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

Sanchez-Aguirre Oscar\(^a\), Cruz-Navarro Antonio\(^a,b,*\), Guevara-Valencia Marina\(^a\), Rengifo-Salgado Elsa\(^c\), Vargas-Arana Gabrield

\(^a\) Faculty of Chemistry, Universidad Veracruzana, 94340 Orizaba, Mexico
\(^b\) Basic sciences and engineering institute, Universidad Autonoma del Estado de Hidalgo, 42183 Pachuca, Mexico
\(^c\) Research Program on Amazonian Biodiversity, The Peruvian Amazon Research Institute, 784 Iquitos, Peru
\(^d\) Laboratory of Natural products chemistry, The Peruvian Amazon Research Institute, 784 Iquitos, Peru

**A R T I C L E  I N F O**

Article History:
Received 8 April 2019
Revised 22 September 2019
Accepted 27 October 2019
Available online 12 November 2019

Edited by GI Stafford

Keywords:
H suaveolens
Phytochemicals
Antioxidant activity
Antibacterial activity
Total phenols

**A B S T R A C T**

*Hyptis suaveolens*(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 *Hyptis suaveolens* 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*, *Escherichia coli* and *Salmonella thyph*, 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.

© 2019 SAAB. Published by Elsevier B.V. All rights reserved.

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).

*Hyptis suaveolens* (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 anti-septic, insecticide, antiparasitic, antifungal 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; Rios Tesch et al., 2015; Tachakittirungrod and Chowwanapoornpohn, 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, suaveanol, methyl suaveolate, as well as steroids such as: \(\beta\)-sitosterol and ursolic acid. According to the literature, phenolic compounds such as rosmarinic 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 determine the phytochemical properties and total phenols content thereof. Results from this work will enlighten the medical aspects of this herb.
2. Materials and methods

2.1. Materials

Absolute ethanol, trichloromethane, hexane, ethyl acetate, gallic acid, 2,2-diphenyl-1-picyrylhydrazyl (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-5100 UV). 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’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 Córdoba, 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 L of 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 flavonoids

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 flavonoids are present.

2.6.5. Determination of tannins

A small 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.

2.6.6. 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.

2.6.7. Determination of phenylpropanoids

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

2.6.8. Determination of phenols

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.

2.7. 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% NaHCO₃ 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 IC₅₀ 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 typhi* at 1 × 10⁸ 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. Antimicrobial 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 dissolved oxygen for dilution was used as a negative control whilst a 20 μg/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

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Hexane</th>
<th>Trichloromethane</th>
<th>Ethylacetate</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenylpropanoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Note:** *+ = positive, - = negative.*

As shown in Table 2, antioxidant activity increases depending of the polarity of the extract. The calculated IC₅₀ 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 70:30 extract presented MIC values for *E. Coli* and *S. typhistrains* 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 forces, which produces reactive oxygen species. The ethanolic extract contains tannins, phenylpropanoids and coumarins, while the hexane and trichloromethane extracts contain 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.

Table 2

<table>
<thead>
<tr>
<th>Extract</th>
<th>mg GAE/g</th>
<th>% reduced DPPH</th>
<th>IC₅₀ (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>336.49 ± 0.005</td>
<td>27.56 ± 5.76</td>
<td>3.57 ± 0.04</td>
</tr>
<tr>
<td>Acidified ethanol</td>
<td>335.66 ± 0.005</td>
<td>36.85 ± 3.17</td>
<td>3.38 ± 0.01</td>
</tr>
<tr>
<td>Ethanol–Water 50:50</td>
<td>330.33 ± 0.01</td>
<td>32.25 ± 4.68</td>
<td>3.47 ± 0.02</td>
</tr>
<tr>
<td>Ethanol–Water 70:30</td>
<td>334.33 ± 0.004</td>
<td>80.76 ± 1.20</td>
<td>2.73 ± 0.005</td>
</tr>
<tr>
<td>Water</td>
<td>253.68 ± 0.03</td>
<td>44.44 ± 3.02</td>
<td>3.22 ± 0.01</td>
</tr>
</tbody>
</table>

Note: Each experiment was replicated three times.

**Table 3**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inhibition zone (cm)</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>0.7 ± 0.05</td>
<td>1.74 ± 0.02</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0.25 ± 0.13</td>
<td>1.17 ± 0.06</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>0.52 ± 0.12</td>
<td>1.49 ± 0.09</td>
</tr>
</tbody>
</table>

Results are expressed in 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 as flavonoids, tannins and other secondary metabolites. Results of the phytochemical screening are similar to those reported in the literature (Gavani and Paarakh, 2008) where is indicated a high concentration of sterols, flavonoids, tannins and alkaloids in ethanol extracts.

The antioxidant activity of different extracts of H. suaveolens was evaluated to determine 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 determined. 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 IC50 value (2.73 μg/mL) according to this parameter, low IC50 values reflect a high activity antioxidant (Feghhi-Najafabadi et al., 2019), indicating that the accurate mixture of solvents 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 μg 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 an inhibition zone of 0.7 ± 0.05 cm, followed by S. typhi with an inhibition zone of 0.52 ± 0.12. The E. coli showed to be the most resistant to treatment with the smallest inhibition zone of 0.25 ± 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 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.


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.

Funding sources

This work was financially supported by the Universidad Veracruzana. The authors would like to thank to the Faculty of Biology (Universidad Veracruzana) for the technical support.

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.

References


