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Original Article

FORMULATION AND STABILITY EVALUATION OF ANTI-OBESITY NUTRACEUITCAL BLEND OF WHITE KIDNEY BEAN EXTRACT *(PHASEOLUS VULGARIS L.),* PROPOLIS ETHANOLIC EXTRACT AND CRPIC$_3$

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ABSTRACT

Objective: This study aimed to formulate and evaluate the stability profile of an anti-obesity nutraceutical combination in different dosage forms.

Methods: Active and inactive ingredients were formulated into pharmaceutical dosage forms. The quality parameters of the dosage forms were determined, followed by accelerated stability testing (40±2 °C and 75±5% Relative Humidity (RH)) for 180 d was completed to evaluate their physical, chemical and microbiological attributes throughout the storage period.

Results: Pre-formulation parameters of the powder blend of active and inactive ingredients for each dosage form showed a satisfactory flowability with Hausner's ratio falling between 1.16 and 1.18, average angle of repose between 22.29° and 22.90° and acceptable compressibility with Carr's index below 25%. Tablets assessments were acceptable with a mean friability value of 0.21±0.03%, hardness of 4.12±0.09 kg/cm^2. The average disintegration time of 5 min 10 sec for tablets and 4 min and 30 sec for capsules. The accelerated stability study revealed that tablet dosage forms are stable for longer period that can reach up to 180 d (24 mo real-time), while sachets and capsules are stable for a period of 135 d (18 mo real-time).

Conclusion: The anti-obesity blend of White Kidney Bean Extract (WKBE), Propolis Ethanolic Extract (PEE) and CrPic$_3$ can be successfully formulated in acceptable and convenient dosage forms that can be stable for 18-24 mo.

Keywords: Obesity, Nutraceutical, WKBE, PEE, CrPic$_3$, Hausner's ratio, Carr's index, Angle of repose, Disintegration, Stability study

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INTRODUCTION

For decades, the search for novel drugs to treat human diseases has focused more on natural products because of the unfavorable side effects, high cost, and inefficiency of some pharmaceutical treatments. Approximately 25% of prescription medications filled in the US contain at least one active ingredient derived from plant material. According to WHO estimates, 60% of people worldwide use herbal medicine and 80% use natural drugs [1-3]. Managing chronic diseases like obesity and averting its chronic consequences using nutraceuticals and dietary supplements have emerged as a cutting-edge and all-inclusive dietary strategy [4]. In addition to lowering people's quality of life, obesity is the leading cause of preventable deaths globally and is linked to several comorbidities, making it a global public health concern [5, 6]. With up to 3.4 million deaths per year, it is the second most dangerous risk factor after smoking [7]. Obesity has been linked by epidemiological research to an increased risk of dyslipidemia, cardiovascular disease, type 2 diabetes, sleep apnea, knee osteoarthritis, and certain cancers [8–11]. Following the COVID-19 pandemic, it was discovered that obesity posed a substantial risk factor for hospitalization and unfavorable clinical outcomes in SARS-CoV2-infected patients [12].

Although there are various approaches to treating obesity, such as calorie restriction, exercise, weight loss surgery, and lifestyle modifications [13], anti-obesity medications still seem like a promising way to lose weight quickly. However, due to potential side effects and drug interactions, the idea of researching new, safe, and effective weight control options from natural products is gaining a lot of attention. Certain natural products that gained popularity included fiber-rich food supplements, polyunsaturated fatty acids, and phytochemicals that increase satiety, energy expenditure, adipocyte differentiation, and the enzymes involved in lipid synthesis in order to help people lose weight [14–16]. As demonstrated in numerous clinical trials, chromium may improve insulin function, lower the amount of glucose converted to fat, increase muscle mass, and increase protein synthesis. These effects may reduce appetite, food intake, and fat-related cravings. Thus, it has been suggested that taking chromium supplements can enhance body composition and aid in weight loss through the reduction of body fat and the increase of lean body mass [17–22]. *Phaseolus vulgaris*, a native South and Central American legume with a well-established anti-obesity profile, is the source of WKBE. The extract binds to the α-amylase enzyme, which is required for the digestion of carbohydrates, acting as a "starch-blocker" [23]. WKBE has been shown in multiple *in vivo* studies to support appetite control, body fat reduction, and weight loss in both animal and human models. With the exception of a few gastrointestinal side effects brought on by the extract's inhibition of the small intestine's ability to digest and absorb carbohydrates, these studies found no significant negative effects of supplementing with it [24, 25]. Propolis, a naturally occurring substance with a range of biological properties used in folk medicine since ancient times, has also attracted attention. Numerous pharmacological effects, including immune-modulatory, antitumoral, antibacterial, antiviral, antifungal, anti-inflammatory, and antioxidant qualities, have been associated with propolis [26]. Propolis's high polyphenol content has been linked to its remarkable anti-obesity efficacy in numerous *in vivo* and *in vitro* studies. According to the study's findings, propolis extract improves lipid metabolism and reduces insulin resistance to control weight loss. Furthermore, in rats with induced diabetes mellitus and insulin resistance, ethanol-extracted propolis was found to alter blood glucose, glucose metabolism, and blood lipid concentration [27–31].

In an earlier study, we demonstrated the theanti-obesity benefits, effectiveness and safety of combining WKBE, PEE, and CrPic$_3$ in one nutraceutical supplement using an *in vivo* model of obesity-induced

Sprague-Dawley rats fed a 45% high-fat diet for a 14-weeks study period. Comparable effects also included nutritional parameters, biochemical parameters, and biomarkers of cardiovascular disease, kidney, liver, and gut health [32]. However, in order to translate the knowledge gained from this research into practical steps towards investigation in a clinical setting, it is still crucial that these benefits be offered in a convenient, safe, and stable formula. In accordance with the FDA's recommendations for the percentage of daily value for each active ingredient, the current study discusses the formulation and assessment of dosage forms that combined PEE, chromium picolinate, and WKBE in the form of sachets, tablets, and capsules. Subsequently, the three dosage forms' stability profiles were assessed after being stored in accordance with the guidelines provided by drug regulatory bodies like the World Health Organization, the European Medicines Agency, and the International Conference on Harmonization (ICH) to assess the nutraceuticals quality, safety, and efficacy study [33–36].

MATERIALS AND METHODS

Ethical permission

The Institutional Ethics Committee of the Faculty of Pharmacy Cairo University, Cairo, Egypt approved this research (protocol code 1143)

Materials

WKBE (Phaseolus vulgaris) was acquired fromXi'an Sentian Biotechnology Co. Ltd. (Shanghai, China). Egyptian propolis was purchased from a local natural product store in Cairo, Egypt. Food-grade chromium picolinate (elemental chromium of 12.5%) was obtained from El-Gomhoria Company, Cairo, Egypt. The following inactive ingredients were acquired from El-Gomhoria Company, Cairo, Egypt; microcrystalline cellulose (INS 460i), Silicon dioxide (INS 551), Citric acid (INS 330), Sucralose (INS 995), Orange juice powder, Orange flavor, Sunset yellow (INS 110), Sodium benzoate (INS 211), Calcium Carbonate (INS* 170i), Microcrystalline cellulose (INS 460i), Colloidal Silicon dioxide (INS 551), Magnesium stearate (INS 572), Menthol crystals, Iron oxide (INS 172ii), Calcium Carbonate (INS* 170i), Microcrystalline cellulose (INS 460i), Colloidal Silicon dioxide (INS 551), Magnesium stearate (INS 572), Menthol crystals, Gelatin, Subset (INS 110) and Ponceau 4R (INS 124).

Methods

Formulation of pharmaceutical dosage forms

Table (1) illustrates the ingredients of the prepared sachets, tablets, and capsules from WKBE (source of alpha-amylase inhibitor), PEE (sources of phenolics and flavonoids), and Chromium picolinate. The capsule shell composition is shown in table 2.

Table 1: Composition of the sachet, tablet, and capsule pharmaceutical dosage forms

Composition		Sachet (mg)	Tablet (mg)	Hard gelatin capsule (mg)	Function
Active ingredients	WKBE (*Phaseolus Vulgaris*) powder Standardization: NLT 3000 α-amylase inhibitory units (AAIU/g)	1000	500	500	Block carbohydrate absorption and weight loss
	PEE Standardization: NLT 137.32±3.16 gallic acid equivalent mg/g dry extract and 41.6±0.68 quercetin equivalent mg/g dry extract	500	250	250	Reduced high-fat diet-induced weight body gain obesity and hepatic, fat accumulation and glucose metabolism, anti-obesity and anti-diabetic
	Chromium Picolinate equivalent to 12.4% elemental chromium	0.200	0.100	0.100	Block insulin effect on fat cells, interfering with its lipogenesis effect
Inactive ingredients	Microcrystalline cellulose (INS 460i)	66.0	33.0	33.0	Bulking agent
	Colloidal Silicon dioxide (INS 551)	20	13.5	13.5	Anticaking agent
	Citric acid (INS 330)	35	––––––	–––––	Acidity regulator
	Calcium Carbonate (INS* 170i)	–––––	65.0	65.0	Acidity regulator
	Magnesium stearate (INS 572)	–––––	15.0	15.0	Softening agent
	Sucralose (INS 995)	50	–––––	–––––	Artificial sweetener
	Orange juice powder	600	–––––	–––––	Juice
	Orange flavor	10	–––––	–––––	Natural flavouring agent
	Sunset yellow (INS 110)	0.5	–––––	–––––	Coloring agent
	Sodium benzoate (INS 211)	5.5	–––––	–––––	Preservative
	Menthol crystals	–––––	5.0	5.0	Natural Flavoring agent
	Iron oxide (INS 172ii)	–––––	1.5	–––––	Yellow color
	Average weight	2.280 g±2%	883 mg±2%	997 mg±2%	––––––––––

Table 2: Composition of the capsule shell

Composition of capsule shell	Cap (cont.)	Body (Conc)	Function
Gelatin	45.5	70	Shell Builder
Subset (INS 110)	0.22 mg	–––––	Yellow color
Ponceau 4R (INS 124)	–––––	0.24 mg	Red color

*INS: International numbers

Pre-formulation parameters of powder blend of active and inactive ingredients for each dosage form

Bulk density and tapped density

Six (6) grams of powder from each of the powder blends displayed in table (1), was weighed using a digital balance (CP2245, HR-120, Sartorius, USA) and then passed through a sieve no.18 (Controls Milano ASTM) to break up any agglomerates. Then, poured in a 10-mL graduated cylinder. The cylinder was dropped onto a wooden surface three times from a height of 3 cm at 2-second intervals. The bulk density (ρ_{bulk}) was then determined using the mass/volume ratio and the volume that was recorded directly from the cylinder ($V°$). The cylinder was tapped until the volume was constant (V_f) to determine the tapped density (ρ_{tap}) [37]. The experiment was conducted in triplicate.

$$\text{Bulk density } (\rho\text{bulk}) = \text{weight of powder}/V°$$

$$\text{Tapped density } (\rho\text{tap}) = \text{weight of powder}/V_f$$

Hausner's ratio (HR) and Carr's index (CI%)

The following formula was used to determine HR and CI (%) based on the bulk and tapped densities [38]

$$HR = (\rho\text{tap})/\rho\text{bulk})$$

$$CI\ (\%) = (\rho\text{tap}) - (\rho\text{bulk})/(\rho\text{tap}) \times 100$$

Angle of repose for each dosage formula

Using the fixed-funnel method, a glass funnel's tip was positioned 2 cm above a level base. While closing the tip of the funnel, 6g of powder was poured to fill the funnel. Then, the funnel was opened and the powder was allowed to flow out of the funnel, forming a pile. The height (h) and diameter (d) of the pile we remeasured and the angle of repose (ø), was determined according to the following equation [37],

$$\text{Tan } ø = 2h/d$$

Preparation of sachets

The sachet powder formula as shown in table (1) was grinded in a porcelain mortar into fine powder and sieved using the analytical dry sieving method [39]. A set of sieves was used where 90% of the fractioned portion was retained on sieve number 20 after it passed through sieve number 50. The retained portion of powder on the lower sieve was packaged in a food-grade aluminum sachets with an average final weight of 2.280 g±2% powder in each sachet.

Formulation of tablets

The tablet powder formula, as presented in table (1), was directly compressed using the compression method to create tablets that were further assessed for weight variation, thickness, hardness, friability, and disintegration time [40].

Formulation of hard gelatin capsules

The hard gelatin capsule powdered formula shown in table (1) was mixed well and sieved through sieve no.50 followed by sieve no.20 where the fractioned portion that passed through was used for encapsulation. Elongated gelatin capsules size 000 with the composition shown in table (2) were used to encapsulate the powder formulation using a hand-held capsule filling system to obtain an average final weight of (997 mg±2%). Capsules were assessed for disintegration time and weight variation [41].

Evaluation of tablets and hard gelatin capsules

Weight variation (tablets and capsules) and thickness (tablets)

To determine weight variation, an electronic balance was used to weighten tablets, and the mean weight was calculated [40]. The weight variation of capsules was determined following the same method. Ten tablets were measured for thickness using a digital Vernier caliper (China).

Hardness test (tablets)

Ten tablets were chosen at random, and their hardness was assessed using a hardness tester (Dr. Schlenger Pharmaton, USA) to calculate their average breaking strength [41].

Friability test (tablets)

Using a friability (Pharma Test, Germany), the friability of tablets was determined. For four minutes, ten pre-weighed tablets were inserted into the friabilator's drum, which was attached to a motor spinning at 25 rpm. After subtracting the tablets and reweighing them, the below formula was used to determine the percentage of weight loss [42].

$$\text{Friability (\%)} = [\text{initial weight (W1)} - \text{final weight (W2)}/\text{initial weight (W1)}] \times 100$$

Disintegration time (tablets and capsules)

Six tablets were used in the test, which was conducted at 37±2 °C using phosphate buffer (900 ml, pH 6.8) as the disintegration medium and the apparatus (Hanson research, USA). The duration in seconds that the tablets took to fully dissolve and leave no trace inside the device was noted [41]. Using the same technique, the capsules' disintegration time was calculated, and the device was operated until the six capsules had broken open and only the gelatin shell remnants remained on the mesh.

Stability study

The experiment was conducted according to the International Council for Harmonization (ICH, 2003) guidelines. Accelerated storage conditions were RH at 75±5% and temperature at 40±2 °C for a period of 6 mo [43].

Certain number of sachets, tablets and capsules were placed on shelf in a stability cabinet (Climacell Medcenter, Einrichtungen GmbH MMM group, Germany) for 6 mo. Initial samples were withdrawn at times 0, 30, 45, 90, 135 and 180 d intervals. All samples were withdrawn and analyzed in triplicates [44]. Samples of withdrawn capsules or sachets were carefully opened, and the contents was collected in full, while withdrawn tablet samples were grounded to a fine powder. The collected content was then mixed, weighed and analyzed.

Determination of moisture content in sachets, tablets, and capsules

Using a Karl-Fischer titrator (787 KF trinitro, Metrohm, Italy), the formula's water content was determined. After calibrating the device with anhydrous methanol, powder samples weighing 0.15 g were put in the titrator. The water content was then determined by measuring the quantity of iodine that was consumed during the reaction of the formula samples with water [45].

Determination of pH in sachets, tablets, and capsules

A pH meter (Mettler-Toledo International Inc., Columbus, OH, USA) was used to measure the pH. After the samples were removed, they were homogenized, and a pH electrode was inserted for analysis. Three duplicate readings of each were recorded.

Microbial contamination test in sachets, tablets, and capsules

To calculate the Total Plate Count (TPC) in the dosage forms, 10 ml of phosphate buffer (pH 7.2) was mixed with 1 g of powder, and the mixture was then further diluted up to 10^{-6}. One ml of each dilution was pipetted into a sterile petri dish. Then, 15-20 ml of Nutrient Agar (45±1 °C) media was poured into the Petridishes, shaken, and rotated to ensure the even distribution of the solution. Blanks were created in a single petri dish that was filled with 1 ml of diluent and agar medium. The TPC was calculated by counting the number of colonies that developed after a 24-hour incubation period at 35–37 °C. The same process was used to determine the yeast mold numbers using Potato Dextrose Agar seed media and the plate method [46].

Determination of alpha-amylase from WKBE in sachets, tablets, and capsules

A technique that had previously been published was used to characterize the extract. Porcine pancreatic α-amylase (40 units/ml) was dissolved in a sodium succinate buffer (15 mmol NaOH, 20 mmol $CaCl_2$, 0.5 M NaCl, pH 5.6). Porcine pancreatic alpha-amylase (100 ul) was added to 100 ul of WKBE, and the mixture was incubated for 30 min at 37 °C in a water bath to measure the amylase inhibition activity. After that 400 ul of soluble starch (2%, w/v) was added, and the mixture was incubated for one minute in 20 mmol sodium phosphate buffer containing 6.7 mmol NaCl (pH 6.9). The reaction was stopped by adding 800 µl of 3,5-dinitrosalicylic acid (0.65% w/v) and the mixture was then incubated for 10 min in a boiling water bath. Lastly, distilled water was added to reach the volume up to 6 ml. The same amount of porcine pancreatic α-amylase was used in a parallel trial, but WKBE was absent. These two results were compared to determine the inhibitory activity of WKBE. The quantity of α-amylase inhibitor required to stop one unit of porcine pancreatic α-amylase enzyme activity was known as an inhibitory unit [47].

Determination of total phenolic content in propolis ethanolic extract in sachets, tablets, and capsules

The total phenolic contents of the propolis ethanolic extract used in the three dosage forms were estimated using the oxidizing agent Folin-Ciocâlteu. A mixture of 1.0 mg was dissolved in 1.0 ml ethanol. A 0.5 ml aliquot of the extract was combined with 2.5 ml of a 10-fold diluted aqueous Folin-Ciocâlteu solution. The mixture was shaken and allowed to stand for 6 min before the addition of 2.0 ml of 7.5% sodium carbonate and mixed until a homogeneous mixture was obtained. After letting the solution sit for half an hour, the absorbance was measured at λ = 765 nm using a spectrophotometer (Lambda 25 UV/vis Systems, PerkinElmer, Washington, USA). By plotting a standard curve using 1 ml aliquots of 50, 100, 150, 200, 250, 300, 400, and 450 mg/ml Gallic acid solutions under the same conditions, the total phenolic content was estimated as equivalent to Gallic acid (mg GA/g of dry weight sample) [48].

The experiment was performed in triplicates, then absorbance was measured and the total phenolic contents in the extract using this formula,

$$C = C1 \times (v/m)$$

Where;

C= total phenolic content in mg/g, in GAE (Gallic acid equivalent),

C1= concentration of Gallic acid established from the calibration curve in mg/ml,

v= volume of extract in ml, and

m= the weight of the dry sample in g

Determination of total flavonoid content in propolis ethanolic extract in sachets, tablets, and capsules

The total flavonoid content in the three dosage forms was estimated by preparing a solution using aluminum chloride at 2.0% in methanol in a 1:1 solution. The solution was left at room temperature for 10 min, then the mixture's absorbance was measured at λ = 415 nm using a spectrophotometer (Lambda 25 UV/vis Systems, PerkinElmer, Washington, USA). To create a standard curve, the identical process was carried out with standard Quercetin (QE) solutions (25–250 ppm), three independent analyses were carried out, and the findings were reported in milligrams of quercetin per gram of propolis [49].

Determination of elemental chromium and calculated chromium picolinate in sachets, tablets, and capsules

The concentration of chromium was carried out through acidic digestion preparation and analysis using graphite furnace atomic absorption spectrometer (PerkinElmer, Washington-USA) using high purity argon as the inert gas and operating chromium hollow cathode lamp at λ = 357.9 nm and spectral bandpass set at 0.7 nm, then chromium picolinate equivalent to 12.4% elemental chromium was calculated [50-53].

Statistical analysis

The mean±Standard Deviation (SD) was used to express all data. One-way Analysis of Variance (ANOVA) was utilized to identify the differences among various time points. The Social Sciences Package for Statistics (SPSS) 28.0 program was employed to carry out the statistical analyses. For differences, a significance threshold of $p<0.01$ was used [54].

RESULTS

Pre-formulation parameters of powder blend of active and inactive ingredients of each dosage form

The granules flowed satisfactorily out of the hopper with no signs of capping, sticking, or ratholing. As can be seen from table 3, all of the formulations showed good flowability, with the mean values for Hausner's ratio falling between 1.16 and 1.18. The formulations' mean compressibility indices ranged from 14.04 to 14.88%, all of which were below 25%. The average angle of repose for each formulation falls between 22.29° and 22.90°. The granules were determined to have good flow properties based on the values of the compressibility index, Hausner's ratio, and angle of repose obtained for the three formulations.

Table 3: Bulk density, tapped density, Hausner's ratio, and Carr's index of prepared formulas

Parameters	Dosage forms		
	Sachets	Tablets	Hard gelatin capsules
Weight (g)	6	6	6
Bulk volume (V°) (ml)	31.67±0.67	31.97±0.68	31.72±0.63
Final volume (V_f) (ml)	27.27±0.55	27.2±0.26	27.05±0.36
Bulk density(ρ_{bulk})	0.19±0.01	0.19±0.01	0.19±0.01
Tapped density (ρ_{tap})	0.22±0.01	0.22±0.01	0.22±0.01
Hausner's ratio (HR)	1.16±0.01	1.18±0.03	1.17±0.01
Carr's index (CI%)	14.04±0.49	14.88±2.54	14.69±1.04
The angleof repose (ø) in degrees	22.29±0.01	22.90±0.46	22±0.44

Results are represented as mean±SD (n=3)

Tablets and capsules evaluation results

The tablet assessment parameters are shown in table (4). Powder has been compressed into tablets with a weight of 883 mg each. They showed a mean weight variation of 2.04±0.07%, a mean friability value of 0.21±0.03%, and a hardness of 4.12±0.09 kg/cm2, which is higher than the minimum of 3.0 kg/cm2. In addition, it having an average tablet thickness of 8.21±0.15 mm, which suggests that the tablets are generally acceptable. The average disintegration time was 5 min and 10 sec for tablets and 4 min and 30 seconds for capsules.

Powder organoleptic results

The organoleptic evaluation results of the powder form of the three dosage forms are presented in table 5. Overall, no changes to these initial characteristics were noticed during the storage period.

Table 4: Tablet evaluation parameters

Evaluation	Parameters			
Dosage form	Hardness (kg/cm^2)	Thickness (mm)	Weight Variation %	Friability (%)
Tablet	4.12±0.09	8.21±0.15	2.04±0.07	0.21±0.03
Hard gelatin capsule	N/A	N/A	2.09±0.36	N/A

Results are represented as mean±SD (n=10)

Table 5: Evaluation results of powder organoleptic

Dosage form	Form	Color	Odor	Taste
Sachets	Fine powder	Faint yellow	Citrus	The sweet, orange flavor
Tablets	Fine powder	White	Aromatic	Peppermint
Hard gelatin capsules	Fine powder	Yellowish red	Aromatic	Peppermint

Changes in the physical, chemical, and microbiological attributes of sachets, tablets, and sachets during storage under temperature (40±2 °C) and RH (75±5%)

The three formulated nutraceutical dosage forms were stored at 40±2 °C and a RH of 75±2% for a period of 180 d (6 mo). The methodology of the accelerated stability study is based on the Arrhenius equation (Equation#1), which explains how temperature affects reaction rate;

$$k = Ae^{-Ea/RT}$$

where;

k is the rate constant,
A is the exponential factor or Arrhenius factor,
Ea is the molar activation energy (J),
R is the universal gas constant (8.3144 J/mol K), and

T the is the absolute temperature.

According to the Arrhenius equation, a 6-months accelerated stability study is equivalent to 2 y real-time. Observations are shown in tables 6, 7, and 8. The stability attributes were analyzed after 0, 30, 45 90, 135, and 180 d of storage at accelerated conditions.

Chemical attributes included measurement of moisture content and pH. The outcomes concerning variations in the level of moisture of the formulated nutraceuticals during storage conditions as observed in tables 6, 7, and 8 and fig. 1, illustrates that over the 180 d of storage, there was a steady rise in the moisture content among the three dosage forms. Values ranged from 4.32% to 4.81%, 4.3% to 4.5%, and 4.35%-4.55% for sachets, tablets, and capsules, respectively. Even though the percentage of moisture in the dosage forms increased during the storage period, this increase in moisture was still within allowable limits. When compared to the initial 0-time, the increase in percentage of moisture content at 90, 135, and 180 d time points were non-significant (p>0.01).

As illustrated in fig. 1 and Tables 6, 7, and 8, the initial pH values of the sachets, tablets, and capsules differed marginally. At the 0-time point, the sachets had the lowest pH value with 5.5. While, the initial pH of the tablets and capsules were 6.53 and 6.4, respectively. At the end of the 6-months stability study, the pH values of the sachets reached 5.9, 6.8, and 6.55 for sachets, tablets, and capsules, respectively. It was also observed an increase in the pH values at the other time points; this increase was significant (p<0.01) for sachets at 45 d but was non-significant (p>0.01) for tablets and capsules throughout the 180 d when compared with the starting initial value.

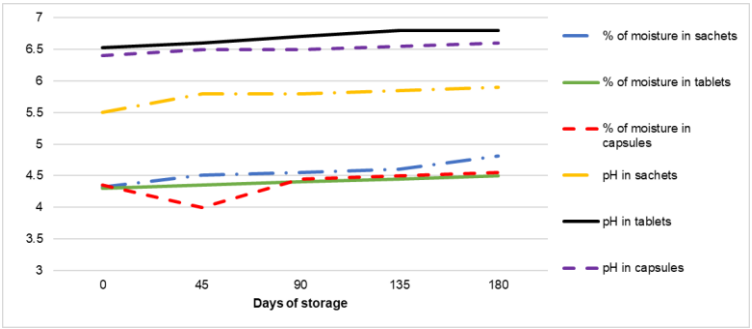

Fig. 1: Effect of accelerated stability study (40±2° and 75±5% RH) on chemical attributes (moisture and pH) of the three formulations

Bioactive ingredients, including α-amylase inhibitor activity of the WKBE, phenolic and flavonoid content in PEE, and chromium content released from chromium picolinate were analyzed initially and at regular intervals of time (30, 45, 90, 135, and 180 d). The results of initial α-amylase inhibitor activity in each dosage form at 0-time showed that the formulations had acceptable values at 3030IU, 1540IU, and 1530IU in sachets, tablets, and capsules, respectively. However, there was a gradual non-significant (p>0.01) decrease during the storage stability tests. In more detail, the decrease was highest in stability samples of capsules (5.23%), less in sachets (4.29%), and minimum in tablets (only 3.90 %) as compared to the 0 d values. While the decrease was non-significant (p>0.01) for tablets until 180 d, capsules and sachets showed a significant decrease (p<0.01) at 180 d with 5.23% and 4.29% less active ingredients as compared to 0-day, respectively.

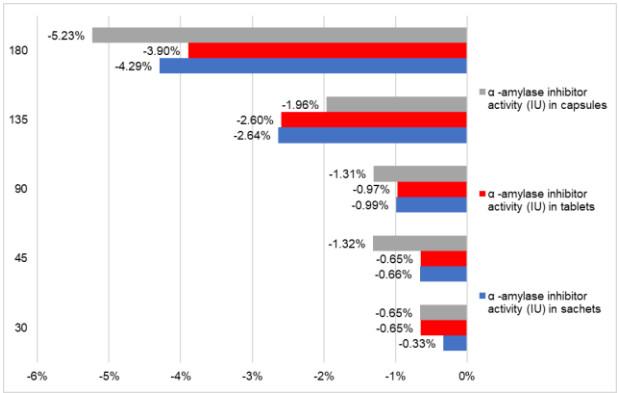

Fig. 2: Effect of accelerated stability study (40±2 °C and RH 75±5%) on the WKBE α-amylase inhibitor activity in the three formulations

Phenolic content in control samples at 0-day was found to be 68.8 mg GA/g for sachets and 34.4 mg GA/g for both tablets and capsules. This value decreased gradually during the 6-months accelerated stability study to reach 62.5, 33, and 32.1 mg GA/g in sachets, tablets, and capsules, respectively. Tablets showed the highest phenolic concentration at 180 d with a value of 3.90% decrease from the initial concentration as opposed to sachets and capsules that lost 9.16% and 6.69% from their initial concentration. The decrease in the Phenolic content concentration was significantly different (p<0.01) at 135 d for sachets (-3.78%) and capsules (-2.91%). Flavonoid content was affected by storage conditions during the 6 mo stability study period, where its levels in sachets showed a decrease from 20.8 to 19.1 mg QE/g, from 10.5 to 10 mg QE/g in tablets and from 10.4 to 9.77 mg QE/g in capsules. Sachets and capsules showed a significant decrease (p<0.01) at 135 d where the concentration decreased by 4.8% of the initial concentration and at 180 d where the concentration decreased by 6.06% of the initial concentration, respectively. On the other hand, tablets showed a non-significant decrease (p>0.01) throughout the period of the stability study.

The smallest change during storage was seen in the chromium content. Initial chromium content concentration at 0-day was 24.8µg, 12.4µg, and 12.4µg for sachets, tablets, and capsules, respectively. Chromium concentration decreased by the end of the 6 mo stability study, where sachets had 24.5µg, while tablets and capsules each contained 12.3µg. However, the decline in chromium concentration among the three dosage forms was non-significant (p>0.01). Similarly, chromium picolinate slightly decreased during the 180-day storage period. For sachets, the initial value was 200µg and ended being 198µg at the end of the study; for tablets and capsules, it ranged from 100µg-99.2µg. However, these changes were non-significantly different (p>0.01).

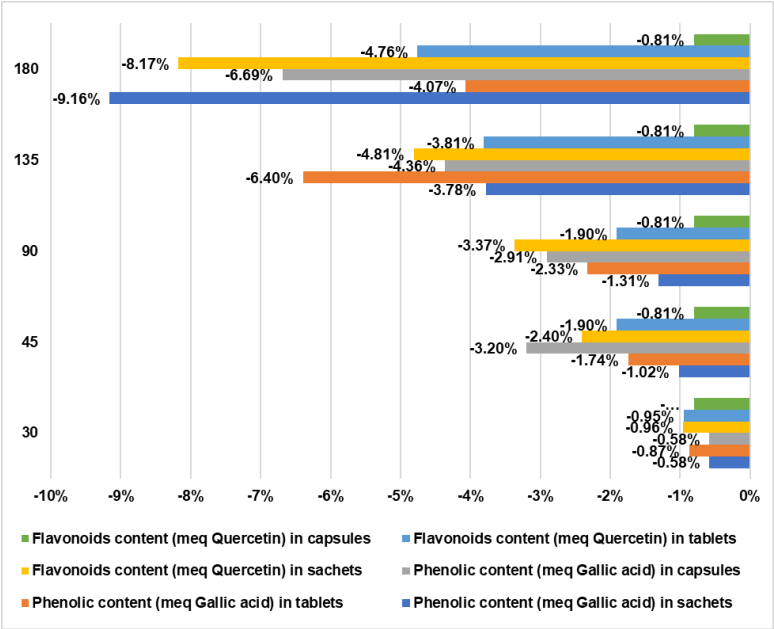

Fig. 3: Effect of accelerated stability study (40±2 °C and RH 75±5%) on the PEE phenolic and flavonoid contents in the three formulations

The samples underwent assessments to determine if their microbiological quality had changed during the storage period interms of total plate count, yeast mold count, and the presence of coliform bacteria, E-coli, or Salmonella. TPC values ranged from 3.1x10^2 to 3.5 x10^{2}CFU/g in sachets and, 3x10^2 to 3.2 x10^{2}CFU/g in tablets and capsules. The results presented in Tables 6, 7, and 8 show that TPC increased gradually during the 6 mo storage period for the three dosage forms. However, during the 180-day storage period, this increase was not statistically significant (p>0.01). Salmonella, E. Coli, and Coliform counts were not detected in any of the samples during the storage period in the current investigation. The samples revealed no count even on the zero-day of storage.

Table 6: Physical, chemical, and microbiological changes in sachets upon storage

Attributes	Test	Storage period (days)					
		0	30	45	90	135	180
	Real-time	Control	4 mo	6 mo	12 mo	18 mo	24 mo
Chemical Analysis	Moisture (%)	4.32±0.11	4.4±0.12	4.51±0.12	4.55±0.12	4.6±0.12	4.81±0.13
	pH	5.5±0.15	5.75±0.15	5.8±0.15	5.8±0.15	5.85±0.15	5.9±0.15
Bioactive compounds	α-amylase inhibitor activity (IU)	3030±80.17	3020±79.90	3010±79.64	3000±79.37	2950±78.05	2900±76.73
	Phenolic content (mg GA/g)	68.8±1.82	68.4±1.81	68.1±1.80	67.9±1.80	66.2±1.75	62.5±1.65
	Flavonoids content (mg QE/g)	20.8±0.55	20.6±0.55	20.3±0.54	20.1±0.53	19.8±0.52	19.1±0.51
	Chromium (III) content (µg)	24.8±0.66	24.9±0.66	24.8±0.66	24.6±0.65	24.5±0.65	24.5±0.65
	Equivalent to $CrPic_3$ content (µg)	200.67±5.03	201.47±5.03	200.67±5.03	199.06±4.99	198.26±4.97	198.26±4.97
Microbiological	Total plate count (TPC) (Log_{10} CUF/g)	3.1±0.08	3.15±0.08	3.2±0.08	3.3±0.09	3.4±0.09	3.5±0.09
	Yeast mold count (CFU/g)	<10^2	<10^2	<10^2	<10^2	<10^2	<10^2
	Coliform	Absent	Absent	Absent	Absent	Absent	Absent
	E. coli	Nil	Nil	Nil	Nil	Nil	Nil
	Salmonella	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

*From White kidney bean extract, **From Propolis ethanolic extract, ***From Chromium picolinate (Cr accountsfor 12.4% of weight $CrPic_3$), Results are represented as mean±SD (n=3)

Table 7: Physical, chemical, and microbiological changes in tablets upon storage

Attributes	Tests	Storage period (days)					
		0	30	45	90	135	180
	Real-time	Control	4 mo	6 mo	12 mo	18 mo	24 mo
Chemical analysis	Moisture (%)	4.3±0.11	4.32±0.11	4.35±0.12	4.4±0.12	4.45±0.12	4.5±0.12
	pH	6.53±0.17	6.55±0.17	6.6±0.17	6.71±0.18	6.8±0.18	6.8±0.18
Bioactive compounds	α-amylase inhibitor activity (IU)	1540±40.74	1530±40.48	1525±40.35	1520±40.22	1500±39.69	1480±39.16
	Phenolic content (mg GA/g)	34.4±0.91	34.1±0.90	33.8±0.89	33.6±0.89	32.2±0.85	33±0.87
	Flavonoids content (mg QE/g)	10.5±0.28	10.4±0.28	10.3±0.2	10.3±0.27	10.1±0.27	10±0.26
	Chromium (III) content (µg)	12.4±0.33	12.6±0.33	12.5±0.33	12.4±0.33	12.3±0.33	12.3±0.33
	Equivalent to $CrPic_3$ content (µg)	100±2.65	100.8±2.67	100.8±2.67	99.2±2.62	99.2±2.62	99.2±2.62
Microbiological	Total plate count (TPC) (Log_{10} CUF/g)	3±0.08	3.05±0.08	3.1±0.08	3.15±0.08	3.2±0.08	3.2±0.08
	Yeast mold count (CFU/g)	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	Coliform	Absent	Absent	Absent	Absent	Absent	Absent
	E. coli	Nil	Nil	Nil	Nil	Nil	Nil
	Salmonella	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

*From White kidney bean extract, **From Propolis ethanolic extract, ***From Chromium picolinate (Cr accountsfor 12.4% of weight $CrPic_3$), Results are represented as mean±SD (*n*=3)

Table 8: Physical, chemical, and microbiological changes in hard gelatin capsules upon storage

Attributes	Tests	Storage period (days)					
		0	30	45	90	135	180
	Real-time	Control	4 mo	6 mo	12 mo	18 mo	24 mo
Chemical Analysis	Moisture (%)	4.35±0.12	4.35±0.12	4±0.11	4.45±0.12	4.5±0.12	4.55±0.12
	pH	6.4±0.17	6.45±0.17	6.5±0.17	6.5±0.17	6.55±0.17	6.6±0.17
Bioactive compounds	α-amylase inhibitor activity (IU)	1530±40.48	1520±40.22	1510±39.95	1510±39.95	1500±39.69	1450±38.36
	Phenolic content (mg GA/g)	34.4±0.91	34.2±0.90	33.3±0.88	33.4±0.88	32.9±0.87	32.1±0.85
	Flavonoids content (mg QE/g)	10.4±0.28	10.3±0.27	10.3±0.27	10.5±0.28	10.2±0.27	9.77±0.26
	Chromium (III) content (µg)	12.4±0.33	12.5±0.33	12.5±0.33	12.3±0.33	12.3±0.33	12.3±0.33
	Equivalent to $CrPic_3$ content (µg)	100±2.65	101.6±2.69	100.8±2.67	100±2.65	99.2±2.62	99.2±2.62
Microbiological	Total plate count (TPC) (Log_{10} CUF/g)	3±0.08	3.05±0.08	3.1±0.08	3.15±0.08	3.2±0.08	3.2±0.08
	Yeast mold count (CFU/g)	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	Coliform	Absent	Absent	Absent	Absent	Absent	Absent
	E. coli	Nil	Nil	Nil	Nil	Nil	Nil
	Salmonella	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

*From White kidney bean extract, **From Propolis ethanolic extract, ***From Chromium picolinate (Cr accounts for 12.4% of weight $CrPic_3$), Results are represented as mean±SD (*n*=3)

DISCUSSION

Herbal medicine has grown exponentially in the past few years in both developed and developing nations due to its natural origins and low side effects. Numerous metabolic disorders, including insulin resistance, type 2 diabetes, dyslipidemia, hepatic steatosis, and cardiovascular diseases, have been related to obesity [55]. Dietary interventions are increasingly being used as a primary strategy in the battle against obesity and its related issues. Our present study serves as a practical application to our previous findings regarding the effectiveness of combining the bioactive substances from WKBE, PEE, and $CrPic_3$ on an obesity-induced rat model in comparison to their anti-obesity effects with normal and obese rats. The present study aimed to formulate different dosage forms combining these natural products, evaluate the formulated dosage forms, and assess their shelf-life in preparation for the next steps toward evaluating them in a clinical trial setting.

The composition of the suggested formulations were based on previous pre-clinical and clinical research investigations. While there is no set dosage for WKBE (*Phaseolus vulgaris L.*), clinical studies used doses ranging from 445-3,000 mg/day [23]. Thus, WKBE supplements can be found in capsule or powdered form with a daily dose of 2,000-3,000 mg for capsules or 4,500-6,000 mg from the powdered form to be taken before meals with water. Research on the recommended dosage of propolis is still ongoing, but clinical studies conducted on mice and humans have revealed that propolis and its components are generally well tolerated and safe, provided that they are not taken in very high doses [56]. In preclinical studies, propolis administration at doses of 200-500 mg/kg body weight/day did not result in toxic deaths in experimental animals and was reported as safe [57, 58]. The U. S. Food and Drug Administration (FDA) which develops the daily value (DV) for nutrients, indicated that the daily value for chromium is 35µg for adults and children age 4 or older [59].

A pharmaceutical excipient's flow property is an essential part of the formulation process. The proper excipients must be used in order for the powder to have the best possible flow property. A material's ability to flow and reorganize under compression is generally better when it has a higher bulk and tapped densities. A powder's bulk density provides an approximation of its flowability, whereas its tapped density indicates how well it can be packed into a small area through repeated tapping. In the present study, we observed that the powder of the three formulations had closer values of bulk and tapped density. Furthermore, powder flowability can also be predicted using Hausner's ratio, which is associated with interparticle friction. Good powder flowability was indicated by a Hausner's ratio of less than 1.25 for the powder of the three formulations. Another measure of powder flowability is Carr's index. Typically, Carr's index values of 5–15, 16–18, 19–21, 22–35, and 36–40 indicate excellent, good, fairly passable, poor, and very poor powder flowability, respectively [38]. In the present study, Carr's indexes of sachets, tablets, and capsules were found to be 14.04, 14.88, and 14.69, respectively. Thus sachets had better flowability than capsules followed by tablets. On the other hand, the angle of repose serves as an indirect method for determining powder flowability because of their relationship with interparticle cohesion. According to USP, Powders that have an angle of repose less than 30° are considered excellent, whereas powders with angle of repose between 31°-35° and 36°-40° demonstrate good and passable flow characteristics, respectively [37], while an angle of repose higher

than 65° denotes extremely poor flowability. The angle of repose of the three formulations was below 35°, suggesting a good flow property. As a result, we can determine that the powder form of the three formulations had good flow property by combining the values of different parameters to evaluate flow property.

To characterize the tablets, we measured several parameters according to the tablet pharmacopeia requirements, such as weight variation, hardness, thickness, friability, and disintegration time. The formulated tablets had a weight variation of±2.04%, with a standard deviation of less than 2%. Friability test which measures the ability to withstand physical force during transportation, showed a weight loss of 0.21%±0.03 and a hardness of 4.12±0.09 kg/cm^2. As per the guidelines set forth by the USP, oral tablets are required to have a minimum hardness of 40 N and a maximum weight loss of 1% [40-42]. Our findings indicated that the suggested formulation used to make the tablets dosage formula produced sufficiently hard tablets which comply with USP specifications. The disintegration apparatus was used to perform *in vitro* disintegration of the formulated tablets and capsules. All of the formulated tablets disintegrated in 5 min 10 sec, while capsules disintegrated in 4 min 30 sec, suggesting that both tablets and capsules had excellent disintegration properties.

Regulatory guidelines recommend accelerated stability studies on nutraceutical products to establish their shelf life. The formulated dosage forms were subjected to accelerated stability testing at 75-±5% RH and 40±2 °C temperature for a period of 6 mo. Control and stability samples were evaluated for chemical, bioactive ingredients, and microbiological attributes [43].

As hypothesized both chemical and bioactive attributes of dosage forms decreased gradually during the accelerated stability study. Chemical attributes included moisture and pH. The moisture content of dried products was identified as a crucial factor in the stability of dietary supplements. Increased moisture content causes high water activity, which encourages microbial growth and reduces shelf life. Moisture was determined not only to affect the stability of pharmaceutical preparations for long-term use but also it is an important factor that can adversely influence the powder characteristics. Since the ingredients used in the formulation and packaging of the product are hygroscopic, there is a possibility that the moisture content of the dietary supplements will increase during progressive storage. Our findings agreed with previous findings about the high moisture content but this increase was within the acceptable limits and did not affect the sensory or physical properties of the dosage forms [60-62].

pH is the measure of the acidity or alkalinity of a product and is among the key elements influencing stability. Careful monitoring and control of pH is critical to ensure efficacy, stability, and safety. Active ingredient degradation frequently happens via hydrolysis, which is influenced by the medium's pH. pH profiles are significant because they can help determine which inactive ingredients are best to use in the formulation and/or what pH range is best to achieve for the finished product [62]. Our observations showed that the initial pH of the three formulations was primarily affected by the ingredients in each dosage form. The initial pH of sachets was observed to be acidic at 5.5, which can be attributed to the presence of orange juice powder as a flavoring agent and citric acid as an acidity regulator, while the initial pH values of tablets and capsules were 6.53 and 6.4 due to the addition of menthol flavoring agent and calcium carbonate as acidity regulator which has an alkaline nature.

The stability of the WKBE was evaluated by assaying the alpha-amylase inhibition activity. Previous reports concluded that the activity of α-AI is influenced by various factors, such as temperature, pH, and various ions. Some studies indicated that a slightly acidic environment (pH range of 4.5–5.5) is the ideal range for inhibitory activity and can be almost inactive outside of this range. However, data showed several discrepancies regarding the optimal operational conditions for inhibition activity and indicated that more research was necessary to assess these interactions more precisely [63]. Our results reported a gradual non-significant (p>0.01) decrease in the α-amylase inhibitor activity of the WKBE, with the decrease being highest in capsules (5.23%), less in sachets (4.29%) and minimum in tablets (only 3.90 %) as compared to the 0 d values. While the decrease was non-significant (p>0.01) for sachets and tablets until 180 d, capsules showed a non-significant decrease (p>0.01) until 135 d when compared to the initial activity. A finding that contradicted the previously reported findings where the α-amylase inhibitor retained some of its activity within the tablets and capsules alkaline medium. However, the previous findings can explain the reason that the percentage of the decrease in activity was lower in sachets that had an acidic medium than the percentage of the decrease in tablets and capsules. Therefore, we can conclude that the pH can affect the rate of decreasing the α-amylase inhibitor activity rather than being inactive outside of the acidic range.

The stability of PEE after the 6 mo study period was determined according to the amounts of the total phenolic and flavonoid contents as its main compounds [64, 65]. The values of total phenolic and flavonoid contents in the dosage forms gradually decreased as a result of storage at 40±2 °C temperature and 75±5% RH. Although tablets were stable for 180 d, however, sachets and capsules were only stable until 135 d. In agreement with a previous study, propolis topical formulation showed both physical and functional stability during the period of study, at 25±2 °C in actual humidity and at 40±2 °C/70% RH for 360 d [66]. Other studies have shown that thermal processes mostly affect the phenolic and flavonoid stability in many dietary supplements. Where total phenolic compound was stable during storage for 180 d at 10 °C, a slight decrease of this stability was observed upon storage at 25 °C [67].

Similarly, our findings regarding the stability of chromium picolinate as judged from the level of chromium, demonstrated it to be stable for the whole period of the study in the three dosage forms under the accelerated stability study conditions. This finding agreed with multiple previous studies that showed that chromium-picolinate is a highly stable molecule with an over 95% recovery under forced degradation [68, 69].

The development and activity of microorganisms, particularly bacteria, is one of the main factors influencing how quickly a product deteriorates while being stored. The chemical makeup of the dietary supplement, moisture content, pH level, humidity, and storage temperature all affect how microorganisms grow. In our study, in agreement with previously reported studies, all samples showed a gradual non-significant (p>0.01) increase in TPC [55]. However, based on the TPC<100 and the Total Combined Yeasts/Molds Count (TYMC<10), all samples fulfilled the requirements for microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use microbiological safety of non-sterile oral preparations [70]. Our findings contradicted with some previous studies that found a decrease trend or no significant change in the TPC during a storage period of 6 mo [60, 71].

During the course of the study, no presence of Coliform, E-coli or Salmonella in the studied samples were observed. This suggested that appropriate hygienic measures were implemented throughout the process of preparation, packaging, and production. The samples, therefore found to be of good microbiological quality that has been maintained throughout the storage period.

CONCLUSION

Our findings indicated that combining the three anti-obesity active ingredients with the inactive ingredients suggested in this study can yield an acceptable dosage form with good flowability, hardness, friability, and disintegration time. According to the ICH guidelines, accelerated stability testing should be carried out for six months at 40 °C/75% RH, while long-term stability testing should be conducted at 25 °C/60% RH. If there is an apparent change at this point, the formulation should be tested at an intermediate temperature of 30 °C/75% RH. Our data revealed that the three formulated nutraceutical dosage forms had different stability statuses at different time points under the accelerated storage conditions of 40±2 °C temperature and 75±5% RH. Sachets and capsules showed that they can be stable until 135 d without appreciable changes in its chemical, active ingredients, and microbiological parameters. While the microbiological characteristics, active ingredients, and chemical content of the

tablets did not significantly decrease over a 180-day period. Based on this accelerated stability test, sachets and capsules may have up to 18 mo of real-time shelf-life, while tablets can be stable for up to 24 mo in real-time. This study will unquestionably build a way for more studies to characterize this anti-obesity nutraceutical blend and confirm the stability of these different natural products in real-time studies.

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AUTHORS CONTRIBUTIONS

Doaa Salaheldin Abdelfattah and Sammar Fathy Alhabal-conceptualization; Doaa Salaheldin Abdelfattah, Mohamed A. El-Nabarawi, Mervat A. Fouad, Aliaa N. ElMeshad-methodology. Doaa Salaheldin Abdelfattah and Sammar Fathy Alhabal-writing, editing and reviewing. Mohamed A. El-Nabarawi, Mervat A. Fouad, Aliaa N. ElMeshad-reviewing and supervision. Doaa Salaheldin Abdelfattah, Mohamed A. El-Nabarawi, Mervat A. Fouad, Aliaa N. ElMeshad and Sammar Fathy Elhabal-results interpretation, data analysis and discussion.

CONFLICT OF INTERESTS

Declared none

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