



In vitro* Evaluation of Antimicrobial Activity from the Methanolic Extracts of *Taraxacum officinale* and *Melissa officinalis* on *Staphylococcus aureus

Noelia Gómez^(✉) , Sandy Gavilanes , Inés Malo , and Mónica Espadero

Universidad Politécnica Salesiana, Cuenca, Ecuador

ggomeza@est.ups.edu.ec, {sgavilanes, imalo, mespadero}@ups.edu.ec

Abstract. The use of plants has been increasing by the presence of bioactives, which is an opportunity in the creation of products such as medicines, food and cosmetics. *Taraxacum officinale* (Dandelion) and *Melissa officinalis* (Toronjil), are cosmopolitan species with great therapeutic potential, traditionally used for their anti-inflammatory, antioxidant and antimicrobial activity. Therefore, this research evaluates the antimicrobial activity of the methanol extracts of *Taraxacum officinale* and *Melissa officinalis* on *Staphylococcus aureus* using *in vitro* tests. Analyses of secondary metabolites were performed by phytochemical screening tests, as well as the identification of possible compounds responsible for antimicrobial activity using the Bioautography technique. The phenolic compounds were quantified by the Folin-Ciocalteu assay, and the antimicrobial capacity of the extracts was evaluated by the Kirby-Bauer method. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, phenols and tannins in both extracts. Using Bioautography, potentially inhibitory metabolites were identified, including chlorogenic acid (Rf 0.45), taraxasterol (Rf 0.65), caffeic acid (Rf 0.87), geraniol (Rf 0.2), citral (0.42) and citronellal (0.8). In addition, a greater number of phenolic compounds (112.20 ± 10.13 mg EAG/g MS) was observed in the extract of *Melissa officinalis*, while the extract of *Taraxacum officinale* showed a greater bacterial inhibition capacity, reaching 65.49%.

Keywords: *Taraxacum officinale* · *Melissa officinalis* · *Staphylococcus aureus* · antimicrobial activity · phenolic compounds

1 Introduction

Plants with healing properties and their derived products have been commonly used by traditional medicine, thus their relevance as raw material in the production of medicines in the pharmaceutical industry; hence, their use has been increasing in recent years [1]. There are several medicinal plants in Ecuador with great therapeutic potential, mainly with anti-inflammatory, antibacterial, antioxidant, analgesic activity, among others. However, some of the species with antimicrobial properties have not been thoroughly investigated to determine all their benefits [2].

Staphylococcus aureus, is considered a pathogenic microorganism associated with localized, invasive and toxin-mediated infections at the level of the dermis, respiratory tract, bone and less common in the urinary tract, and can cause serious infections if it enters the bloodstream, raising concern because strains of *Staphylococcus aureus* are resistant to several commercial antibiotics [3]. Facing these challenges, contemporary medicine has directed its efforts to exploring and creating innovative treatments, allowing the investigation of several bioactive compounds present in plant species, which have been extracted by different methods to be applied in various cosmetic, food and pharmaceutical formulations.

Due to the growing market demand in the use of medicinal plants, there is the need to conduct research of introduced plants such as *Taraxacum officinale* commonly called “dandelion” and *Melissa officinalis* known as “toronjil” and compare the antimicrobial activity of its methanolic extracts on the bacterium *Staphylococcus aureus*, enabling the use of existing resources in the country. In addition, it is intended to generate and provide essential data that may be useful for future research. In this way, the idea is to take advantage of the richness of the country’s biodiversity, maximizing its possible applications in various industries, as well as promoting its conservation and use as alternatives for treating various conditions.

2 Materials and Methods

2.1 Plant Material Conditioning

Twelve kilograms of aerial parts of *Taraxacum officinale* and *Melissa officinalis* were collected and were washed with distilled water. Then, were dried at room temperature and put in a stove at $40\text{ }^{\circ}\text{C} \pm 2$; both processes were carried out for 24 h. Once dried, the leaves were pulverized, weighed and placed in paper cases to preserve their biological characteristics until their use.

2.2 Preparation of Extracts

Cold maceration was done with constant stirring of dry and ground plant material with absolute methanol in a 1:6 ratio for 72 h. Subsequently, a double filtration was performed; the macerates concentrated in rotavapor at 337 mbar at $50\text{ }^{\circ}\text{C}$ until the complete elimination of the solvent and the raw extracts were obtained [2]. The concentrated pure extracts were stored in amber jars and placed in refrigeration for 7 days to ensure the stability of the secondary metabolites [2, 4].

2.3 Qualitative Characterization

The phytochemical screening of *Taraxacum officinale* and *Melissa officinalis* extracts was carried out by preliminary tests that qualitatively determined the presence of bioactive compounds that were identified by their coloration and precipitation [5]; the following process was carried out from the tests described in Table 1.

Table 1. Phytochemical testing of extracts of *Taraxacum officinale* and *Melissa officinalis* leaves.

Compounds	Testing
Alkaloids	Dragendroff's essay
Flavonoids	Shinoda's essay
Saponins	Fehling reagent test + Na ₂ CO ₃
Phenols	Folin reagent + Na ₂ CO ₃ al 5%
Tannins	Ferric Chloride Test

2.4 Quantification of Phenolic Compounds

The phenolic content of the extracts was determined by the Folin-Ciocalteu assay [6, 7]; a calibration curve was carried out with different concentrations of gallic acid from 10 to 100 ppm. The *Taraxacum officinale* and *Melissa officinalis* extracts were diluted with distilled water, the Folin-Ciocalteu 1 N reagent was placed, and the sodium carbonate solution Na₂CO₃ (20% w/v) was added. It was kept in the dark for two hours; absorbance at 760 nm was measured on a UV-Vis spectrophotometer. Results are expressed as (mg EAG/100 g DM).

2.5 Antimicrobial Activity Assessment Using Bioautography Method

A strain of *Staphylococcus aureus* ATCC 25923 was used in nutrient broth + agar agar 0.3% [8]. It was proceeded to read the optical density of the culture with wavelength of 600 nm; silica gel plates were prepared, using the thin layer chromatography (TLC) procedure, they were arranged in saturated chromatographic cameras with mobile phase of ethyl acetate + methanol (9:1 v/v) [9]. The bands were observed by fluorescence at 254 nm and 366 nm. The plates were prepared in humidity chambers, incubating them at 25 °C for 90 min. After this time, they were immersed in the bacterial suspension of *Staphylococcus aureus* ATCC 25923 for 10 s. They were placed in steam chambers at 37 ± 2 °C of incubation for 24 h. Finally, the plates were immersed in an aqueous solution of 0.1% MTT + Triton X-100 dye for 60 s and were incubated in the water vapor chambers at 37 °C for 1 h. An immersion in 70% ethanol was carried out for 10 s.

2.6 Determination of Antimicrobial Capacity by Kirby-Bauer Method

The antimicrobial sensitivity test was carried out by means of the disk diffusion technique [10, 11]. A pure strain of *Staphylococcus aureus* ATCC 25923 was used in blood agar, colonies were taken and suspended in sterile physiological serum up to a concentration of 0.5 on the McFarland scale. Sowing was performed with the standardized sample in Mueller Hinton agar. The disks were placed in the pre-seeded Petri boxes, including positive control (Vancomycin), and negative control with sterile distilled water. Finally, it was incubated at 37 °C ± 2 for 24 h. The diameter of the halos and the percentage of the inhibition effect were expressed.

2.7 Statistical Analysis

The normal distribution of the data was evaluated by the Shapiro-Wilk normality test and Q-Q graph; the ANOVA model was applied with $p < 0.05$. The assumptions of the model, assumption of normality and assumptions of homocedasticity of variances were evaluated by the Shapiro-Wilk and Levene test. When the assumptions were met, the best treatment was finally determined by Tukey Test.

3 Results

3.1 Extraction of Methanol Extracts

Out of the 100 g of the dried and ground leaves of *Taraxacum officinale* and *Melissa officinalis* were obtained by cold maceration with methanol 30 mL of the extract with greenish brown coloring and 20 mL of the extract with dark green appearance, respectively.

3.2 Qualitative Characterization

The presence of alkaloids in the extracts was identified in *Taraxacum officinale*, as well as a moderate presence of saponins, and low presence of flavonoids, and high presence of flavonoids was observed in *Melissa officinalis*. Additionally, moderate presence of phenolic compounds and tannins was identified in both extracts.

The results obtained from the different phytochemical screening assays are presented in Table 2.

Table 2. Results of Phytochemical Screening

Compounds	Methanolic Extracts	
	<i>Taraxacum officinale</i>	<i>Melissa officinalis</i>
Alkaloids	+++	+++
Flavonoids	+	+++
Saponins	+ ++	++
Phenols	++	++
Tannins	+++	+++

Note: (+++): high evidence; (++) : medium evidence; (+): low evidence; (–): negative.

3.3 Antimicrobial Activity Assessment on *Staphylococcus aureus* by the Bioautography Method

Using the Bioautography method it was possible to identify the compounds potentially responsible for the biological activity. Tables 3 and 4 detail the distances traveled by the substances, the solvent front, the reference front (Rf), the suggested antimicrobial compound and the color detected at a wavelength of 366 nm (Figs. 1 and 2).

Compounds showing antimicrobial activity were identified using the reference front value (Rf) [9, 12, 13].

Table 3. Results obtained in the Bioautography with extract of *Taraxacum officinale*.

	Distance traveled (cm)	Solvent Front	Reference Front (RF)	Compound	Color Detected
1	2.7	6	0.45	Chlorogenic acid	Blue
2	3.9	6	0.65	Taraxasterol	Orange
3	5.22	6	0.87	Caffeic acid	Reddish brown

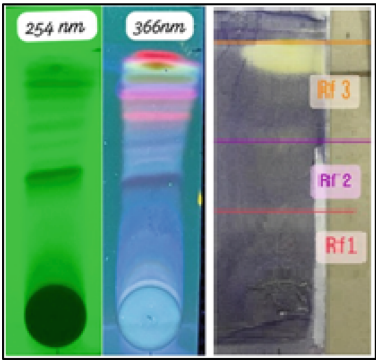


Fig. 1. TLC and Bioautography of *Taraxacum officinale* extract.

Table 4. Results obtained in the Bioautography with extract of *Melissa officinalis*.

	Distance traveled (cm)	Solvent front	Reference Front (RF)	Compound	Color Detected
1	1.2	6	0.2	Geraniol	Blue
2	2.52	6	0.42	Citral	Blue violet
3	4.8	6	0.8	Citronella	Reddish coffee

3.4 Quantification of Polyphenolic Compounds

The following results were obtained (Table 5) from the quantification of phenolic compounds.

3.5 Determination of Antimicrobial Capacity (Kirby-Bauer)

The results of the antimicrobial susceptibility assays revealed that both the disks containing the extract of *Taraxacum officinale* and *Melissa officinalis* generated inhibition halos in the presence of *Staphylococcus aureus* ATCC 25923 (Table 6), indicating an inhibitory activity on the bacterium.

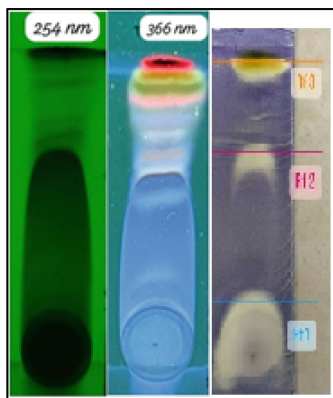


Fig. 2. TLC and Bioautography of *Melissa officinalis* extract

Table 5. Quantification of polyphenolic compounds in methanol extracts.

Extracts	Content of phenolic compounds (mg EAG/g DM)
<i>Melissa officinalis</i>	112,20 $\pm$ 10,13
<i>Taraxacum officinale</i>	23,07 $\pm$ 6,87

Note: the values used are the average of 15 samples ($\pm$ DS).

Table 6. Antimicrobial activity of extracts on *Staphylococcus aureus*.

Treatments (Methanol extracts)	Inhibition Halo Diameter (mm)	% Inhibition	Groups
<i>Taraxacum officinale</i>	11,22 $\pm$ 0,50	65,49 $\pm$ 5,61	a
<i>Melissa officinalis</i>	10,44 $\pm$ 1,26	53,39 $\pm$ 1,63	b

Note: the values used are the average of 15 samples ($\pm$ DS).

The analysis of variance (ANOVA) showed significant difference between treatments (P value = 0.0231). There were no significant deviations from the model assumptions (normality and homogeneity of variances). The treatment with *Taraxacum officinale* extract (Fig. 3) obtained the highest inhibition percentage, because *Staphylococcus aureus* is sensitive to the group of phenolic compounds present in the extract.

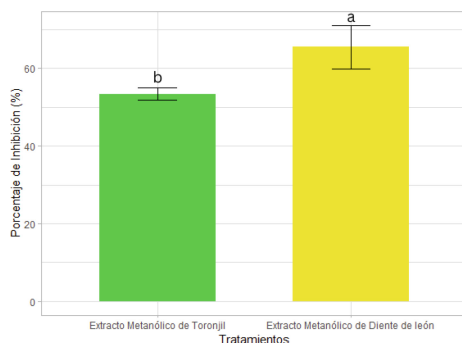


Fig. 3. Effect of methanol extracts of *Melissa officinalis* and *Taraxacum officinale* on *Staphylococcus aureus*.

4 Discussion

Methanol is known to be a highly effective solvent for extracting a wide range of secondary metabolites from plant material, and it presents a high solubility for a wide variety of compounds, concluding that a higher extraction efficiency is achieved on the third day of maceration [14, 15].

According to studies, the use of solvents such as methanol, ethanol and chloroform favor the entrainment of the alkaloid taraxine, which are found in both species [16]. In a research that evaluates plant species against pathogenic microorganisms [17], flavonoids were observed in *Taraxacum officinale* extracts, results that are consistent with the ones obtained in this paper, but in low concentrations. This variation could be due to various factors such as the percentage of the solvent, the chemical composition of the extracts, the age of the plant or even climatic conditions. On the other hand, in the trial conducted with *Melissa officinalis*, a high number of flavonoids was detected, also supported by studies where they were identified as modifiers of antibiotic activity [14].

From the detection of compounds with antimicrobial properties, in the chemical analysis of *Taraxacum officinale* [12] chlorogenic acid is classified as a flavonoid and is credited with numerous properties, including anti-inflammatory, enzyme inhibitor and antimicrobial properties. Taraxasterol, a compound mostly present in *Taraxacum officinale*, belongs to the group of triterpenoids. This compound has anti-inflammatory, antioxidant and antimicrobial activity, especially against *Staphylococcus aureus* with significant value [18]. In addition, another investigation determined that the main components of *Melissa officinalis* extract were Geraniol, Citronella and Citral [19]. These components are responsible for antimicrobial activity, indicating the potential of *Melissa officinalis* as a promising natural preservative for the food industry.

According to the quantification of polyphenolic compounds, values of 90.1 mg of GAE/g of dry matter are exposed in extracts of *Melissa officinalis* obtained by maceration, being similar to the results presented in this research [20]. When *Melissa officinalis* is compared with other species studied such as *Agastache foeniculum* (27.19 mg AG/g), *Lavandula angustifolia* (12.44 mg AG/g) and *Nepeta cataria* (14.66 mg AG/g), this extract presents a higher concentration [21]. This suggests that the antioxidant properties of *Melissa officinalis* leaves may be because of the presence of various phenolic

compounds (rosmarinic acid, caffeic acid, among others), and vary according to regions. Therefore, it is essential to consider factors such as the variety used, the region of origin, extraction conditions, and temperature, as these influence the presence or absence of compounds with antioxidant capacity.

5 Conclusions

The extracts of *Taraxacum officinale* and *Melissa officinalis* contain a variety of secondary metabolites, including alkaloids, flavonoids, saponins, phenols and tannins, in varying proportions. From the tests carried out by the Bioautography method, it was determined that the possible compounds with antimicrobial activity are: chlorogenic acid, taraxasterol and caffeic acid, in the extract of *Taraxacum officinale*. On the other hand, in the extract of *Melissa officinalis* (grapefruit), geraniol, citral and citronellal were identified.

Melissa officinalis extract had the highest content of phenolic compounds. In addition, it was determined that methanol extracts of *Taraxacum officinale* and *Melissa officinalis* showed antimicrobial activity on *Staphylococcus aureus*. However, through statistical analysis with a 95% confidence level, it was determined that the methanolic extract of *Taraxacum officinale* achieved the highest inhibition percentage.

References

1. Lozada, M.: ESTUDIO FIQUQUÍMICO Y EVALUACIÓN DE ACTIVIDAD ANTIBACTERIANA SOBRE *Staphylococcus aureus* ATCC:25923, *Streptococcus mutans* ATCC: 25175, *Streptococcus pneumoniae* ATCC: 49619, *Streptococcus pyogenes* ATCC: 19615DE EXTRACTOS APOLARES (CLOROFORMO-HEXANO) DE *Croton elegans* KUNTH (mosquera), Quito (2016)
2. Azuero, A., Jaramillo, C.J., San Martín, D., D'Armas, H.: Análisis del efecto antimicrobiano de doce plantas medicinales de uso ancestral en Ecuador/Analysis of antimicrobial effect of twelve medicinal plants of ancient use in Ecuador. *CIENCIA UNEMI* 9(20), 11–18 (2016). <https://doi.org/10.29076/issn.2528-7737vol9iss20.2016pp11-18p>.
3. Cervantes-García, E., García-González, R., María Salazar-Schettino, P.: Características generales del *Staphylococcus aureus*. *Rev. Latinoam. Patol. Clin. Med. Lab.* 61(1), 28–40 (2014). www.medigraphic.com/patologiaclinica www.medigraphic.org.mx
4. Vélez, R., D'Armas, H., Jaramillo-Jaramillo, C., Vélez, E.: Metabolitos secundarios, actividad antimicrobiana y letalidad de las hojas de *Cymbopogon citratus* (hierba luisa) y *Melissa officinalis* (toronjil). *FACSALUD-UNEMI* 2(2), 31–39 (2018). <https://doi.org/10.29076/issn.2602-8360vol2iss2.2018pp31-39p>
5. Rodríguez, J., Hernández, M., Méndez, L.: Manual de prácticas de farmacognosia. Xalapa (2020)
6. Noreen, H., Semmar, N., Farman, M., McCullagh, J.S.O.: Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant *Coronopus didymus*. *Asian Pac. J. Trop. Med.* 10(8), 792–801 (2017). <https://doi.org/10.1016/j.apjtm.2017.07.024>
7. Vargas, C.: Determinación y cuantificación de compuestos fenólicos en flores de *Taraxacum officinale*, mediante HPLC-DAD-MS y ensayos colorimétricos UV-vis. Bogotá (2020)
8. Dewanjee, S., Gangopadhyay, M., Bhattacharya, N., Khanra, R., Dua, T.K.: Bioautography and its scope in the field of natural product chemistry. *J. Pharm. Anal.* 5(2), 75–84 (2015). <https://doi.org/10.1016/J.JPHA.2014.06.002>

9. Wagner, H., Bladt, S.: Plant Drug Analysis, 2nd edn. Springer, New York (1996)
10. Bernal, M., Guzman, M.: EL ANTIBIOGRAMA DE DISCOS. NORMALIZACION DE LA TÉCNICA DE KIRBY-BAUER. BIOMEDICA **4**(3–4), 114–121 (1984)
11. Hudzicki, J.: Kirby-Bauer Disk Diffusion Susceptibility Test Protocol (2009). www.atcc.org
12. Cortés, N., et al.: Microscopical descriptions and chemical analysis by HPTLC of *Taraxacum officinale* in comparison to *Hypochaeris radicata*: a solution for mis-identification. Rev. Bras. **24**(4), 381–388 (2014). <https://doi.org/10.1016/J.BJP.2014.07.018>
13. Senguttuvan, J., Subramaniam, P.: HPTLC fingerprints of various secondary metabolites in the traditional medicinal herb *Hypochaeris radicata* L. J. Bot. 1–11 (2016). <https://doi.org/10.1155/2016/5429625>
14. De Sousa, A.T.L., et al.: Evaluation of the antibacterial activity of methanolic and hexanic extracts of puff pastry stem, *Melissa Officinalis* L. Revista Ciencias de la Salud **14**(2), 201–210 (2016). <https://doi.org/10.12804/revsalud14.02.2016.05>
15. Najda, A., Sugier, D.: Selected secondary metabolites content in the roots of *Taraxacum officinale* depending on the method of plantation establishment. Herbal Polonica **53**(3), 152–156 (2007). <https://www.researchgate.net/publication/258519306>
16. Muñoz, M., Montes, M., Wilkomirsky, T.: Plantas medicinales de uso en Chile: química y farmacología, 2nd ed. Universidad de Chile, Vicerrectoría de Asuntos Académicos, Universitaria, Chile (2004). <https://bibliotecadigital.infor.cl/handle/20.500.12220/19111>. Accessed 04 February 2024
17. Rodríguez, C., Zarate, A., Sánchez, L.: Actividad antimicrobiana de cuatro variedades de plantas frente a patógenos de importancia clínica en Colombia. NOVA **15**(27), 119–129 (2017)
18. Jiao, F., Tan, Z., Yu, Z., Zhou, B., Meng, L., Shi, X.: The phytochemical and pharmacological profile of taraxasterol. Front. Pharmacol. **13**(2022). <https://doi.org/10.3389/FPHAR.2022.927365>
19. Yu, H., Pei, J., Qiu, W., Mei, J., Xie, J.: The antimicrobial effect of *Melissa officinalis* L. essential oil on *Vibrio parahaemolyticus*: insights based on the cell membrane and external structure. Front. Microbiol. **13**(2022). <https://doi.org/10.3389/FMICB.2022.812792>
20. Moacă, E.A., et al.: A comparative study of *melissa officinalis* leaves and stems ethanolic extracts in terms of antioxidant, cytotoxic, and antiproliferative potential. Hindawi **2018**, 1–12 (2018). <https://doi.org/10.1155/2018/7860456>
21. Duda, S.C., Mărghițaș, L.A., Dezmirean, D., Duda, M., Mărgăoan, R., Bobiș, O.: Changes in major bioactive compounds with antioxidant activity of *Agastache foeniculum*, *Lavandula angustifolia*, *Melissa officinalis* and *Nepeta cataria*: effect of harvest time and plant species. Ind. Crops Prod. **77**, 499–507 (2015). <https://doi.org/10.1016/J.INDCROP.2015.09.045>

Open Access This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

