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Article

Bio-modulation of Post-Harvested Potato: LC-ESI-MS/MS, GC-MS Analysis, Antioxidant, and α-amylase Activity of Treated Potato Peels

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Mariam M. Hewala[1], Sara Abouzeid[1], Yhiya Amen[1], Farid A. Badria[1]*

[1]Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.

ABSTRACT

Potato peel may play a mixed blessing role via providing useful and economic compounds, in contrast, it contains very toxic glycoalkaloids. However, massive amounts of potato peel waste are generated from industrial and personal uses. The evaluation of the four used bio-modulator responses on potato peel was estimated by monitoring the chemical contents by GC-MS, LC-ESI-MS/MS, reducing sugar, total phenolics, and total flavonoid contents as well as α-amylase and antioxidant activities by the comparison to control untreated peels. The in vitro α-amylase assay indicated that treatment with red beetroot (0.0025% *w/v*) methanolic extract exhibited the maximum α-amylase inhibitory activity by 91.7%. In addition, the treatment with red beetroot's different aqueous extract concentrations significantly minifies the reduced sugar content three-fold in the peel in comparison to the control untreated. The treated peel with methanolic extract (0.0025% *w/v*) of pomegranate outer peel retained the highest total flavonoid content while treated with olive leaves (0.0025% *w/v*) methanolic extract exhibited the highest total phenolic content. GC-MS revealed that solanidine-5-en-ol was suppressed by pomegranate outer peel (0.05% *w/v*) aqueous extract, lantana camara leaves (0.05% *w/v*) aqueous extract, and red beetroot (0.0025% *w/v*) methanolic extract. LC-ESI-MS/MS analysis interestingly determines the food safety effectiveness of olive leaves (0.1% *w/v*) aqueous extract among all applied bio-modulators in the reduction of toxic glycol and steroidal alkaloids in potato peel. In conclusion, these findings may indicate the versatile applications of bio-modulators via boosting and/or modifying potato peel's chemical and biological properties in either food safety or pharmaceutical industries.

Keywords: Potato peel, α-solanine, α-amylase, Food safety, Pharmaceutical applications.

1. INTRODUCTION

Potato (*Solanum tuberosum L.*) is overstated as one of the most consumed crops after rice, wheat, and maize. Potato shape, peel color, texture, size, and phytochemicals differ from one variety to another as there are diverse potato phenotypic species distributed all over the world [1]. Besides that, the potato crop is also correlated with one of the major food processing sectors worldwide [2]. Aside from potato consumption values, massive amounts of potato peel of about 15% to 40% of the fresh original weight are generated as industrial and individual waste products [3,4].

**Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt.*
E-mail address: faridbadria@gmail.com

On the other hand, potato peel which is often discarded and mentioned as a food waste product has substantial nutritional and medicinal value. Potato peel is a rich source of phenolic compounds (chlorogenic acids, flavonoids, etc.) which lowers cholesterol and controls diabetes; hence it can be used in achieving functional foods and nutraceuticals (Figure 1). Glycoalkaloids occur naturally in potatoes and are concentrated in the potato peel. Glycoalkaloids are toxic to humans and are not broken down by cooking or frying (Figure 1) [5-8]. The nutraceutical effect of potato peel arises from its glycoalkaloid contents which act as natural and safe steroidal hormone precursors [9,10].

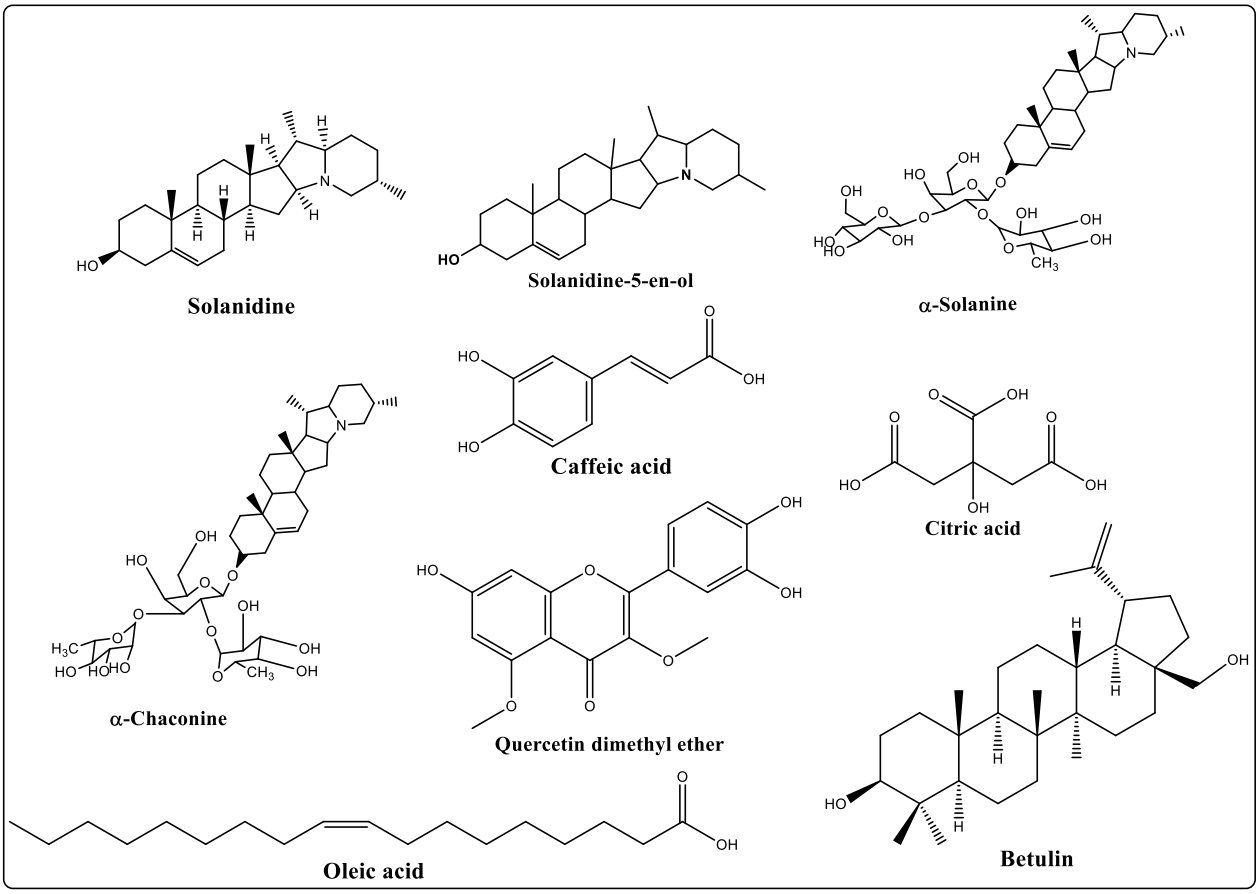

Figure 1. Chemical structure of some different compounds identified in potato peels.

Potato peel is characterized by its food and non-food uses. Concerning food uses, potato peel is recognized as a source of dietary fibers which replaced wheat flour utilization in baking [11,12]. Potato peel is considered a safe antioxidant source as synthetic antioxidants have been reported to have carcinogenic effects on humans [13]. Also, potato peel is characterized by its safe antimicrobial activities with non-mutagenic bacteriostatic effects [14,15]. Non-food uses of potato peel are summarized in being utilized in the production of some enzymes such as alpha-amylase and alkaline protease productions [16,17]. Potato peel is used in nano-crystal synthesis [18], biogas [19], biofuel [20], and animal feed [21]. Therefore, the underutilization of food waste turned into increasingly valuable, minimizing throwing and yielding a leading multiple values utilization strategy. In recent years, food security has become an economic priority in the production of safe, healthy, and functional foods [22].

Keeping in view, physiologically functional food production is of great interest because of its effects on the body's biological responses with benefits for health and disease prevention [23]. Functional safe food and medicinal values can be estimated by improving and enhancing secondary metabolite production in potato peel by applying various modulators (elicitors) [24,25]. Bio-modulators are a subtype of elicitors characterized by their availability, safety, and cost-effectiveness in the tendency of the production of green products [26]. Different modulators (either elicitors or suppressors) to either help in the accumulation or halt the production of secondary metabolites either in medicinal or edible plants were determined successfully [27-30]. For example, investigating the influence of dark conditions on the levels of total phenolic and total flavonoid Contents as well as total antioxidant capacity in germinated sprouts derived from edible plant seeds, specifically lettuce, and linseed. The findings of the study revealed a notable improvement in antioxidant activity. Nevertheless, it was observed that the germination process led to a significant reduction in both the total phenolic and flavonoid contents [29].

This study aimed to modulate the contents of potato peel to comply with food safety regulations and open a new

horizon for the utilization of toxic compounds in developing new anti-cancer drugs [7]. Specifically, post-harvested potatoes were treated with several plant extracts for a certain period and the treated peel metabolites were identified by GC-MS and LC-ESI-MS/MS profiling. Reduced sugar, total phenolic, and total flavonoid contents, as well as antioxidant and *α*-amylase inhibitory activities, were also evaluated for the treated potato peels.

Selected four bio-modulators were used as they are known to be rich in valuable phytochemical classes such as flavonoids, tannins, glycosides, and alkaloids which exert different biological activities such as antiviral, antimicrobial, antiparasitic, and antifungal activities [31-35]. Using bio-modulators indicates a safe, economic, and services for a green environment. For that, this study aimed to enhance the antioxidant and *α*-amylase inhibitory activity of the waste potato peel by the application of different bio-modulators, in addition to total phenolic, and total flavonoids, and reducing sugar contents. At least to our knowledge, there are no previous studies on the modulation of total phenolic, total flavonoid, and reducing sugar contents as well as antioxidant and *α*-amylase enzyme inhibitory activities of potato peel by using bio-modulators. This finding can be significant in presenting functional food for various chronic disease patients. This implies that individuals can derive greater benefits from their food choices, as these products offer a higher concentration of essential nutrients.

2. METHODS

2.1. Chemicals, solvents, enzymes, reagents and standards

All used solvents and chemicals were of analytical grade: aluminum chloride (Sigma Aldrich, St.Louuis, USA), folin-ciocalteau reagent (Sigma Aldrich, St.Louuis, USA), acetic acid HPLC 99.9% (Sigma Aldrich, St.Louuis, USA), Sodium carbonate (El-Nasr Pharmaceuticals and chemicals Co., Egypt), gallic acid (Sigma Aldrich, St.Louuis, USA), DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Sigma Aldrich, St.Louuis, USA), *α*-amylase from *Aspergillus oryzae* (Sigma Aldrich, St.Louuis, USA), Ascorbic acid (Sigma Aldrich, St.Louuis, USA), quercetin (Sigma Aldrich, St.Louuis, USA), soluble starch (BDH chemicals, UK), 3,5-dinitro salicylic acid 98% (DNS, Euromedex, France), sodium potassium tartrate tetrahydrate (Oxford, UK), DMSO HPLC 99.9% (dimethyl sulfoxide) (Sigma Aldrich, St.Louuis, USA), formic acid MS-grade (Sigma Aldrich, St.Louuis, USA), acetonitrile HPLC 99.9% and methanol HPLC 99.9% (Sigma Aldrich, St.Louuis, USA),). D- (+) - Glucose anhydrous (HPLC) 99% (El-Nasr Pharmaceuticals and Chemicals Co., Egypt), Ultra-pure water was purified by Milli-Q purification system (Millipore, Billerica, MA).

2.2. Plant material

Freshly harvested cara potato (*Solanum tuberosum*) tubers, pomegranate (*Punica granatum*) fruits, and red beetroot (*Beta vulgaris*) were purchased from a local market in Mansoura, El-Dakhlia. *Lantana camara* and *Olea europaea* (olive) leaves were collected in November 2022 from the farm of the Faculty of Pharmacy, Mansoura University, Egypt. Samples were identified by staff members of the Agricultural Botany Department, Faculty of Agriculture, Mansoura University, and voucher specimens were deposited in the Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University with numbers: Olive leaves (22M-12), lantana leaves (23M-12), pomegranate outer peel (24M-12), red beetroot (25M-12) and Cara potato (26M-12).

2.3. Preparation of plant extracts (bio-modulators)

Olive leaves (OL) and lantana leaves (LC), pomegranate outer peel (PM), and red beetroot (RB) were thoroughly washed with distilled water and allowed to dry in the shade for 24 hours. The dried organs of 100 g were subjected to extraction by 70% (*v/v*) methanol till exhaustion. The obtained methanolic extracts (meth. ext) with a yield of 7-10 g of each were concentrated under a vacuum at 45°C using a rotary evaporator. The concentrated methanolic residues were kept in clean and tight containers at -20° till use. The aqueous extracts (aqua. ext) were prepared by 100 g dried plants in 750mL Ultra-pure water. The aqueous extracts were lyophilized using a freeze dryer (Taisite, model LY-10N, China) for 72 hours at -65°C, packaged in an airtight container, and stored at -20°C ready for use. The yield of lyophilized samples was OL 10.9%, PM 33.7%, RB 28.5%, and LC 17.0%.

2.4. Experimental design

Briefly, cara potatoes were cleaned well with tap water, then sterilized with 5% (*v/v*) sodium hypochlorite for 2 min then washed with tap water till neutral then let to dry overnight (16 hrs). The preserved aqueous and methanolic extracts at different concentrations as shown in **Table 1** were prepared using Ultra-purified water and placed into sprayers. After that potatoes were sprayed till immersion only once during the experiment for 15 min and retained for, placed, and grouped over plates with control untreated only sprayed with Ultra-pure water. The plates containing potatoes were stored at an ambient temperature of 23±1°C with 65–75 % relative humidity (RH) for 30 days under diurnal light (12 hrs of light

and 12 hrs of dark). While flipping potatoes every 3 days to ensure that all sides were exposed to the same experimental conditions. After that potatoes were washed several times with Ultra-pure water. At the end potato peel of about 5 g was collected and extracted with 70% (*v/v*) methanol till exhaustion giving a yield of approximately 200 mg. The methanolic extract was concentrated at 45℃ and then stored at -20°C till use.

2.5. Total phenolic content (TPC)

The total phenolic assay was conducted according to Li et al [36]. Briefly, one mg of each potato peel methanolic extract was dissolved in 1mL methanol and mixed with 5 mL 10% (*v/v*) Folin-Ciocalteau reagent and set aside for 5 min. Four mL from 7% (*w/v*) Na_2CO_3 was added to the reaction mixture and incubated for 30 min at 40°C. The reaction mixture after cooling for 30 min was measured using a 4-mm path-length quartz cuvette with a slit width of 1.0 nm at 760 nm with a UV spectrophotometer (Shimadzu 1601 PC, model TCC240, Japan). A gallic acid standard calibration curve with concentrations 20, 40, 60, 80, and 100 µg/mL was prepared. The results were expressed as µg gallic acid equivalent (GAE) and calculated from the calibration plot y = 0.0119x + 0.0365.

2.6. Total flavonoid content (TFC)

The total flavonoid assay was conducted according to Li et al with minor modifications [36]. Briefly, 100 µL of methanolic extracts (5mg dissolved in 500 mL 70% ethanol) was reacted with 100 µL of 20% $AlCl_3$, and a drop of acetic acid was added then volume was completed to 5mL with 70% ethanol. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured using a 4-mm path-length quartz cuvette with a slit width of 1.0 nm at 415 nm with a UV spectrophotometer (Shimadzu 1601 PC, model TCC240, Japan). Similarly, a quercetin standard calibration curve with concentrations of 800, 600, 400, and 200 µg/mL was prepared. The concentration of TFC in the tested samples was calculated from the calibration plot y = 0.0002x + 0.066 and expressed as mg quercetin equivalent (QE)/mg of dried plant material.

2.7. *In vitro* antioxidant assay

The antioxidant assay was conducted according to Aliabadi et al [31]. Briefly, A stock solution of DPPH was prepared by dissolving 3.9 mg of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) reagent in 100 mL of methanol and finally, incubated overnight at -4°C. In a testing tube, 2.5 mL from DPPH previously prepared solution was mixed with 500 µL of plant extract (1mg/ mL). A mixed solution of 2.5mL of DPPH solution and 500 µL of methanol is used as a control. Finally, the tubes were incubated for 30 min in complete darkness. After that, the absorbance was measured using a 4-mm path-length quartz cuvette with a slit width of 1.0 nm at 517 nm with a UV spectrophotometer (Shimadzu 1601 PC, model TCC240, Japan) The percentage of antioxidants was calculated using the formula:

$$\% \text{ of antioxidant activity} = \frac{A\ control - A\ sample}{A\ control} \times 100$$

2.8. Reducing sugar content

The reducing sugar was quantified by the dinitro-salicylic acid (DNS) method according to Miller with some modifications [37]. For the procedure, Briefly, 1 mg of peel methanolic extract. was added to 1 mL of methanol and shaken for 1 h. In the experiment, 950 µL of DNS was added to 50 µL of sample in a Wassermann tube, and 200 µL of the mixture was placed in 96-well plates and incubated at 100 °C in a water bath for 2 min. After cooling for 10 min, the absorbance was measured at 570 nm in a Biotek plate reader (Gen5™), and the absorbance was measured on power Wave XS (Winooski, VT, USA). The samples were analyzed against glucose standards calibration curve of concentrations ranging from (2 -10 µL), y=0.165x-0.02.

2.9. *In vitro* α-amylase inhibitory assay

The α-amylase inhibitory assay was conducted according to Badria *et al* [38]. Briefly, 50 µL of each methanolic extract (4 mg/ mL) was mixed with 50 µL of 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M NaCl) added to a 96-well plate and incubated for 10 min at room temperature. Fifty microliters of 1% of soluble starch solution was added and the reaction mixture was incubated for 3 min at room temperature. Finally, 50 µL of DNS was added to the 96 well plate and was placed for 5 min over a boiling water bath. After cooling for 10 min the activity was measured at 570 nm in a Biotek plate reader (Gen5™), and the absorbance was measured on power Wave XS (Winooski, VT, USA). Quercetin was used as a positive control. The α-amylase inhibitory activity of the potato peel extracts was calculated using the formula:

$$\% \text{ of inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where Abs control is the 100% enzyme activity absorbance and Abs sample is the reaction mixture containing potato peel meth. ext. or quercetin as positive control.

2.10. Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS was performed by using a GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column temperature initially was 60°C and then 250°C by 5°C /min to then elevated to 300 °C by 30 °C /min. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The injector temperature is 270°C. The solvent delay was 4 min. Samples of alcoholic extract were dissolved in High-Performance Liquid Chromatography solvent grade and filtered using a membrane syringe filter (0.22 µm, Sartorius, Göttingen, Germany) and 1 µL was injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 50–650 in full scan mode. The ion source and transfer line were set at 200 °C and 280 °C, respectively [39]. The components of the extracts were identified by comparison of their mass spectra with those of WILEY 09 and NIST14 mass spectral database.

2.11. Liquid Chromatography - Electrospray Ionization Mass Spectrometry (LC-ESI-MS/MS)

LC-ESI-MS/MS experiments for compound identification were performed from the meth.ext., using an Agilent 1200 HPLC system with a quaternary pump, column heater, DAD detector, and fraction collector (Santa Clara, CA). The LC-ESI-MS/MS system consisted of a Shimadzu LC-20 AD HPLC chromatograph (Canby, OR) and an Applied Biosystems 4000 Q Trap mass spectrometer (Foster City, CA, USA). A 150 x 4.6 mm i.d., 3.0 µm, superficially porous column (Agilent Infinity Lab Poroshell 120 EC-C18, 3.0 mm × 100 mm, 1.9 µm) was used with a flow rate of 0.5 mL/min. Samples of alcoholic extract were dissolved in High-Performance Liquid Chromatography solvent grade and filtered using a membrane syringe filter (0.22 µm, Sarorius, Göttingen, Germany) after that volume of 10 µL using a Harvard syringe pump and collected every half minute to obtain a high-resolution masses of identified compounds with a Waters LCT Premier XE mass spectrometer (Milford, MA, USA) in negative electrospray ionization mode. The column oven temperature was set to 30 ºC. A gradient mobile phase elution was composed of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), varying linearly from 2 to 35% B (*v/v*) for 35 min, from 35 to 98% until 45 min. A time of 10 min was allowed between samples, which were held at the initial mobile phase composition (2% B). MS spectra were obtained in both negative and positive ion electrospray (ESI) modes and processed using Analyst 1.4.2 software. The ESI probe was operated at - 3500 V (negative) or 3500 V (positive), using a fragmentor voltage of 110 V. The source and temperature were 325 º C. The nitrogen nebulizing and drying gas pressures were set to 55 psi. Each spectrum was recorded between *m/z* 100 and 1300 Da in centroid mode with a duration of 3s/scan and an interscan time of 0.2 s[40]. The instrument was controlled by Masslynx 4.1 software.

2.12. Statistical analysis

Data were represented as mean ± standard deviation (SD). All data were performed in triplicate and were represented using Graph Pad Prism 8 version 8.4.2 software and Microsoft Excel 365 MSO version 16.0.13.

3. RESULTS AND DISCUSSION

3.1. Effect of bio-modulators on flavonoids and phenolic contents of potato peel

Potato peel is known for its health benefits and is a good source of phenolic and flavonoid compounds [5,6]. Varying bio-modulators and their concentrations significantly indicate differences among results as shown in Table 1. In this study, the effect of the bio-modulators on the total flavonoid content and total phenolic contents of treated potato peel were evaluated, and we noticed that total phenolic and total flavonoid contents varied considerably among treated potato peel when compared to control untreated. The total phenolic content for treated potato peel ranged from 11.32 to 49.12 µg/mg GAE. The total flavonoid content for treated cara potato peel ranged from 231.66 to 651.66 µg/mg QE. The methanolic extract (0.0025% *w/v*) of PM retained the highest TFC 651.6 µg/mg QE while the aqueous RB (0.005% *w/v*) extract exhibited the lowest value 231.6 µg/mg QE. OL methanolic (0.0025% *w/v*) and aqueous (0.1% *w/v*) extracts showed an observed increase in TPC with values 49.12 and 43.35 µg/mg GAE, respectively in comparison to the control (38.84 µg/mg GAE). The phenolic compound accumulations in plants were reported to be induced by the influence of different abiotic and biotic modulators [41]. Our finding indicated the significance of certain applied bio-modulators in the induction of phenolics in potato peel while being stored for a month during the experiment. Although Singh and Saldaña reported the degradation of phenolic compounds during storage conditions [42]. This reflects the effect of the applicated bio-modulators on the modulation and enhancement of TFC and TPC of potato peel.

3.2. Antioxidant activity of potato peel

The antioxidant activity of treated cara potato peel was evaluated according to their free radical scavenging activity by using the DPPH reagent (Table 1). Interestingly, significant elevation in the antioxidant activity of cara potato peel after 30 days from the application of bio-modulator PM (0.2% *w/v*) aqueous, (0.0025% *w/v*) methanolic, (0.05% *w/v*) aqueous and (0.01% *w/v*) methanolic extracts by values: 85.17, 76.8, 69.1، and 51.10%, respectively in comparison to control untreated 42.31%. Our study illustrates the effect of the applied bio-modulators on potato peel antioxidant activity in addition to the effect of storage. On the other hand, RB (0.2% *w/v*) aqueous extract, OL (0.1% *w/v*) methanolic extract, and OL (0.05% *w/v*) aqueous extract resulted in a high decline in the potato peel antioxidant activity by values: 11.06, 12.73 and 12.92%, respectively. These results will help in providing a natural safe eco-friendly source of antioxidants modulators for food and pharmaceutical industries purposes.

3.3. *α*-Amylase enzyme inhibitory activity of potato peel

α-Amylase is deemed to be one of the main hydrolyzing carbohydrate enzymes. Inhibiting this enzyme can result in the reduction of postprandial blood glucose and improve tolerance of glucose in diabetic patients [43,44]. In this study, the *α*-amylase inhibitory activities of potato peel treated with different bio-modulators and control untreated were investigated (Table 1). The percentage of *α*-amylase enzyme inhibition of potato peel varied from 8.49% to 91.74%. The peel treated with RB methanolic extract (0.0025% *w/v*) effectively exhibited the maximum inhibitory activity of 91.74% towards *α*-amylase in comparison to the standard quercetin and control peel 71.17% and 26.1%, respectively. On the other hand, the treatment with PM methanolic extract (0.0025% *w/v*) retained the least *α*-amylase inhibitory activity (8.49%). Treated peel with PM aqueous (0.05% *w/v*) extract showed acceptable *α*-amylase (71.35%) very close to the standard quercetin (71.17%). *α*-Amylase inhibitors from natural sources give a novel therapeutic approach to diabetes management other than synthetic drugs with many side effects [45]. Interestingly, our finding indicated the effectiveness of bio-modulators in enhancing potato peel as *α*-amylase enzyme inhibition activity. Potato peel is also reported as a superior substrate for amylase production [46,47]. Thus, potato peel is not only an *α*-amylase enzyme inhibitor but also a substrate. In addition, potato peel was reported by Arun *et al* for its potential *α*-glucosidase inhibitory activity [48]. For that, this study aimed to enhance the inhibitory activity of the waste potato peel by the application of different bio-modulators, in addition to total phenolic, total flavonoids, and antioxidant activity. The significance of this finding lies in its potential to offer valuable insights into the creation of functional food alternatives that cater to the dietary needs of diabetic patients and those afflicted with various chronic diseases. To the best of our understanding, there have been no prior investigations conducted on the manipulation of TFC, TPC, and antioxidant activity, as well as the inhibitory effects on *α*-amylase enzyme, in potato peel through the utilization of bio-modulators.

Table 1. Potato peel total flavonoid content (TFC), total phenolic content (TPC), antioxidant activity, *α*-amylase inhibitory activity, and reduced sugar content.

Treatment	Extract	Concentration % *w/v*	TFC (µg/mg QE)	TPC (µg/mg GAE)	Antioxidant activity (%)	*α*-Amylase inhibitory activity (%)	Reducing Sugar content (µg/mg)
LC	meth. ext.	0.01	330 ± 5	24.45 ± 0.04	31.94	32.86 ± 3.20	4.46±0.60
		0.005	253.33 ± 2.88	17.60 ± 0.07	14.30	47.85 ± 4.40	3.94±0.19
		0.0025	383.33 ± 2.88	30.93 ± 0.03	37.98	53.69 ± 2.90	4.3±0.95
	aqu. ext.	0.1	363.33 ± 5.77	31.28 ±0.06	21.71	17.62 ± 2.20	3.89±0.22
		0.05	278.33 ± 2.88	23.40 ± 0.36	14.91	65.06 ± 3.90	3.89±0.10
2. OL	meth. ext.	0.01	328.33 ±7.63	14.46 ± 0.04	12.73	76.89 ± 5.30	4.17±0.25
		0.005	363.66 ± 7.76	26.63 ± 0.04	16.51	56.71 ± 5.02	4.29±0.14
		0.0025	313.33 ± 2.88	49.12± 0.1	47.25	57.89 ± 2.90	4.67±0.38
	aqu. ext.	0.1	258.33 ± 2.88	43.35 ± 0.04	29.99	48.39 ± 3.60	4.52±0.15
		0.05	358.33 ± 5.77	23.10 ± 0.04	12.92	63.29 ± 3.02	4.49±0.09
3. PM	meth. ext.	0.01	378.33 ± 2.88	19.81 ± 0.05	51.10	51.97 ± 5.60	5.05±0.32
		0.005	308.33 ± 5.77	26.39 ± 0.04	14.98	76.14 ± 5.90	4.77±0.33
		0.0025	651.66 ±2.88	30.68 ± 0.04	76.80	8.49 ± 0.97	3.55±0.02
	aqu. ext.	0.2	370.66 ± 6.02	24.49 ± 0.08	85.17	63.75 ± 6.78	3.77±0.07
		0.1	486.66 ± 2.88	11.32 ± 0.02	43.50	27.55 ± 2.80	3.86±0.05
		0.05	500 ± 5	32.1 ± 0.04	69.10	71.35 ± 6.40	3.95±0.19
4. RB	meth. ext.	0.01	310 ± 5	24.31 ± 0.01	24.34	72.60 ± 6.10	3.93±0.05
		0.005	231.66 ± 5.77	18.4 ± 0.04	33.26	34.15 ± 4.08	4.22±0.08
		0.0025	310 ± 5	16.44 ± 0.02	41.70	91.74 ± 6.30	4.04±0.08
	aqu. ext.	0.2	268.33 ± 2.89	20.71 ± 0.08	11.06	30.38 ± 0.90	1.70±0.27
		0.1	435 ± 5	17.22 ± 0.04	16.51	58.54 ± 3.30	1.76±0.20
		0.05	NC [a]	32.85 ± 0.039	57.06	38.58 ± 4.50	1.92±0.50
5. Control (Untreated)			391.66 ± 2.88	38.84 ± 0.10	42.31	26.16 ± 2.50	4.22±0.64
6.Quercetin[b]		-	-	-	-	71.17 ± 5.64	-
7. Ascorbic acid [c]		-	-	-	91.23	-	-

*Data represented as mean ±SD. The experiment was conducted in triplicate., [a] Not conducted, [b] Quercetin was used as a positive control for *α*-amylase, and [c] Ascorbic acid used as a positive control for antioxidant activity

3.4. Estimation of the reduced sugar content of potato peel

The effect of the applied bio-modulators on potato peel on the reduced sugar content was measured by using the dinitro salicylic acid (DNS) method as it's a rapid and cost-effective procedure and presented in Table 1 [49]. The reduced sugar content in treated potato peel by different concentrations from aqueous RB extract indicated a significant reduction by three-fold in comparison to the control (4.22 µg/mg). On the other hand, other applied bio-modulators did not significantly lower the reduced sugar content in potato peel. Since the reducing sugars are the limiting precursor in the formation of acrylamide in food upon frying or exposure to temperatures above 120°C. Acrylamide is a possible carcinogenic compound and formed from the reaction between asparagine amino acid and existing reducing sugars [50,51]. Moreover, its synthesis reduction or inhibition exerts an efficacious goal for improving food safety. Accordingly, decreasing the reduced sugar will play a significant role in improving the food safety of potato peel.

3.5. GC-MS analysis

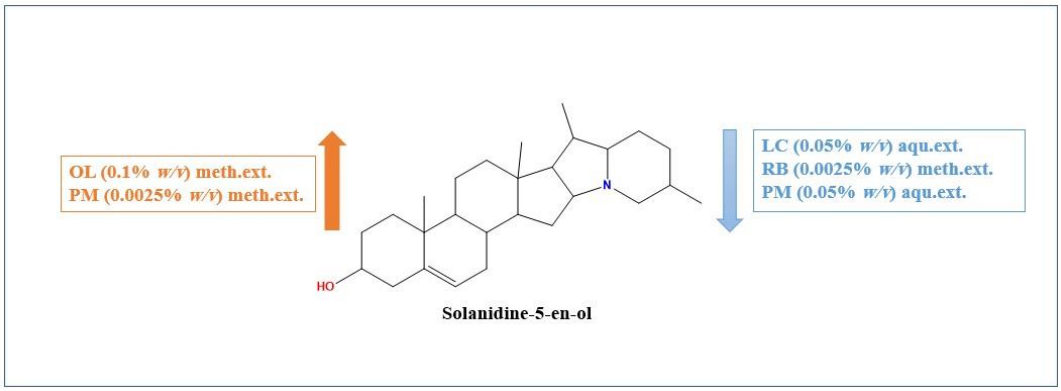

Figure 2. Modulation of toxic solanidine-5-en-ol content in potato peels after treatment with bio-modulators (LC (0.05% *w/v*) aqueous, RB (0.0025 % *w/v*) methanolic, OL (0.1% *w/v*) aqueous, PM (0.0025% *w/v*) methanolic and PM (0.05% *w/v*) aqueous extracts).

The GC-MS profile revealed serious modulations in the potato peel secondary metabolites when comparing the control with the treated group as mentioned in Table 2 and Supplementary chromatograms (Figure 8 -13). The decision to use specific treated potato extracts for GC-MS and LC-ESI-MS/MS was based on their performance in different conducted assays. Interestingly the treatment with LC (0.05% *w/v*) aqueous, RB (0.0025 % *w/v*) methanolic, and PM (0.05% *w/v*) aqueous extracts suppressed the production of toxic solanidine-5-en-ol steroidal alkaloid. On the other hand, treated potato peel with OL (0.1% *w/v*) aqueous and PM (0.0025% *w/v*) methanolic extracts revealed a significant increase in its concentration by two-fold and three-fold, respectively (Figure 2).

Moreover, the presence of newly identified metabolites such as 1-Hexadecanol, 2-methyl, and 11-Octadecenal in peel treated with OL (0.1% *w/v*) aqueous extract. While 9-Octadecenoic acid (E)- and Dodecanoic acid were identified only in peel treated with LC (0.05% *w/v*) aqueous extract. On the other hand, Hexadecanoic acid was expressed only in the control peel with a % peak area of 1.55.

The GC-MS profile (Table 2) revealed that most of the identified fatty acids were of saturated chains. Interestingly, RB (0.0025 % *w/v*) methanolic and LC (0.05% *w/v*) aqueous extracts induced inhibition to most of the mentioned saturated fatty acids as listed in Table 2. This finding indicates cutting down saturated fatty acid which in role will help in reducing our risk of cardiovascular disease [52]. On the other hand, OL (0.1% *w/v*) aqueous extract and control contained the highest saturated fatty acid content.

3.6. LC-ESI-MS/MS profiling

The identification was conducted by comparing the *m/z* values and the fragmentation patterns with spectral data taken from the literature or by searching the databases (NIST14, MassBank). The target metabolites are shown in Table 3. belong to different classes as follows: flavones, flavonols, hydroxycinnamic acids, tricarboxylic acids, polyhydroxy carboxylic acids, oxylipin, phenolic amines, steroidal alkaloids, and glycoalkaloids. The total ion chromatogram of tandem mass spectrometry of the peel potato sample is represented in Supplementary (Figure 2-7) where (blue) chromatogram of positive ion signal intensity; and (black) chromatogram of negative ion signal intensity.

Table 2. Secondary metabolites in potato peel identified by GC-MS

	Compound	Peak Area (%)					
		Control	OL[a]	PM[b]	PM[c]	LC[d]	RB[e]
1	1-Hexadecanol, 2-methyl	-	0.38	-	-	-	-
2	Dodecanoic acid	-	-	-	-	1.76	-
3	Hexadecanoic acid, methyl ester	5.40	4.82	2.75	7.72	1.29	2.02
4	n-hexadecanoic acid	9.67	9.85	2.75	9.75	16.11	10.68
5	9,12-Octadecadienoic acid (Z, z)-, methyl ester	4.73	5.87	3.81	1.09	3.94	2.83
6	9-Octadecenoic acid (Z)-, methyl ester	5.54	8.89	9.21	9.15	7.80	4.14
7	Heneicosane	1.06	-	-	1.09	-	-
8	Methyl stearate	2.24	3.23	0.51	4.68	-	-
9	Oleic Acid	53.03	41.14	64.59	36.68	59.48	75.85
10	9-Octadecenoic acid, (E)-	-	-	-	-	4.96	-
11	Octadecanoic acid	1.37	1.98	1.23	1.87	3.44	2.94
12	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.40	0.66	-	-	-	-
13	1-Heptatriacotanol	1.54	1.48	-	-	-	-
14	Cis-13-Eicosenoic acid	0.32	0.51	0.77	-	-	-
15	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl] methyl] methyl]-, methyl ester	0.79	0.77	-	-	-	
16	Linoleic acid ester	0.50	0.51	-	-	-	-
17	11-Octadecenal	-	0.53	-	-	-	-
18	2-Monolinolenin, 2TMS derivative	-	1.30	-	-	-	-
19	Hexadecanoic acid	1.55	-	-	-	-	-
20	1,2-Benzene dicarboxylic acid	0.92	1.73	1.46	1.91	-	-
21	Arabinitol, pentaacetate	0.51	0.53	-	1.13	-	-
22	Betulin	0.33	0.87	0.62	1.01	-	-
23	Ethyl iso-allocholate	0.40	0.96	0.81	1.84	-	-
24	9,12-Octadecadienoic acid (Z, Z)-,2,3-Bis[(trimethyl silyl)oxy]propyl ester	0.52	1.42	1.15	1.69	-	-
25	Solanidine-5-en-ol	1.27	2.78	-	4.70	-	-
26	Stigmast-5-en-ol, (3a, 24S)-	0.91	0.93	1.30	-	-	-
27	Methyl-hydroxy-11-hydroxy (180)-3,11-dimethyl-2,6-Tridecadienoate	0.49	-	-	1.50	-	-

Potato peel treated with [a] OL 0.1% *w/v* aqueous; [b] PM 0.05% *w/v* aqueous; [c] PM 0.0025% *w/v* methanolic; [d]LC 0.05% *w/v* aqueous and [e] RB 0.0025 % *w/v* methanolic extracts.

The identified *α*-solanine, *α*-chaconine, and N-feruloyl octopamine exhibited ionization in both positive and negative modes as mentioned in Supplementary Table 1. *α*-solanine positive molecular ion *m/z* 868.5 while 912.4 *m/z* negative molecular ion and with major fragmental ions *m/z* 398; 706; 560; 383; 327; 253 and 157. The molecular ions of *α*-chaconine in the positive and negative modes are 852.5 and 896.4 *m/z*, respectively with major fragmental ions *m/z* 706; 560; 398, and 253 [53,54]. N-feruloyl octopamine exhibits positive molecular ion *m/z* 330.1 and negative molecular ion *m/z* 328.1 with major fragmental ions *m/z* 310 295; 161 and 135 [55]. The LC-ESI-MS/MS analysis revealed major variations between the control and treated samples. Steroidal alkaloids tomatidinol and solanidine were suppressed by the applied bio-modulators although their expression in the control untreated potato peel. Although in GC-MS solanidine-5-en-ol was expressed by OL (0.1% *w/v*) aqueous and PM (0.0025% *w/v*) methanolic extracts. Leptinine II and Leptinine I glycoalkaloids were suppressed by OL (0.1% *w/v*) and LC (0.05% *w/v*) aqueous extracts. Leptinine II and Leptinine I exhibit positive molecular ions 884.5 and 868.4 *m/z*, respectively [54]. The treated potato peel with OL (0.1% *w/v*) aqueous extract treatment indicates only the expression of *α*-solanine and *α*-chaconine glycoalkaloids. Moreover, our finding interestingly determines the food safety effectiveness of OL (0.1% *w/v*) aqueous extract among all applied bio-modulators in the reduction of toxic glycoalkaloid in potato peel while exhibiting higher TFC 43.3 µg/mg GAE. On the other hand, PM (0.0025% *w/v*) methanolic and (0.05% *w/v*) aqueous extracts. Although containing higher glycoalkaloid metabolites such as *α*-solanine, *α*-chaconine, *β*-chaconine, Leptinine II, and Leptinine I, their TFC as presented in Table 1 was lower than that with OL (0.1% *w/v*) with values 30.6 and 11.3 µg/mg GAE. This contrasts with what was reported by Valcarcel *et al.* 2015 that there is a significant correlation between TFC and glycoalkaloids in potato peel[64].

Table 3. The LC-ESI-MS/MS identified metabolites in potato peel.

No	Compound Class	Compound identified	Formula	Selected potato peel					
				Control	OL[a]	PM[b]	PM[c]	LC[d]	RB[e]
1	Glycoalkaloids	Leptinine II	$C_{45}H_{73}NO_{16}$	√		√	√		√
2		Leptinine I	$C_{45}H_{73}NO_{15}$	√		√	√		√
3		*α*-solanine	$C_{45}H_{73}NO_{15}$	√	√	√	√	√	√
4		α-chaconine	$C_{45}H_{73}NO_{14}$	√	√	√	√	√	√
5		*β*-chaconine	$C_{39}H_{63}NO_{10}$			√	√	√	
6		Tomatidinol	$C_{27}H_{43}NO_2$	√					
7		Solanidine	$C_{27}H_{43}NO$	√					
8	Flavonoids Flavonol	Quercetin dimethyl ether	$C_{17}H_{14}O_7$				√	√	√
9	Flavone	Chrysoeriol [Chryseriol]	$C_{16}H_{12}O_6$	√	√	√	√	√	√
10	Phenylpropanoid	Triandrin [Sachaliside]	$C_{15}H_{20}O_7$	√			√	√	√
11	Phenolic amine	N-feruloyloctopamine	$C_{18}H_{19}NO_5$	√		√	√	√	√
12	Polyhydroxycarboxylic acid	Quinic acid	$C_7H_{12}O_6$			√			
13	Tricarboxylic acid	Citric acid [Anhydrous; Citrate]	$C_6H_8O_7$		√	√	√	√	
14	Oxylipin	13-TrihydroxyOctadecenoic acid [THODE]	$C_{18}H_{34}O_5$					√	
15		9,12,13- Trihydroxytrans-10-octadecenoic acid	$C_{18}H_{34}O_5$					√	
16	Hydroxycinnamic acid	Chlorogenic acid [3-O-Caffeoylquinic acid]	$C_{16}H_{18}O_9$			√	√	√	√
17		Caffeic acid	$C_9H_8O_4$	√		√			

Potato peel treated with [a] OL0.1% aqu. ext.; [b] PM 0.05% aqu. ext.; [c] PM 0.0025% meth. ext.; [e]LC 0.05% aqu. ext., [e] RB 0.0025 % meth. ext.

As shown in Table 3. Some metabolites were recently expressed as a) 13- TrihydroxyOctadecenoic acid and 9,12,13- Trihydroxytrans-10-octadecenoic acid of the fatty acid Oxylipin class were only expressed by LC (0.05% *w/v*) aqueous extract with negative molecular ions 329 m/z differing in major fragmental ions 309; 229; 171; 127; 153 m/z and171; 201; 311; 153 *m/z*, respectively and b) Quinic acid was only expressed by PM (0.05% *w/v*) aqueous extract with negative molecular ions 191 *m/z* having major fragmental ions173; 129 and 111 *m/z*.

4. CONCLUSION

In Summary, Potato peel although mentioned as a byproduct waste represents a good source for economic compounds and nutraceuticals. Modulation of potato peel contents is a beneficial tool in serving food safety, drugs, and pharmaceutical purposes. Using bio-modulators indicates a safe, economic and serves for a green environment. The evaluation of bio-modulator response on potato peel was estimated by monitoring the chemical contents by GC-MS, LC-ESI-MS/MS, antioxidant, reducing sugar, total phenolics, total flavonoids, and *α*-amylase activity. Thus, this result will help in establishing a safe functional food as in the case of diabetes. In addition, significantly affects the reduced sugar content in the peel which in role helps in reducing carcinogenic acrylamide formation upon frying. This finding indicates that the applied bio-modulators boost and improve potato peel's different properties with the aim of food safety, drug, and pharmaceutical purposes. Moreover, this strategy might be a significant safety tool in different medicinal and edible plants' important metabolites modulation. The significance and influence of bio-modulators in the field of functional food are immense. By incorporating modified food rich with important bioactive compounds into everyday diets, these specialized food products can help prevent and manage chronic diseases, reducing the strain on healthcare systems and promoting overall population health.

Conflicts of Interest

The authors declare no conflict of interest.

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