

Physicochemical, functional and sensory characteristics of a filtering infusion based on lemon balm leaves (*Melissa officinalis* L.) and orange peel (*Citrus sinensis* (L.) Osbeck)

Características fisicoquímicas, funcionales y sensoriales de una infusión filtrante basada en hojas de toronjil (*Melissa officinalis* L.) y cáscara de naranja (*Citrus sinensis* (L.) Osbeck)

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Abstract

Melissa officinalis L., a medicinal herb, is a source of a large number of bioactive compounds, as well as orange peels, mainly owing to phenolic compounds widely studied for their biological properties. The present investigation focused on studying the physicochemical, sensory and biological characteristics of lemon balm leaf and orange peel infusions. The analysis was carried out through the quantification of total phenolic content (TPC), antioxidant activity by DPPH and ABTS. In addition, the optimization of the best infusion was assessed by response surface methodology (RSM) and analysis of variance (ANOVA). The results showed a TPC between 4.50-4.80 µg GAE/ mL, an antioxidant activity in the range of 53-74 % by DPPH and 73-83 % by ABTS. On the other hand, the RSM reported the optimal conditions of the infusion with the best sensory acceptability and desirability resulted from combining 150 g of lemon balm leaves with 60 g of orange peel. Nevertheless, the extracts showed limited antioxidant activity, less than 80 % inhibition and with halos of less than 8 mm by disk diffusion.

Keywords: Antimicrobial capacity. Antioxidant capacity. Infusion. Lemon balm. Orange peel.

Resumen

Melissa officinalis L., una hierba medicinal, es fuente de un gran número de compuestos bioactivos, al igual que las cáscaras de naranja, principalmente debido a los compuestos fenólicos ampliamente estudiados por sus propiedades biológicas. La presente investigación se centró en el estudio de las características fisicoquímicas, sensoriales y biológicas de las infusiones de hojas de toronjil y cáscaras de naranja. El análisis se llevó a cabo mediante la cuantificación del contenido fenólico total (TPC), la actividad antioxidante por DPPH y ABTS. Además, se evaluó la optimización de la mejor infusión mediante la metodología de superficie de respuesta (RSM) y el análisis de varianza (ANOVA). Los resultados mostraron un TPC entre 4.50 – 4.80 µg GAE/ mL, una actividad antioxidante en el rango de 53 - 74 % por DPPH y 73 - 83 % por ABTS. Por otra parte, el RSM informó que las condiciones óptimas de la infusión con la mejor aceptabilidad y deseabilidad sensorial resultaron de combinar 150 g de hojas de melisa con 60 g de cáscara de naranja. No obstante, los extractos mostraron una actividad antioxidante limitada, inferior al 80 % de inhibición y con halos de menos de 8 mm por difusión en disco.

Palabras clave: Capacidad antimicrobiana. Capacidad antioxidante. Infusión. Toronjil. Cáscara de naranja.

1. Introduction

Plant biodiversity in Peruvian territory is vast, as evidenced by the wide variety of medicinal plants and fruits¹. On the one hand, medicinal plants such as oregano (*Origanum vulgare*), lemon balm (*Melissa officinalis* L.), lemon verbena (*Aloysia citrodora*), among many others, have long been used in infusions as ancestral remedies for the prevention and treatment of various diseases, and even as muscle relaxants². Today, however, they are also incorporated into the food, cosmetic, pharmaceutical, and other industries for their preservative and sensory properties, as well as for their health benefits³. On the other hand, citrus fruits such as orange, lemon, and grapefruit are consumed daily in large quantities because they provide significant amounts of vitamin C, minerals, and other micronutrients. However, they also generate hundreds of tons of fruit peels, considered a waste product, which is why there is growing interest in transforming these residues into valuable products with greater social and environmental benefits^{4,5}.

Many researchers have noted that, although orange peels are not edible like lemon balm leaves, they exhibit significant biological activities, including antibacterial, antioxidant, and anticarcinogenic effects^{3,6}. These properties have been linked to the wide variety of bioactive compounds they contain, such as flavonoids, phenolic acids, pectins, and polysaccharides, which are also capable of reducing oxidative stress. For centuries, orange peels have likewise been used in the treatment of neurodegenerative, cardiovascular, autoimmune, and respiratory diseases, as well as the common cold, among others^{3,7,8}.

Melissa officinalis, or lemon balm, is a traditional medicinal plant listed in several pharmacopoeias for its potential therapeutic effects^{9,10}. Recent pharmacological studies have shown that it exhibits diverse biological activities, including antibacterial, antioxidant, antidepressant, anti-diabetic, antispasmodic, anxiolytic, anti-inflammatory, and anti-Alzheimer effects^{11–13}. The literature also reports its use in treating stress or insomnia associated with anguish, melancholy, and sadness. Many of these pharmacological activities have been attributed to its phenolic constituents (such as rosmarinic acid, caffeic acid, thymol, and gallic acid), as well as to terpenes and flavonoids—mainly present in the leaves—along with vitamins and minerals^{9,13,14}.

Many researchers argue that extracts and infusions of plants or fruit residues such as peels and leaves possess multiple metabolites (polyphenols, flavonoids, etc.) with strong antimicrobial activity; however, this depends on the extraction factors such as solvent, temperature, time, particle size, and pH^{15–17}. Moreover, there are various usable forms for these bioactive compounds, such as those present in lemon balm leaves and orange peels; however, one of these forms is through infusions by direct contact with water at boiling temperature¹³. Additionally, infusing dried plant leaves combined with fruit peels in hot water helps to release its bioactive components that promote relaxation and relieve tension and anxiety¹⁸. At the same time, sustainability is emphasized, and agricultural by-products such as fruit peels are valorized. In addition, the combination produces a synergy between its bioactive principles that, when are consumed, help to release stress and improve sleep quality.

Moreover, extracts in different solvents, such as water, ethanol, or the combination of both, as referred to by Draginic and coworkers, have shown slightly better phenolic and flavonoid content, making them potential antioxidants and anti-inflammatories¹⁹. Similarly, Abd El-Aziz and coworkers state that orange peel extracts obtained by maceration in water for 3 days may exhibit protective potential against neurodegenerative diseases such as Alzheimer's²⁰.

Nowadays, considering the biological activities reported for orange peels and lemon balm leaves, many researchers have emphasized the study of antiviral effects against several viruses, such as human papilloma, among others. Moreover, the effects against gram-negative bacteria (e.g., *Escherichia coli*, *Salmonella*, etc.) and gram-positive bacteria (e.g., *Staphylococcus aureus*, *Bacillus cereus*, etc.), respectively. Moreover, the physiological and metabolic effects of their infusions or extracts in food diet^{11,13,21}. Thus, the present work aims to study the physicochemical and sensory characteristics, antioxidant activity (by DPPH and ABTS), and antimicrobial activity of six different formulations based on orange peel (*Citrus sinensis* (L.) Osbeck) and lemon balm leaves (*Melissa officinalis* L.).

2. Materials and methods

2.1 Materials

2, 2' - diphenyl - 1- picrylhydrazyl (DPPH, Lt: STBH6773), 2, 2' - azino - bis (3 -ethylbenzothiazolin) - 6 - sulfonic acid (ABTS, Lt: SLBV6099), Folin Ciocalteu reagent (2N), sodium bicarbonate, gallic acid, methanol and analytical grade Trolox (Lt: BCBW5446) purchased from Merck-Sigma Aldrich. Likewise, lemon balm leaves (*Melissa officinalis* L.) and orange peel (*Citrus sinensis* (L.) Osbeck) obtained from the wholesale market of Caquetá, Lima-Peru.

2.2 Obtaining the material and preparing the infusions

Lemon balm leaves (*Melissa officinalis* L.) and Valencia orange peel (*Citrus sinensis* (L.) Osbeck) were previously washed with abundant water. In the case of orange peel, they were cut into thin strips, then into small pieces of 0.5 cm x 0.5 cm, and were dried in a food dehydrator (Boxa®, DH-20V) for 8 h at 40 °C, whereas the lemon balm leaves were dried for 5 h at 35 °C. After drying, the samples were ground using an electric grinder (Oster® model BVSTBMH23), followed by sieving through a 16-mesh sieve. Finally, the powders were stored at room temperature under vacuum in a desiccator and labeled until further use²².

From the previously dried samples, in the present study, a 2x3 factorial design was selected to evaluate the effect of the factors: lemon balm leaves (X_1) and orange peel (X_2), at different levels, especially on the functional and sensory properties of the infusions. Nevertheless, this factorial design is used to optimize the number of treatments generated, using the response surface methodology (RSM). Likewise, the levels were determined from preliminary trials, considering both the sensory acceptability and the yield of bioactive compounds. Therefore, six formulations were prepared by mixing lemon balm leaves and ground orange peels (**Table 1**); these were packaged in small filter

Table 1. Infusions based on 2-factor factorial design: lemon balm leaves with orange peel.

Sample	Lemon balm leaves	Orange peel
	g (%)	g (%)
F1	100 (83.3 %)	20 (16.7 %)
F2	150 (88.2 %)	20 (11.8 %)
F3	100 (71.4 %)	40 (28.6 %)
F4	150 (78.9 %)	40 (21.1 %)
F5	100 (62.5 %)	60 (37.5 %)
F6	150 (71.4 %)	60 (28.6 %)

bags with a capacity of 1g. Subsequently, the infusions were obtained by direct contact extraction, solid-liquid. For this, the filter bags were placed inside a glass beaker with 100 mL of bottled water at 98 °C and macerated for 5 minutes. The preparation process was carried out simultaneously for all six formulations.

2.3 Total phenolic content

Total phenolic content (TPC) was determined using the Folin Ciocalteu colorimetric method described by Ordoñez-Gómez *et al.*²³, with slight modifications. 100 µL of sample (filtering infusion) was mixed with 860 µL of deionized water, then added 40 µL of Folin Ciocalteu's reagent (2N), 5 minutes later 100 µL of sodium carbonate (200 g L⁻¹) was added. Finally, 900 µL of deionized water was added and left to stand for 1 h, then TPC was determined by Uv-Visible spectroscopy at 725 nm. Polyphenol quantification was done using the calibration curve of the standard gallic acid (GA) ($Y = 0.0039X + 0.0165$, $R^2 = 0.9902$). The results were expressed as micrograms equivalents of GA per milliliter of filtering sample (µg GAE/ mL sample).

2.4 Antioxidant activity

2.4.1 DPPH method

The antioxidant activity of the extracts (infusions) was carried out by the DPPH method described by Jurado Teixeira *et al.*²⁴ and Mokoroane *et al.*²⁵ with some modifications, using ascorbic acid as a reference standard. A solution of DPPH 20 mg L⁻¹ in methanol with an absorbance adjusted to 0.693 at 517 nm was prepared. The different samples were diluted by taking 1 mL of sample and 2 mL of water. Subsequently, 100 µL of diluted sample was mixed with 1.9 mL of the DPPH solution and left in complete darkness for 30 minutes. Finally, the absorbance was determined at 517 nm, and the antioxidant activity was calculated as a percentage of the DPPH radical scavenging capacity using the equation (1).

$$\% \text{ DPPH radical scavenging activity} = \left(1 - \frac{A_{SP}}{A_{DPPH}}\right) * 100 \quad (1)$$

Where: A_{SP} , the absorbance of the sample and A_{DPPH} , the absorbance of the free radical DPPH.

2.4.2 ABTS method

The antioxidant activity of the infusions was determined using the ABTS assay described by Ordoñez-Gómez *et al.*, with some modifications and using Trolox® as a reference standard²³. The ABTS^{•+} radical was prepared by oxidation of 10 mL of ABTS (7 mmol L⁻¹) with 176 µL of potassium persulfate (0.14 mol L⁻¹), the mixture was left to react for 24 h in complete darkness at room temperature. Subsequently, the mixture was diluted to record an absorbance between 0.634 and 0.638 at 734 nm. A 20-µL aliquot of the diluted samples (1 mL of sample with 2 mL of water) was blended with 2 mL of the ABTS^{•+} radical prepared above and allowed to stand for 6 minutes. Finally, absorbance was measured at 734 nm, and the antioxidant activity was calculated as a percentage of the ABTS radical scavenging capacity using the equation (2).

$$\% \text{ ABTS radical scavenging activity} = \left(1 - \frac{A_{SP}}{A_{ABTS}}\right) * 100 \quad (2)$$

Where: A_{SP} , absorbance of the sample and A_{ABTS} , the absorbance of the free radical ABTS.

2.5 Sensorial analysis

The overall acceptability of the infusions was assessed using a 10-point scale anchored by the terms "unpleasant" at the low, "Neither pleasant, nor unpleasant" at the middle, and "very pleasant" at the high end. The evaluation was conducted voluntarily on a group of 112 untrained panelists²⁶. 20 mL of a total of 6 samples were individually filled in polypropylene cups, randomly coded, and served sequentially to the panelists for evaluation at room temperature.

2.6 Antimicrobial activity

The antimicrobial activity was evaluated using the disc diffusion method and broth microdilution, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI)²⁷, with certain modifications. The assays were conducted against four types of microorganisms: *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella* spp. For the disc diffusion tests, inocula were adjusted to 0.5 McFarland standard, equivalent

to $1-2 \times 10^8$ CFU/mL. Subsequently, 100 μ L of each inoculum was spread onto Mueller–Hinton (MH) agar plates. Six wells were then made using a punch, and 50 μ L of sample was placed in each well. The plates were incubated at 37 °C for 24 h, after which the inhibition diameters were measured.

For broth microdilution assays, 90 μ L of MH was added to the wells of the first column of a 96-well plate, to those of the second column 180 μ L of the extract was added. From the latter, 1:1 serial dilution was performed, 90 μ L was transferred from one column to the other, up to the eleventh column. On the other hand, the initial inoculum was diluted to an equivalent concentration of 10^6 CFU ML^{-1} of each strain and 10 μ L was added to all wells except the first column, having a final volume of 100 μ L in each well. Subsequently, it was incubated at 37 °C for 24 h and the minimum inhibitory concentration was determined by turbidity at 600 nm in a microwell reader (Biotek® Model Power Wave XS2). Likewise, all absorbance values for the first and twelfth column represent the blank and positive control, respectively. Meanwhile, the values obtained for the other columns represent the antimicrobial effect of a concentration of 100 % to 0.20 % against a microorganism. The assays were performed by triplicate and the antibacterial activity was expressed as percentage inhibition through equation (3).

$$\% \text{ inhibition} = \left(1 - \frac{A_S - A_{CB}}{A_{CP} - A_{CB}} \right) * 100 \quad (3)$$

Where: A_S , A_{CB} y A_{CP} , the average absorbance of the sample, blank and positive control, respectively.

2.7 Statistical analysis

The formulated infusions (based on lemon balm leaves and orange peel) were used to determine the optimum combinations of the process variables on the response variables, using the response surface methodology (RSM). The selected process variables were: dry mass of lemon balm leaves (X1) and orange peel (X2). The experimental design was carried out using Statgraphics Centurion® 19 statistical software at a confidence level of 95 % ($P < 0.05$). The experimental data were fitted to a second-degree linear mathematical model, equation (4), considering the parameters X1, X2 and their interactions. On the other hand, the analysis of variance test (ANOVA) was performed to evaluate the model fit considering the regression coefficients, probability values (P) and fit loss, on the response variables: Total polyphenols (TPC), antioxidant capacity (by DPPH and ABTS), acceptability and desirability.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_2^2 \quad (4)$$

Where: Y is the response variable, β_0 is a constant, β_1 , β_2 , β_3 y β_4 coefficients for the main effects and interactions, respectively.

3. Results and discussions

Figure 1 (a) shows the TPC of each formulated infusion, with infusions F1 (4.80 μ g GAE/mL) and F4 (4.74 μ g GAE/mL)

exhibiting the highest phenolic content. This is partly attributed to the presence of caffeic acid derivatives, catechins, and coumarin in both lemon balm leaves and orange peel, which display greater thermal stability than more volatile phenols such as cadinol and carvacrol^{2,28}. The slight differences observed between the samples and the values reported in the literature are associated with the physicochemical conditions of the infusion, such as its pH of 7.18 ± 0.08 . According to some researchers, at alkaline or neutral pH, polyphenols tend to undergo degradation reactions²⁹. Additionally, variations have been linked to the presence of polyphenol oxidase in orange peel, as this enzyme can degrade and oxidize polyphenols, thereby reducing their phenolic content and biological activity^{2,12,17,30}. However, during infusion processes using water at approximately 100 °C, the enzyme's activity is inactivated, and therefore its effect is disregarded in quantification³¹.

The activity of plant-origin natural infusions is often assessed by their antioxidant activity using established methods such as DPPH or ABTS. Two methods that allow to determine the antioxidant activity of a broad diversity of substances, such as hydrogen-donating antioxidants or scavengers of aqueous phase radicals and chain-breaking antioxidants or scavengers of lipid peroxyl radicals³². In the DPPH method, **Figure 1 (b)**, results have shown the highest antioxidant activity in infusions F1 (73.3 %) and F4 (69.1 %), a result consistent with the TPC found for the same samples since both factors are directly correlated (**Figure 1c**). On the contrary, by ABTS it was observed that F5 (82.4 %) showed the highest radical scavenging capacity compared to the others of similar values (~ 76 %). Moreover, an inverse statistical correlation, i.e., it shows a tendency to decrease the antioxidant activity as the total phenolic content increases. On the other hand, the difference with the results by DPPH, is because of the better consistency for polyphenols than ABTS³⁰, where the radical can also react with minerals and vitamins present in the extracts. Nevertheless, with DPPH the reaction of the antioxidant is faster and more stable, since it does not cause subsequent lipid oxidation and does not lead to the formation of unstable end products. In contrast, with ABTS the reaction occurs mainly in terms of the reduction of the ABTS+ radical with the antioxidants; which, contain both hydrophilic and lipophilic compounds^{6,17}. Results through this method were slightly higher than those reported for single infusions of lemon balm leaves and orange peel by the same methods, since for the first a 39.5 % by DPPH has been found, whereas for the second of 67.9 %, respectively. Similar results have been determined by ABTS for orange peel extracts of 60.48 %^{6,13}.

The effects of the extraction factors, lemon balm leaves (X1) and orange peel (X2), above the preparation of the infusions, as well as the response surfaces obtained, were studied in order to establish the optimal conditions of the best infusion. **Table 2** shows the regression coefficients of the fitted quadratic polynomial model and the relevance of the described factors on the response variables. The results displayed that the TPC is directly dependent on the combination of factors X1 and X2, since the associated regression coefficient as well as that of the independent term contribute significantly ($P < 0.05$), being corroborated

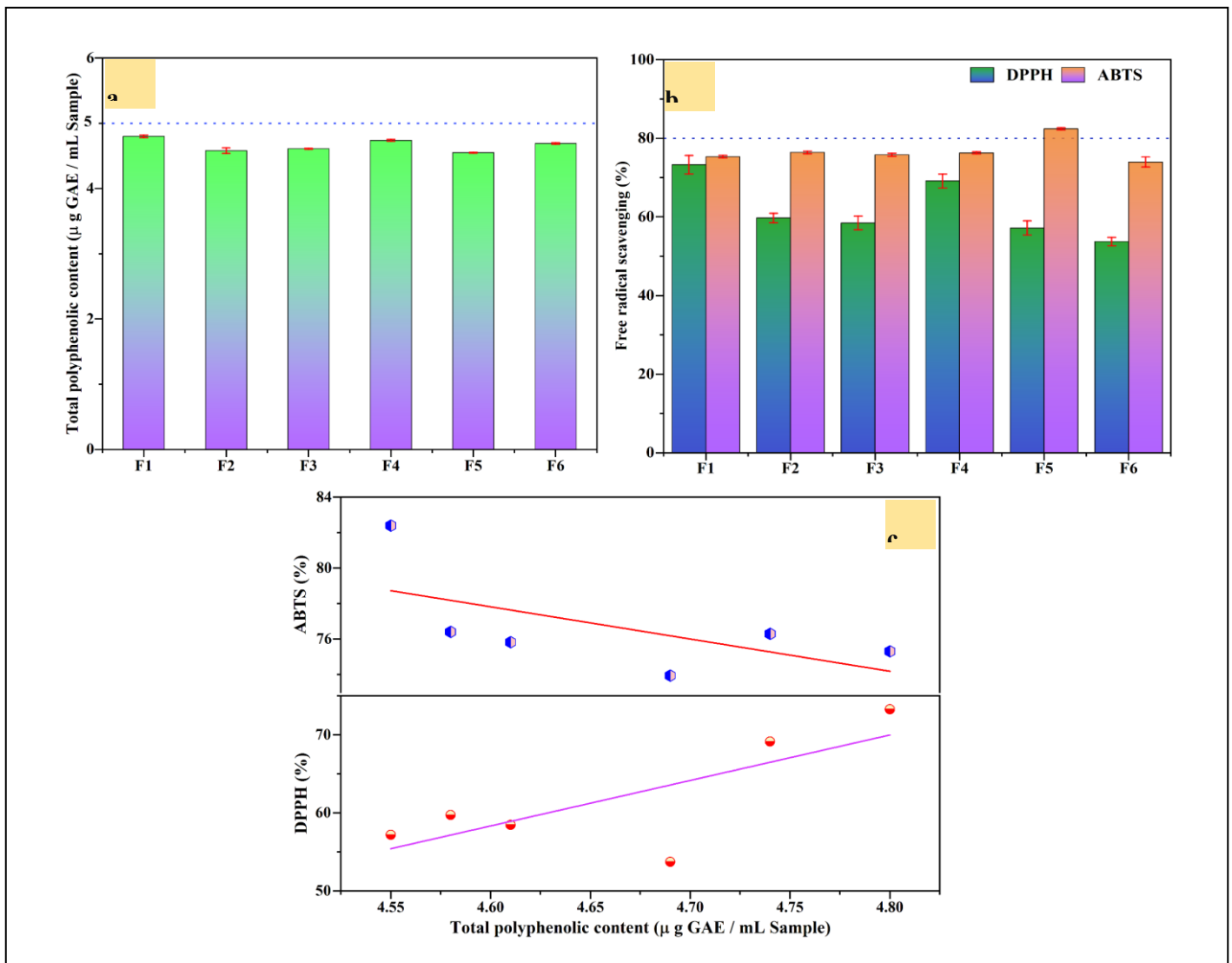


Figure 1. a) Total polyphenolic content, b) antioxidant activity by DPPH and ABTS of the filtering infusion and c) correlation matrix.

Table 2. Estimated regression coefficients of the second-order polynomial equation and analysis of variance (ANOVA) of the model for the response variables.

Regression coefficients	TPC		DPPH		ABTS		ACCEPTABILITY	
	Effect	P	Effect	P	Effect	P	Effect	P
β_0	5.5083	0.0000*	94.0488	0.0000*	59.4301	0.0000*	7.6658	0.0000*
$\beta_1(X_1)$	-0.00677	0.5456	-0.24413	0.5985	0.14525	0.0385*	-0.00357	0.6101
$\beta_2(X_2)$	-0.02025	0.1422	-0.34131	0.0553	0.46336	0.0732	-0.012	0.0105*
$\beta_3(X_1X_2)$	0.00018	0.0049*	0.00505	0.3197	-0.00478	0.0042*	0.000102	0.1425
$\beta_4(X_2^2)$	-0.00005	0.5975	-0.00707	0.509	0.00240	0.3385	0.000059	0.6675
R^2	0.7920	0.0304*	0.5612	0.2267	0.8456	0.0130*	0.7397	0.0568

X₁: Lemon balm leaves, X₂: Orange peel, * significant in $\alpha \leq 0.05$.

by the analysis of the Pareto diagram and its standardized effect (Figure 2 a). Furthermore, the model correlated and properly explained 79.20 % of the data (R^2 : 0.792). On the other hand, the response surface plot (Figure 2 b) showed that the most optimal combination contributing in higher TPC (4.77 μ g GAE/mL) was the mixture of 100 g lemon balm leaves with 20 g dried orange peel (F1). However, the model response indicates a slight decrease in TPC as the ratio between X1 and X2 is greater than 5, contrasting with what is reported in the literature and the presence of polyphenol oxidase commonly found in orange peel extracts¹². For antioxidant activity, it was found that

X2 has a partial effect on DPPH, a result that is corroborated by the standardized Pareto analysis (Figure 2 c-d), likewise the model did not display a direct autocorrelation between the parameters and the response variable, predicting values with a certainty of 56.12 %. It can also be indicated that the optimum value was 70.10 %, obtained by combining X1 and X2 in proportions similar to F1 and F4. On the other hand, it has been seen that X1, as well as its combination with X2 have a considerable influence on the antioxidant activity, determined by ABTS, since the associated regression coefficients and the independent term showed a significant contribution ($P < 0.05$). Results that

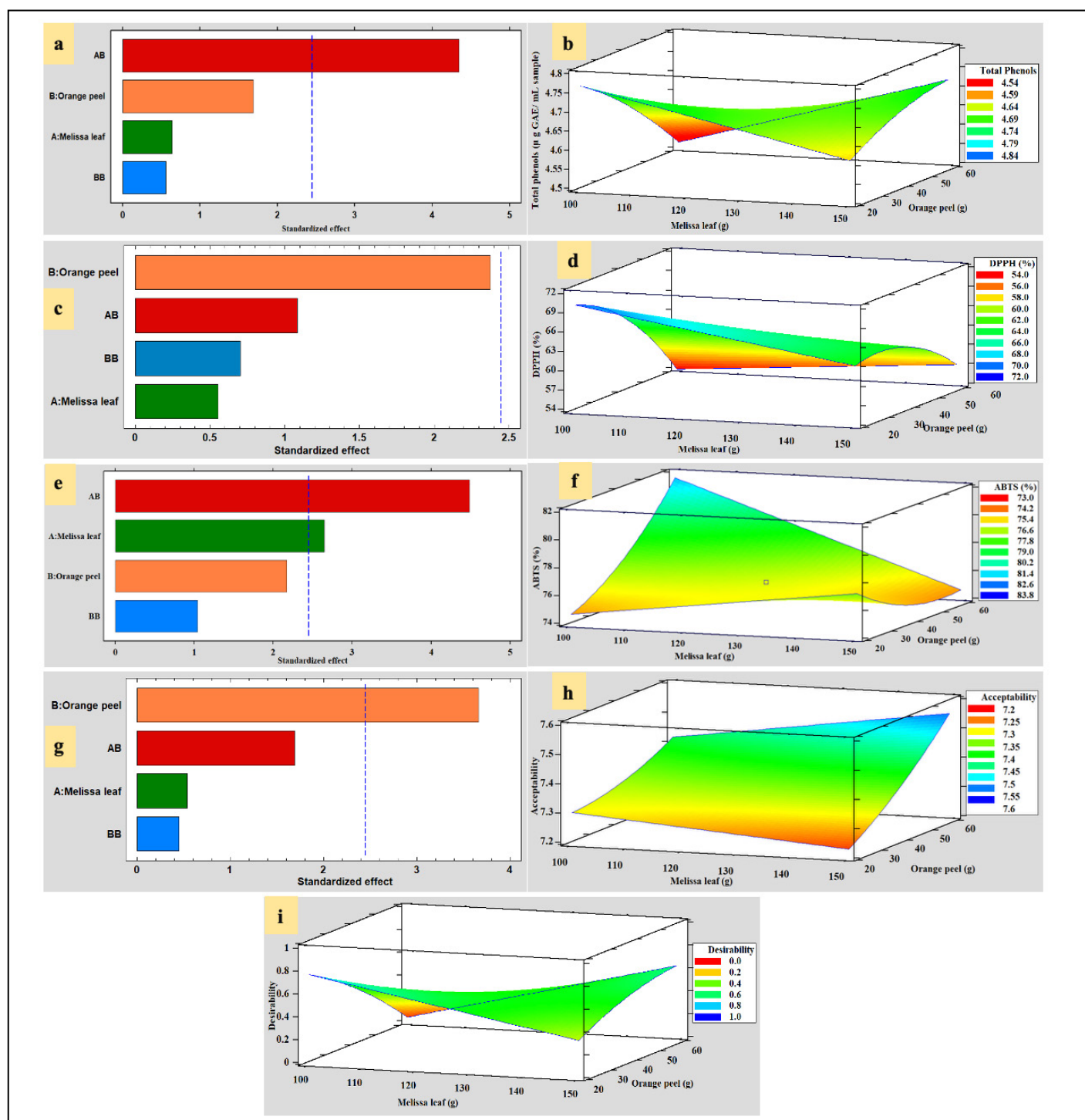


Figure 2. Pareto and response surface graphs of total phenolic content (a- b), antioxidant activity by DPPH (c- d), ABTS (e- f), sensory acceptability (g- h) and desirability (i) of the six filtering infusion.

were demonstrated by the Pareto diagram (**Figure 2 e**), in addition to a strong regression coefficient between the antioxidant activity by ABTS and the factors (R^2 : 0.8556), corresponded with a significant model fit ($P < 0.05$). Nevertheless, the response surface (**Figure 2 f**) showed an optimum value of 81.7 % antioxidant activity, similar to the F5 formulation. The results are comparable with what has been reported in other investigations for orange peel and lemon balm leaf extracts, as well as the strong correlation observed between TPC and antioxidant capacity^{6,33,34}. On the other hand, to understand the sensory acceptability of the different infusions, a sensory study was conducted, and the results were analyzed by surface response. The test strongly suggested that the independent variable X2 has a significant effect on acceptability, as well as the independent term of the model ($P < 0.05$), which explains and predicts with an accuracy of 73.97 %. Moreover, the role played by each factor on acceptability was also demonstrated by the Pareto analysis (**Figure 2 g**). However, in **Figure 2 (h)** it was observed that acceptability generally increases as the amount of orange peel rises, obtaining an acceptable optimum score of 7.55, corresponding to F6.

This result has been linked to compounds such as organic acids (oxalic, citric, gallic, ferulic, *p*-coumaric, etc.) and flavonoids (e.g., naringin, hesperidin, flavones) present in orange peels (8,30), which are slightly more thermally stable than certain compounds found in lemon balm leaves. The latter contain monoterpenes, such as citronellal; sesquiterpenes, such as β -caryophyllene and β -caryophyllene oxide; monoterpene alcohols, such as nerol, geraniol, and citronellol; as well as phenolic acids (10,35). The optimization results were integrated using the desirability function, as shown in **Figure 2(i)**, revealing that the optimal conditions were achieved in formulation F6 (0.78), corresponding to the combination of 150 g of X1 with 60 g of X2. These findings highlight both the influence of orange peels on the response variables and their important role in the preparation of infusions when combined with lemon balm leaves.

All analyses were conducted on infusions, each prepared using 1 g of the corresponding formulation (F1–F6). The formulations were designed following a 2×3 factorial

scheme, which allowed us to evaluate the individual and combined effects of the two ingredients on the response variables. Using the same amount for the infusion preparation ensured a more accurate comparison of the effects of the independent variables.

In the present work, the antibacterial activity was evaluated for the optimal infusion (F6), as well as for extracts of lemon balm leaves and pure orange peels. The assays were conducted against Gram-positive and Gram-negative microorganisms. As shown in **Table 3**, the lemon balm extract exhibited mild antimicrobial activity against *E. coli* (26.73%), *Salmonella* spp. (32.37%), *B. cereus* (39.75%), and *S. aureus* (40.46%). Although these values are below the threshold considered significant (<80%) (27), some researchers have reported that antimicrobial activity can occur through several mechanisms, including disruption of microbial cell membranes, inhibition of key enzymes, and interference with DNA replication, among others. These results are consistent with the literature, which indicates that the antimicrobial properties of lemon balm are primarily associated with its essential oil^{35,36}, showing noteworthy activity against these bacteria⁹.

In contrast, the orange peel extracts and the optimal formulation (F6) showed no positive response against any of the Gram-positive or Gram-negative bacteria. This may be due to the solvent used for extraction, as literature reports indicate that orange peel extracts better concentrate stable bioactive compounds in ethanol-based media³⁷. The disc diffusion assay revealed inhibition diameters below 8 mm for all microorganisms, indicating a lack of substantial inhibitory activity (**Figure 3**)²⁷. Nevertheless, these results are comparable to those reported by other researchers for aqueous extracts of lemon balm leaves and orange peels^{21,38}.

Although lemon balm leaves and orange peel contain antimicrobial compounds, the low activity observed in infusions can be attributed to factors such as temperature and preparation time, which limit the extraction of these compounds in concentrations sufficient to exert a strong effect. According to Hilal and Engelhardt³⁹, infusions prepared from commercial tea bags for 3 or 5 minutes at 100 °C contain lower concentrations of phenolic compounds

Table 3. Antibacterial activity of lemon balm leaves, orange peel and the mixture extracts on Gram-positive and Gram-negative bacteria.

Methods	Bacterial cultures		Lemon balm leaves extract	Orange peel extract	Mixture
Broth microdilution	Gram negatives	<i>E. coli</i>	26.73 %	0.00 %	0.00 %
		<i>Salmonella</i> spp	32.37 %	0.00 %	0.00 %
	Gram positives	<i>B. Cereus</i>	39.75 %	0.00 %	0.00 %
		<i>St. aureus</i>	40.46 %	0.00 %	0.00 %
Disc diffusion	Gram negatives	<i>E. coli</i>	< 5 mm	< 5 mm	< 5 mm
		<i>Salmonella</i> spp	< 5 mm	< 5 mm	< 5 mm
	Gram positives	<i>B. Cereus</i>	< 5 mm	< 5 mm	< 5 mm
		<i>St. aureus</i>	< 5 mm	< 5 mm	< 5 mm

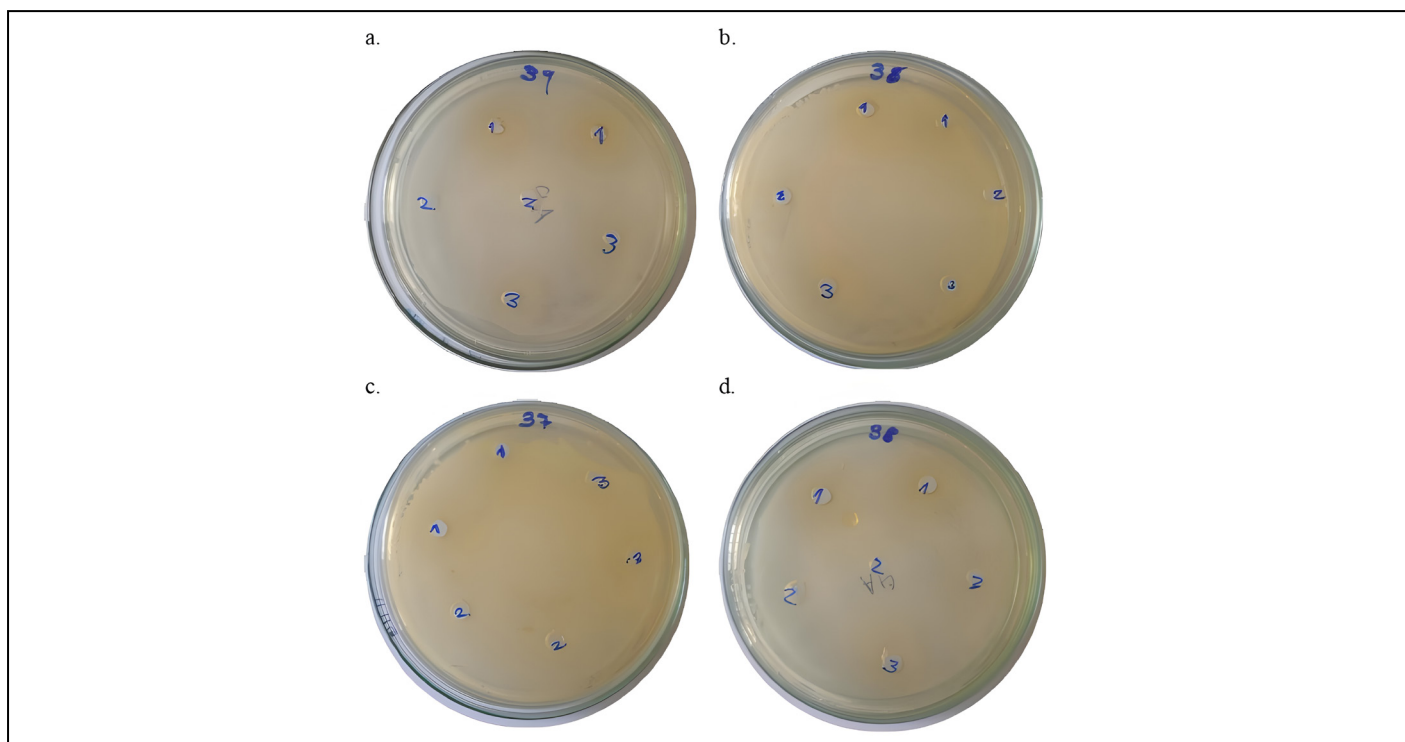


Figure 3. Disc diffusion assay of orange peel extracts and formulation F6 (a–d).

compared to infusions extracted for 15 minutes at 80 °C. Therefore, the present results indicate that infusions do not have strong antimicrobial effects. Nonetheless, they remain valued and irreplaceable beverages due to their carminative, digestive, and diuretic effects, as well as their appealing sensory qualities.

4. Conclusions

The results of the present work revealed that the extracts (infusions) have a maximum TPC of 4.80 $\mu\text{g GAE/ mL}$ (F1), while an antioxidant activity by DPPH of 73.30 % (F1) and 82.40 % (F5) by ABTS. On the other hand, ANOVA statistical analysis and response surface showed that the combination of lemon balm leaves and orange peel significantly influence on TPC, as well as antioxidant activity by ABTS ($P < 0.05$), with a high correlation coefficient ($R^2 > 0.79$). Additionally, the concentration of orange peel had a significant impact on sensory acceptability of the infusions, as indicated by Pareto diagram. As revealed by the desirability analysis, the optimal formulation was identified as F6 (0.78), corresponding to a mixture of 150 g of lemon balm leaves and 60 g of orange peel. Finally, the extracts of lemon balm leaves displayed low activity against gram-positive and gram-negative bacteria, whereas those of orange peels and F6 did not inhibit bacterial growth. Overall, this work aimed to valorize citrus by-products such as orange peels and its combination with lemon balm leaves as a potential drinkable infusion, rich in bioactive compounds with outstanding antioxidant activity and acceptability.

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Conflict of interest

The authors declare no conflicts of interest.

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