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# INFLUENCE OF THE TEMPERATURE AND THE DURATION OF EXTRACTION ON THE COMPOSITION AND THE PROPERTIES OF EXTRACTS FROM *ALCHEMILLA MOLLIS*

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***Abstract***
*The influence of the temperature and the duration of the extraction on the composition and the properties of extracts of Alchemilla mollis were investigated. The extraction of lady's mantle (A. mollis) was carried out with 20 % (v/v) aqueous ethanol solution, at a temperature from 15 to 90 °C and duration from 1 to 72 hours. The raising of the extraction temperature was accompanied by an increase in the concentration of total phenolic compounds, phenolic acids, flavonoids, tannins and total extractable compounds in the extracts. The increase of the process duration more than one hour led to a reduction in the amount of the extracted tannins.*

***Key words:*** *Alchemilla mollis, extracts, time, temperature, phenolics*

## Introduction

*Alchemilla mollis* (Buser) Rothm. (Rosaceae) is a herbaceous perennial plant that grows in the mountainous regions of the Balkan Peninsula (Romania, Greece and Bulgaria) [10]. The genus *Alchemilla* (Rosaceae) is represented in the Bulgarian flora by 35 species, which include *A. vulgaris L., A. mollis, A. achtarowii, A. bundericensis*, etc [3, 16].

*Alchemilla* is an aggregate of species collectively referred as Lady's mantle, all possessing similar medicinal properties: a mild astringent, anti-inflammatory, diuretic, menstrual cycle regulator, a treatment for digestive disorders and a relaxant for muscular spasms [2, 8]. Different studies showed that the phenolic compounds (tannins, flavonoids, etc.) presented in the plant are responsible for the pharmacological activity of the Lady's mantle [6, 9, 11, 14].

The studies on *Alchemilla* species were mainly associated with isolation and identification of the compounds in the drug [3, 4, 5, 13, 15, 16, 17] and botanical surveys [19, 20, 21]. There is limited data in the literature concerning the investigations of the different methods for extraction of the biologically active compounds from *A. mollis* [18]. The high antioxidant activity and the significant content of phenolic compounds in *Alchemilla* species make them suitable for application in functional foods and beverages production. There is absence of data on the use of the Lady's Mantle extracts in the food industry. An effective method for extraction of the biologically active compounds from *Alchemilla* species and application of extracts in the functional beverages is important to be developed.

Consequently, the aim of the present work is to study the influence of the temperature and the duration of extraction on the composition and the properties of extracts of *Alchemilla mollis*.

## Materials and methods

The experiments were carried out with whole dry stalks from lady's mantle (*A. mollis*), vintage 2014, which were obtained from the experimental station of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences. The raw material was collected during the mass

flowering. The dried drug was milled to a particle size below 1 mm.

The solvent was prepared with deionized water and ethyl alcohol. Caffeic and gallic acid were supplied by Merck - Germany. Albumin from bovine serum was purchased from Fluka - Germany. Quercetin was supplied by Alfa Aesar USA. All others were analytical reagents.

The extraction was made by maceration for 1, 3, 6, 24, 48 and 72 hours at 15 °C and 30 °C and 1, 3 and 6 hours at 60°C and 90 °C. The aqueous ethanol solution with concentration 20 % (v/v) and 1:10 solid/solvent ratio were used. After the drug separation, the extracts were filtered through Machinery and Nagel filter paper. Each variant was carried out in duplicate.

The extracts were analyzed for total phenolic compounds (expressed as gallic acid in g/dm$^3$), phenolic acids (expressed as caffeic acid in g/dm$^3$) and flavonoid phenolic compounds (expressed as qercetin in g/dm$^3$) according to the method of Glories, modified by Mazza et al. [12] total extractable compounds and ash according to the weight method [1]; tannins, as catechins in g/dm$^3$ – spectrophotometric method after precipitation with protein according to the method of Hagerman and Butler [7].

## Results and discussion

The total phenolic compounds at a various time - temperature regimes (Figure 1) ranged between 6,35 and 7,48 g/dm$^3$ at 15 °C, from 7,91 to 8,45 g/dm$^3$ at 30 °C, from 8,17 to 9,65 g/dm$^3$ at 60 °C and from 10,27 to 10,38 g/dm$^3$ at 90 °C. The temperature increase from 15 °C to 30 °C led to an increase in the amount of total phenolic compounds between 6 and 28%. In the range from 30 °C to 60 °C the increase was between 4 and 15%, and in the range from 60 °C to 90 °C it was between 7 and 27%.

The content of phenolic acids (Figure 2) derived from *Alchemilla mollis* by extraction at different time and temperature, ranged between 1,28 and 1,59 g/dm$^3$ at a temperature of 15 °C; 1,56 and 1,73 g/dm$^3$ at a temperature of 30 °C; 1,63 and 1,95 g/dm$^3$ at 60 °C and from 2,10 to 2,16 g/dm$^3$ at 90 °C. The temperature increase from 15 °C to 30 °C led to an increase of the amount of the phenolic acids between 19 and 29%. Only at the first hour, the growth was negligible. In the range of 30 °C - 60 °C the increase was less (up to 13 %) and in the range of 60 °C - 90 °C it was between 8 and 29 %.

A similar trend was also observed in the flavonoid phenolic compounds (Figure 2). The temperature raise from 15 °C to 30 °C led to their increase between 24 and 34 %, except for the extraction for an hour, where the rise was negligible (3%). In the range of 30 °C - 60 °C the enhancement was 12 – 20 %. The increase of flavonoid phenols was more significant in the range of 60 °C - 90 °C (24 – 45 %). Their concentrations varied between 1,33 and 1,47

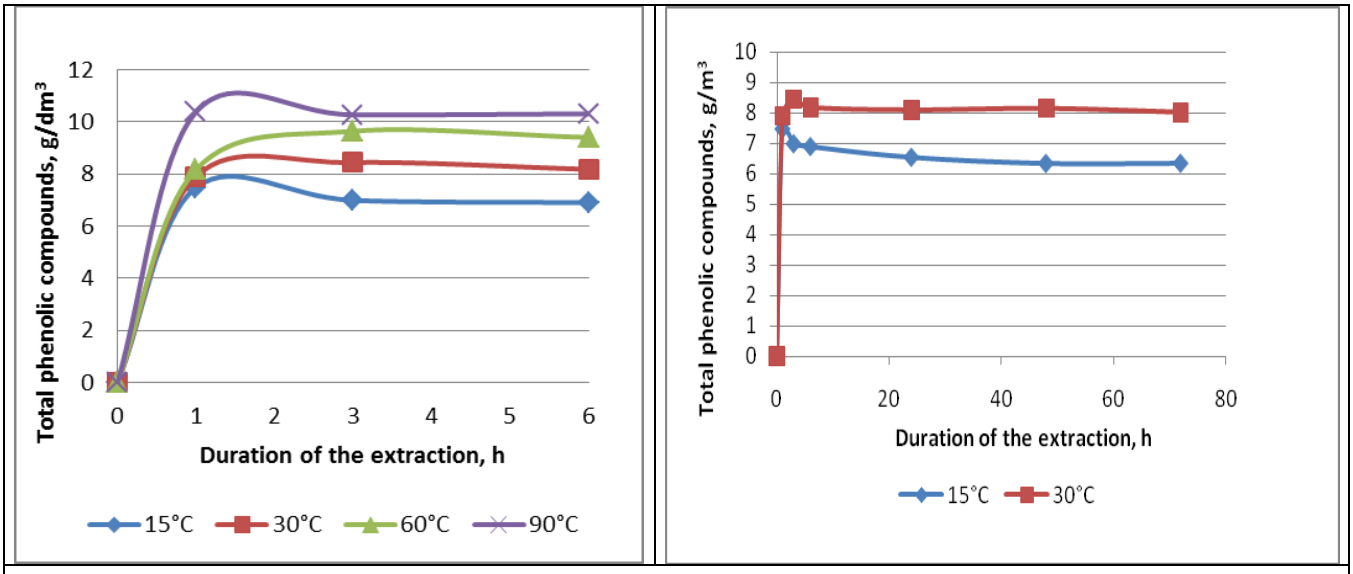


**Figure 1. Influence of the time and the temperature of extraction on the content of total phenolic compounds in extracts of *Alchemilla mollis***

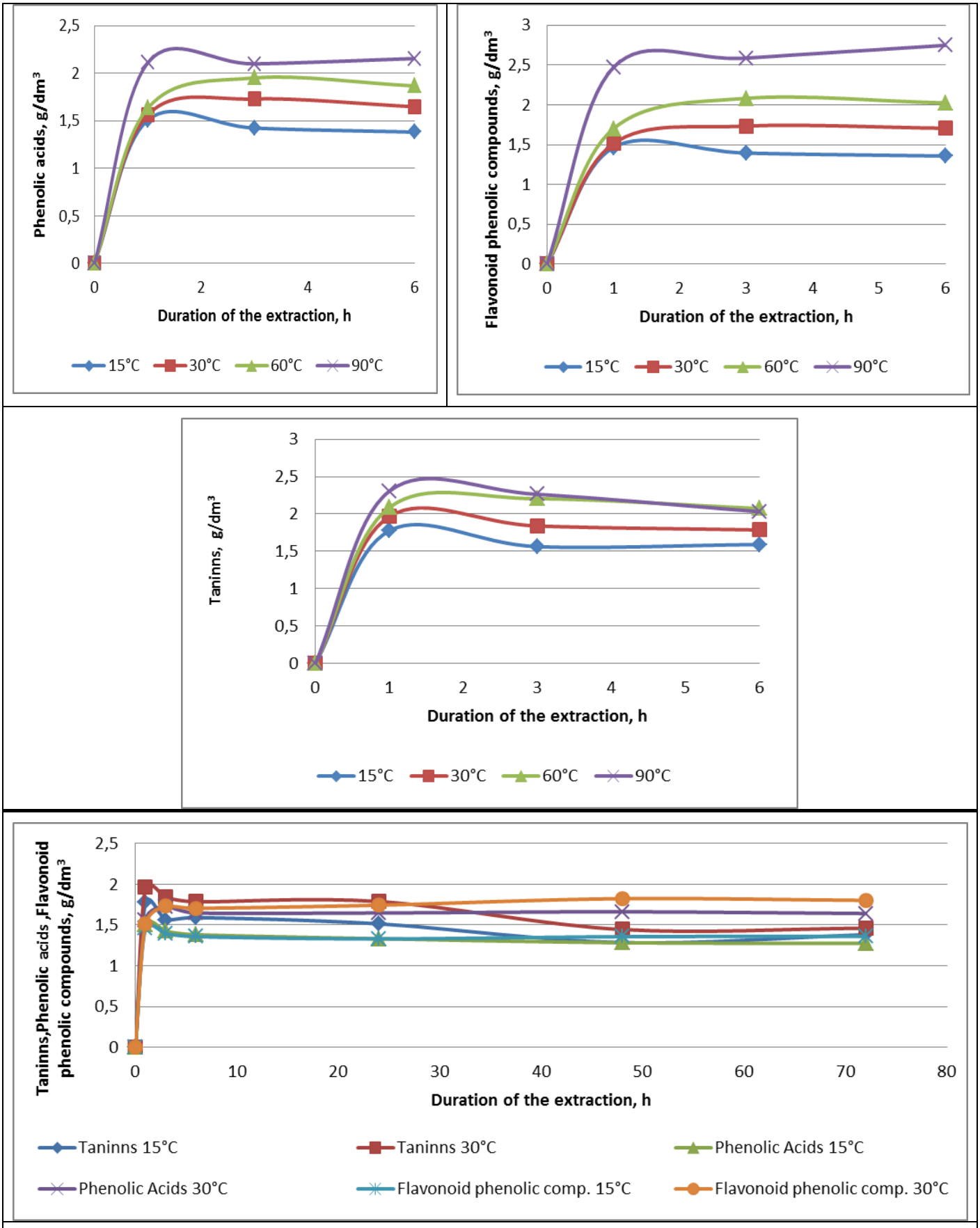


**Figure 2. Influence of the time and the temperature of extraction on the content of phenolic acids, flavonoids phenolic compounds and tannins in extracts of *Alchemilla mollis***

g/dm$^3$ at 15 °C; from 1,51 to 1,82 g/dm$^3$ at 30 °C; from 1,70 to 2,08 g/dm$^3$ at 60 °C and from 2,47 to 2,75 g/dm$^3$ at 90 °C.

The tannins content derived from *Alchemilla mollis* by extraction at a different time and temperature (Figure 2), ranged from 1,28 to 1,78 g/dm$^3$ at a temperature of 15 °C; from 1,45 to

1,97 g/dm$^3$ at 30 °C; from 2,07 to 2,20 g/dm$^3$ at 60 °C and from 2,03 to 2,30 g/dm$^3$ at 90 °C. The data showed that the amount of tannins was increased between 12 and 18 %, when the temperature was raised from 15 °C to 30 °C.

The enhancement of temperature from 30 °C to 60 °C led to 16 – 20 % growth of the tannins. Less growth (2 – 10 %) was observed when the temperature was raised from 60 °C to 90 °C.

The temperature enhancement led to an increase not only of the phenol compounds, but also of the total extractable compounds (Figure 3). The total extractable compounds varied between 26,0 and 28,0 g/dm$^3$ at 15 °C; 28,4 and 30,6 g/dm$^3$ at 30 °C; 29,2 and 31,7 g/dm$^3$ at 60 °C and from 33,1 to 36,2 g/dm$^3$ at 90 °C. The enhancement of temperature from 15 °C to 30 °C was accompanied by an increase in the total extractable compounds between 8 and 10 %. In the range of 30 °C - 60 °C the growth was minimal (5 - 7 %). A substantial increase (15 – 20 %) was observed in the range of 60 °C - 90 °C.

The amount of mineral elements (ash) in the *Alchemilla mollis* extracts (Figure 3) rose slightly with the increasing of the extraction temperature. The values of the ash varied between 3,71 and 3,86 g/dm$^3$ at a temperature of 15 °C; from 3,76 to 3,96 g/dm$^3$ at 30 °C; from 3,74 to 4,05 g/dm$^3$ at 60 °C and from 4,07 to 4,73 g/dm$^3$ at 90 °C.

It was found that for all investigated compounds, the extraction rate was the greatest during the first hour (Figure 1, Figure 2 and Figure 3). The enhancement of the extraction time over one hour was accompanied by minor changes in the concentration of total extractable compounds and mineral components (ash). The coefficient of variation between the different variants was commensurate with this of the experiment replica. A similar trend was observed for the total phenolic compounds, phenolic acids and flavonoid phenolic compounds. The equilibrium state was reached between the first and third hour of the extraction. After that no significant changes in

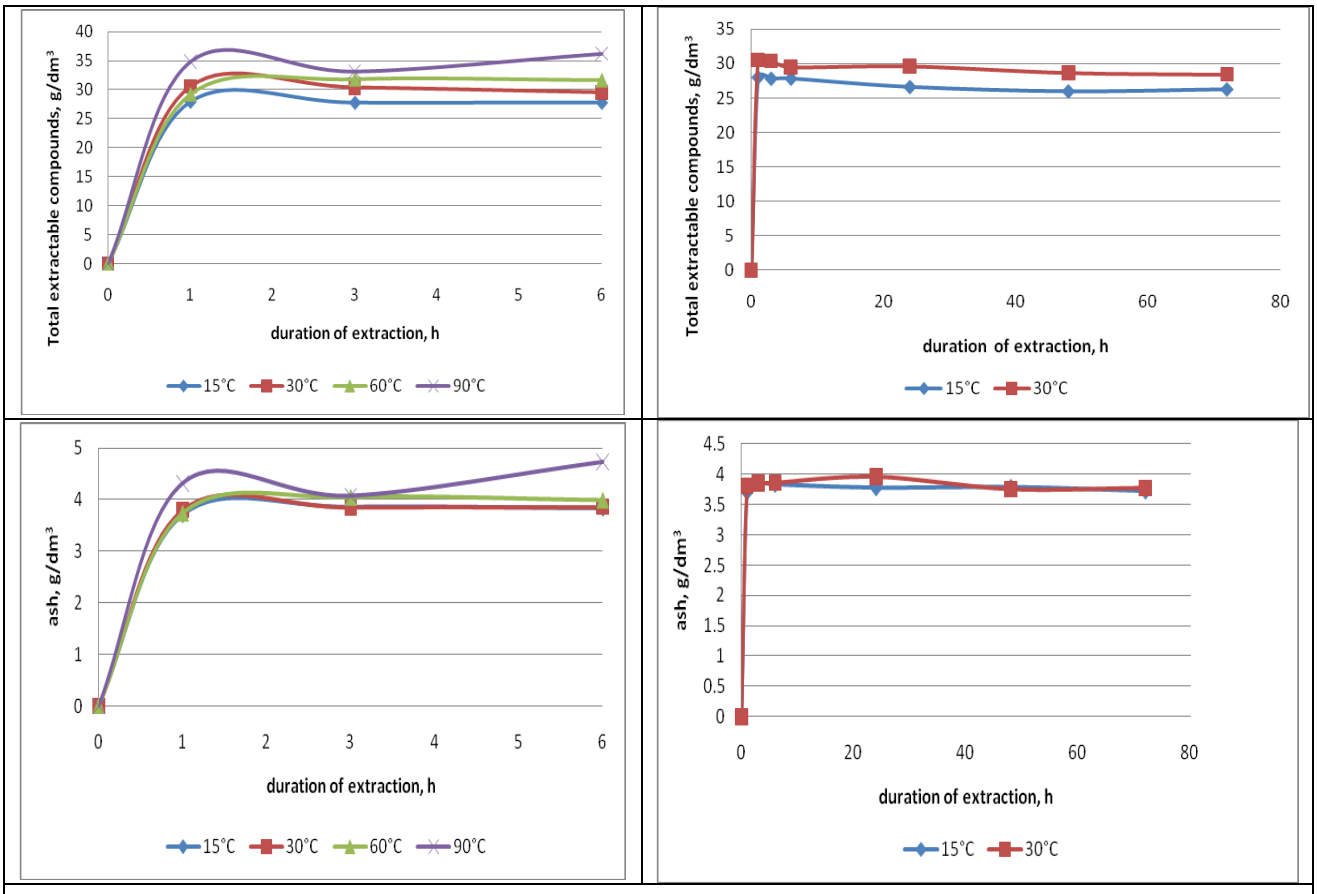


**Figure 3. Influence of the time and the temperature of extraction on the content of total extractable compounds and mineral elements (ash) in extracts of *Alchemilla mollis***

the concentration of the phenolic compounds were observed.

A different trend was found between the quantity of tannins and the extraction time. The duration of the extraction over one hour was accompanied by a reduction of the tannins in the tested samples. This reduction was more noticeable after the twenty fourth hour.

As it is known, the temperature is a factor that significantly influences the plant materials extraction. The higher temperature is accompanied by a greater extextactivity of the various components, due to the improved solubility and mass transfer. Moreover, the viscosity and the surface tension of the solvent are decreased when the temperature increases and the solvent reaches more easily to the plant tissues, improving the rate of extraction. The obtained results in the present study are fully consistent with mentioned above.

It is important to note that certain phenolic compounds can be easily hydrolyzed and oxidized. The long extraction time and the high extraction temperature increase the ability of the phenolic compounds to be oxidized, which reduces their quantity in the extract. Moreover, the phenolic compounds react with other components derived from the plant materials, such as proteins, polysaccharides and certain metals. As a consequence of these reactions, the compounds with low solubility are formed. That is another reason for the loss of already extracted compounds, including phenolics. Probably, such processes caused the observed trend of tannins reduction at a longer extraction time.

The organoleptic analysis of the extracts diluted with water, showed that even at high extraction temperatures (60 and 90 °C), the samples had good organoleptic profile. It is important to note that the variants obtained at both temperatures (60 and 90 °C) for 6 hours extraction time had a bit rough taste. Based on this it can be concluded that the combination of high temperature and long extraction period is not desirable.

## Conclusion

The present work studied the influence of the temperature and the extraction duration on the composition and the properties of extracts from *Alchemilla mollis*.

It was found that at a temperature between 15 °C and 90 °C, the highest extraction rate was observed at the first hour of process. The raise of extraction time over an hour was accompanied by minor changes in the concentration of total extractable compounds and mineral components (ash). The amount of tannins was decreased when the extraction time was over one hour.

The values of all investigated compounds increased when the temperature was raised between 15 and 90 °C. The ash content was an exception. It did not change substantially.

The present study gave basic information and opportunity to optimize the time-temperature regime of extraction of Lady's mantle (*A. mollis*) using response surface methodology.

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