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Article

# Preformulation studies for the development of a microemulsion formulation from *Ambrosia peruviana* All., with anti-inflammatory effect

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Natural products are considered an important source of the therapeutic arsenal currently available. Among these alternatives are the seeds of *Ambrosia peruviana* (altamisa), whose extract has shown an anti-inflammatory effect. The main objective of this work was to perform a preformulation study of *Ambrosia peruviana* seeds ethanolic extract, where the main factors that affect the physical, chemical, and pharmacological stability of the extract were evaluated, as well as a compatibility study by differential scanning calorimetry (DSC) analysis against different excipients. A dry extract was obtained by rotary evaporation of the seeds macerated with 96% ethanol. The anti-inflammatory activity was determined by measuring its effect on NO production in RAW 264.7 macrophages, stimulated with LPS. The results showed that the dry extract maintained its stability over time when stored at a temperature of 4 and 25ºC, demonstrating its biological activity, the content of phenolic compounds, and its physicochemical parameters remain practically invariable. However, when exposed to high temperatures (60 ºC) it was affected. The thermal analysis revelated that the behavior of most of the selected excipients and the dry extract was maintained, which indicates that it did not present incompatibilities, therefore they can be candidates for formulating a microemulsion.

**Keywords:** *Ambrosia peruviana*. Preformulation. Stability test. Excipient compatibility study. Microemulsion.

**Conflict of interest:** All authors have none to declare.

## INTRODUCTION

Nowadays a large part of the research aimed at the development of new and powerful therapeutic agents is focused on the study of plants used in traditional medicine. In fact, in defining what is considered a drug derived from a natural product, it is estimated that between 25–50% of pharmaceutical products currently used are derived from natural products (Kingston, 2010). Those products have taken hold in the market because the population believes that they have fewer adverse effects than synthetic drugs. The WHO recognizes their great relevance in primary care programs since they represent effective alternatives to prevent or treat several diseases (OMS, 2013).

Colombia is a country with a wide variety of ecosystems, ranking among the fourteen countries with the highest biodiversity index, with a fundamental role in sustaining living beings and also representing a source with excellent research potential, making use of plants for the development of new products, such as

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phytopharmaceuticals (Andrade, 2011), especially those that present a high demand, such as phytopharmaceuticals with anti-inflammatory activity. The high demand for compounds with this activity is because many studies carried out in recent decades have established a clear relationship between the inflammatory response and the physiopathology of chronic non-transmissible diseases (Pan, Lai, Ho, 2010; Prasad, Sung, Aggarwa, 2012). The inflammation in these diseases may appear to different degrees and could be associated with different factors such as age, genre, genetic susceptibility, and modifiable risk factors related to lifestyle (Medzhitov, 2008; Meetoo, 2008). The most clinically meaningful drugs for the treatment of these diseases are steroids and non-steroidal anti-inflammatory drugs (NSAIDs), which have a high incidence of side effects. (Nagai *et al*., 2017; Vonkeman, Van de Laar, 2010). These reasons have encouraged the search for promising, safer, and more effective substances to treat diseases involving inflammation.

*Ambrosia peruviana* (Asteraceae), is an aromatic plant, used in traditional medicine in several South American countries for the treatment of different diseases that cause inflammation such as: colic, chronic pain, arthritis, spasms, and infections, among others. (Cicció, Chaverri, 2015; Jimenez-Usuga *et al*., 2016). *In vitro* studies on this plant species have shown that the ethanolic extract of seeds has showed significant inhibition on inflammatory markers in RAW 264.7 macrophages stimulated with LPS. (Castro, Franco, Diaz, 2021). Those antecedents make it an important starting point for the development of herbal medicine with anti-inflammatory properties.

The development of a phytopharmaceutical product involves objectives aimed at obtaining a physically and chemically stable, technologically feasible, and biologically available product, for which the concept of "Rational Design of Medicines" must be applied, which in its first stage includes a preformulation study. It is a useful tool in the development of conventional medicines and turn have many elements in common with the preformulation of phytopharmaceuticals (Wyttenbach *et al*., 2005). However, some of these elements are difficult to standardize, due to the complex composition that characterizes these preparations (Bernal, Ramos, Baena, 2019; Matiz, Cárdenas, Rincon, 2007). These studies must be carried out as a stage prior to starting the formulation phase. At this stage, the characterization of the physicochemical properties and the study of the interaction of the extract with possible excipients to be used in the formulation should be performed. At this level, there must be analytical methodologies to be able to ensure the safety, efficacy, and stability of the product in the subsequent stages. In addition, because of the collection of all this information, the guidelines to consider in the implementation of the product manufacturing process in the later stages are obtained (Bernal, Ramos, Baena, 2019; Kopelman, Augsburger, 2002; Wu *et al*., 2009). Considering the panorama previously described, in this work a preformulation study of the ethanolic extract of *Ambrosia peruviana* seeds was carried out with focus on obtaining a microemulsion for topical use with anti-inflammatory effects.

## MATERIAL AND METHODS

### Reagents

Ethanol and dimethyl sulfoxide (DMSO) were purchased from Thermo Fisher Scientific (Pittsburgh, PA). Dulbecco's modified eagle medium (DMEM), lipopolysaccharide from *Escherichia coli* (LPS), Folin–Ciocalteu, 4-aminobenzenesulfonamide, N-[1,1-naphthyl] ethylenediamine dihydrochloride, sodium nitrite, sodium carbonate, N′-[[3-(aminomethyl)phenyl]methyl] ethanimidamide dihydrochloride (1400W), and gallic acid were obtained from Sigma Aldrich (St Louis, MO). Fetal bovine serum (FBS) from Gibco (São Paulo, Brazil). (Macrophages RAW 264.7 (TIB-71™) were acquired from the American Type Culture Collection, ATCC. (Manassas, VA). Bromide of 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) was obtained from Calbiochem (San Diego, CA).

### Excipients

Castor oil, vegetable oil and coconut oil supplied by Sigma-Aldrich; sodium laureth sulfate (Sulforokanol® L270/1A) and ceteareth-12 (Rokanol® T12) supplied by PCC Exol; ceteareth-20 (Eumulgin® B2), PEG-150 distearate

(Eumulgin® EO 33), poloxamer 188 (Kolliphor® P188), PEG-40 hydrogenated castor oil (Cremophor® RH-40), cocamide DEA (Comperlan® KD), oleth-5 (Eumulgin® O5), PPG-1-PEG-9 lauryl glycol ether (Eumulgin® L), PPG-5-laureth-5 (Eumulgin® ES), PEG-35 castor oil (Kolliphor® EL), [PEG-7 coconutglycerides, PEG-40 hydrogenated castor oil, glycerin, and glyceryl oleate] (Eumulgin® PRO-CL), [MIPA-laureth sulfate, laureth-4 and cocamide DEA] (Plantapon® WW 1000), poloxamer 407 (Kolliphor®P407), [tocopherol and hydrogenated glycerides palm citrate] (Controx® KS C) and EDTA supplied by BASF; polysorbate 80, polyethylene glycol 400, glycerin and propylene glycol supplied by DOW; benzyl alcohol, methylchloroisothiazolinone and methylisothiazolinone (Biosure® MBC) supplied by Petrocarbono.

## Plant material

*Ambrosia peruviana* seeds were collected in Turbana, Bolívar, Colombia (10°24′0″ N, 75°30′0″ O), in July 2018. A voucher specimen of the plant was identified in the herbarium of the Universidad de Antioquia, (Medellin–Colombia) by the biologist Felipe Cardona and deposited with the identification code HUA 214539.

## Plant extraction and phytochemical screening

Dried and powdered seeds of *A. peruviana* were exhaustively extracted with ethanol (96% v/v) by maceration at room temperature (25±3 °C). The extract obtained was dried in a rotary evaporator (Heidolph, Germany) at constant temperature of 40 °C. The dry extract was chemically characterized by the presence of alkaloids, flavonoids, tannins, coumarins, cardiotonic glycosides, saponins, triterpenes/steroids, and quinones. The results were consistent with a previous report (Castro, Franco, Diaz, 2021).

## Preformulation study

### *Stability testing*

The reactivity of the extract was evaluated against different conditions, according to the recommendations of the ICH and the WHO on stability studies for drugs (ICH, 2003; WHO, 2008). Table I specifies the parameters that were considered and the determinations that were made for each of the dry extract samples (Wu *et al*., 2009).

The dry extract was conditioned in type I amber vial bottles. All the vials were closed, one part of them was stored under refrigeration, another part of them was placed under room temperature conditions and the rest were placed under temperature stress conditions. (See Table I). Each bottle corresponds to one sampling unit. For each of the sampling intervals and conditions, three samples were removed to analyze the response variables in triplicate and have a fourth, in case a re-analysis was needed (Wu *et al.*, 2009). The response variables established for the study were: organoleptic characteristics, pH, biological evaluation (NO inhibition), and the total phenolic content (TPC) (ICH, 2003; Wu *et al.*, 2009).

**TABLE I -** Conditions and variables of the stability testing

| Condition | Sampling time (weeks) | Number of samples | Response variables |
|---|---|---|---|
| Refrigerator (4°C) | 0, 4, 8, 12, 24 | 4 for each sampling time 16 total. | Organoleptic characteristics pH Pharmacological evaluation. Content of total polyphenols |
| Room temperature (25°C) | 0, 4, 8, 12, 24 | 4 for each sampling time 16 total. | |
| Stress temperature (60°C) | 0, 4, 8, 12, 24 | 4 for each sampling time 16 total. | |

*Organoleptic characteristics*

Various organoleptic characteristics were considered, such as the appearance, color, smell, and texture of the ethanolic extract of *A. peruviana* seeds. These characteristics were analyzed in each of the sampling times of the stability study (ICH, 2003; Wu *et al.*, 2009).

*pH*

A stock solution of *A. peruviana* seeds extract ($1\times10^5$ µg/mL) in DMSO was prepared. A portion of this solution was diluted with deionized water to obtain a solution of 4000 µg/mL; the pH of this solution was measured using an OHAUS starter 3100 potentiometer. Measurements were performed on all samples at each of the sampling times of the stability study.

*Determination of total phenolic content (TPC)*

Total phenolic content was determined using the Folin–Ciocalteu's method with slight modifications (Mejia *et al.*, 2020). Briefly, 30 µL of different concentrations of the extracts under study were mixed with 150 µL of a Folin–Ciocalteu solution (0.1 M) and 120 µL of a sodium carbonate solution (7.5%). The mixture was incubated for 2 hours and the $DO_{760}$ was determined in a microplate reader (Multiskan GO, Thermo). The results of TPC were presented as milligrams of gallic acid equivalent per gram of dry extract (mg GAE·g-1).

*Effect on nitric oxide production and cell viability*

RAW 264.7 macrophages were maintained routine in DMEM enriched with 10% inactivated FBS at 37 °C and 5% $CO_2$. The effect on the release of nitric oxide (NO) was determined as a function of the accumulation of nitrites in the culture medium using the Griess reaction. Briefly, RAW 264.7 cells were plated in 96-well plates ($2\times10^4$ cells/well), incubated for 48 hours, and treated with the higher non-toxic concentration of *A. peruviana* extract. Thirty minutes later, cells were stimulated with LPS (1 µg/ml) and incubated for 24 h. Later, the medium was collected and stored at -20 ºC for the subsequent quantification of nitrite. To determine the effect on the viability, MTT solution (0.25 mg/mL) in fresh medium was added to the cells and incubated for 4 hours, after which the medium was carefully aspirated, and the resulting formazan crystals were dissolved in 100 µL of DMSO. Optical density ($DO_{550}$) was determined using a microplate reader (Multiskan GO, Thermo), and the percentage of cell survival relative to the control group was calculated. NO concentration was determined by measuring the amount of nitrite in the cell culture supernatant using the Griess reaction (Green *et al.*, 1982), equal volumes of Griess reactive and supernatants were mixed and the absorbance of samples was determined in a Multiskan GO microplate reader ($DO_{550}$), the concentration of nitrites was calculated using a standard $NaNO_2$ curve (1–200 µM). For all experiments, fresh culture medium was used as blank control, and 1400 W (10 µM), a selective inhibitor of iNOS, was used as positive control.

*Compatibility study between the dry extract and excipients*

Different binary mixtures of the dry extract of *A. peruviana* and excipients were evaluated to determine their compatibility. The excipients were selected considering a composition of a microemulsion formulation and their availability. Samples of 300 mg of dry extract were mixed with each one of the excipients at a 1:1 ratio. The excipients used were: oil phase castor oil, vegetable oil and coconut oil; surfactant sodium laureth sulfate; ceteareth-12; ceteareth-20; PEG-150 distearate; poloxamer 188; PEG-40 hydrogenated castor oil; cocamide DEA; oleth-5; PPG-1-PEG-9 lauryl glycol ether; PPG-5-laureth-5, PEG-35 castor oil; [PEG-7 coconutglycerides, PEG-40 hydrogenated castor oil, glycerin, and glyceryl oleate]; [MIPA-laureth sulfate, laureth-4 and cocamide DEA]; cosurfactant polysorbate 80; polyethylene glycol 400; glycerin and propylene glycol; poloxamer 407; antioxidant tocopherol and hydrogenated glycerides palm citrate; preserving agent benzyl alcohol, methylchloroisothiazolinone and methylisothiazolinone; stabilizing agent EDTA and vehicle (water).

The binary mixtures between the dry extract of *A. peruviana* and the required amount of excipient were placed in type I amber glass bottles. Finally, 20 mg of water (5%) were added to each of the samples and were mixed for 1 minute. to facilitate interactions between excipients and the extract. Subsequently, the samples were placed in a circulating air oven at a temperature of 50 °C for 15 days. As a negative control for degradation, the same procedure was carried out for the extract without excipients, placing a sample at 50 °C and another at 4 °C, to determine the true effect that excipients could have on degradation (Bernal, Ramos, Baena, 2019; Kopelman, Augsburger, 2002; Serajuddin *et al.*, 1999; Sims *et al.*, 2003; Wu *et al.*, 2009; Wyttenbach *et al.*, 2005).

Upon completing the 15 days of study, each one of the samples was analyzed to find possible incompatibilities in the appearance and the compatibility was determined by evaluating the thermal behavior by means of DSC, in a Shimadzu DSC-60 equipment. During this process, they were subjected to heating starting with an approximate temperature of 25° C until reaching 200 °C, with a heating ramp of 10 °C/min. The samples were placed in an aluminum sample holder, covered, and hermetically sealed under a nitrogen atmosphere at a flow rate of 50 mL/min. The results were analyzed using the TA Analysis Software program.

### Statistical analysis

Assay results of three independent experiments were expressed as the means ± SEM and analyzed using one-way analysis of variance (ANOVA) with post-hoc Dunnet or Tukey comparison. *P*-values < 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

### Stability testing

#### *Organoleptic characteristics*

Dry seeds of *Ambrosia peruviana* were macerated with ethanol until 51.3 g of total extract (yield of 12.73%). The ethanolic extract of *A. peruviana* seeds showed dark green color as shown in Figure 1 A, characteristic odour and appearance of viscous fluid, what makes handling difficult.

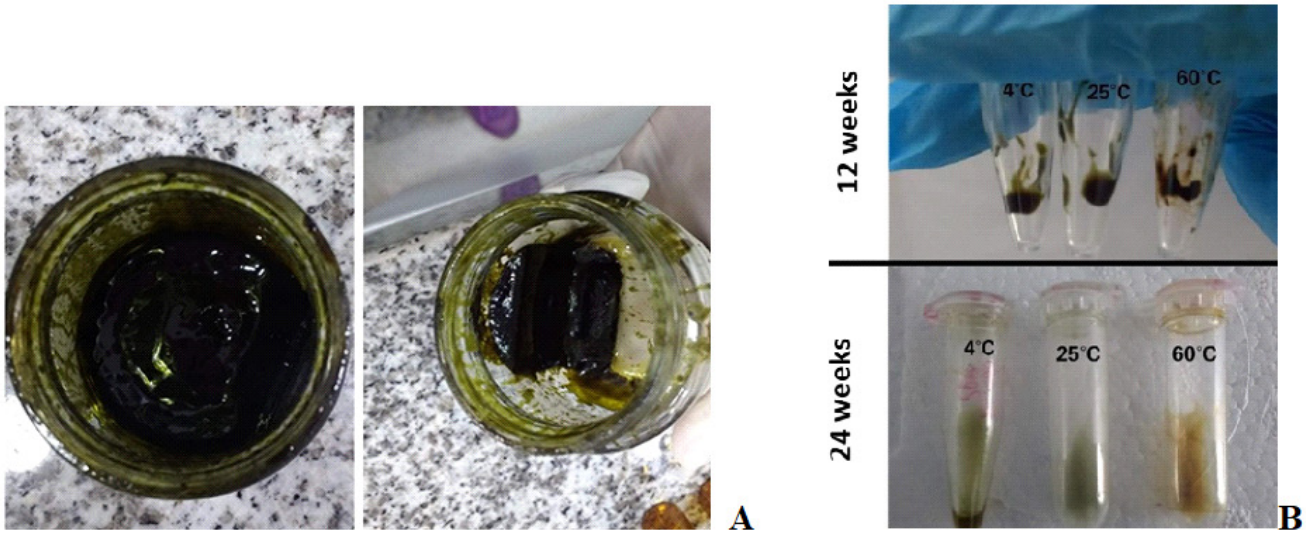


**FIGURE 1 -** *Ambrosia peruviana* seeds dry extract. A. at time zero. B. At time 12 and 24 weeks in different conditions.

Regarding the organoleptic characteristics observed at each time of the stability test under the different conditions, the extract that must keep under refrigeration (4 °C) or at room temperature (25 °C) had not significant changes; however, at 60 °C had slight changes in the color at the final of the study. In weeks 12 and 24 of the stability study, as shown the Figure 1 B, the *A. peruviana* extract at 60ºC, presented dark brown coloration.

There are pigments in plants to which their coloration is attributed; among these could be found anthocyanins, carotenoids, and chlorophyll; the latter oversees providing the green coloration to the various plant species. However, the color can be affected by different factors such as light, pH, time, and temperature. Some authors indicated that the conversion of chlorophylls to pheophytins represents the main cause of the loss of green colour in plants. When they are subjected to heat or acidic conditions, said coloring of the vegetables turns into a brownish-green color (Ahmed, Kaur, Shivhare, 2002).

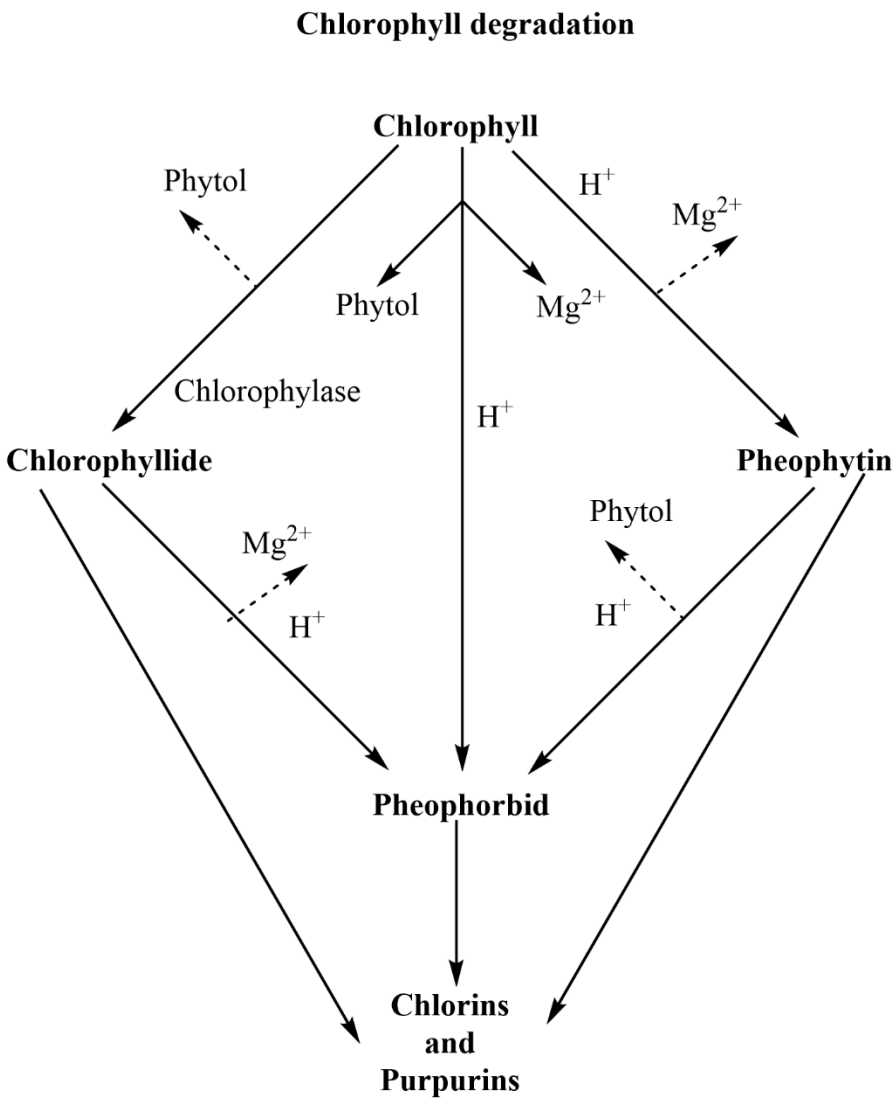


**FIGURE 2 -** Chemical degradation of chlorophylls. (Adapted from Rodríguez, Gallego, 1999).

During the degradation process, hydronium ions can transform chlorophyll into pheophytin by substituting magnesium ions in the porphyrin ring. Figure 2 shows the degradation process of chlorophyll; when heating a plant extract, it may change in color to a brown-green that indicates the degradation of chlorophyll to pheophytin, which is subsequently degraded to pheophorbides which are brown compounds (Ahmed, Kaur, Shivhare, 2002).

*pH*

At the beginning of the stability test, the *A. peruviana* seed dry extract had a pH of 5.35 and was maintained at an interval of 5.2 to 5.6 as is observed in Figure 3. When these pH values obtained throughout the stability study were reviewed, no statistically significant differences were found between them at the different temperatures used and the different analysis times.

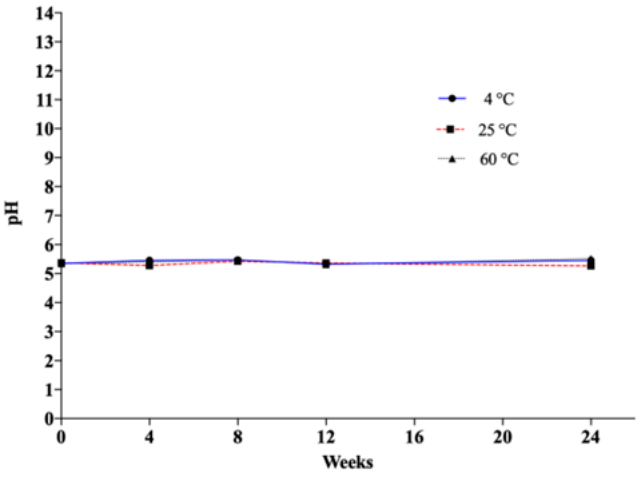


**FIGURE 3 -** Changes of pH of the extract in the stability study.

*Total phenolic content (TPC)*

The Folin-Ciocalteu method was used to quantitatively determine phenolic compounds, based on the reaction of phenolic groups with the Folin-Ciocalteu reagent, a mixture of phosphomolybdate and phosphotungstate that forms the yellow phosphomolybdotungstate acid which, when it interacts with phenolic groups, reduces it causing a blue coloration. These blue pigments have a maximum absorption according to the quality and/or quantitative composition of phenolic mixtures in addition to the basic pH of the solution, which is generally obtained by adding sodium carbonate (Cicco *et al*., 2009). To determine the influence of the storage conditions on the *A. peruviana* seeds extract, the total phenolic content was evaluated at different temperatures and times, protecting the samples from light by using amber flasks to prevent this factor from having a significant influence on the extract.

The content of total phenolic compounds throughout the stability study shows that when evaluating the time at the stipulated storage conditions for the *A. peruviana* seeds dry extract, the degradation of these compounds is significant over 4 weeks (Figure 4). The extracts stored at 4°C and 25°C have similar behavior (reducing total polyphenols to 18.4 and 20.3%, respectively, of the initial concentration), while at 60 °C the decrease in phenols content in the extract is significantly greater (47.7%).

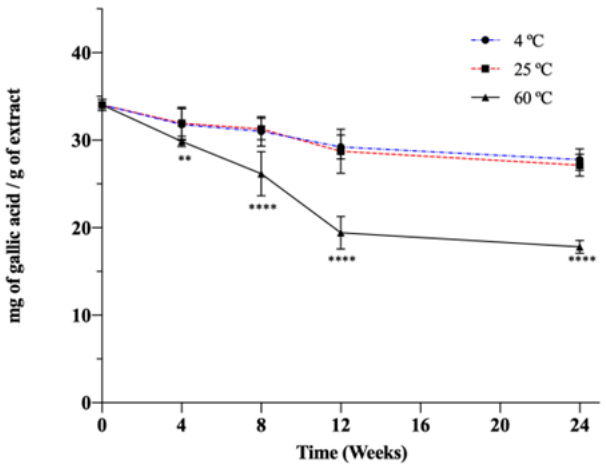


**FIGURE 4 -** Changes of the total phenolic content in *Ambrosia peruviana* seeds extract during storage at different temperatures. ***P*<0.01; *****P*<0.0001 statistically significant one-way ANOVA and Dunnett's test against the 4 °C group.

This behavior was previously described in extracts with high phenolic compound content, which presents a significant decrease in phenols after 30 days of storage at temperatures of 6 °C and 23 °C, which are close to those used for us in this assay. This is in addition to agreeing with the authors regarding the fact that the higher the temperature, the greater the

reduction of total phenols in the extract (Srivastava *et al.*, 2007).

The presence of phenolic compounds in plants attribute antioxidant properties due to the ability to capture free radicals due to the presence of the hydroxyl group. However, the stability, biosynthesis, and degradation of this type of compounds can be affected by various factors such as storage conditions and light that can negatively influence the quantity of phenolic compounds present in the plant (Lattanzio, 2003; Valenzuela *et al.*, 2014).

The Folin-Ciocalteu method is widely used to determine the effect of the storage conditions due to the possibility that some of the degradation products could react with the Folin-Ciocalteu reagent, affecting the content of phenolic compounds (Camelo, Sotelo, 2012). In plant species, in addition to bioactive compounds, there are also enzymes such as polyphenol oxidases (PFOs), which are metalloenzymes present in the different organs of plants (roots, seeds, leaves and fruits) and which are responsible for the oxidative degradation of polyphenols, also called enzymatic browning.

The degradation of phenolic compounds by enzymatic oxidation or enzymatic browning may partially explain the change in the color of the extract under heat stress conditions, as phenolic compounds oxidize and polymerize to a brown color (Morante *et al.*, 2014). On the other hand, the possibility of simultaneous degradation of the phenolic and chlorophyll yielding a color change of the extract from dark green to brown can not be ruled out; Regarding the influence of temperature and exposure time on the stability of phenols, significant degradation was observed at high temperatures (60 °C), which increased with exposure time in this condition (12 and 24 weeks). Other authors have already observed this relationship between exposure time and temperature in the degradation of bioactive constituents (Camelo, Sotelo, 2012; Uurrea *et al.*, 2012), corroborating the close relationship between them.

*Biological evaluation*

The Griess method is a colorimetric test by which organic nitrites are detected. It is carried out using two solutions (A and B). Reagent A consists of sulfanilamide with the addition of a strong acid and reagent B of N (1-naphthyl) ethylenediamine. (Malinski, Mesaros, Tomboulian, 1996). The Griess reaction is based on the formation of azo dyes. The test consists of two reactions, which result in the formation of a colored reagent. In the first reaction the nitrite reacts with sulfanilamide under acid conditions (diazotization reaction) and at the second stage a diazotization product reacts with N-(1- naphthyl) ethylenediamine (diazonium coupling) to form an azo derivative dye. This azo derivative is readily quantifiable by means of a spectrophotometer. Due to its simplicity, speed and satisfactory sensitivity, the Griess reaction has become a widely used colorimetric method for the determination of nitrite in various matrices (Malinski, Mesaros, Tomboulian, 1996).

The biological activity shown by the extract of *A. peruviana* (15 µg/mL) was conserved during the time of the stability study for the temperatures of 4 ºC and 25 ºC, while the extract subjected to high temperatures (60ºC) showed a significant reduction of the inhibition of NO production in macrophages which is evident from 8 weeks where the inhibition of nitric oxide production has decreased more than 50% (Figure 5).

Macrophages play a critical role in the immune response, its activation with LPS releases large amounts of nitrites which are essential precursors of cytotoxic agents (Hibbs, Vavrin, Taintor, 1987; Nathan, 1992). The excessive production of NO by macrophages during a chronic inflammatory response causes disturbance in essential molecules such as membrane lipids, nucleic acids, proteins, which leads to oxidative stress affecting cellular homeostasis. Hence, the development of products that have substances to prevent the overproduction of NO has become the objective of research to treat chronic inflammation (Desai, Park, 2005; Taira, Nanbu, Ueda, 2009).

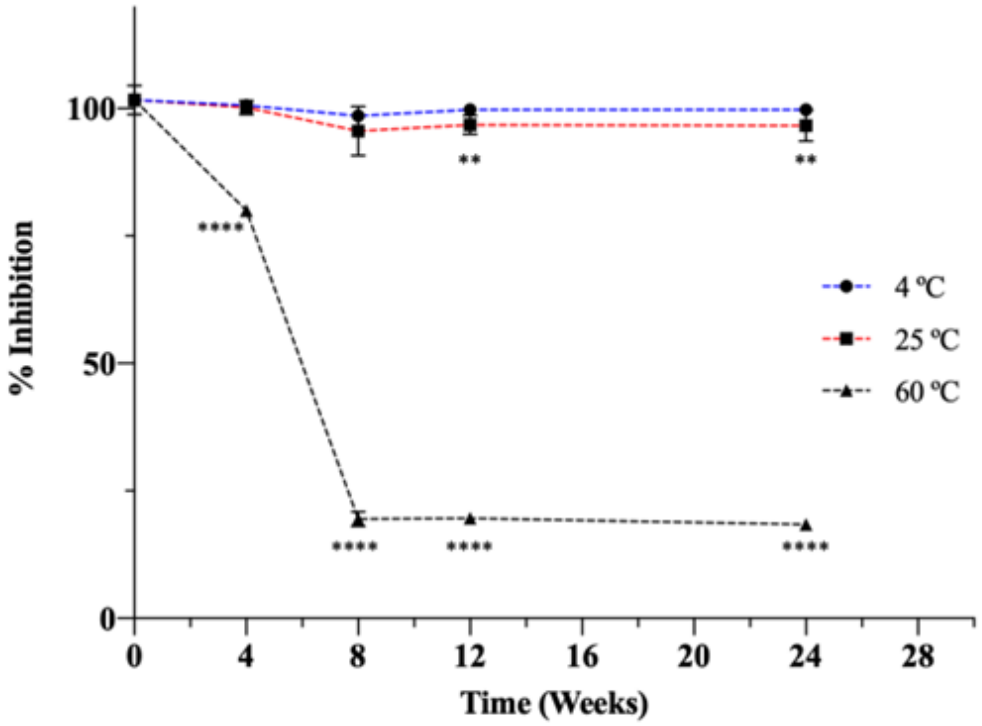


**FIGURE 5 -** Inhibition of NO production by *Ambrosia peruviana* seed extract during storage at different temperatures for 24 weeks.

### Compatibility study between the dry extract and excipients

Figure 6 shows the thermogram of the extract stored at different temperatures (4 and 50 °C). In these curves, two endothermic transitions were detected between approximately 130 and 150 °C. It is generally said that natural products whose composition consists of a great variety of substances, such as the extract under study, present similar thermal behavior (Bernal, Aragón, Baena, 2016, 2019). It should be noted that this behavior is maintained at both temperatures, with the difference that at 50 °C the endothermic transitions are smaller in size compared to what is observed at 4 °C, concluding that the storage temperature influences the thermal behavior of the extract but not significantly.

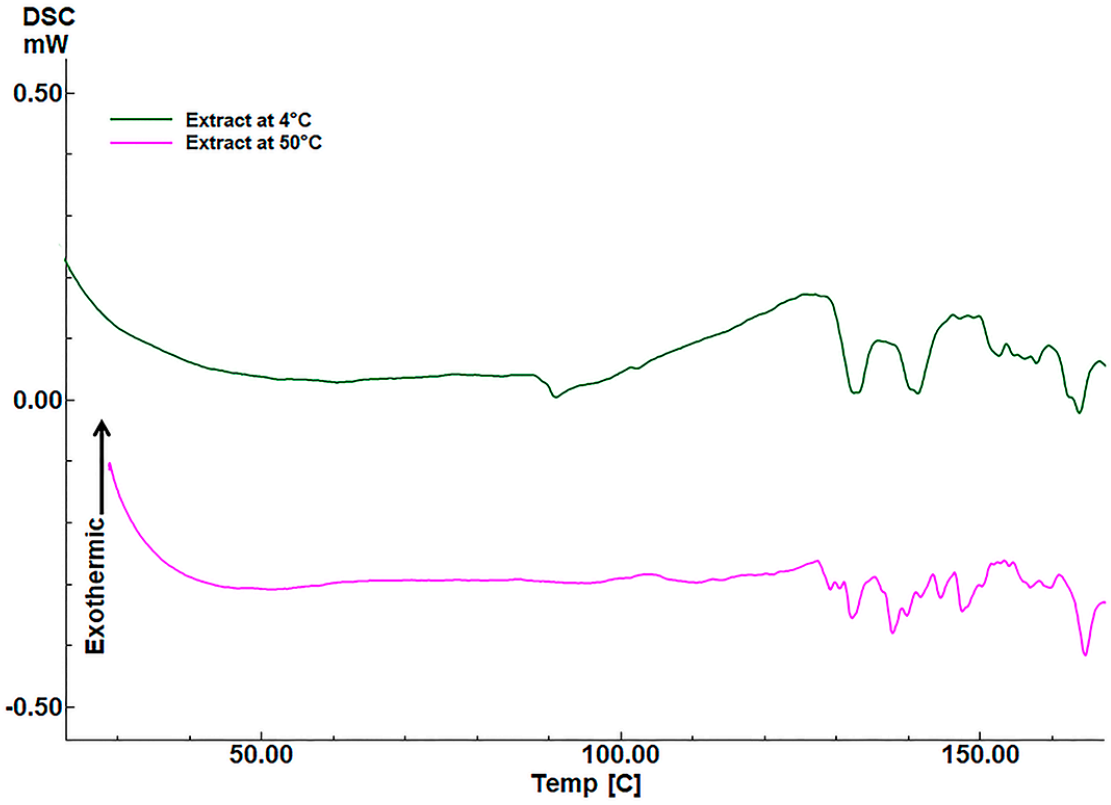


**FIGURE 6 -** DSC curves of *Ambrosia peruviana* seeds extract stored at different temperatures.

However, the aim is to incorporate the extract into a microemulsion, therefore, a series of excipients were selected according to the chosen pharmaceutical form, hoping that when mixing with the extract the thermal behaviors of both would be maintained, indicating that they are compatible and that therefore both it is possible to use them in a formulation (Uurrea *et al.*, 2012).

The thermal behavior of the extract, the respective excipient and the extract-excipient combination are compared in the DSC curves. For them to be considered to be compatible, the thermal behavior of the extract and the excipient in question must be maintained. The example in Figure 7 shows the thermal behavior of the extract, the excipient 1 (Castor Oil) which is highly thermostable as it does not present peaks, and the combination of both, from which it can be said that castor oil is compatible with the extract, because the mixture maintains the two endothermic transitions characteristic of the extract, and that in this case the excipient that does not present thermal transitions in this temperature range. It does not alter the behavior of the extract when mixed with it.

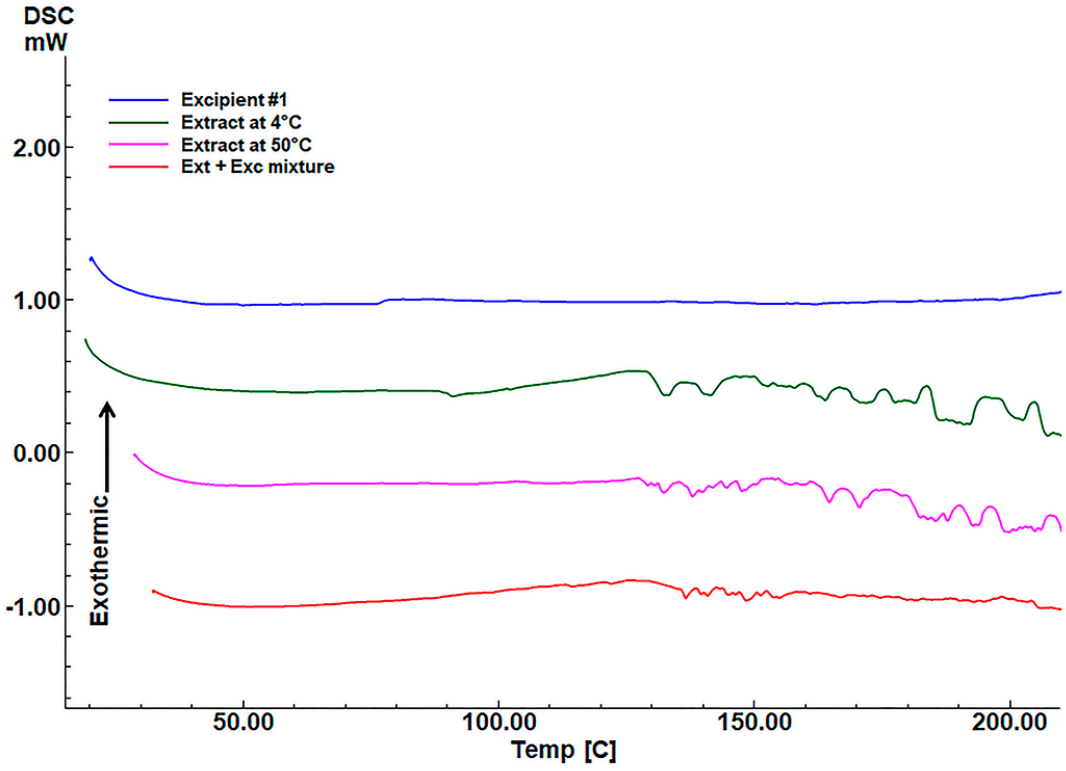


**FIGURE 7 -** DSC curves of *Ambrosia peruviana* seed extract and castor oil (excipient # 1).

After carrying out the analysis of the different thermograms of the mixtures and comparing them with the thermal behavior that the extract and the excipient presented separately, it was found that within the working conditions used during the study by DSC there is no chemical incompatibility between the extract of *A. peruviana* seeds and a large part of the selected excipients, (Data not shown). Here the thermal behavior of the components is maintained separately or they suffer variations such as widening or displacement of endothermal peaks, which are not significant since this type of phenomenon can be typical of the decrease in the purity of each component due to the mixture (Kiss *et al*., 2006; Ranjan *et al*., 2012).

As an example of interactions, Figure 8 shows the thermogram of the extract and excipient 4 (sodium lauryl ether sulfate) which presents an endothermic peak between approximately 100 and 120 ° C and that when mixed with the extract, the disappearance of this thermal peak is observed, which may indicate a possible interaction among them. Nevertheless, when observing the thermograms of excipients 2, 4, 5, 12, 14, 16, and 20 (Data not shown), it is possible to appreciate marked differences in the mixture curve, which probably lead to interactions among them. These differences may be due to various factors, among which we find the disappearance or appearance of peaks in the curves, due to possible chemical extract-excipient interactions when the mixture is heated.

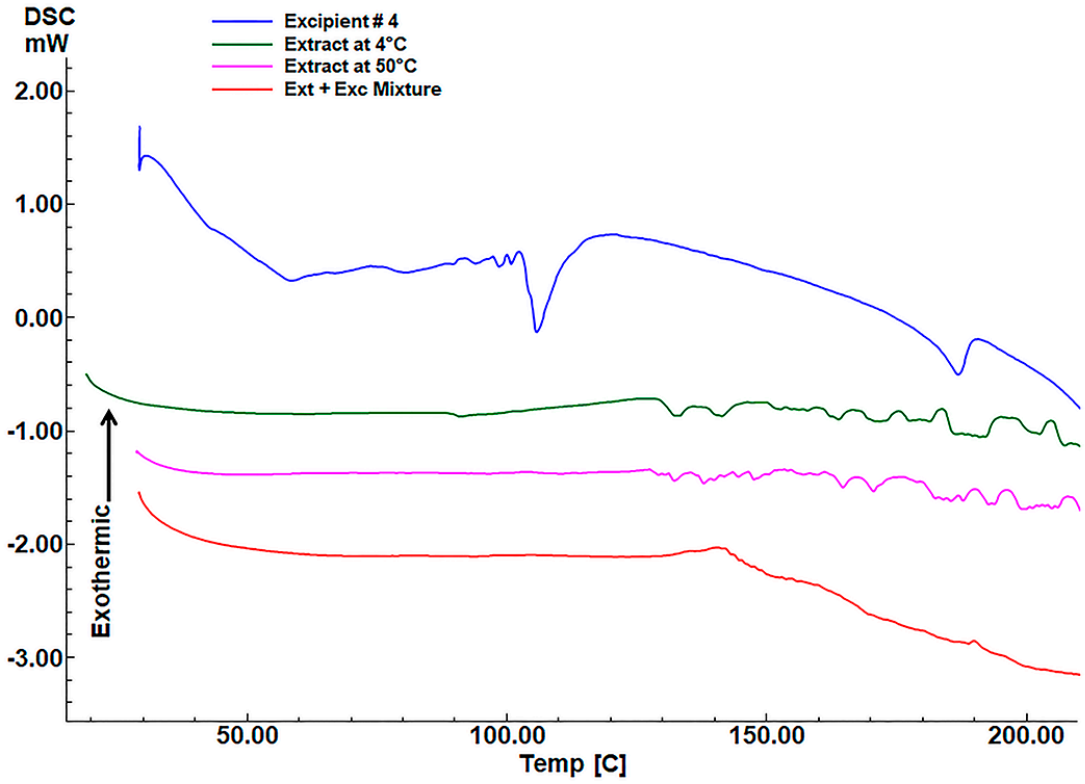


**FIGURE 8 -** DSC curves of *Ambrosia peruviana* seed extract and sodium laureth sulfate (excipient # 4).

It should also be noted that in some of the mixtures previously mentioned as incompatible, there are significant widening and displacement of the peaks of both the extract and the excipient. Said thermal behavior may indicate an alteration in the characteristic of some of the components, and that could compromise the stability and pharmacological activity of the extract. However, most excipients turn out to be compatible for this reason; it can be said that they are considered potential candidates to be included in microemulsion formulations that contain the extract of *A. peruviana* seeds. The results of the excipient compatibility are shown in the Table II.

**TABLE II -** Results of the excipient compatibility evaluated by DSC

| # | Name | Function | Result |
|---|---|---|---|
| 1 | Castor oil | Oil phase | C |
| 2 | Vegetable oil | | I |
| 3 | Coconut oil | | C |
| 4 | Sodium laureth sulfate | Surfactant | I |
| 5 | Ceteareth-12 | | I |
| 6 | Ceteareth-20 | | C |
| 7 | PEG-150 distearate | | C |
| 8 | Poloxamer 188 | | C |
| 9 | PEG-40 hydrogenated castor oil | | C |
| 10 | Cocamide DEA | | C |
| 11 | Oleth-5 | | C |
| 12 | PPG-1-PEG-9 lauryl glycol ether | | I |
| 13 | PPG-5-laureth-5 | | C |
| 14 | PEG-35 castor oil | | I |
| 15 | MIPA-laureth sulfate, laureth-4 and cocamide DEA | | C |
| 16 | PEG-7 cocoglycerides, PEG-40 hydrogenated castor oil, glycerin, and glyceryl oleate | | I |
| 17 | Polysorbate 80 | Cosurfactant | C |
| 18 | Propylene glycol | | C |
| 19 | Poloxamer 407 | | C |
| 20 | Polyethylene glycol 400 | | I |
| 21 | Glycerin | | C |
| 22 | Tocopherol and hydrogenated glycerides palm citrate | Antioxidant | C |
| 23 | Benzyl alcohol, methylchloroisothiazolinone and methylisothiazolinone | Preserving agent | C |
| 24 | EDTA | Stabilizing agent | C |
| 25 | Water | Vehicle | C |

**#:** Number of excipients; **C:** Compatible excipient; **I:** Incompatible excipient.

## CONCLUSION

The preformulation studies of the ethanolic extract from seeds of *Ambrosia peruviana* all suggested it was stable at temperatures of 4 °C and 25 °C during the 24 weeks, presenting losses of approximately 21% of the initial phenolic content. On the other hand, it was unstable at 60 °C losing approximately 50% of the initial phenolic content after 12 weeks. Moreover, the anti-inflammatory effect evaluated on nitric oxide production

and cell viability demonstrated the excellent activity of the extract, which did not change at 4 °C and 25 °C.

The compatibility study among the dry extract and excipients showed that the extract was compatible against different excipients: oils, surfactants, cosurfactants, antioxidants, preserving agents, stabilizing agents, and vehicles. This study is the first step for formulating *Ambrosia peruviana* seeds ethanolic extract as a standardized microemulsion dosage form.

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