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Ultrasonic-assisted extraction of condensed tannin from acron, gland, leaf and gall of oak using response surface methodology

Reza Tabaraki*[1],Alimohammad Safari[2], Ali Yeganeh Faal[3]

[1]*Department of Chemistry, Faculty of Science, Ilam University, Ilam, Iran.*

[2]*Department of Chemistry, University of Payame Noor, Hamedan, Iran.*

[3]*Department of Chemistry, University of Payame Noor, Qom, Iran.*

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Abstract

Ultrasonic technology and response surface methodology (RSM) wereused for optimization of extraction of condensed tannins from the leaf, acron, gland and gall of oak. Three independent variables such as solvent percentage (%), temperature (°C) and time (min) were studied. Effect of methanol concentration was found to be significant on all responses. Optimal ultrasonic-assisted extraction (UAE) conditions were identified as 74–82% methanol, 60°C and45 min.The experimental values agreed with those predicted by RSM models, thus indicating suitability of the models employed and the success of RSM in optimizing the extraction conditions. Condensed tannins can be used as wood adhesives.

Keywords**:** *Ultrasonic, Condensed tannin, Oak, Response surface methodology.*

Introduction

Zagros forests cover 5 million hectares and consist 40% of Iran's forests. These forests consist mainly of *Quercuspersica, Quercusinfectoria, Quercuslibani*. Oaks are one of the important trees, distributed in many regions of temperate zone in the world. They are source of raw materials for some useful products. Oaks contain about 25-28 chemical compounds. These include tannic acid, gallic acid, ellagic acid, mono terpens, p-coumarin, vanillic acid, toluene and kaempferol [1].

Tannins have the ability to precipitate proteins[2], which made them valuable to the leather tanning industry and gave them their name. In fact, the word "tannin" comes from an ancient Celtic word for oak. Tannins, secondary metabolites of higher plants, are oligomeric compounds with multiple structure units with free phenolic groups and molecular weight

***Corresponding author:** Dr. Reza Tabaraki, Department of Chemistry, Faculty of Science, Ilam University, Ilam, Iran, Tel: 98 841 2227022, fax: 98 841 2227022, E-mail address: rezatabaraki@yahoo.com; r.tabaraki@ilam.ac.ir.*

ranging from500 to > 20000 [3]. The main groups of tannins are hydrolyzable tannins and condensed tannins [4, 5]. Condensed tannins (or proanthocyanidins) comprise a group of polyhydroxy-flavan-3-ol oligomers linked by carbon-carbon bonds between flanvanol subunits. Condensed tannins (CTs) are known to inhibit protein digestibility by formingirreversible complexes with proteins, therefore reduced the bioavailability of amino acids.

The reactivity of condensed tannins with molecules of biological significance has important nutritional and physiological consequences [6, 7]. Tannins are used as antihelmintics [8], antioxidants, antimicrobials and antivirals [9-11]. Tannins are involved in defense mechanisms of plants against bacteria, fungi, and herbivores [12]. Different parts of plants such as bark, wood, fruit, pods, leaves, roots, and plant galls contain tannins [13]. Tanninsare used for tanning leather, hardening the fibers of paper, preventing corrosion of fishing nets [14]. Also galls are used for tannin dyeing, and in the manufacture of inks [15].

Up-to now, several extraction techniques havebeen reported for the extraction of tannin like solvent extraction [8], Soxchlet extraction [7]and microwave-assisted extraction [16]. In the most of works, acetone, ethanol, petrol-ether and water was used as extraction solvent. Although studies have been published on the extraction of tannin, these not have evaluated the influence of the extraction variables or interaction of them. The disadvantages of the conventional solvent extraction include long extraction times, and large solvent consumption. With the development of the green chemistry concept during the last years, environment-friendly techniques are becoming more and more attractive. The extraction of bioactive compounds under ultrasound irradiation (20–100 KHZ) is one of the upcoming extraction techniques that can offer high reproducibility in shorter times, simplified manipulation, reduced solvent consumption and temperature and lower energy input [17]. However, the economical feasibility of an industrial process also requires working in such a way that high extraction efficiency is attained. Many factors have been established to influence the extraction efficiency, such as extraction methods, solvent type, solvent concentration, temperature and extraction time [18].

In general, optimization of process could be achieved by either empirical or statistical methods. The empirical method is known as one-factor-at-a-time approach, in which one factor is varying at a time while all other factors are kept constant. Its major disadvantage is that it does not include the interactive effects among the variables studied. Another disadvantage of the one-factor optimization is the increase in the number of experiments necessary to conduct the research. This leads to an increase of time and expenses as well as an increase

in the consumption of reagents and materials. Response surface methodology (RMS) enables evaluation of variables effects and their interactions on response variables. Thus, RSM is a collection of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing processes[19, 20].

The aim of this study was to optimize ultrasonic-assisted extraction variables such as temperature, time and concentration of methanol as modifier for the maximum extraction of condensed tannins from different parts of oak by response surface methodology.

Experimental

Plant material

Quercus samples were collected during the autumn in 2010 at several locations in the Zagros Mountains of Ilam province, Iran, and included leaves, galls, acrons and glands. After drying at room temperature, the samples were ground to pass a 0.4 mm sieve and stored in dark environment at an ambient temperature for further experiments.

Chemicals

Butanol, ferric ammonium sulfate and HCl were purchased from Merck. Catechin was purchased from Sigma–Aldrich.Butanol-HCl reagent (butanol-HCI 95:5 v/v) was prepared by mixing 950 ml n-butanol with 50 ml concentrated HCl (37%). Ferric reagent (2% ferric ammonium sulfate in 2N HCl) was prepared by dissolving2.0 g ferric ammonium sulfate in 2N HCl. This reagent should be stored in a dark bottle.

Extraction procedure

The process of condensed tannin (CT) extraction from acron, leaf, gland and gall by ultrasonic was performed in an ultrasonic bath RK103H (BANDELIN SONOREX, Germany) with a maximum capacity of 4 L (35 KHZ, 140 W). Acron, leaf, gland and gall powders (0.2 g) were sonicated in the solvent (10 ml) for different times at required temperature. After the extraction, the extract centrifuged at 3000 rpm at 4°C for 20 min. The extracts were concentrated by rotary evaporation under vacuum to dryness and the yield of extraction was determined.

Optimization of solvent type and solvent-to-solid ratio

The preliminary experiments were carried out in order to select a suitable solvent and solvent-to-solid ratio. Samples were extracted with ethanol, methanol, acetone, water and n-hexane, respectively, and kept for sonication at 45°C for 20 min. The best solvent was selected according to the values of responses. The influence of the solvent-to-solid ratio on the extraction was investigated by considering four ratios (30:1, 50:1, 70:1, 90:1; v:m). Different weights (0.333, 0.200, 0.143 and 0.111 g) of

each sample (acron, leaf, gland and gall) were sonicated in fixed volume of 10 ml of methanol (50%, v:v) solutions at 45 °C for 20 min.

Condensed tannin content (CT)

The condensed tannin content of the acron, leaf, gland and gall extracts was determined by butanol-HClassay [21]. Five hundred microliters of properly diluted extract solution were mixed with3 ml of butanol-HCl reagent and 100 microliters ferric reagent. The mixture was kept for 1 h at100°C. The solution was cooled to room temperature. Finally, the absorbance was measured at550 nm, using a UV–visible spectrophotometer. A calibration curvewas prepared, using a standard solution of catechin. The results of condensed tannin content were expressed as mg catechin equivalents per g dry weight of acron, leaf, gland and gall.

Experimental design

A three-factor (X_1, X_2, and X_3) and three levels (-1, 0 and +1)central composite design (CCD) was used to achieve maximal information about the process from a minimal number of possible experiments (Table1).The independent variables were methanol percentage(X_1, %), extraction temperature (X_2, °C) and extraction time (X_3,min) while the dependent variables (response variables) were CT (mg catechin/g of acron, leaf, gland and gall of oak).

Table 1. Coded and uncoded levels of the independent variables.

Independent variables	**Code units**	**Coded levels**		
		-1	0	1
Methanol concentration (%)	X_1	50	70	90
Temperature (^{0}C)	X_2	30	45	60
Time (min)	X_3	15	30	45

Each experiment was performed in replicate and the average values were taken as the response, y. Experimental data were fitted to the following second order polynomial model and regression coefficients were obtained. The generalized second-order polynomial model proposed for the response surface analysis was as follows:

$$y = \beta_o \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ij} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \qquad (1)$$

where β_0, β_i, β_{ii} and β_{ij} are regression coefficients for intercept, linear, interaction terms, respectively. $X_i$and $X_j$are coded value of the independent variables while k equals to the number of the tested factors (k = 3).

All experiments were carried out in triplicates and the results were expressed as means ± SD. Statistical analysis was performed using the Minitab 15.1 (Minitab Inc., State College, PA, USA) software and fitted to a second-order polynominal regression model containing the coefficients of linear, quadratic and interaction

terms. An analysis of variance (ANOVA) with 95% confidence level was then carried out for each response variable in order to test the model significance and suitability. The significances of all terms in the polynomial were analyzed statistically by computing the F-value at a probability (p) of 0.1 or 0.05.

Results and discussion

Effect of solvent type and solvent-to-solid ratio on tannin extraction

At the beginning of this study, effects of solvent type and solvent-to-solid ratio were investigated. The selection of solvent can play an important role for extraction of condensed tannins from acron, leaf, gland and gall of oak samples. Researchers have been used hot water, acetone, ethanol and petrol-ether for tannin extraction. As shown in figure 1, methanol was the best solvent for tannin extraction from acron, leaf, gland and gall of oak (20:1(v:m), 45 °C and 20 min). So methanol was chosen as the extraction solvent for the next experiments.

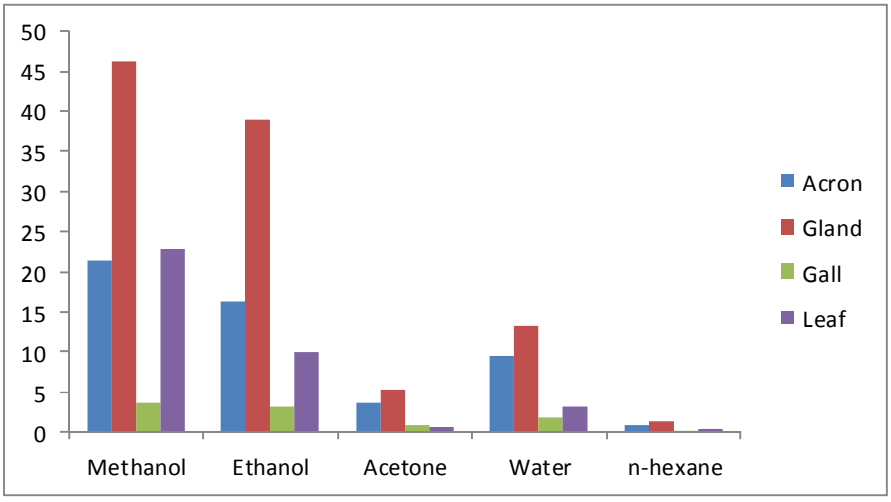

Figure 1. Effect of solvent type on the extraction of condensed tannin from acron, gland, gall and leaf (mg catchin equivalent /g dw).

The effect of the solvent-to-solid ratio on the extraction of condensed tannins from oak samples was also studied with four ratios (30:1, 50:1, 70:1, 90:1; v:m) over a 20 min extraction period, with a 60% methanol solution, at 45 °C. A marked increase was observed up to 50:1 solvent–solid ratio and then decreased until 90:1 (Figure 2). In order to minimize the solvent requirement and without compromising on the responses, solvent–solid ratio of 50:1 (ml:g) was selected.

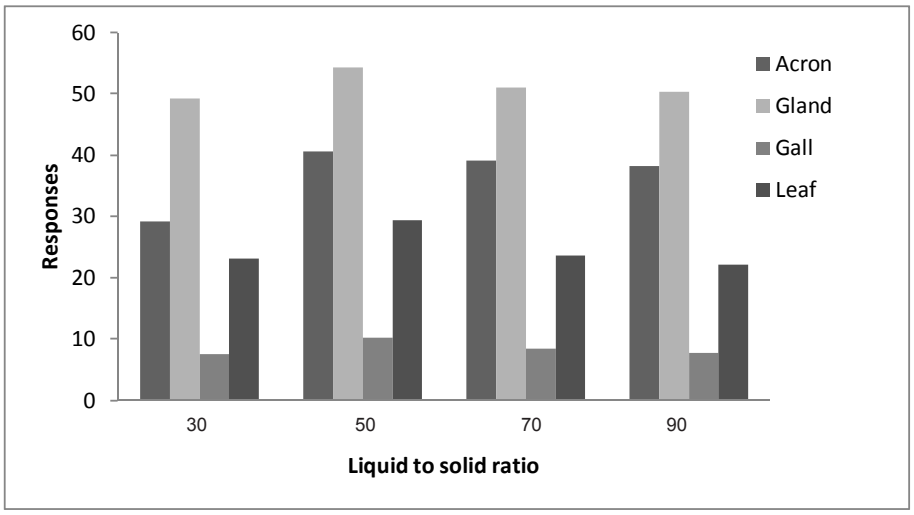

Figure 2. Effect of the liquid-to-solid ratio on the responses(mg catchin equivalent/g dw) at 20 min extraction period and 45 ^{o}C.

Modeling of the extraction process

The responses (condensed tannins content of acron, leaf, gland and gall extract) of each run of the experimental design were presented inTable2. The coded and decoded values of independent variables for each experiment are also presented. Condensed tannins content of leaf, gall, gland and acron extracts varied from 15.52 to 32.98, 1.71 to 11.51, 28.12 to 64.49 and 23.82 to 42.55mg catechin equivalent/g of dry sample, respectively. Extraction yields ranged from 13.3 to 25.3%, 17.3 to 26.7%, 15.2 to 26.1% and 14.3 to 24.7% for leaf, gall, gland and acron, respectively.

Table 2. Central composite design of three variables with their observed responses.

				Condensed tannin				Yield%			
Exp. no	**MOH (%)**	**T (^{0}C)**	**t (min)**	**Leaf**	**Gall**	**Gland**	**Acron**	**Leaf**	**Gall**	**Gland**	**Acron**
1	90	60	45	32.13	11.51	64.49	42.55	25.3	26.7	26.1	24.7
2	90	30	45	30.64	10.3	64.31	36.94	20.8	24	24	22.1
3	50	30	15	15.52	9.16	28.92	23.82	14.9	17.3	17.2	14.3
4	70	30	30	25.25	7.15	42.21	30.18	20.02	22.3	21.9	19.6
5	70	45	30	30.69	10.71	59.85	40.37	21.8	20.1	23.1	20.7
6	50	60	45	22.05	2.34	36.42	25.25	21.1	20.4	22.7	20.9
7	90	30	15	31.21	3.83	54.35	41.8	16.07	19.2	19.31	16.1
8	70	45	15	26.63	6.24	53.89	30.69	21.7	22.3	22.1	24.1
9	70	45	45	25.88	6.98	53.26	29.32	22.6	24.1	23.9	21.9
10	50	60	15	18.78	1.71	28.12	25.25	14.3	20.1	16.9	19.4
11	50	45	30	19.75	2.06	31.55	27.14	13.7	18.8	15.6	18.5
12	90	60	15	32.98	7.78	53.26	42.09	24.1	25.5	25	23.6
13	90	45	30	31.55	7.15	50.17	41.8	22.02	20.3	24.1	22.4
14	50	30	45	19.18	2.8	32.13	23.82	13.3	20.7	15.2	19.2
15	70	45	30	30.01	6.52	48.16	39.91	21.6	22.4	22.9	20.5
16	70	60	30	29.03	7.04	46.96	38.08	24.3	25.7	25.1	23.9

*mg catchin equivalent /g dry weight

Regression coefficients and analysis of variance of the second-order polynomial models for condensed tannins contents and extraction yield are summarized in Tables 3 and 4.The absence of any lack of fit ($p > 0.05$) also strengthened the reliability of all models. The models were used for the construction of three dimensional response surface plots to predict the relationship between independent and dependent variables.

Table 3. Regression coefficients of predicted polynomial models for condensed tannin content.

Coefficients	Condensed tannin			
	Leaf	Gall	Gland	Acron
β_0	-40.24	15.88	-95.12	-5.50
β_1	1.11	0.33	3.15	1.07
β_2	0.31	-0.71	1.48	-0.04
β_3	0.62	-0.56	-1.68	0.30
β_{11}	-0.45×10^{-2}	-0.48×10^{-2}	-0.18×10^{-1}	-0.19×10^{-1}
β_{22}	-0.15×10^{-2}	0.26×10^{-2}	-0.16×10^{-1}	-0.03×10^{-2}
β_{33}	-0.54×10^{-2}	0.05×10^{-2}	0.24×10^{-1}	0.07×10^{-2}
β_{12}	-0.12×10^{-2}	0.54×10^{-2}	-0.18×10^{-2}	0.30×10^{-2}
β_{13}	-0.35×10^{-2}	0.66×10^{-2}	0.40×10^{-2}	-0.18×10^{-2}
β_{23}	-0.04×10^{-2}	0.24×10^{-2}	0.35×10^{-2}	0.13×10^{-2}
Model	*	**	*	*
Linear	*	ns	ns	ns
Quadratic	**	ns	ns	ns
Cross-product	ns	**	ns	ns
Lack of Fit	ns	ns	ns	ns
R^2	0.965	0.810	0.912	0.908

* $p \leq 0.05$.** $p \leq 0.1$.
ns, no significant

Effect of process variables on condensed tannin contents and yield

Condensed tannin contents of extracts obtained by ultrasonic-assisted extraction are shown in Table 2. Regression analysis was performed on the experimental data and the coefficients of models were evaluated for significance. The effect of methanol concentration was significant on the extraction of condensed tannin. Condensed tannin content of extracts gradually mounted up with the increase of methanol concentration and achieved optimum value at about 80%, before it began to decrease. In general, the polarity of methanol–water mixture would decrease continuously with the addition of methanol. Methanol concentration demonstrated a pronounced influence on condensed tannin in linear and quadratic manner (Tables3 and 4). Longer extraction times had positive effects on the condensed tannin. Mild

heating might soften the plant tissue, weaken the cell wall integrity, hydrolyze the bonds of bound compounds as well as enhance solubility, thus more compounds would distribute to the solvent [22].

Table 4. Regression coefficients of predicted polynomial models for yield.

coefficients	Condensed tannin			
	Leaf	Gall	Gland	Acron
β_0	-25.95	-0.27	-19.37	-17.31
β_1	1.52	0.90	1.16	0.64
β_2	-0.59	-0.66	-0.24	0.38
β_3	0.24×10^{-1}	-0.65×10^{-1}	-0.14	0.06
β_{11}	-0.12×10^{-1}	-0.68×10^{-2}	-0.74×10^{-2}	-0.45×10^{-2}
β_{22}	-0.22×10^{-2}	0.76×10^{-2}	0.31×10^{-2}	-0.22×10^{-2}
β_{33}	-0.22×10^{-2}	0.40×10^{-2}	0.08×10^{-2}	0.34×10^{-2}
β_{12}	0.97×10^{-2}	-0.37×10^{-2}	0.02×10^{-2}	0.14×10^{-2}
β_{13}	-0.72×10^{-2}	0.96×10^{-3}	0.08×10^{-2}	0.03×10^{-2}
β_{23}	0.13×10^{-1}	-0.37×10^{-2}	0.23×10^{-2}	-0.46×10^{-2}
Model	*	*	*	*
Linear	ns	*	Ns	ns
Quadratic	*	*	Ns	ns
Cross-product	*	ns	Ns	ns
Lack of Fit	ns	ns	Ns	ns
R^2	0.922	0.931	0.895	0.861

* $p \leq 0.05$. ** $p \leq 0.1$.
ns, no significant.

The yields of extractions are presented in Table 2. Solvent was removed from the extracts by evaporation under vacuum at 40 °C by a rotary evaporator. The regression analysis of the data showed that the extraction yield was significantly affected by the methanol concentration and extraction time and temperature. The relationship of the extraction yield and process variables is depicted in Figure 3. Increase of each variable improved the extraction yield.

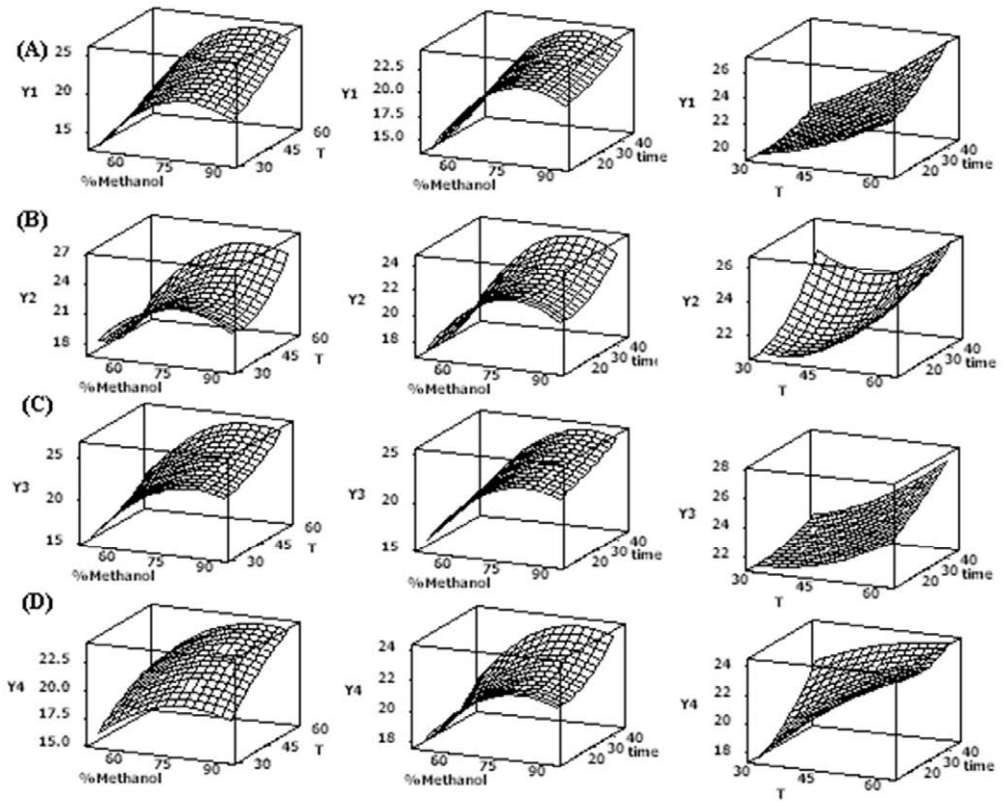

Figure 3. Response surface plots for the effect of MeOH/temperature, MeOH/time and temperature/time on the yield (%)of condensed tannin from A) leaf, B) gall, C) gland and D) acron.

Table 5indicates the optimum UAE conditions for the condensed tannins contents of acron, leaf, gland and gall and yield of extraction using response surface methodology. Temperature of 60°C, extraction time of 45 min and concentrations 74–82% of methanol as modifier were optimal conditions. The predicted results matched well with the experimental results and validated the RSM models.

Conclusions

In this study, ultrasonic technology was used for extraction of the condensed tannins from oak. Response surface methodology (RSM) was used to optimize the experimental variables such as methanol concentration (% v/v), temperature (°C) and time (min). The application of ultrasound-assisted extraction (UAE) offers many advantages including the reduction of solvents, temperature and time. Therefore, ultrasound-assisted extraction of condensed tannins from acron, leaf, gland and gall of oakis an environment-friendly or green process for the preparation of condensed tannins. Condensed tannins can be used as wood adhesives [23].

Table 5. Estimated optimum conditions, predicted and experimental values of responses under these conditions.

Response variables	Optimum UAE conditions			Maximum values	
	MeOH(%)	T(^{0}C)	t (min)	Predicted	Actual
CT* (leaf)	90	60	26	33.59	33.23
CT (gall)	90	60	45	11.64	11.51
CT (gland)	90	46	45	65.80	64.46
CT (acron)	70	60	45	47.04	42.50
Yield % (leaf)	74	60	45	28.3	25.1
Yield % (gall)	81	60	45	27.3	26.5
Yield % (gland)	82	60	45	28.2	26.4
Yield % (acron)	82	60	45	24.8	24.6

*CT, condensed tannin content (mg catchin equivalent /g dry weight).

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