



OPTIMIZATION OF PHENOLIC COMPOUNDS EXTRACTION CONDITIONS FROM LADY'S BEDSTRAW (*GALIUM VERUM L.*) USING HISTORICAL DATA DESIGN



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INTRODUCTION

Lady's bedstraw (*Galium verum L.*) are often used in the traditional medicine of the Balkan countries. In previous studies it has been shown that the main components of *G. verum* extracts, phenols and flavonoids are in the form of heterosides with different saccharides. Also, different types of terpenes, which are the main components of essential oil, are present.

METHODS

Lady's bedstraw (*Galium verum L.*) plant material was collected on the Zlatibor mountain during June and July, when the plant is in the flowering stage. After drying, the plant is crushed in a mill. Thus prepared crude material was used to extract phenolic compounds. For extraction methanol, 96% ethanol and 70% ethanol were used at five time intervals (15, 30, 60, 90 and 120 minutes). Extraction was carried out in conical flasks, on a shaker, at room temperature (25°C). The total phenolic content in the extracts was determined spectrophotometrically, using the method with the Folin–Ciocalteu reagent, and the results were expressed as gallic acid equivalents (GAE – mg of gallic acid/g of crude extract). Total phenolic content in crude *Galium verum L.* extract by solvent and extraction time is presented in Table 1. Historical data design (HDD) in Design Expert 7.0 software was used to identify optimal extraction conditions.

Table 1: Total phenolic content in crude *Galium verum L.* extract by solvent and extraction time

Time (min)	Methanol	96% ethanol	70% ethanol
	M±SD (GAE)	M±SD (GAE)	M±SD (GAE)
15	12,99±0,99	24,41±1,72	33,36±1,94
30	18,65±1,30	38,25±1,37	64,057±1,51
60	25,06±1,69	79,67±2,43	112,36±3,23
90	32,19±2,13	97,76±2,56	141,4±3,06
120	34,88±1,52	100,36±2,65	142,77±3,28

M – Mean, SD – standard deviation, GAE – gallic acid equivalents (mg of gallic acid/g of crude extract)

References:

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RESULTS

ANOVA analysis showed that there is a statistically significant difference in the amount of extracted phenols in between all time intervals except between 90 and 120 minutes. This is the case with all three used solvents. The results of the optimization analysis showed that the highest yield of total phenols (145.78 GAE) would be obtained using 70% ethanol as a solvent in a time of 107.03 minutes (desirability level = 0.996), while the lowest yield was obtained using methanol as a solvent. Equation of model when 70% ethanol is used as a solvent is:

$$\text{Total phenols} = 0.26 + 2.30 * \text{time} + 4.74^{-3} * \text{time}^2 - 3.70^{-5} * \text{time}^3.$$

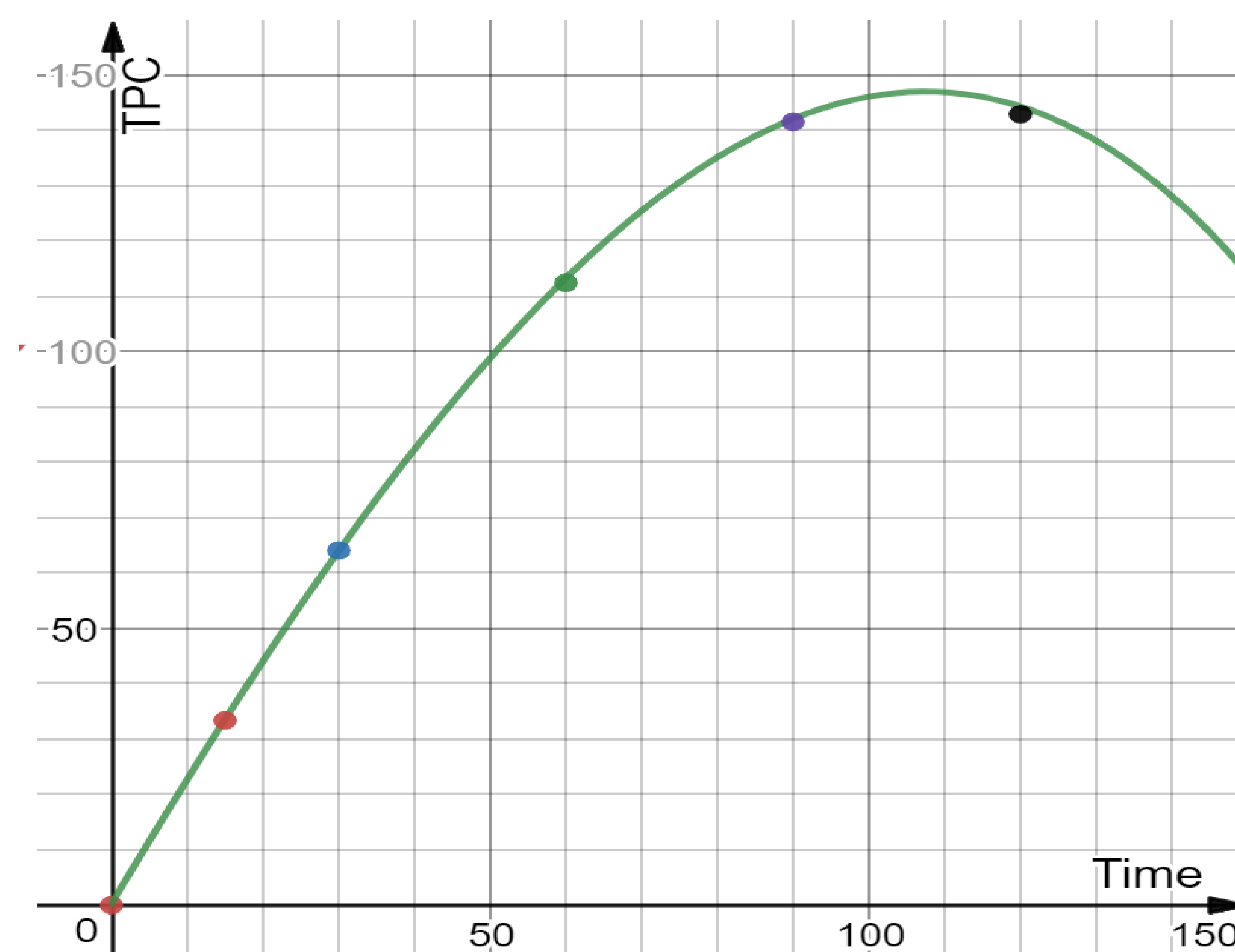


Figure 1: Obtained optimized cubic function (green line) and experimentally measured values of total phenolic content (dots)

CONCLUSION

The experimental values agreed with those predicted, thus indicating suitability of the model employed and the success of HDD in optimizing the extraction conditions.

