Experimental design for the determination of the antioxidant activity of the essential oil of "Toronjil blanco" (*Agastache mexicana* subsp. *xolocotziana*)

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Introduction

Agastache mexicana subsp. *xolocotziana* is found only in a cultivated state in central Mexico. It is known in traditional Mexican medicine as "toronjil blanco" and is used to treat heart diseases. Due to its therapeutic properties, it is commercially traded internationally (Zielinska & Matkowski, 2014; Najar et al., 2019; Palma-Tenango et al., 2021). Recent phytochemical and pharmacological studies correlate with its ethnomedicinal uses; the organic extract has been reported to have spasmolytic, analgesic, and anti-inflammatory effects. Regarding its essential oil, it has been reported to contain 38 compounds, with estragole and methyl eugenol being the most abundant. Despite its economically valuable and scientifically interesting properties, there are no reports on the antioxidant activity of its essential oil (Estrada-Reyes et al., 2014; Juárez et al., 2015).

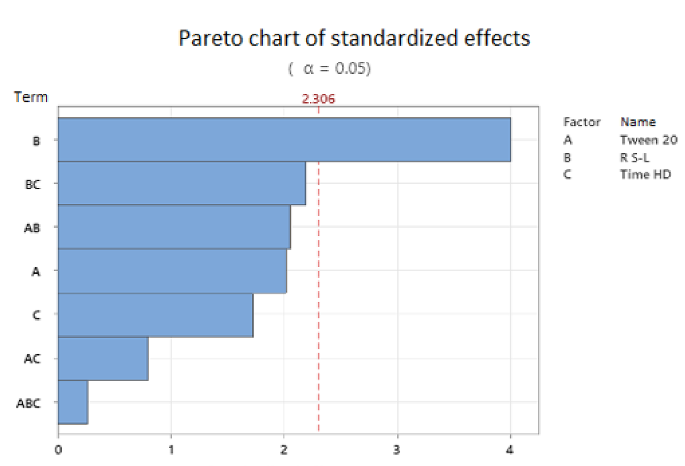
Methodology

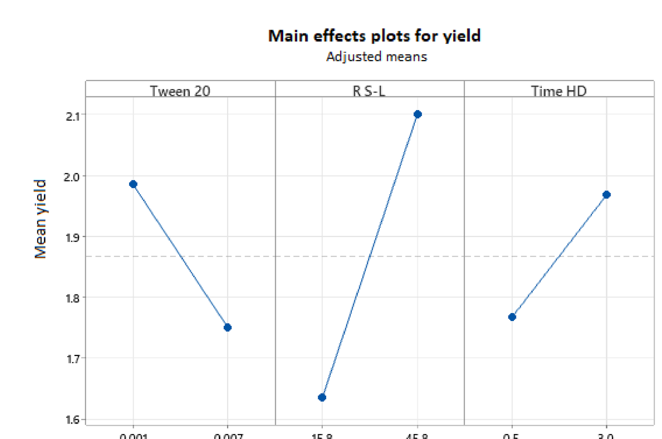
The essential oils were obtained by hydrodistillation. Antioxidant Activity Determined by the DPPH (Rashidi et al., 2018) and ABTS Methods (Re et al., 1999). Chemical Composition by Gas Chromatography-Mass Spectrometry (GC-MS).

Results and Discussion

ANOVA for yield

Source	DF	SS	MS	F Value	P Value
Model	7	1.77630	0.253757	4.69	0.023
Lineal	3	1.24917	0.416389	7.70	0.010
Tween 20	1	0.22027	0.220274	4.07	0.078
R S-L	1	0.86769	0.867692	16.05	0.004
Time HD	1	0.16120	0.161202	2.98	0.122
Two-Term Interactions	3	0.52321	0.174402	3.23	0.082
Tween 20*R S-L	1	0.22832	0.228325	4.22	0.074
Tween 20*Time HD	1	0.03478	0.034782	0.64	0.446
R S-L*Time HD	1	0.26010	0.260100	4.81	0.060
Three-Term Interactions	1	0.00393	0.003927	0.07	0.794
Tween 20*R S-L*Time HD	1	0.00393	0.003927	0.07	0.794
Error	8	0.43251	0.054064		
Total	15	2.20882			

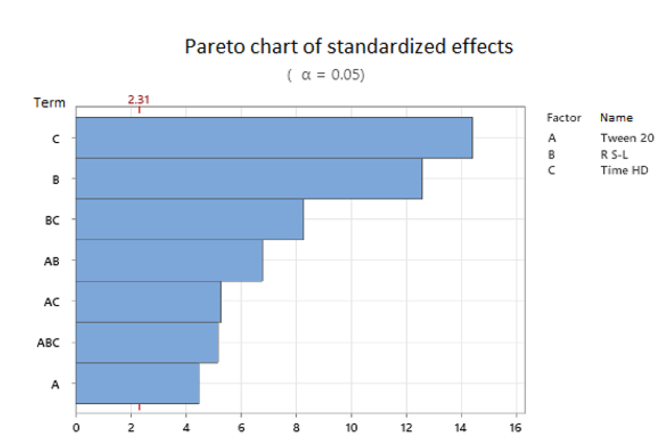


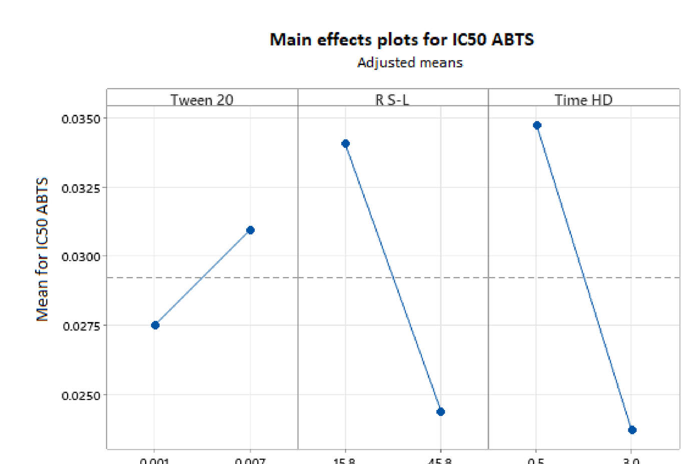


As can be observed in the ANOVA and Pareto chart, the variable that most affects the yield is the solid-liquid ratio. This is also confirmed in the main effects plot. The interaction that most affected the yield was the solid-liquid ratio-time HD.

ANOVA for IC50 ABTS

Source	DF	SS	MS	F Value	P Value
Model	7	0.001311	0.000187	79.26	0.000
Lineal	3	0.000911	0.000304	128.54	0.000
Tween 20	1	0.000047	0.000047	20.04	0.002
R S-L	1	0.000374	0.000374	158.19	0.000
Time HD	1	0.000490	0.000490	207.39	0.000
Two-Term Interactions	3	0.000337	0.000112	47.49	0.000
Tween 20*R S-L	1	0.000109	0.000109	46.00	0.000
Tween 20*Time HD	1	0.000066	0.000066	27.89	0.001
R S-L*Time HD	1	0.000162	0.000162	68.57	0.000
Three-Term Interactions	1	0.000063	0.000063	26.75	0.001
Tween 20*R S-L*Time HD	1	0.000063	0.000063	26.75	0.001
Error	8	0.000019	0.000002		
Total	15	0.001330			

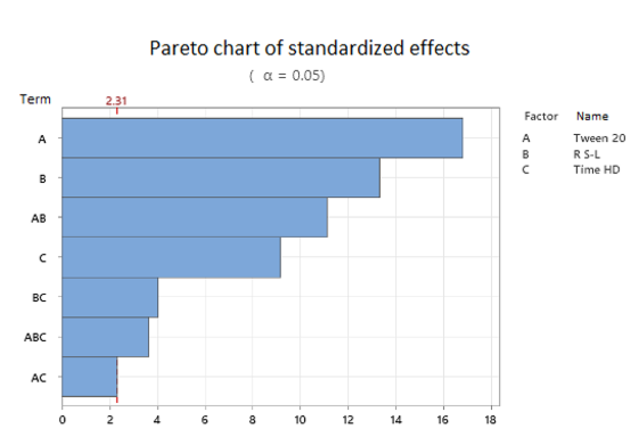


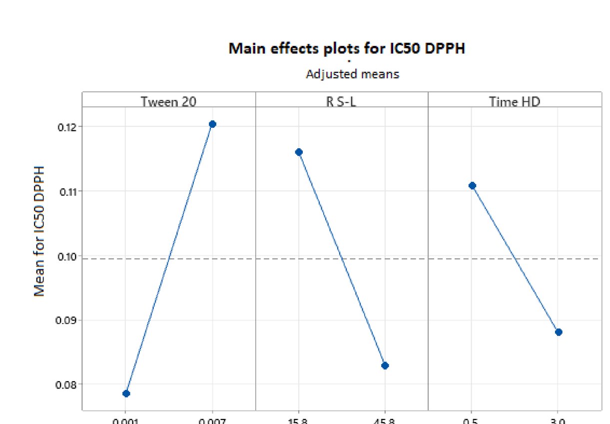


Regarding the antioxidant activity by the ABTS method, the variable that most affected it was the HD time, followed by the solid-liquid ratio, and finally the Tween 20. This is also confirmed by the Pareto chart. As with the yield, the interaction that most affected the antioxidant activity was the solid-liquid ratio-HD time interaction.

ANOVA for IC50 DPPH

Source	DF	SS	MS	F Value	P Value
Modelo	7	0.017448	0.002493	100.45	0.000
Lineal	3	0.013513	0.004504	181.54	0.000
Tween 20	1	0.007018	0.007018	282.84	0.000
R S-L	1	0.004406	0.004406	177.58	0.000
Time HD	1	0.002089	0.002089	84.19	0.000
Interacciones de 2 términos	3	0.003602	0.001201	48.39	0.000
Tween 20*R S-L	1	0.003065	0.003065	123.51	0.000
Tween 20*Time HD	1	0.000132	0.000132	5.31	0.050
R S-L*Time HD	1	0.000406	0.000406	16.36	0.004
Interacciones de 3 términos	1	0.000332	0.000332	13.40	0.006
Tween 20*R S-L*Time HD	1	0.000332	0.000332	13.40	0.006
Error	8	0.000199	0.000025		
Total	15	0.017647			





Finally, for the antioxidant activity determined by the DPPH method, it was observed that the solid-liquid ratio, HD time, and Tween 20 all had an effect. The interaction that most affected the antioxidant activity by the DPPH method was the solid-liquid ratio-Tween interaction.

The main compounds found in the essential oil were: nerol (36.83%), isopulegone (18.39%), and geranyl acetate (15.39%). Nerol is a common constituent in essential oils such as Citrus aurantium (De Cássia da Silveira e Sá et al., 2017) and Amomum tsaoko Crevost et Lemarie (Liao et al., 2022). This component was also identified in the extract of Agastache mexicana subsp. xolocotziana (Palma-Tenango et al., 2021) and was associated with antinociceptive and anti-inflammatory activities (De Cássia da Silveira e Sá et al., 2017) attributed to the plant.

When compared with synthetic antioxidants, the results shown in Table 1 were obtained. The results are similar to those of EO from the aerial parts of Clinopodium sericeum (473.030 mg/mL and 106.060 mg/mL for DPPH and ABTS, respectively) reported by Benites et al. (2021). Moderate antioxidant activity was also found for EO from the aerial parts of Thymus algeriensis Boiss (41.090 mg/mL and 10.840 mg/mL for DPPH and ABTS, respectively), which could be attributed to a long extraction time (Ouakouak et al., 2021) since, according to Cui et al. (2018), the bioactive compounds and thermolabile components that confer antioxidant characteristics to essential oils can decompose, hydrolyze, and dissolve in water during a prolonged extraction period and high boiling water temperature in the hydrodistillation process.

Table1. IC50 Synthetic antioxidants

Synthetic Antioxidant	IC50 (mg/mL) DPPH	IC50 (mg/mL) ABTS
Ascorbic Acid	0.092	0.0983
BHT	0.358	0.261
Trolox	0.225	0.121

Conclusions

The yield values were found to be between 1.336% and 2.626%. The IC50 obtained by the ABTS method was found to be between 0.0204126 and 0.0500870 mg/mL, and the IC50 obtained by the DPPH method was found to be between 0.0575880 and 0.166600 mg/mL. The variable that most affected both the yield and the IC50 by the ABTS and DPPH methods was the solid-liquid ratio.

The study concluded that the *Agastache mexicana* subsp. *xolocotziana* EO showed significant antioxidant activity. It is important to know the chemical properties of the essential oil of *Agastache mexicana* subsp. *xolocotziana* since they have not been reported so far and because it is consumed as tea in certain regions of Mexico and is a plant of commercial importance

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