An Experimental study exploring Box-Behnken Design for Optimal Extraction of Phenolics from *Olea europaea* leaves

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Background

- ✓ *Olea europea* is commonly known as Zaytoun in Arabic. Arabian Peninsula is famous for its intake of olives with food and it is well known for its nutritional and antioxidant benefits.
- ✓ The main constituents of the olive plant are oleuropein, oleoside and dimethyl oleuropein and flavonoids, phenolic compounds, such as tyrosol and caffeic acid[1].
- ✓ Extraction method plays a critical role in the study of medicinal plants. **Microwave-assisted extraction (MAE)** is an advanced extraction approach that was formed to reduce the extraction time and to increase yields with minimal consumption of solvents[2].

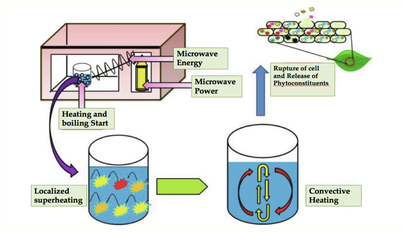


Figure 1: Microwave-assisted extraction Mechanism

Research Problem

- ✓ Currently, process optimization with **response surface methodology** for extraction parameters in herbal infusions has drawn consideration to maximize antioxidant phenolic compounds[3].
- ✓ *Olea europaea* is reported to possess wide area of pharmacological and therapeutic attributes. There are many reported studies on the health influences of olive leaves, still, there are limited research on the efficient extraction method.

Aim & Objectives

Aim: Exploration of Box-Behnken Design for Optimal Extraction of Phenolics from *Olea europaea* leaves.

Specific objectives:

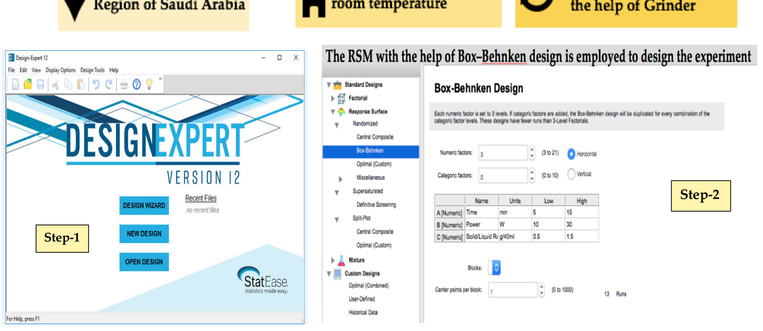
✓ To prepare the aqueous extracts of leaves of *Olea europaea* using the Box-Behnken Design (BBD) with microwave assisted extraction.

✓Estimation of total phenolic compound in each extract.

✓MAE method development to obtain the highest extraction yield and Total Phenolic content from *Olea europaea* leaves.

Materials

Plant sample Collection and preparation



Acknowledgement

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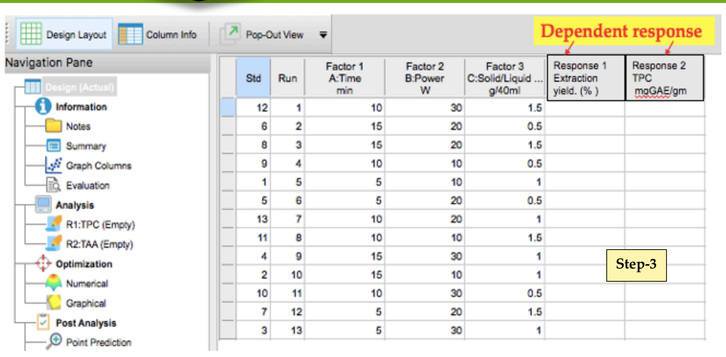
Methodology



Microwave-assisted extraction (MAE)

✓Based on the generated experimental values, all the 13 extracts were made with the help of MAE.

✓Each extract was evaporated and % yield of recovered dried extract was calculated with the formula:

Yield (%)= (W1/W2) * 100

where W1 = weight of the extract after evaporation, and W2 = weight of the plant

Result and discussion

Figure 2: Macroscopical Characters of olive leaf

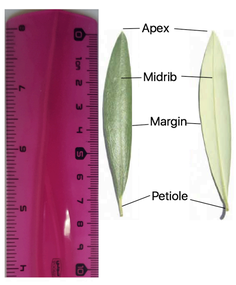


Table 1: Length and width Ratio of olive leaves

S.No.	Characters (Measurements in cm scale)		
	Leaf-length	Leaf-width	Length/ width ratio
1	6.8	1.5	4.5
2	5.9	2	2.95
3	5	1	5
4	6	1.5	4
5	6.4	1.7	3.8
6	5.7	1.5	3.8
7	6.8	1.4	4.8
8	6.3	1.6	3.9

Standard Graph of Gallic acid

Figure2:

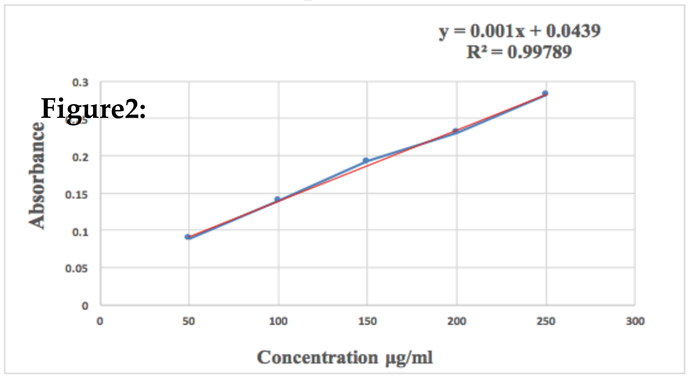


Table 2: Ranges of Independent parameters, % Yield and Total Phenolic content of olive leaves

Run	Time	Power	Solid/ Liquid	%Yeild	TPC (Total phenolic content (mg GAE/g DW)
1	15	20	0.5	24	64.1 ± 1.7
2	10	30	0.5	26	28.76 ± 1.52
3	15	10	1	16	107.76 ± 1.15
4	5	10	1	26	51.76 ± 0.57
5	15	20	1.5	26	100.43 ± 2.08
6	15	30	1	28	90.76 ± 0.57
7	5	30	1	33	199.43 ± 2.51
8	10	20	1	24.6	102.76 ± 1.15
9	5	20	0.5	16	192.1 ± 3.60
10	5	20	1.5	33	157.77 ± 1.14
11	10	10	0.5	48	32.1 ± 4.35
12	10	10	1.5	24.6	221.43 ± 2.3
13	10	30	1.5	37	121.43 ± 0.57

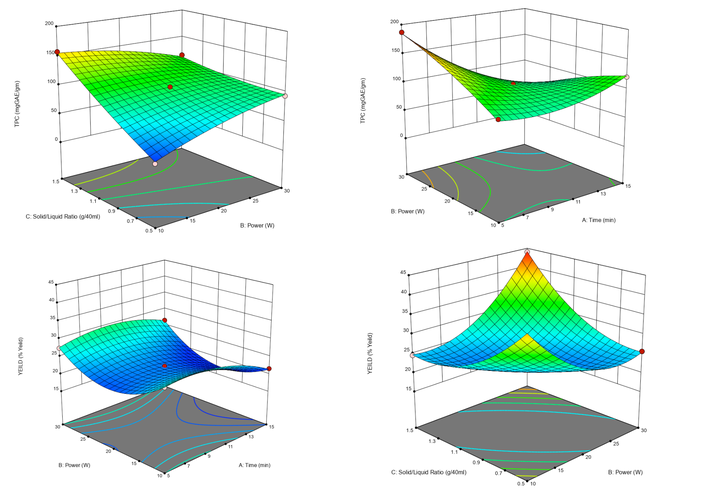
The predicted Second order Polynomial equation explaining the effect of each factor on the response

Total Phenolic Content = + 102.76 - 27.45 A + 3.81 B + 33.52 C - 41.17 AB + 22..27AC - 24.95 BC + 16.69 A^2 - 7.01B^2 + 4.54C^2

Yield = + 22.31 - 1.87 A + 1.22 B + 0.8975 C + 1.25 AB - 3.52 AC + 8.44 BC 2.80 A^2 + 6.24 B^2 + 5.01 C^2

Result and discussion

Figure 3: Three-dimensional response surfaces plots showing effect of solid/liquid ratio, time of extraction (min) and power (W) using microwave assisted extraction for total phenolic content and extraction yield.

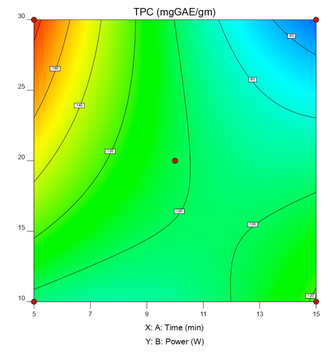


Optimization of MAE Parameters

✓ The optimal conditions for the aqueous extraction of phenolic compounds from olive leaves were proposed to be at 10W power for 10 min time at a sample-to-solvent ratio of 1.5g/40mL solvent.

✓Power and ratio were shown to have a positive influence on the extraction of TPC and yield of biomass.

✓A quadratic regression model was the most appropriate model for understanding the contribution of maximum yield of biomass and TPC.



Observation and Conclusion

✓This study attempted to find the most effective conditions of MAE through Box–Behnken design for high TPC recovery by considering water as green solvent.

✓Results showed that the obtained % yield ranges from 16 to 48 %, range of TPC was found to be from 28.76 to 221.43 mg GAE/g DW.

✓The significance of regression coefficients was statistically examined by analysis of variance (ANOVA). It was analysed that the lack of fit value of the quadratic model was insignificant but the model was significantly fitted.

✓So the second order polynomial model could be used to optimize extraction of phenolic compounds from olive leaves

✓This study provides ideas with scientific basis of utilization of olive leaves as a rich source of phenolic compounds to be extracted using MAE.

Future Plan

✓ Optimization of total flavonoid content and antioxidant activity of olive leaves through Microwave-assisted extraction.

✓ Detection of major bioactive compounds in the optimized extract of *Olea europea* leaves through GC–MS analysis.

References

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✓ Oluwaseun R. A, Abdurahman,N.H., Ukaegbu,C.I., & Kabbashi, N.A., 2019, 'Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave assisted extraction techniques, *Journal of Taibah University for Science*, vol 13 no. 1, pp 414-422.