

## Pharmacognostic, chemical and mucolytic activity study of *Malva pseudolavatera* Webb & Berthel. and *Malva sylvestris* L. (Malvaceae) leaf extracts, grown in Ecuador

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**Abstract.** Sarmiento-Tomalá GM, Miranda-Martínez M, Chóez-Guaranda IA, Gutiérrez-Gaitén YI, Delgado-Hernández R, Carrillo-Lavid G. 2020. Pharmacognostic, chemical and mucolytic activity study of *Malva pseudolavatera* Webb & Berthel. and *Malva sylvestris* L. (Malvaceae) leaf extracts, grown in Ecuador. *Biodiversitas* 21: 4755-4763. Among the cultivated and/or native plant species of Ecuador, are those belonging to Malvaceae. Of these, the most traditionally used are *Malva pseudolavatera* Webb & Berthel. and *Malva sylvestris* L., which are grown and sold in indigenous markets. Various articles have been published for *M. sylvestris* about its chemical composition and pharmacological properties; however, *M. pseudolavatera* lacks references and is the most commercialized in Ecuador. Therefore, this work sets the following objective: To carry out a comparative study of the pharmacognostic, chemical and mucolytic activity of the species *M. pseudolavatera* and *M. sylvestris*. The species studied were collected in the province of Chimborazo. Extracts were obtained with different solvents: water, hexane and 80% ethanol. The aqueous extract was used to determine the mucolytic activity; the hexane and alcoholic extracts were analyzed by the coupled gas chromatography-mass spectrometry system. It was found that *M. pseudolavatera* has a very similar chemical composition to *M. sylvestris*; The presence of fatty acids, di and triterpenoids, phytosterols and abundant amino acids was detected. Both species showed an important mucolytic effect, the activity of *M. pseudolavatera* being higher than the highest doses tested. These studies provide scientific data that allow demonstrating the high potentiality of extracts from the leaves of two *Malva* species as sources of plant material for possible research and development of phytotherapeutic products with mucolytic and gastroprotective activity in correspondence with their uses in traditional Ecuadorian herbal medicine.

**Keywords:** Chemical composition, gas chromatography-mass spectrometry, pharmacognosy, physicochemical parameters

### INTRODUCTION

Among the 10 countries with the greatest biodiversity in the world, Ecuador is home to 10% of the flora existing on the planet, where 64 of every 100 Ecuadorian species grow in the Andes mountain range. This great diversity of Ecuadorian flora has been recognized and studied, documenting the presence of more than 16,000 species of plants, although it is estimated that they exceed 20,000. Among the diversity of flora found in the country, many are considered medicinal due to their effects. Pharmacological attributes attributed to traditional medicine and the uses are given to it by the Andean communities (de la Torre et al. 2008). Among these species, those belonging to the genus *Malva* stand out, which includes some 30 species distributed in temperate, subtropical, and tropical zones of Africa, Asia, and Europe and has been introduced throughout the American continent (Gimeno 2000).

Many species of the genus are traditionally used for their medicinal use, but among them, *Malva sylvestris* stands out for its use as medicinal and consumption as food (Razavi et al. 2011; Gasparetto et al. 2012; Nasiri et al. 2015; Mofid et al. 2015). It is traditionally used in the treatment of skin conditions or damage due to its carminative, antimicrobial, and anti-inflammatory activities, in injuries and internal or external inflammation against edema, burns, helping to improve contraction and wound healing (Barros et al. 2010; Pardo et al. 2014; Saad et al. 2017; EMA/HMPC 2018). It is also attributed to mucolytic properties, such as a liver cleansing tonic (hepatoprotective), hypoglycemic, etc. (Prudente et al. 2013; Matera et al. 2015; Lim 2016). It is also used as food, in certain places in the Mediterranean region and in the form of infusions or herbal teas (Hussain et al. 2014; Ahmad et al. 2016; Jabri et al. 2017).

Several chemical components have been reported for *M. sylvestris*, with the use of high-performance liquid chromatography, the presence of amino acids was detected including alanine, threonine, hydroxyproline, serine, glutamine, asparagine and arginine, trigonelline and glycine, as well as betaine in the leaves, flowers, and roots, respectively (Blunden et al. 2001). The presence of anthocyanosides, vitamins A, B1, B2, C, carotenes, coumarinic, chlorogenic and caffeic acids has also been reported, flavonoids, tannins, anthraquinone derivatives, and oleic, palmitic and stearic acids (Hiçsönmez et al. 2009; Zohra et al. 2013). The flowers and leaves contain mucilages (greater than 10%) that by hydrolysis provide arabinose, glucose, rhamnose and galacturonic acid. In addition, the presence of terpenoids such as: sesquiterpenes, diterpenes, and monoterpenes has been reported (Classen and Blaschek 2002; Dipak 2016).

Among the species that are marketed in Ecuador, the one with the highest consumption is *Malva pseudolavatera* (de la Torre et al. 2008), however, so far there is no information on its chemical composition and biological properties, so the proposed objective of present work is to carry out a comparative study of the pharmacognostic, chemical and mucolytic activity of the species *M. pseudolavatera* and *M. sylvestris*.

## MATERIALS AND METHODS

### Collection and drying

The plant material (leaves of *M. sylvestris* and *M. pseudolavatera*), were collected in the month of September 2019 from the city of Riobamba (1°40'15.5"S, 78°38'49.6"W), Chimborazo province, located in the Andes mountains at 2750 meters above sea level, having average area temperature of 13°C, average humidity 62% and 561mm annual precipitation

A sample of each species was herborized in the GUAY herbarium of the Faculty of Natural Sciences of the University of Guayaquil, Ecuador, where the herbarium numbers were assigned, 13118 *M. sylvestris* and 13119 *M. pseudolavatera*. Genetic characterization was also carried out on these species (Sarmiento-Tomalá et al. 2020).

The leaves of both species were washed with drinking water and dried in a Mettler Toledo brand oven at 40° C, until constant weight, after which they were crushed in a Pulvex mill with blades at a particle size of 2 mm and stored in amber glass flasks.

### Determination of the physicochemical parameters of drugs: Qualitative chemical study

The powder from the leaves of the two species were determined in triplicate and the physicochemical parameters, residual moisture (azeotropic method), content of soluble substances (water and hydroalcoholic mixtures at 30, 50, 80, and 98%), total ash, ash soluble in water and ashes insoluble in 10% hydrochloric acid were measured through the procedures described by WHO (2011). Phytochemical screening was performed on dry tubers, according to the procedure described by Miranda and Cuéllar (2000). An

extraction system was used with a battery of solvents, of increasing polarity with ethyl ether, ethanol, and water, on the same plant material to obtain the corresponding extracts which were subjected to the different tests.

### Obtaining and analyzing extracts

Three types of extracts were obtained: aqueous, hexane, and hydroalcoholic. The aqueous extract was prepared from 100 g of dry leaves crushed in 500 mL of distilled water. The mucilage was obtained by assisted precipitation, adding three volumes of 96% ethanol per 100 mL of the aqueous extract. Once filtered, the precipitate obtained was dehydrated at 50° C for 3 hours in a recirculating air oven (Vargas-Rodríguez et al. 2016).

The extraction with hexane was carried out by Soxhlet. Hexane (Merck) was used and an extraction time of two hours. 20 g of leaves of each species and 200 mL of hexane started and 3 extractions were made.; Subsequently, they were distilled in a Heildoph Laboratory model 4001 efficient HB digital rotary evaporator at reduced pressure, 50 rpm, and a temperature of 40° C. The ethanolic extracts were made from the plant material at a rate of 20g of drug/100 mL of solvent, by the maceration method with sporadic stirring, for a period of seven days at a temperature of 30° C  $\pm$  2° C, using an 80% hydroalcoholic mixture as solvent (Miranda and Cuéllar 2000).

Quality determinations were made on these extracts, three replicates for each experiment, the following parameters being evaluated: organoleptic properties (odor and color), pH, refractive index (ABBE digital refractometer), relative density (by pycnometer), and solids total (Miranda and Cuéllar 2000). These extracts were also determined the content of total phenols by the Folin-Ciocalteu method (Pourmorad et al. 2006; Memnune et al. 2009; Chlopicka et al. 2012) and total flavonoids by the colorimetric method of aluminum chloride (Chang et al. 2002; Pourmorad et al. 2006).

### Saponification of the hexane extract and methylation of fatty acids

The oils obtained from the hexane extract were independently saponified according to the technique of Bombón and Albuja (2014). Sodium hydroxide, concentrated hydrochloric acid, anhydrous sodium sulfate (Sigma-Aldrich), ethanol, and diethyl ether (Merck) were used. For the methylation of the fatty acids, a methanol/oil molar ratio of 11.66: 1 was used. The mixture was stirred at a temperature of 35° C for 3 minutes (Salimon et al. 2014).

### Analysis by gas chromatography-mass spectrometry

The fractions of the methylated saponifiable compounds and the unsaponifiable ones were analyzed in an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometer with electron impact ionization source and simple quadrupole analyzer. Injector temperature: 280° C, injection volume 2  $\mu$ L, helium carrier gas at 1 mL/min. The working conditions were: HP-5 column, 5% Phenyl Methyl Siloxan of 30 m x 0.30 mm  $\times$  0.25  $\mu$ m, initial temperature: 150° C for 4 minutes increasing 2° C/min up to 300° C for 2 min. Analysis time: 79 min.

Mass spectrometer operated at 70 eV in full scan mode from 50 to 600 mass units. Source temperature 230° C, quadrupole temperature 150°C. Compounds were identified by comparing their mass spectra and the Wiley 9th mass reference with the NIST 2011 MS Library taking into account those with a percentage similarity of 95% or higher.

Extract ethanolic: Dried samples were mixed with N, O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) and heated in a water bath at 80°C for 2 h to allow the silylation of metabolites (Saitta et al. 2002). Next, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed in GC-MS equipment of the brand Agilent Technologies (7890A GC system and 5975C inert XL MSD with triple-axis detector). A capillary column DB-5MS (30 m × 0.25 mm) with phenyl dimethylpolysiloxane was used as stationary phase (0.25-micron film thickness) and helium as the carrier gas (1.2 mL/min). The injection of 1 µL of derivatized sample was performed at 250° C with splitless mode. The oven temperature was started at 70° C for 2 minutes, then it was increased to 300°C at 5°C/min, and it was maintained at 300°C for 6 minutes. The compounds identification was done by comparison of mass spectra based on the ninth version of the Wiley software, and NIST 2011 MS Library. An electron ionization of 70 eV at 230°C was used in the ion source and the data compounds were collected with the full scan mode (40-600 amu) in the quadrupole mass analyzer.

### Mucolytic activity

The precipitate of the aqueous extracts of *M. sylvestris* and *M. pseudolavatera* (mucilage) and the phenol red method (Engler and Szelenyi 1984) were used. 8 test groups were formed with 5 male mice each, of the CD1 strain with a weight between 25 and 30 g, identified with letters from A to H at the different concentrations tested (Table 1). The administration of the precipitates and bromohexine was carried out orally and phenol red intraperitoneally.

After 24 hours of animal fasting, the administration of the 300 mg/kg phenol red indicator was administered to all groups of animals by the intraperitoneal route, except for the control group (-). 1 hour after the administration of phenol red, all groups of animals were sacrificed, followed by the removal of their tracheas, for their respective evaluation. All the animals used received care and attention according to the established international regulations, following Bioethics and Biosafety Standards established by (The World Medical Association 2016).

**Table 1.** Test groups for mucolytic activity

Groups	Description
A	Control (-) Phenol red 300 mg/kg (bw)
B	Control (+) Bromhexine 100 mg/kg (bw)
C	<i>Malva pseudolavatera</i> 250 mg/kg (bw)
D	<i>Malva pseudolavatera</i> 500 mg/kg (bw)
E	<i>Malva pseudolavatera</i> 1000 mg/kg (bw)
F	<i>Malva sylvestris</i> 250 mg/kg (bw)
G	<i>Malva sylvestris</i> 500 mg/kg (bw)
H	<i>Malva sylvestris</i> 1000 mg/kg (bw)

Note: bw: body weight

The phenol red concentration was determined spectrophotometrically (Genesys 10S UV VIS Spectrophotometer), using a wavelength of 546 nm. For the determination, a blank composed of 1 mL of 0.9% sodium chloride and 0.1 mL of 0.1N NaOH solution was used. The percentages of mucolytic activity were determined by the following formula:

$$P = \frac{T - C}{C} \times 100$$

Where:

P : Percentage (%) of mucolytic activity.

T : Tracheal secretion of phenol red in mice treated with the extracts every day.

C : Average tracheal secretion of phenol red in the group treated as control.

### Statistical analysis.

Data were expressed as arithmetic mean/standard deviation. The one-way analysis of variance (One-way ANOVA) was used to determine if there was a statistically significant difference for the evaluated variable and then Kruskal-Wallis was applied, followed by the Friedman test. The level of significance set was  $P < 0.05$ . The data obtained in each trial were processed using the SPSS statistical package for Windows version 8.0.

## RESULTS AND DISCUSSION

### Physicochemical parameters to drugs: Qualitative chemical study.

The moisture content was within the range established by the literature for medicinal plants (8-14%), although significant differences were observed between both species, with *M. pseudolavatera* presenting higher moisture content, which may be in correspondence with the dimensions of the leaves that are superior in this species. Of the solvents tested for the determination of extractable substances, the highest extractive power was achieved with water and the 50% hydroalcoholic mixture, which indicates a high concentration of polar substances.

Regarding ash, some Pharmacopoeias suggest a total ash index of up to 5% (Lou 1980; WHO 2011) and another, such as the Chinese Pharmacopoeia (ChP 2015), refers up to 15%. In the experience carried out, the percentage was found below the maximum allowed limit. On the other hand, the ashes insoluble in acid and soluble in water were higher than 2%. Between both species, significant differences were found in this parameter (Table 2).

For the species *M. sylvestris*, it has been reported that it has the property of accumulating nutrients such as phosphorus, potassium, nitrogen, and magnesium, as well as heavy metals such as cadmium, copper, nickel, lead, and zinc (Elsagh et al. 2015; Ahmad et al. 2016), which may justify the high values found for ash.

In the phytochemical analysis, there were no differences in the chemical composition of the drugs under study. The presence of phenolic compounds, triterpenes/steroids, and mucilage should be highlighted (Table 3).

**Table 2.** Physicochemical parameters of raw drugs from *Malva sylvestris* and *M. pseudolavatera* leaves

Parameters (%)	Results ( $\bar{x}$ /SD)	
	<i>M. sylvestris</i>	<i>M. pseudolavatera</i>
Moisture content (%)	873/013 <sup>a</sup>	1014/014 <sup>b</sup>
Water-soluble extractive	3045/119 <sup>c</sup>	2738/101 <sup>d</sup>
Alcohol-soluble extractive at 30%	1877/149 <sup>e</sup>	1951/059 <sup>e</sup>
Alcohol-soluble extractive at 50%	2305/026 <sup>f</sup>	1710/010 <sup>g</sup>
Alcohol-soluble extractive at 80%	1579/023 <sup>h</sup>	1092/032 <sup>i</sup>
Alcohol-soluble extractive at 98%	1006/013 <sup>j</sup>	739/033 <sup>k</sup>
Total ash content	1424/032 <sup>l</sup>	1525/014 <sup>m</sup>
Water-soluble ash	435/02 <sup>n</sup>	417/006 <sup>n</sup>
Acid-insoluble ash	1261/017 <sup>o</sup>	1302/008 <sup>p</sup>

Note:  $\bar{x}$ /SD: Average value of determinations (n = 3)/standard deviation. Different letters in a row show significant differences (p < 0.05)

**Table 3.** Phytochemical screening of raw drugs from *Malva sylvestris* and *M. pseudolavatera*

Metabolites	Name of test	Extracts					
		Ethereal		Alcoholic		Aqueous	
		MS	MP	MS	MP	MS	MP
Fats or oil	Sudan III	+	+				
Mucilages	Mucilage test					++	++
Coumarins/lactones	Baljet test	-	-	+	-		
Triterpenes/steroids	Liebermann-Burchard	++	++	++	++		
		Green dark	Green dark	Green dark	Reddish green dark		
Saponins	Foam test			+	+	±	±
Resins	Resin test			-	-		
Amino acids	Ninhydrin			++	++		
Reducing compounds	Fehling test			++	++	++	++
Phenols/tannins	Ferric chloride test			++	++	++	+
Anthocyanins	HCl conc./pentanol			++	++		
				Brown	Brown		
Quinones	Börntrager			+	+		
Flavonoids	Shinoda (Mg-HCl)			++	++	++	++
				Brown	Brown	Yellow	Yellow
Cardiac glycosides	Kedde test			-	-		
Catechins	Catechin test			+	+		
Bitter & astringent principles						+	+

Note: + : positive, ++ : Highly positive, - : negative, ± : undefined test, MS: *Malva sylvestris*, MP: *Malva pseudolavatera*

### Analysis of the extracts

To the hydroalcoholic extracts of the species under study, the quality parameters were determined (Table 4), for the parameters pH and total solid significant differences (p < 0.05) were found between the species. For *M. sylvestris* the pH value was closer to neutral values, while for *M. pseudolavatera* the pH was close to acid values. On the other hand, total solids were somewhat higher for *M. sylvestris*.

The content of total phenols was determined by the Folin-Ciocalteu method, in the quantification of total flavonoids the colorimetric method of aluminum chloride was used. In both quantifications, calibration curves with good correlation between the tested concentrations of the reference substances (gallic acid and quercetin) and the absorbances were achieved. The correlation coefficient ( $R^2$ ) was  $\geq 0.99$ , this is indicative of the good fit of the model equation to the experimental data (Figure 1).

Table 5 shows that the species *M. sylvestris* presented a higher content of phenols and total flavonoids with significant differences between them. Phenolic compounds and flavonoids in particular are associated with a wide spectrum of health benefits, which is due to the wide range of pharmacological properties, associated with their ability to modulate the key functions of many cellular enzymes (Panche et al. 2016; Najjaa et al. 2020). The concentration of these metabolites in plants is very sensitive to environmental and climatic conditions, geographical location, and taxonomic variety (Najjaa et al. 2020).

### Analysis by gas chromatography-mass spectrometry

#### Fractions of saponifiable and unsaponifiable compounds

As can be seen in Figure 2 and Table 6, for the fraction of saponifiable compounds, three fatty acids were identified, the majority being linolenic acid. These three compounds presented higher abundance for the species *M. pseudolavatera* and have been reported for the species *M. sylvestris* (Mofid et al. 2015).

**Table 4.** Physicochemical parameters of the hydroalcoholic extracts from *Malva sylvestris* and *M. pseudolavatera*

Parameters (%)	Results ( $\bar{x}/SD$ )	
	<i>M. sylvestris</i>	<i>M. pseudolavatera</i>
pH	6.20/0.01 <sup>a</sup>	5.83/0.02 <sup>b</sup>
Total solids (%)	1.88/0.09 <sup>c</sup>	1.40/0.05 <sup>d</sup>
Refraction index	1.3601/0.0001 <sup>e</sup>	1.3593/0.0002 <sup>f</sup>
Relative density (mg/mL)	0.8740/0.0005 <sup>g</sup>	0.8743/0.0015 <sup>g</sup>

Note:  $\bar{x}/SD$  = Average value of determinations (n = 3)/standard deviation. Different letters in a row show significant differences (p < 0.05)

In the unsaponifiable compound fractions, 10 components were identified for each species (Figure 3, Table 6). For both fractions, an abundance of hydrocarbons is observed, the majority of them being nonacosane for the species *M. pseudolavatera* and triclosan for *M. sylvestris*. With the exception of nonacosane, the rest of the hydrocarbons presented a higher percentage of abundance in *M. sylvestris*. Hydrocarbons are part of plant waxes that are modifications of the cell wall by deposit and serve as protection against excessive evaporation of water (Miranda and Cuéllar 2012).

The diterpenoids neophytadiene and phytol, the acyclic triterpenoid squalene, and the sterol stigmasterol were also found in these extracts, all with greater abundance in *M. pseudolavatera* and stigmasterol not detected in the *M. sylvestris* species, possibly due to concentration problems. Terpenoids have also been reported metabolites for the species *M. sylvestris*, linked to different pharmacological activities (Mohajer et al. 2016).

**Table 5.** Total phenols and total flavonoids content of the hydroalcoholic extracts from *Malva sylvestris* and *M. pseudolavatera*

Extracts	Total phenols (mmg/mL) $\bar{x}/SD$	Total flavonoids (mmg/mL) $\bar{x}/SD$
<i>M. sylvestris</i>	1.88/0.03 <sup>a</sup>	1.65/0.02 <sup>c</sup>
<i>M. pseudolavatera</i>	1.47/0.04 <sup>b</sup>	1.22/0.01 <sup>d</sup>

Note:  $\bar{x}/SD$  = Average value of determinations (n = 3)/standard deviation. Different letters in a row show significant differences (p < 0.05)

#### Hydroalcoholic extracts

Figure 4 shows the analytical gas chromatograms of the hydroalcoholic extracts of the two species under study. Great complexity is observed in both extracts with appreciable qualitative differences. Table 7 shows the compounds identified for both extracts.

For the species *M. sylvestris*, the major component was  $\alpha$ -linolenic acid (30.61%), followed by palmitic acid (12.14%) and stearic acid (8.31%). For the species *M. pseudolavatera*, the main component was glucitol sugar (14.91%), although the fatty acids  $\alpha$ -linolenic acid (13.75%), palmitic acid (13.40%), and stearic acid (14.98%) were also present with different abundance percentages.

An important aspect to highlight is that in addition to fatty acids and sugars, the presence of abundant amino acids in both extracts and some triterpenes and sterols were detected, all these compounds previously reported for *M. sylvestris* (EMA/HMPC 2018).

The differences that are qualitatively observed may be due to concentration problems not detectable by the equipment or to biogenetic differences between species.

**Table 6.** Compounds identified in the fractions of saponifiable and unsaponifiable compounds of *Malva sylvestris* and *M. pseudolavatera*

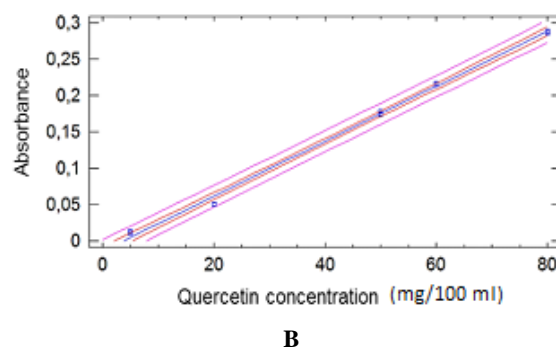
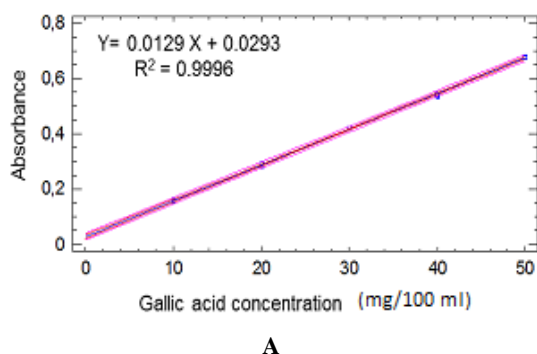
No.	RT (min)	Compound	Content % ̄/SD	RT (min)	Compound	Content % ̄/SD
		<i>M. sylvestris</i>			<i>M. pseudolavatera</i>	
		<b>Saponifiable fraction</b>				
1	20.07	Hexadecanoic acid (palmitic acid)	23.17/1.64	20.08	Hexadecanoic acid (palmitic acid)	27.42/7.65
2	26.95	9,12-octadecadienoic acid (linoleic acid)	6.81/0.49	26.96	9,12-octadecadienoic acid (linoleic acid)	11.02/3.46
3	27.16	9,12,15-octadecatrienoic acid (linolenic acid)	35.46/3.03	27.17	9,12,15-octadecatrienoic acid (linolenic acid)	61.56/6.75
		<b>Unapponable fraction</b>				
1	26.17	neophytadiene	4.80/0.06	26.17	neophytadiene	5.00/0.20
2	31.42	phytol	7.12/0.12	31.43	phytol	26.95/0.63
3	32.60	docosane	0.50/0.01			
4	34.84	tricosane	35.85/0.25	34.82	tricosane	4.81/0.13
5	36.47	tetracosane	1.73/0.01	36.47	tetracosane	0.44/0.02
6	38.06	pentacosane	11.28/0.06	38.06	pentacosane	3.59/0.11
7	41.07	heptacosane	16.33/0.02	41.06	heptacosane	9.66/0.36
8	42.48	octacosane	1.41/0.08	42.48	octacosane	2.97/0.03
9	42.62	squalene	1.49/0.05	42.60	squalene	1.62/0.10
10	43.86	nonacosane	19.49/0.07	43.88	nonacosane	42.99/1.17
11				48.96	Stigmasta-5-en-3β-ol	1.98/0.22

Note:  $\bar{x}/SD$  = Average value of determinations/standard deviation (n=3)

**Table 7.** Compounds identified in the hydroalcoholic extracts of *Malva sylvestris* and *M. pseudolavatera*

No.	RT (min)	Compound	Content % x̄/SD	RT (min)	Compound	Content % x̄/SD
Hydroalcoholic extracts <i>M. sylvestris</i>				Hydroalcoholic extracts <i>M. pseudolavatera</i>		
1	8.32	L-alanine	5.26/0.03			
2	11.22	L-valine	1.48/0.01	11.20	L-valine	4.47/0.02
3				13.00	Nicotinic acid	0.27/0.04
4	13.91	Butanedioic acid, Succinic acid	1.39/0.16	13.65	Butanedioic acid, Succinic acid	1.65/0.07
5				14.42	Uracil	0.41/0.06
6				14.87	2-butenedioic acid, Fumaric acid	2.41/0.35
7				15.75	L-threonine	1.92/0.28
8	19.15	L-aspartic acid	7.16/0.03	19.12	L-aspartic acid	2.61/0.38
9	19.97	L-threonine acid	1.43/0.08	19.68	L-threonine acid	1.92/0.28
10				22.03	L-arabinopyranose	0.76/0.11
11	23.41	L-lysine	1.02/0.01	23.41	L-lysine	1.00/0.15
12	25.80	Citric acid	0.69/0.03	25.78	Citric acid	0.89/0.13
13	26.60	Adenine	1.75/0.02	26.60	Adenine	2.61/0.35
14	28.02	Glucitol	2.31/0.01	28.03	Glucitol	14.91/0.04
15	28.15	L-tyrosine	4.80/0.01			
16	29.30	Hexadecanoic acid ethyl ester	1.66/0.00	29.29	Hexadecanoic acid ethyl ester	1.72/0.00
17	29.65	Purine	3.14/0.04			
18	30.32	Hexadecanoic acid	12.14/0.05	30.32	Hexadecanoic acid	13.40/0.02
19	31.61	Guanina	0.78/0.01	31.61	Guanina	0.48/0.00
20	32.44	9,12,15-octadecatrienoic acid ethyl ester	3.74/0.01	32.32	9,12,15-octadecatrienoic acid ethyl ester	3.55/0.00
21	33.16	L-tryptophan	6.60/0.00	33.16	L-tryptophan	9.42/0.03
22	33.23	9,12-octadecadienoic acid	2.91/0.00	33.22	9,12-octadecadienoic acid	2.27/0.01
23	33.41	A-linolenic acid	30.61/0.08	33.36	A-linolenic acid	13.75/0.04
24	33.46	Oleic acid	0.46/0.00	33.45	Oleic acid	1.17/0.01
25	33.85	Octadecanoic acid	8.31/0.04	33.86	Octadecanoic acid	14.98/0.03
26	49.23	B-sitosterol	1.34/0.01	49.23	B-sitosterol	1.79/0.01
27				49.49	B-amyirin	0.48/0.01
28	50.09	A-amyirin	1.02/0.00	50.09	A-amyirin	0.62/0.01

Note: RT= retention time, x̄/SD = Average value of determinations/standard deviation (n=3)

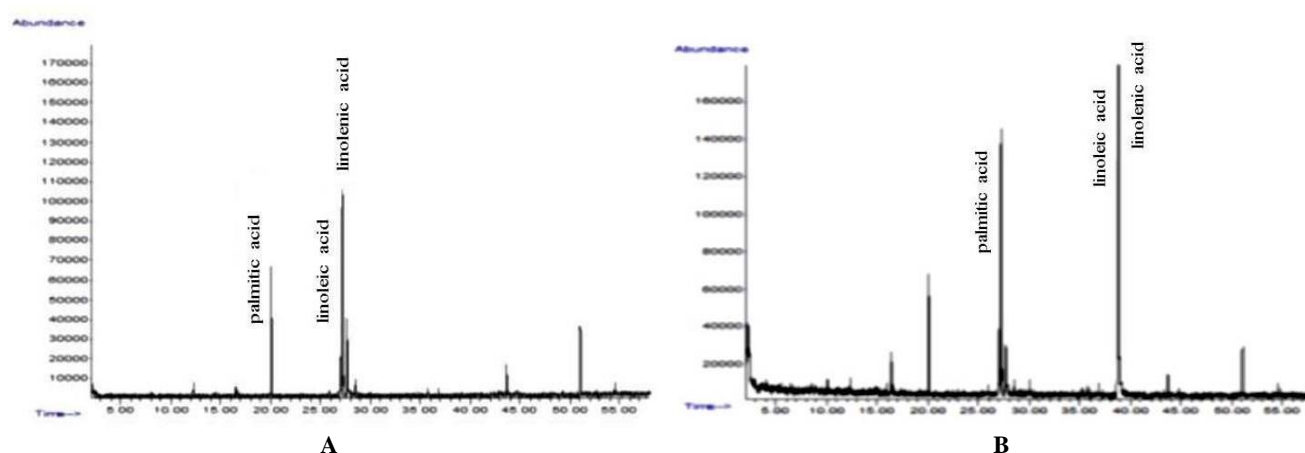
**Figure 1.** Calibration curves of the gallic acid (A) and quercetin (B) for the determination of total phenols and total flavonoids

### Mucolytic activity

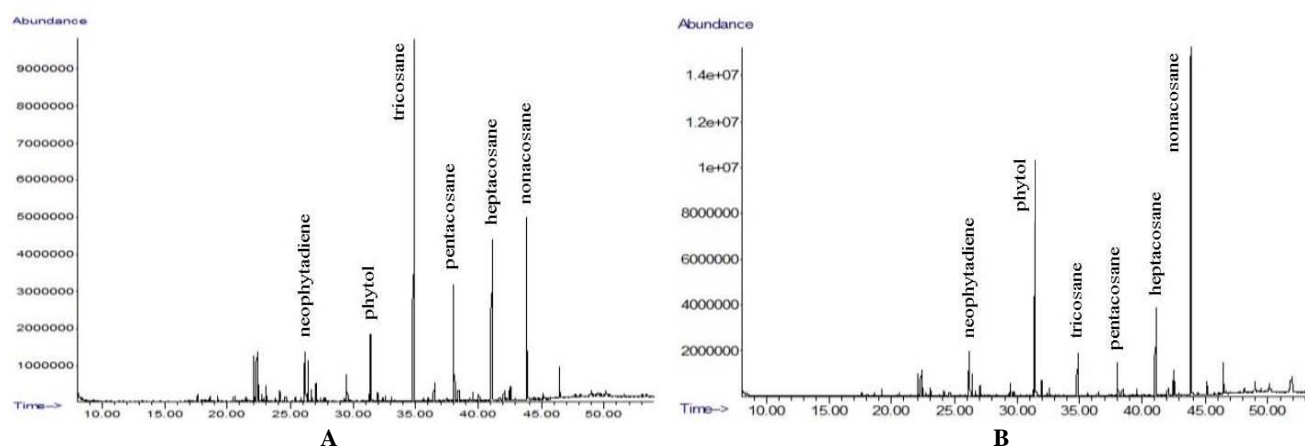
Mucolytic drugs change the biophysical properties of airway secretions, lowering their viscosity, preventing a large amount of phlegm that could obstruct airflow and ultimately lead to the development of respiratory diseases, such as bronchitis, asthma, and chronic obstructive pulmonary disease. Although a different approach to mucolytic medication has been investigated for decades, only a few mucolytic agents from medicinal plants have been defined (Wisastira and Arifin 2020). In this context, the mucolytic activity of *M. sylvestris* and *M. pseudolavatera* was evaluated using the Phenol Red

method, since it is an indicator that has an adequate study of drugs that modify tracheobronchial secretions (Engler and Szelenyi 1984).

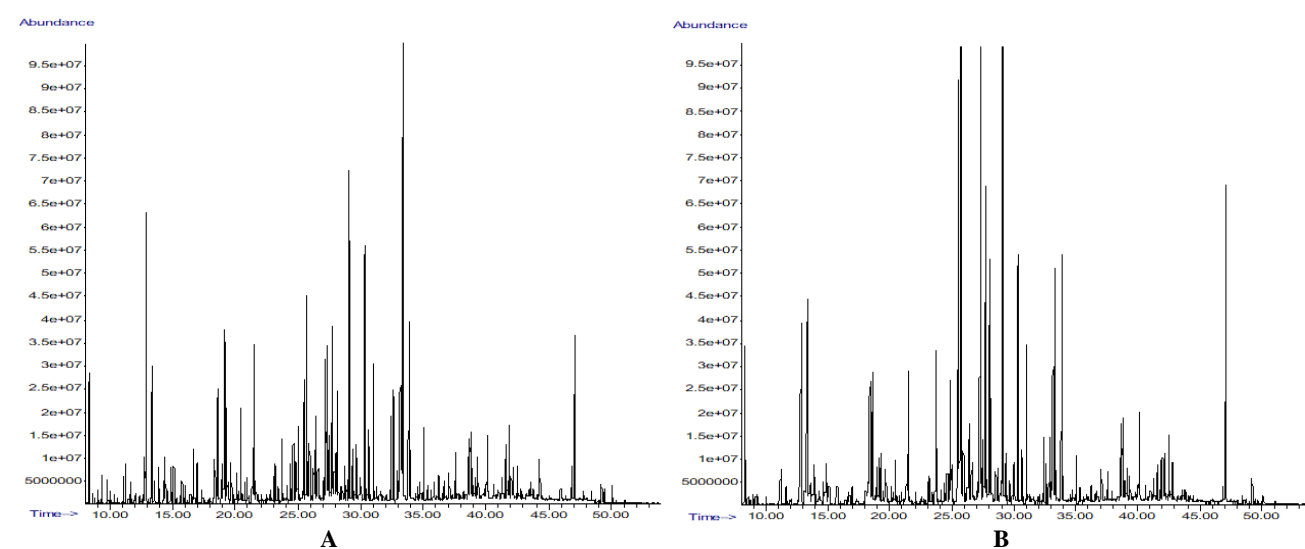
The results for the determination of mucolytic activity are illustrated in Figure 5. It is possible to show the highest percentages of activity in the higher doses, which indicates that there is a directly proportional relationship, dose-response; the higher the dose, the greater the effect; presenting a better percentage of efficacy for both *M. pseudolavatera* and *M. sylvestris* in the doses of 500 and 1000 mg/kg against the reference drug bromhexine.



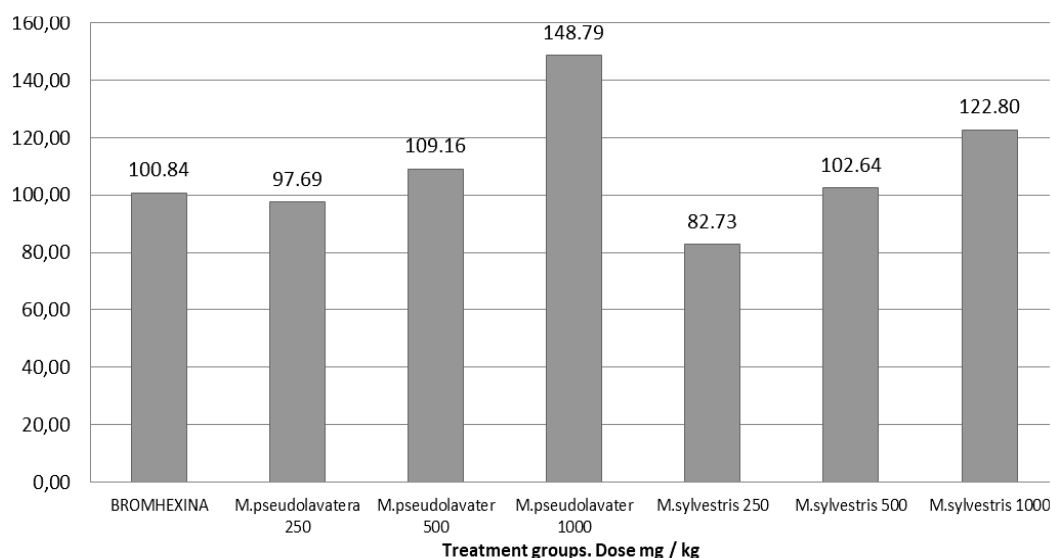
**Figure 2.** Analytical gas chromatogram of the fraction of saponifiable compounds of the leaves. A. *Malva sylvestris* and B. *M. pseudolavatera*



**Figure 3.** Analytical gas chromatogram of the fraction of unsaponifiable compounds of the leaves. A. *Malva sylvestris* and B. *M. pseudolavatera*



**Figure 4.** Analytical gas chromatogram of the hydroalcoholic extract of the leaves. A. *Malva sylvestris* and B. *M. pseudolavatera*



**Figure 5.** Percentages of mucolytic activity presented by the different groups tested

Bromhexine is a mucolytic agent with antioxidant properties that is used as a secretolytic expectorant for the effective treatment of cough with phlegm. The mechanism of action is based on the breakdown of phlegm, improves the production of serous mucus in the respiratory tract, and makes the phlegm less viscous. This contributes to a secretomotor effect by helping the cilia to expel phlegm out of the lungs (Bhagat and Rachana 2018). This activity was demonstrated when there was an increase in the concentration of phenol red in the tracheobronchial secretions of the experimental animals, which is reflected by the different absorbances.

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