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Comparison of Different Extracts of Nettle in Quercetin and Evaluation of its Antimicrobial Activity

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ABSTRACT

Nettle (*Urtica dioica L.*) is one of the most well-known medicinal plants. It has been mostly used for medical purposes. This study summarizes extensive results on the antimicrobial effect of *Urtica dioica L.* The aim of this study was to investigate several extraction methods of nettle extract for more access to flavonoid materials especially quercetin. For this purpose, three methods (soaking, sonication, soxhlet) and three solvents (chloroform, methanol, deionized water) were used for extraction. Finally, the highest amount of quercetin determined by HPLC was sonication for 90 min with methanol solvent. The effect of this extract was investigated on several pathogenic microbes including *Escherichia coli, Entrecoccus faecalis, Klebsiella pneumoniae, Acinetobacter calcoaceticus, Candida albicans*. Antibiogram testing revealed that *Escherichia coli* and *Candida albicans* were resistant to nettle extract, *Acinetobacter calcoaceticus* and *Klebsiella pneumonia,* were sensitive and *Enterococcus faecalis* was semi-susceptible.

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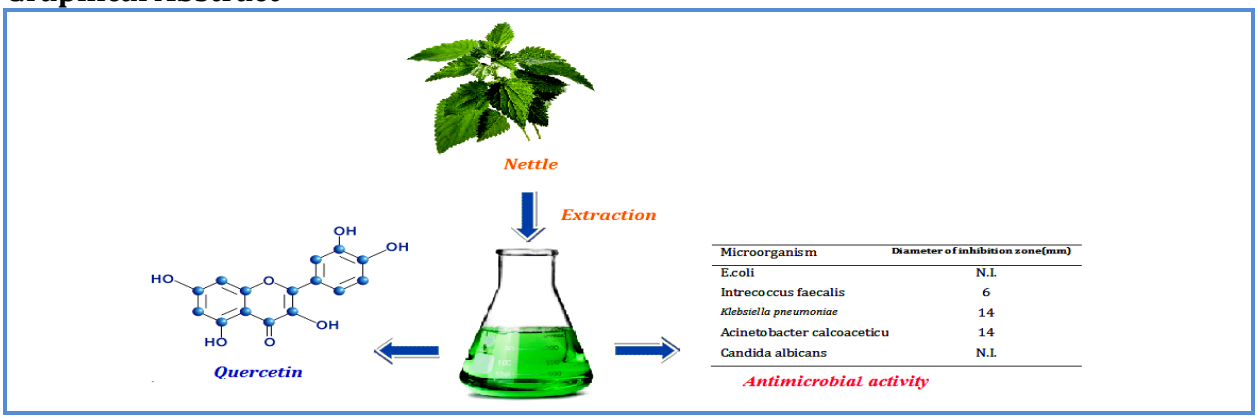
Graphical Abstract



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Introduction

The genus *Urtica* is a member of the Urticaceae family, belonging to main group of angiosperms. The main members of this species are nettle *Urtica dioica L.* and the small nettle *U. urens L.,* which are generally native to Europe, Africa, Asia and North America [1]. It is also traditionally used as a medicine in West Asia [2]. Nettle (*Urtica dioic*) has many therapeutic properties and its extract has been traditionally used in the world for hundreds of years to treat diseases such as eczema, digestion (digestive disorders), joints pain and anemia [3]. Nettle is a very effective nutrient that is easily digestible and high in minerals such as iron and vitamins such as C and A [4]. Nettle and its extract are used with antioxidant properties to improve animal performance and quality of meat products [5]. The healing properties of many plant spices and herbs are due to the presence of antioxidant and antimicrobial compounds in their tissues. Antioxidants such as flavonoids, phenolic acids, and diterpenes can be used to counteract the harmful effects of free radicals [6]. Infectious and inflammatory diseases are currently one of the leading causes of mortality in developing countries and around the world and are the second leading cause of death after heart disease. The emergence of new pathogens with the potential of rapid global expansion coupled with antibiotic resistance worsens this problem [7] and increases the need to look for new bioactive agents. Despite the many drugs available against all of these diseases, unfortunately due to their significant negative side effects, their use in the population is limited [8, 9].

Today, antibiotic resistance among pathogenic bacteria has increased due to the overuse of antimicrobial drugs for the treatment of diseases, especially urinary tract infections. Therefore, the use of herbs as antibacterial drugs is very important [10]. Urinary tract infection (UTI) is one of the most common and prevalent infections of any age [11] and is the second most common infection after upper respiratory tract infection. *Escherichia coli, Proteusvulgaris, Klebsiella pneumoniae, Staphylococci, Enterobacter, Citrobacter* and *Pseudomonas aeruginosa* are major UTI-producing bacteria [12]. Antibiotics are commonly used to treat the UTI however, due to the increased antibiotic resistance in pathogenic bacteria, the treatment has become difficult [12-14]. Fresh and dried nettle leaves can be used to treat UTI [15-17]. This plant contains compounds such as chlorophyll, vitamins (C, K, B1, B2), pantothenic acid, carotenoids, proteins, tannins, essential oils, minerals (iron, manganese, copper and nickel), acetylcholine, histamine, flavonoids [18] phenolic acids and alkaloids [19] which are located in different parts of it. Antioxidant, antibacterial and anti-inflammatory properties are the most important biological functions of phenolic compounds and flavonoids [20, 21].

Experimental

Materials and methods

Fresh nettle (*Urtica dioica*) leaves were obtained from the markets of Shahrekord (Iran), then dried and powdered.

Extract preparation

Three methods including soaking, ultrasonic, soxhlet and three solvents including chloroform (Merck-Germany), 80% methanol (Merck-Germany), deionized water, were used in this study. Nettle leaf powder in each solvent was mixed separately at a ratio of 1:6 ratio and then samples were prepared for each extraction operation.

Soaking extract

5 grams of nettle leaf powder was soaked in 30 mL of chloroform, 80% methanol, and deionized water at a ratio of 1:6. The specimens were then kept in sealed containers for 24 h at room temperature. After 24 h, the samples were filtered by whatman filter paper and stored at 4 °C [22].

Ultrasonic extract

15 grams of nettle leaf powder was soaked in 90 mL of chloroform, 80% methanol, and deionized water at a ratio of 1:6, and the samples were then placed in an ultrasonic bath at 40 kHz for 30, 60, 90 min. Then, the samples were filtered with whatman filter paper and stored at 4 °C [22].

Soxhlet extract

5 grams of nettle leaf powder was soaked in 30 mL of chloroform, 80% methanol, and deionized water at a ratio of 1:6, and the samples were individually immersed in soxhlet and subjected to six extraction cycles. The samples were then stored at 4 °C [23].

Determination of flavonoid content (Quercetin) of nettle by high performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) (SY-8100) with C18 column (COSMOSILMS-II; 4.6/250 mm; Nacalai Tesque Co. Kyoto, Japan) at alow ratio 0.9 mL/min was used to determine and compare the flavonoid content of the extracts. The mobile phase consisted of 50% acetonitrile (Merck-Germany) and 50% methanol (Merck-Germany) and the wavelength used was 265 nm. The quercetin standard ($C_{15}H_{10}O_7$) standard (Merck-Germany, ≥95% (HPLC), solid) was used to determine the retention time.

Antimicrobial test

The microorganisms used in this study were: *Escherichia coli* (PTCC 1338), *Candida albicans* (PTCC 5027), *Klebsiella pneumoniae* (PTCC 1290), *Enterococcus faecalis* (PTCC 1237), *Acinetobacter calcoaceticus* (PTCC 1318), which was purchased from the microbial collection of Iranian Research Organization for Science and Technology (IROST, Karaj, Iran).

A suspension was prepared from 24 hour culturing of each microorganism in normal saline, containing 1.5×10^8 CFU/mL bacteria and fungi and in accordance with the 0.5 McFarland standard. Then, based on the CLSI (2014) protocol [24], the standard diffusion disk method was used. The bacteria were cultured in Mueller-Hinton agar (Merck-Germany) and the fungus in Sabouraud dextrose agar (Merck-Germany). Cellulose discs (6 mm) were impregnated with nettle extract (6 mg/mL) and placed in the culture medium. Bacteria were incubated at 37 °C for 24 h and *Candida albicans* was incubated at 25 °C for 48 h. Gentamicin (120 µg) (Mast) for *Enterococcus faecalis*, Gentamicin (10 µg) and Cefepime (30 µg) (Rosco) for other bacteria, Fluconazole (25 µg) (Rosco) for *Candida albicans* and quercetin standard were used as positive controls. Disks impregnated with 85% methanol were used as a negative control.

Results and discussion

The flavonoid content (Quercetin) of the extracts prepared from three methods, including succulent, ultrasonic and soaking with three different solvents, was analyzed by high performance liquid chromatography (HPLC), the results of which are described below. Quercetin standard was used as control. As seen in Figure 1, the retention time in HPLC for the quercetin standard was between 3–4 min.

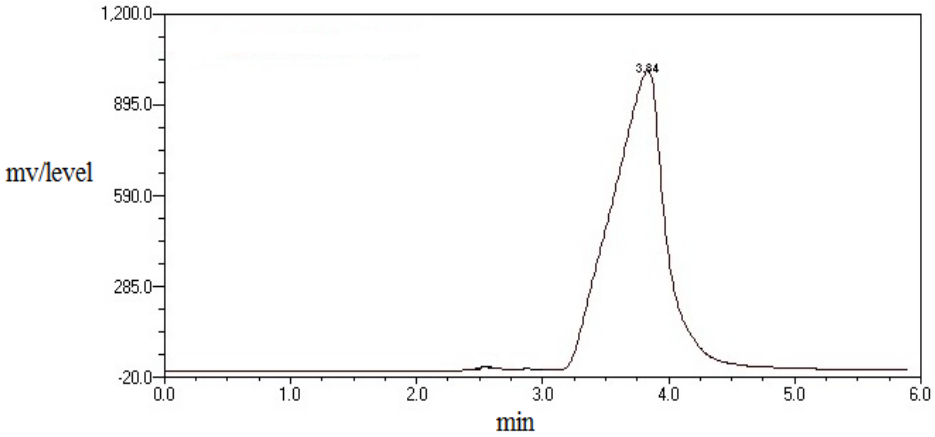

Figure 1. High performance liquid chromatography chart of the quercetin standard

The HPLC results of the soaking method, with three solvents (methanol, chloroform and deionized water), are shown in Figure 2. The highest amount of the quercetin was observed in methanol and the lowest in chloroform solvents.

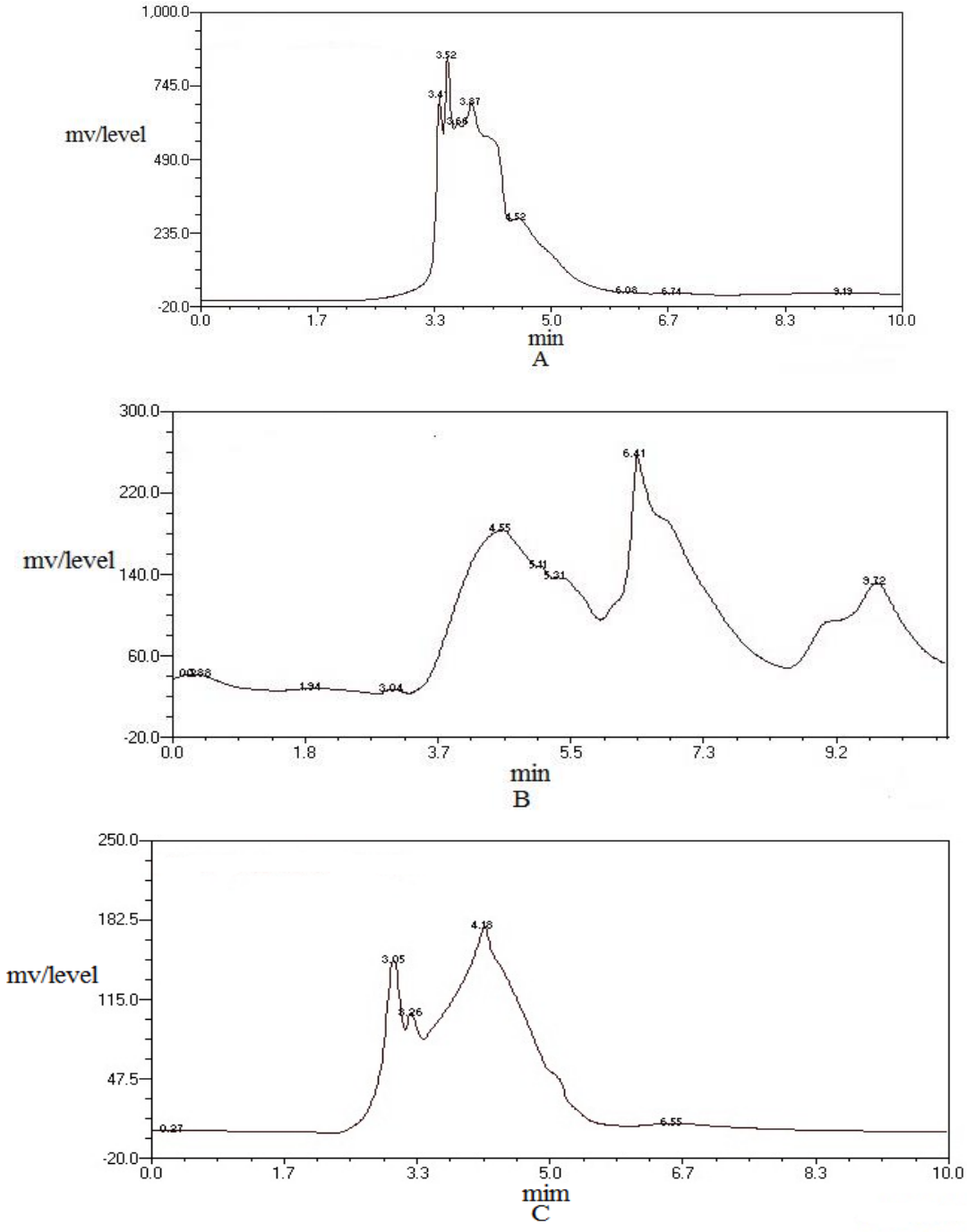

Figure 2. High performance liquid chromatography of extraction by soaking method: A: with methanol solvent, B: with chloroform solvent, C: with water solvent

HPLC results were obtained by ultrasonic extraction with three solvents of chloroform and deionized water and methanol for each solvent at three different times (30, 60 and 90 min). For water solvent the highest amount of quercetin was at 60 min, for methanol solvent was highest at 90 min and for chloroform solvent at 90 min. In general, the highest amount of quercetin was observed in the methanol solvent at 90 min and the lowest in chloroform at 30 min. The results are shown in Figures 3-5.

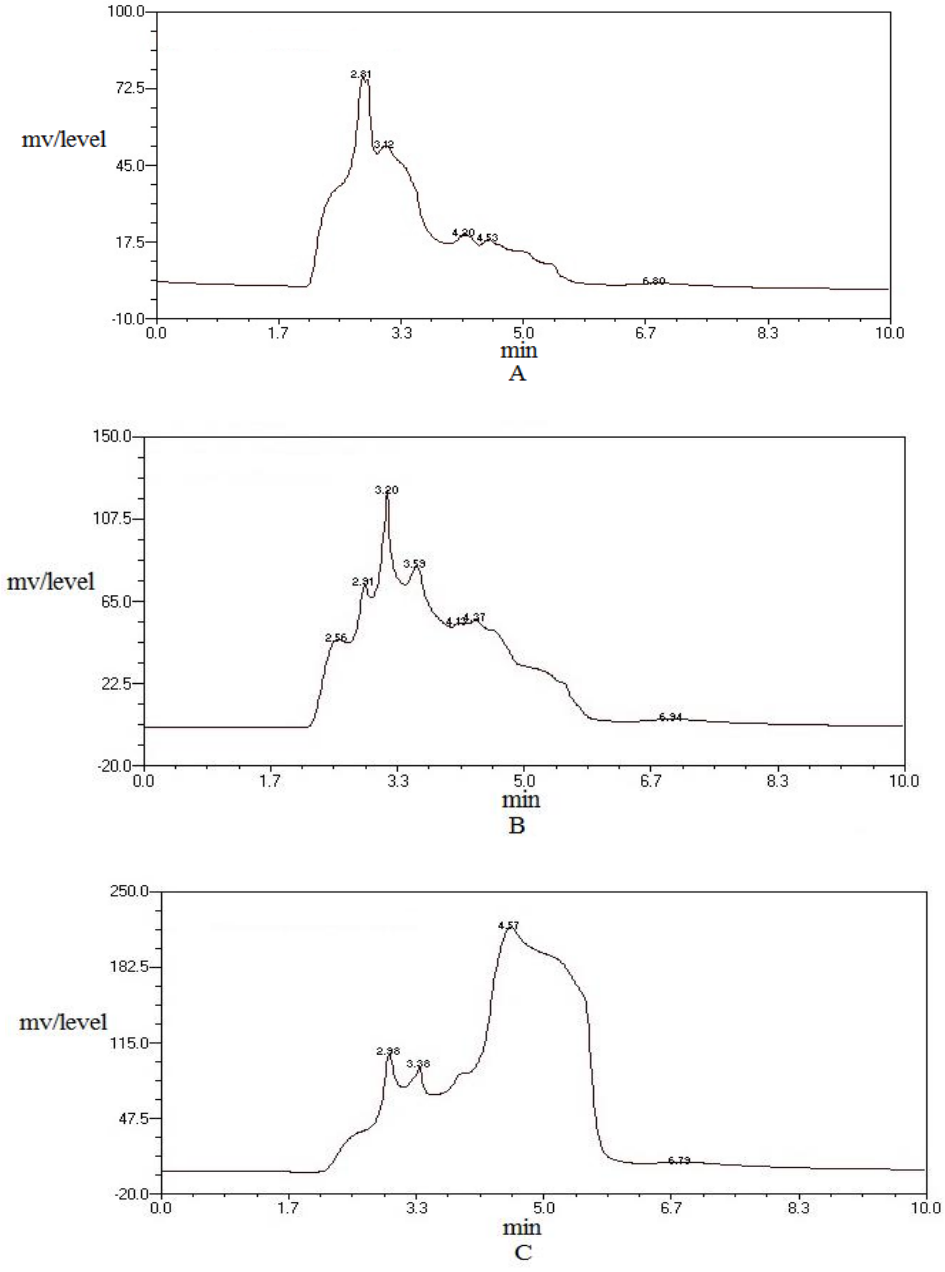

Figure 3. HPLC results by ultrasonic extraction with deionized water solvent for three different durations. A: 30 min, B: 60 min, C: 90 min

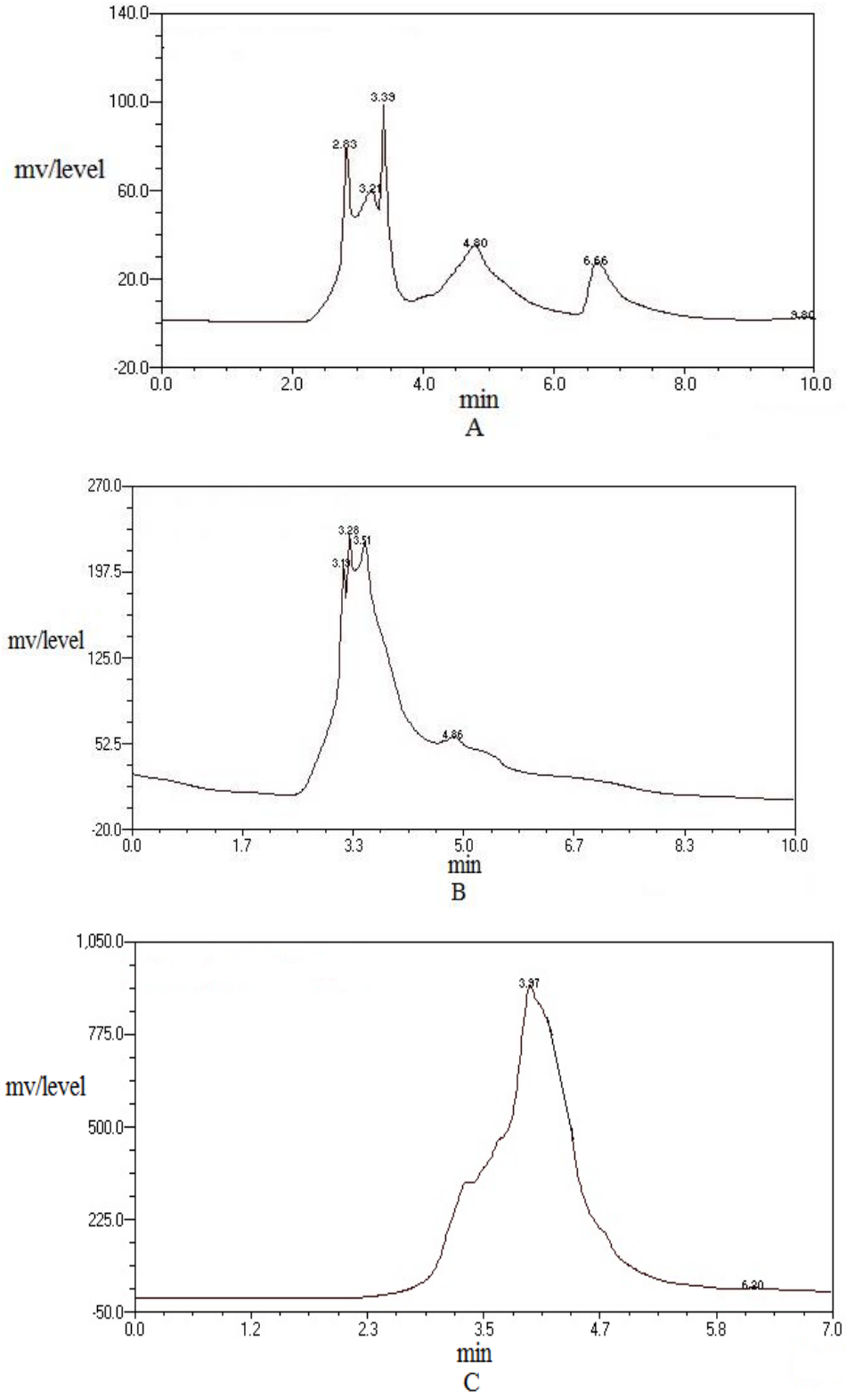

Figure 4. HPLC results by ultrasonic extraction with methanol solvent for three different durations. A: 30 min, B: 60 min, C: 90 min

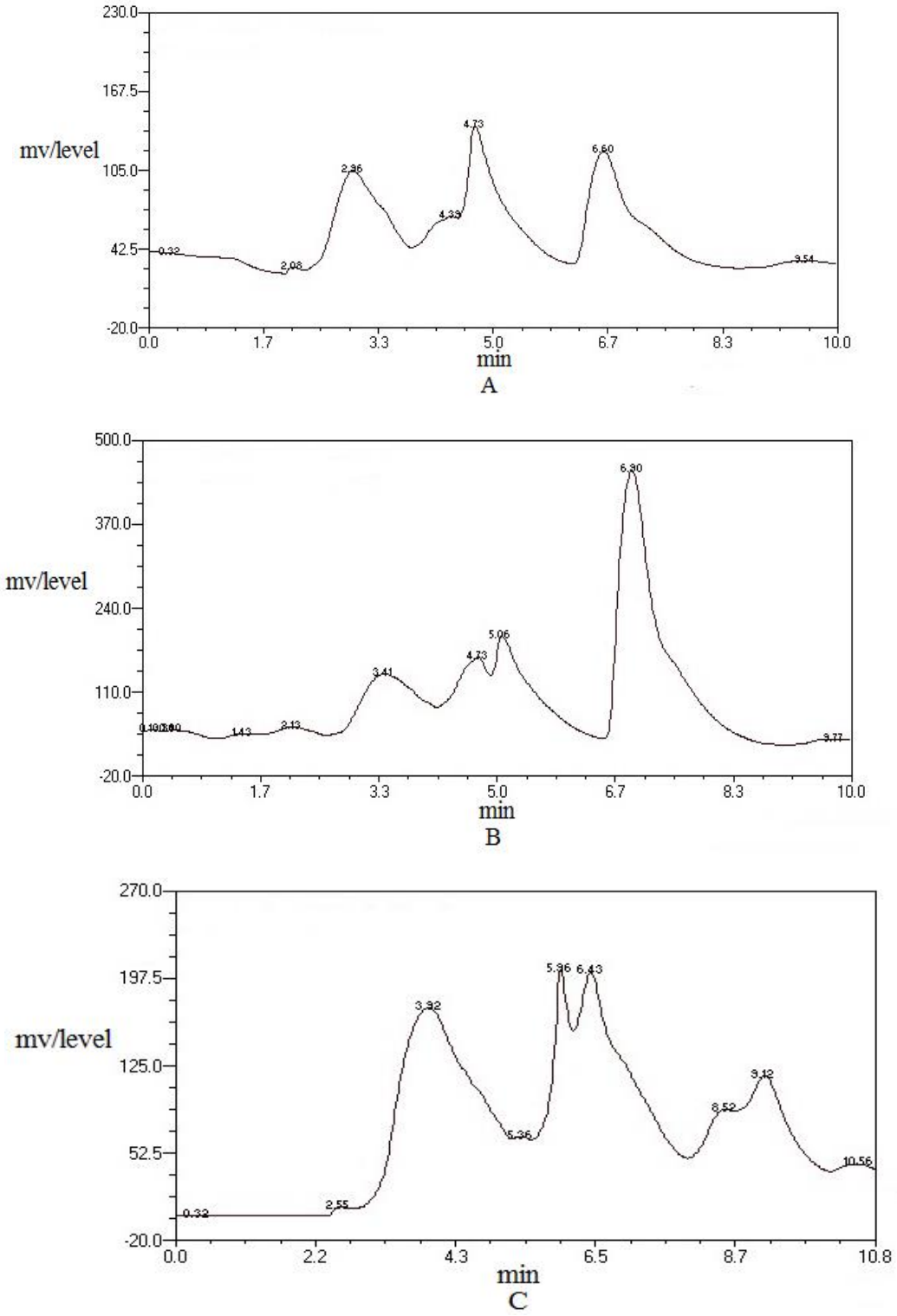

Figure 5. HPLC results by ultrasonic extraction with chloroform solvent for three different durations. A: 30 min, B: 60 min, C: 90 min

The results of HPLC in Figure 6. are shown by soxhlet extract with three chloroform solvent and deionized water. The highest amount of quercetin was observed in methanol solvent and the lowest in water solvent.

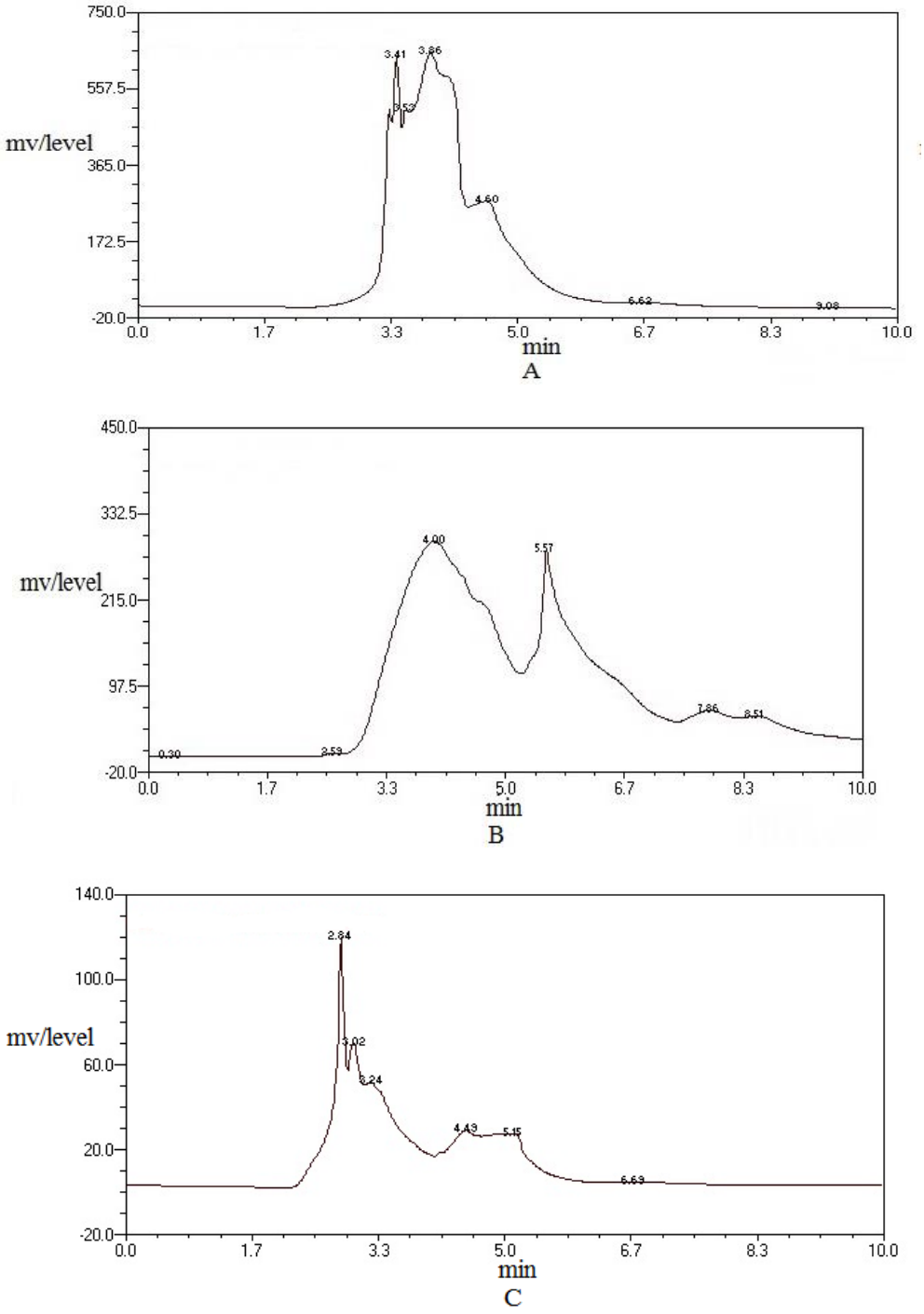

Figure 6. High performance liquid chromatography of extraction by soxhlet method: A: with methanol solvent, B: with chloroform solvent, C: with water solvent

It was reported that, a polyphenolic extract of *Urtica dioica L.*, a traditional medicinal plant, was synthesized and characterized by the production of enriched and functional chocolate [25]. Various extraction techniques have been employed to obtain valuable natural compounds from plants for commercialization have been extensively reviewed [26], with emphasis on extracted polyphenolic compounds using innovative extraction techniques and organic solvents. However, the usual methods of water-based extraction and the potential impact of other bioactive compounds extracted in this way have not been well characterized [25].

To investigate the antimicrobial activity of nettle methanol extract obtained by ultrasonication over a period of 90 min was used and its effect on microbial samples including *Escherichia coli, Entrecoccus faecalis, Klebsiella pneumoniae, Acinetobacter calcoaceticus* and *Candida albicans* was determined as shown in Table 1. *Escherichia coli* and *Candida albicans* were resistant to nettle extract but *Acinetobacter calcoaceticus* and *Klebsiella pneumoniae* were susceptible and *Entrecoccus faecalis* was semi-susceptible.

Table 1. Antimicrobial activity of nettle extract on some pathogenic microbes

	Diameter of inhibition zone (mm)					
Microorganism	**Nettle extract (6 mg/mL)**	**Quercetin (10 mg/mL)**	**Gentamicin (10 µg)**	**Gentamicin (120 µg)**	**Cefepime (30 µg)**	**Fluconazole (25 µg)**
Escherichia coli	N.I.	5	15	-	12	-
Enterococcus faecalis	6	12	-	6	-	-
Klebsiella pneumoniae	14	20	12	-	11	-
Acinetobacter calcoaceticus	14	18	10	-.	10	-
Candida albicans	N.I	5	-	-	-	14

N.I. = No inhibition

In a similar study, *E. coli* reported moderate sensitivity to acetyl acetate extract of nettle (10 mm) [27]. In another study, *Acinetobacter calcoaceticus* reported good and moderate sensitivity to chloroform and methanol extract of nettle however, the *Escherichia coli* was resistant to these extracts [2]. Currently, one of the reasons for the increased attention to medicinal plants is to avoid the adverse side effects of the synthetic drugs. For this reason, the analysis of the antimicrobial activity of medicinal plants has become increasingly the focus of the scientific experiments and one of the most prominent of these is the nettle plant.

Conclusions

Currently, one of the reasons for the greater attention of medicinal plants is to avoid the side effects of synthetic drugs. Therefore, the analysis of the antimicrobial activity of the medicinal plants is

increasingly being the focus of scientific experiments and one of the most prominent is the nettle plant. In this research study, three extraction methods were utilized to gain more access to flavonoid materials, especially quercetin in nettle (soaking, sonication, soxhlet) with three solvents (chloroform, methanol, deionized water). The highest amount of quercetin detected by HPLC was the ultrasonic method with a time of 90 min and methanol solvent. The level of inhibition of methanolic extract of nettle on pathogenic microorganisms (*Escherichia coli, Entrecoccus faecalis, Klebsiella pneumoniae, Acinetobacter calcoaceticus, Candida albicans*) was investigated. Antibiogram tests showed that *Escherichia coli* and *Candida albicans,* were resistant to nettle extract could not control them, *Acinetobacter* and *Klebsiella pneumoniae* were Susceptible and *Entrecoccus faecalis* was semi-susceptible.

Acknowledgments

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Conflict of Interest

We have no conflicts of interest to disclose.

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