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Biocatalysis Meets Green Extraction: A Case Study on *Origanum dictamnus* L.

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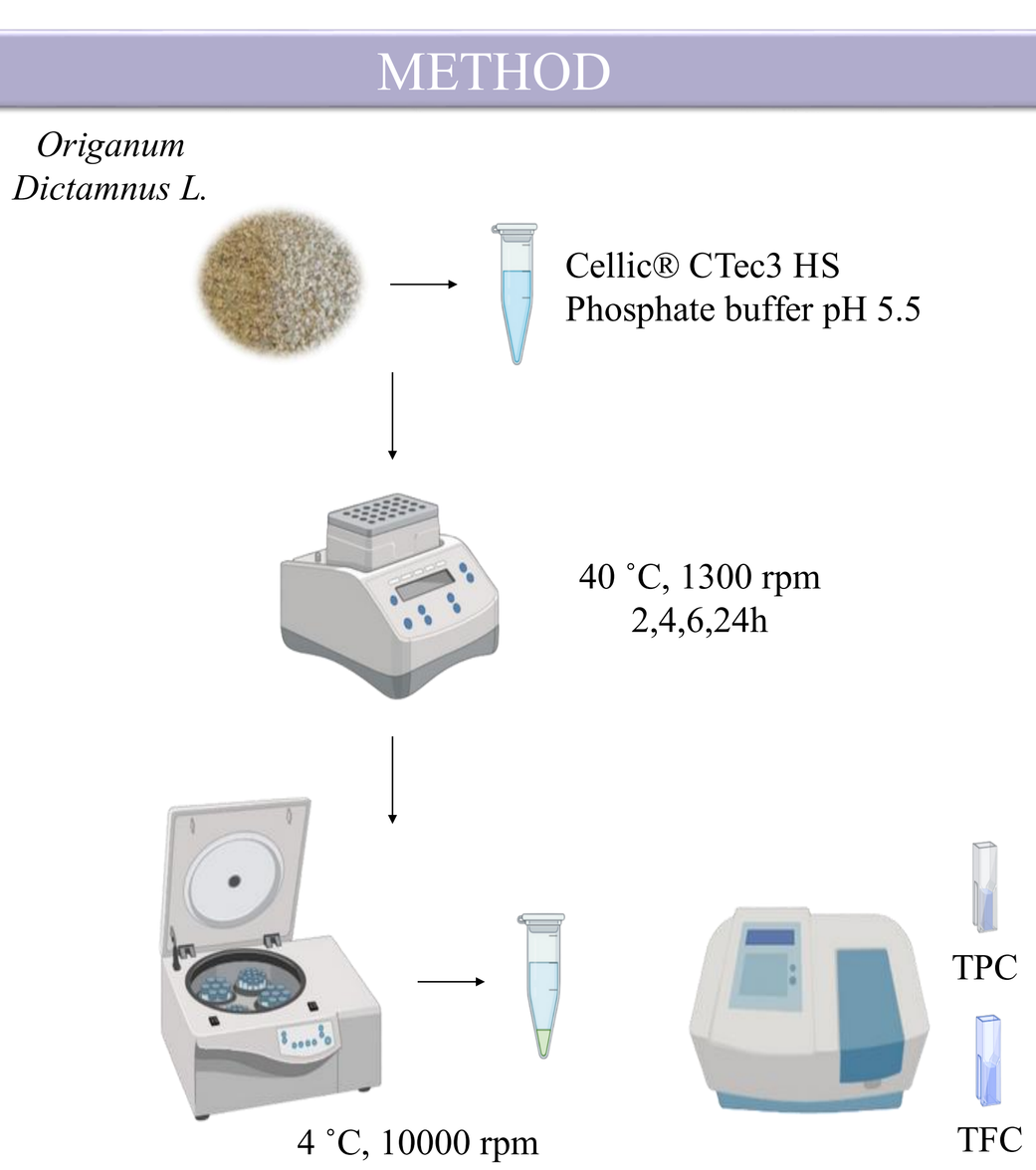
INTRODUCTION & AIM

Origanum dictamnus L. is a medicinal plant known for its rich content in bioactive compounds. The plant cell wall consists of structural polysaccharides such as cellulose, hemicellulose, pectin, along with lignin, proteins and bioactive compounds. These compounds are trapped within the plant cell wall or free in the cytocol of the plant cell. Enzyme-assisted extraction (EAE) is a green technology that relies on the enzymes ability to selectively degrade the plant cell wall, thereby facilitating the release of the bioactive compounds. In the present study, EAE of bioactive compounds from the leaves of *Origanum dictamnus* L. was applied using the commercial enzyme preparation Pectinex® Ultra Color (Novozymes).

A Taguchi experimental design was employed to determine the optimal EAE conditions. The variables were enzyme loading (EL, 50, 100, and 200 U/mg), solid-to-liquid ratio (SLR, 1, 4, and 7% w/v), and extraction time (t, 1, 3, and 6 h). The responses were total phenolic content (TPC) and total flavonoid content (TFC). Kinetic modelling of the extraction process for the optimum extract was carried out using second-order, Peleg's, and power law models. EAE achieved the highest TPC yield 153.4 ± 3.4 mg GAE/g DW and TFC yield 81.3 ± 3.7 mg CAE/g DW at 1% w/v, 200 U/mg, and 1h.

METHOD



RESULTS & DISCUSSION

Table 1. Results of total phenolic content (TPC) and total flavonoid content (TFC) of Taguchi design.

Solid to liquid ratio (% w/v)	Enzyme loading (Units/g)	Extraction time (h)	TPC (mg GAE/g)	TFC (mg CAE/g)
1	50	1	122,0 ± 2,0	63,9 ± 1,6
1	100	3	131,0 ± 6,2	62,1 ± 1,3
1	200	6	124,3 ± 1,3	61,1 ± 7,9
4	50	3	90,5 ± 2,7	76,4 ± 1,4
4	100	6	102,8 ± 2,1	69,7 ± 4,8
4	200	1	106,9 ± 2,6	81,4 ± 2,7
7	50	6	88,6 ± 1,4	86,1 ± 0,7
7	100	1	72,3 ± 3,7	80,6 ± 2,5
7	200	3	85,9 ± 1,0	87,3 ± 1,0

$$TPC\ (mg\ GAE/g) = 128{,}8 - 10{,}8SLR + 0{,}029EL + 1{,}1t + 0{,}4SLR^2 + 0{,}02t^2 \quad (\mathbf{R^2 = 0{,}918})$$

$$TFC\ (mg\ CAE/g) = 68{,}4 + 5{,}7SLR - 0{,}2EL + 0{,}7t - 0{,}2SLR^2 + 0{,}2t^2 \quad (\mathbf{R^2 = 0{,}973})$$

Optimal conditions
Solid to liquid ratio 2,2% w/v,
Enzyme loading 200 Units/g
Extraction time 1h

Optimal yields
highest TPC 106,0 ± 4,8 mg GAE/g, at 1% w/v (100 U/mg, 6h)
highest TFC 83,1 ± 2,9 mg CAE/g, 7% w/v (100 U/mg, 1h)

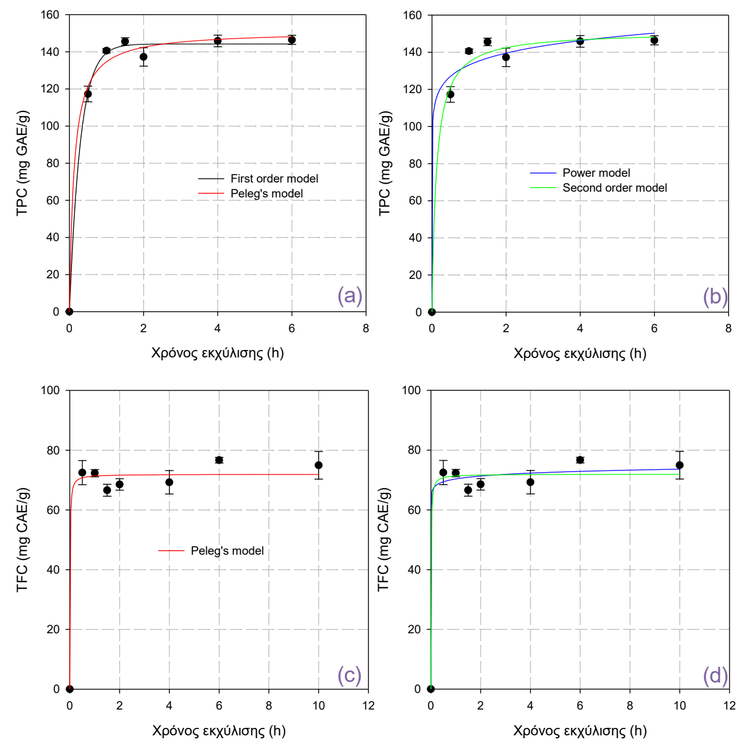


Figure 1. The lines represent the fitting of the experimental data to the indicated kinetic models. (a) & (b) TPC vs. time, (c) & (d) TFC vs. time.

Model		TPC	TFC
Power law	B	133,1	70,4
	n	$6{,}78 \times 10^{-2}$	$1{,}97 \times 10^{-2}$
	R^2	0,986	**0,986**
	Adj – R^2	0,983	**0,983**
	NRMSD	8,94	**8,59**
Peleg's model	k_1	$8{,}00 \times 10^{-4}$	$1{,}00 \times 10^{-4}$
	k_2	$6{,}60 \times 10^{-3}$	$1{,}39 \times 10^{-2}$
	R^2	**0,993**	0,983
	Adj – R^2	**0,992**	0,980
	NRMSD	**6,23**	9,33
2nd order	Cs (mg/g)	151,2	71,9
	k (g/mg.min)	0,05	1,66
	R^2	0,993	0,983
	Adj – R^2	0,992	0,980
	NRMSD	6,23	9,33

Best performing model
TPC: Peleg's model
TFC: Power's law model

CONCLUSION

- The EAE outperformed the conventional ethanol–water extraction up to 20%.
- The TPC and TFC data fitted differently to the kinetic models.
- These findings highlight EAE as an efficient technique with strong potential for scale-up and integration into industrial processes for the production of natural bioactive-rich extracts from *Origanum Dictamnus* L.

FUTURE WORK

- Combination of EAE with other green extraction methods (ultrasound or microwave-assisted extraction to improve efficiency)
- Evaluation of extracts' biological activities, namely antioxidant, antimicrobial, antiaging, antidiabetic, and anticancer
- Life cycle assessment (LCA) of the EAE process to evaluate the environmental impact and sustainability of EAE compared to conventional extraction methods

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