REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.11.e1585

ISSN 2007-3380

Original article / Artículo original

Antioxidant and Biofunctional Potential of Botanical-based Dietary Supplements Through *In vitro* and *In Silico* Analysis

Potencial antioxidante y Biofuncional de Suplementos Alimenticios de Origen Botánico Mediante Análisis *In vitro* e *In Silico*

Jiménez-Ortega, L.A., López-Romero, B.J., Heredia-Batiz, J.M., Bastidas-Bastidas, P.J., Heredia, J. Basilio*

Centro de Investigación en Alimentación y Desarrollo, AC, Carretera a Eldorado Km 5.5, Campo el Diez, 80110, Culiacán, Sinaloa, México.

Please cite this article as/Como citar este artículo: Jiménez-Ortega, L.A., López-Romero, B.J., Heredia-Batiz, J.M., Bastidas-Bastidas, P.J., Heredia, J. B. (2024). Antioxidant and Biofunctional Potential of Botanical-Based Dietary Supplements Through *In Vitro* and *In Silico* Analysis. *Revista Bio Ciencias*, 11, e1585.
https://doi.org/10.15741/revbio.11.e1585

Article Info/Información del artículo
Received/Recibido: October 09th 2023.
Accepted/Aceptado: January 24th 2024.
Available on line/Publicado: February 23th 2024.

ABSTRACT

Dietary supplements can help minimize the appearance of pathologies; however, biofunctional and toxicological studies are necessary to determine efficacy and toxicity. The objective of the present investigation was to evaluate the antioxidant and biofunctional potential of commercial food supplements made from herbal extracts, medicinal plants, and fruits such as blueberry, bitter melon, citrus, *Gingko biloba*, bearberry, goyi and joconostle, among others. For this, the antioxidant capacity, total reducing capacity, total flavonoids, and toxicity *in vitro* in a model of *Artemia salina* were determined. In addition, volatile organic compounds were identified by gas chromatography coupled to mass spectrometry, and their biofunctionality was evaluated through bioinformatics studies. The supplements showed antioxidant capacity due to their content of compounds with total reducing capacity, polyphenols, flavonoids, terpenes, fatty acids (hexadecenoic acid), and phytosterols. The product with the highest antioxidant activity did not present toxicity in the *A. salina* model. The bioinformatic study showed that the possible targets in the body are related to inhibiting the pathogenesis of some chronic-degenerative diseases with the highest incidence and prevalence in Mexico, such as diabetes and hypertension.

KEY WORDS: Food safety, biological activity, phytochemicals, herbalist, flavonoids.

***Corresponding Author:**

Heredia, J. Basilio. Centro de Investigación en Alimentación y Desarrollo, AC, Carretera a Eldorado Km 5.5, Campo el Diez, 80110, Culiacán, Sinaloa, México. E-mail: jbheredia@ciad.mx. Teléfono: (667)480-6950 https://www.ciad.mx/

Revista Bio ciencias
ISSN 2007-3380

RESUMEN

Los suplementos alimenticios pueden coadyuvar a minimizar la aparición de patologías, sin embargo, son necesarios estudios biofuncionales y toxicológicos para determinar su eficacia y toxicidad. El objetivo de la presente investigación fue evaluar el potencial antioxidante y biofuncional de suplementos alimenticios, hechos a base de extractos botánicos, plantas medicinales y frutos como arándano, melón amargo, cítricos, *Gingko biloba*, gayuba, goyi y joconostle, entre otros. Para esto se determinó la capacidad antioxidante, capacidad reductora total, contenido de flavonoides totales y toxicidad mediante el bioensayo de *Artemia salina*. Además, se identificaron los compuestos orgánicos volátiles mediante cromatografía de gases acoplado a espectrometría de masas y se evaluó su biofuncionalidad mediante estudios bioinformáticos. Los suplementos mostraron capacidad antioxidante debido al contenido de compuestos con capacidad reductora total, como polifenoles, flavonoides, terpenos, ácidos grasos (ácido hexadecanoico) y fitoesteroles. El producto con mayor actividad antioxidante no presentó toxicidad en el modelo de *A. salina*. El estudio bioinformático arrojó que los posibles objetivos de los compuestos en el organismo se encuentran relacionados con la inhibición de la patogénesis de algunas de las enfermedades crónico-degenerativas con mayor incidencia y prevalencia en México como la diabetes e hipertensión.

PALABRAS CLAVE : Inocuidad, funcionalidad biológica, fitoquímicos, herbolaria, flavonoides.

Introduction

According to the United States Drug Administration (FDA), dietary supplements are those products that are ingested orally and contain a dietary ingredient. These may include vitamins, minerals, amino acids, herbal, and botanical extracts among other compounds that can supplement the daily diet (FDA, 2022a). The demand for these products intensified considerably due to the arrival of SARS-CoV-2. Hamulka *et al*. (2021) point out that during the COVID-19 outbreak in 2020, the population's interest in inquiring about dietary supplements that help improve the immune system increased. The main compounds searched on the web were vitamins (C and D) and medicinal plant extracts (garlic, ginger, and turmeric). Dietary supplements can help strengthen the immune system and treat and prevent the main non-communicable diseases in the world, such as cardiovascular and respiratory diseases, cancer, diabetes, and obesity, among others (Bruins *et al*., 2019; OMS, 2022).

Revista Bio ciencias
ISSN 2007-3380

Likewise, the appearance of chronic-degenerative diseases is closely associated with oxidative stress (O S), which can be defined as an imbalance between antioxidants and oxidants such as free radicals (FR) and reactive oxygen species (ROS), which cause damage to macromolecules, and trigger a cascade of signaling involved with pro-inflammatory factors, which subsequently give rise to a wide variety of non-communicable diseases (Byrne *et al*., 2021; Charlton *et al*., 2021; Pizzino *et al*., 2017; Ramachandra *et al*., 2021; Sabbatino *et al*., 2021; Sies, 2020). Therefore, a balanced diet, rich in phytochemicals and nutraceuticals, can minimize the impact of OS (Prior & Cao, 2000), since it has been observed that they have the capacity to provide antioxidant activity against FR and ROS, in addition to exerting anti-inflammatory (Leyva-López *et al*., 2016), anticancer (Criollo-Mendoza *et al*., 2022), hypoglycemic (Gutiérrez-Grijalva *et al*., 2022), and hypocholesterolemic activities (Das *et al*.,2022), among others.

The dietary supplement industry and market are constantly changing as consumers increasingly demand products that meet quality and safety standards and also satisfy the nutritional and functional needs sought. The dietary supplements with the greatest demand are those that contain vitamins, minerals, amino acids, enzymes, and phytochemicals. These are presented individually or as mixtures, which can come from herbal extracts from chemical synthesis or by isolating and purifying the compounds of interest (Hassan *et al*., 2020; Lordan, 2021).

The mixture of phytochemicals and extracts from plants, medicinal fruits, and other nutraceuticals can enhance its biological activity, creating a synergy between the compounds. However, on some occasions, they are made with ethnopharmacological knowledge, lacking tests that guarantee their functionality and safety, so it is important to inquire about these products. According to Brzezicha *et al*. (2021), it is estimated that more than 80 % of the world's population uses some type of dietary supplement or herbal remedy, so it is necessary to study its safety and effectiveness. For this, study models have been proposed in cell lines, microorganisms, and living organisms. In the present work, the use of the biological model of *Artemia salina* was suggested since it has been widely used to evaluate the toxicity of natural compounds. It is economical and reproducible and allows the possible toxicity of the compounds to be elucidated before an evaluation in cell lines or murine models (Karchesy *et al*., 2016; Wanyoike *et al*., 2004). For this study, dietary supplements made from extracts of botanical origin, such as medicinal plants and edible fruits abundant in phytochemicals such as flavonoids, phenolic acids, terpenes, phytosterols, fatty acids, vitamins, and minerals with antioxidant, anti-inflammatory, antidiabetic, and antihypercholesterolemic therapeutic indications, were selected, so this research aimed to evaluate the antioxidant, biofunctional and toxicological potential of dietary supplements marketed in Mexico, made from medicinal plants, botanical extracts and edible fruits.

Materials and Methods

Ten dietary supplements based on medicinal plants, natural extracts, and edible fruits of different chemical nature were analyzed (Table 1). They were purchased in stores that commercializes dietary supplements in Culiacan, Sinaloa, Mexico. Green tea leaves (*Camellia sinensis* L.) were used as a reference for the antioxidant and toxicological assays.

Revista Bio ciencias
ISSN 2007-3380

Table 1. Characteristics of the dietary supplements analyzed.

Code	Ingredients	Therapeutic indication**	Serving/day	Weight (mg)*	Dose/ day (mg)	Origin
Suppl.1	Bioflavonoid extract from *Citrus limon, C. sinensis, C. paradisi, C. reticulata, C. aurantifolia,* and *Citrus sinensis* peel hesperidin extract.	Antioxidant	1-3 capsules	650	1,950	Canada
Suppl.2	Standardized concentrate of *Vaccinium corymbosum* 36:1 and anthocyanins.	Antioxidant	1-3 capsules	512.5	1,537.5	Canada
Suppl.3	Dry extract of: *C. longa, C. sinensis, Arthrospira platensis*, *L. ododes, R. officinalis, S. hispanica, T. officinale, R. rosea, A. muricata, E. oleracea, M. citrifolia, G. mangostana, A. propinquus, Aphanizomenon flos-aquae*, seed of *V. vinifera, P. africana*. Mix of amino acids, carotenoids, vitamins, and minerals.	Antioxidant	1 tablet	1,200	1,200	Mexico
Suppl.4	Dry extract of *G. biloba* leaves. Vitamins and minerals.	Mental focus	1-2 tablets	445	890	Mexico
Suppl.5	*M. charantia* extract, of which 10% are bitter phytochemicals such as charantin	Glycemic control	1 capsule	500	500	EUA
Suppl.6	A mixture of standardized phytosterols (β-sitosterol, campesterol, stigmasterol). *G. monogyna* extract, *A. hippocastanum, V. vinifera, V. myrtillus, A. sativum, C. annuum, C. sinensis, P. cuspidatum* root resveratrol. Mix of vitamins and minerals.	Cardiovascular health	3 tablets	1,003.5	3,010.5	EUA

Revista Bio ciencias
ISSN 2007-3380

Continuation

Table 1. Characteristics of the dietary supplements analyzed.

Code	Ingredients	Therapeutic indication**	Serving/day	Weight (mg)*	Dose/ day (mg)	Origin
Suppl.7	Powder of *O. ficcus, O. joconostle, A. sativum, C. sinensis, C. longa, C. limon, C. cyminum, I. sonorae*.	Glycemic control	6 capsules	400	2,400	Mexico
Suppl.8	*J. communis* berries, *P. alba* leaves, and *P. silvestris* leaves.	Anti-inflammatory	6 capsules	300	1,800	Mexico
Suppl.9	Extract of *C. vulgaris, C. sempervirens, A. uva-ursi, C. pubescens.*	Anti-inflammatory and antioxidant	2 tablets	500	1,000	Mexico
Suppl.10	Dried fruit of organic *L. barbarum*	Antioxidant	3 capsules	500	1,500	Mexico
Control	*C. sinensis* leaves	Antioxidant	N/D	N/D	N/D	N/D

Note: Suppl. (Supplement); USA (United States of America); N/D (Not declared). *Weight in mg of each tablet/ capsule. **Noted by the manufacturer.

Extraction

500 mg of each dietary supplement was weighed and dissolved with 10 mL of 80 % ethanol (EtOH) assisted by ultrasound, with a power of 50 Hz and a frequency of 40 KHz (Cole Parmer Ultrasonic Bath EW-08895-15) at 45 °C, for 2 h. After time, the extracts were centrifuged at 10,000 rpm, at 4°C for 15 min, and the supernatant was recovered and used for the following determinations.

Revista Bio ciencias
ISSN 2007-3380

For the determination of volatile organic compounds (VOCs), hexane and methanolic extracts of supplement 8 and methanolic extracts of supplement 3 were obtained. These solvents were used to extract the hydrophilic and hydrophobic compounds. The same extraction conditions mentioned above were used.

Determination of Total Reducing Capacity (TRC) and Total Flavonoids (TF)

For the determination of TRC, the Folin-Ciocalteu method described by Swain & Hillis (1959), was used with slight modifications. A calibration curve of gallic acid (0-0.4 mg/mL) was carried out, which was used as a standard. 80 % EtOH was used as blank. In a 96-well Costar® microplate, 10 µL of the extract was mixed with 230 µL of distilled water and 10 µL of the 2N Folin-Ciocalteu reagent and incubated for 3 min, then 25 µL of Na_2CO_3 was added and kept 2 h in darkness. The absorbance was read at 725 nm in a microplate reader Synergy HT (BioTek, Inc, EUA). The results were expressed in mg equivalents of gallic acid (GAE) per gram.

The TF was determined following the method of Ebrahimzadeh *et al*. (2018), which consisted of adding 10 µL of the extracts to a 96-well microplate, followed by 250 µL of distilled water, 10 µL of 10% $AlCl_3$ and 10 µL of 1M CH_3CO_2K. It was allowed to incubate for 30 min in the darkness, and the absorbance was read at 415 nm using a Synergy HT microplate reader (Biotek, Inc, USA). 80% EtOH was used as blank, and a quercetin standard curve from 0 to 1.0 mg/mL was constructed. The results were expressed in mg quercetin equivalents (EQ) per gram.

Antioxidant activity (AOX)

Oxygen Radical Absorbance Capacity (ORAC)

It was carried out following the method described by Huang *et al*. (2002) with slight modifications. A 75 mM phosphate buffer adjusted to pH 7.4 was prepared, a 22.5 mg/mL fluorescein solution in phosphate buffer (stock solution), of which 100 µL was taken and made up to 10 mL with the phosphate buffer (intermediate solution). From this last solution, 400 µL was taken and volumetric to 25 mL with phosphate buffer (working solution). A solution of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) 2.6 mg/mL was prepared with phosphate buffer and a calibration curve of 400 µM Trolox in phosphate buffer.

In a 96-well microplate, 230 µL of distilled water was deposited in the outer rows and columns to maintain the working wells at a stable temperature (37 °C). The working solution of fluorescein and AAPH was deposited in the microplate reader (Synergy HT, BioTek, Inc, USA) to be automatically dispensed. 25 µL of blank (phosphate buffer), calibration curve (Trolox), and samples were deposited, and the microplate was placed inside the reader at a temperature of 37 °C, and the experiment was carried out. The microplate reader automatically dispensed 150 µL fluorescein and 50 µL AAPH and took readings for 70 min at 60 s intervals with an excitation wavelength of 485 nm and emission at a wavelength of 580 nm. The results were expressed as equivalent µmol of Trolox per gram.

Revista Bio ciencias
ISSN 2007-3380

ABTS radical scavenging capacity

It was carried out using the method proposed by Thaipong *et al*. (2006), with slight modifications. A 7.4 mM ABTS (2,2'-Azinobis-3-ethyl-benzo-thiazoline-6-sulfonic acid depletion) stock solution and a 2.6 mM potassium persulfate stock solution were prepared; both were mixed in equal volumes, and They were left to react, protected from light, at room temperature for 16 h. From this mixture, the working solution was prepared, which consisted of diluting it with absolute EtOH until obtaining an absorbance of 0.7 at 734 nm. A 1 mM Trolox stock solution was prepared, with a calibration curve from 0.1 to 1.0 mM. For the assay, 10 µL of the blank (80 % EtOH), calibration curve, and sample were deposited in a 96-well microplate, followed by 190 µL of the ABTS radical, and allowed to react for 2 h at room temperature, in the darkness. Once the time had elapsed, the absorbance was read at 734 nm in a Synergy HT microplate reader (BioTek, Inc., USA). The results were expressed as equivalent mmol of Trolox per gram.

DPPH radical scavenging capacity

It was carried out according to the methodology proposed by Karadag *et al*. (2009), with slight modifications. A 100 µM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution and a 1 mM Trolox stock solution were prepared. A calibration curve was prepared from 0.1 to 1.0 mM Trolox. For the assay, 10 µL of the blank (80 % EtOH), calibration curve, and sample were deposited in a 96-well microplate, followed by 190 µL of the DPPH radical, and allowed to react for 30 min at room temperature, in the darkness. Once the time had elapsed, the absorbance was read at 540 nm in a Synergy HT microplate reader (BioTek, Inc., USA). The results were expressed as equivalent mmol of Trolox per gram.

Determination of volatile organic compounds (VOCs) by GC-IT-MS/MS

The VOCs present in the methanolic and hexanic extracts of supplement No. 8 and methanolic extracts of supplement No. 3 were identified. Identification was performed on an Agilent 7890B gas chromatograph with an ion trap tandem mass spectrometry detector (CG-IT-MS Agilent 240). A VF-5 MS column, 30 m x 0.25 mm x 0.25 µm, was used. The mass spectra were compared to the NIST Mass Spectral Library using the NIST MS search or probability-based match search format as part of Agilent Technologies Workstation MS Software Version 7.0.1; those compounds with a similarity percentage greater than 80 were considered present in the extracts.

Toxicity test in *Artemia salina* model

It was carried out following the method described by Meyer *et al.* (1982). *A. salina* eggs were obtained from a local aquarium in Culiacan, Sinaloa. 3 g of eggs were placed in one L of brine adjusted to pH 7 with 1N sodium hydroxide. They were allowed to hatch for 12 h at 28 °C, with oxygenation and incandescent light. Once the eggs hatched, 10 nauplii were transferred to 6-well microplates. For the bioassay, the nauplii were placed in contact with the evaluated

Revista Bio ciencias
ISSN 2007-3380

concentrations (100, 200, 300, 400, 500, 1,000, 1,500, 2,000, and 2,500 µg/mL) (only from supplement 8). After 24 h, the surviving crustaceans were counted with a stereoscope. 99 % caffeine obtained from Sigma Merck® was used as a positive control and distilled water as a negative control.

Bioinformatic study

The SuperPred platform (https://prediction.charite.de/) was used to predict the biofunctional potential that the metabolites identified in the extracts of supplements 3 and 8 could potentially have. Targets related to oxidative stress pathologies were obtained from the Comparative Toxicogenomics Database (CTD) (http://ctdbase.org/).

Statistical analysis

All assays were performed by triplicate. The results were expressed as means and standard deviation. A completely randomized one-way ANOVA was performed for the AOX, TRC, and TF analyses, and the means were contrasted using the Tukey test with an $\alpha \leq 0.05$. The Minitab18 program (Minitab Inc. State College, Pa., USA) was used for this. For the toxicity study, the IC_{50} of extract No. 8 was determined by linear regression, represented by the logarithm of the concentration against the lethality percentage.

Results and discussion

Determination of Total Reducing Capacity (TRC) and Total Flavonoids (TF)

Highlights the TRC of supplements 3 and 8 (Table 2). Likewise, the TF content was higher in supplement 3, followed by supplements 4, 5, and 2 (Table 2). In contrast, the TF content in supplement 8 was 3.15 ± 0.31 mg EQ/g, so its TRC may be due to other phytochemicals and compounds present, such as phenolic acids, fatty acids, terpenes, saponin sugars, among others. The Folin-Ciocalteu method is frequently used to estimate the total content of phenolic compounds. However, it has been shown that other compounds, such as terpenes, sterols, vitamins, sugars, alkaloids, ketones, aldehydes, and fatty acids with TRC can interfere with the assay (Magalhães *et al*., 2010). Therefore, various investigations indicate that it is also a method to estimate the AOX of natural extracts due to the electron transfer that the TRC of an antioxidant compound measures. Additionally, it correlates with other electron transfer assays such as DPPH and ABTS methods (Everette *et al*., 2010; Lamuela-Raventós, 2018; Magalhães *et al*., 2006). Also, the molecular structure of phenols can interfere with the test, mainly in the amount and position of the hydroxyl groups present (Magalhães *et al*., 2010; Platzer *et al*., 2021). This may explain the very low TF content of supplement 8, which has the highest TRC.

TRC correlates closely with phytochemical content. The composition of supplement 8 stands out for its majority content of *Juniperus communis* fruits, *Pinus sylvestris* leaves, and *Populus alba* leaves. In the fruits of *J. communis*, a higher content of monoterpenes, diterpenes,

Revista Bio ciencias
ISSN 2007-3380

and flavonoids has been observed (Ben Mrid *et al*., 2019; Falasca *et al*., 2014; Jegal *et al*., 2017). While in *P. sylvestris,* mainly monoterpenes, sesquiterpenes, diterpenes, flavan-3-ol type flavonoids, acetylated and glycosylated flavonoids, neolignans, and condensed tannins have been identified (Allenspach *et al*., 2020; Tegelberg *et al*., 2018). Likewise, the bark has significant phenolic compounds (Pap *et al*., 2021). The content of total phenolic compounds in *P. sylvestris* leaf extracts has also been reported, providing 0.19 mg GAE/g and 51.09 mg QE/g of total flavonoids (Fierascu *et al*. 2018).

The presence of phenolic acids, lignans, aglycone, and glycosylated flavonoids has been demonstrated in *P. alba* leaves (Danise *et al*., 2021; Elsbaey *et al*., 2019; Tawfeek *et al*., 2019). Likewise, *P. alba* leaf extracts contain total phenolic compounds (139.55 ± 8.81 mg GAE/g) and TF (46.12 ± 1.19 mg QE/g) (Elsbaey *et al*., 2019).

On the other hand, supplement 3 was the one that presented the greatest amount of TF (Table 2). These results are due to the great diversity of phytochemicals present in the product, which have been shown to exert a powerful AOX (Memarzia *et al*., 2021), with *Curcuma longa* extracts standing out for their higher concentration in the product followed by extract of *Camelia sinensis* (Zhang *et al*., 2019), *Arthrospira platensis* (Braune *et al*., 2021) and *Rosmarinus officinalis* (Ali *et al*., 2019). It is worth mentioning that this product is a mixture of at least 16 herbal extracts, amino acids, vitamins, minerals, and carotenoids, which also provide TRC and AOX.

Supplement 4 was the third with the highest TRC and TF (Table 2). This product is mainly composed of dry extract of *Gingko biloba* leaves. It is well known that *G. biloba* is a good source of phytochemicals, particularly glycosylated flavonoids derived from quercetin, kaempferol, and isorhamnetin (Liu *et al*., 2015). In contrast, supplements 1 and 2, containing flavonoid extracts, such as hesperidin (flavanone) (Supplement 1), and blueberry concentrate (*Vaccinium corymbosum*) (Supplement 2), which is abundant in anthocyanins, phenolic acids, and flavonoids (Sun *et al*., 2018). Manufacturers recommend a daily serving of 1,950 and 1,535.5 mg, respectively. According to our results, they only provide 2.70 and 5.98 mg EQ/g of total flavonoids. These results, lower than those reported by the manufacturers, may be due to the type of solvent used for the extraction (EtOH 80%) and the extraction method (ultrasound), in addition to the reference standard (quercetin), which is a flavonol. At the same time, hesperidin is a flavanone, and the compounds in blueberry are anthocyanins and phenolic acids mainly. Likewise, there may be irregularities in the production processes of this type of product (Wolsko *et al*., 2005).

Supplement 9 presented a low content of TRC and TF (Table 2). Its main ingredient is *Chlorella vulgaris*, followed by *Cupressus sempervirens*, *Arctostaphylos uva-ursi,* and *Capsicum pubescens*. *C. vulgaris* is characterized by presenting phenolic acids, flavonoids, tannins, and triterpenoids, among other compounds (Habashy *et al*., 2018). It also stands out as a source of hydrophobic compounds, so the solvent used for the extraction probably could not extract them (Ho & Redan, 2022). The leaves of *C. sempervirens* contain flavonoids and phenolic acids with reducing capacity (Ibrahim *et al*., 2007). Likewise, the leaves of *A. uva-ursi* contain arbutin, gallic acid, gallotannins, quercetin glycosides, kaempferol, and myricetin, which have been shown to TRC (Panusa *et al*., 2015).

Revista Bio ciencias
ISSN 2007-3380

Similarly, supplement 10, composed of goji berries, did not present a significant content of TRC and TF (Table 2). This may be because it mainly comprises hydrophobic metabolites such as carotenoids and fatty acids; However, flavonoids and phenolic acids have also been identified (Amagase & Farnsworth, 2011). These significant differences may be due to growing conditions, harvest, variety, origin, biotic and abiotic stress, climate, and handling (Figueiredo *et al*., 2008). However, it leaves a lack of credibility for the manufacturer since, according to their ingredients, they should have a higher TRC and TF content. Due to the above, it is correct to mention that a specialist should always recommend this type of products, monitor their consumption, and purchase from companies registered with the corresponding health commissions.

AOX *in vitro*

The AOX results are shown in Table 2. Supplement 3 was the one that showed the highest AOX in the ABTS and DPPH methods, showing significant differences from the other supplements. Phenolic compounds and terpenes are the two phytochemicals that contribute majorly to the AOX of vegetables and medicinal plants (Zhang *et al*., 2015). In supplement 3, the main ingredient is the rhizome of *C. longa*, which is abundant in phenolic compounds and terpenes, which is why it exerts a powerful AOX and considerable antiradical capacity *in vitro* (Altir *et al*., 2021).

Among the other components of supplement 3 is green tea (*C. sinensis*), which is recognized for its ability to capture free radicals and reduce biomarkers of oxidative stress (Thitimuta *et al.*, 2017). It also contains *Arthrospira plantensis*, whose protective properties against oxidative damage have been previously reported (Gutiérrez-Rebolledo *et al*., 2015). Finamore *et al*. (2017), reported the antioxidant effects of *A. platensis*, relating them mainly to its content of phenolic compounds, phycocyanins, and polysaccharides. Regarding the results of the ORAC method, supplement 6 was the one that showed the highest AOX, followed by supplement 4 and 1 (Table 2).

Revista Bio ciencias
ISSN 2007-3380

Table 2. Total reducing capacity, total flavonoids, and antioxidant activity of hydroethanolic extracts of food supplements.

Sample	TRC (mg GAE/g)	TF (mg EQ/g)	AOX (µmol ET/g)		
			ABTS	DPPH	ORAC
Suppl.1	7.47 ± 0.48^{D,E,F}	2.70 ± 0.02^{E,F}	0.9 ± 0.00^{E,F}	0.07 ± 0.01^{C}	311,720 ± 16,840^{C,D}
Suppl.2	5.03 ± 0.38^{E,F}	5.98 ± 0.05^{C,D,E}	0.074 ± 0.00^{E,F,G}	0.18 ± 0.00^{D}	124,980 ± 2,230^{E}
Suppl.3	28.82 ± 2.09^{C,D}	11.82 ± 0.83^{A,B}	4.02 ± 0.04^{A}	1.91 ± 0.12^{A}	263,430 ± 24,220^{D}
Suppl.4	18.50 ± 2.05^{C}	9.66 ± 1.14^{A,B,C}	0.22 ± 0.01^{D}	0.16 ± 0.01^{D}	333,770 ± 11,160^{B,C}
Suppl.5	8.19 ± 0.54^{D,E,F}	7.53 ± 0.50^{B,C,D}	0.12 ± 0.01^{E}	0.03 ± 0.01^{D}	45,900 ± 4,840^{F}
Suppl.6	11.82 ± 0.75^{C,D,E}	1.61 ± 0.24^{E,F}	0.26 ± 0.03^{D}	0.05 ± 0.00^{D}	384,220 ± 9,340^{B}
Suppl.7	7.70 ± 0.75^{D,E,F}	1.56 ± 0.06^{E,F}	0.08 ± 0.01^{E,F,G}	0.5 ± 0.01^{D}	150,010 ± 680^{E}
Suppl.8	43.08 ± 3.13^{B}	3.15 ± 0.31^{D,E,F}	2.43 ± 0.02^{C}	1.28 ± 0.00^{B}	308,140 ± 10,040^{C,D}
Suppl.9	0.62 ± 0.16^{F}	0.13 ± 0.01^{F}	0.009 ± 0.00^{G}	0.03 ± 0.02^{D}	25,560 ± 3,210^{F}
Suppl.10	1.32 ± 0.17^{E,F}	0.12 ± 0.01^{F}	0.01 ± 0.00^{F,G}	0.02 ± 0.00^{D}	12,810 ± 640^{F}
Control*	78 ± 10.92^{A}	14.64 ± 2.87^{A}	3.57 ± 0.04^{B}	1.62 ± 0.55^{B}	681,210 ± 50,710^{A}

Note: **C. sinensis* leaves. The results are the means and standard deviation of three replicates. GAE (gallic acid equivalent); EQ (quercetin equivalent); TRC (Total Reducing Capacity); TF (total flavonoids); ET (Trolox equivalent). Different letters in columns indicate statistical differences according to Tukey's test at $\alpha \leq 0.05$.

Supplement 6 is a mixture of phytosterols, which have a high radical scavenging capacity and act mainly by protecting cell membranes (Vezza *et al*., 2020; Yoshida & Niki, 2003). On the other hand, supplement 4 is made from *G. biloba*, which has AOX, which is related to its content of phenolic compounds (Liu *et al*., 2007). Supplement 1 is made from plants abundant in flavonoids, whose pharmacological properties are attributed to the inhibition of enzymes that participate in the production of free radicals and their capture capacity, as well as iron chelation (Russo *et al*., 2000). The results obtained are supported by what was obtained by Ferretti *et al*. (2010), who previously reported the ability of plant phytosterols to reduce lipid peroxidation of LDL cholesterol, demonstrating their antioxidant activity. The differences in AOX obtained with the different methods could be because the ABTS method allows measuring the antioxidant activity of compounds with a hydrophilic and lipophilic nature. At the same time, DPPH can only dissolve in organic media (Kuskoski *et al*., 2005), and the ORAC method measures the oxygen radical absorbance capacity, making it one of the methods that provides the most information on the antioxidant potential in biological systems (Zapata *et al*., 2014).

Revista Bio ciencias
ISSN 2007-3380

Free radicals have been related to the incidence and development of chronic diseases such as cardiovascular diseases, various types of cancer, and neurodegenerative diseases, among others (Ginter *et al*., 2014). This is why products rich in antioxidants are highly attractive to consumers since they consider that consuming this type of supplement does not cause any harm to health (Schroder & Navarro, 2006). However, due to its high doses of phytochemicals, it is necessary to continue evaluating the doses and periods in which this supplementation is appropriate and safe so that possible adverse effects and health damage can be avoided when consumed.

Determination of Volatile Organic Compounds (VOCs)

Only supplements 3 and 8 were selected to determine VOCs because they had the highest nutraceutical content (Table 2). Fatty acids, terpenoids, and phytosterols were identified (Table 3). In supplement 3, aromatic terpenes derived from turmeric stand out. While in supplement 8 a wide variety of terpenes with biological activities were identified.

Revista Bio ciencias
ISSN 2007-3380

Table 3. Volatile organic compounds identified in hexanic and methanolic extracts of supplements 3 and 8.

Supplement	Extract	Compound	Retention time	# CAS	% Similarity
Suppl. 3	MeOH	o-Ethylhydroxylamine	2.41	624-86-2	91.1
		Methenamine, N-hydroxy-N-methyl-	2.44	5725-96-2	95.9
		N-allyl-N, N-dimethylamine	2.47	2155-94-4	90.6
		3(2H)-Pydazinone	3.04	504-30-3	86.0
		1H-Tetrazole	3.15	288-94-8	81.3
		2(5H)-furanone	3.16	597-23-4	88.0
		1,2-Cyclopentanedione	3.21	3008-40-0	94.05
		Methanamine, N-methoxy-	3.54	1117-97-1	93.9
		2-pyrrolidinone	3.71	616-45-5	92.8
		2,4,5-trihydroxypyrimidine	3.90	496-76-4	82.5
		Benzoic acid hydrazide	3.97	613-94-5	86.8
		1,azabicycle[3.1.0] hexane	3.99	285-76-7	84.3
		1, methyl-5-fluoruracil	4.22	1000427-92-0	86.3
		Camphor	4.24	76-22-2	91.5
		Catechol	4.40	120-80-9	85.6
		4-vinylphenol	4.48	2628-17-3	96.0
		4-Chloro-1-azabicyclo[2.2.2] octane	4.76	5960-95-2	92.1
		1,2-benzenediol, 3-methoxy-	4.76	934-00-9	91.2
		2-propen-1-ol, 3-phenyl-	4.96	104-54-1	93.1
		2-methoxy-4-vinylphenol	5.00	7786-61-0	95.1
		Phenol, 2, 6-dimethoxy-	5.17	91-10-1	85.1
		Benzaldehyde, 2,4-dihydroxy-6-methyl-	5.48	487-69-4	85.1
		Isobisabolene	6.10	1000-424-85-5	80.1
		Pentanoic acid	6.70	109-52-4	80.5
		aR-Turmerone	6.92	5-32-65-0	82.0
		Turmerone	7.26	180315-67-7	95.8
		Heptadecane	7.33	629-78-7	96.4
		(E)-atlantone	8.08	108645-54-1	90.1
		Neophytadiene	8.46	504-96-1	80.4
		Caffeine	8.78	58-08-2	84.9
		Hexadecanoic acid, methyl ester	9.18	112-39-0	91.0
		Turmeronol A	9.38	13165-37-1	87.1
		n-hexadecanoic acid	9.49	57-10-3	89.0
		Hexadecanoic acid, ethyl ester	9.75	628-97-7	90.8
		Methyl.gamma.-linolenate	10.53	16326-32-2	95.4
		9,12-Octadecanoic acid(Z,Z)-,methyl ester	10.65	112-63-0	94.3
		9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-	10.72	301-00-8	82.2
		Phytol	10.80	150-86-7	94.9
		Methyl stearate	10.89	112-61-8	91.1
		Linoleic acid, ethyl ester	11.22	544-35-4	94.0
		Octadecanoic acid, ethyl ester	11.45	111-61-5	87.2
		Curlone	13.06		90.9
		Campesterol	19.45	474-62-4	83.0
		Stigmasterol	19.72	83-48-7	86.5
		Gamma-sitosterol	20.26	83-47-6	83.9

Revista Bio ciencias
ISSN 2007-3380

Continuation

Table 3. Volatile organic compounds identified in hexanic and methanolic extracts of supplements 3 and 8.

Supplement	Extract	Compound	Retention time	# CAS	% Similarity
Suppl. 8	MeOH	o-ethylhydroxylamine	2.41	624-86-2	89.9
		Hydrazine, propyl-	2.53	5039-61-2	83.8
		1H-imidazole,1-methyl-	2.90	616-47-7	88.0
		1H-pyrazole,1 methyl-	2.90	930-36-9	81.8
		Methanamide, N-hydroxy-N-methyl-	3.04	5725-96-2	83.6
		3-amino-1,2,4-triazine	3.05	1120-99-6	80.7
		1H-tetrazole	3.16	288-94-8	85.2
		1,2-cyclopentanedione	3.19	3008-40-0	95.0
		N-methoxy-N-methylacetamide	3.45	78191-00-1	84.6
		Thymine	3.85	65-71-4	85.8
		Dihydro-3-methylene-5-methyl-2-furanone	3.88	62873-16-9	91.0
		Phenol, 2-methoxy-	3.94	90-05-1	91.9
		Benzoic acid, methyl ester	3.96	93-58-3	83.5
		Ethanone, 1-(2-hydroxy-6-methoxyphenyl)-	4.11	703-23-1	80.8
		Catechol	4.38	120-809	95.1
		Beta.-D-glucopyranoside-methyl	6.82	709-50-2	80.2
		Neophytadiene	8.46	504-96-1	85.9
		Hexadecanoic acid, methyl ester	9.17	112-39-0	92.6
		n-hexadecanoic acid	9.47	57-10-3	91.2
		9-octadecanoic acid, methyl ester, (E)-	10.69	1937-62-8	82.6
		Phytol	10.80	150-86-7	87.1
		Octadecanoic acid	11.17	57-11-4	80.9
		Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	14.02	23470-00-0	80.4
		Squalene	16.37	111-02-4	92.9
		Hentriacontano	16.79	630-04-6	88.8
		Stigmasterol	19.72	83-48-7	86.6
		γ-sitosterol	20.26	83-47-6	84.1
		Olean-12-en-3-ol, acetate, (3.beta.)-	20.71	1616-93-9	
		α-amyrin	44.74		83.5
		β-amyrin	20.72	559-70-6	84.9
		D-friedoolean-14-en-3-ol	20.55	81654-73-1	80.5
	Hexane	2-pyrrolidinone	2.11	616-45-5	82.2
		(-)-spathulenol	11.70	-	84.5
		Cubenol	12.21	-	80.2
		7-epi-*cis*-sesquisabinene hyd	12.39	-	74.3
		Tau-muurolol	12.54	-	79.9
		Cis-verbenol	7.63	-	84.3
		(-)-myrtenol	8.27	-	82.1
		4-epi-cubedol	11.09	-	82.2
		Trans-calameno	11.14	-	81.6
		1,3-propanediamine	2.72	109-76-2	86.6
		Neophytadiene	8.45	504-96-1	94.9
		2-pentadecanone, 6,10,14-trimethyl-	8.51	502-69-2	89.8
		n- hexadecanoic acid	9.44	57-10-3	82.4
		Phytol	10.79	150-86-7	90.1
		Heneicosan	13.89	629-94-7	83.5
		Hentriacontano	15.38	630-04-6	90.8
		Eicosan	15.38	112-95-8	90.7
		Squalene	16.37	111-02-4	94.3
		Triacontane	19.66	638-68-6	81.5
		Stigmasterol	19.71	83-48-7	81.6
		γ-sitosterol	20.25	83-47-6	83.3
		D-Friedoolean-14-en-3-ol	20.53	81654-73-1	80.5
		β-amyrin	20.71	559-70-6	87.8

Revista Bio ciencias
ISSN 2007-3380

The compounds identified in supplement No. 3 aR-turmerone, turmerone, (E)-atlantone, turmeronol A, and curlone, are curcuminoids and sesquiterpenes derived from turmeric, the main ingredient of the product (Salem *et al*., 2022). These metabolites suggest presenting biological activity, mainly anti-inflammatory, anti-cancer, and antioxidant (Jayaprakasha *et al*., 2005). Likewise, phytosterols identified in *C. longa,* such as campesterol, stigmasterol, and gamma-sitosterol, have been shown to reduce cholesterol levels in individuals with hypercholesterolemia (Ferguson *et al*., 2018).

The hexane and methanolic extract of supplement No. 8 extracted a great diversity of metabolites due to the different solvents' polarities. The predominant compounds extracted were terpenes and triterpenes, such as squalene, hentriacontane, α-amyrin, and β-amyrin. Within the product's composition, the content of white poplar leaves, scots pine leaves, and juniper berries stand out, which are abundant in these compounds. In addition, terpenes are the secondary metabolites with the greatest presence and distribution in plants. They stand out for their antioxidant, anti-inflammatory, anticancer, and antibacterial activity (Bajac *et al*., 2023; Guleria *et al.,* 2021; Ji & Ji, 2021).

Cytotoxicity assays

Extracts with an IC_{50} less than 1,000 µg/mL were considered toxic, while an IC_{50} greater than 1,000 µg/mL was considered non-toxic (Meyer *et al*., 1982). Our results indicated that extract number 8 had an IC_{50} of 1,562.5 µg/mL, which suggests that it is not toxic for the study model used. The IC_{50} of the positive control (caffeine) was 800 µg/mL, which is why it is considered slightly toxic. Acute toxicity was evaluated with the *A. salina* model because it is practical, easy, and economical and provides quick guidance on the toxic potential of organic samples (Aydın *et al*., 2016).

As previously mentioned, extract 8 is composed mainly of *J. communis* berries. Various investigations corroborate our results since the safety of extracts from different species of *Juniperus* has been demonstrated (Miceli *et al*., 2020; Taviano *et al*., 2011). Schneider *et al*. (2004), reported IC_{50} values lower than those reported in our research; however, it may be due to the type of extract used (methylene chloride and ethyl acetate); both solvents are extremely toxic for living organisms *(*Kimura *et al*., 1971). To our knowledge, there are no investigations on the toxicity of extracts of *P. alba* leaves and *P. silvestris* leaves (complementary ingredients of supplement 8); however, a slight cytotoxicity was observed in the latter in NIH 3T3 fibroblasts (Smirnova *et al*., 2020). The manufacturer of supplement 8 recommends a daily intake of 1,800 mg, so according to our results, caution is advised in its consumption since, although it was not toxic at the concentrations evaluated, this recommendation exceeds them, so manufacturers must assess the recommended doses *in vivo* models.

Bioinformatic study

The biofunctional potential of the phytochemicals present in supplements No. 3 and 8 was elucidated in Table 4. Only the identified medicinally relevant compounds were selected. In

Revista Bio ciencias
ISSN 2007-3380

supplement 3, fatty acids and terpenoids derived from turmeric (aR-turmerone, turmerone, (E)-atlantone, turmeronol A, and curlone) were identified, which, according to the bioinformatic analysis, mostly share the targets related to cancer, inflammation and attention deficit hyperactivity. Likewise, phytosterols (campesterol, stigmasterol, and γ-sitosterol) were identified, which are associated with preventing cardiovascular diseases. Regarding the phytochemicals of supplement 8, the terpenes and triterpenes stand out (squalene, hentriacontane, α-amyrin, β-amyrin, D-friedoolean-14-en-3-ol, cubenol, tau-muurolol, *cis*-verbenol, (-)-myrtenol and 4-epi-cubedol).

According to the analysis carried out in the CTD database (Therapeutic Toxicogenomics Database), ar-turmerone is mainly associated with diabetes involving the genes BAX, CASP3, CYP1A1, PPARG, and TP53. Squalene is associated as a therapeutic agent against coronary conditions (BAX, BCL2, CASP3, CAT, MMP2, and TNF), renal carcinoma (EPAS1, RELA), and diabetes (BAX, BCL2, CASP3, CAT, MMP2, RELA, and TNF). (-)-Myrtenol is associated as a therapeutic agent to treat liver damage, edema, hypocholesterolemia, hyperglycemia, infertility in women, and pancreatic diseases. α-amyrin is a terpene identified in both supplements, associated with treating hyperalgesia, pain, and inflammation. γ-sitosterol is used to treat hypercholesterolemia and liver damage (Davis *et al*., 2021). The results obtained from the SuperPred database (Table 5) indicate that the compounds exert activity on pathologies related to inflammation, oxidative stress, and the cardiovascular system. According to the present results, the possible mechanisms of action associated with the consumption of these compounds can be elucidated, in addition to providing a broader overview of the use and safety of the use of dietary supplements since these are consumed simultaneously with medications, can cause drug interactions (FDA, 2022b).

Tabla 4. Targets moleculares e Indicaciones terapéuticas predichas por la base de datos de toxigenómica comparativa (CTD) de los fitoquímicos presentes en los extractos.

Compound	Interacting genes	Disease	Inference score
Ar-Turmerone	ABCB1, AHR, BAX, CASP3, CYP1A1, PPARG, TP53	Diabetes Mellitus (Experimental)	25.62
		Infertility (Male)	20.89
		Breast neoplasms	18.78
		Colorectal neoplasms	17.23
		Esophageal Neoplasms	14.98
α-Amyrin	TACR1	Bronchial diseases	6.28
		Neurogenic inflammation	5.76
		Alcoholism	5.45
		Attention deficit disorder with hyperactivity	5.31

Revista Bio ciencias
ISSN 2007-3380

Continuation

Tabla 4. Targets moleculares e Indicaciones terapéuticas predichas por la base de datos de toxigenómica comparativa (CTD) de los fitoquímicos presentes en los extractos.

Compound	Interacting genes	Disease	Inference score
γ-Sitosterol	AKT1, APOA1, APOB, BAX, BCL2, BIRC2, CASP3, CASP9, CAT, CDH1, CLEC4E, CYCS, EEIG1, EGFR, ESR1, ESR2, IL10, IL1B, IL6, LDLR, PARP1, TNF, VEGFA	Breast neoplasms	45.18
		Prostatic neoplasms	37.74
		Adenocarcinoma	34.89
		Brain ischemia	33.81
		Reperfusion injury	32.02
		Diabetes Mellitus	31.83

Table 5. Molecular targets and therapeutic indications predicted by SuperPred of the phytochemicals present in the extracts.

Compound	Predicted Target	TTD ID	Indication of predicted targets	Probability (%)	Model accuracy (%)
Ar-Turmerone	Cathepsin D	T67102	Hypertension	95.31	98.95
		T67102	Multiple sclerosis	95.31	98.95
	Pregnane receptor X	T82702	Arteriosclerosis	93.29	94.73
	ADN- liase	T13348	Glioma	88.97	91.11
			Melanoma	88.97	91.11
			Eye cancer	88.97	91.11
			Solid tumor/cancer	88.97	91.11
	G protein-coupled to receptor 55	T87670	Attention deficit hyperactivity	84.19	78.15
	Formyl peptide receptor 1	T87831	Inflammation	83.01	93.56
Curlone	DNA liase	T13348	Glioma	93.48	91.11
			Melanoma	93.48	91.11
			Eye cancer	93.48	91.11
			Solid tumor/cancer	93.48	91.11
	Cathepsin D	T67102	Hypertension	91.48	98.95
			Multiple sclerosis	91.48	98.95
	Formyl peptide receptor 1	T87831	Inflammation	83.31	93.56
			Peptic ulcer	83.31	93.56
	G protein-coupled to receptor 55	T87670	Attention deficit hyperactivity	81.9	78.15

Revista Bio ciencias
ISSN 2007-3380

Continuation

Table 5. Molecular targets and therapeutic indications predicted by Superpred of the phytochemicals present in the extracts.

Compound	Predicted Target	TTD ID	Indication of predicted targets	Probability (%)	Model accuracy (%)
Squalane	Cathepsin D	T67102	Hypertension	82.96	98.95
			Multiple sclerosis	82.96	98.95
	Pregnane X receptor	T82702	Arteriosclerosis	82.68	94.73
	ADN liase	T13348	Glioma	80.29	91.11
			Melanoma	80.29	91.11
			Eye cancer	80.29	91.11
			Solid tumor/cancer	80.29	91.11

Note: TTD (Therapeutic Target Database ID).

Conclusions

The dietary supplements analyzed were shown to contain flavonoids and exert antioxidant activity. The metabolites identified were mostly triterpenes, terpenes, sterols, and fatty acids, which were shown to be related to biomarkers of oxidative stress and inflammation, so their consumption could help minimize the appearance of chronic degenerative diseases. It was possible to elucidate the biofunctional potential of the dietary supplements evaluated, according to the bioinformatic analysis, the phytochemicals present can potentially exert activity in the cardiovascular system and protection against different types of cancer and type 2 diabetes. However, it is necessary to carry out *in vivo* studies to determine its clinical functionality. Supplement 8 did not show toxicity in the *A. salina* model; despite this, it is recommended to evaluate acute and chronic toxicity in other biological models since the doses recommended by the manufacturers may present side effects to the consumer's health. The consumption of this type of product is recommended to be supervised by a health specialist, taking extreme precautions if you are undergoing pharmacological treatment since, according to our results, supplements can exert more than one bioactivity due to the diversity of ingredients and compounds present interacting with different molecular targets.

Revista Bio ciencias
ISSN 2007-3380

Authors' contribution

"Conceptualization of work, Jiménez-Ortega, L.A.; Heredia, J.B.; development of the methodology, Jiménez-Ortega, L.A.; López-Romero, B. J.; Heredia-Batiz, J.; Bastidas-Bastidas, P.J.; software management, Bastidas-Bastidas, P.J, Jiménez-Ortega, L.A.; experimental validation, Heredia, J. Basilio.; analysis of results, Jiménez-Ortega, L.A.; data management, Jiménez-Ortega, L.A.; writing and preparation of the manuscript, Jiménez-Ortega, L.A, López-Romero, B. J.; Heredia-Batiz, J.; writing, review and editing, Heredia, J. Basilio, Bastidas-Bastidas, P .J.; project administrator, Heredia, J. Basilio.; acquisition of funds, Heredia, J. Basilio.

"All authors of this manuscript have read and accepted its published version."

Funding

This research did not receive external funding.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Ali, A., Chua, B. L. & Chow, Y. H. (2019). An insight into the extraction and fractionation technologies of the essential oils and bioactive compounds in *Rosmarinus officinalis* L.: Past, present and future. *Trends in Analytical Chemistry*, 118, 338-351. https://doi.org/10.1016/j.trac.2019.05.040

Allenspach, M., Valder, C., Flamm, D., Grisoni, F. & Steuer, C. (2020). Verification of Chromatographic Profile of Primary Essential Oil of *Pinus sylvestris* L. Combined with Chemometric Analysis. *Molecules*, 25(13), 2973. https://doi.org/10.3390/molecules25132973

Altir, N. K. M., Ali, A. M. A., Gaafar, A.-R. Z., Qahtan, A. A., Abdel-Salam, E. M., Alshameri, A., Hodhod, M. S. & Almunqedhi, B. (2021). Phytochemical profile, in vitro antioxidant, and anti-protein denaturation activities of *Curcuma longa* L. rhizome and leaves. *Open Chemistry*, 19(1), 945-952. https://doi.org/10.1515/chem-2021-0086

Amagase, H. & Farnsworth, N. R. (2011). A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (Goji). *Food Research International*, 44(7), 1702-1717. https://doi.org/10.1016/j.foodres.2011.03.027

Aydın, A., Aktay, G. & Yesilada, E. (2016). A Guidance Manual for the Toxicity Assessment of Traditional Herbal Medicines. *Natural Product Communications*, 11(11), 1763-1773. https://doi.org/10.1177/1934578X1601101131

Bajac, J., Zengin, G., Mitrović, I., Antić, I., Radojković, M., Nikolovski, B. & Terzić, M. (2023).

Juniper berry essential oils as natural resources of biological and pharmacological high-valuable molecules. *Industrial Crops and Products*, 204, 117248. https://doi.org/10.1016/j.indcrop.2023.117248

Ben Mrid, R., Bouchmaa, N., Bouargalne, Y., Ramdan, B., Karrouchi, K., Kabach, I., El Karbane, M., Idir, A., Zyad, A. & Nhiri, M. (2019). Phytochemical Characterization, Antioxidant and *In Vitro* Cytotoxic Activity Evaluation of *Juniperus oxycedrus* Subsp. oxycedrus Needles and Berries. *Molecules*, 24(3), 502. https://doi.org/10.3390/molecules24030502

Braune, S., Krüger-Genge, A., Kammerer, S., Jung, F. & Küpper, J.-H. (2021). Phycocyanin from *Arthrospira platensis* as Potential Anti-Cancer Drug: Review of *In Vitro* and *In Vivo* Studies. *Life*, 11(2), 91. https://doi.org/10.3390/life11020091

Brzezicha, J., Błażejewicz, D., Brzezińska, J. & Grembecka, M. (2021). Green coffee VS dietary supplements: A comparative analysis of bioactive compounds and antioxidant activity. *Food and Chemical Toxicology*, 155, 112377. https://doi.org/10.1016/j.fct.2021.112377

Bruins, M. J., Van Dael, P. & Eggersdorfer, M. (2019). The Role of Nutrients in Reducing the Risk for Noncommunicable Diseases during Aging. *Nutrients*, 11(1), 85. https://doi.org/10.3390/nu11010085

Byrne, N. J., Rajasekaran, N. S., Abel, E. D. & Bugger, H. (2021). Therapeutic potential of targeting oxidative stress in diabetic cardiomyopathy. *Free Radical Biology and Medicine*, 169, 317-342. https://doi.org/10.1016/j.freeradbiomed.2021.03.046

Charlton, A., Garzarella, J., Jandeleit-Dahm, K. A. M. & Jha, J. C. (2021). Oxidative Stress and Inflammation in Renal and Cardiovascular Complications of Diabetes. *Biology*, 10(1), 18. https://doi.org/10.3390/biology10010018

Criollo-Mendoza, M. S., Ramos-Payán, R., Contreras-Angulo, L. A., Gutiérrez-Grijalva, E. P., León-Félix, J., Villicaña, C., Angulo-Escalante, M. A. & Heredia, J. B. (2022). Cytotoxic Activity of Polyphenol Extracts from Three Oregano Species: *Hedeoma patens, Lippia graveolens* and *Lippia palmeri*, and Antiproliferative Potential of *Lippia graveolens* against Two Types of Breast Cancer Cell Lines (MDA-MB-231 and MCF-7). *Molecules*, 27(16), 5240. https://doi.org/10.3390/molecules27165240

Danise, T., Innangi, M., Curcio, E., Piccolella, S., Fioretto, A. & Pacifico, S. (2021). White poplar (*Populus alba* L.) leaf waste recovery and intercropping outcome on its polyphenols. *Industrial Crops and Products*, 171, 113866. https://doi.org/10.1016/j.indcrop.2021.113866

Das, G., Gonçalves, S., Basilio Heredia, J., Romano, A., Jiménez-Ortega, L. A., Gutiérrez-Grijalva, E. P., & Patra, J. K. (2022). Cardiovascular protective effect of cinnamon and its major bioactive constituents: An update. *Journal of Functional Foods*, 97, 105045. https://doi.org/10.1016/j.jff.2022.105045

Davis, A. P., Grondin, C. J., Johnson, R. J., Sciaky, D., Wiegers, J., Wiegers, T. C. & Mattingly, C. J. (2021). Comparative Toxicogenomics Database (CTD): update 2021. *Nucleic Acids Research*, 49(1), 1138-1143. https://doi.org/10.1093/nar/gkaa891

Ebrahimzadeh, M. A., Khalili, M. & Dehpour, A. A. (2018). Antioxidant activity of ethyl acetate and methanolic extracts of two marine algae, *Nannochloropsis oculata* and *Gracilaria gracilis* - an i*n vitro* assay. *Brazilian Journal of Pharmaceutical Sciences,* 54(1), 6. https://doi.org/10.1590/s2175-97902018000117280

Elsbaey, M., Amen, Y., Nakagawa, T. & Shimizu, K. (2019). White poplar: Targeted isolation of pancreatic lipase inhibitors. *Industrial Crops and Products*, 141, 111778. https://doi.

Revista Bio ciencias
ISSN 2007-3380

org/10.1016/j.indcrop.2019.111778

Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W. & Walker, R. B. (2010). Thorough Study of Reactivity of Various Compound Classes toward the Folin−Ciocalteu RGAEent. *Journal of Agricultural and Food Chemistry*, 58(14), 8139-8144. https://doi.org/10.1021/jf1005935

Falasca, A., Melck, D., Paris, D., Saviano, G., Motta, A. & Iorizzi, M. (2014). Seasonal changes in the metabolic fingerprint of *Juniperus communis* L. berry extracts by 1H NMR-based metabolomics. *Metabolomics*, 10(1), 165-174. https://doi.org/10.1007/s11306-013-0566-1

Ferguson, J. J. A., Stokanovski, E., MacDonald-Wicks., Garg, M. L. (2018). Curcumin potentiates cholesterol-lowering effects of phytosterols in hypercholesterolaemic individuals. A randomised controlled trial. *Metabolism Clinical and Experimental*, 82, 22-35. https://doi.org/10.1016/j.metabol.2017.12.009

Ferretti, G., Bacchetti, T., Masciangelo, S. & Bicchiega, V. (2010). Effect of phytosterols on copper lipid peroxidation of human low-density lipoproteins. *Nutrition*, 26(3), 296-304. https://doi.org/10.1016/j.nut.2009.04.015

Fierascu, I., Ungureanu, C., Avramescu, S. M., Cimpeanu, C., Georgescu, M. I., Fierascu, R. C., Ortan, A., Sutan, A. N., Anuta, V., Zanfirescu, A., Dinu-Pirvu, C. E. & Velescu, B. S. (2018). Genoprotective, antioxidant, antifungal and anti-inflammatory evaluation of hydroalcoholic extract of wild-growing *Juniperus communis* L. (Cupressaceae) native to Romanian southern sub-Carpathian hills. *Complementary and Alternative Medicine*, 18(3). https://doi.org/10.1186/s12906-017-2066-8

Figueiredo, A. C., Barroso, J. G., Pedro, L. G. & Scheffer, J. J. C. (2008). Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour and Fragrance Journal*, 23(4), 213-226. https://doi.org/10.1002/ffj.1875

Finamore, A., Palmery, M., Bensehaila, S. & Peluso, I. (2017). Antioxidant, Immunomodulating, and Microbial-Modulating Activities of the Sustainable and Ecofriendly Spirulina. *Oxidative Medicine and Cellular Longevity*, 2017, 3247528-3247528. https://doi.org/10.1155/2017/3247528

Food and Drug Administration [FDA]. (2022a) Introducción básica a los suplementos dietéticos. https://www.fda.gov/consumers/articulos-para-el-consumidor-en-espanol/introduccion-basica-los-suplementos-dieteticos

Food and Drug Administration [FDA]. (2022b) Mixing Medications and Dietary Supplements Can Endanger Your Health. https://www.fda.gov/consumers/consumer-updates/mixing-medications-and-dietary-supplements-can-endanger-your-health#:~:text=Certain%20dietary%20supplements%20can%20change,of%20the%20medication%20you%20need.

Ginter, E., Simko, V. & Panakova, V. (2014). Antioxidants in health and disease. *Bratislava Medical Journal*, 115(10), 603-606. https://doi.org/10.4149/bll_2014_116

Guleria, I., Kumari, A., Lacaille-Dubois, M. A., Nishant, Kumar, V., Saini, A. K., Dhatwalia, J. & Lal, S. (2021). A review on the genus *Populus*: a potential source of biologically active compounds. *Phytochemistry Reviews*, 21, 987-1046. https://doi.org/10.1007/s11101-021-09772-2

Gutiérrez-Grijalva, E. P., Leyva-López, N., Vazquez-Olivo, G. & Heredia, J. B. (2022). Oregano as a potential source of antidiabetic agents. *Journal of Food Biochemistry*, 46(12), e14388. https://doi.org/10.1111/jfbc.14388

Gutiérrez-Rebolledo, G. A., Galar-Martínez, M., García-Rodríguez, R. V., Chamorro-Cevallos, G. A., Hernández-Reyes, A. G. & Martínez-Galero, E. (2015). Antioxidant Effect of *Spirulina* (Arthrospira) maxima on Chronic Inflammation Induced by Freund's Complete Adjuvant in Rats. *Journal of Medicinal Food*, 18(8), 865-871. https://doi.org/10.1089/jmf.2014.0117

Habashy, N. H., Abu Serie, M. M., Attia, W. E. & Abdelgaleil, S. A. M. (2018). Chemical characterization, antioxidant and anti-inflammatory properties of Greek *Thymus vulgaris* extracts and their possible synergism with Egyptian *Chlorella vulgaris*. *Journal of Functional Foods*, 40, 317-328. https://doi.org/10.1016/j.jff.2017.11.022

Hamulka, J., Jeruszka-Bielak, M., Górnicka, M., Drywień, M. E. & Zielinska-Pukos, M. A. (2021). Dietary Supplements during COVID-19 Outbreak. Results of Google Trends Analysis Supported by PLifeCOVID-19 Online Studies. *Nutrients*, 13(1), 54. https://doi.org/10.3390/nu13010054

Hassan, S., Egbuna, C., Tijjani, H., Ifemeje, J. C., Olisah, M. C., Patrick-Iwuanyanwu, K. C., Onyeike, P. C. & Ephraim-Emmanuel, B. C. (2020). Dietary Supplements: Types, Health Benefits, Industry and Regulation. In: Egbuna, C., Dable Tupas, G. (eds.). Functional Foods and Nutraceuticals: Bioactive Components, Formulations and Innovations (pp. 23-38). Springer, Cham. International Publishing. https://doi.org/10.1007/978-3-030-42319-3_3

Ho, K. K. H. Y. & Redan, B. W. (2022). Impact of thermal processing on the nutrients, phytochemicals, and metal contaminants in edible algae. *Critical Reviews in Food Science and Nutrition*, 62(2), 508-526. https://doi.org/10.1080/10408398.2020.1821598

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A. & Prior, R. L. (2002). High-Throughput Assay of Oxygen Radical Absorbance Capacity (ORAC) Using a Multichannel Liquid Handling System Coupled with a Microplate Fluorescence Reader in 96-Well Format. *Journal of Agricultural and Food Chemistry*, 50(16), 4437-4444. https://doi.org/10.1021/jf0201529

Ibrahim, N. A., El-Seedi, H. R. & Mohammed, M. M. D. (2007). Phytochemical investigation and hepatoprotective activity of Cupressus sempervirens L. leaves growing in Egypt. *Natural Product Research*, 21(10), 857-866. https://doi.org/10.1080/14786410601132477

Jayaprakasha, G. K., Rao, M. L. & Sakariah, K. K. (2005). Chemistry and biological activities of *C. longa. Trends in Food Science & Technology,* 16 (12), 533-548. https://doi.org/10.1016/j.tifs.2005.08.006

Jegal, J., Chung, K. W., Chung, H. Y., Jeong, E. J. & Yang, M. H. (2017). The Standardized Extract of *Juniperus communis* Alleviates Hyperpigmentation *in Vivo* HRM-2 Hairless Mice and *in Vitro* Murine B16 Melanoma Cells. *Biological and Pharmaceutical Bulletin*, 40(9), 1381-1388. https://doi.org/10.1248/bpb.b17-00122

Ji, W. & Ji, X. (2021). Comparative Analysis of Volatile Terpenes and Terpenoids in the Leaves of *Pinus* Species—A Potentially Abundant Renewable Resource. *Molecules*, 26(17), 5244. https://doi.org/10.3390/molecules26175244

Karadag, A., Ozcelik, B. & Saner, S. (2009). Review of Methods to Determine Antioxidant Capacities. *Food Analytical Methods*, 2, 41-60. https://doi.org/10.1007/s12161-008-9067-7

Karchesy, Y. M., Kelsey, R. G., Constantine, G. & Karchesy, J. J. (2016). Biological screening of selected Pacific Northwest forest plants using the brine shrimp (*Artemia salina*) toxicity bioassay. *Springer Plus,* 5. https://doi.org/10.1186/s40064-016-2145-1

Kimura, E. T., Ebert, D. M. & Dodge, P. W. (1971). Acute toxicity and limits of solvent residue for sixteen organic solvents. *Toxicology and Applied Pharmacology*, 19(4), 699-704. https://doi.

Revista Bio ciencias
ISSN 2007-3380

org/10.1016/0041-008X(71)90301-2

Kuskoski, E. M., Asuero, A. G., Troncoso, A. M., Mancini-Filho, J. & Fett, R. (2005). Aplicação de diversos métodos químicos para determinar atividade antioxidante em polpa de frutas. *Food Science and Technology*, 25(4), 726-732. https://doi.org/10.1590/S0101-20612005000400016

Lamuela-Raventós, R. M. (2018). Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. In *Measurement of Antioxidant Activity & Capacity*. https://doi.org/10.1002/9781119135388.ch6

Leyva-López, N., Gutierrez-Grijalva, E. P., Ambriz-Perez, D. L. & Heredia, J. B. (2016). Flavonoids as Cytokine Modulators: A Possible Therapy for Inflammation-Related Diseases. *International Journal of Molecular Sciences*, 17(6), 921. https://doi.org/10.3390/ijms17060921

Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X. & Yan, G. (2007). Antioxidant activity and phenolics of an endophytic Xylaria sp. from *Ginkgo biloba*. *Food Chemistry*, 105(2), 548-554. https://doi.org/10.1016/j.foodchem.2007.04.008

Liu, X.-G., Wu, S.-Q., Li, P. & Yang, H. (2015). Advancement in the chemical analysis and quality control of flavonoid in *Ginkgo biloba*. *Journal of Pharmaceutical and Biomedical Analysis*, 113, 212-225. https://doi.org/10.1016/j.jpba.2015.03.006

Lordan, R. (2021). Dietary supplements and nutraceuticals market growth during the coronavirus pandemic – Implications for consumers and regulatory oversight. *PharmaNutrition,* 18, (100282), 2213-4344. https://doi.org/10.1016/j.phanu.2021.100282

Magalhães, L. M., Santos, F., Segundo, M. A., Reis, S. & Lima, J. L. F. C. (2010). Rapid microplate high-throughput methodology for assessment of Folin-Ciocalteu reducing capacity. *Talanta*, 83(2), 441-447. https://doi.org/10.1016/j.talanta.2010.09.042

Magalhães, L. M., Segundo, M. A., Reis, S., Lima, J. L. F. C. & Rangel, A. O. S. S. (2006). Automatic Method for the Determination of Folin−Ciocalteu Reducing Capacity in Food Products. *Journal of Agricultural and Food Chemistry*, 54(15), 5241-5246. https://doi.org/10.1021/jf060324s

Memarzia, A., Khazdair, M. R., Behrouz, S., Gholamnezhad, Z., Jafarnezhad, M., Saadat, S. & Boskabady, M. H. (2021). Experimental and clinical reports on anti-inflammatory, antioxidant, and immunomodulatory effects of *Curcuma longa* and curcumin, an updated and comprehensive review. *BioFactors*, 47(3), 311-350. https://doi.org/10.1002/biof.1716

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. & McLaughlin, J. L. (1982). Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Medica*, 45(05), 31-34. https://doi.org/10.1055/s-2007-971236

Miceli, N., Marino, A., Köroğlu, A., Cacciola, F., Dugo, P., Mondello, L. & Taviano, M. F. (2020). Comparative study of the phenolic profile, antioxidant and antimicrobial activities of leaf extracts of five *Juniperus* L. (*Cupressaceae*) taxa growing in Turkey. *Natural Product Research*, 34(11), 1636-1641. https://doi.org/10.1080/14786419.2018.1523162

Organización Mundial de la Salud [OMS]. (2022) Noncommunicable diseases. https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases#:~:text=Cardiovascular%20diseases%20account%20for%20most,disease%20deaths%20caused%20by%20diabetes).

Panusa, A., Petrucci, R., Marrosu, G., Multari, G. & Gallo, F. R. (2015). UHPLC-PDA-ESI-TOF/MS metabolic profiling of *Arctostaphylos pungens* and *Arctostaphylos uva-ursi*. A comparative study of phenolic compounds from leaf methanolic extracts. *Phytochemistry*, 115, 79-88.

https://doi.org/10.1016/j.phytochem.2015.01.002

Pap, N., Reshamwala, D., Korpinen, R., Kilpeläinen, P., Fidelis, M., Furtado, M. M., Sant'Ana, A. S., Wen, M., Zhang, L., Hellström, J., Marnilla, P., Mattila, P., Sarjala, T., Yang, B., Lima, A. d. S., Azevedo, L., Marjomäki, V. & Granato, D. (2021). Toxicological and bioactivity evaluation of blackcurrant press cake, sea buckthorn leaves and bark from Scots pine and Norway spruce extracts under a green integrated approach. *Food and Chemical Toxicology*, 153, 112284. https://doi.org/10.1016/j.fct.2021.112284

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D. & Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, 2017, 8416763. https://doi.org/10.1155/2017/8416763

Platzer, M., Kiese, S., Herfellner, T., Schweiggert-Weisz, U. & Eisner, P. (2021). How Does the Phenol Structure Influence the Results of the Folin-Ciocalteu Assay? *Antioxidants*, 10(5), 811. https://doi.org/10.3390/antiox10050811

Prior, R. L. & Cao, G. (2000). Analysis of Botanicals and Dietary Supplements for Antioxidant Capacity: A Review. *Journal of AOAC International*, 83(4), 950-956. https://doi.org/10.1093/jaoac/83.4.950

Ramachandra, C. J. A., Cong, S., Chan, X., Yap, E. P., Yu, F. & Hausenloy, D. J. (2021). Oxidative stress in cardiac hypertrophy: From molecular mechanisms to novel therapeutic targets. *Free Radical Biology and Medicine*, 166, 297-312. https://doi.org/10.1016/j.freeradbiomed.2021.02.040

Russo, A., Acquaviva, R., Campisi, A., Sorrenti, V., Di Giacomo, C., Virgata, G., Barcellona, M. L. & Vanella, A. (2000). Bioflavonoids as antiradicals, antioxidants and DNA cleavage protectors. *Cell Biology and Toxicology*, 16(2), 91. https://doi.org/10.1023/A:1007685909018

Sabbatino, F., Conti, V., Liguori, L., Polcaro, G., Corbi, G., Manzo, V., Tortora, V., Carlomagno, C., Vecchione, C., Filippelli, A. & Pepe, S. (2021). Molecules and Mechanisms to Overcome Oxidative Stress Inducing Cardiovascular Disease in Cancer Patients. *Life*, 11(2), 105. https://doi.org/10.3390/life11020105

Salem, M. A., El-Shiekh, R. A., Fernie, A. R. & Alseekh, S. (2022). Metabolomics-based profiling for quality assessment and revealing the impact of drying of Turmeric (*Curcuma longa* L.). Scientific Reports, 12, 10288. https://doi.org/10.1038/s41598-022-13882-y

Schneider, I., Gibbons, S. & Bucar, F. (2004). Inhibitory Activity of *Juniperus communis* on 12(S)-HETE Production in Human Platelets. *Planta Medica*, 70(5), 471-474. https://doi.org/10.1055/s-2004-818980

Schroder, H. & Navarro, E. (2006). Effects and safety of antioxidant supplementation. *Current Topics in Nutraceutical Research*, 4(1), 23-33.

Sies, H. (2020). Oxidative Stress: Concept and Some Practical Aspects. *Antioxidants*, *9*(9), 852. https://doi.org/10.3390/antiox9090852

Smirnova, I. E., Kazakova, O. B., Loesche, A., Hoenke, S. & Csuk, R. (2020). Evaluation of cholinesterase inhibitory activity and cytotoxicity of synthetic derivatives of di- and triterpene metabolites from *Pinus silvestris* and *Dipterocarpus alatus* resins. *Medicinal Chemistry Research*, 29(8), 1478-1485. https://doi.org/10.1007/s00044-020-02566-9

Sun, Y., Li, M., Mitra, S., Hafiz Muhammad, R., Debnath, B., Lu, X., Jian, H. & Qiu, D. (2018). Comparative Phytochemical Profiles and Antioxidant Enzyme Activity Analyses of the Southern Highbush Blueberry (*Vaccinium corymbosum*) at Different Developmental Stages.

Revista Bio ciencias
ISSN 2007-3380

Molecules, 23(9), 2209. https://doi.org/10.3390/molecules23092209

Swain, T. & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, 10(1), 63-68. https://doi.org/10.1002/jsfa.2740100110

Taviano, M. F., Marino, A., Trovato, A., Bellinghieri, V., La Barbera, T. M., Güvenç, A., Hürkul, M. M., Pasquale, R. D. & Miceli, N. (2011). Antioxidant and antimicrobial activities of branches extracts of five *Juniperus* species from Turkey. *Pharmaceutical Biology*, 49(10), 1014-1022. https://doi.org/10.3109/13880209.2011.560161

Tawfeek, N., Sobeh, M., Hamdan, D. I., Farrag, N., Roxo, M., El-Shazly, A. M. & Wink, M. (2019). Phenolic Compounds from *Populus alba* L. and *Salix subserrata* Willd. (Salicaceae) Counteract Oxidative Stress in *Caenorhabditis elegans*. *Molecules*, 24(10), 1999. https://doi.org/10.3390/molecules24101999

Tegelberg, R., Virjamo, V. & Julkunen-Tiitto, R. (2018). Dry-air drying at room temperature – a practical pre-treatment method of tree leaves for quantitative analyses of phenolics?. *Phytochemical Analysis*, 29(5), 493-499. https://doi.org/10.1002/pca.2755

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19(6), 669-675. https://doi.org/10.1016/j.jfca.2006.01.003

Thitimuta, S., Pithayanukul, P., Nithitanakool, S., Bavovada, R., Leanpolchareanchai, J. & Saparpakorn, P. (2017). *Camellia sinensis* L. Extract and Its Potential Beneficial Effects in Antioxidant, Anti-Inflammatory, Anti-Hepatotoxic, and Anti-Tyrosinase Activities. *Molecules*, 22(3), 401. https://doi.org/10.3390/molecules22030401

Vezza, T., Canet, F., de Marañón, A. M., Bañuls, C., Rocha, M. & Víctor, V. M. (2020). Phytosterols: Nutritional Health Players in the Management of Obesity and Its Related Disorders. *Antioxidants*, 9(12), 1266. https://doi.org/10.3390/antiox9121266

Wanyoike, G. M., Chhabra, S. C.,Lang'at-Thoruwa, C. C. & Omar, S. A. (2004). Brine shrimp toxicity and antiplasmodial activity of five Kenyan medicinal plants. *Journal of Ethnopharmacology*, 9(1), 129-133. https://doi.org/10.1016/j.jep.2003.09.047

Wolsko, P. M., Solondz, D. K., Phillips, R. S., Schachter, S. C. & Eisenberg, D. M. (2005). Lack of herbal supplement characterization in published randomized controlled trials. *The American Journal of Medicine*, 118(10), 1087-1093. https://doi.org/10.1016/j.amjmed.2005.01.076

Yoshida, Y. & Niki, E. (2003). Antioxidant effects of phytosterol and its components. *Journal of Nutritional Science and Vitaminology,* 49(4), 277-280. https://doi.org/10.3177/jnsv.49.277

Zapata, S., Piedrahita, A. M. & Rojano, B. (2014). Capacidad atrapadora de radicales oxígeno (ORAC) y fenoles totales de frutas y hortalizas de Colombia. *Perspectivas en Nutrición Humana*, 16(1), 25-36. https://doi.org/10.17533/udea.penh.20310

Zhang, L., Ho, C.-T., Zhou, J., Santos, J. S., Armstrong, L. & Granato, D. (2019). Chemistry and Biological Activities of Processed Camellia sinensis Teas: A Comprehensive Review. *Comprehensive Reviews in Food Science and Food Safety*, 18(5), 1474-1495. https://doi.org/10.1111/1541-4337.12479

Zhang, Y.-J., Gan, R.-Y., Li, S., Zhou, Y., Li, A.-N., Xu, D.-P. & Li, H.-B. (2015). Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules*, 20(12), 21138-21156. https://doi.org/10.3390/molecules201219753