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The anti-amylolytic effects of polyphenols extracted from bracts and buds of Egyptian banana blossom wastes monitored by multispectral and molecular docking approaches.

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Abstract

Banana blossoms (Bb) are by-products of bananas planting that are underutilized. We aimed at evaluating the nutritional value, polyphenolic content, antioxidant capacity, and anti-amylolytic effects of the Egyptian Bb, particularly buds and bracts. Results showed that bracts had a higher content of moisture, total ash, reducing and non-reducing sugars and crude fiber, while buds had a higher content of fat, protein, Na, K and P. Ca was the same in both buds and bracts. Both parts were rich in antioxidative polyphenols and crude dietary fiber. HPLC-PDA analysis showed that chlorogenic acid was the highest phenolic acid in both bracts and buds with values of 24.53 and 18.39 mg/g dry weight, respectively, followed by ferulic and gallic acids. The higher concentration (5 mg/mL) of bud extract inhibited both α-glu and α-amy by 79.63 and 93.58%, respectively; while bract extract inhibited α-glu and α-amy by 83.21 and 97.68%, respectively. Molecular docking results indicated that Bb ex-tract might work as an inhibitor to competition on both enzymes, with a single high-affinity binding site. In conclusion, promising results of this work imply that Bb are a valuable by-product of the banana industry and could be utilized as functional substances.

Upcycling; Banana blossom; Polyphenols; Anti-amylolytic; Buds; Bracts

1. Introduction

The banana is a highly common fruit in the global market, with a significant trade economically, being the fourth largest food crop in the world [1]. Bananas have probably been consumed as food since ancient times and are significant parts of the diet worldwide [2, 3]. It is an essential element in many different foods which are either consumed raw or processed. Several bioactive substances as phenolics, carotenoids, and phytosterols, are found in bananas, which are very desirable in the diet because of their health promising effects [4]. Therefore, upcycling such substances in food and/or foodstuffs is demanding. Banana output in the globe has expanded over the years, with the quantity produced increasing from around 68 to 135 M tons between 2000-2022, respectively [5]. Recent production statistics report that Africa's output provides for around 16% of the world banana production, whereas Egypt's production is approximately 1.3 M tons according to FAOSTAT (2023). Bananas are grown for their fruit, but banana trees are composed of other parts which generate underutilized by-products and waste. Based on the harvesting circumstance, handling conditions, processing steps, and other related factors, each ton of bananas harvested creates around 4 tons of solid

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waste, which includes approximately half a ton of inflorescences [6].

The plant stem upon maturity gives rise to a bunch of rolled leaves that evolve into a dark purple bud, elongated and hearth-shaped, known as inflorescence or banana blossom (Bb). This consists of two parts: the outer sheath, reddish to purple in color, known as a bract; the inner small white flowers arranged along the stacks, known as florets. After harvesting, the inflorescence and the other by-products of the banana plants are thrown down.

The Bb, particularly the florets, are eaten raw or steamed or as an ingredient in preparing curry, particularly in South and South-East Asia. The Bb has also been used in folk medicine to treat various ailments [7]. It is commonly consumed also for its health benefits due to the presence of several distinct bioactive compounds including polyphenols, triterpenes, sterols, tannins, saponins, as well as a list of nutrients as P, Cu, Ca, Fe, K, vitamins A, E, and C [8]. However, the nutritional value of the inflorescence may differ based on the variety or the consumed part, as it consisted of buds and crimson red bracts.

Few studies spotlighted the impact of the varieties, usable parts, and age of the banana plant on the overall composition, bioactive components, and their biological effects of the by-products, including Bb. Muchahary and Deka [9] compared the chemical composition of the internal and external parts of the Bb. They found that the phytochemicals of the inner part of Bb had bottomed out, whereas their outer part had peaked. Meanwhile, phytochemicals of the Bb are variety-, geographical-, and growth depended [10]. Sheng, et al. [10] reported that blossoms of the Baxijiao cultivar have superior antioxidant capacity than that of the Paradisiaca under the Chinese growing conditions, thereby this cultivar was recommended as a powerful food supplement and/or additive. No studies focused on the composition of the Bb of the Egyptian variety. Furthermore, it is essential to compare the blossom parts in terms of categorizing their applications as food supplements.

Th The major group of secondary metabolites present in Bb are polyphenols [11]. Epidemiological evidence reported that this class of compounds is beneficial to the regulation of obesity and to the prevention and control of the obesity-induced type 2-diabetes (T2D) [12]. The possible mechanisms by which polyphenols exert their action can be attributed to the regulation of lipid and carbohydrate metabolism; to the regulation of gut microbiota; to the scavenging of ROS and AGEs, reducing inflammation. Furthermore, some polyphenols showed inhibitory activity against enzymes that break down carbohydrates., i.e., α-amylase (α-amy) and α-glucosidase (α-glu) [13]. There are also no studies to figure out the effect of polyphenols found in Bb on these carbohydrates' degradation enzymes. Thus, this study aims at: (a) measuring the Bb whole chemical composition, mineral components, polyphenols, and antioxidant properties and (b) studying the impact of its key polyphenols against α-amy and α-glu by spectral and molecular docking approaches.

2. **Experimental:**

Chemicals and reagents

Oxalic acid, hydrochloric acid, sulphuric acid, sodium hydroxide, potassium sulfate, copper sulfate, aluminum chloride, sodium acetate (pH 4.5; 0.4 M), potassium chloride (pH 1.0: 0.025 M), and petroleum ether were purchased from Alnser Co., Cairo, Egypt. Folin-Ciocalteu's reagent, gallic acid, and 1, 1-diphenyl-2- picrylhydrazyl (DPPH) were acquired from Sigma-Aldrich, Germany. Human pancreatic α-glucosidase (#G9001) and α-amylase (#A9000) were acquired from Sigma-Aldrich Chemical Co. (St. Louis, MO, US). The 3, 5-dinitrosalicylic acid and p-nitrophenyl-α-D-glucopyranoside (PNPG) obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals and reagents were of analytical grade. MillQ-H2O (Millipore, Bedford, MA, US) was used in the current study.

Preparation of Bb (buds and bracts) powder and extract

Bb were collected from the Egyptian banana (cv Baladi) in the Sahel Dijwa Village, Benha, Qalyubia, Egypt. The damaged leaves were removed, then blossoms were thoroughly washed with tap water. The de-browning was then done by manually separating buds and bracts, blanching in water containing oxalic acid (1.5±0. 5%) at 93 °C for 3 min to control enzymatic browning. After the samples had cooled with cold water, they were strained, and dried in an oven (GEC.x4733, England) at 60±1 °C until the moisture was lower than 5%. Afterwards they were ground in a mill (F.A.P.A ARDILLA mill, Italy), and sieved (Xinxiang Xin Ming De Machinery Co., Ltd.). Extracts were prepared macerating dried powder samples in acidified MeOH 80% (HCL 1%) for 3 times (1 sample: 20 solvent) for 60 min each time, and then filtered and rotary evaporated at 38 °C. Those extracts were then used for measuring the bioactive substances and polyphenols fraction (Fig. 1).

Chemical composition

Moisture was determined using hot air oven by weighing accurately 5-7 g of well mixed sample, then let it in an air oven (Precision Scientific Group, US) at 105±2 °C for at 4-5 h. Samples were cooled and weighed, then the moisture rate was calculated [14].

Ash was determined based on the assay of Harris and Marshall [15] using muffle furnace (Neytech 9493308 Vulcan Muffle Furnace, 550 cu in, 3-Stage Programmable; 120 VAC, US), where 5-10 g sample were ashed for 12-18 h / 550 °C. The ash was calculated as follows:

Ash (%) = (weight after ashing-tare weight of crucible)/ (original sample weight) ×100 (1)

Fat was determined using Soxhlet assay as based on the method of Nisar, et al. [16] as follow: A 5-10 g of each sample was in a thimble, and fat was extracted using petroleum ether in the Soxhlet apparatus (Precision Scientific Group, US) for 6-8 h at 60 °C. After the syphoning procedure finished, the leftover solvent was evaporated within a drier, and the lipid percentage was calculated.

The crude protein was estimated with Kjeldahl (VELP Scientifica, Italy) method. Approximately 5 g of sample was digested with appropriate volume of concentrated sulfuric acid, then the sample was distilled using NaOH solution and received into HCl solution: the nitrogen content was determined by titrating sample with NaOH, crude protein content calculated using a conversion factor of 6.25 [17].

Crude fiber was determined using acid alkali digested method as described by Unuofin, Otunola and Afolayan [18] as follow: A 2 g of each sample was digested by boiling with 100 mL of 1.25% H_2SO_4 solution for 30 min, then filtered under pressure. The residue was rinsed several times with boiling water. This process was repeated on the residue using 100 mL of 1.25% NaOH or KOH solution. The final residue was then dried at 100 °C (Precision Scientific Group, US), cooled in a desiccator and weighed (C1). It was thereafter incinerated in a muffle furnace (Neytech 9493308 Vulcan Muffle Furnace, 550 cu in, 3-Stage Programmable; 120 VAC, US) at 550 °C for 5 h, then transferred to cool in a desiccator and reweighed (C2). Percentage of crude fiber was then calculated using the following equations:

Crude fiber (%) = (C2-C1)/ (Weight of original sample) ×100 (2)

Reducing and total sugars were determined using the standard, official method as described by Lane and Eynon [19]. Minerals were determined using atomic absorption spectrophotometer (Perkin-ELMER, 2380, England).

Bioactive substances and antioxidant capacity

Total monomeric anthocyanin (TMA) was measured according to Khalifa, Li, Mamet, and Li [20] using a pH-differential technique with buffers of sodium acetate (pH 4.5, 0.4 M) and potassium chloride (pH 1.0, 0.025 M). In brief, 0.4 mL of each sample was combined with 3.6 mL of appropriate buffers and read at wavelength 510 and 700 nm against a blank. The following formulae were used to determine TMA as mg cyanidin 3-glucoside equivalents mg (CGE /g) using the following equations:

$$A = (A510 - A700)\ pH1.0 - (A510 - A700)\ pH4.5 \quad (3)$$

$$TMA = (A \times MW \times DF) \times 100 / MA \quad (4)$$

Where: A: Absorbance – MW: 449.2 Molecular weight – DF: Dilution factor – MA: 26.900

The total phenolic compounds (TPC) in samples extracts were determined according to Khalifa, et al. [20]. Briefly, 1 mL of a 10-fold diluted Folin-Ciocalteu reagent was added to 200 µL of each sample. After 5 min, 1 mL of 75 g /L Na_2CO_3 was added to stop the reaction, followed by 1.5 mL of dH_2O. After incubating the solutions in the dark for 60 min, the absorbance was measured at 760 nm. Based on the calibration curve prepared with gallic acid at concentration range (10-50 mg/100 mL), the TPC was expressed as gallic acid equivalents (mg of GAE /100 g dw).

The total flavonoids content (TF) of the extracts was measured using the method reported in Khalifa, et al. [20]. A 0.5 mL aliquot of 20 g /L $AlCl_3$ EtOH solution was mixed thoroughly with 0.5 mL of extracts. After 1 h at room temperature, the absorbance was measured at 420 nm. The final concentration of TFC was represented as quercetin equivalent (mg QE /100 g dw), which was determined using the calibration curve of quercetin (10-50 mg/100 mL range of concentration)

The antioxidative capacity of Bb buds and bracts extracts was estimated by the DPPH radical scavenging method. A 3.9 mL of DPPH was added to 0.1 mL of each extract solution, and the absorbance was measured at 517 nm using spectrophotometer (UVS-90 Single Beam Spectrophotometer, Acculab, US) after the solution was incubated in the dark for 1 h. Trolox calibration curve was prepared (1-20 mg/ mL range of concentration) and the results were expressed as Trolox equivalents (µmoL TE/ g dw) [20].

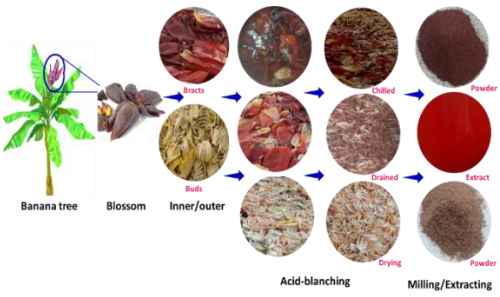


Fig 1. The overall steps of the preparation of both buds and bracts powder and extract.

Polyphenols characterization

The identification of the polyphenols of bracts and buds of Egyptian Bb was performed using HPLC-PDA apparatus (Agilent -series 1240) equipped with auto sampling injector, solvent degasser, and quaternary micro pump (series 1209A). The column used was RP-C18 BDS column (250 L X 4.6 ID, 5 µm particle size). The column was kept at room temperature. Gradient separation was done with solvent conditions initially composed of aqueous 0.5% formic acid: acetonitrile at 10:90 (v/v), then changed in acetonitrile content to 20, 25, 40, 70, 70, 10, and 10% by volume acetonitrile content at 10, 15, 23, 40, 45, 53, and 60 min, respectively, at a flow rate of 1 mL /min. Authentic phenolics and flavonoids were dissolved in mobile phase and injected into HPLC-PDA. The injection volume was 20 µL. The retention time and peak area were used to calculate the phenolics and flavonoids concentrations by the data analysis of ChemStation software (NODCAR. PHYTO.M32-2004) [21]. The methanolic extract of each Bb part (as prepared in section 3. 2.) was rotary evaporated and freeze-dried to get a fine powder. After that, 100 mg of this lyophilized extract was dissolved with MeOH (60 mL) into a volumetric flask. It was then sonicated for 10 min, diluted to 100 mL with mobile phase, shaken well, and finally filtered through a 0.22 µm Millex-GV filter (Millipore, Merk). The standard curve of rutin, quercetin, kaempferol, caffeic acid, p-coumaric acid, chlorogenic acid, ferulic acid, gallic acid, were prepared [22].

Inhibition activity on α-glucosidase and α-amylase

The anti-α-glu activity of extracts from buds and bracts of Egyptian Bb was determined using the method of Sobhy, et al. [13] with some adjustments. Concisely, each extract's stock solution (5 mg based on TPC) was sonicated till completely dissolved and diluted with PBS (pH 6.8, 0.02 M). Next, various extract concentrations (0.5, 1, 2.5, and 5 mg/mL from the stock solution) were combined with 0.5 mL of the same PSB and 100 µL of 10 mg/L of α-glu (50 U/mg, produced in PBS, pH 6.8, 0.02 M). The reaction was started by adding 0.5 mL of PNPG (2.5 mM, produced in PBS, pH 6.9, 0.1 M) as a substrate after incubation at 37 °C for 15 min. Titrating 1 mL of 0.2 M Na2CO3 halted the reaction after a further 15 min of incubation at 37 °C. The unconstrained p-nitrophenol (PNP) was detected using a Multiskan GO microplate reader (Thermo-Scientific, US) at λ = 405 nm. Acarbose was used as a control and the anti-α-glu action of each extract was calculated using the following equation:

Inhibition ratio " ("%")" = "(〖"1-Abs" 〗 _"sample" "- " 〖 "Abs" 〗 _"background")/ 〖 "Abs" 〗 _"control" × 100 (5)

Where $_{Abs}$control: the acarbose 's absorbance; $_{Abs}$sample: each extract 's absorbance after reacting with α-glucosidase; $_{Abs}$background: the extract solution 's absorbance without α-glucosidase.

Furthermore, the extracts' dose-dependent effects on α-amylase were examined by estimating the reducing sugar release using Sheng, et al. [23] with some changes. The α-amylase (40 µL, 10 mg/mL, 50 U/mg prepared in PBS, pH 6.9, 0.02M) was combined with 0.5 mL of each extract, as previously made, and 0.5 mL of PBS (0.02 M, pH 6.9) was also totaled. Substrate starch (40 µL, 1%, w/v) was diluted in PBS (20 mM, pH 6.9) and incubated for 15 min after pre-incubating for 10 min at 37 °C. After that, a colored reagent solution containing 20 µL of 1% w/v 3, 5-dinitrosalicilic acid was added to complete the reaction. After that, the mixture was cooled to 25 °C and incubated for 10 minutes at 100 °C in a water bath. A microplate reader scanned the mixture at an absorbance of λ = 595 nm. The same equation (5) used for α-glu was utilized to compute the inhibitory ratio of each extract's anti-α-amylase activity.

The effects of each extract on tyrosine fluorescence spectra of α-amy and α-glu were performed on an F-4600 fluorescence spectrometer (Hitachi) using a previous method with some modifications [24]. The mixture of α-amy and α-glu were both prepared in PBS (20 mM, pH 7.4) with a concentration of 1 mg/mL and mixed with each extract (0, 0.5, 1, 2.5, and 5 mg/mL). The 2-D fluorescence emission was noted in a 1 cm quartz-cell at λ_{ex} of 280 nm, and λ_{em} of 290 to 450 nm. Both excitation and emission slit widths were set at 5 nm with a scanning rate of 1,200 nm/min.

Molecular docking modeling

The basic procedure by which quercetin and chlorogenic acid limit their respective activities, as the key polyphenols fraction in both bracts and buds extracts of Egyptian Bb against α-glucosidase and α-amylase were subsequently explained by means of a docking study. Using the Surflex-dock model in SYBYL X-2.0 software on a Windows operating system, the docking study was conducted. The α-amylase's X-ray structure (PDB ID: 1HNY) [25] and α-glucosidase (PDB ID: 2QMJ) [26] was recovered from the RCSB. Both the structures of chlorogenic acid (PubChem: 1794425) and quercetin (PubChem: 5280343) were linked as ligands independently. Based on how well the docked composite fit into the receptor structure, it was modified for van der Waals forces, electrostatics, entropy, discrete and continuum hydrophobicity, and H-bonding. Prior to docking, the structures of α-glucosidase and α-amylase were

cleaned to remove any defective residues, correct uncommon amino acid names, remove substitute conformations, and insert hydrogens. This involved removing water from these structures. According to the docking results, out of ten attempts, the best docked model was chosen to represent its most satisfactory attaching technique.

Statistical analysis

Every experiment, unless specified differently, was conducted in triplicate. The mean ± standard deviation of the data was shown, and a value of $p<0.05$ was considered statistically significant. ANOVA was used to compare the parameters, and Tukey's multiple assessment post-test was then performed using SPSS version 27 (IBM, US). OriginPro 23 was used to handle post-collection data (Origin Lab, Co., US).

Results and Discussion

Chemical constituents of bracts and buds

The chemical constituents of dehydrated buds and bracts samples were estimated, as shown in Table 1. The results spotlighted a significant differentiation ($p<0.05$) in each determined chemical characteristics between buds and bracts. No significant differentiation ($p>0.05$) was noted between the content of total ash and Ca of both buds and bracts, showing the impact of Ca on the total ash content, followed by K, in both Bb by-products. These results agreed with those obtained by Wickramarachchi and Ranamukhaarachchi [27], while they only determined iron and calcium in the whole blossom before and after blanching. Meanwhile, both blossom by-products showed a moderate content of Na, K, and P. The concentration of minerals differs from what was mentioned by earlier studies [28-30]. Authors found that potassium is the highest element, mostly due to the difference in the cultivars and the parts in which the elements were estimated. Meanwhile, the difference in the chemical composition of the interior and exterior parts of the Bb in different species was also recently reported [29], but no reports were found about such difference in case of the Egyptian one. The results also showed that the moisture content was around 4-6.5%, showing the efficiency of the dried method used. In general, the water content in the dried foods should be around 5-7%, agreeing well with our previous results [31]. Fiber was the most significant component in the bracts, whereas protein was the highest one in the buds, representing that both parts could be considered good sources for fiber and protein as well. These findings are consistent with related studies [32, 33], which reviewed the complete bloom without separating its parts and found crude fiber was around 16%. Interestingly, the buds also have a higher fat content, almost a double ratio with the bracts, that may be attributable to them containing pollen grains, which are abundant sources of fat and protein [34]. Reducing sugars were mostly found in both buds and bracts at low ratio. As indicated by Edwards, et al. [35] less than 10% of total energy intake should come from free sugars in foods, according to the World Health Organization (WHO) guideline released in March 2015 [36], and this contribute to reducing the possibility of developing T2D alongside other diseases.

Table 1. The proximate chemical composition of Bb (buds and bracts) on dry basis. (Mean±SD)

Items (%)	Buds	Bracts
Moisture	4.65±0.85^{b}	6.49±0.96^{a}
Fat (ether extract)	8.66±1.02^{a}	4.93±0.58^{b}
Total ash	7.71±0.93^{a}	8.02±0.90^{a}
Protein	13.01±1.17^{b}	11.38±1.28^{a}
Crude fiber	11.83±1.74^{a}	18.24±2.01^{b}
*Available carbohydrate	54.14^{a}	50.94^{b}
Total sugars	4.02±0.25^{a}	8.03±1.01^{b}
Reducing sugars	3.77±0.01^{a}	4.37±0.62^{b}
Non-reducing sugars	0.24±0.03^{a}	3.48±0.41^{b}
Ca	3.82±0.5^{a}	3.8±0.4^{a}
Na	0.32±0.7^{b}	0.19±0.1^{a}
K	2.82±0.2^{b}	2.57±0.6^{a}
P	0.43±0.2^{b}	0.28±0.3^{a}

*Available carbohydrates were expressed by difference. a & b: there is no significant variance ($p>0.05$) between any two means, inside the same row have the same superscript letter.

Polyphenolic components in bracts and buds' extract

Phenolics are secondary metabolites found in plants, commonly present in both edible and inedible plant parts, and have been linked to several biological impacts, including antioxidant, anti-obesity, and anti-enzymes capabilities [37]. TPC of buds and bracts is shown in Fig. 2. Buds and bracts contain polyphenols, with values of 21.84 and 20.85 mg GAE /100 g dw, respectively. Thaweesang [38] reported that fresh and blanched Bb had a great content of TPC that reached to 1091.30 and 1380.58 µg GAE /g, respectively; Manaois, Zapater and Morales [39] also noted that TPC in boiled Bb was 8.0 mg GAE /g.

Flavonoids are significant antioxidants, which have been proven to be efficient at lowering the risk of many diseases. TFC content in buds and bracts was 8.29 and 10.07 mg QE /100 g dw, respectively (Fig. 2). These outcomes well matched with the findings of Ravindran, John and Jacob [28], who reported that the amounts of phenols and flavonoids differ from the outside to the inside of the blossom. Sheng, Ma, Jin, Bi, Sun, Dou, Li, and Han [30] also found that TFC ranged 5.27-5.90 mg /100 g in Bb. Meanwhile, Sarker, Shuvo, Takey, Akter, Hadaytullah, Al Reza and Zubair [40] reported that TFC of the whole blossom were 4.27 mg /100 g. The TFC in two cultivars of Bb ranged 5.53-6.4 and 4.27 mg /100 g

[40, 41], showing the difference of TFC among varieties and cultivars. These results suggested that the total polyphenols of both parts of Bb, the Egyptian cultivar, are mainly anthocyanidin-based polyphenols, as anthocyanins represented the main polyphenols. Meanwhile, little quantities of non-anthocyanidins polyphenols occurred which were fractionized and identified later by HPLC-PDA.

Anthocyanins are a group of natural pigmented flavonoids that have plenty of biological activity alongside their coloring effect [42]. Both Bb parts (buds and bracts) contained anthocyanins. However, bracts showed a greater content of TMA compared to buds with values of 80.73 mg CGE /100 g dw, while in buds it was 16.26 mg CGE /100 g dw (Fig. 2). The difference is mostly explained by the crimson red color of bracts compared to buds. Begum and Deka [43] reported that TMA was 56.98 mg /100 g in culinary banana bract, where cyanidin 3-rutinoside was identified as major anthocyanin, followed by cyanidin 3-glucoside, and peonidin 3-glucoside.

The AOA of the methanolic extract of both Bb parts was measured by DPPH free radical scavenging assay. This method is extensively used for determining the antioxidant capability of extracts from various plant materials [29]. As shown in Fig 2, AOA in buds is higher than that in bracts extracts with values of 4.92 and 4.32 µmoL TE /g, respectively. However, both parts are considered a rich source of antioxidative phytochemicals compared with other flowers like squash, and many other fruits and vegetables according to Manaois, Zapater and Morales [39].

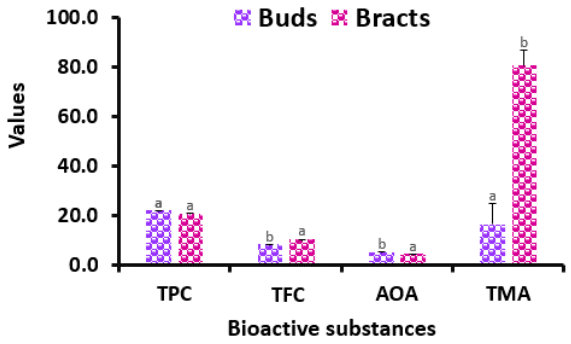
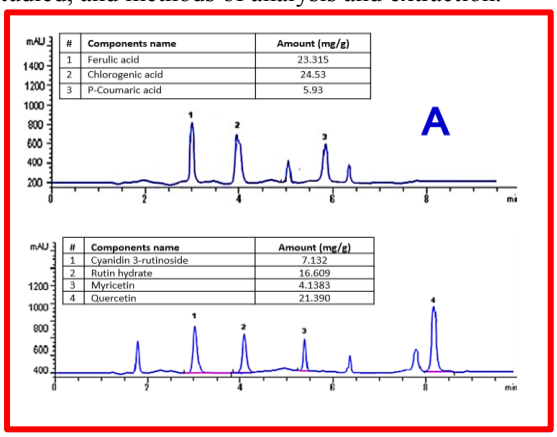


Fig 2. The bioactive components found in Bb (buds and bracts), that include total phenolic contents (TPC, mg GAE /100 g dw), total flavonoids (TFC, mg QE /100 g dw), antioxidant activity by DPPH assay (AOA, µmoL TE / g dw), and total monomeric anthocyanins (TMA, mg CGE /100 g dw) (Mean ± SD). Different letters imply significantly different means at P<0.05.

Profiling for polyphenols of both bracts and buds

The polyphenol profiles of buds and bracts were fractionized by HPLC-PDA (Fig. 3). In bract extract, three phenolic acids were identified, namely ferulic, chlorogenic, and p-coumaric acids, while four flavonoids were also identified including cyanidin 3-rutinoside, rutin hydrate, myricetin, and quercetin. In bud extract, three phenolic acids and two flavonoids were identified including chlorogenic acid, caffeic acid, gallic acid, rutin, and kaempferol. The identification of those components was based on spectra and retention time comparison with authentic standard, when available. Anthocyanin cyanidin 3-rutinoside was tentatively identified based on literature [28, 29]. In detail, chlorogenic acid was mainly found in both parts of Bb, where the highest content was estimated in bracts (24.53 mg /g). Rutin was found in the two parts in a significant content with values of 16.6 and 18.7 mg /g in bracts and buds, respectively. Our results confirm the previous results, with the finding of gallic, ferulic, p-coumaric, and chlorogenic acids, as phenolic acids; rutin and quercetin, as flavonoids [9, 15, 30]. These results signified that both Bb parts are a source of polyphenols, namely phenolic acids, and flavonoids. The variation in studies arise from differences in climate condition, locations and agriculture environments, stages of maturity, varieties, parts studied, and methods of analysis and extraction.



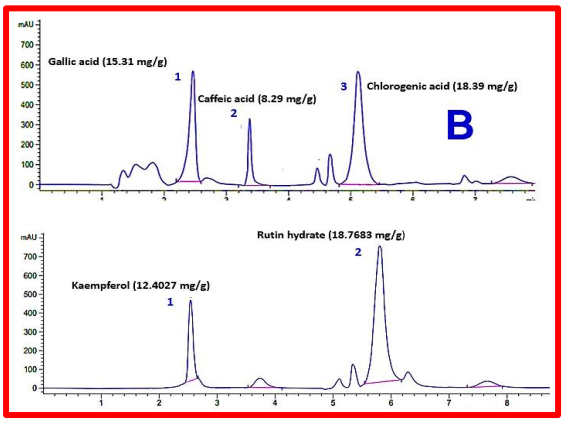


Fig 3. The HPLC-PDA chromatographs of Bb bracts **(A)** and buds **(B)** with the quantitative analysis (mg/g) of their main key phenolic acids and flavonoids.

The anti-enzymes effects of Bb bracts and buds' extracts

In this study, we estimated the inhibitory capability of extracts of Egyptian Bb buds and bracts against α-glu and α-amy enzymes in vitro. As illustrated in Fig. 4, α-amy and α-glu were both inhibited in a dose-dependent way by both bud and bract extracts. In details, the higher concentration (5 mg/mL) of bud extract inhibited both α-glu and α-amy by 79.63 and 93.58%, respectively; while bract extract inhibited α-glu and α-amy by 83.21 and 97.68%, respectively. These inhibitory levels resemble those from a comparable study, when Sobhy, et al. [13] found that phytosterols inhibited both enzymes activity with values around 63-97%; also, Sakulnarmrat and Konczak [44] found a-amylase inhibitory activity in poly-phenolic-rich fractions of different Australian herbs. Sheng, et al. [45] noted that phytosterols isolated from Bb inhibited both enzymes activity. Polyphenols of persimmon fruits also displayed a greater anti-α-amylase and α-glucosidase effects [45], mostly via binding with the catalytic-closed sides of these enzymes.

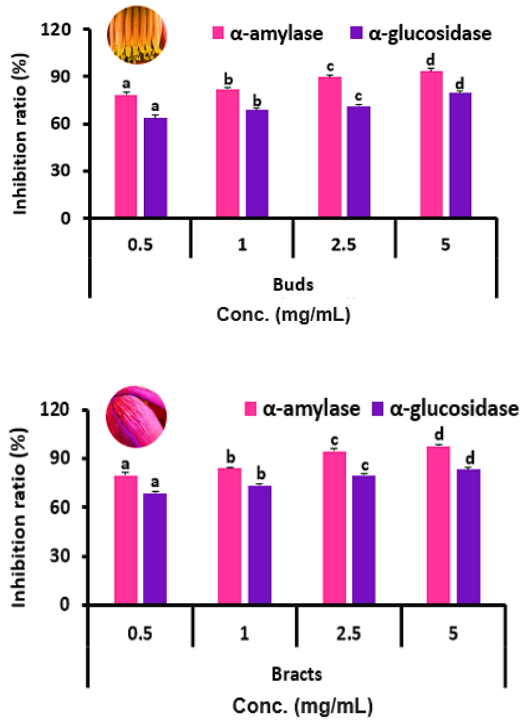


Fig 4. Dose inhibitory effects ratio (%) of both brace and bud extracts (0.5, 1, 2.5, and 5 mg/mL) against α-glu and α-amy enzymes. Different letters imply significantly different means at $p<0.05$.

The foregoing findings implied that Bb extracts could inhibit α-glu and α-amy enzymes, and therefore, we theoretically and experimentally measured the binding affinity between both extracts and enzymes. Fluorometric analysis was then utilized to classify the binding between extracts and proteins based on the intrinsic fluorescence of tyrosine (Tyr) and tryptophan (Trp) subunits of both enzymes. The enzymes controlled the fluorescence curve with a highest point at 325 nm (Fig. 5). At the same time, the active components of each extract showed a stronger inhibition effect on the fluorescence intensity of both enzymes. For ex-ample, 5 mg/mL of Bb bud extract declined the fluorescence strength of α-amy and α-glu by around 73.21 and 79.68%, orderly with a shift of the maximum peak (Fig. 5A), referring to their binding simultaneously. Likewise, 5 mg/mL of bracts extract declined the fluorescence strength of α-amy and α-glu by 81.52 and 84.86%, orderly (Fig. 5B). The outcomes from fluorescence spectral analysis proposed that Bb extracts could conjugate with α-amy and α-glu to form enzyme-Bb complexes. The slight red shift of the emission peak imitated the polarity fluctuations of microenvironment of Tyr and Try residues, signifying that the con-formations of α-amy and α-glu might change [24]. Furthermore, either bud or bract extracts significantly inhibited α-amylase, more than α-glucosidase. This difference is probably due to the difference in their polyphenol structures, where the anti-amylolytic effects of natural plant extracts were shown to be structurally dependent.

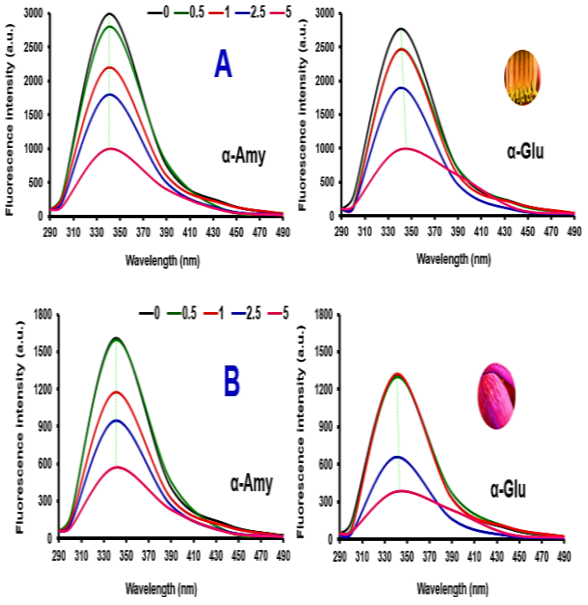


Fig 5. The fluorescence intensity of both bud **(A)** and bracts **(B)** extracts (0.5, 1, 2.5, and 5 mg/mL) against α-glu and α-amy enzymes.

Polyphenolics, which were found in both extracts, could deliver OH-groups and electron delocalization

systems, thereby showing its anti-amylolytic activity. he inhibition of our extracts against α-glu and α-amy might be the relation between the active components of each extract and the structure of enzymes. Molecular docking experiments between extracts and enzymes were conducted to study the potential residues involved in the interaction between both. As displayed in Figs. 6A-B, quercetin and chlorogenic acid, which are the key polyphenols of bud and bract extracts, might form a complex when docked to α-amy and α-glu via different bonds. For example, quercetin interacted with His299 and Asp197 of α-amy structure and Asp193 of α-glu structure via H-bonds. Meanwhile, it also could bind with Trp59 and His91 of α-amy and α-glu structures via π–π interaction. On the other hand, chlorogenic acid interacted with Asp300, Glu233, Tyr151, and Asp197 of α-amy structure and Glu432 of α-glu structure via H-bonds. It was clearly charted that the polyphenols of Bb could interact with α-amy than α-glu, agreeing well with our inhibition results (Fig. 4). Furthermore, various α-amy and α-glu residues were found to surround the polyphenols in Bb, around 22 and 17 amino acids for α-amy and α-glu, orderly. The amino acid residues of Asp and Glu were found to be essential to both enzymes' catalytic activity, according to a previous study [46]. The interaction's residues that are involved between Bb extracts and enzymes were observed to be close to the active sites based on the docking data (Fig. 6). These results indicated that Bb extract might work as an inhibitor to competition on both enzymes, with a single high-affinity binding site. Several subsites formed up of residues bridging the α-helix and β-sheet make up the amylolytic enzymes. According to the 3D docking diagrams, Bb was encircled by numerous α-helix and β-sheet structures. This suggested that Bb extracts might attach themselves to catalytic sites or regions around them, changing the structure of α-amy and α-glu in the process and causing both enzymes to lose their catalytic function. Additionally, the inhibition activity of Bb polyphenols is possibly owing to their intense sensitivity of the allylic secondary carbon centers. The pairwise correlation analysis among the parameters measured in both Bb buds and bracts extracts was also done using heatmapper as portrayed in Fig. 7. The results concluded that each nutrient and bioactive substance is highly correlated with anti-amylolytic effects of both bracts and bud extracts versus both α-amy and α-glu enzymes. TMA was also well-correlated with each component, alongside available carbohydrates.

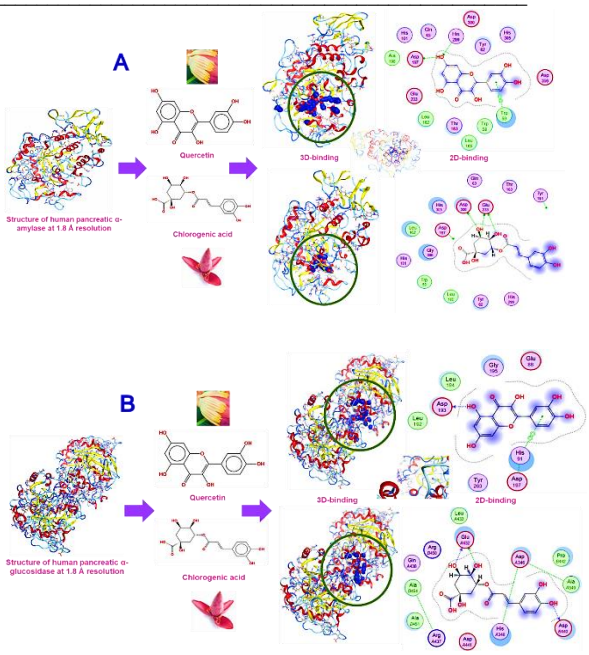


Fig 6. The 2D and 3-D homology models of the docking process of the key polyphenols in both bracts and buds extracts versus α-amy (**A**) and α-glu enzymes (**B**).

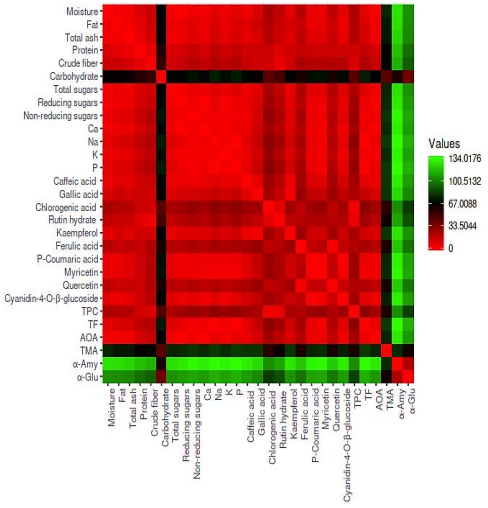


Fig 7. The pairwise correlation analysis among the parameters measured in both buds and bracts of Bb.

3. Conclusions

For the first time, the antioxidant and anti-amylolytic effects of banana blossom extracts (both from buds and bracts) from the Egyptian cultivar were studied. The chemical composition and bioactive substances were also quantitively measured. Results showed that both parts have adequate amounts of polyphenolic compounds, (phenolic acids, flavonoids, anthocyanins) and dietary fiber. They also showed a good source of Ca, K, and proteins, and a powerful antioxidant capacity. Most importantly, both parts

effectively mitigated the activity of α-amylase and α-glycosidase with a dose dependent-effect. The results implied that the banana blossom parts could be utilized, by the meaning of food wastes recycling, to fortify and/or formulate different models of functional foods in term of reducing and/or controlling obesity and diabetes; however, future in vivo confirmation of the potential of the extract ought to be considered. This helps in making optimal uses of agricultural waste instead of wasting it, and this has many economic and environmental benefits.

4. Conflicts of interest

There are no conflicts to declare.

5. Formatting of funding sources

No funding sources.

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