Chapter 7 Obtaining and characterization of the ethanolic extract of the leaves of the *Tradescantia Spathacea* SW

Capítulo 7 Obtención y caracterización del extracto etanólico de las hojas de *Tradescantia Spathacea* SW

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Abstract

In the last 20 years, chemical studies of plants in México have increased notably, intending to provide society with alternative mechanisms without damaging the atmosphere and the environment, ensure effectiveness and efficiency. There is a great diversity of native plants in the Mexican southeast, such as *Tradescantia Spathacea SW*, which has antioxidant and antibacterial properties. For this reason, this work presents the obtaining and characterization of the ethanolic extract of the leaves of the Tradescantia *Spathacea SW* (Purple Maguey) plant. The leaves were obtained from Nayarit Castellot, Champotón Campeche, and the ethanolic extract was obtained by the traditional method using purification processes. As a result, the ethanolic extract was obtained without purification, which was characterized by phytochemical and spectroscopic techniques. Phytochemical tests and thin layer chromatography showed polyphenols, and UV-VIS and FTIR spectroscopy showed the presence of the phenol group. The extract obtained in this work will be subsequently evaluated as a corrosion inhibitor in API 5L-X52 steel.

Extract, Purple Maguey, Maceration, *Tradescantia spathacea* SW

Resumen

En los últimos 20 años, los estudios químicos de las plantas en México han incrementado notoriamente, con el objetivo de proporcionar a la sociedad mecanismos alternos sin la nocividad a la atmósfera y el medio ambiente asegurando efectividad y eficiencia. En el sureste mexicano se tiene una gran diversidad de plantas nativas, como es la *Tradescantia Spathacea SW,* la cual tiene propiedades antioxidantes, y antibacterianas. Por esta razón, en este trabajo se presenta la obtención y caracterización del extracto etanólico de las hojas de la planta *Tradescantia Spathacea SW* (Maguey morado). Las hojas se obtuvieron en la localidad Nayarit Castellot, Champotón Campeche y el extracto etanólico se obtuvo mediante el método tradicional empleando procesos de purificación. Como resultado, se obtuvo el extracto etanólico sin purificación, el cual se caracterizó mediante técnicas fitoquímicas y espectroscópicas. Las pruebas fitoquímicas y cromatografía en capa fina mostraron la presencia de polifenoles y la espectroscopia UV-VIS y FTIR mostraron la presencia de grupo fenol. El extracto obtenido en el presente trabajo será evaluado posteriormente como inhibidor de corrosión en el acero API 5L-X52.

Extracto, Maguey Morado, Maceración, *Tradescantia spathacea* SW

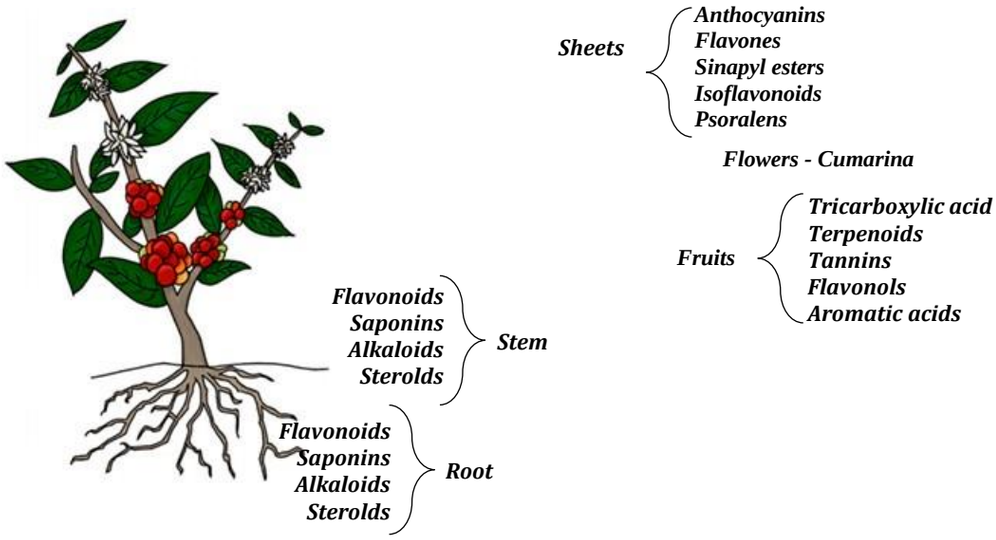
7.1 Introduction

In order to find practical and sustainable solutions to everyday problems in the industrial sector, such as internal and external corrosion problems in the oil industry (offshore platforms). Corrosion inhibitors have been used as an internal protection measure for metals.

The inhibitor is a chemical substance added in small concentrations to a corrosive electrolyte whose function is to slow down the corrosion rate of the exposed metal (Umoren et al., 2019). Commercial inhibitors used in practice are expensive, highly toxic and environmentally damaging. For this reason, scientists have focused their research on the search for natural or green inhibitors. Novel green inhibitors have been synthesised from a variety of natural resources, mainly using plants, as they are easy to obtain and non-toxic.

Plants are composed of leaves, fruits, flowers, stems and roots, so they can contain different organic compounds such as flavonoids, polyphenols and polysaccharides that act as inhibitors (see figure 7.1). These have been obtained from flowers, leaves and roots of different plants showing the ability to inhibit the corrosion process (Kesavan et al., 2012; Mazumder & Abstract, 2013; Miralrio & Vázquez, 2020a; Tejeda Benítez et al., 2014).

Figure 7.1 Basic parts of a plant and their common organic compounds



Source:(Miralrio & Vázquez, 2020b)

Particularly, *Tradescantia spathacea SW* also known as Rhoeo discolor, *Rhoeo spathacea (Sw.)*, *Tradescantia discolor* and commonly called maguey morado, is a herbaceous species belonging to the commelinaceae family and native to Belize, Guatemala, Gulf of Mexico and Southeast Mexico (see figure 7.2). Its study has focused on medicinal, food and, to a lesser extent, energy and textiles (Lai et al., 2008; Reyes-Munguía et al., 2009).

However, its main use has been in the field of medicine, as it has properties to inhibit the growth of cancer cells, antibacterial, stomach pain, headache, anti-inflammatory, asthma and cough, among others (Prakash & Rajesh, 2014). It is important to mention, Tradescantia Spathacea SW is very abundant in the southeastern region of Mexico, it is easy to reproduce due to the environmental conditions. It has also been reported to contain phenolic compounds and flavonoids showing antioxidant activities (Tan et al., 2015).

Figure 6.2 *Tradescatia Spathacea* SW plant

Source: (Own elaboration)

For this reason, the present work presents the extraction of Tradescantia Spathacea SW plant leaves by the maceration method and identifies the organic compounds it contains by means of phytochemical tests, thin layer chromatography and spectroscopic techniques such as ultraviolet-visible spectroscopy (UV-Vis) and Fourier transform infrared spectroscopy (FTIR).

7.2 Methodology to be developed

7.2.1 Harvesting, cleaning and drying of leaves

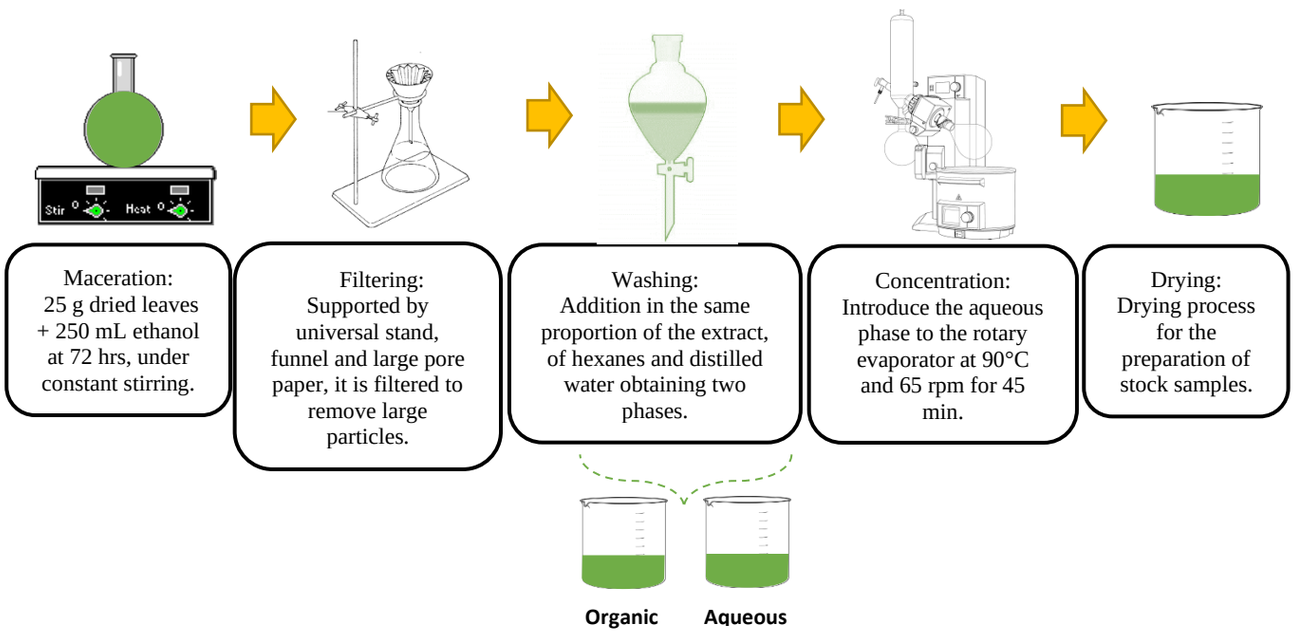
The leaves of the Tradescatia Spathacea SW plant were obtained on 27 November 2020 in the locality of Nayarit Castellot (Xnoha) located in the municipality of Champotón in the state of Campeche, a place that according to the literature has optimal conditions such as: soil type, temperature, pH, humidity, altitude, etc., which guarantee proper development and growth as well as quality in all components of the plant (Villarreal-Ibarra et al., 2015). Subsequently, the leaves obtained were washed with water and taken to the oven to dry for 4 days at 68 °C.

7.2.2 Preparation of the extract

The leaves of the dried Tradescantia spathacea SW plant were weighed to obtain the decrease in grammage. The extract was obtained by the maceration method. In a round flask equipped with a Vigraux column and magnetic stirrer, 25 g of dried leaves of the Tradescantia spathacea SW. plant and 250 mL of 95 % ethanol were added to the mixture, which was left at room temperature (25C°), with constant stirring for approximately 72 hours. At the end of this time, the mixture was filtered using large pore filter paper to obtain the ethanolic extract (EA). Finally, the EA is placed in the rotary evaporator to concentrate the extract.

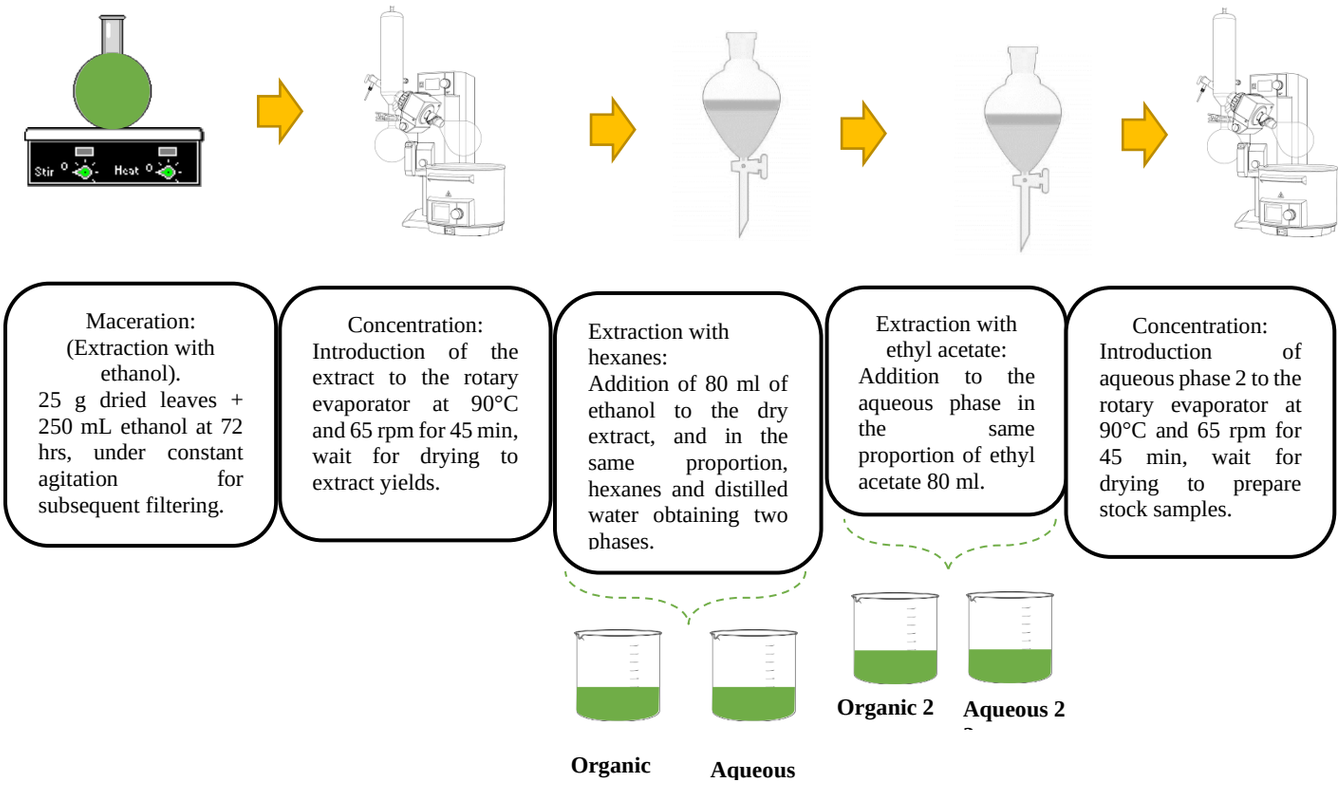
As a complement to the previous process, it was proposed to include the purification of the extract through successive washes with hexanes and ethyl acetate, in this way 3 different extracts are obtained for subsequent analysis, the description of the procedure is shown in figures 7.3 and 7.4.

Figure 7.3 Hexane purification process



Source of consultation: (Own elaboration)

Figure 7.4 Purification procedure with hexanes and ethyl acetate



Source of consultation: (Own elaboration)

7.2.3 Characterisation of the extract

In order to identify the organic compounds present in the extract, different analyses were carried out, which are described below:

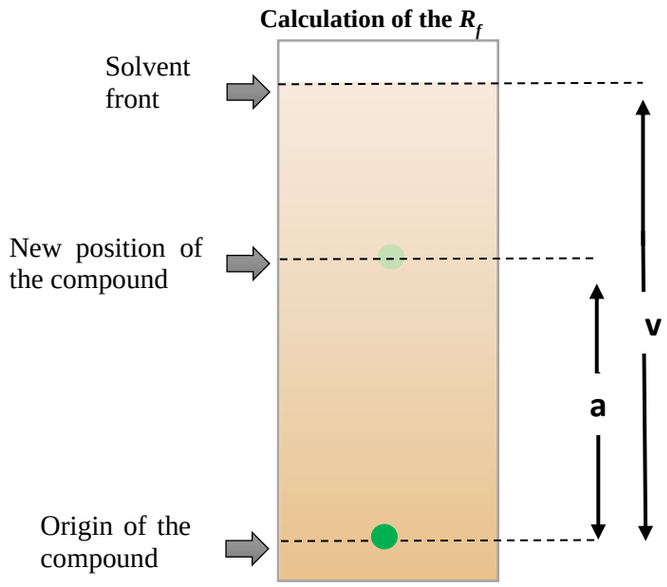
- Thin layer chromatography

A large pore filter paper, 9 cm long and 3 cm wide, was used, the upper end (in front of the solvent) and the lower end (origin of the compound) are marked, see Figure 7.5. Subsequently, the filter paper is placed in a flask containing the eluent (iodine solution dissolved in ethanol), taking care that the solution does not touch the lower mark, and left to act for 10 minutes. Once the eluent rises by capillary action until it reaches the upper edge, it is removed from the flask. Finally, the filter paper is allowed to dry and the sample is revealed by forming coloured complexes. The retention factor (Rf) is used to determine the presence of the compound of interest. The Rf is calculated by the following equation:

$$R_f = \frac{a}{v} \quad (1)$$

Where a is the distance travelled from the origin of the compound to the new position and v is the distance from the origin along the eluent front.

Figure 7.5 Image of the filter paper used for the thin layer chromatography test



Source of consultation: (Own elaboration)

R_f is a physical constant for a given compound, assuming that the chromatographic conditions (e.g. solvent, temperature and nature of the stationary phase) are specified to a dimensionless value between the length of the plate and the extent of the major components, the certain organic compounds contained in the sample can be determined.

- Phytochemical tests

They consist of colouring tests that will allow the identification of the chemical compounds in the extract, and the yield of the extracts is evaluated.

a) **Identification of flavonoids:** Dissolve 0.5 mL of the total extract (ethanolic extract) in 2 mL of absolute ethanol and divide into three test tubes. The third test tube is taken as the control and the following tests are then carried out:

- Shinoda reaction: 2 drops of concentrated hydrochloric acid are added to the first test tube, then a piece of magnesium metal is placed in the test tube for 5 minutes at room temperature between 25 and 26 C°.

- 10% sodium hydroxide reaction: 3 drops of sodium hydroxide are added to the second test tube and observations are noted.

b) **Identification of Saponins:** To identify this compound, the foam height and stability test is carried out, which consists of placing 1 mL of total ethanolic extract in a test tube, shaking it vigorously, waiting 15 minutes, and measuring the height of the foam that appears (the height of the foam obtained after 15 minutes is measured).

c) **Identification of Tannins:** Take 1 mL of the ethanolic extract in a flask and add 2 mL of distilled water and 3 drops of 2% sodium chloride, heat to boiling for one minute, cool and filter. The filtrate is divided into three test tubes, the third test tube is used as a control. To identify tannins, the following tests are carried out:

- Gelatine reaction: 2 drops of gelatine reagent (0.5 g of pure grenetin gauged to 50 mL of distilled water) are added, if no changes are observed, no tannins are present.

- Ferric chloride reaction (gallic acid derivatives or catechol); to the second test tube is added a drop of 1% ferric chloride (0.5 g of ferric chloride to 50 mL of distilled water), the sample tends to have a strong blue colour in the presence of polyphenols.

d) **Lead acetate reaction**: In order to identify the presence of phenols in the extract, this test is carried out. To 1 ml of ethanolic solution, 1 mL of 10% lead acetate solution was added.

- Spectroscopic techniques

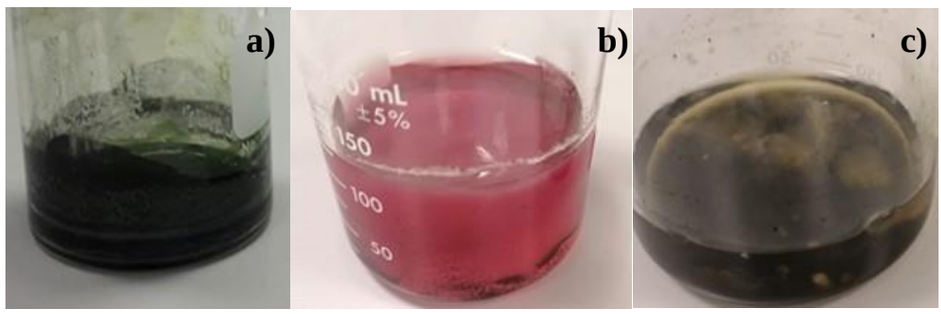
The extract was analysed by Ultraviolet-Visible Spectroscopy (UV-Vis) to identify the organic compounds contained in the ethanolic extract and Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups present in the extract.

7.3 Results

In order to determine the optimal conditions for obtaining organic compounds from Tradescantia spathacea Sw leaves, three procedures were considered: 1) extraction of the leaves using the maceration process without a purification process, 2) maceration process with hexane purification and 3) maceration process with hexane and ethyl acetate purification (see figure 7.6).

Figure 7.6a shows the extract obtained without a purification process. As can be seen, the dry extract 22 days after leaving the rotary evaporator shows a good appearance with a characteristic odour of the plant. On the other hand, the extracts obtained with hexane and hexane purification with ethyl acetate that were left to dryness after being concentrated in the rotary evaporator and taken to dryness show after 20 days the presence of fungi due to the decomposition of the sample (figure 7.5b). Similarly, fungi and bacteria were observed in the extract purified with hexanes and ethyl acetate (figure 7.5c). At the end of the process of obtaining the extract using different purification processes, only the extract obtained by maceration without purification was considered for subsequent characterisation.

Figure 7.6 Image of the extract obtained by maceration process: a) without purification process, b) purification with hexanes and c) purification with hexanes and ethyl acetate



Source of consultation: (Own elaboration)

7.3.1 Extract yield

Table 7.1 shows data obtained before and after the process of obtaining the ethanolic extract from the leaves of Tradescantia Spathacea SW. With respect to the volume measured, 40% of the volume recovered was obtained. On the other hand, the yield of the ethanolic extract was 26%, showing that a small amount of dry extract is obtained after concentration at the rotary evaporator.

Table 7.1 Recording of the extraction conditions of the extract from the leaves of the plant *Tradescantia spathacea* SW

Data	Value
Weight of dried leaves	25 g
Volume of solvent	250 mL
Temperature	25 °C
Volume of extract recovered	100 mL
Weight of the extract after the concentration process using the rotary evaporator	6.5 g

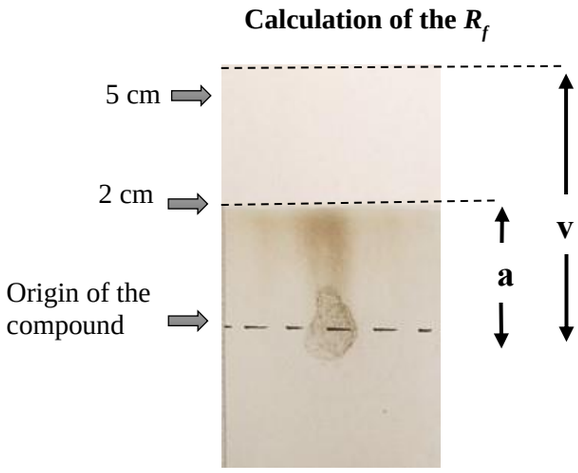
Source of consultation: (Own elaboration)

Plants contain primary metabolites (lipids and gradates, proteins, nitrogenous compounds, etc.) and secondary metabolites (alkaloids, isoprenoids and phenolic derivatives) (Auwal et al., 2014). In order to identify the presence of polyphenols, the evaluation of the extract by phytochemical tests and spectroscopic techniques is presented.

7.3.2 Thin layer chromatography (CCF)

In order to identify the major compound in the Tradescantia Spathacea SW extract, thin layer chromatography was performed. Figure 7.7 shows the evidence of the test. A retention factor (Rf) of 0.40 was determined. This technique allows revealing the amount of polyphenols present in the sample, however, to identify which polyphenols are present, other techniques are required, which are shown below.

Figure 7.7 Thin layer chromatography of the ethanolic extract

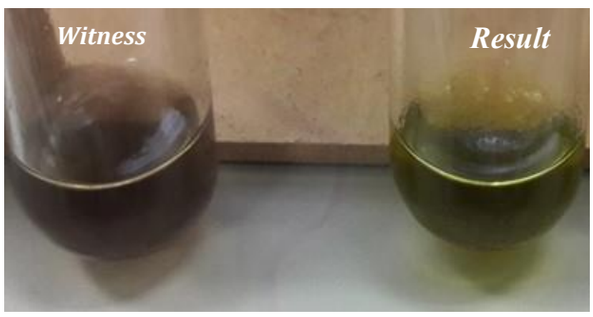


Source: (Own elaboration)

7.3.3 Phytochemical characterisation

Phytochemical tests are used to identify organic compounds, in this case, they were used to identify the polyphenolic compounds present in the extract. The Shinoda reaction allows the identification of flavonoids, *i.e.* the presence of polyphenolic compounds such as flavonols, flavones, flavonones, chalcones and aurones in the extract. Some flavonoids called anthocyanidins react with concentrated hydrochloric acid at boiling temperature and show a reddish colour change as shown in figure 7.8.

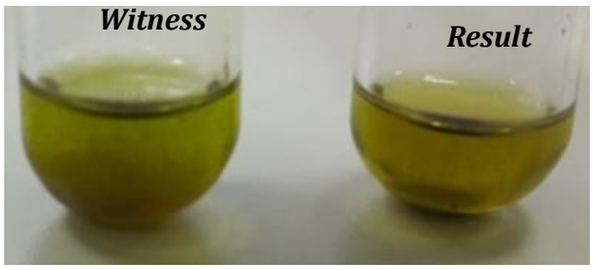
Figure 7.8 Shinoda reaction



Source: (Own elaboration)

Another specific reaction of phenols is their acidic capacity, which was demonstrated by reacting the polyphenolic compounds with a basic NaOH solution, where the presence of polyphenolic compounds was revealed when adding the solution to the ethanolic extract showed turbidity and suspension of particles (see figure 7.9).

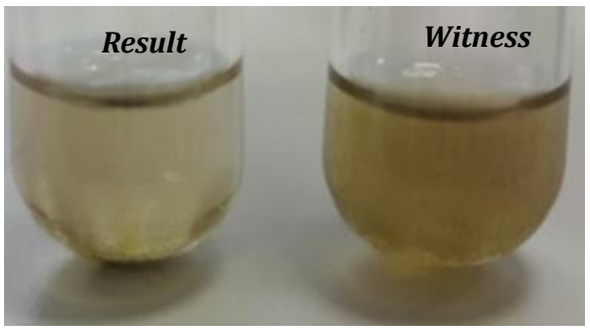
Figure 7.9 Reaction with NaOH



Source: (Own elaboration)

The grenetin test was positive and showed the existence of tannins (polymeric structures of flavones or flavonoids). When these compounds reacted with grenetin, they showed precipitate in solution and white colouring as shown in figure 7.10.

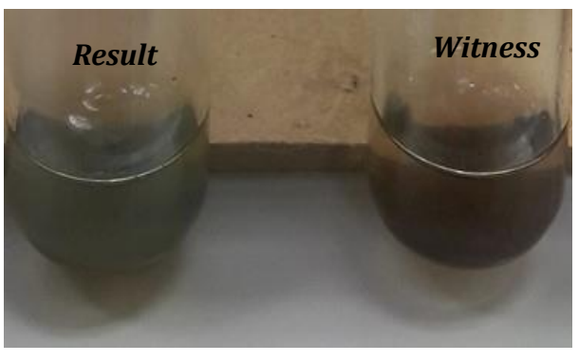
Figure 7.10 Reaction with grenetin



Source: (Own elaboration)

The ferric chloride reaction with phenols is a general test that allowed to identify the presence of polyphenols, by colour changes to strong blue as shown in figure 7.11.

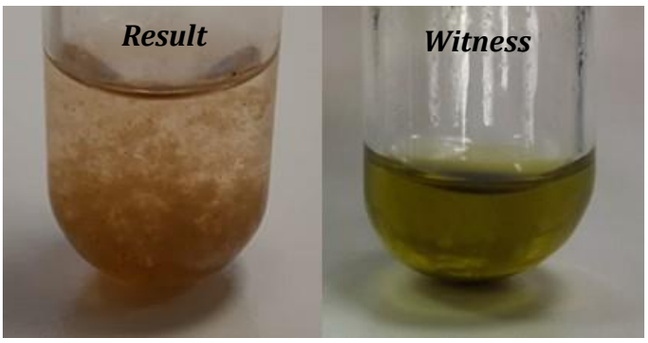
Figure 7.11 Ferric chloride reaction



Source: (Own elaboration)

In the reaction with lead acetate, the presence of polyphenols was identified, ranging from large size such as tannins; medium structure such as flavones, flavonols or as small as caffeic acid, where the increase in particle size was observed (see figure 7.12).

Figure 7.12 Lead acetate reaction



Source: (Own elaboration)

Table 2 shows a summary of the results obtained from the ethanolic extract by different phytochemical tests.

Table 7.2 Phytochemical tests on ethanolic extract

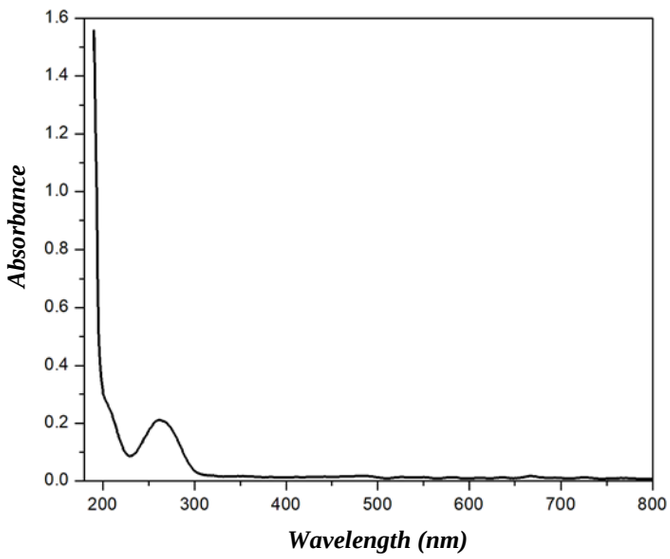
Phytochemical tests	Resulted	
Shinoda reaction	Positive	Negative
10% sodium hydroxide	X	
1% ferric chloride	X	
Reaction with grenetin	X	
Foam height test		X

Source: (Own elaboration)

Ultraviolet-Visible Spectroscopy (UV-Vis)

UV-Vis spectroscopy is widely used to identify the characteristic bands associated with organic compounds present in the range of 100-400 nm (UV) and the visible spectrum of 400-700 nm. Figure 7.13 shows the UV-Vis absorption spectrum of the total ethanolic extract. A maximum peak is observed at 270 nm corresponding to flavonoid and phenolic group structures (Porras & López, 2009).

Figure 7.13 UV-Vis spectrum of the extract

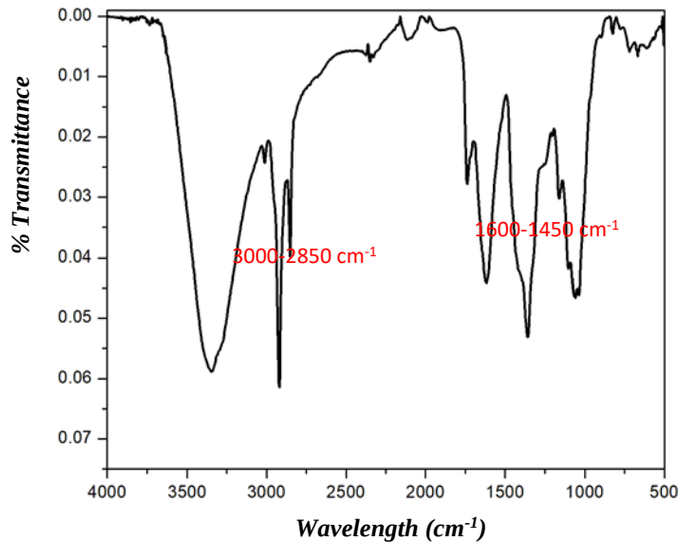


Source: (Own elaboration)

Fourier Transform Infrared Spectroscopy (FTIR)

Figure 7.14 shows the response of the ethanolic extract obtained by infrared spectroscopy. A maximum peak at 3344 cm^{-1} associated with the O-H band characteristic of the presence of an alcohol, phenol, polyphenols is observed in the range 3700-3600 cm-1, if hydrogen bridge bonds are present there is a broadening of the band and a slight decrease in the absorption frequency (3600-3000 cm^{-1}). The vibrations corresponding to the C-H stretching of the methyl and methylene groups appear between 3000-2850 cm-1. Bands corresponding to the carbonyl C=O group appear in the range (1830-1650 cm^{-1}), possibly corresponding to flavonoids and/or coumarins and the aromatic C=C double bond, occurring in pairs at 1600 cm-1 and 1450 cm^{-1}. (Interaction & Sales, 2010).

Figure 7.14 FTIR spectrum obtained from ethanolic extract *Tradescantia*



Source of consultation: (Own elaboration)

7.4 Acknowledgement

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7.5 Conclusions

The ethanolic extract of the leaves of the Tradescantia Spathacea SW plant was obtained by the maceration process. The purification of the extract with hexanes and hexanes with ethylene could not be obtained because the samples showed decomposition before the natural drying process for the production of a concentrate. On the other hand, the ethanolic extract without purification was able to evaluate the compounds it contains. The results of the phytochemical tests showed the presence of polyphenols in the extract obtained from the leaves of the Tradescantia Spathacea SW plant. Finally, the UV-Vis spectroscopy test confirmed the presence of polyphenolic groups with flavonoid-type structures and the FTIR results showed the presence of different functional groups, mainly the characteristic bands of the phenol group. It is important to mention that this study has an impact on a current topic and opens up a wide research panorama, promoting the study and use of native resources.

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