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# Phytochemical screening, antioxidant activity and *in vitro* biological evaluation of leave extracts of *Hyptis suaveolens* (L.) from south of Mexico

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ABSTRACT

*Hyptissuaveolens*(L.) is a medicinally plant available in the south and southeast region of Mexico, Latin America and the south and west region of Africa. Infusion of this plant is used to treat diarrhea and as a muscle relaxant. In this study the antioxidant and antibacterial activity, and the phenolic compounds content of *Hyptissuaveolens* leaves was evaluated. The antioxidant activity was performed by DPPH (1,1-diphenyl-2-picryl-hydrazyl) method, obtaining an $IC_{50}$ range of 2.73–3.58 $\mu$g/mL for the different prepared extracts. The content of phenolic compounds was carried out by Folin-Ciocalteu method, obtaining for the extracts an oscillation of 253.68–336.50 mg of GAE/g of extract. The antibacterial activity was evaluated by disc diffusion method against *Staphylococcus aureus*, *Escherechia coli* and *Salmonella thipy*, of the three pathogenic microorganisms evaluated, *S. aureus* showed a higher sensitivity to treatment with a minimum inhibitory concentration (MIC) of 0.25 $\mu$g/mL, for *E. coli* and *S. typhi* was 0.5 $\mu$g/mL. The results confirm the great potential of this species as an antioxidant and antibacterial representing the first contribution to the chemical and biological knowledge of *H. suaveolens* from Mexican mountain range.



## 1. Introduction

Antioxidants are substances that delay, control or prevent the initiation and propagation of degenerative diseases in the body and reduce oxidative process (Shah et al., 2014). Phenolic compounds from plant extracts had a strong correlation with antioxidant and antimicrobial activities (Pham et al., 2018) and might have health benefiting properties such as protection against oxidative damage to the cell, minimize tissue damage and improve cell survival (Elghandour et al., 2018; Junsathian et al., 2018). Phenolic compounds inhibit the chain reaction of oxidation by acting as hydrogen donors or free radical acceptors and generation of more stable radicals. The inhibition reaction is considered to be in competition with the propagation step of lipid oxidation and yields stable products that will not initiate new free radicals or bring about a rapid oxidation via a chain reaction (Shahidi and Zhong, 2015).

*Hyptissuaveolens* (L.)Poit (Fig. 1), is an aromatic plant that was widely cultivated in the pre-Hispanic era of Mexico because its seeds are edible, it is currently cataloged as a "pseudocereal." This species is traditionally known as the donkey's herb and has a wide geographical distribution, in most places where it is found, it is a component of natural vegetation (Vergara-Santana et al., 2005).

This plant possesses a huge variety of medical uses such as antiseptic, insecticide, antiparasitic, antifungical and among other applications (Azevedo et al., 2002; Grassi et al., 2006; Jesus et al., 2013; Malele et al., 2003) as well as, the antioxidant and antibacterial properties of essential oil of *H. suaveolens*has been previously reported by (Gavani and Paarakh, 2008; Mandal et al., 2007; Nantitanon et al., 2007; Ríos Tesch et al., 2015; Tachakittirungrod and Chowwanapoonpohn, 2007; Tafurt-García et al., 2015). *H. suaveolens* is an important source of essential oils that have identified alkaloids, flavonoids, phenols, saponins, terpenes and sterols. For example, diterpenes have been reported: suaveolic acid, suaveolol, methyl suaveolate, as well as steroids such as: $\beta$-sitosterol and ursolic acid. According to the literature, phenolic compounds such as rosamarinic acid and rosmarinate have been identified (Sharma et al., 2013).

The aim of this study was to evaluate the antioxidant and antibacterial activities of the leaves extracts from the plant, and determinate the phytochemical properties and total phenols content thereof. Results from this work will enlighten the medical aspects of this herb.

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**Fig 1.** Picture of Hyptissuaveolens(L.) POIT.

## 2. Materials and methods

### 2.1. Materials

Absolute ethanol, trichloromethane, hexane, ethyl acetate, galic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent and ciprofloxacin were purchased from Sigma-Aldrich Ltd, Germany, and used without purification. Nutrient agar and plastic petri dish were purchased from MCD Lab, Mexico.

### 2.2. Instruments

UV spectrophotometer was obtained from VeLab Company, Mexico (Model VE-5100UV). The rotary evaporator was from Büchi Instruments, Germany (Model R-100). The incubator was purchased from Felisa company, Mexico (Model: FE-131).

### 2.3. Microbial material

*Staphylococcus aureus, E. coli* and *Salmonella thypi*strains were provided and cultured by the Microbiology Department of Faculty of Chemical Sciences, Universidad Veracruzana, Mexico.

### 2.4. Plant material

*Hyptissuaveolens* (L.) was collected from the central region of Veracruz, Mexico (lowland rainforest, latitude 18°45′38″N, longitude 96°27′38″O, altitude 27 m) in October 2018. After collection, the specimen was deposited at the herbarium of the Faculty of Biology (Universidad Veracruzana) in Cordoba, Mexico.

### 2.5. Leave extract preparation

500 g of leaves were dried at 32 °C in a closed room for two weeks and crushed in a fine powder. The powder was poured into a 4 L round bottom flask for maceration at room temperature for three days with 2 Lof the corresponding dissolvent (ethanol, ethyl acetate, trichloromethane, hexane and water).

### 2.6. Phytochemical screening

All the extracts were evaluated using qualitative chemical analysis for the identification of phytochemical species according to the literature (Sarker and Nahar, 2012).

#### 2.6.1. Detection of alkaloids

A sample (5 mL) was stirred with dilute sulfuric acid and four drops of Dragendorff was added. An orange-red precipitated is an indication of the presence of alkaloids.

#### 2.6.2. Determination of coumarins

A small sample of 3 mL was treated with three drops of 5% v/v ethanolic solution of *p*-dimetilaminobenzaldehyde and hydrogen chloride was bubbled through the mixture. The solution turns orange with the presence of coumarins.

#### 2.6.3. Determination of saponins

An extract sample (5 mL) was diluted with 15 mL of destilled water. The resultant mixture was shaken strongly, the apparition of foam indicates the presence of saponins

#### 2.6.4. Determination of phenylpropanoids

A mixture of 2 mL of HCl (0.5 N) and 2 mL of sodium nitrite (10% w/v) was treated with 1 mL of extract and 2 mL NaOH (2 mL). Production of violet color means phenylpropanoids are present.

#### 2.6.5. Determination of flavonoids

An aqueous solution of extract was treated with 10% v/v ammonium hydroxide solution. Apparition of yellow fluorescence color means flavonoids are present.

#### 2.6.6. Determination of tannins

A sample of 5 mL was evaporated and the resultant residues were dissolved in 5 mL of distilled water. The aqueous solution was filtered and a small sample (0.5 mL) was treated with few drops of 5% w/v ferric chloride solution. Production of dark blue color indicates the presence of hydrolysable tannins, and the production of dark green color means condensed tannins are present.

#### 2.6.7. Determination of terpenoids

A small sample of the extract was treated with 1 mL of acetic anhydride, 1 mL of trichloromethane and 1 mL of sulfuric acid. Production of violet color indicates the presence of terpenoids.

### 2.7. Determination of total phenols on the extracts

Total phenol determination was carried out in extracts of ethanol, acidified ethanol (with 1% HCl), ethanol-water (50:50 and 70:30) and water. Extracts were obtained by maceration of leaves powder (30 g) with 200 mL of the corresponding dissolvent for 72 h at room temperature in a dark room. An extract sample of 0.05 g was taken and dissolved in 10 mL of methanol. 0.35 mL of the extract solution was taken in a container and added 1.72 mL of 10% Folin–Ciocalteu's reagent, 1.7 mL of deionized water and 3.45 mL of 7.5% $NaHCO_3$ solution. The mixture was stirred in a Vortex and heated in a water-bath at 40 °C for 15 min. Similarly, the blank sample was prepared without the addition of extract samples. Absorbance of the resultant solution was measured at 760 nm, the same procedure was applied to Gallic acid standard solutions for the preparation of calibration curve. Total phenols content was quantified interpolating the absorbance of the sample on the calibration curve. Results were expressed in terms of Gallic acid equivalent (mg of GAE/g of dried extract).

### 2.8. Antioxidant activity

Antioxidant activity of the leaves extracts was measured by using the DPPH method. 1 mL of extract sample (5 mg/mL) was treated with 2 mL of 24 ppm DPPH solution. The mixture was shaken and incubated in a dark place for 20 min at room temperature. The absorbance of the sample was measured at 515 nm against a blank. The calibration curve was prepared following the same procedure

without adding extract. Results are expressed in% of reduced DPPH. The $IC_{50}$ of the antioxidant activity was calculated from logarithmic regression of results. The percentage of reduced DPPH was calculated according to the reported method (Mishra et al., 2012).

### 2.9. Determination of minimum inhibitory concentration (MIC)

A sample of 1 mL (0.5 mg) of 70:30 ethanol/water extract was diluted with nutrient broth to obtain solutions with different concentrations (0.5, 0.25, 0.175, 0.0875, 0.043, 0.022 mg/mL). The solutions were inoculated with 2 mL of 0.5 McFarland turbidity standards of *Staphylococcus aureus, E. coli* and *Salmonella thypi* at $1 \times 10^8$ CFU/mL. The resultant solution was incubated for 24 h at 36 °C. After incubation, absorbance was measured at 590 nm. MIC was obtained from the lowest absorbance value observed as indicated in the procedure reported (Bhuyan et al., 2017).

### 2.10. Antibacterial activity

The antibacterial activity of 70:30 ethanol/water extract was evaluated by disc diffusion method. A sample of the extract was diluted in ethanol to give a 1 mg/mL solution. The dissolvent employed for dilution was used as a negative control whilst a 20 $\mu$g/mL solution of ciprofloxacin was used as a positive control drug. Filter paper disc with 5 mm of diameter were dipped with the extract solution and the controls. The discs were placed on Mueller-Hinton agar plates inoculated with the bacteria strains and incubated for 24 h at 37 °C. The antibacterial activity was evaluated according the diameter of the inhibition zone on the bacterial strains.

## 3. Results

### 3.1. Phytochemical screening

The results of phytochemical screening (Table 1) indicated that the ethanolic extract contains tannins, phenylpropanoids and coumarins and the water extract contains saponins, tannins and flavonoids. However, the hexane and trichloromethane extracts only contains terpenoids and the ethyl acetate contains alkaloids and coumarins. In base of the results obtained, the next studies are focused on the ethanolic extract due their phytochemical properties.

### 3.2. Antioxidant activity and total phenols content

A calibration curve of different concentrations of Gallic acid was used to calculate the amount of total phenols of each extract. The antioxidant activity and the quantification of total phenols were carried out only in ethanolic and different ethanol/water extracts. The highest concentration of phenols was found in the ethanolic extract with 336.49 mg of GAE/g and the lowest concentration was present on water extract. The maximum antioxidant activity was found on 70:30 ethanol/water extract, reducing DPPH radicals at 80.76 ± 1.2%.

**Table 1**
Phytochemical screening of the extracts.

| Secondary metabolites | Hexane | Trichloromethane | Ethylacetate | Ethanol | Water |
|---|---|---|---|---|---|
| Alkaloids | – | – | + | – | – |
| Coumarins | – | – | + | + | – |
| Flavonoids | – | – | – | – | + |
| Phenylpropanoids | – | – | – | + | – |
| Saponins | – | – | – | – | + |
| Tannins | – | – | – | + | + |
| Terpenoids | + | + | – | – | – |

+ = positive, - = negative.

**Table 2**
Evaluation of antioxidant activity on Hyptis suaveolens extracts.

| Extract | mg GAE/g | % reduced DPPH | $IC_{50}$ ($\mu$g/mL) |
|---|---|---|---|
| Ethanol | 336.496 ± 0.005 | 27.56 ± 5.76 | 3.57± 0.04 |
| Acidifiedethanol | 335.664 ± 0.005 | 36.85 ± 3.17 | 3.38± 0.01 |
| Ethanol–Water 50:50 | 330.33± 0.01 | 32.25 ± 4.68 | 3.47± 0.02 |
| Etanol–Water 70:30 | 334.33± 0.004 | 80.76 ± 1.20 | 2.73± 0.005 |
| Water | 253.68 ± 0.03 | 44.44 ± 3.02 | 3.22± 0.01 |

Note: Each experiment was replicated three times.

As shown in Table 2, antioxidant activity increases depending of the polarity of the extract. The calculated $IC_{50}$ is represented in Table 2.

### 3.3. Antibacterial activity

Due it antioxidant activity, antibacterial activity was measured only on ethanol-water 70:30 extract. The results (Table 3) show a potential activity against *S. aureus*and *S. typhi*, however, the extract does not have activity on *E. coli.*

### 3.4. Minimum inhibitory concentration

The *H. suaveolens* ethanol-water 70:30extract presented MIC values for *E. Coli* and *S. typhi*strains at 0.5 mg/mL, and for *S. aureus* at 0.25 mg/mL. The Extract is considered strong inhibitor when it MIC greater than 0.25 mg/Ml. Results are resumed in Fig. 2.

## 4. Discussion

The most abundant secondary metabolites are phenolic compounds, enhancing the antioxidant activity against free radicals and others reactive oxygen species. Plants are exposed to highly oxidative

**Table 3**
Antibacterial activity of ethanol–water 70:30 extract.

| Strain | Inhibitionzone (cm) | Ciprofloxacine |
|---|---|---|
| S. aureus | 0.7 ± 0.05 | 1.74 ± 0.02 |
| E. coli | 0.25 ± 0.13 | 1.17 ± 0.06 |
| S. typhi | 0.52 ± 0.12 | 1.49 ± 0.09 |

Results are expresedin terms of SD ($n$ = 3).

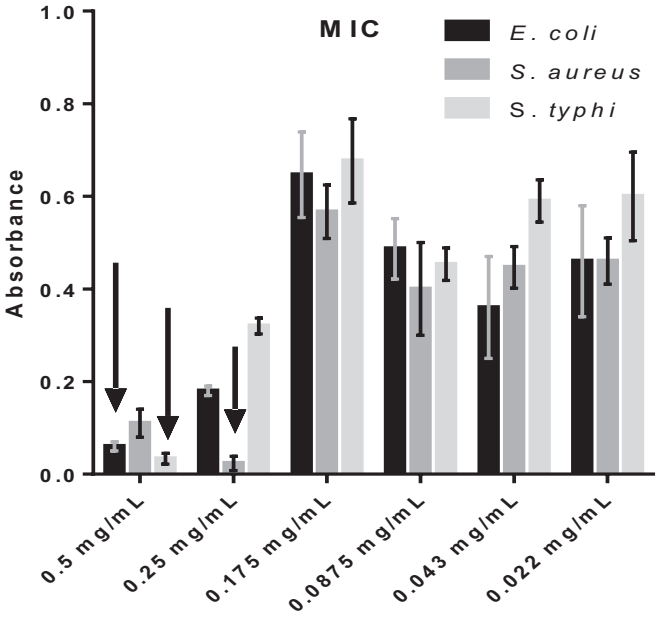


**Fig 2.** Evaluation of the MIC of the ethanol–water 70:30 extract.

environments that induce the production of radicals, for this reason, they have developed an antioxidant system based on the synthesis of phenolic compounds such flavonoids, tannins and other secondary metabolites. Results of the phytochemical screening are similar to reported in the literature (Gavani and Paarakh, 2008) where is indicated a high concentration of steroids, flavonoids, tannins and alkaloids in ethanol extracts.

The antioxidant activity of different extracts of *H. suaveolens*was evaluated to determinate the efficacy against free radicals. This property is associated with the capability of secondary metabolites to neutralize all the effects related to free radicals and oxygen reactive species in the body. For that, the total amount of phenolic compounds was determinated. The ethanolic extract showed the highest concentration of phenols while water has the lowest value. The best antioxidant activity was present on the ethanol-water 70:30 extract with the lowest $IC_{50}$ value (2.73 $\mu$g/mL), according to this parameter, low $IC_{50}$ values reflect a high activity antioxidant (Feghhi-Najafabadi et al., 2019), indicating that the accurate mixture of dissolvents allowed to obtain huge amount of metabolites with antioxidant activity.

As a result of the antioxidant activity of the extract, it was used to evaluate its antibacterial activity on important strains in food science. The evaluation of the antimicrobial activity was carried out using the disc diffusion method, where 20 $\mu$g of the ethanol-water extract 70:30 of *H. suaveolens* was used against pathogenic microorganisms such as *E. coli*, *S. typhi* and *S. aureus*. The obtained results showed that the highest inhibition was manifested in strains of *S.aureus* with a inhibition zone of $0.7 \pm 0.05$ cm, followed by *S. typhi* with an inhibition zone of $0.52 \pm 0.12$. The *E. coli* showed to be the most resistant to treatment with the smallest inhibition zone of $0.25 \pm 0.13$ cm.

Due the capability of the extract of *H. suaveolens* against Gram positive and Gram negative bacteria, the minimum inhibitory concentration (MIC) was determined for find the lowest concentration of the 70:30 ethanol−water extract of *H. suaveolens* that is capable of completely inhibit microbial growth in liquid medium. According to the obtained results, ethanol−water 70:30 extract has a MIC of 0.5 mg/mL against *E. coli* and *S. typhi,* whilst *S.aureus* presented a MIC of 0.25 mg/mL. This behavior is because of the presence of phenolic compounds with high activity against Gram positive bacteria.

Results obtained in this work indicate that *S. aureus* is the microorganism that showed greater sensitivity to the treatment with the ethanol-water extract 70:30 of *H. suaveolens*, developing inhibition zones of greater diameter compared with that of the other two bacterial strains under study. These results are corroborated with studies previously conducted by Mozhiyarasi and Anuradha (2016) Prasanna and Koppula (2012) for extracts obtained from *H. suaveolens*, where the polar extracts showed the best antimicrobial activity against Gram-positive bacteria. Also, in studies carried out by Tachakittirungrod and Chowwanapoonpohn (2007) is reported that the essential oil of *H. suaveolens* has a better antibacterial activity against the Gram positive bacteria *S. aureus* and *S. suis*, than against the Gram negative bacteria *P. aeuroginosa* and *E. coli*. The low activity in Gram negative bacteria is due to the presence of a bacterial cell structure composed of liposaccharides in addition to peptidoglycan, which prevents the lipophilic passage of essential oils (Mann et al., 2000). It is also known that the mechanisms of resistance are related to the activation of synthesis of the cell wall, with hyperproduction of penicillin-binding proteins, thickening of the wall and the imprisonment of drugs by overproduction of the wall components.

On the contrary, Ríos-Tesch (2015), in a study conducted in Venezuela, point out that the essential oil is active against Gram negative bacteria, showing MIC values that ranged between 300 $\mu$g/mL and 450 $\mu$L/mL. These differences are observed with respect to the antioxidant and antibacterial activity of *H. suaveolens*, these differences are attributed to the difference in geographical distribution, climate, soil type, time of year and chemical composition, which varies according to the origin of the species.

## 5. Conclusion

These results confirm the great potential of *H. suaveolens* as antioxidant and antibacterial, and could be used in the treatment of diseases related to oxidative stress and for common infectious diseases caused by bacterial species. This research represents the first contribution to the chemical and biological knowledge of *H. suaveolens* from Mexico.

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## Conflict of interest

Authors of this work have any conflicts of interest

## Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2019.10.016.

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