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Effects of aqueous and ethanolic extracts of *Lepidium meyenii* Walp (red and black maca) on *Caenorhabditis elegans*

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ABSTRACT. *Lepidium meyenii* (maca) is a Peruvian nutraceutical plant, whose hypocotyl has a variety of colors ranging from black to white. The black and red varieties of maca have been the most studied since their extracts are associated with effects such as increased sperm count, decreased glucose levels, reversal of prostatic hyperplasia, among others. However, the properties related to reduction of oxidative stress, metabolic diseases and anti-aging have not yet been confirmed. The aim was to evaluate the effects of aqueous and ethanolic extracts, obtained from spray-dried hydroalcoholic extract of hypocotyl of black and red maca (*Lepidium meyenii*), on mortality, growth, reproduction, lipid accumulation and the expression levels of genes related to oxidative stress and heat shock, in the *in-vivo* model, *Caenorhabditis elegans* (*C. elegans*), using different concentrations. The results showed that maca extracts were not toxic to the model at concentrations below 100 mg L^{-1}. However, higher concentrations caused high mortality, growth disturbances, oxidative stress and lipid accumulation. Black maca extracts increased the reproduction of *C. elegans* by increasing the number of offspring in *C. elegans*, both in aqueous and ethanolic extracts. On the other hand, ethanolic extracts produced an increase in the expression of genes related to oxidative stress, indicating a mild stressor behavior of the same. *C. elegans* represents an established model for evaluating the biological properties of nutraceutical plants of biological interest and can be used in the search for antioxidant activity of *L. meyenii* (hypocotyl), as well as it can be used in future studies to identify some metabolites involved in each biological property and to understand the biochemical and molecular mechanisms involved in these properties.

Keywords: Reproduction; lipid accumulation; *Lepidium meyenii*; *Caenorhabditis elegans*; ethanolic extract; aqueous extract.

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Introduction

Lepidium meyenii is an herbaceous plant of the family Brassicaceae, commonly known as maca, which grows in the Peruvian Andes at an altitude between 4,000 and 4,500 meters (Gonzales, 2011; Marin-Bravo, 2003). This plant is made up of a small aerial part and a voluminous reserve root, called hypocotyl. This present color variations, called ecotypes, ranging from white to black and each one has different biological properties. Color variation is due to a difference in the proportion and content of secondary metabolites (Yábar, Pedreschi, Chirinos, & Campos, 2011; Gonzales, Villaorduña, Gasco, Rubio, & Gonzales, 2014).

The main metabolites present in extracts of *L. meyenii* are: alkaloids, glucosinalates, tannins, saponins, phenols, flavonoid steroids, glycosides and other chemical components: uridine, macanes and macamides (Gonzales et al., 2006a; Gonzales, 2012; Leitão et al., 2020). The Peruvian natives attribute to these metabolites biological properties, such as: treatment of stress, fatigue, fertility enhancer for both humans and animals, analgesic effects, treatment of respiratory disorders, laxative effect and cure anemia (León, 1964; Gonzales, 2011; Gonzales, Gonzales, & Gonzales-Castañeda, 2009). Nowadays, there is a wide variety of pharmaceuticals and food products derived from *L. meyenii*, such as powders, pills, capsules, flour, liquor and extracts, distributed and marketed worldwide, However, the scientific evidence is still insufficient to corroborate all these properties. (Borrelli, Colalto, Delfino, Iriti, & Izzo, 2018; Leitão Peres et al., 2020).

The most studied ecotypes have been black, red and yellow. Aqueous and hydroalcoholic extracts of black maca improved the daily production and mobility of sperm in rodents (Gonzales, Gasco, Córdova, & Chung, 2004; Gonzales, Nieto, Rubio, & Gasco, 2006; Gonzales, Gonzales-Castañeda, & Gasco, 2013), reduced by 50%

the value of blood glucose in diabetes induced rats (Gonzales et al., 2013; Wang & Zhu, 2019) and reverse cognitive damage induced by scopolamine in male mice (Liu et al., 2011). On the other hand, lyophilized aqueous extracts of red maca were able to reverse benign prostatic hyperplasia in male rats (Gonzales et al., 2006a; Wang & Zhu, 2019), and ethanolic extracts of red maca prevented bone loss in ovariectomized rats (Gonzales et al., 2010). The aqueous extracts of yellow maca have been shown to increase the physical endurance of male rats, an effect similar to that of an energizer (Choi et al., 2012).

Biological models, such as rodents (mice and rats), have been useful in searching for the biological properties of *L. meyenii* (Ruiz-Luna et al., 2005; Gonzales et al., 2006a; Gonzales et al., 2006; Liu et al., 2011); however, these models are characterized by long life cycles that make it difficult to assess the effect of *L. meyenii* on oxidative stress, metabolic diseases and anti-aging (Beharry & Heinrich, 2018). In contrast, *Caenorhabditis elegans* is a free-living, hermaphrodite nematode, with a transparent body, easily manipulated and contains approximately 40% human orthologous genes (Andersen et al., 2012), which makes it an *in-vivo* model, allowing the evaluation of different substances distributed in the environment, such as environmental toxins (García-Espiñeira et al., 2018a), antioxidant and neuroprotective drugs (Thabit et al., 2018) and in some plants extracts, such as *Polygonum multiflorum*, *Cassia fistula*, *Paullinia cupana*, and *Gracilaria lemaneiformis* (Ferreira Boasquívis et al., 2018; Saier, Büchter, Koch, & Wätjen, 2018; Thabit et al., 2018; Wang et al., 2019).

For this reason, this study used the *C. elegans* model, to evaluate the effect of aqueous and ethanolic extracts of the black and red ecotypes of *L. meyenii* on different endpoints biological and molecular of mortality, growth, reproduction, lipid accumulation, expression of oxidative stress and heat shock genes.

Material and methods

Preparation of aqueous and ethanolic extracts of *L. meyenii*

Spray-dried hydroalcoholic extract of black and red *L. meyenii* packed in sachets containing each 3 g maca extract, was diluted in K medium (milli-Q water, NaCl, KCl), and subsequently, solutions at different concentrations (10,000; 1,000; 100; 10; 1 and 0.1 mg L^{-1}) were prepared in 10 mL. To obtain the ethanolic extract of *L. meyenii*, 3 g spray-dried extract of black and red maca were exposed to ethanol for 24 hours, and then filtered (Whatman filter), for recovery using a rotary evaporator. All extracts were diluted in 5 mL K medium to be subsequently used at the concentrations of 100; 10; 1 and 0.1 mg L^{-1} (Gonzales-Arimborgo et al., 2016; Zevallos-Concha et al., 2016).

Biological model

Caenorhabditis elegans N2 Bristol Wild-type strain and transgenic strains with green fluorescent protein (GFP) introduced into genes encoding heat shock protein (hsp-3) and oxidative stress (sod-4, gpx-4, gpx-6), CGC strains owned by the Laboratory of the BIOTOXAM Research Group, were cultivated in Petri dishes at 20°C with NMG, nematode growth medium, (NaCl, agar, peptone, Cholesterol, KCl, $CaCl_2$, $MgSO_4$) and *Escherichia coli* OP50 as a source of food (Hunt, 2017). To obtain a homogeneous population, nematodes were synchronized by suspension in an alkaline solution composed of 5.25% hypochlorite and NaOH, whose function is to oxidize the organism without destroying the eggs (Tejeda-Benitez & Olivero-Verbel, 2015).

Mortality assay

L4 nematodes were aliquoted in K medium and exposed for 24 hours to solutions of ethanolic extracts of black maca (EBM) and red maca (ERM) and aqueous extracts of black maca (ABM) and red maca (ARM) in 96-well plates, adding approximately 10±3 nematodes per well. Subsequently, living and dead nematodes were counted by visual inspection, using a dissecting microscope. Nematodes were classified as dead by not responding to physical stimuli. The results were compared to a control group exposed to K medium (Wu, Qu, Li, & Wang, 2012).

Growth assay

For the growth assay, L1 nematodes were exposed for 48 hours to different concentrations of EBM, ERM, ABM and ARM, with inoculation of *E. coli* OP50, every 24 hours. After exposure, nematodes were heated to 50°C, thus their bodies adopt a straight position. Approximately 30 nematodes per treatment were analyzed under Leica ICC50 microscope, under a 10X objective lens and body length was determined using Image J software (Yu, Zhang, & Yin, 2016; Woodruff, Johnson, & Phillips, 2019).

Lipid accumulation assay with 'quick oil red O' (qORO)

Synchronized Bristol N2 wild type nematodes at the L4 larval stage were exposed for 24 hours to solutions of EBM, ERM, ABM and ARM. For this, the dye solution was prepared from a high quality 0.5% red oil stock solution in 100% isopropanol, incubated at room temperature for one day, and then filtered through a 0.45 µm pore membrane, according to the manufacturer's recommendations. The stock solution was diluted to 60% with milli-Q water the day before use, and then incubated at room temperature overnight. To perform the assay, 200 µL high-quality 60% isopropanol was added to nematodes previously exposed to maca solutions. Nematodes were settled at the bottom of the well and aspirated 175 µL buffer. Then, 200 µL qORO working solution was added. Nematodes were exposed for 18-24 hours, at 20°C. Finally, 100 µL 0.01% Triton X-100 was used as a preservative. Images were captured using a Leica ICC50 microscope, under a 10X objective lens (García-Espiñeira et al., 2018b; Wählby et al., 2014).

Reproduction assay

The L4-synchronized N2 nematodes were transferred individually to Petri dishes containing NGM and *E. coli* OP50 exposed to different concentrations of EBM, ERM, ABM, ARM and control. They were observed under the microscope at 10X every day for five days, and the number of offspring at all stages was counted after 24 hours. Approximately 10 nematodes were examined per treatment (Paiva et al., 2015).

Gene expression

Effects of *L. meyenii* on gene expression were determined using GFP transgenic *C. elegans* strains containing the tagged genes 'hsp-3, sod-4, gpx-4 and gpx-6'. Equal aliquots of nematodes at all larval stages were placed in 96-well microplates exposed to solutions of EBM, ERM, ABM, ARM and control. Plates were incubated at 20°C for 24 hours, the absorbance was read using a 620 nm filter plate reader (Multiskan FC, Thermo Scientific). The relative absorbance was calculated as the ratio of the solution absorbance to the control absorbance (Equation 1) (García-Espiñeira et al., 2018b).

$$\text{Relative absorbance} = \frac{\text{Absorbance after 24 hours of exposure} - \text{Initial absorbance}}{\text{Control absorbance}} \quad (1)$$

Statistical analysis

The GraphPad Prism 6 software was used to test significant differences between the means by two-way ANOVA. In addition, Dunnett's test was applied to compare each dilution with the control. The level of significance was set at $p < 0.05$.

Results

Results of mortality assays are shown in Figure 1; only nematodes exposed to concentrations above 1,000 mg L^{-1} aqueous extracts of black and red maca showed a significant difference from control. The highest mortality was found at 1,000 mg L^{-1} ABM, followed by ARM at this same concentration (Figure 1A). Nematodes exposed to concentrations below 100 mg L^{-1} showed a behavior like the control (Figure 1A). On the other hand, the mortality results for ethanolic extracts of *L. meyenii* showed no significant difference from the control (Figure 1B).

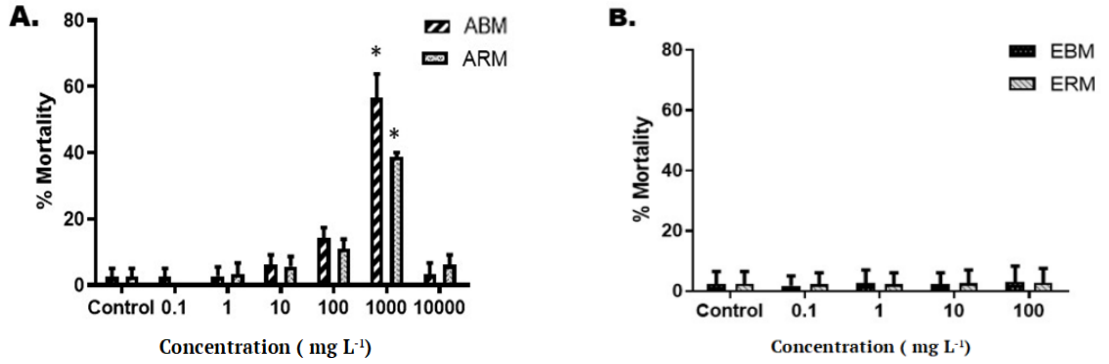


Figure 1. Mortality of *Caenorhabditis elegans* exposed to extracts of *Lepidium meyenii*. A) Mortality of *C. elegans* exposed to aqueous extracts of black and red maca. B) Mortality of *C. elegans* exposed to ethanolic extracts of black and red maca. *Significant difference from the control ($p < 0.05$).

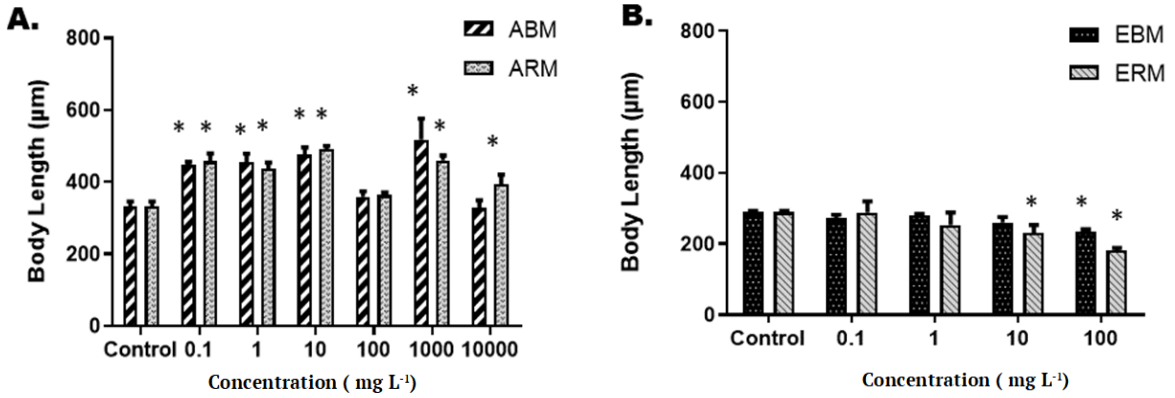


Figure 2. Body length of *Caenorhabditis elegans* exposed to extracts of *Lepidium meyenii*. A) body length of *C. elegans* exposed to aqueous extracts of black and red maca; B) body length of *C. elegans* exposed to ethanolic extracts of black and red maca. *Significant difference from the control ($p < 0.05$).

Variations in the nematode body length after treatment with aqueous extracts of *L. meyenii* were similar in both ecotypes, showing a significant increase in all concentrations (except for the concentrations of 100 mg L^{-1} and 10,000 mg L^{-1} black maca). The maximum growth was found for nematodes exposed to the concentration of 1,000 mg L^{-1} black maca, followed by nematodes subjected to 10 mg L^{-1} red maca. None concentration inhibited nematode growth (Figure 2A).

Ethanolic extracts caused a decrease in the nematode body length at the highest concentrations of both maca ecotypes (100 mg L^{-1}), particularly red maca also caused a decrease in body length at the 10 mg L^{-1} concentration. For the other concentrations tested, there were no significant differences from the control group (Figure 2B).

In Figure 3, the significant difference in the brood size of nematodes exposed to different concentrations of *L. meyenii* extracts and the control was considerable. An increase was observed in the brood size of nematodes exposed to ABM and EBM, at the lowest concentrations (0.1; 1; 10 mg L^{-1}) mainly at 0.1 mg L^{-1} EBM, as illustrated in Figures 3A and 3C, while ARM and ERM showed no significant differences from the control (Figures 3B and 3D).

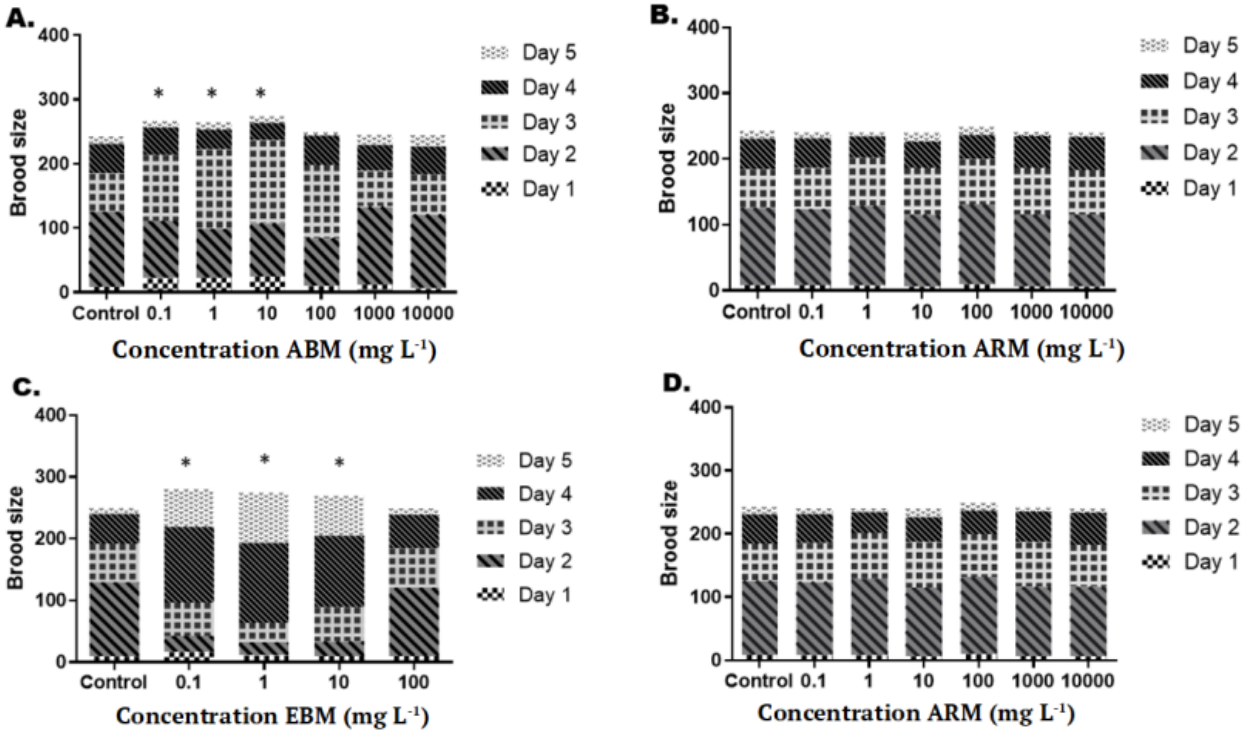


Figure 3. Reproduction of *Caenorhabditis elegans* exposed to extracts of *Lepidium meyenii*. A) Brood size of *C. elegans* exposed to aqueous extracts of black maca. B) Brood size of *C. elegans* exposed to aqueous extracts of red maca. C) Brood size of *C. elegans* exposed to ethanolic extracts of black maca. D) Brood size of *C. elegans* exposed to ethanolic extracts of red maca. *Significant difference from the control ($p < 0.05$).

Nematodes exposed to the concentrations where the brood size was increased, the maximum egg-laying was observed on the third day, for aqueous extracts, and on the fourth day, for ethanolic extracts. In turn, for the other nematodes, the maximum egg-laying was found on the second day, with no significant differences from the control, as evidenced in Figure 3.

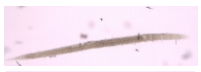
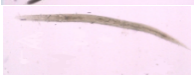
Results for lipid accumulation assays with quick oil red O (qORO), after exposure to aqueous and ethanolic extracts of maca, are presented in Tables 1 and 2. Lipid accumulation was visible in nematodes exposed to concentrations of 1,000 mg L^{-1} ABM and 10,000 mg L^{-1} of both aqueous extracts, the lipid deposit was more pronounced in nematodes exposed to red maca (Table 1).

Table 1. qORO staining of nematodes exposed to aqueous extracts of black and red maca.

Concentrations (mg L^{-1})	ABM	ARM
0		
0.1		
1		
10		
100		
1,000		
10,000		

Similarly, nematodes exposed to ethanolic extracts showed lipid accumulation only at the highest concentration of red maca (Table 2). The other nematodes showed a pattern similar to the control.

Table 2. qORO staining of nematodes exposed to ethanolic extracts of black and red maca.

Concentrations (mg L^{-1})	EBM	ERM
0		
0.1		
1		
10		
100		

All genes related to oxidative stress and heat shock (gpx-6, sod-4, gpx-4 and hsp-3) in nematodes exposed to both extracts showed a similar pattern, with high expression levels at concentrations of 1,000 and 10,000 mg L^{-1} of both varieties (Figure 4).

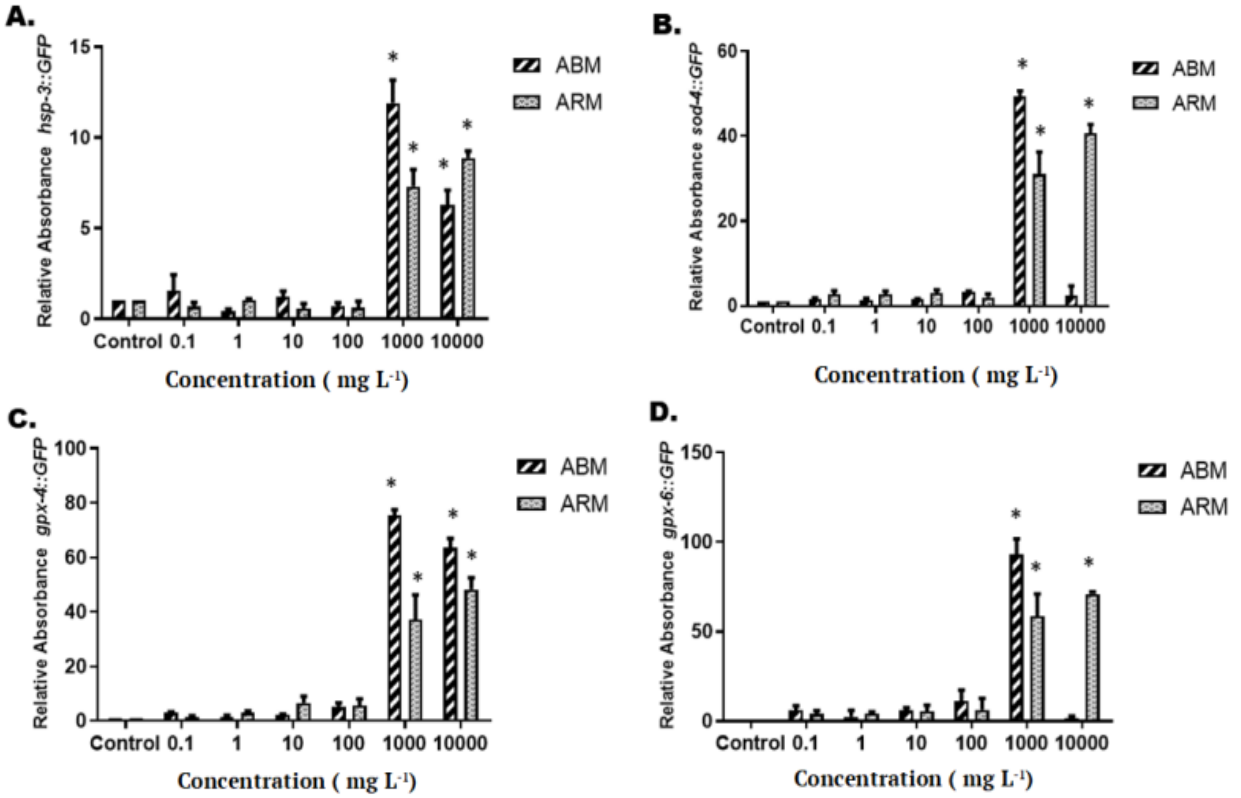


Figure 4. Changes in levels of gene expression in *Caenorhabditis elegans* exposed to aqueous extracts of black and red maca compared to the control (K medium). A) Gene expression levels of the hsp-3 GPF gene. B) Gene expression levels of the sod-4 GPF gene. C) Gene expression levels of the gpx-4 GPF gene. D) Gene expression levels of the gpx-6 GPF gene. *Significant difference from the control ($p < 0.05$).

The markers for oxidative stress, 'gpx-4' and 'sod-4', showed significant differences in levels of expression at all concentrations of ethanolic extracts of both maca varieties, except for the concentrations 10 and 1 mg L^{-1} black maca in the transgenic strain sod-4 (Figures 5B and 5C). Changes in the expression of the 'gpx-6' strain were not significant for red maca, however for black maca, it increased in all concentrations, except for the 10 mg L^{-1} (Figure 5D). Regarding the expression of the gene related to heat shock, both ethanolic extracts of maca showed high levels of gene expression at concentrations of 10 and 100 mg L^{-1} (Figure 5A).

Discussion

The composition of different commercial presentations of maca can be affected by preparation and prosecution, causing an increase or decrease in the concentration of specific metabolites, that is, there is a variation in the intake of these compounds, depending on the way maca is processed processed (flours, extracts, capsules, liqueurs, etc.) and therefore in the therapeutic effects associated (Gonzales, 2011). The products evaluated in the present study are spray-dried extracts, prepared from dry maca hypocotyls using 70% ethanol as solvent and characterized by Gonzales-Arimborgo et al., 2016 using nuclear magnetic resonance. In this same study, the effect of oral ingestion of the product on the population health was evaluated, demonstrating that prolonged consumption of these extracts improves mood, energy and health. It also proposed the use of these extracts for clinical therapies, for which toxicity studies in animal models are necessary (Gonzales-Arimborgo et al., 2016; Wang & Zhu, 2019; Yábar & Reyes, 2019; Leitão Peres et al., 2020; Tafuri et al., 2021).

C. elegans has been used as a model for toxicological tests, providing data from a complete animal with metabolically active digestive, reproductive, endocrine, sensory and neuromuscular systems, in addition to its low cost and easy handling *in vitro*. Further, the toxic mode of action of some substances on *C. elegans* is conserved in mammals (Clavijo et al., 2016; Tejeda-Benitez & Olivero-Verbel, 2016; Honnen, 2017; Hunt, 2017).

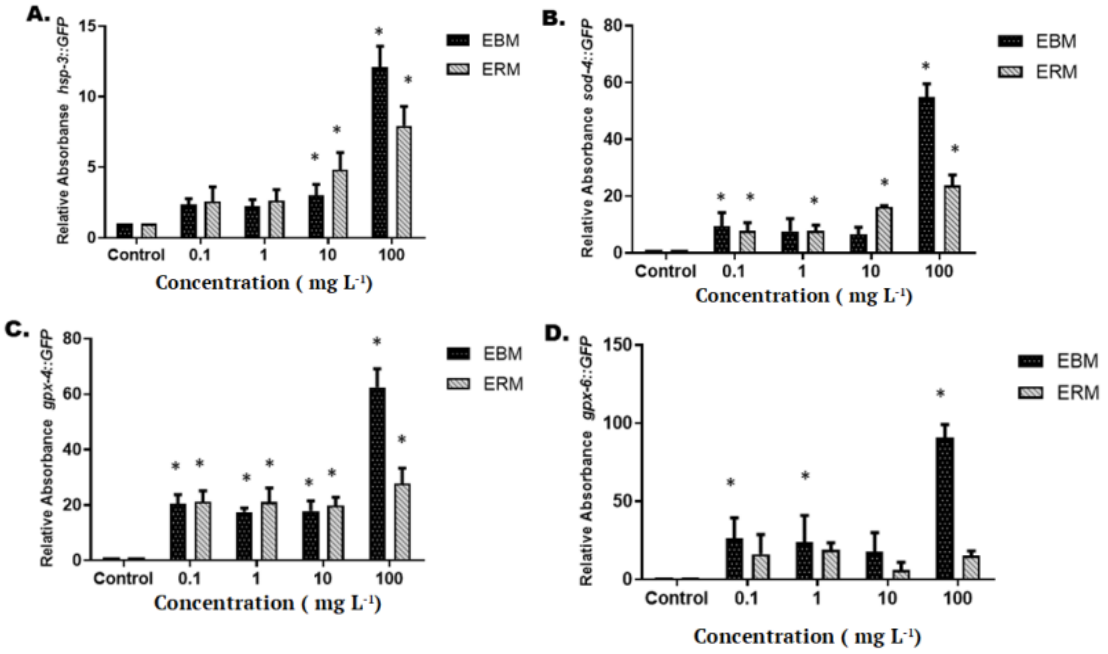


Figure 5. Changes in levels of gene expression in *Caenorhabditis elegans* exposed to ethanolic extracts of black and red maca compared to the control (K medium). A) Gene expression levels of the hsp-3 GPF gene. B) Gene expression levels of the sod-4 GPF gene. C) Gene expression levels of the gpx-4 GPF gene. D) Gene expression levels of the gpx-6 GPF gene. *Significant difference from the control ($p < 0.05$).

In this study, toxicity of extracts of *L. meyenii* was evaluated; ethanolic maca extracts showed no significant mortality in the *C. elegans* model, as did nematodes exposed to concentrations equal to or less than 100 mg L^{-1} aqueous maca extracts. However, at high concentrations it threatens the vitality of the model causing high mortality, increased body length, lipid accumulation and induction of oxidative stress (García-Espiñeira et al., 2018a; García-Espiñeira et al., 2018b; Cai et al., 2020; Miao et al., 2020).

The growth in *C. elegans* consequently can be affected by environmental factors, where energy resources can be redirected to different developmental processes, such as growth (Lingala & Ghany, 2016). *C elegans* growth is highly regulated by transcription factors, such as nhr-86, hlh-12, nhr-66 and nhr-31 and genes related to the migration of cells associated the neural channels. Some food or environmental substances are capable of interfering with the correct expression of these factors, resulting in changes in body length (Forrester & Garriga, 1997; Lingala & Ghany, 2016). In this case, aqueous extracts of *L. meyenii* increased the body length of *C. elegans*, thus indicating that transcription factors responsible for growth could be positively regulated. Ethanolic extracts showed a decrease in body length, at high concentrations, showing that these extracts can interfere with the expression of transcription factors or expression of genes related to neuronal channels (Lee & Kang, 2017; Nagar, Singh, & Parveen, 2020; Parra-Guerra & Olivero-Verbel, 2020). Nevertheless, this last result is comparable with the evaluation of the toxicity of ethanolic extracts of *Peganum harmala* (Chinese nutraceutical plant), where similar concentrations decreased the body length of the nematode. In this case, anomalies in the development of nematodes exposed to *Peganum harmalase* extracts are ascribed to combined effects on the nervous system and the Insulin/IGF-1 signaling pathway (Miao et al., 2020), for which these may be the pathways affected by ethanolic extracts of *L. meyenii,* however, additional studies are required to check this hypothesis.

It has been reported that the increase in nematode length is related to lipid accumulation (Lingala & Ghany, 2016; Clark, Meade, Ranepura, Hall, & Savage-Dunn, 2018; Yang, Shao, Wu, & Wang, 2020), however, nematodes showing greater lipid retention showed no significant increase in body length, so it is unlikely that lipid accumulation is the cause of growth. Lipid metabolism in *C. elegans* can be affected by factors like temperature and food, where the excess of lipids are stored in the intestine of the worm, in the form of droplets (Watts & Ristow, 2017). Extracts of plants like cranberries (Sun, Yue, Shen, Yang, & Park, 2016) or plants metabolites like hesperidin (Peng et al., 2016) have a positive effect on the regulation of lipid metabolism. For the particular case of *Lepidium meyenii*, no study has assigned obesogenic properties to this plant, however, we reported that at high concentrations, mainly red maca, can cause a slight accumulation of lipids in the model compared to the control (K medium).

The reproduction of *C. elegans* exposed to aqueous and ethanolic extracts of black maca showed an increase in brood size at the same concentrations (0.1, 1 and 10 mg L^{-1}), indicating that this ecotype can increase brood size of the nematode (Figure 3). This result corroborates Ruiz-Luna et al. (2005) who found that the aqueous extract of black maca increased the pregnancy rate, the litter size and the survival rate of rodent offspring (Ruiz-Luna et al., 2005). Additionally, *L. meyenii* has effects on spermatogenesis in mice and rats, increasing the sperm count and protecting against damage caused by altitude, environmental pollutants, and drugs, such as malathion and cyclophosphamide. In all cases, black maca extracts showed a better biological activity on spermatogenesis than other varieties (Gonzales et al., 2004; Bustos-Obregón, Yucra, & Gonzales, 2005; Chung, Rubio, Gonzales, Gasco, & Gonzales, 2005; Gonzales et al., 2006a; Melnikovova et al., 2021; Tafuri et al., 2021). The extracts of *L. meyenni* has also been combined with plants with similar biological functions, resulting in a significantly higher frequency of ejaculation in mice (Zhang, Zhou, & Ge, 2019).

In Figures 3A and 3C, a change in the egg-laying pattern is shown in those nematodes that increased the brood size. A similar phenomenon occurred with mice treated with aqueous extracts of black maca, where changes in the spermatogenesis pattern were reported (Gonzales et al., 2004; Bustos-Obregón et al., 2005; Chung et al., 2005; Gonzales et al., 2006). In addition, an *in vivo* study on bovines showed that maca has the ability to increase sperm motility with an increase in ejaculate volume without increasing sperm concentration, that is, for bovines, maca extracts improve semen quality, without increasing the sperm count (Clément et al., 2010).

Double-blind clinical studies conducted in male, infertile patients showed no significant increases in sperm concentration in those treated with extracts of *L. meyenii* and placebo. (Melnikovova, Fait, Kolarova, Fernandez, & Milella, 2015; Alcalde & Rabasa, 2020; Melnikovova et al., 2021). Therefore, the effect of black maca on reproduction are still dubious regarding the ability of *L. meyenii* extracts to improve human reproduction.

The improvement in fertility caused by *L. meyenii* is attributed to macamides, secondary amides composed of benzylamine and fatty acids, the main bioactive compound of this plant (Zheng et al., 2000; Gonzales, 2012; Gonzales-Arimborgo et al., 2016; Zhu et al., 2020). The presence of these compounds in aqueous and ethanolic extracts was already reported (Sun et al., 2018; Wang & Zhu, 2019; Zhou et al., 2018; Zhu et al., 2020). For the particular case of atomized extracts of *L. meyenii* used in this investigation, they were characterized by Gonzales-Arimborgo et al. (2016) who showed the presence of macamides, in a greater proportion in black maca (Gonzales-Arimborgo et al., 2016). The foregoing may justify that only this ecotype caused the improvement in reproduction in *C. elegans*.

Finally, results of oxidative stress and heat shock were very different between the two types of extracts evaluated. The aqueous extract did not cause the increase in the expression of genes responsible for regulating free radicals, at concentrations below 100 mg L^{-1}, while ethanolic extracts increased the expression of genes susceptible to oxidative stress in *C. elegans*, acting as a mild expressor (Zevallos-Concha et al., 2016).

Conclusion

This is the first study evaluating the effect of *L. meyenii* extracts on *C. elegans*. *L. meyenii*, does not cause toxicity on *C. elegans* at concentrations below 100 mg L^{-1}. The most revealing effect of *L. meyenii* (black maca) on the biological model increase the offspring of nematodes and alter the egg laying pattern. It is recommended for future studies to analyze the mechanisms by which these extracts improve fertility and the possible effect on endocrine disruption of the model. Despite being an uncomplicated nematode, *C. elegans* is a model for the *in vivo* evaluation of presumptive biological properties of nutraceutical plants, such as *L. meyenii*, and the possible adverse effects.

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References

Alcalde, A. M., & Rabasa, J. (2020). Does *Lepidium meyenii* (Maca) improve seminal quality ? *Journal of Andrology, 5*2(10), e13755. DOI: https://doi.org/10.1111/and.13755

Andersen, E. C., Gerke, J. P., Shapiro, J. A., Jonathan, R., Ghosh, R., Bloom, J. S., ... Monod, J. (2012). Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. *HHS Public Access*, *44*(3), 285-290. DOI: https://doi.org/10.1038/ng.1050

Beharry, S., & Heinrich, M. (2018). Is the hype around the reproductive health claims of maca (*Lepidium meyenii* Walp.) justified? *Journal of Ethnopharmacology*, *211*(1), 126-170. DOI: https://doi.org/10.1016/j.jep.2017.08.003

Borrelli, F., Colalto, C., Delfino, D. V., Iriti, M., & Izzo, A. A. (2018). Herbal dietary supplements for erectile dysfunction: a systematic review and meta-analysis. *Drugs*, *78*(6), 643-673.

Bustos-Obregón, E., Yucra, S., & Gonzales, G. F. (2005). *Lepidium meyenii* (Maca) reduces spermatogenic damage induced by a single dose of malathion in mice. *Asian Journal of Andrology, 7*(1), 71-76. DOI: https://doi.org/10.1111/j.1745-7262.2005.00006.x

Cai, H., Xu, Y., Tang, S., Yang, X., Zou, Y., Wang, X., ... Meng, L. (2020). Toxicology in vitro systemic toxicity evaluation of novel tobacco products in *Caenorhabditis elegans*. *Toxicology in Vitro*, *62*(1), 104671. DOI: https://doi.org/10.1016/j.tiv.2019.104671

Choi, E. H., Kang, J. I, Cho, J. Y., Lee, S. H., Kim, T. S., Yeo, I. H., & Chun, H. S. (2012). Supplementation of standardized lipid-soluble extract from maca (*Lepidium meyenii*) increases swimming endurance capacity in rats. *Journal of Functional Foods*, *4*(2), 568-573. DOI: https://doi.org/10.1016/j.jff.2012.03.002

Chung, F., Rubio, J., Gonzales, C., Gasco, M., & Gonzales, G. F. (2005). Dose-response effects of *Lepidium meyenii* (Maca) aqueous extract on testicular function and weight of different organs in adult rats. *Journal of Ethnopharmacol, 98*(1-2), 143-147. DOI: https://doi.org/10.1016/j.jep.2005.01.028

Clark, J. F., Meade, M., Ranepura, G., Hall, D. H., & Savage-Dunn, C. (2018). *Caenorhabditis elegans* DBL-1 / BMP regulates lipid accumulation via interaction with insulin signaling. *Genes Genomes Genetics*, *8*(1), 343-351. DOI: https://doi.org/10.1534/g3.117.300416

Clavijo, A., Kronberg, M. F., Rossen, A., Moya, A., Calvo, D., Salatino, S. E., ... Munarriz, E. R. (2016). The nematode *Caenorhabditis elegans* as an integrated toxicological tool to assess water quality and pollution. *Science of the Total Environment*, *569-570*(1), 252-261. DOI: https://doi.org/10.1016/j.scitotenv.2016.06.057

Clément, C., Diazgrados, D. A., Avula, B., Khan, I. A., Mayer, A. C., Aguirre, D. D. P., ... Kreuzer, M. (2010). Influence of colour type and previous cultivation on secondary metabolites in hypocotyls and leaves of maca (*Lepidium meyenii* Walpers). *Journal of the Science of Food and Agriculture*, *90*(5), 861-869. DOI: https://doi.org/10.1002/jsfa.3896

Ferreira Boasquívis, P., Silva, Martins, M. G., Aparecida Paiva, F., Marinho Cavalcanti, R., Nunez, C. V., & Paula Oliveira, R. (2018). Guarana (*Paullinia cupana*) Extract protects *Caenorhabditis elegans* models for Alzheimer disease and Huntington disease through activation of antioxidant and protein degradation pathways. *Oxidative Medicine and Cellular Longevity*, *2018*(1), 9241308. DOI: https://doi.org/10.1155/2018/9241308

Forrester, W. C., & Garriga, G. (1997). Genes necessary for *C. elegans* cell and growth cone migrations. *Development*, *124*(9), 1831-1843. DOI: 10.1242/dev.124.9.1831

García-Espiñeira, M. C., Tejeda-Benitez, L., & Olivero-Verbel, J. (2018a). Ecotoxicology and environmental safety toxicity of atrazine- and glyphosate-based formulations on *Caenorhabditis elegans*. *Ecotoxicology and Environmental Safety*, *156*(1), 216-222. DOI: https://doi.org/10.1016/j.ecoenv.2018.02.075

García-Espiñeira, M. C., Tejeda-Benítez, L. P., & Olivero-Verbel, J. (2018b). Toxic effects of bisphenol A, propyl paraben, and triclosan on *Caenorhabditis elegans*. *International Journal of Environmental Research and Public Health*, *15*(4)., 684. DOI: https://doi.org/10.3390/ijerph15040684

Gonzales, C., Cárdenas-Valencia, I., Leiva-Revilla, J., Anza-Ramirez, C., Rubio, J., & Gonzales, G. F. (2010). Effects of different varieties of Maca (*Lepidium meyenii*) on bone structure in ovariectomized rats. *Forschende Komplementarmedizin, 17*(3), 137-143. DOI: https://doi.org/10.1159/000315214

Gonzales, C., Rubio, J., Gasco, M., Nieto, J., Yucra, S., & Gonzales, G. F. (2006a). Effect of short-term and long-term treatments with three ecotypes of *Lepidium meyenii* (MACA) on spermatogenesis in rats. *Journal of Ethnopharmacology*, *103*(3), 448-454. DOI: https://doi.org/10.1016/j.jep.2005.08.035

Gonzales, G. F. (2011). Maca, producto de bandera del Perú : de la tradición a la ciencia. *Acta Andina*, *11*(1), 56-67.

Gonzales, G. F. (2012). Ethnobiology and ethnopharmacology of *Lepidium meyenii* (Maca), a plant from the peruvian highlands. *Evidence-Based Complementary and Alternative Medicine*, *2012*(1), 1-10. DOI: https://doi.org/10.1155/2012/193496

Gonzales, G. F., Gasco, M., Córdova, A., & Chung, A. (2004). Effect of *Lepidium meyenii* (Maca) on spermatogenesis in male rats acutely exposed to high altitude (4340 m). *The Journal of Endocrinology, 180*(1), 87-95. DOI: https://doi.org/10.1677/joe.0.1800087

Gonzales, G. F., Gonzales, C., & Gonzales-Castañeda, C. (2009). *Lepidium meyenii* (Maca): A plant from the highlands of peru - From tradition to science. *Forschende Komplementarmedizin*, *16*(6), 373-380. DOI: https://doi.org/10.1159/000264618

Gonzales, G. F., Gonzales-Castañeda, C., & Gasco, M. (2013). A mixture of extracts from Peruvian plants (black maca and yacon) improves sperm count and reduced glycemia in mice with streptozotocin-induced diabetes. *Toxicology Mechanisms and Methods*, *23*(7), 509-518. DOI: https://doi.org/10.3109/15376516.2013.785656

Gonzales, G. F., Nieto, J., Rubio, J., & Gasco, M. (2006). Effect of Black maca (*Lepidium meyenii*) on one spermatogenic cycle in rats. *Andrologia*, *38*(5), 166-172. DOI: https://doi.org/10.1111/j.1439-0272.2006.00733.x

Gonzales, G. F., Villaorduña, L., Gasco, M., Rubio, J., & Gonzales, C. (2014). Maca (*Lepidium meyenii* Walp), a review of its biological properties. *Revista Peruana de Medicina Experimental y Salud Publica*, *31*(1), 100-110.

Gonzales-Arimborgo, C., Yupanqui, I., Montero, E., Alarcón-Yaquetto, D. E., Zevallos-Concha, A., Caballero, L., ... Gonzales, G. F. (2016). Acceptability, safety, and efficacy of oral administration of extracts of black or red maca (*Lepidium meyenii*) in adult human subjects: a randomized, double-blind, placebo-controlled study. *Pharmaceuticals*, *9*(3), 49. DOI: https://doi.org/10.3390/ph9030049

Honnen, S. (2017). *Caenorhabditis elegans* as a powerful alternative model organism to promote research in genetic toxicology and biomedicine. *Archives of Toxicology, 91*(1), 2029-2044.

Hunt, P. R. (2017). The *C. elegans* model in toxicity testing. *Journal of Applied Toxicology*, *37*(1), 50-59. DOI: https://doi.org/10.1002/jat.3357

Lee, S. Y., & Kang, K. (2017). Measuring the effect of chemicals on the growth and reproduction of *Caenorhabditis elegans*. *Jornal of Visualized Experiments*, *128*(1), 1-5. https://doi.org/10.3791/56437

León, J. (1964). The 'Maca' (*Lepidium meyenii*), a little known food plant of peru. *Economic Botany*, *18*(1), 122-127. DOI: https://doi.org/10.1007/BF02862707

Lingala, S. M., & Ghany, M. G. M. (2016). Many transcription factors contribute to *C. elegans* growth and fat storage akihiro. *HHS Public Access*, *25*(3), 289-313. DOI: https://doi.org/10.1007/s11065-015-9294-9.Functional

Liu, X., Rubio, J., Qiong, W., Jiang, Z., Dang, H., Chen, S. L., & Gonzales, G. F. (2011). Aqueous extract of Black maca (*Lepidium meyenii*) on memory impairment induced by ovariectomy in mice. *Evidence-Based Complementary and Alternative Medicine, 2011*(1), 253958. DOI: https://doi.org/10.1093/ecam/nen063

Marin-Bravo, M. (2003). Histología de la maca, *Lepidium meyenii* Walpers (Brassicaceae). *Revista Peruana de Biologia*, *10*(1), 101-108. DOI: https://doi.org/10.15381/rpb.v10i1.2489

Melnikovova, I., Fait, T., Kolarova, M., Fernandez, E. C., & Milella, L. (2015). Effect of *Lepidium meyenii* Walp. on semen parameters and serum hormone levels in healthy adult men: a double-blind , randomized , placebo-controlled pilot study, *Evidence-Based Complementary and Alternative Medicine, 2015*(1), 324369. DOI: https://doi.org/10.1155/2015/324369

Melnikovova, I., Russo, D., Fait, T., Kolarova, M., Milella, L., Tauchen, J., ... Falabella, R. (2021). Evaluation of the effect of *Lepidium meyenii* walpers in infertile patients : a randomized , double-blind , placebo-controlled trial. *Phytotherapy Research*, *35*(1), 6359-6368. DOI: https://doi.org/10.1002/ptr.7287

Miao, X., Zhang, X., Yuan, Y., Zhang, Y., Gao, J., Kang, N., ... Wu, J. (2020). The toxicity assessment of extract of *Peganum harmala* L. seeds in *Caenorhabditis elegans*. *BMC Complementary Medicine and Therapies*, *20*(1), 256.

Nagar, Y., Singh, R., & Parveen, T. (2020). Chemosphere toxicity assessment of parabens in *Caenorhabditis elegans*. *Chemosphere*, *246*(1), 1-11. DOI: https://doi.org/10.1016/j.chemosphere.2019.125730

Paiva, F. A., Bonomo, L. F., Boasquivis, P. F., Paula, I. T. B. R., Guerra, J. F. C., Leal, W. M., ... Oliveira, R. P. (2015). Carqueja (*Baccharis trimera*) protects against oxidative stress and β-amyloid-induced toxicity in *Caenorhabditis elegans*. *Oxidative Medicine and Cellular Longevity*, *2015*(1), 740162. DOI: https://doi.org/10.1155/2015/740162

Parra-Guerra, A., & Olivero-Verbel, J. (2020). Ecotoxicology and environmental safety toxicity of nonylphenol and nonylphenol ethoxylate on *Caenorhabditis elegans*. *Ecotoxicology and Environmental Safety*, *187*(1), 1-9. DOI: https://doi.org/10.1016/j.ecoenv.2019.109709

Peng, H., Wei, Z., Luo, H., Yang, Y., Wu, Z., Gan, L., & Yang, X. (2016). Inhibition of fat accumulation by hesperidin in *Caenorhabditis elegans*. *Journal of Agricultural and Food Chemistry, 64*(25), 5207-5214. DOI: https://doi.org/10.1021/acs.jafc.6b02183

Peres, N. S. L., Bortoluzzi, L. C. P., Marques, L. L. M., Formigoni, M., Fuchs, R. H. B., Droval, A. A., & Cardoso, F. A. R. (2020). Medicinal effects of Peruvian maca (*Lepidium meyenii*): a review. *Food and Function*, *11*(1), 83-92. DOI: https://doi.org/10.1039/c9fo02732g

Ruiz-Luna, A. C., Salazar, S., Aspajo, N. J., Rubio, J., Gasco, M., & Gonzales, G. F. (2005). *Lepidium meyenii* (Maca) increases litter size in normal adult female mice. *Reproductive Biology and Endocrinology*, *3*(1), 1-6. DOI: https://doi.org/10.1186/1477-7827-3-16

Saier, C., Büchter, C., Koch, K., & Wätjen, W. (2018). *Polygonum multiflorum* extract exerts antioxidative effects and increases life span and stress resistance in the model organism *Caenorhabditis elegans* via DAF-16 and SIR-2.1. *Plants, 7*(3), 60. DOI: https://doi.org/10.3390/plants7030060

Sun, Q., Yue, Y., Shen, P., Yang, J. J., & Park, Y. (2016). Cranberry Product decreases fat accumulation in *Caenorhabditis elegans*. *Journal of Medicinal Food, 19*(4), 427-433. https://doi.org/10.1089/jmf.2015.0133

Sun, Y., Dai, C., Shi, S., Zheng, Y., Wei, W., & Cai, D. (2018). Composition analysis and antioxidant activity of essential oils, lipids and polysaccharides in different phenotypes of *Lepidium meyenii*. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *1099*(40), 25-33. DOI: https://doi.org/10.1016/j.jchromb.2018.09.010

Tafuri, S., Cocchia, N., Vassetti, A., Carotenuto, D., Esposito, L., Maruccio, L., ... Carotenuto, D. (2021). *Lepidium meyenii* (Maca) in male reproduction. *Natural Product Research*, *32*(22), 4550-4559.

Tejeda-Benitez, L., & Olivero-Verbel, J. (2015). *Caenorhabditis elegans*, a biological model for research in toxicology. *Reviews of Environmental Contamination and Toxicology*, *237*(1), 1-35.

Tejeda-Benitez, L., & Olivero-Verbel, J. (2016). *Caenorhabditis elegans*, a biological model for research in toxicology. In W.Voogt (Ed.), *Reviews of Environmental Contamination and Toxicology* (Vol. 237, p. 1-35). Cham, CH: Springer.

Thabit, S., Handoussa, H., Roxo, M., El Sayed, N. S., Azevedo, B. C., & Wink, M. (2018). Evaluation of antioxidant and neuroprotective activities of *Cassia fistula* (L .) using the *Caenorhabditis elegans* model. *PeerJ Journal*, *6*(1), e5159. DOI: https://doi.org/10.7717/peerj.5159

Wählby, C., Lee Conery, A., Bray, M. A., Kamentsky, L., Larkins-Ford, J., Sokolnicki, K. L., ... O'Rourke, E. J. (2014). High- and low-throughput scoring of fat mass and body fat distribution in *C. elegans*. *Methods, 68*(3), 492-499. DOI: https://doi.org/10.1016/j.ymeth.2014.04.017

Wang, S., & Zhu, F. (2019). Chemical composition and health effects of maca (*Lepidium meyenii*). *Food Chemistry*, *288*(1), 422-443. DOI: https://doi.org/10.1016/j.foodchem.2019.02.071

Wang, X., Zhang, Z., Zhou, H., Sun, X., Chen, X., & Xu, N. (2019). The anti-aging effects of *Gracilaria lemaneiformis* polysaccharide in *Caenorhabditis elegans*. *International Journal of Biological Macromolecules*, *140*(1), 600-604. DOI: https://doi.org/10.1016/j.ijbiomac.2019.08.186

Watts, J. L., & Ristow, M. (2017). Lipid and carbohydrate metabolism in *Caenorhabditis elegans*. *Genetics*, *207*(2), 413-446. DOI: https://doi.org/10.1534/genetics.117.300106

Woodruff, G. C., Johnson, E., & Phillips, P. C. (2019). A large close relative of *C. elegans* is slow-developing but not long-lived. *BMC Ecology and Evolution, 19*(1), 74. DOI: https://doi.org/10.1186/s12862-019-1388-1

Wu, Q., Qu, Y., Li, X., & Wang, D. (2012). Chromium exhibits adverse effects at environmental relevant concentrations in chronic toxicity assay system of nematode *Caenorhabditis elegans*. *Chemosphere, 87*(11), 1281-1287. DOI: https://doi.org/10.1016/j.chemosphere.2012.01.035

Yábar, E., Pedreschi, R., Chirinos, R., & Campos, D. (2011). Glucosinolate content and myrosinase activity evolution in three maca (*Lepidium meyenii* Walp.) ecotypes during preharvest, harvest and postharvest drying. *Food Chemistry, 127*(4), 1576-1583. DOI: https://doi.org/10.1016/j.foodchem.2011.02.021

Yábar, E., & Reyes, V. (2019). La maca (*Lepidium meyenii* walpers) alimento funcional andino: bioactivos, bioquímica y actividad biológica. *Revista de Investigaciones Altoandinas, 21*(2), 139-152. DOI: http://dx.doi.org/10.18271/ria.2019.457

Yang, Y., Shao, H., Wu, Q., & Wang, D. (2020). Lipid metabolic response to polystyrene particles in nematode. *Environmental Pollution*, *256*(1), 113439. DOI: https://doi.org/10.1016/j.envpol.2019.113439

Yu, Z. Y., Zhang, J., & Yin, D. Q. (2016). Multigenerational effects of heavy metals on feeding, growth, initial reproduction and antioxidants in *Caenorhabditis elegans*. *PLoS ONE*, *11*(4), 1-13. DOI: https://doi.org/10.1371/journal.pone.0154529

Zevallos-Concha, A., Nuñez, D., Gasco, M., Vasquez, C., Quispe, M., & Gonzales, G. F. (2016). Effect of gamma irradiation on phenol content, antioxidant activity and biological activity of black maca and red maca extracts (*Lepidium meyenii* walp). *Toxicology Mechanisms and Methods*, *26*(1), 67-73. DOI: https://doi.org/10.3109/15376516.2015.1090512

Zhang, Y., Zhou, F., & Ge, F. (2019). Effects of combined extracts of *Lepidium meyenii* and *Allium tuberosum* Rottl. on erectile dysfunction. *BMC Complementary and Alternative Medicine, 19*(1), 135. DOI: https://doi.org/10.1186/s12906-019-2542-4

Zheng, B. L., He, K., Kim, C. H., Rogers, L., Shao, Y., Huang, Z. Y., ... Zheng, Q. Y. (2000). Effect of a lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. *Urology*, *55*(4), 598-602. DOI: https://doi.org/10.1016/s0090-4295(99)00549-x

Zhou, Y., Wang, H., Guo, F., Si, N., Brantner, A., Yang, J., ... Bian, B. (2018). Integrated proteomics and lipidomics investigation of the mechanism underlying the neuroprotective effect of n - benzylhexadecanamide. *Molecules, 23*(11), 2929. DOI: https://doi.org/10.3390/molecules23112929

Zhu, H., Hu, B., Hua, H., Liu, C., Cheng, Y., Guo, Y., & Yao, W. (2020). Macamides: a review of structures , isolation , therapeutics and prospects. *Food Research International*, *138*(Part B), 109819. DOI: https://doi.org/10.1016/j.foodres.2020.109819