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Phytochemical Analysis and Antioxidant Potential of Gel Plus EXO As a Food Supplement

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Abstract— **The modern fast-paced lifestyle, characterized by long working hours, high stress levels, poor diet, and lack of physical activity, has led to an increase in oxidative stress. This phenomenon is a significant external factor contributing to the development of various chronic diseases such as cancer, type 2 diabetes, hypertension, cardiovascular diseases, and neurological and gastrointestinal disorders. Oxidative stress occurs when there is an imbalance between the production of free radicals and the body's ability to neutralize them with antioxidants.**

To counteract the harmful effects of oxidative stress, functional foods and nutraceutical supplements, enriched with antioxidants, have gained popularity. These products provide not only essential nutrients but also additional health benefits, such as disease prevention. Antioxidants, which are specific types of nutraceuticals, play a key role in neutralizing free radicals and protecting cells from oxidative damage.

Polyphenols, a group of bioactive compounds, are among the most studied antioxidants due to their health benefits. Found in plant-based foods like fruits, vegetables, tea, and red wine, polyphenols include flavonoids such as quercetin, gallic acid, and epigallocatechin gallate. These compounds have been linked to reduced risks of various diseases.

This study aimed to quantify the phenol and flavonoid content in a commercial product, Gel Plus EXO, a supplement containing antioxidants. The antioxidant potential of this product was evaluated using three spectrophotometric methods: DPPH, ABTS, and FRAP, which assess the product's ability to neutralize free radicals and protect against oxidative stress.

The results indicated that the content of antioxidant compounds in the product is around 30%, considering this potential as intermediate and, finally, it is considered a good complement to a healthy diet.

Keywords— **food science; nutrition; flavonoid, phenols, free radicals; energy drink.**

I. INTRODUCTION

The modern lifestyle, characterized by a fast-paced rhythm, long working hours, high exposure to stress, poor diet, and lack of physical activity, has resulted in harmful consequences for human health. One of the most significant effects of this lifestyle is the increase in oxidative stress. This phenomenon is considered one of the most influential external factors in the development of various chronic and degenerative diseases. It has been shown that oxidative stress plays a crucial role in the onset of serious pathologies such as cancer, type 2 diabetes, hypertension, cardiovascular diseases, as well as neurological and gastrointestinal disorders [1].

Oxidative stress is defined as an imbalance between the production of free radicals and the body's ability to counteract their effects through antioxidants. Free radicals are highly reactive molecules that, in excess, can damage cells, tissues, and molecular structures such as lipids, proteins, and DNA. Antioxidants, on the other hand, are compounds capable of neutralizing these free radicals, preventing or minimizing oxidative damage, and thereby protecting the body from various diseases [2].

To mitigate the harmful effects of oxidative stress, functional foods and supplements enriched with nutraceuticals have been used. These products not only provide essential nutrients but can also exert additional health benefits, contributing to disease prevention. Nutraceuticals encompass a wide range of bioactive substances, among which antioxidants play a prominent role due to their ability to neutralize free radicals and protect cells from oxidative damage [3].

Among the most studied antioxidants are polyphenols, bioactive compounds that have demonstrated a wide variety of health benefits. These compounds are found in many plant-based foods, such as fruits, vegetables, tea, red wine, and certain grains and nuts. Polyphenols include subgroups such as flavonoids, which possess potent antioxidant properties. Some of the most notable flavonoids include gallic acid, quercetin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate, and (-)-epicatechin, which are found in various nutraceutical products and have been associated with reduced risks of cardiovascular diseases, cancer, and neurodegenerative disorders [4].

Given the growing interest in the beneficial effects of antioxidants, the main objective of this study was to quantify the content of phenols and flavonoids in a commercial product called Gel Plus EXO, a dietary supplement commonly used for its antioxidant properties [5]. Oxidative stress, caused by factors such as the modern lifestyle, is an important trigger for numerous diseases. Antioxidants found in nutraceutical products, such as polyphenols, play a crucial role in mitigating this damage [6]. The study of products like Gel Plus EXO can

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provide valuable information about their potential to combat oxidative stress and, therefore, help prevent various associated diseases.

This study aimed to determine the antioxidant potential of this product by applying three spectrophotometric methods, which allow for the assessment of its ability to neutralize free radicals and provide an objective measure of its effectiveness as a nutraceutical supplement.

The spectrophotometric methods used include measuring antioxidant capacity through the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, the ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) assay, and the DMPD (N, N-dimethyl-p-phenylenediamine dihydrochloride) assay. These tests help evaluate different aspects of antioxidant activity, such as free radical scavenging ability and reduction of reactive oxygen species, contributing to a comprehensive assessment of the antioxidant activity of the product.

II. MATERIALS AND METHODS

A. Sample and reagets

The Gel Plus EXO (Purple Grape Flavor) product sample was purchased at a local food supplement and homeopathic medicine store. One serving of the nutritional supplement consists of one 28g sachet.

Reagents: standards quercetin, and gallic acid were brand Meyer (CDMX, Mexico), Folin-Ciocalteau was brand Golden Bell (Quezon, Philippines), sodium carbonate (Na_2CO_3), 2,2'-azino-bis-(3-ethylbenzothiazolin)-6-sulfonic acid (ABTS), 2,2-diphenyl-1- picrylhydrazyl (DPPH) and N, N-dimethyl-p-phenylenediamine dihydrochloride (DMPD) were brand Sigma-Aldrich (Dorset, UK).

B. Sample preparation

For the phytochemical screening and antioxidant activity quantification, the crude extract was prepared from 1 mL of sample and 10 mL of 4% acetic acid in acetonitrile (solution A) was added and kept in a water bath at 30 °C with constant stirring for 1 hour; the sample was made up to 25 mL with solution A, this mixture was filtered through a nylon filter with a 0.2 µm pore (Acrodisc®). The test sample was stored in amber bottles at room temperature [7].

C. Flavonoid content

The aluminum chloride colorimetric method was applied to determine the presence of flavonoids [8]. 0.5 mL of the crude extract were added with 1.5 mL of 96% (v/v) ethanol, 0.1 mL of 10% (w/v) $AlCl_3$ solution, 0.1 mL of 1 M CH_3COOK solution and 2.8 mL of distilled water. The mixture was incubated for 30 min at room temperature. Absorbance was read on a Genesys 10s spectrophotometer Thermo-Fisher Scientific (Florida, USA) at a wavelength of 415 nm. For the quantification of flavonoids, a standard curve was made based on the flavonol quercetin [9]. The results were expressed in mg equivalents of quercetin per 100 g of fresh weight (mg EQ 100 g-1 f.w.).

D. Poliphenols determination

The spectrophotometric method developed by Folin and Ciocalteau [10] was used, which is based on the reducing nature of the Folin-Ciocalteau reagent. To 0.5 mL of the crude extract, 0.5 mL of the Folin-Ciocalteu reagent (0.2 N) and 4 mL of Na_2CO_3 (0.7 N) were added. The mixture was vortexed and incubated in the dark at room temperature for 2 hours. After the incubation period, absorbance was read on a Genesys 10s spectrophotometer Thermo-Fisher Scientific (Florida, USA) at a wavelength of 765 nm. Water was used as blank, and gallic acid as positive control. The results were expressed as mg of gallic acid per g of crude extract [11].

E. DPPH method

For the antioxidant activity determination, a spectrophotometric method described by [12] was used. This method consists of measuring the absorbency of the $DPPH^+$ radical at a wavelength of 515 nm. A solution 0.1 mM of $DPPH^+$ was dissolved with 80% (w/v) of aqueous methanol. Each test consisted of 2.9 mL of the free radical solution and 0.1 mL of the sample or reference. The sample was shaken and incubated at room temperature and in the dark for 30 min and 60 min. Methanol was used as blank, quercetin and gallic acid as references. The results are expressed as antioxidant activity equivalent to the reference.

F. ABTS method

The spectrophotometric method described by [13] was used. This method is based on the oxidation and reduction of the $ABTS^+$ free radical in the presence of potassium persulfate ($K_2S_2O_8$). The $ABTS^+$ free radical was obtained by mixing ABTS 7 mM with $K_2S_2O_8$ 2.45 mM and allowing the solution to react for 16 hours at room temperature and in the dark. The $ABTS^+$ radical was diluted with ethanol until obtaining an absorbance value of 0.70 ± 0.1 read at 734 nm. For the test, 1 mL of the $ABTS^+$ free radical was used with 10 µL of the test sample or the reference solution. The absorbance of this mixture was determined after 7 minutes of reaction at a wavelength of 734 nm. Ethanol was used as blank, quercetin and gallic acid as references. The results are expressed as antioxidant activity equivalent to the reference.

G. DMPD method

The method proposed by [14] was used, which is based on the reduction of the free radical by the action of the transfer of hydrogen atoms from the antioxidant compound. A solution 100 mM of $DMPD^+$ free radical was prepared, this solution was diluted with a buffer solution of acetic acid/ sodium acetate (pH = 5.25) and 0.05 M $FeCl_3*6H_2O$, until obtaining an absorbance of 0.9 ± 0.1 at a wavelength of 506 nm. For the test, 1 mL of the free radical and 50 µL of the sample or reference were added and mixed in a tube. The absorbance of this sample was determined after 10 min of reaction at a wavelength of 506 nm. Acetate buffer was used as blank, quercetin and gallic acid as references. The results are expressed as antioxidant activity equivalent to the reference.

H. Data analysis

Each analysis was carried out in quadruplicate and analyzed using a one-way analysis of variance (ANOVA) and Tukey's mean comparison test ($p<0.5$). Descriptive statistics were conducted using SAS System for Windows version 9.0 (SAS Institute, NC) [15].

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III. RESULTS AND DISCUSSION

The results obtained in the determination of flavonoids and phenols are presented in Fig. 1 where high values of flavonoids and phenols are observed.

Some studies carried out with natural grapes (*Vitis vinifera* L.) where both the phytochemical profile and the antioxidant activity were studied show that it exists, although they depend on the concentration used, the reaction duration and the dose-response analysis [16]. Around this topic, the authors suggest the potential of this sample to strengthen the immune system [17], to control blood pressure [16], antitumoral activity [18], helps improve eye problems [19] and other biological activities.

According to literature, the presence of polyphenols and flavonoids in a sample are indicators of the probable antioxidant activity measured *in vitro* with oxidation-reduction reactions of free radicals of chemical origin [19, 20, 21, 22].

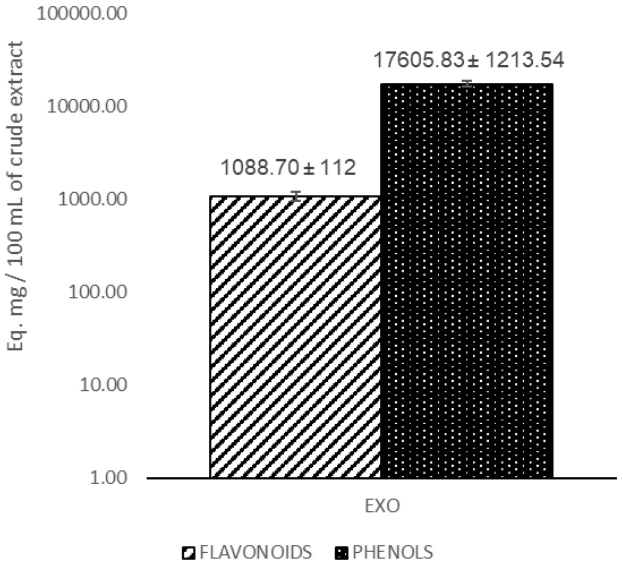

Figure 1. Total flavonoid and polyphenol content in the Gel Plus EXO commercial sample. Flavonoids and polyphenols are reported as Eq. mg of reference /100 mL of sample. Values are presented as mean ± standard deviation, n=4.

In the present work, considerable values were obtained in the determination of polyphenols. It should be noted that these values are similar than those of fresh fruits and natural juices [18]. This phenomenon is probably because it is a supplement that contains various components of chemical origin which provide stability over time. This is how [21] reinforce this information, since they reported several polymerized phenolic compounds that increase their polyphenol content and consequently their antioxidant activity by up to 350%. Among the compounds that were studied, the following stand out biocompatible polymers such as chitosan, poly(allylamine), poly(ε-lysine). Otherwise, the results reported by [22] who published values of the content of total polyphenols with reference to gallic acid of various red fruits resulted in values between 150 and 460 mg/100mL of acetonic extract.

In a study, is reported the flavonoids and polyphenols, also the antioxidant capacity of some food supplements, including some that contain grapes, such as the case of the sample analyzed in this work, and within the results it was observed that the antioxidant activity of the samples is dependent on the concentration analyzed, and its importance is denoted since the grape extracts stand out from the rest of the samples that mainly contain citrus fruits and some vegetables such as tomato and ginger [20]. It is important to highlight that the samples where the activity was higher are a mixture of several different components, which suggests a synergy between the compounds with antioxidant potential contained.

When carrying out the study and analysis of results with respect to antioxidant activity, it was observed that it is at values around 30% (Table 1), which indicates moderate activity. It should be noted that only a significant difference was observed between the methodologies, with DMPD and DPPH being those with the highest results. However, it must be remembered that these are colorimetric tests where chemical oxidation depends on various factors and not only on the sample analyzed. These data indicate that the free radical inhibitory compounds in the analyzed sample could be related to molecules like gallic acid or quercetin [21].

TABLE I. ANTIOXIDANT ACTIVITY QUANTIFIED IN THE GEL PLUS EXO

Method	*Standard* [z]	*Antioxidant activity*	*Inhibition (%)*
ABTS[Z]	Galic acid	1654.80±60.45	27.84±0.85[b]
	Quercetin	4693.40±155.65	
DMPD[Z]	Galic acid	4256.09±93.61	31.95±0.70[a]
	Quercetin	17694.79±282.1	
DPPH[Z]	Galic acid	2763.85±122.23	33.40 ± 1.39[a]
	Quercetin	2998.08±131.50	

[Z] $AAE_{Ref.}$ (Antioxidant activity equivalent to reference) expressed in mg of reference/100 mL of sample. Values are presented as mean ± standard deviation), n=4. Evaluated concentration: 100 mL/L. [a,b,c] Means with the same letter by columns are statistically equal (P ≤ 0.05).

They could even relate to each other to form a synergistic antioxidant action. In a study on the antioxidant activity of grape juice, results were reported from 16 to 60% of antioxidant activity in which it is likely that a mixture of components within the juice is generating the inhibition of the free radical DPPH, in addition to concentration [18]. For their part, the [20] working group agrees that the percentages of free radical inhibition reach up to 60% when the concentration of the sample increases.

It is important to consider that the result of antioxidant activity is quantified based on a reference chemical compound, which can generate different results for the same sample. In a study where the antioxidant activity of synthetic compounds was quantified, it was determined that the antioxidant activity was around 26%, measured by both the DPPH and ABTS methods. However, in the case of quercetin it was indicated that pH is a factor to consider, since the inhibition of the DPPH free radical increases in a range of 4.5 to 6.5 [21].

An important fact of the sample analyzed, Gel plus EXO, is that the antioxidant compounds contained have greater stability over time in relation to natural juices, most likely because they are of synthetic origin [23].

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And this fact is reported in several research works where the intake of antioxidant compounds, both natural and from food supplements and supplements, is suggested to prevent diseases caused by free radicals. For this reason, the sample analyzed provides the consumer with enough antioxidant compounds that, together with an adequate diet and a healthy life, can be beneficial for humans.

IV. CONCLUSIONS

The product Gel Plus EXO is a commercially manufactured supplement widely promoted for consumption among the population due to the health benefits it claims to offer, according to the information provided on its packaging. It is highlighted as a rich source of antioxidants and is presented as an effective option for combating the effects of oxidative stress, improving overall health, and preventing various diseases associated with cellular aging, such as cardiovascular diseases, cancer, and neurodegenerative disorders. However, upon analyzing the results obtained through scientific testing, it is observed that the free radical inhibition percentage exhibited by the product is relatively intermediate, reaching 27 - 33%. This result suggests that, although the supplement contains certain antioxidant compounds, its ability to neutralize free radicals is not as high as one might expect based on the marketing claims.

It is important to highlight that, despite Gel Plus EXO showing a moderate inhibition percentage, this does not necessarily mean it lacks value as a supplement. The antioxidants in the product can still play a role in protecting against oxidative damage, but the benefits may not be as significant as advertised. Therefore, the consumption of this supplement is recommended as an additional complement to a balanced diet and a healthy lifestyle, with the aim of increasing the intake of antioxidant compounds that, combined with other dietary and health factors, may contribute to improving general well-being and preventing diseases associated with oxidative stress. It is crucial, however, for consumers to understand that dietary supplements should be used as support and not as a sole solution for health issues, and that the key to a healthy life lies in a varied diet, regular exercise, and proper stress management.

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