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EXTRACTION OF α-SOLANINE AND α-CHACONINE FROM GREEN POTATO TUBERS AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

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Abstract

The main glycoalkaloids (GAs) in the flesh of two varieties Iranian and Iraqi potatoes were extracted with 5% aqueous acetic acid. This study aimed to investigate the GAs content and antibacterial activity of flesh samples from turned green potatoes. In addition, the results showed that both types of potato contain high toxic GAs, solanidine, α-solanine and α-chaconine. The amount of glycoalkaloids in Iranian potato was higher than that of Iraqi potato. However, the main alkaloid was α-solanine with concentrations of 931.333 and 923.16µg/ml for both Iranian and Iraqi potato, respectively. The antimicrobial activities of glycoalkaloids were successfully examined against *Staphylococcus aureus, Pseudomonus aeruginosa* and *E. coli* by cup plate method. The GAs showed effective inhibition against three pathogenic bacteria *Staphylococcus aureus, Pseudomonus aeruginosa* and *E. coli* while the highest effect was 9mm on *E.col*. The outcome suggests that large amounts of GAs could be used as antibacterial from both extracted samples. As well as GAs levels are detrimental to human health, these potatoes should be systematically set aside for safety consideration. Furthermore, the technology of herbal drugs has become very important because they are deemed to have very little or no side effects and toxic impacts.

***Key words*:** Potato flesh, glycoalkaloids, HPLC analysis, antimicrobial activity

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important essential products consumed by humans (Mattila and Hellstrom 2007). Potatoes are an excellent source of carbohydrates, elevated protein quality, vitamins, minerals and antioxidant polyphenols (Schieber *et al.,* 2009). The potatoes consider as a rich source of food, however, they have a group of toxic compounds known as steroidal glycoalkaloids (GAs) which are found mainly in the skin (Friedman, 2006). GAs are secondary metabolites commonly present in plants of the *Solanaceae* family, including tomato, potato, pepper and eggplant (Passam and Karapanos, 2008; Majeed *et al.,* 2014; Friedman, 2015). Due to their significance in food safety, the determination of GAs in potato tissues have drawn considerable attention and different analytical methods have been reported (Friedman *et al.,* 1998; Simonovska and Vovk, 2000).

The GAs including two major compounds (Fig. 1) which are α-solanine and α-chaconine presented as 95% of total amount of potatoes (Edwards *et al.,* 1996; Friedman, 2004). These compounds are made of aglycone solanidine plus a nonpolar lipophilic steroid nucleus united to the two heterocyclic rings containing nitrogen. At C-3, the glycosides have soluble polar to the water of trisaccharide (Lachman *et al.,* 2001). The carbohydrate moiety in β-solanine is comprised of glucose, galactose and rhamnose (β-solatriose), while glucose and two moieties of rhamnose (βchacotriose) are present in α-chaconine. The proven ratio for the α-chaconine and α-solanineare always respectively, about 60:40, greater ratio always occupied by α-chaconine from GAs of potatoes (Roddick *et al.,* 1988; Slanina, 1990). Moreover, in gemmiparous or green potato there is a lot of α-solanine due to improper storage, especially the green or germination site (Affleck *et al.,* 2017).

Additionally, potato tubers typically contain about 4 to 12mg of GAs per 100g in total weight of fresh potato and 20 to 60mg of GAs per 100g of total weight of freeze

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or dried potato (Sotelo and Serrano, 2000). However, in case of increase GAs more than the normal value (4 to 12mg fresh potato and 20 to 60mg for freeze potato) could be considered as a toxic and gives a bitter taste. The toxicity GAs can cause diarrhea, vomiting, nausea, stomach and abdominal pains, fever, headache, rapid and weak pulse, hurried breathing, delirium, hallucination and in extreme cases, coma (Mondy and Seetharaman 1990; Friedman and McDonald, 1997). Generally, the concentration of GAs accumulates to high levels in response to a number of factors, like exposure to light, mechanical injury, poor conditions of growth, fungal attack, environmental factors and storage conditions (Edwards *et al.,*1996; Dao and Friedman, 1994; Machado *et al.,* 2007). Furthermore, α-chaconine and α-solanine molecules are heat stable and the structure of these chemicals do not change by frying, home cooking, baking and microwaving, thus, they are fairly heat-stable with melting points in the range of 190-285°C (Tajner-Czopek *et al.,* 2012; Lachman *et al.,* 2013). For these reasons, the concentration of GAs is critical and should be monitor before consumption of potatoes by human (Bushway *et al.,* 1981).

However, in pharmaceutical industry, α-solanine and α-chaconine are suitable for utilization. The aglycone-solanidine is a precursor for the synthesis of hormones like progesterone, cortisone and testosterone (Nikolic & Stankovic, 2003). Some *in vitro* studies indicate certain beneficial effects for instance anti-allergic, anti-diabetic, antipyretic, antibiotic properties and anti-inflammatory (Friedman, 2006; Kenny *et al.,* 2013). GAs has some inhibitory effects similar to natural pesticide and insecticide, as well as GAs can be used against fungi, bacteria and insect pests (Jadhav *et al.,* 1991; Boulogne *et al.,* 2012). Moreover, α-chaconine and α-solanine, both are functioning as antifungal activities (Fewell and Roddick, 1997). As indicated by these beneficial and harmful properties of GAs, it is necessary for enforcing the regulation of GAs in potatoes and their products. Therefore, the main objective of this study was develop a rapid, sensitive and accurate method for determination of α-solanine and α-chaconine in the flesh of turned green, *Solanum tuberosum* potatoes. As well as, the antibacterial activity of toxic glycoalkaloids were investigated against *Staphylococcus aureus, Pseudomonus aeruginosa* and *E. coli* and then comparison of GAs which have been extracted from two types of potatoes, in Erbil province.

Materials and Methods

Materials

All chemical which have been used in this investigation were analytical grade, ordered and purchased from commercial source in Kurdistan-Erbil province. Acetic acid (CH_3COOH), ammonium hydroxide (NH_4OH), butanol $CH_3(CH_2)_3OH$ and methanol (CH_3OH). All the solutions were prepared using distilled water.

The collection of samples

Two types of potato (Iraqi and Iranian) were taken as the sample for the extraction of GAs. These tubers were purchased from a local market in Erbil, Kurdistan.

Sample preparation

Ten tubers of uniform shape and size of each variety with greening were washed with tap water in order to remove the soil. After cleaning, potato samples were peeled, sliced green area manually with a knife and then placed in a laboratory oven at 40°C for 48h. both dried samples were ground to a powder and stored in dark glass color containers, firmly closed, in a refrigerator at 4°C.

Extraction of Glycoalkaloids

To prepare the extract, 1g of powdered potato from each varieties of potato was weighed and dissolved in 15ml of 5% aqueous acetic acid. The mentioned solution of each potato has been transferred to 50ml sized conical flask and stirred with a magnetic stirrer for 30 minutes. What man (No. 4) filter paper was used to separate the un-dissolved sample particles. The purpose for the addition of 30% ammonium hydroxide was to adjust the pH of mixture to the 10 which facilitate in the precipitation of the GAs. After that, with 15ml of water saturated butanol, the alkaline extract was partitioned twice. The combined butanol extracts were evaporated using water bath at 40°C. The residue was dissolved in 2ml of methanol, again the organic solvent was evaporated until it was dry. The precipitate was collected and kept in dark vials tightly stopper in a refrigerator at 4°C and then tested for their GAs content.

High Performance Liquid Chromatography (HPLC*)*

The extraction of alkaloids were done according to enclosed procedure on FLC (Fast Liquid Chromatographic) column, 3μm particle size, phenomenex C-18 (50×4.6mm I.D) column, Mobile phase, were 0.01M phosphate buffer pH 6.2:acetonitrile (75:25, V/V) detection UV set at 330nm, flow rate 1.4ml/min.

Extraction

Plant samples 1g of powder homogenized, grinding to fine powder, dissolved in 3% H_2SO_4 for 2h. at room temperature. Filtration on 2.5um filter paper, 25% NH_4OH (pH 9.5) for the adjustment of supernatants and addressed to Extrelut (Merck) columns. The alkaloids were eluted by CH_2Cl_2 (6ml/g Extrelut) and the extracts

Table 1: The glycoalkaloid (µg/ml) content in potato samples as determined by the HPLC.

Seq	Subjects	Rt (min)	Area uv	Conc. of of standard	Conc. Of Sample (1)	Conc. Of Sample (1)X 20=	Conc. Of Sample(2)	Conc. Sample (2)X 20=
1	Solanidine	2.03	218728	26.48	14.519	290.39	15.873	317.46
2	α-Solanine	3.19	294997	35.71	46.566	931.333	46.158	923.16
3	α-chaconine	4.148	312258	37.8	40.763	815.277	25.736	514.72

mixtures were evaporated using nitrogen stream to the final stage of dryness. Thus obtained residues were resolved in 1ml CH_3OH for the further analysis by HPLC according the optimum separation of authentic standard, then the concentration was determined by comparison between area of standard with that of sample under the same separation condition and each standard was 25µg/ml.

Equipment

The processes of separation took place by liquid chromatography Shimadzu 10AV-LC which optioned with binary delivery pump model LC-10A Shimadzu, the elute peak illustrated and interpreted by UV-Vis 10A-SPD spectrophotometer (Mohsen and Rouini, 2008).

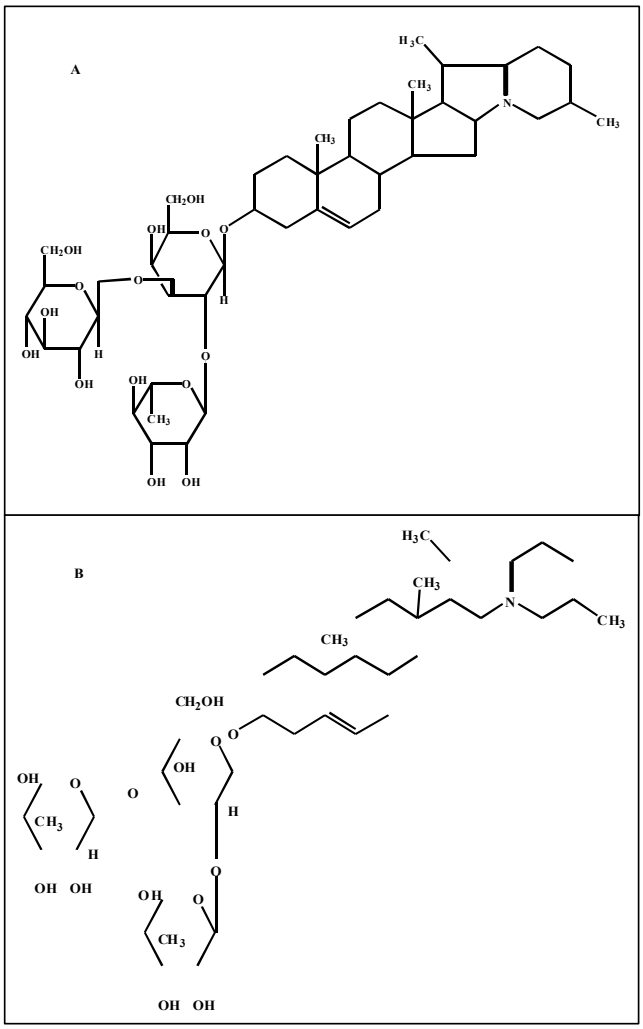

Fig. 1: Chemical structures of GAs in potato: A α-solanine and B α-chaconine.

Screening of extracts by disk diffusion technique

Disk diffusion techniques guided by standard procedure from previous investigation (Kamel *et al.,* 2013; Kamel and Jarjes, 2015). The pathogenic strains of these bacteria (*S. aureus*, *E. coli* and *Pseu. aeruginosa*) were inoculated on the entire surface of Mueller-Hinton agar. Sterile disc papers (sized of 6-mm) with ethanoic extraction and essential oils 1 volume added to disc in 20µl then discs were placed on the surface of Mueller-Hinton agar, 10% DMSO 20-µl aliquot was also added as an adverse control to a sterile paper disk. Agar plates were left at room temperature for 15min before the time of incubation at 37°C for 24h. finally, overnight incubation the plates have been examined the growth and inhibition zones were measured. The whole process has been repeated 2 times for elimination of errors.

Results and Discussion

HPLC analysis of potato flesh extracts

The separation of three glycoalkaloids in turned green of two potato varieties were analyzed by HPLC (Fig. 2 and 3). Three major peaks with retention times (Rt) of 2.03, 3.19 and 4.148 min. were identified and were consistent to those of authentic samples of solanidine (alkaloidalaglycone), α-solanine and α-chaconine, respectively. The content of the Iranian (sample 1) and the Iraqi (sample 2) potatoes studied (Table 1) and the results showed that both types of potato contain solanidine, α-solanine and α-chaconine in their flesh. The concentration of solanidine, α-solanine and α-chaconine reported in table 1, was 290.39, 931.333 and 815.277µg/ml of Iranian potato, respectively. While in the Iraqi potato, the amounts were 317.46, 923.16 and 514.72µg/ml of

Table 2: Antimicrobial activity of potato extract.

		Zone of inhibition in mm.	
No.	Isolated pathogenic (bacteria)	Antibiotic control (Tetracycline) inhibition zone	Potato extract (30 micro.gm)
1	*Staphylococcus aureus*	9	5
2	*Pseudomonus aeruginosa*	8	4
3	*E. coli*	9	9

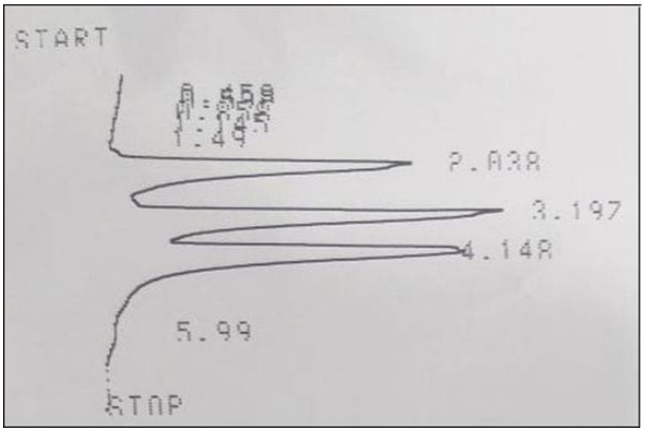

Fig. 2: The HPLC chromatogram of standard compound.

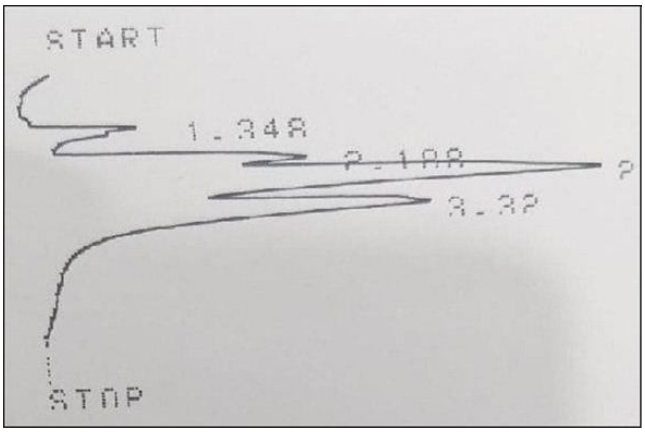

Fig. 3: The HPLC chromatogram of extract potato.

solanidine, α-solanine and α-chaconine, respectively".

The main glycoalkaloid in the potato was α-chaconine, which accounts about 65-71% of the total GAs. The high concentration of α-chaconine in potatoes is physiological significance as this alkaloid, is more toxic than α-solanine (Friedman, 2006; Dao and Friedman, 1994). However, as observed in table 1, both these samples α-solanine and α-chaconine content was irregular (Fig. 4), α-solanine was the principal glycoalkaloids. The same result has been recently reported by Ping *et al*., (2017) many external factors like damage of potato led to increase the production of enzymes to repair the harm and in consequence the production of α-solanine increase as well.

The present study showed that Iranian potato contain highest amount of α-solanine and α-chaconine compare to the Iraqi potato, but the concentration of the solanidine in the Iranian potato was low, which due to the last hydrolytic product of α-chaconine is solanidine. The difference in the amount of GAs between the samples of potatoes evaluated due to factors like variety, conditions of growth and postharvest storage. Previous study confirmed that genetically different potatoes have

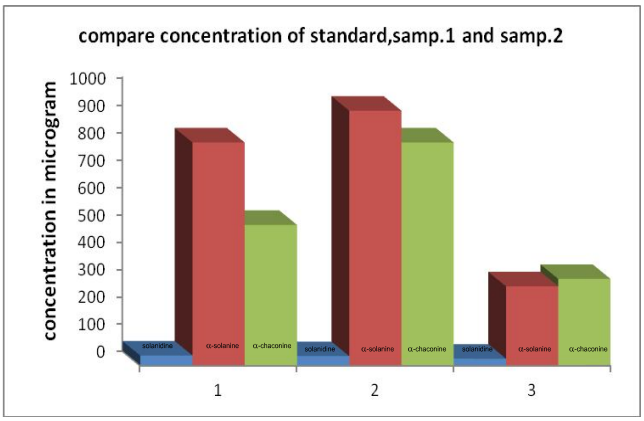

Fig. 4: Solanidine, α-solanine and α-chaconine contents of potato samples extract from two varieties.

different concentration of glycoalkaloids (Deahl *et al.,* 1993; Lampitt *et al.,* 1943; van Gelder *et al.,* 1985). In the present study, increased glycoalkaloid synthesis is probable from the result exposure of potato tubers to light during storage in fields, market places or at home. Similar results have shown by Percival and Baird (2000), the temperature of storage, is also a critical parameter such as that found in tropical countries.

The concentration of Glycoalkaloids and it is compounds which were obtained in this research are greater compare to fresh potato flesh than recommended range by FAO/WHO (1999). Post-harvest treatment and long-term storage of potatoes result in the drastically increase of GAs. Indeed, the concentration of glycoalkaloids found in this work is much higher than that reported from fresh potato flesh by Eltayeb *et al.,* (2003) and Devkota *et al.,* (2015) which always remained within the safety limit (<200mg/kg fresh weight).

Furthermore, high quantities of GAs in potatoes have been ascribed to a more effective protective impact of this metabolite against pathogens (Friedman and McDonald, 1997). Although, potatoes have green colored are un-edible for human due to high ratio of α-solanine and α-chaconine, these molecules have the ability to accumulate in the body as reported by Mensinga *et al*., (2005) and their toxicity do not decrease even during cooking and frying, the diagnosis of this toxin is somehow complicate and confusable because symptoms are resembling those caused by other common gastrointestinal disorders. As a consequence, HPLC provided a high GAs content in flesh of 2 varieties of green color potatoes and test them with animal and human models for their antibacterial activity.

Antibacterial activity of potato flesh sample extracts

The inhibitory effect of potatoes extract against three pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonus*

aeruginosa and *E. coli*) has been illustrated in (Table 2). The microorganisms which used for this test have been isolated from medical laboratory, the inhibitory intensity of potatoes extract were different on each microorganism compare to another, highest effect was on *E. coli* then *Staphylococcus aureus* and *Pseudomonus aeruginosa*, respectively.

Bactericidal effects of potato extract due to the damage of hydrogen bond in the backbone of DNA (Donald Mabhiza and Mukanganyama, 2016). Recently, some investigations confirmed that Alkaloids or potatoes extract could limit the growth of microorganisms through the damage of cell wall (Burdiek, 1971).

Conclusion

This study shows that steroidal alkaloids solanidine, α-solanine and α-chaconine were successfully extracted in the flesh of two types of green potato and the extracted samples analyzed using HPLC. The analysis indicated both Iranian and Iraqi potatoes contain the high levels of GAs. The results also demonstrated that α-solanine was the main glycoalkaloid followed closely by α-chaconine in samples. Moreover, large amounts of GAs were obtained from Iranian compared to the Iraqi potatoes. This might be explained as the presence of GAs depends on the variety, growing conditions and genotypes. However, some environmental variables such as wounding and light exposure can enhance the amount of GAs in potatoes tuber. The current research clearly showed that turned green flesh contains high amounts of toxic solanine and chaconine, exceeding the recommended limit and indicating that even after peeling, these potatoes are unsuitable for human use. The relationship between GAs compounds and their antibacterial activities were calculated. Furthermore, cup plate method was used to investigate antimicrobial activity. The GAs showed effective inhibition against three pathogenic *Staphylococcus aureus*, *Pseudomonus aeruginosa* and *E. coli* while it was the greatest effect on *E. coli*. Therefore the flesh of green colored potato can be considered to be the promising source of antimicrobial compounds.

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