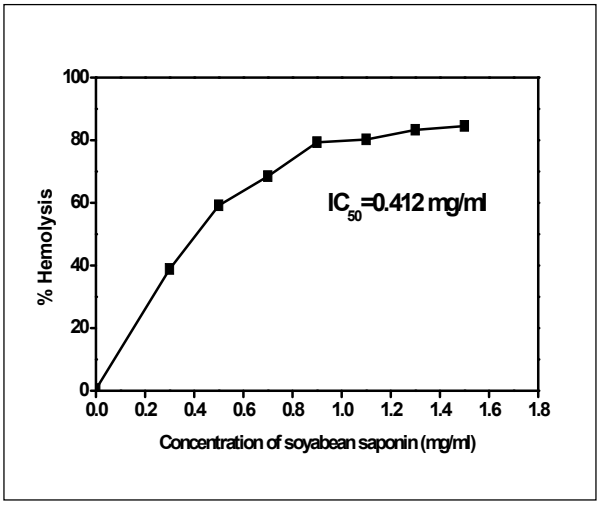

Fig. (6): Hemolytic activity of soybean saponin

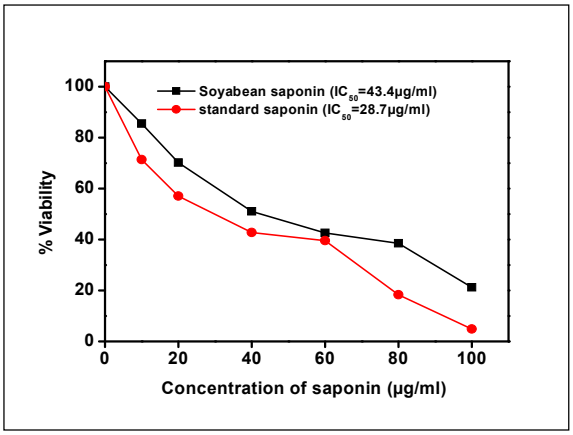

Fig. (8): Effect of standard saponin and soybean saponin on Coca cell viability by the MTT assay after 48 h incubation. The mean percent of control (± standard deviation) was calculated for each cell viability and compared with the viability of untreated cell, which was considered to corresponding 100% viability

Discussion:

In the past, legumes were consumed as the main staplefoods in traditional dietary patterns; they are rich in high-biological value proteins, bioactive peptides, essential amino acids and other bioactive compounds such as functional fatty acids, isoflavones, phenolic acids and saponins **(Messina, 1991; Zahra and Parvin, 2015)**

Saponins are the main bioactive compounds that exhibit a wide range of biological activities and have many therapeutic effects **(Sottorff et al., 2013)**. These compounds have been studied in many plant sources and also in some marine animals. For example, **Silchenko et al. (2008)** identified saponins in Mediterranean, North Atlantic, and North Pacific sea cucumber species.

Hu et al., 2002 extracted and isolated saponin from soybean, they found that the soyasaponin concentration and composition varied in soybeans, soy foods, and soy ingredients and this may depend on the variety of soybeans and the processing conditions used to produce a particular product.

In the present study, saponins were extracted using ethanol followed by precipitation using excess volume of acetone. The main saponin that present in legumes are soya saponins which are triterpenoid saponin of oleanan type so the determination of total triterpenoid saponin content in crude legumes extract was performed by colorimetric method of **Xiang *et al.* (2001)**. Soya bean was the richest source of saponin with concentration 185.3mg/ml ± 0.77. The concentration of saponin in soya bean was higher than previously reported by **Murakami *et al.* (2013)** who extracted saponin from Ilex paraguariensis St. Hil and determined its concentration was 0.366 mg/ml ± 14.01 and **Pasaribu *et al.* (2014)** who determined the concentration of saponin extracted from *Sapindus rarak* Pericarp which was 40.27 mg/ml. The other five legumes, *lupine, cowpea, chickpea, kidney bean and bean* were 45.96, 82.71, 57.6, 35.16 and 55.4mg/ml, respectively.

In this study TLC was applied to confirm the presence of saponin in different legumes extracts as simple and qualitative method. In the present study, saponin extract from lupine, cowpea, chickpea, kidney bean bean and soybean before applying to the column has been separated into six fractions. **Khalil and El-Adawy (1994)** showed that saponin extract from peas and soybean has been separated into seven and six fractions each. The obtained results showed that there are violet spots in each extract with R_f value 0.4 when the plate was sprayed with ethanol and sulfuric acid. These results are nearly in accordance with the results reported by **Jamuna and Subramanium, (2014)** and **Payal *et al.* (2010)** when they extracted saponin from the leaf of *Hypochaeris radicata* L and *Sesbania*

sesban (L.) Merr in which TLC showed violet spots with R_f values 0.56 and 0.61 respectively by using methanol and chloroform as solvent system.

Column chromatography is a method used to purify individual compounds from mixtures of compounds in biology and biochemistry. Silica gel is the most common solid adsorbents that used in column chromatography **(Ranjith *et al*., 2014).** In this study, the soybean saponin was separated and partially purified from soybean crude extract using silica gel column chromatography. Saponin from soybean after elution showed blue violet spots without impurities after running with TLC plate. The results showed that Rf of soybean from thin layer after elution from silica gel column was 0.4 as before elution.

Fourier transform infrared spectroscopy (FTIR) technique was performed as highly specific method for saponin identification. Recognition of saponin in soybean after partial purification with silica gel column chromatography was confirmed by infrared absorptions recorded. FTIR spectrum of the reference sample and partial purified soybean saponin indicated characteristic absorbtion peaks of saponin. It showed the presence of the long sharp peak at 3400cm^{-1} indicates the presence of hydroxyl groups (-OH) and the peak at 2927cm^{-1} represents alkyl groups (C-H). (–C=C) groups at 1628 cm^{-1}. C-O-C bond at 1052 cm^{-1}. Existence of -OH, C-H, and C=C bands in absorptions peak of FTIR spectrum was characteristic of saponins. The C-O-C absorptions indicated glycoside linkages to the sapogenins. Therefore, FTIR spectra represented indicative sign of presence saponin like compounds in soybean **(Kareru *et al*., 2008).**

Some studies were used FTIR for saponin characterization. For instance, **Amini *et al*.** in **2014** performed FTIR spectroscopy for direct identification of saponin like compounds isolated from Persian Gulf brittle star (*Ophiocoma erinaceus*) which revealed the presence of broad and strong signal of hydroxyl group (339 8.78 cm^{-1}), C-H (285 1.54 cm^{-1}), signal olefinic (C=C) (167 3.33 cm^{-1}) , strong absorption signals sulfate group (C-O-C) noted at 121 3.97 cm^{-1}, 105 5.14 cm^{-1}.

The partial purified soybean saponin was identified on reverse phase high performance liquid chromatography. Due to their poor chromophores, most of the soybean triterpenoid saponins can only be detected at wavelength of 205 nm which indicated that the peaks obtained before 5 minutes were saponins as compared with the standard. In HPLC chromatogram of standard and soybean saponin there were high peaks obtained at retention time 1.7 and 1.8 respectively with concentration 20.6 mg/ g dry weight. These results are in agreement with the previous study reported by **Rupasinghe *et al*** in **2003** in which the concentration of saponin in soya bean was 15.6mg/g dry weight in three different cultivars of soya bean by using reverse-phase high performance liquid chromatography (RPHPLC) with external standards.

The unique chemical structure of saponins is related to their hemolytic effects. Pore formation and cell permeabilization of saponins is due to the presence of cholesterol on the target membrane that is essential for the pore formation by interacting with saponins to form micelle-like complex and the hydrophilic sugar moieties are thought to be located in the central of the complex and leads to the development of aqueous pores. Such pores can increase the permeability of membrane and enabling the macromolecules and ions to pass through the membrane bilayer. This pore formation cause alteration of negatively charged carbohydrate portions on the erythrocyte cell surface which lead to lyse erythrocyte. Complexity of sugar moiety and the number of side chain in saponin have influenced hemolytic activity **(Keukens *et al*., 1995 and Man *et al*., 2010**).

In this study, the hemolytic activity of saponin was evaluated on human blood erythrocyte and the results showed that the hemolytic percents of red blood cell treated with soybean saponin was 50% at a concentration 417.73µg/ml. These results are agree with **(Amini *et al*., 2014)** and (**Mozhgan *et al*., 2014**) when they evaluated the hemolytic activity of saponin like compounds isolated from Persian Gulf brittle star (Ophiocoma erinaceus) and purified saponin from *Holothuria leucospilota* sea cucumber on human blood erythrocyte, who found that HD_{50} value was 500µg/ml that indicated for high hemolytic activity. Therefore, **Sun. (2008)** assessed hemolytic activitiy of saponins isolated from the rhizoma of Anemone raddeana and demonstrated that saponins extracted exhibited a low hemolytic effect, with 16.50% and 3.56% at the concentrations of 500 and 250 µg/ml, respectively (**Sun *et al*., 2008).**

Due to the increasing attention paid to the cancer prevention, the term "chemoprevention" has made a career in scientific community (**Steward and Brown, 2013**). Some edible plants have been shown to reduce the risk of various types of cancer in humans (**Ting *et al*., 2014**). Epidemiological data suggest that the risk of cancer rates are lower in the populations consuming the products largely of "natural" origin (unprocessed) (**Saxe *et al*., 2008**).

In this study, the antitumor activity of standard saponin and soybean saponin on colon cancer cells in vitro was evaluated by using MTT cytotoxicity assay. The results of the present study demonstrated that standard saponin and soybean saponin are cytotoxic to human colon cancer cells in which they decreased the viability of Coca cells in a dose dependant manner after 48hr treatment with IC_{50} value 28.7 and 43.4µg/ml respectively. Other study showed that crude saponin isolated from Platycodi Radix induce apoptosis in HT-29 colon cancer cell line with IC_{50} value 37.07µg/ml in 24h treatment (**Kim *et al*., 2008).**

Also in this regard, saponins isolated from Solanum trilobatum leaf *is* preferentially cytotoxic to human larynx carcinoma cells in a dose dependent manner with IC_{50} value of 1mg/ml after 24hr treatment **(Kanchana and Balakrishna, 2011).** Moreover, **Lu *et al*. (2011)** found that the viability of human breast cancer cells (MCF-7) was decreased when Rhizoma Paridis saponins concentration was raised in a dose-dependent manner with IC_{50} value of 71.2µg/ml after 48hr treatment.

Conclusion:

Our data demonstrated presence of saponin in legumes with high concentration in soybean and indicated that with comparation to above research reports saponin extracted from soybean exhibited hemolytic and cytotoxic activity on colon cancer cells in vitro. Based on these findings, for clinical application saponin isolated from soybean must be examined as anticancer chemopreventive drug against colon cancer cells in vivo.

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دراسه التاثير الانحلالى والسمى للصابونين المستخلص من الفول الصويا

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يعتبر الصابونين من المنتجات الطبيعيه الهامه الموجوده فى كثير من النباتات وكذلك فى بعض الحيوانات البحريه. يمتلك الصابونين مجموعه متنوعه من الخصائص والتى تشمل الخصائص الدوائيه والطبيه وخصائصه الانحلاليه القويه كما انه مضاد للحشرات والميكروبات وله نشاط مضاد للسرطان. تهدف هذه الدراسه الى عزل الصابونين من مصادر نباتيه محليه مختلفه من البقوليات وتقييم النشاط الانحلالى والسام للصابونين الموجود فى اعلى مصدر. فى هذه الدراسه تم استخلاص الصابونين من البقوليات باستخدام الكلوروفورم والايثانول. الصابونين

المستخلص من الفول الصويا تم تنقيته جزئيا عن طريق الكروماتوجرافي باستخدام السيليكا ثم التاكد من وجوده باستخدام تقنيه TLC, FTIR و HPLC. بالاضافه الى ذلك تم تقييم قدرة الصابونين المستخلص من الفول الصويا على تحلل كرات الدم الحمراء البشريه وكذلك تاثيره السام على خلايا سرطان القولون البشريه باستخدام تحليل MTT. لقد اوضحت نتائج تحليل TLC الى ظهور بقعه بنفسيجيه فى صابونين الفول الصويا كما اظهرت نتائج تحليل IR الى وجود رابطه بين C-H عند طول 2927سم$^{-1}$وبين C-O عند 1052سم$^{-1}$وبين C=C عند 1628سم$^{-1}$وبين O-H عند طول 3400سم$^{-1}$. كما اظهرت النتائج ان تركيز الصابونين المستخلص من الفول الصويا القادر على تحلل 50% من كرات الدم الحمراء البشريه هو 0.412مل جرام لكل مل. بالاضافه الى ذلك لقد اوضحت النتائج ان حضانه خلايا سرطان القولون البشريه مع تركيزات مختلفه من صابونين الفول الصويا لمده 48 ساعه ادى الى تقليل حيويه هذه الخلايا حيث ان تركيز صابونين الفول الصويا القادر على تثبيط نمو 50% من هذه الخلايا هو 43.4 ميكروجرام لكل مل. ويمكن من هذه الدراسه ان نستنتج ان صابونين الفول الصويا يثبط نمو خلايا سرطان القولون لذلك من الممكن ان يكون له تطبيقات علاجيه فى المستقبل.