Phytochemical characterization of *Phytolacca Americana* leaves and determination of their antifungal potential

Caracterización fitoquímica de las hojas de Phytolacca americana y determinación de su potencial antifúngico

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Resumen

La *Phytolacca Americana* es una especie de planta de la familia *Phytolaccaceae* nativa del Norte de América utilizada como tratamiento autóctono en enfermedades comunes y controversiales como el cáncer, debido a sus propiedades analgésicas, antiinflamatorias y antimicóticas. El objeto de este estudio fue caracterizar a nivel fitoquímico los compuestos presentes en las hojas de esta planta y determinar su potencial antifúngico. Para ello, se evaluaron tres condiciones de secado: temperatura ambiente (12 ± 2 °C), 22 °C y 40 °C; técnica de infrarrojo con trasformada de Fourier *(FTIR)* y espectrofotometría UV/VIS donde se evidenció que las condiciones de secado no influyeron en la estabilidad de los grupos biosintéticos. Además, se identificaron metabolitos secundarios mediante *screening* fitoquímico como flavonoides, saponinas, cumarinas y taninos en extractos etanólicos que se analizaron mediante cromatografía de gases acoplada a espectrometría de masas con bombardeo electrónico, en donde se reconocieron ácidos esenciales con fines comerciales como el ácido oleico. Finalmente, se evaluó el potencial antifúngico de los extractos etanólicos con concentraciones de 60 a 300 mg/mL, en cuatro tipos de cepas: *F. solani, A. brasiliensis, S. kudriavzevii* y *C. albicans*, los resultados mostraron mayores diámetros de inhibición de 18 y 19 mm contra las cepas *F. solani* y *C. albicans*, respectivamente.

Palabras clave: screening fitoquímico; metabolitos secundarios; actividad antifúngica; ácido oleico.

Abstract

Phytolacca Americana is a species of the Phytolaccaceae family native to North America. *P. Americana* is used as a folk medicine to treat inflammation, fungal infections, rashes, and breast problems; moreover, recent publications support possible anti-cancer properties. The purpose of this study was to characterize and compare the phytochemical constituents of the leaves of *P. Americana* and determine their antifungal potential.

For this, three temperatures were evaluated to dry the leaves: ambient temperature (12 ± 2 °C), 22 °C and 40 °C, then extracts of the leaves were obtained by ethanol extraction. FTIR and UV/VIS spectroscopy analysis of the extracts shows that the drying temperatures do not affect the stability of the biosynthetic groups. Main secondary metabolites such as flavonoids, saponins, coumarins, and tannins were determined by phytochemical screening. Furthermore, extracts were analyzed using gas chromatography coupled to mass spectrometry (GCMS), essential acids such as octadecanoic and some alcohols were identified. Finally, the antifungal potential of ethanolic extracts with concentrations of 60 to 300 mg/mL was evaluated in four types of strains: *F. solani* (ATCC 36031), *A. brasiliensis, S. kudriavzevii* and *C. albicans*. Results show inhibition diameters of 18 and 19 mm for *F. solani* and *C. albicans* strains respectively.

Keywords: phytochemical characterization; antifungal potential; *Phytolacca Americana*; oleic acid.

1. Introduction

P. Americana is a species of plant in the *Phytolaccaceae* family native to North America, widely cultivated in China, and currently found easily in the Andean region of Colombia, specifically in the southern part of the department of Nariño. Although it is a highly toxic plant to livestock and humans, in 1820 the U.S. Pharmacopoeia declared it a pain reliever and anti-inflammatory (Lady Bird Johnson Wildflower Center, cited July 11, 2017; Natural Remedies cited June 14, 2017), since some parts of *P. Americana* are used as medicine if they are prepared co).

Native Americans used the tea from the berries of *P. Americana* to heal rheumatism, arthritis, dysentery, and sore breasts, the root for rheumatism, nerve pain, and bruising (Hernández-Niño, 2010). In all parts of the plant, especially in the root, the presence of phytolaccatoxin has been determined, related to the presence of triterpenoid saponins, Phytolaccin alkaloids, histamines and an enzymatic protein called Phytolacaina G. The protein extracts obtained from the roots of This plant has been compared to the commercial mitogenic activity of pokeweeedTM; likewise, they have a powerful cytotoxic activity once it enters the cytoplasm of a cell (Yang; Wieczorck; Allen; Nett, 2003).

The population of the municipality of Túquerres, the department of Nariño, Colombia, has traditionally depended on medicinal plants for their cures. However, it is unknown many properties that these plants can offer in the treatment of diseases. For this reason, this work involves the study of secondary metabolites of plants such as *P. Americana* that is easily found in the rural area of this municipality, to contribute to the taxonomic determination and biological activity of this plant. For example, triterpenic saponins called *Phytolacca-saponins*, isolated from the root of *P. Americana* were included in research processes against ovarian cancer and other adipose tumors or for weight loss purposes (Hernández-Niño, 2010; Rojas-Quintero, 2011; Sanabria, 1983). It can contribute to the production of drugs with clinical potentials from plant extracts.

Regarding antifungal activities, there are currently commercial drugs to treat different types of mycoses, such as amphotericin B, ketoconazole, fluconazole, among others; however, due to their high toxicity they can cause damage to human host cells, hence the interest in natural products and their possible applications in the pharmaceutical industry (Treviño *et al*., 2012). Although there are studies regarding the antimicrobial activity of plant extracts (Navarro-García *et al*., 2003; Kim; Chom; Han, 2013; Davicino *et al*., 2007); In *P. Americana* there is minimal evidence of activity against microorganisms, since only preliminary information was found that due to its hydrophobic characteristics on the surface of the leaves, antimicrobial proteins can be found, which provided a clue to perform a determination of the antifungal potential of this plant (Peng *et al*., 2005). On the other hand, to guarantee the benefits of the healing properties of these plants, it is necessary to determine certain physical parameters such as temperature, which does not alter the internal structures of the components, especially volatile compounds. In this sense, the drying conditions of the plant material must be evaluated, due to possible losses of essential oil, volatile substances, degradation of thermolabile substances or for the isolation of purely natural substances. Therefore, Sharapin (2000) suggests drying at temperatures below 40 ºC, where good air circulation is guaranteed. In this order of ideas, in this work, three suitable drying conditions were evaluated for the conservation of the molecules of interest present in the leaves of *P. Americana.*

Also, the presence of secondary metabolites was determined by phytochemical screening and a Gas Chromatography-Mass Spectrometry Analysis was performed with electron bombardment to qualitatively identify some essential acids. Finally, antifungal activity was evaluated in four types of strains such as *F. Solani* and *A. Brasiliensis, S. kudriavzevii* and *C. albicans,* to contribute to the knowledge of the properties of native plants in danger of extinction as *P. American* is and promote the use of the same in the therapeutic field, in agriculture and the pharmaceutical and food industries.

2. Methodology

2.1. Plant material collection

The fresh leaves of *P. Americana* were collected in winter, between May and July, in the district of Pinzón, Sabana de Túquerres, Nariño (Colombia). This plant was botanically identified in the Luis Sigifredo Espinal-Tascón Herbarium - CUVC of the Universidad del Valle. The leaves were carefully washed with distilled water until further analysis.

2.2. Preparation of plant extracts

The leaves washed with distilled water were subjected to a drying process in a BINDER FD 53-UL forced convection oven, where two temperature conditions 22 °C and 40 °C were evaluated (Martínez, 2006), and a third condition of drying at average room temperature of the Tuquerres, Nariño savanna (12 ± 2 °C). Regarding the extraction, the methodology proposed by Sanabria (1983) was followed, 50 g of dry plant material was macerated for 12 to 15 hours using 600 mL of 95 % ethanol (Merck) and n-hexane (Merck), respectively. Subsequently, it was refluxed for 1 hour, then it was vacuum filtered and the residue was washed with 100 mL of the solvent, the time was also evaluated. The resulting solutions were filtered and then concentrated in vacuo on a rotary evaporator at 40 °C. The extracts obtained were kept in amber-type bottles until their subsequent analysis.

2.3. Assessment of drying conditions

The ethanolic extracts obtained from the three drying conditions (22 °C, 40 °C and room temperature), were characterized by the spectroscopic technique, the methods applied were: infrared with Fourier transform (FTIR for its acronym in English) in a 600 to 4000 cm-1 range and UV/VIS in the entire wavelength range (250 - 900 nm) (Skoog, 1998).

2.4. Phytochemical screening

The extracts of ethanol and hexane obtained at a drying temperature of 40 °C from the leaves of *P. Americana,* were subjected to different tests, to determine the presence of tannins, saponins, coumarins, flavonoids, phenols and alkaloids following the following methodology with some modifications:

Tannin test: The extract was stirred separately with distilled water (10 mL), then filtered and five drops of 5 % $FeCl_3$ added. Black or blue-green or precipitated coloration indicated the presence of tannins (Andriani *et al*., 2015).

Saponin test: 0.5 g of extract was taken in a test tube, 10 mL of distilled water was added and stirred vigorously. The formation of 2 cm of a foam layer that persisted in heating in a water bath for 5 min, showed the presence of saponins (Andriani *et al*., 2015; Khanam; Wen; Bhat, 2015).

Coumarin test: from the extracts, a dilution was made following the 9: 1 ratio, extract: water, from which 2 mL was taken in a glass tube with a lid, with a strip of filter paper inside the tube previously soaked in an alkaline solution NaOH (0.06 g/mL). Without touching the extract inside the tube, it was covered and heated in a Bunsen burner until steam evolved, then the filter paper was taken to a UV lamp (UVP, UVLS-26), fluorescent spots (greenish-yellow) were observed, Therefore, the sample was considered positive for coumarins (Hinojosa-Dávalos *et al*., 2013).

Flavonoid test: To 1 mL of each extract in test tubes, 0.5 g of magnesium powder, and five drops of 10 % hydrochloric acid was added until hydrogen evolution. Pink, orange or strawberry colors indicated a positive test; the test is known as the Shinoda reaction (Khanam *et al*., 2015; Rondón *et al*., 2018).

Phenol test: To 50 mg of extract, 5 mL of distilled water and a few drops of 5 % $FeCl_3$ were added. The bluish-black color indicated the presence of phenolic compounds (Andriani *et al*., 2015).

Alkaloid test: To 15 mg of each extract, 6 mL of 1 % HCl was added, the mixture was left in a water bath for 5 min and filtered. The filtrate was divided into three equal parts (approximately 0.5 mL); To the first, five drops of Drangerdorff's reagent were added. If it was red-orange, it indicated the presence of alkaloids; To the second, 1 mL of Mayer's reagent was added, the formation of a cream-colored precipitate indicated a positive test, and to the last filtrate five drops of Wagner's reagent solution were added, the formation of a brown precipitate indicated the presence of alkaloids (Iqbal; Salim; Lim, 2015).

2.5. Chromatographic analysis

The use of Gas Chromatography-Mass Spectrometer requires special connection systems. In principle, these are two techniques that work in the gas phase and require a very small amount of sample for analysis, so they are highly compatible. Therefore, identification of possible components present in the ethanol and hexane extracts from the leaves of *P. Americana* was carried out and was carried out through a gas chromatograph coupled to a Shimadzu CG-EM QP-2010 mass spectrometer, equipped With an AOC-20i autoinjector, AOC-20s autosampler, Split/splitless injection, EI/SCI/NCI ionization mode and direct insertion probe controlled by GCMS-solution software, the column used was a DB-5MS column.

The chromatographic and spectrometric method used corresponds to an oven temperature of 200 ºC, injection temperature of 300 ºC, split injection mode that varies from 10: 1 to 30: 1 depending on the concentration of the species, the control mode of linear velocity flow, column flow 0.60 mL/min, linear velocity 30 cm/s, helium stripping gas, ion source temperature of 230 ºC, and interface temperature of 280 ºC. The method's confidence interval was 95 %. The team has the Nist Research Librar library attached.

2.6. Evaluation of antifungal activity

The evaluation of antifungal activity was performed by the agar well diffusion method. From the culture of each strain with mycelium, the spores of these were taken with a loop and diluted in a test tube with previously sterilized Merck KGaA soy flour peptone broth. Subsequently, they were mixed by stirring on a mechanical tube shaker (Vortex Mixer, Model: VM-300P). Then, the inoculum was streaked in the 9.1 cm diameter plates with potato dextrose agar and 4 % labor and dextrose (according to the type of strain) previously solidified, in each plate, perforations distributed properly, according to the concentrations of the extracts. Then, 20 µL of each of the diluted extract concentrations were incorporated into the 6 mm diameter wells (Sanabria; Mantilla, 1986; Quiroga; Sampietro; Vattuone, 2001). As a positive control, thiabendazole was added in one of the wells and 96 % ethanol was used as a negative control in another well.

Subsequently, the S. kudriavzevii and C. albicans plates were incubated aerobically at 30 °C and the F. solani and A. brasiliensis plates at 25 °C, according to their datasheets. In this way, three repetitions were made

for each concentration of extract (60, 120, 180, 240, and 300 mg/mL). Regarding the diameter of inhibition of the mycelia, in all the plates, everything required for visible growth was verified 72 h after incubation (Navarro-García *et al*., 2003; Parveen; Ghalib; Khanam; Mehdi; Ali, 2010). The results were measured and expressed in millimeters in terms of growth inhibition diameter around each disc, where different conditions related to activity were determined: no activity (1-6 mm), moderate activity (7-10 mm), high activity (11-15 mm), very high activity (16-20 mm) (Parveen *et al*., 2010).

The antimicrobial index was calculated using equation 1.

$$Antimicrobial\ Index = (1-Da / Db) * 100 \quad (1)$$

Where Da is the growth diameter of the fungus in the extract, and Db is the growth diameter of the fungus in the positive control.

2.7. Statistic analysis

Results were analyzed using the STATGRAPHICS Centurion XVI Version 16.1.15 program, where an ANOVA was calculated, with which means were evaluated and, also, it was identified if there were significant differences in the concentrations of the extract evaluated for each strain.

3. Results and discussion

3.1. Drying conditions

Plants with medicinal uses contain volatile compounds of pharmaceutical interest. These compounds are produced by biosynthetic pathways of non-volatile secondary metabolites, which explains their diversity. Terpenes are one of these compounds and are the most relevant for their biological activity, followed by those derived from fatty acids (saturated and unsaturated hydrocarbons), benzenes, and phenylpropanoids; although sulfur and nitrogen substances have also been reported in medicinal plants (Dudareva *et al*., 2005).

In the *FTIR* results of the samples that were processed at each of the temperatures that were evaluated, bands close to 3000 cm^{-1} correspondings to the presence of established groups = CH- were observed, common peaks corresponding to –OH between 2300 and 3700 cm^{-1} (usual in these plants, represent groups corresponding to alcohol), carboxylic and phenolic acids, which shows that low molecular weight volatile compounds are maintained. On the other hand, peaks between and 1053-1062 cm^{-1} corresponding to alkyl groups were evident. Relative to the CO bond of nonionic carboxylic acids, close observation was observed in 1735-1738 and 1244-1248 cm^{-1}, along with the asymmetric vibration of ionic carboxylate groups in 1618-1645 and 1375-1385 cm^{-1}. In other words, the qualitative results indicated that the temperature did not influence the extraction process, likewise, the *FTIR* analyzes showed similar peaks independent of the drying temperature (see Figure 1).

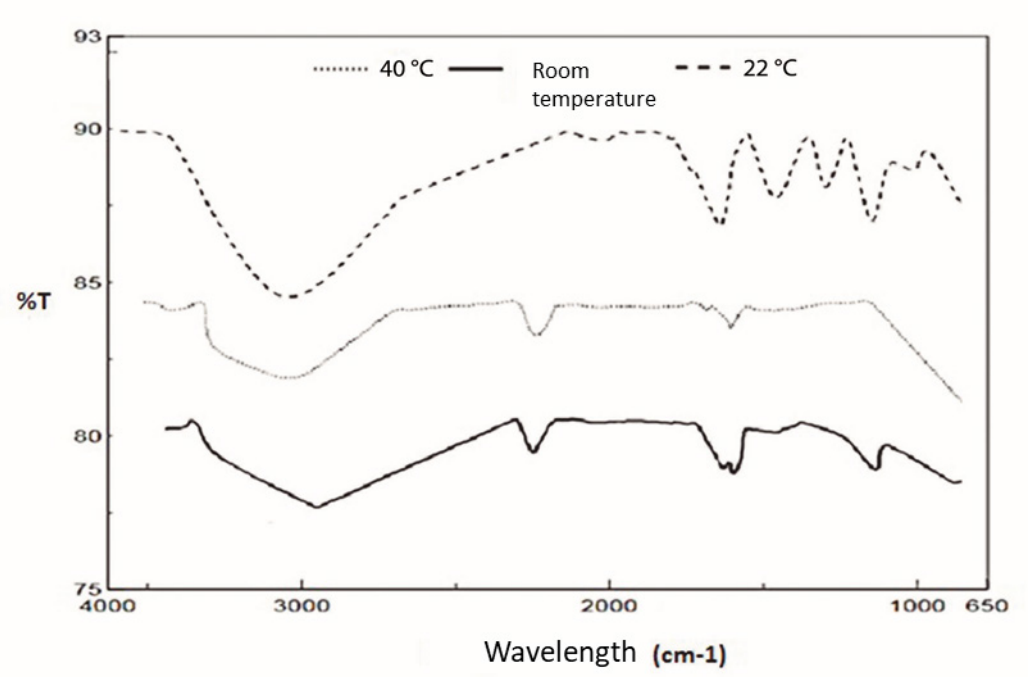

Figure 1. FTIR spectra of dry samples at 22 ° C, 40 ° C and room temperature
Source: self-made.

Regarding the UV/VIS spectra, absorption maxima were evident in the ultraviolet range: 260 to 365 nm for the three ethanolic extracts obtained at 22 °C, 40 °C and room temperature for drying the leaves of *P. Americana.* On the other hand, in the visible spectrum region, only small peaks appeared at 663 and 664 nm for the ethanolic extracts obtained at temperatures of 22 °C and 40 °C. According to the visible ultraviolet analysis of the extracts, maximum absorption bands between 200 and 300 nm were observed, corresponding to the benzoyl band or band II characteristic for the aromatic ring A of flavonoids.

On the other hand, the displacement of the last bands at a wavelength greater than 500 nm is characteristic of flavonoids from the group of anthocyanins. In this sense, the presence of anthocyanins was verified in addition to other phenolic substances in the sample; likewise, peaks close to 663 nm may correspond to cyanine $(CH_3)_2N$- (CH = CH) n-CH = N + $(CH_3)_2$ compounds, which is an unsystematic name of a synthetic family of dyes belonging to the polymethine group. On the other hand, the peaks close to 365 nm correspond in high probability to merocyanines, which could be evidenced in the extract obtained from the samples with a drying temperature of 40 °C from the *P. Americana* leaves (see Figure 2) (Ren; Tian, 2007; Martínez-Cruz *et al*., 2011).

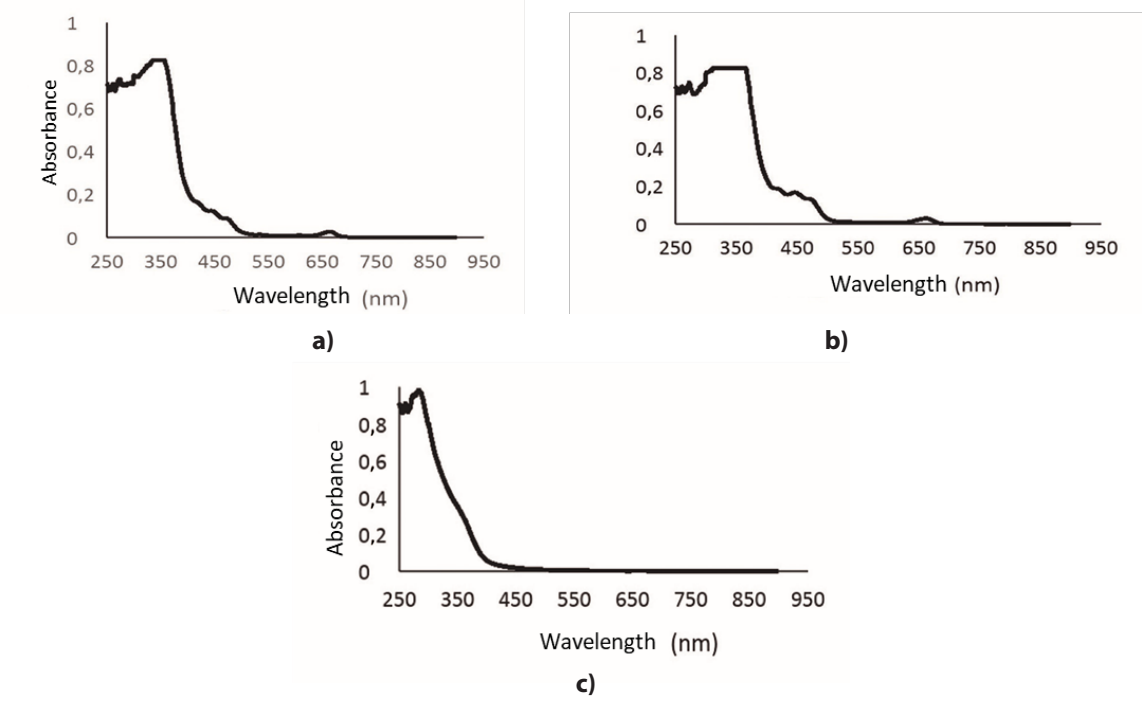

Figure 2. Absorption spectra of ethanolic extracts obtained at different drying temperatures of the leaves of *P. Americana:*

a) 22 °C **b)** 40 °C **c)** Temperatura environment

Source: own elaboration.

3.2. Phytochemical screening

In the results obtained from the phytochemical screening, the presence of flavonoids, saponins, coumarins, and tannins was evidenced; on the other hand, in the hexane extracts the presence of secondary metabolites was not evidenced. Comparing with other studies of leaves of Phytolacca dodecandra belonging to the family of Phytolaccaceae, these revealed the presence of phenolic compounds, although in low concentrations. Likewise, they evidenced the presence of alkaloids and terpenoids; the latter was shown in high concentrations (see Table 1) (Ogutu; Lilechi; Mutai; Bii, 2012).

Table 1.

Phytochemical analysis of the leaves of Phytolacca americana

Component / Test / Solvent	Test	Ethanol extract	Hexane extract
Phenols	Ferric Chloride Test	-	-
Flavonoids	Shinoda	+	-
Saponins	Foam	+	-
Coumarins	Fluorescence reaction	+	-
Alkaloids	Mayer's, Drangerdorft and Wagner's reagent	-	-
Tannins	Test with potassium ferrocyanide	+	-

Note: +, indicates the presence of metabolites; -, indicates the absence of metabolites

Source: own elaboration (Laboratory log).

Regarding the flavonoids evidenced in the ethanolic extracts, they belong to the group of polyphenolic compounds and are typically known to promote health due to their antioxidant, antiallergic, anti-inflammatory, antimicrobial and anticancer properties; they exist widely in the plant kingdom and show a positive correlation between increased flavonoid consumption and reduced cardiovascular risk and cancer (Khanam *et al*., 2015; Miranda-Da Gama; Guimarães; de Abreu; Armando-Junior, 2014; Qadir; Paul; Ganesh, 2015; Chigayo; Mojapelo; Mnyakeni- Moleele; Misihairabgwi, 2016). Likewise, tannins are oligomeric and polymeric flavonoids widely distributed in vegetables and fruits, they are attributed antioxidant, anti-inflammatory and in the prevention of atherosclerosis (Mamet; Ge; Zhang; Li, 2018). As for saponins, they have in their chemical structure, triterpene or steroid aglycone; that is why the consumption of saponins increases protection against the risk of cancer, decreases the level of cholesterol and glucose in the blood, besides, it is attributed anti-inflammatory, hypocholesterolemic and immunostimulatory properties. (Augustin; Kuzina; Andersen; Bak, 2011). Regarding the coumarins present in the leaves of *P. Americana*, these give aroma to the plants and have vitamin properties, decrease capillary permeability and increase the resistance of the capillary walls (protect capillary fragility and act as a venous tonic), also antibacterial properties are attributed to it (Singh; Singh; Singh; Kaur, 2017).

Other studies carried out identified the following compounds in the leaves of *P. Americana*: kaempferol 3-O-β-D- glucopyranoside, kaempferol 3-O-β-D-xylopyranosyl (1 → 2) - β-D-glucopyranoside, kaempferol 3 -O-α-l-rhamnopyranosyl (1 → 2) - β-D-glucopyranoside, kaempferol 3-O-diglucoside and quercetin 3-O-glucoside (Bylka; Matlawska, 2001); this may be as a consequence of the different geographical locations in which the minerals of the soil and the environmental factors have a great influence on the phytochemical contents of the plant (Borokini; Ayodele, 2012).

3.3. Gas chromatography

Plant-based volatile compounds are mainly produced by different biosynthetic pathways, among which the fatty acid/lipoxygenase pathway stands out (Paré and Tumlinson, 1999), where these are synthesized from linoleic and octadecanoic acid (identified by chromatography of masses). For this reason, it is important to ensure that the drying temperature does not alter the nature of these compounds, guaranteeing their presence in the final extracts.

Table 2 lists the compounds identified by the Nist-05 database in the hexane extracts of *P. American*, the chromatographic analysis carried out on the ethanolic extracts did not show results with retention times greater than 0.5 min that could be considered relevant.

Table 2.

Possible compounds found in the hexane extract of P. Americana

Identification factor %	Name	Retention time (min)
8,46	9,9-dimetoxibicyclo[3.3.1]non ane-2,4-diona	2,44
3,88	9-Ácido octadecenoico (Z)-	1,529
83,40	9-Ácido octadecenoico (Z)-	0,740
4,64	10-metoxy-nb-alfa-metilcorynanteol ($C_{21}H_{29}N_2O_2$)	4,508
0,39	Eritro-9,10-Dibromopentacosane ($C_{25}H_{50}Br_2$)	4,891

Source: database. The National Institute of Standards and Technology (NIST), license acquired by the Universidad del Valle (2018).

Fatty acids, such as 9-octadecenoic acid (Z) or oleic acid are monounsaturated fatty acids of the omega 9 series, their IUPAC name is cis-9-octadecenoic acid, this compound was evidenced in the hexane extracts of *P. Americana* and its chromatogram (see Figure 3).

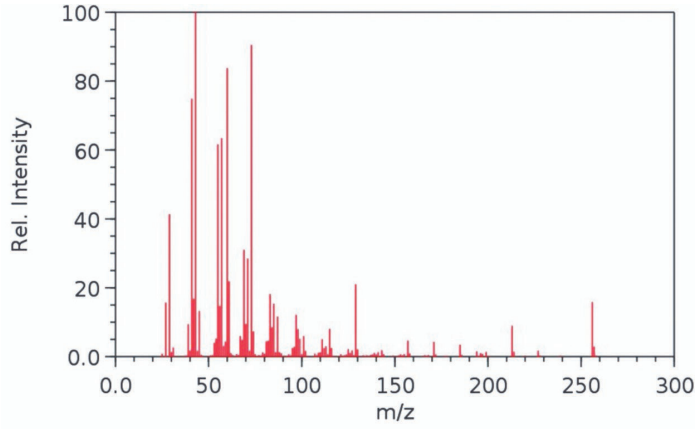

Figure 3. Mass spectrum for oleic acid
Source: self-made.

In Figure 4 it was evidenced that the identified compound has a carboxyl group in its structure, this acid is used mainly in the food industry for its various health benefits.

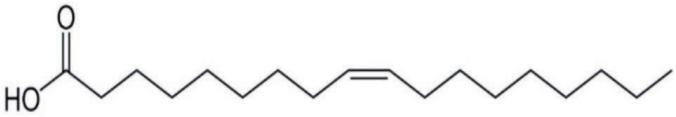

Figure 4. Oleic acid structure
Source: self-made.

On the other hand, the fragmentation in the mass spectrum of some functional groups that are part of the composition and molecular structure of the extracts was contemplated, where molecular ions with mass charge ratios (m/z) were observed that have not yet been published in the literature. Some molecules have a McLafferty arrangement (see Figures 5, 6, 7, 8, and 9).

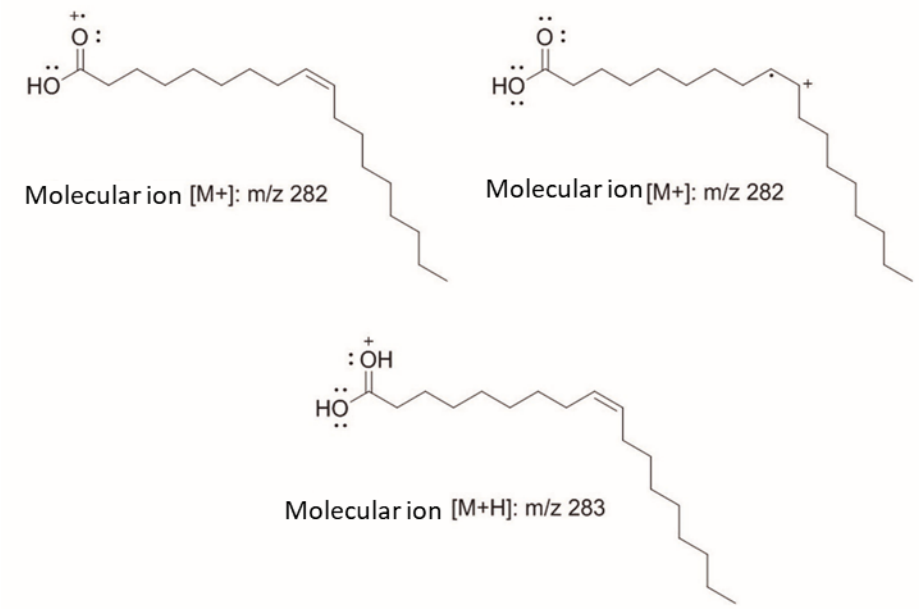

Figure 5. Possible molecular ions in the mass spectrum
Source: self-made.

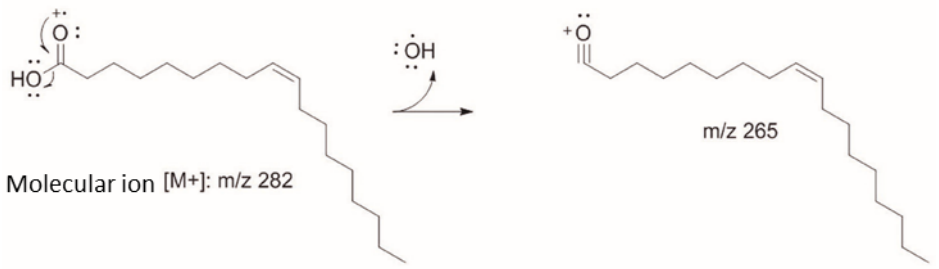

Figure 6. Loss of · OH:
Source: self-made.

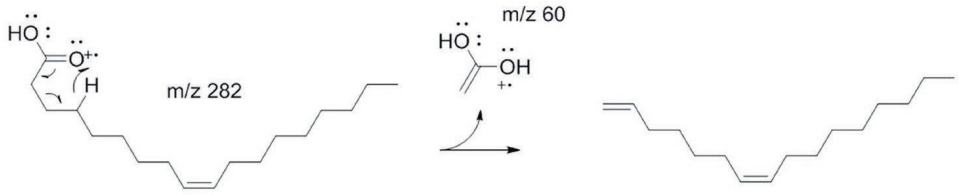

Figure 7. McLafferty arrangement
Source: self-made.

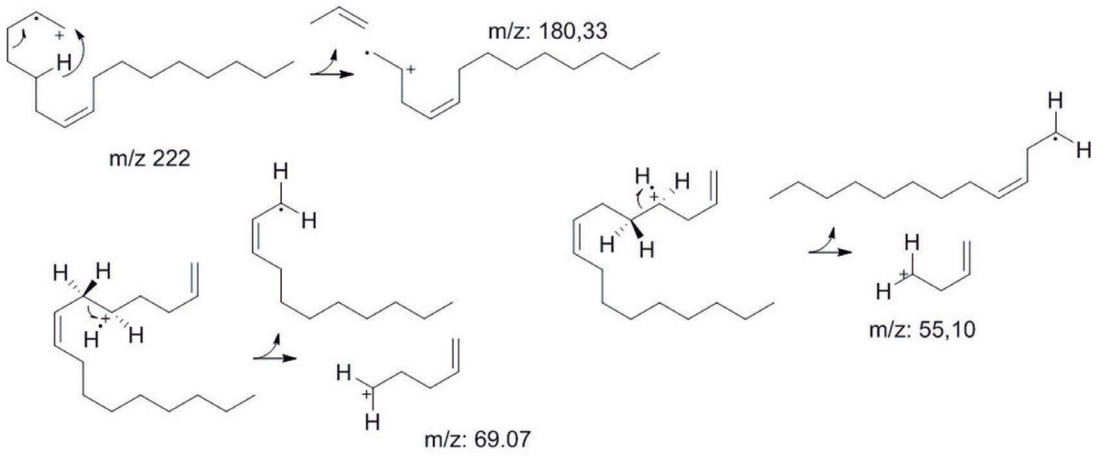

Figure 8. Generation of new molecular ions and breaks in double bonds
Source: self-made.

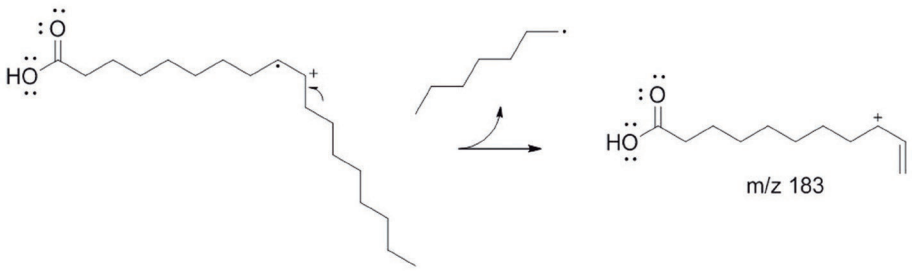

Figure 9. Double bond break
Source: self-made.

Consequently, the compound identified with a carboxyl group in its structure in the *P. Americana* extracts is of agro-industrial importance, since most of the biologically derived unsaturated fatty acids are derived from oleic acid, linoleic acid, and linolenic acid. ; These acids are found in biomass and natural extracts with oleaginous characteristics. Finding them in these species is because plants accumulate lipids when growth is limited, due to the lack of nitrogen or other types of nutrients. Compounds derived from fatty acids such as cholesterol, ergosterol, and others, are common to find in vegetative cells and are classified as secondary metabolites (Samson; de Boer, 1995).

Oleic acid participates in the synthesis of prostaglandins, in the generation of the membrane, as well as in other biological processes related to cell regeneration; fatty acid supplementation can cure these skin symptoms and stimulate epithelialization (Salari; Bakhshi; Sharififar; Naseri; Almani, 2016). Similarly, the consumption of oleic acid promotes good functioning of the human nervous and visual system (Teichmann; Dutta; Staffas; Jägerstad, 2007; Horst, 2012; Nouripour-Sisakht *et al*., 2015; Cerqueira-Sales; Barcellos -Costa; Machado-Bueno; Aires-Ventura; Dummer Meira, 2016). Therefore, the identification of these compounds for exploitation purposes is important, since it would give added value to the supply of plants such as *P. Americana*, abundant in peasant regions dedicated to agriculture such as the department of Nariño.

3.4. Antifungal activity

Preliminary antifungal tests were carried out with five concentrations (60, 120, 180, 240 and 300 mg/mL) of the ethanolic extracts of the leaves of *P. Americana*, thus showing the average inhibition diameters with which it was possible to observe that concerning the *Aspergillus brasiliensis* strain, there was no antifungal activity on the part of *P. Americana* extracts towards this strain compared to the positive control (47 mm), Aspergillus brasiliensis is a fungus of the *Aspergillus* family causing a wide range of diseases in humans, such as invasive or allergic aspergillosis, aspergilloma, sinusitis, otomycosis, onychomycosis and keratitis (Nouripour-Sisakht *et al*., 2015), for which an inhibitory effect would have been desired by the study plant.

On the other hand, it was evidenced that there is a greater inhibition for the *F. solani* and *C. albicans* strains at extract concentrations of 240 and 300 mg/mL, respectively. These results agree with those obtained by Peng *et al*., (2005) in the *F. Solani* fungus, where it obtained a great inhibiting effect against this strain and mentions that the antimicrobial action of the leaves of *P. Americana* is possibly due to the hydrophobic surface of these and the plasma membrane that contains the lipid bilayer, with antimicrobial proteins that are also found in the seed and root of the plant.

Concerning the S. kudriavzevii strain, a family of Saccharomyces cerevisiae, which is a common mucosal colonizer and produces both superficial and invasive visceral infections (Davicino *et al*., 2007), a high inhibition effect was also observed (13 ± 7mm) at the highest concentrations of the plant extract evaluated (see Table 3).

The mentioned results are in agreement with the study carried out by Shao *et al.,* (1999) that mentions that the defense mechanisms of plants such as *P. Americana* are due to the presence of a wide range of antimicrobial peptides or proteins and the particular interest is to isolate these components for therapeutic purposes; therefore, it isolated a highly basic peptide with 38 residues (amino acids) and three disulfide bridges called PAFP-s, from the seeds of this plant and verified from this, a significant broad spectrum of antifungal activity against various fungi, except towards *Escherichia coli*.

Table 3.
Antifungal activity of the leaves of Phytolacca americana

CEPA / MIC Minimum Inhibitory Concentration (mg / mL)	Inhibition diameter (mm)						
	60	120	180	240	300	Control negative Ethanol to 95%	Control positive thiabendazole (500 mg/mL)
Aspergillus brasiliensis (ATCC 16404)	6 (N/A)	6 (N/A)	6 (N/A)	6 (N/A)	7 (N/A)	6 (N/A)	47
Fusarium Solani (ATCC 36031)	13	16	12	18	16	6 (N/A)	18
Candida albicans (ATCC 90028)	9 ±	7	18	16	19	6 (N/A)	21
Saccharomyces kudriavzevii (ATCC 260)	7	9	12	13	13	6 (N/A)	21

N/A: No activity
Source: own elaboration (Laboratory log).

Therefore, it can be affirmed that, compared to other studies (low concentrations of extracts), it must be taken into account that we worked with crude extracts that contain metabolites or impurities without antifungal activity. Regarding the antimicrobial index, a low index was evident, due to the high susceptibility of the fungi concerning the positive control, this was corroborated with the values of diameters of inhibition of concentrations of the extracts, such as the values of the F strain. Close Solani with the positive control (18 mm). Regarding the negative control in which the raw extracts of the plant were found, it was shown that ethanol is not responsible for the inhibitory effect against the evaluated strains, since no inhibition diameters were obtained, therefore the effect of inhibition directly to the extracts (Pérez; Rojas; Chamorro; Pérez, 2011).

Antifungal activity of the plant due to the presence of secondary metabolites such as flavonoids, as it was found in both the characterization UV/VIS as phytochemical *screening*. The evaluation of the antifungal activity of *P. Americana* evidenced the presence of flavonoids compared to other studies related to the *Phytolaccaceae* species, which also obtained favorable results regarding the antimicrobial activity of other species, indicating that the study plant has potential microbial agents that would be very important to isolate (Ogutu *et al*., 2012). Also, it was corroborated with the presence of tannins that are secondary metabolites naturally produced in medicinal and traditional herbs, rich in polyphenols, likewise, this antifungal activity can be derived from the expression of substances with polar characteristics, such as saponins and bioflavonoids that promote anti-radical action free synergistic biological activity, which is driven by maceration of the ethanol extraction plant (Cerqueira-Sales *et al*., 2016).

Regarding the analysis of variance, this was performed for several samples, with the *C. albicans* strain, the P-value of the F-test was less than 0.05 ($p \leq 0.0242$), which showed that there is a difference Statistically significant between the means of the five concentrations evaluated of the extract, the confidence level reached was 95%, so it can be concluded that the ethanolic extract of the leaves of *P. Americana* was more effective in this strain, compared to the others. strains evaluated. These species, such as *Candida*, are human pathogenic fungi that cause systemic and mucous infections that can become serious life-threatening infections with increased mortality among immunocompromised patients. Regarding the antifungal activity of *P. Americana* towards *F. solani*, which is a filamentous fungus that causes the elimination of many crops in fields and greenhouses and, also, produces infections in humans such as fungal peritonitis (Wang; Zheng; Xiang; Li; Yang, 2016; Mayr;

Rasch; Schmid; Huber; Lahmer, 2017) would be an effective and sustainable alternative to synthetic fungicides in the control of pathogenic fungi.

The results of the present work indicated that *P. Americana* has antifungal properties. This explains the use of this plant in popular medicine for the treatment of diseases whose symptoms may involve fungi, infections, among others, and underline the importance of phytochemistry in the discovery of new bioactive compounds to identify the active principles responsible for the effects. antifungals, with the added benefits of a safe environment besides, a contribution to the rescue of this endangered plant, giving it applications in the pharmaceutical and agricultural sectors.

4. Conclusions

The presence of phytochemicals such as flavonoids, saponins, coumarins, and tannins is evident in the ethanolic extracts of the leaves of *P. Americana*, this information may serve as a potential source in the use of useful drugs in the future. Regarding chromatographic analysis, the presence of high added value essential fatty acids was identified, such as oleic acid, 9,9 - dimethoxybicyclo [3.3.1] non-ane-2,4-dione, 10-methoxy-nb-alpha- methylcorynanteol ($C_{21}H_{29}N_2O_2$), erythro-9,10-Dibromopentacosane ($C_{25}H_{50}Br_2$), among others, with low retention times, with percentages of less than 1%. A greater antifungal potential was determined from the ethanolic extracts against the *F. solani* and *C. albicans* strains, which would boost the use of this plant in the agricultural and pharmaceutical sectors. Experimental tests with other types of bacterial and fungal species are recommended to determine a greater microbial efficacy of the *P. Americana* extracts.

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