

Polyphenol content, antioxidant capacity and protein level effects of beetroot and carob-based pre-workout

Conteúdo de polifenóis, capacidade antioxidante e efeitos do nível de proteína do pré-treino à base de beterraba e alfarroba

Efectos del contenido en polifenoles, la capacidad antioxidante y el nivel de proteínas de los preentrenamientos a base de remolacha y algarroba

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ABSTRACT

The present study aimed to assess the effect of mixing beet root (*Beta vulgaris* L) and carob (*Ceratonia siliqua*) on the antioxidant capacity and the protein level in the pre-workout. We proceeded to use the distilled water to extract the pre-workout powder, Spectrophotometry technique was employed to assess the total phenol content and protein level using the Folin-Ciocalteu test, and Bradford method respectively, Flavonoids were quantified using UV-Visible spectrometry in the presence of aluminum trichloride (AlCl₃). For the antioxidant capacity, anti-radical activity (DPPH test), assay of total antioxidant capacity (CAT) and potassium ferricyanide reducing power test (FRAP) were used. It is observed that the yield of the aqueous extract was found to be 10%. The concentration of polyphenols in the aqueous extract was estimated at 224.8 ± 31.88 µg EAG/mg E, while the flavonoid content was measured at 1.84 ± 0.90 µg EQ/mg E. The IC₅₀ value obtained from the DPPH test was 313.07 µg/ml. Additionally, the CAT and FRAP assays indicated values of 55% and an EC₅₀ of 249.859 µg/ml, respectively. Furthermore, the extract contained a protein concentration of 11.90 ± 0.143 g per 100 g of dry weight. In conclusion, aqueous extract is high in phenol

and flavonoids, and demonstrated a significant antioxidant capacity. The high protein level makes it a great choice for athletes.

Keywords: beet root, carob, antioxidant capacity, aqueous extract, pre-workout

RESUMO

A capacidade antioxidante e o nível de proteína foram investigados em *Beta vulgaris L* (raiz de beterraba) e *Ceratonion siliqua* (alfarroba), duas plantas diferentes coletadas no leste da Argélia. Precisou-se a utilização da água destilada para extração do pó pré-treino, a técnica de espectrofotometria foi empregada para avaliar o teor de fenóis totais e o nível de proteína usando o teste de Folin-Ciocalteu e o método de Bradford, respectivamente, os flavonóides foram quantificados usando espectrometria UV-Visível na presença de tricloreto de alumínio (AlCl_3). Para a capacidade antioxidante foram utilizados: Atividade anti-radicalar (teste DPPH), Ensaio de capacidade antioxidante total (CAT) e Teste de poder redutor de ferricianeto de potássio (FRAP). Observa-se que o rendimento do extrato aquoso foi de 10%. A concentração de polifenóis no extrato aquoso foi estimada em $224,8 \pm 31,88 \mu\text{g EAG/mg E}$, enquanto o teor de flavonóides foi medido em $1,84 \pm 0,90 \mu\text{g EQ/mg E}$. O valor de IC_{50} obtido no teste DPPH foi de $313,07 \mu\text{g/ml}$. Além disso, os ensaios CAT e FRAP indicaram valores de 55% e uma CE_{50} de $249,859 \mu\text{g/ml}$, respectivamente. Além disso, o extrato continha uma concentração proteica de $11,90 \pm 0,143 \text{ g por } 100 \text{ g de peso seco}$. O que nos permitiu concluir que o extrato aquoso é rico em fenol e flavonóides, e demonstrou uma capacidade antioxidante significativa. O alto nível de proteína o torna uma ótima opção para atletas

Palavras-chave: raiz de beterraba, alfarroba, , capacidade antioxidante, extrato aquoso.

RESUMEN

Se investigó la capacidad antioxidante y el nivel de proteínas en *Beta vulgaris L* (raíz de remolacha) y *Ceratonion siliqua* (algarroba), dos plantas diferentes recolectadas en el este de Argelia, Este estudio tuvo como objetivo evaluar el efecto de la mezcla de raíz de remolacha y algarroba sobre la capacidad antioxidante y el nivel de proteína en el pre-entrenamiento. Se procedió a utilizar el agua destilada para extraer el polvo pre-entrenamiento, se empleó la técnica de espectrofotometría para evaluar el contenido total de fenol y el nivel de proteínas mediante la prueba de Folin - Ciocalteu y el método de Bradford respectivamente, los flavonoides se cuantificaron mediante espectrometría UV-Visible en presencia de tricloruro de aluminio (AlCl_3). Para la capacidad antioxidante se utilizó: Prueba de actividad antirradicales (DPPH), Ensayo de capacidad antioxidante total (CAT) y Prueba de poder reductor de ferricianuro de potasio (FRAP). Se observa que el rendimiento del extracto acuoso fue del 10%. La concentración de polifenoles en el extracto acuoso se estimó en $224,8 \pm 31,88 \mu\text{g EAG/mg E}$, mientras que el contenido de flavonoides se midió en $1,84 \pm 0,90 \mu\text{g EQ/mg E}$. El valor de IC_{50} obtenido de la prueba DPPH fue de $313,07 \mu\text{g/ml}$. Además, los ensayos CAT y FRAP indicaron valores de 55% y una CE_{50} de $249,859 \mu\text{g/ml}$, respectivamente. Además, el extracto contenía una concentración de proteína de $11,90 \pm 0,143 \text{ g por cada } 100 \text{ g de peso seco}$. Lo que nos permitió concluir que el extracto acuoso es alto en fenol y flavonoides, y demostró una importante capacidad antioxidante. El alto nivel de proteínas lo convierte en una excelente opción para los atletas.

Palabras clave: raíz de remolacha, algarroba, capacidad antioxidante, extracto acuoso.

1 INTRODUCTION

The use of dietary supplements has long been a widespread practice aimed at optimizing training outcomes and addressing the specific nutritional needs of athletes. These supplements, whether in the form of proteins, creatine, vitamins, or pre-workouts, are often integrated into athletes' routines with the goal of enhancing their endurance, strength, and recovery.

However, with a growing awareness of the importance of health and well-being, as well as increasing concerns about the potential side effects of chemical and synthetic products, there is a trend towards more natural and organic alternatives. This shift is particularly notable in the field of sports dietary supplements, where athletes are increasingly turning to options that offer both performance benefits and assurances regarding their composition and origin (Akhila et al., 2022).

Antioxidants serve as a crucial component of nutrition aimed at preventing or mitigation the effects of reactive oxygen and nitrogen species (RONS) that are produced during and following intense physical activities. Athletes, who seek to reduce oxidative stress, facilitate recovery and improve performance, have adopted antioxidant supplements (Neubauer and Yfanti, 2015), and those that are high in protein, which is important in the molecular mechanisms that facilitate muscle recovery and adaptation to training. It supplies the necessary amino acids to replace oxidative losses incurred during exercise (Wang et al., 2021).

Beetroot, scientifically known as *Beta vulgaris L.*, is commonly referred to as chuker, containing significant amounts of sugar while maintaining low calorie content (Khyati and Kirti, 2022), Beet root juice is notable for its high content of polyphenol and inorganic nitrate, can also influence oxygen utilization and improve blood circulation (Ceclu and Oana-Viorela, 2020). The carob is a hard-shelled fruit belongs to the legume family and is botanically called "*Ceratonia siliqua*" (Ali et al., 2023). The pods and seeds of carob are widely used as raw materialism various industries such as in food, pharmaceutical, and cosmetic sectors, as a natural source of polyphenols (Barracosa et al., 2007).

In the field of sportif supplements beet root and carob are widely known for their benefits and uses. Consuming beets has the potential to boost athletic performance through the enhancement of oxygen using and reduction in exhaustion time. Beets are rich in betalains, pigments that exhibit various anti-inflammatory properties (Akhila et al., 2022). Carob is known for its various health benefits, including anti-inflammatory, antimicrobial, anti-diarrheal, antioxidative, anti-ulcer, anti-constipation, and absorption-inhibitory activities in the gastrointestinal tract. These beneficial effects are attributed to the presence of bioactive compounds like phenolic acids, flavonoids, and tannins (Mansour et al., 2024). These compounds possess functional properties that contribute to health advantages such as anti-inflammatory, antioxidative, anti-aging, and anti-diabetic effects (Gaamouri et al., 2019 ; Berroukeche et al., 2024). However, no study has been done on the health properties of beet root and carob based pre-workout intended for athletes. Therefore, the purpose of the present study is to assess the polyphenol content, anti-oxidant capacity and protein level of aqueous extract from the pre-workout.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

Beets were purchased from the vegetable seller, as for carob it was acquired from the botanical merchant both was purchased at the same day (February 2024) in El-Eulma region, Algeria. After, beetroot and carob were washed, beet was peeled then dried using a draying oven and grounded into a fine powder using grinder. The dried powder was combined with 100 ml distilled water, heated for 20 min, filtered, dried and stored until use.

2.2 REAGENTS

Reagents used in the current study: sodium carbonate NaCO_3 Folin-Ciocalteu, Aluminum Trichloride, 2,2-Diphenyl-1-Picrylhydrazyl, Gallic Acid, Quercetin, Sulfuric Acid, Sodium Phosphate, Ammonium Molybdate, Ascorbic Acid, Iron Chloride, Coomassie Blue, Phosphate Buffer, Potassium Ferricyanide, Trichloroacetic Acid were from chemical company Sigma-Aldrich (Germany).

2.3 PHYTOCHEMICAL ASSESSMENT

2.3.1 Total phenolic content

Total phenolic content was estimated by the Folin–Ciocalteu method (Singleton and Rossi, 1965). Using a spectrophotometer, 200 μl of aqueous extract with a concentration of 1 mg/ml was combined with 1ml of 1:10 Folin-Ciocalteu reagent Following a four minute incubation period, 200 μl of 20% sodium carbonate (NaCO_3) was added, and then incubated for an additional 90 minutes at room temperature. The absorbance was recorded at 765 nm using a blank for reference. Gallic acid was used for the standard calibration curve and the results were expressed as microgram gallic acid equivalent ($\mu\text{g GAE}$)/mg extract.

2.3.2 Total flavonoids content

To determine the total flavonoids content, the aluminum chloride assay was used (Bahorun et al., 1996). Briefly, 1ml of the aqueous solution (1 mg/ml) was combined with the same volume of the aluminum trichloride solution (2%) and incubated for 30 minutes at room temperature. The absorbance at 430 nm was read in contrast to the blank sample consisting of 1 ml extract solution with 1 ml methanol without AlCl_3 . Quercetin was used for the standard calibration curve and the data were expressed as microgram quercetin equivalents ($\mu\text{g QE}$)/mg extract.

2.3.3 Antioxidant activity *in vitro*

2.3.3.1 Anti-radical activity (DPPH test)

The donation ability of electrons or hydrogen atoms of compounds was measured by the bleaching of a purple colored ethanol solution of DPPH (Blois, 1958). one ml of DPPH solution was added to 1 ml of aqueous extract at different concentrations. Following a 30 minute incubation period in darkness at room temperature, the absorbance was recorded at 517 nm using a blank for reference. The control was created by combining 1ml of ethanol with 1ml of DPPH solution. BHA was used as a reference at various concentrations (Boudoukha et al., 2019). The percentage of DPPH inhibition was determined using the following formula:

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$$

With: A₀: Absorbance of the negative control.
A₁: Absorbance of tested samples.

2.3.3.2 Assay of total antioxidant capacity (CAT)

The total antioxidant capacity of the extract was evaluated. 300 µl of extract with 3000 µl of phosphomolybdate mixture, consisting of sulfuric acid (0.6M), sodium phosphate (28 mM), and ammonium molybdate (4mM) was mixed in the test tubes. Tubes were then sealed and placed in a water bath at 95°C for 90 minutes. After cooling, the absorbances were measured at 695 nm against a blank solution (0.3 ml of methanol + 3 ml of reagent). The overall antioxidant capacity was quantified in terms of quercetin equivalents (300 µl of quercetin with 3000 µl of reagent) or ascorbic acid (300 µl of ascorbic acid with 3000 µl of reagent) used as control references, representing 100% of the total antioxidant capacity. The values of the total antioxidant capacities of the extract determined by the following equation (Ben Haj Koibaier et al., 2014):

$$\% \text{ inhibition} = (A_t / A_c) \times 100$$

A_t: the absorbance of the sample at 695 nm
A_c: absorbance of quercetin or ascorbic acid

2.3.3.3 Potassium ferricyanide reducing power test (FRAP)

In the present test, 500 µl of the extract at different concentrations were mixed with 1.25 ml of phosphate buffer (0.1 M; pH 6.6) and 1.25 ml potassium ferricyanide solution K₃Fe(CN)₆ to 1% (m/v), then incubated at 50°C for 20 minutes. After that, 1.25 ml trichloroacetic acid (TCA 10%) was added. Next, 1.25 ml aliquots from each tube were combined with 1.25 ml distilled water and

0.25 ml 0.1% of ethanolic FeCl_3 solution. Finally, measure the absorbance at 700 nm against a blank. Quercetin and BHA were used as reference standards at varying concentrations (Amarowicz and Pegg, 2019)

2.3.4 Protein quantification

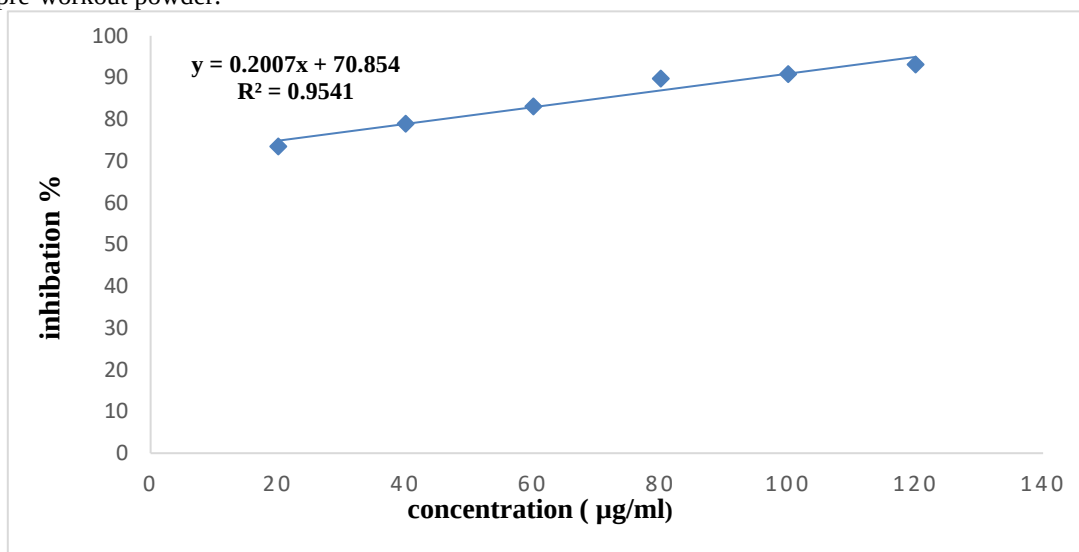
The determination method for total protein content relies on the protein binding ability of Coomassie brilliant blue dye G-250 (Bradford, 1976). Six standard solutions were prepared with different concentrations from a protein standard solution (BSA) with a mass concentration of 20 mg/ml. 50 μl of assay standard solution were added to 2.5 ml of Coomassie blue reagent and incubated for 30 minutes. The absorbance was measured at 540 nm against a blank. The protein concentration of the aqueous extract was assessed by comparing it with a standardized range of bovine serum albumin conducted under identical conditions.

3 RESULTS AND DISCUSSION

The spectrophotometric quantitative analysis of beetroot and carob-based pre-workout powder extract aiming to determine the content of total polyphenols, total flavonoids. These substances were chosen primarily due to the fact that the majority of antioxidant properties in plants are attributed to them. The outcomes of the total polyphenol analysis demonstrate that the level of polyphenols in the aqueous extract was $224.8 \pm 31.88 \mu\text{g GAE/mg E}$. Flavonoids were measured through a colorimetric method that relied on the utilization of aluminum trichloride (AlCl_3). Quercetin was selected as the standard substance. The outcomes were illustrated in a calibration curve. The total quantity of flavonoids was expressed in micrograms of quercetin equivalents per milligram of extract ($\mu\text{g QE/mg E}$). Based on the calibration curve, the estimation of the total concentration of flavonoids was $1.84 \pm 0.90 \mu\text{g QE/mg E}$.

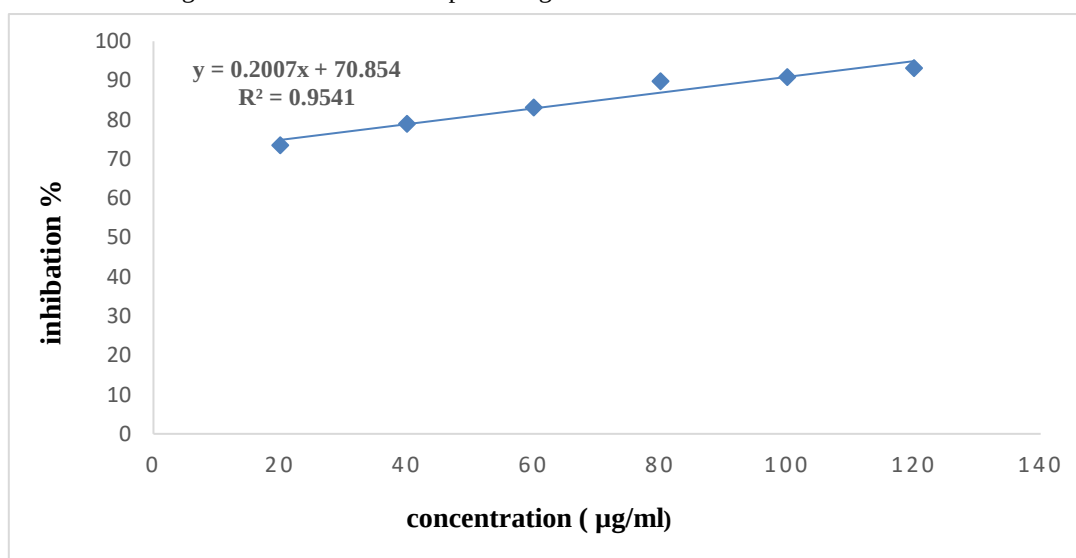
Anti-radical activity (DPPH test), According to the obtained result, the aqueous extract represents an IC_{50} value of $313.07 \mu\text{g/ml}$. The extract exhibited a moderate antioxidant activity compared to the standard BHA, which demonstrated a powerful antioxidant activity with an IC_{50} in the range of $11.90 \mu\text{g/ml}$ (Figure 1, 2), the level of inhibition increased with increasing concentration. The antiradical activity is correlated with the levels of polyphenols and flavonoids, which possess a strong ability to trap radicals. The polyphenols appear to be effective hydrogen donors to DPPH radicals due to their ideal chemical structure (Turkmen et al., 2007) and the presence of hydroxyl groups and the effects of conjugation and resonance of double bonds (Kyriacou et al., 2021).

Figure 1: DPPH inhibition percentage as a function of the concentration of the aqueous extract from beetroot and carob-based pre-workout powder.



Source: Chahra Boudoukha (2024)

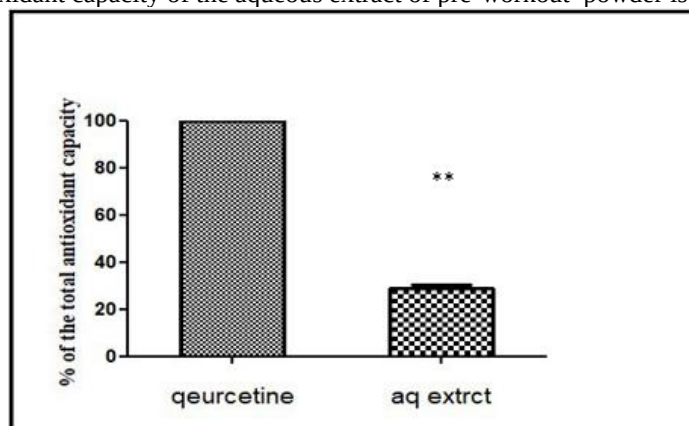
Figure 2: DPPH inhibition percentage as a function of BHA concentration.



Source: Chahra Boudoukha (2024)

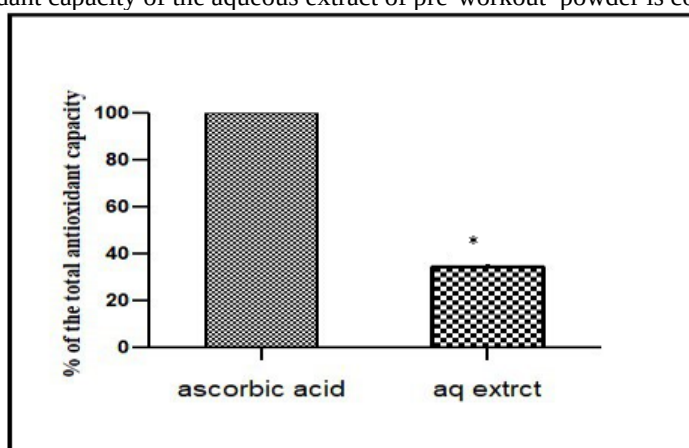
In order to assess the total antioxidant capacity, phosphomolybdate was used. Obtained results indicated that the studied pre-workout extract exhibited a moderate reducing activity of the phosphomolybdate reagent. The aqueous extract represented a percentage of the reducing activity of 55%. The results of the total antioxidant capacity (TAC) of pre-workout powder using the phosphomolybdate reagent showed that the aqueous extract exhibited significantly a lower percentage of reducing activity 28 %, compared to quercetin , which is considered to have 100% reducing activity (Figure 3). When compared to ascorbic acid, which also represents 100% reducing activity (Figure 4), the pre-workout powder extract presented a low percentage (33%) of antioxidant capacity.

Figure 3: Total antioxidant capacity of the aqueous extract of pre-workout powder is compared to quercetin



Source: Chahra Boudoukha (2024)

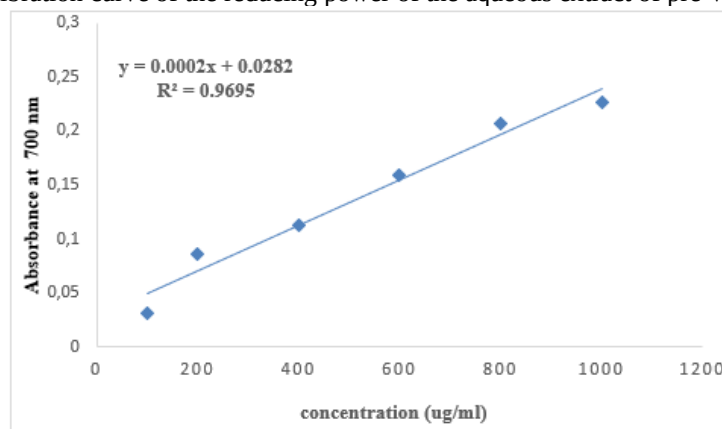
Figure 4: Total antioxidant capacity of the aqueous extract of pre-workout powder is compared to ascorbic acid.



Source: Chahra Boudoukha (2024)

For iron reducing power test, the evaluation of the antioxidant activity of pre-workout aqueous extract was conducted using the FRAP method. Obtained results showed that the ferric reducing antioxidant power of aqueous extract of pre-workout powder exhibited a significant reducing power (Figure 5) with an EC_{50} of 249.859 $\mu\text{g/ml}$.

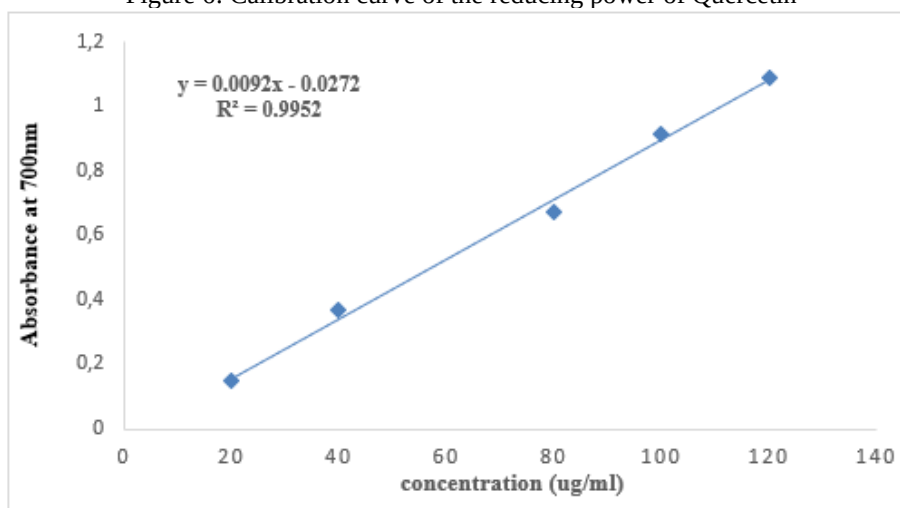
Figure 5: Calibration curve of the reducing power of the aqueous extract of pre-workout powder



Source: Chahra Boudoukha (2024)

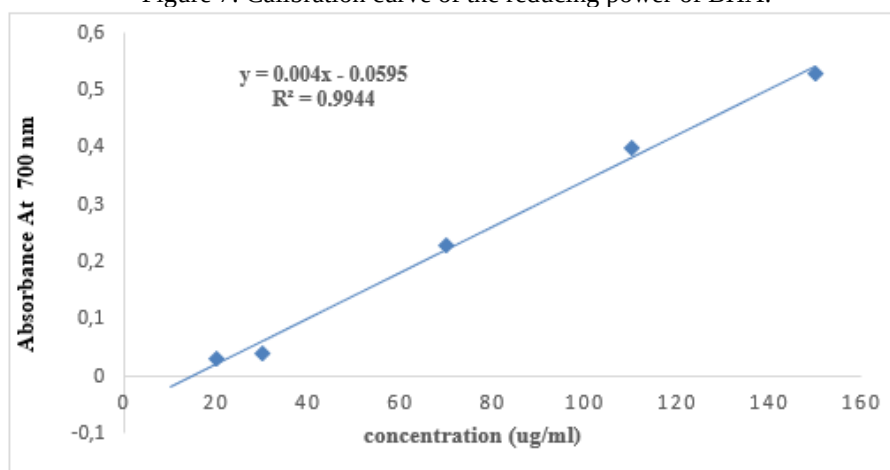
Furthermore, it is evident that the extract demonstrates a lower reducing capacity in comparison to quercetin and BHA, with an EC_{50} value of 25.51 $\mu\text{g/ml}$, and EC_{50} of 37.84 $\mu\text{g/ml}$ (Figure 6,7) respectively. According to the results, the evaluation of antioxidant activity using the three methods indicated that the aqueous extract of beetroot and carob-based pre-workout powder demonstrated an antioxidant activity that may be linked to its polyphenol and flavonoid content.

Figure 6: Calibration curve of the reducing power of Quercetin



Source: Chahra Boudoukha (2024)

Figure 7: Calibration curve of the reducing power of BHA.



Source: Chahra Boudoukha (2024)

The Bradford protein assay, also referred to as the Coomassie protein assay, was used to determine the protein level the aqueous extract of beetroot and carob-based pre-workout powder. The obtained result showed that the value of the protein content in the extract was determined to be 11.90 ± 0.143 g protein per 100 g dry weight. Our findings are slightly higher than the protein content of carob pods and beet root powder, which was measured at 4.45 ± 0.40 and 5.974 ± 0.001 g protein per 100 g dry weight by Ayaz et al., (2009) and Martha et al., (2020), respectively.

4 CONCLUSION

The present study indicated that the aqueous extract of beetroot and carob-based pre-workout powder exhibited significant bioactive properties, offering a natural and potentially safer alternative to conventional pre-workout supplements. The analysis of total polyphenol and flavonoid content revealed notable concentrations of antioxidant compounds in beetroot and carob extract. These compounds play a crucial role in neutralizing free radicals, as evidenced by the *in vitro* antioxidant activity measured. The anti-radical activity of the extract is inversely proportional to the IC₅₀ value, indicating high efficacy at relatively low concentrations. Additionally, the ferric reducing antioxidant power (FRAP) assays showed a notable reducing capacity, comparable to that of quercetin, a well-known antioxidant. The phosphomolybdate assay revealed that the total antioxidant capacity of the extract, expressed in quercetin and ascorbic acid equivalents, is also substantial. These results not only highlight the efficacy of beetroot and carob extract as antioxidant agents but also their potential to enhance athletic performance in a natural way. By offering an alternative to chemical and synthetic products, this extract can address the growing health and wellness concerns of athletes.

For future research, it would be pertinent to explore the underlying molecular mechanisms of the observed effects, as well as to conduct clinical trials to confirm the efficacy and safety of the extract in human. Moreover, investigating the potential synergy between beetroot and carob extract and other natural ingredients could open new perspectives in the development of innovative pre-workout supplements.

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