Bulgarian Chemical Communications, Volume 56, Issue 3 (pp. 282-287) 2024 DOI: 10.34049/bcc.56.3.EA-SR

Evaluating the efficacy of cherry stem extracts against calcium oxalate monohydrate crystallization in kidney stone treatment

E. Akyol[1]*, M. Danisman[2]

[1]*Department of Chemical Engineering, Yildiz Technical University, Davutpasa Campus Esenler, Istanbul 34210, Turkey*

[2]*Department of Chemistry and Chemical Processing Technologies, Bayramic Vocational School, Çanakkale 18 Mart University, Çanakkale 17700, Turkey*

Accepted: June 16, 2024

Urethritis is one of the oldest known diseases of urinary stone formation. Urinary system stone disease ranks third among urinary diseases after urinary infection and prostate diseases. Calcium oxalate monohydrate (COM) crystals are known to be the main cause of urinary tract and kidney stones. In addition to the fact that surgical methods and drug treatments used in the treatment of kidney stones are painful and costly, the fact that traditionally used herbs are thought to be natural and harmless, has led to an increased interest in herbal medicines in recent years. Dried cherry stems have diuretic properties and can be used in the treatment of kidney stones reported in Iranian Traditional Medicine documents. When infused in hot water, they can be used as an herbal remedy in the treatment of high blood pressure and kidney stone disease. In the light of this information, in this study, the effects of cherry stem extracts as a natural additive on the growth of calcium oxalate monohydrate (COM) crystals, which is of great importance in investigating the crystallization mechanism in terms of biomineralization, was investigated *in vitro*. Structural characterizations of CaOx crystals were performed by FT-IR analyses, and morphological characterization and morphological changes were investigated by SEM images. The *in vitro* inhibitory effect of extracts of natural additives on calcium oxalate crystallization was determined by the time course of concentration measured in solution at extract concentrations of 0.5, 1, 5, 10 and 50 mL. Our findings demonstrate that cherry stem extracts significantly inhibit COM crystal growth and promote the formation of calcium oxalate dihydrate (COD) crystals, which are more easily excreted from the body.

Keywords: kidney stone; calcium oxalate; crystallization; inhibition; cherry stem

INTRODUCTION

Urinary tract and kidney stone formation is one of the most common and important clinical problems known since ancient times [1]. Records of bladder and kidney stones were found in Egyptian mummies dating back to 4800 BC [2]. Kidney stones are a major health issue [3]. Urinary stone development is a prevalent worldwide health issue that can impact individuals of all ages [4]. Urinary system stone disease is the third most common urinary disease, following urinary infection and prostate diseases [5]. In mature men, the likelihood of developing stones is 20%, whereas in women it ranges from 5% to 10%. The incidence of nephrolithiasis recurrence is predicted to range from 50% to 80% within 5 years after the first formation of the stone [6]. The global incidence of this condition appears to be rising in both males and females mostly as a result of a sedentary lifestyle, eating patterns, and issues related to global warming [7, 8]. The United States incur an estimated annual economic impact of over $5 billion due to kidney stones [9].

Calcium oxalate monohydrate (COM) crystals are known as the main cause of urinary tract and kidney stones [10]. Calcium oxalate accounts for approximately 70-80% of urinary stones [10, 11]. Calcium oxalate stones are usually small, rough, hard, yellow-brown-gray colored stones with spiny protrusions and are the most abundant, most difficult to treat and most difficult to understand among calcium oxalate crystals [10-12]. Two main steps in stone formation are clearly distinguished. The first is the formation of a nucleus by a number of particles in the solid phase retained in the kidney, and the second is the development of a kidney stone as a result of the continuous deposition of CaOx on the nucleus. Under these conditions, molecules that prevent crystal growth (inhibitors) significantly reduce the formation of nuclei [13, 14]. Preventing the formation and enlargement of calcium oxalate monohydrate (COM) crystals, or the transformation of COM nuclei into calcium oxalate dihydrate (COD) crystals which are less stable, is effective in stopping the formation of kidney stones [15]. In many previous studies it has been determined that

* To whom all correspondence should be sent:
E-mail: eakyol@yildiz.edu.tr

the crystals in the COD structures can be readily eliminated from the body by urine [16]. Although there are now effective treatments that can eliminate the vast majority of kidney stones, this issue persists and impacts on a significant number of individuals. For this reason, many studies have been conducted to prevent the formation of calcium oxalate stones and to elucidate the impact of various additives on calcium oxalate crystallization. In this context, plants attract attention as natural additives [17, 18].

Plants, algae and fungi have been used as medicines for the treatment of various diseases throughout human history [19]. The use of plants for relief and treatment of various diseases dates back to ancient times [20]. Especially in recent years, the clinically proven immunomodulatory, adaptogenic and antimutagenic properties of plants and herbal medicines have led to an increased interest in herbal medicines among people [21]. Plants, on the other hand, are considered to be very safe with little or no toxic properties. Their costs are low and they are readily available [6]. All these reasons have increased the popularity of herbal medicines in recent years [21].

In the preparation of herbal medicines, various parts of plants, even whole plant, root, leaf, stem are used, while tablets, powders, boiling of fresh or dry parts and extracts are the most common methods [20]. Cherry is a plant belonging to the genus *Prunus* of the *Rosaceae* family whose Latin name is *Prunus Avium*. In addition to its rich mineral content, it contains high amounts of vitamins A, B group, C and anthocyanin, which show antioxidant properties [22]. The skin and stem of cherries are a natural source of biogenic substances and antioxidants [23]. Dried cherry stem has a unique simple chemical structure and excellent biochemistry. It has an uncomplicated, regular and distinct cellulosic structure. The low and independent content of active substances makes cherry stem a powerful therapeutic herb. Dried cherry stem facilitates both circulation and removal of toxins from the body. Apart from these, it is effective against prostate hyperthyroidism causing residual urine and difficulty in urination caused by bladder neck obstruction [24]. The stems of the cherry fruit are sold as herbal medicine in Iran and are used against kidney stones, edema and hypertension. Due to the high flovanoid and potassium content of cherry stems, they have a mild diuretic effect and can be used in the treatment of kidney stones, reported in Iranian Traditional Medicine documents [24-26].

For all these reasons, calcium oxalate crystallization has been an important topic of interest to researchers in biomineralization. In this study, the effects of cherry stem as a natural additive to calcium oxalate crystallization, which is very important in terms of biomineralization, were investigated.

MATERIALS AND METHODS

Materials

Calcium chloride ($CaCl_2.2H_2O$) and sodium oxalate ($Na_2C_2O_4$) obtained from J. T. Baker were used in the experiments. Dried cherry stalks were used as natural additives. 0.2 μm polyamide filter paper was used for filtration. Plant extracts were frozen and stored. They were thawed and used immediately before the experiments.

Methods

Spontaneous crystallization method was used in this study to evaluate the effect of cherry stem extract on calcium oxalate monohydrate crystallization process. First of all, 10 g of cherry stem used as a natural additive was heated at 100 °C for 15 min. Then, the solution obtained was filtered through 0.2 μm polyamide filter paper and extracted to obtain cherry stem extract. The plant extracts obtained were frozen and stored. They were thawed immediately before the experiments.

Crystallization experiments were initiated by mixing equal molarities of $CaCl_2$ and $Na_2C_2O_4$ solutions. An automatic temperature-controlled water bath was used to keep constant temperature of the 1 L double walled glass reactor used in the experiments. During the reaction, the temperature was kept constant at 37±0.1ºC based on human body temperature. Stirring throughout the reaction was achieved with a magnetic stirrer.

During the experiments, conductivity, temperature and pH values were recorded using a computer with the "Multi-Lab pilot" program. Using the data obtained, conductivity *vs.* time graphs were plotted without and with additives. The experiments were repeated three times for consistency of the results.

The crystallization rate obtained without additives was defined as (R_0) and the crystallization rate obtained with additives was defined as (R) and the R_0/R ratios were calculated by finding the slope values from the graphs drawn.

Each of the experiments was carried out for 6 h. At the end of the 6^{th} h, the crystals formed in the solution were filtered through a 0.2 μm polyamide membrane filter. The crystals were washed with distilled water to remove the saturated solution. The obtained samples were dried in a 60ºC vacuum oven for 24 h and stored in a desiccator.

The morphology and size of the crystals obtained were examined by scanning electron microscopy (SEM) images.

RESULTS AND DISCUSSION

Effect of the amount of cherry stem extract on the crystallization rate at constant initial supersaturation

In order to investigate the effect of cherry stem extract on calcium oxalate crystallization, 2 series of experiments were performed for constant initial supersaturation experiments. The first series of experiments were carried out at concentrations of $CaCl_2.2H_2O$ and $Na_2C_2O_4$ at $3.25.10^{-4}$ M and the second series of experiments at $6.5.10^{-4}$ M. 0.1 ml, 0.5 ml, 1 ml, 5 ml, 10 ml and 50 ml of cherry stem extract, respectively.

Conductivity *vs.* time graphs and the effect of cherry stem extract on the crystallization rate at $3.25.10^{-4}$ and $6.5.10^{-4}$ initial supersaturation are given in Figures 1 and 2, respectively. In the experiment with 0.1 ml of cherry stem extract, the delay time was 1 min, in the experiment with 0.5 ml of cherry stem extract the delay time was 1.5 min, in the experiment with 1 ml and 5 ml of cherry stem extract the delay time was 2 min. In the experiment with 10 ml of cherry stem extract, it was observed that the delay time was spread over almost the entire 6 h, and in the experiment with 50 ml of cherry stem extract, crystallization was completely stopped.

Effect of initial supersaturation on the crystallization rate

In the experiments carried out to investigate the effect of initial supersaturation on the crystallization rate, the temperature and stirring conditions were not changed. In the experiments, 10 ml of cherry stem extract was used for easy observation of the results. $CaCl_2.2H_2O$ and $Na_2C_2O_4$ starting solutions of $3.5.10^{-4}$ M, $4.5.10^{-4}$ M, $5.5.10^{-4}$ M and $6.5.10^{-4}$ M, respectively, were used. Conductivity vs. time graphs obtained as a result of the experiments are given in Figure 3.

When the initial supersaturation was increased in the experiments without additive, an increase was observed in the rate of crystallization. However, in the experiments with a constant cherry stem extract additive, it was observed that increasing the initial supersaturation reduced the inhibitory effect of the cherry stem extract on the crystallization rate.

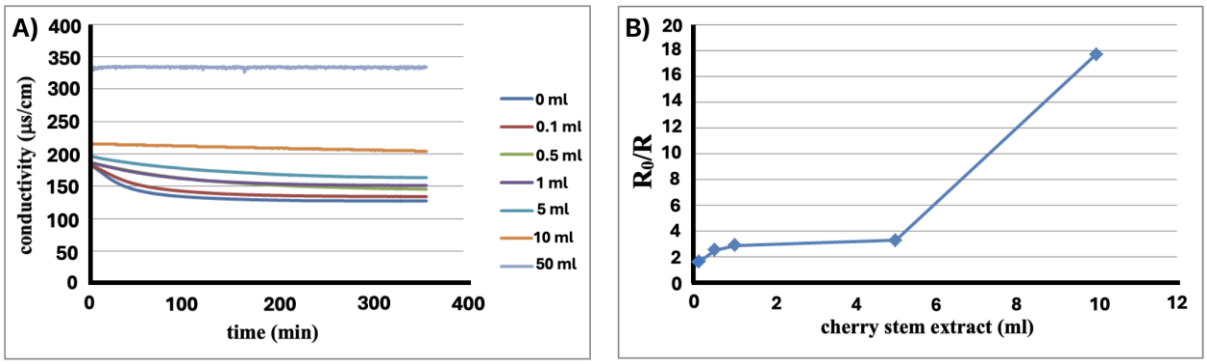

Figure 1. Graphs of conductivity *vs.* time (A) and R_o/R-cherry stem extract (B) at experiments at $3.25.10^{-4}$ M initial supersaturation

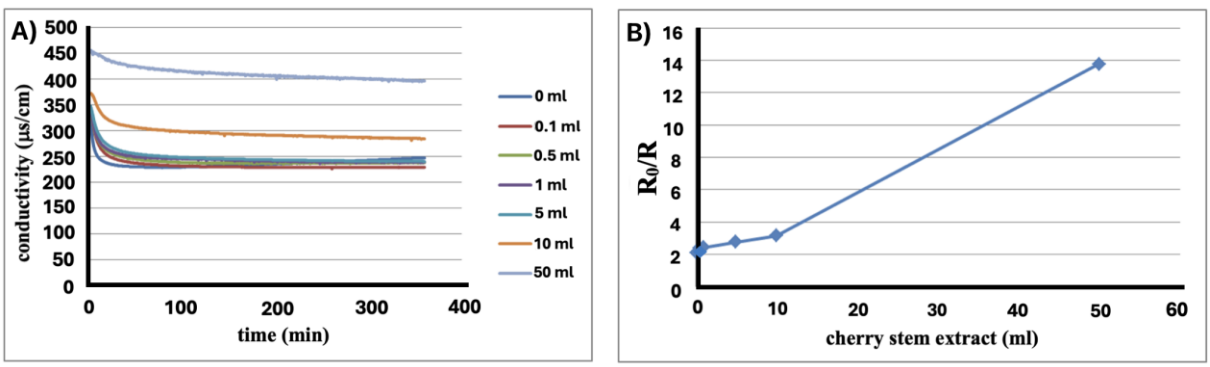

Figure 2. Graphs of conductivity *vs.* time (A) and R_o/R-cherry stem extract (B) at experiments at $6.5.10^{-4}$ M initial supersaturation

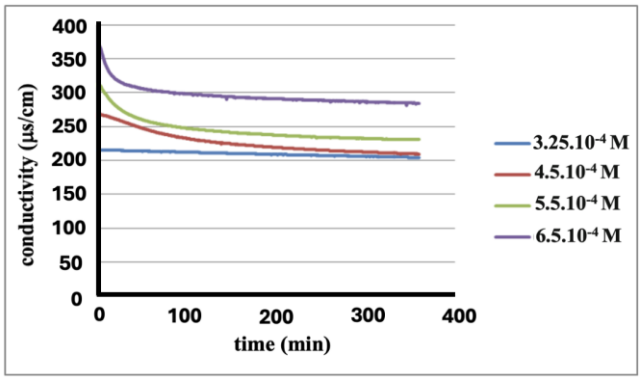

Figure 3. Conductivity *vs.* time graphs of experiments with 10 ml of cherry stem extract added to calcium oxalate solution at different initial supersaturations

SEM analysis

The crystals obtained as a result of the experiments with natural additives were photographed using scanning electron microscopy (SEM) to examine their morphology and size. Measurements were made by averaging between 50 and 150 crystal samples taken from different parts of the SEM photographs.

SEM images of the crystals obtained as a result of the experiments performed with 1 ml, 5 ml, 10 ml and 50 ml cherry stem extract additives in order to observe the effect of cherry stem extract additive on crystal morphologies and sizes during the formation of calcium oxalate crystals at $3.25.10^{-4}$ M initial supersaturation, are given in Figure 4. The size and deviation of the crystals were calculated from these images and the calculated values are given in Table 1.

In the SEM images it is seen that the crystals obtained as a result of the experiments performed with 1 ml and 5 ml cherry stem extract additives are in COM structure. It is seen that some of the crystals obtained by increasing the additive amount to 10 ml started to form in COD structure and all of the crystals obtained after 50 ml additive amount were in COD structure. It is also seen in Table 1 that the crystal sizes decreased with the increase in cherry stem extract additive.

SEM images of the crystals obtained from the experiments performed with 1 ml, 5 ml and 50 ml cherry stem extract at $6.5.10^{-4}$ M initial supersaturation are given in Figure 5. The size and deviation of the crystals were calculated from these images and the calculated values are given in Table 2.

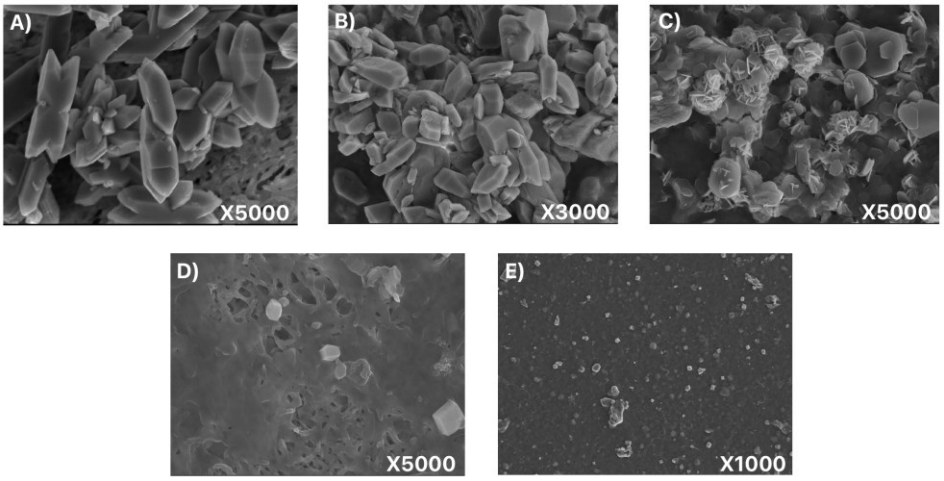

Figure 4. SEM images of the crystals obtained with 0 ml (A), 1 ml (B), 5 ml (C), 10 ml (D) and 50 ml (E) cherry stem extract additives at $3.25.10^{-4}$ M initial supersaturation

Table 1. Size values of calcium oxalate crystals obtained from crystallization experiments with different amounts of cherry stem extract at $3.25.10^{-4}$ M initial supersaturation

Cherry stem extract amount	COM		COD		
	Width(µm)	Length(µm)	Width(µm)	Length(µm)	Diagonal(µm)
	W_{av}	L_{av}	W_{av}	L_{av}	D_{av}
1 ml	4.05(±1.1)	7.70(±2.04)	-	-	-
5 ml	2.50(±0.60)	3.15(±0.75)	-	-	-
10 ml	2.20(±0.29)	2.74(±0.56)	2.71(±0.82)	3.07(±0.70)	3.98(±1.16)
50 ml	-	-	2.63(±0.8)	2.75(±0.66)	3.42(±1.02)

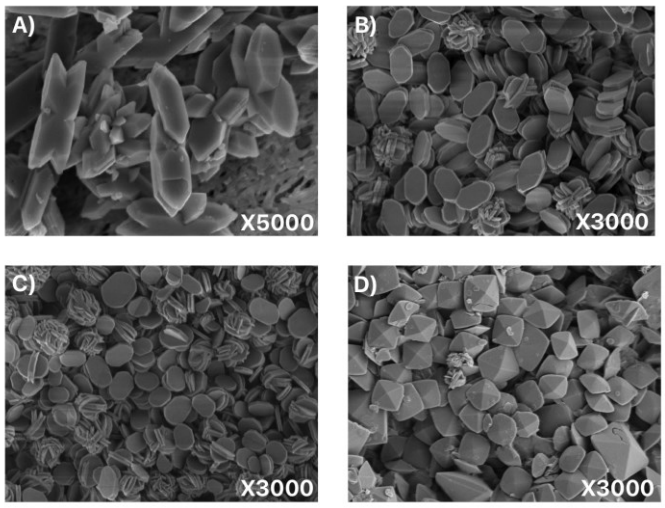

Figure 5. SEM images of the crystals obtained with 0 ml (A), 1 ml (B), 5 ml (C) and 50 ml (D) cherry stem extract additives at $6.5.10^{-4}$ M initial supersaturation

Table 2. Size values of calcium oxalate crystals obtained from crystallization experiments with different amounts of cherry stem extract at $6.5.10^{-4}$ M initial supersaturation

Cherry stem extract amount	COM		COD		
	Width(µm)	Length(µm)	Width(µm)	Length(µm)	Diagonal(µm)
	W_{av}	L_{av}	W_{av}	L_{av}	D_{av}
1ml	5.00(±0.73)	9.43(±1.32)	-	-	-
5 ml	4.22(±0.89)	6.21(±1.07)	-	-	-
50ml	-	-	5.87(±1.95)	7.88(±2.85)	9.60(±2.53)

In the SEM images obtained it is observed that the structure of the crystals obtained from the experiments using 1 ml and 5 ml of additive is COM, while the structure of the crystals obtained in the experiment with 50 ml of additive is COD. In addition, as seen in Table 2, as the amount of additive increases, the size of the crystals decreases.

FT-IR analysis

FT-IR spectra of the crystals obtained by adding 0 ml and 50 ml of cherry stem extract at an initial supersaturation of $6.5.10^{-4}$ M are given in Figure 6.

In the first spectrum, peaks belonging to the O-H band are seen around 3480 cm^{-1} – 3000 cm^{-1}. The peaks seen at 1623 cm^{-1}, 662 cm^{-1} and 599 cm^{-1} belong to the H-O-H vibration. The peaks seen around 1366 cm^{-1} and 1317 cm^{-1} belong to the C-O bonds in the COM structure. In the second spectrum, the peaks belonging to the O-H band became widespread around 3472 cm^{-1} -3250 cm^{-1}. Peaks belonging to H-O-H are seen around 1619 cm^{-1} and 614 cm^{-1}. The peaks belonging to the C-O bond are seen at 1325 cm^{-1}. These characteristic changes between the spectra support that the first spectrum corresponds to the COM structure and the crystals turn into the COD structure in the second spectrum [27].

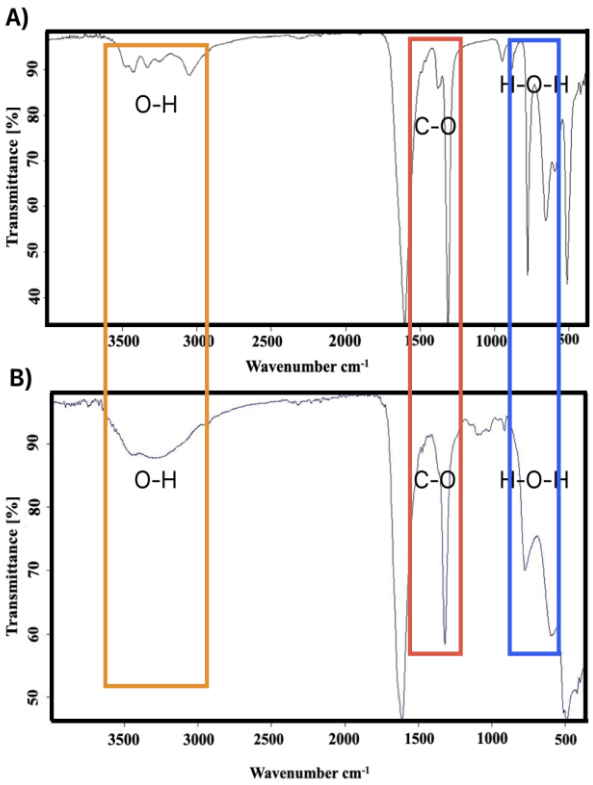

Figure 6. FT-IR spectra of crystals obtained by adding 0 ml (A) and 50 ml (B) of cherry stem extract at $6.5.10^{-4}$ M initial supersaturation

CONCLUSION

In order to inhibit the crystal growth of calcium oxalate monohydrate, studies were carried out with cherry stalks in a laboratory environment under conditions similar to the human body and positive results were obtained from these studies. As a result of the experiments carried out using different ratios of natural additives at constant initial supersaturation, a decrease in crystal size was observed with increasing additive concentration. It was observed that the formation of COM crystals gradually decreased and COD crystals were formed as the additive concentration increased and the formation of COM crystals was completely prevented at high concentrations.

In the experiments performed with and without cherry stem extracts at different initial supersaturations, an increase in crystallization rate and size was observed with increasing initial supersaturation. It was observed that crystallization rates and crystal sizes were lower in the experiments with natural additives compared to the experiments without additives.

As a result of the studies, it was observed that cherry stem extract used in sufficient concentrations was successful in preventing calcium oxalate crystallization. The fact that the additives used are natural, is very important for biomineralization studies. The shrinking effect of the additives on CaOx crystals and the conversion to COD structure support the view that these natural additives may be useful in the treatment of kidney stone disease.

Acknowledgement: *The current study was supported by Yildiz Technical University (Project No: 2012-07-01-YL05).*

REFERENCES

1. S. Atanassova, *J. Cryst. Growth*, **312,** 1940 (2010).
2. M.E: Salem, G. Eknoyan, *Am. J. Nephrol.,* **19(2)**, 140 (1999).
3. J. Medetognon-Benıssan, S. Tardıvel, C. Hennequın, M. Daudon, T. Drücke, B. Lacour, *Urol. Res.,* **27**, 69 (1999).
4. A. Nimavat, A. Trivedi, A. Yadav, P. Patel, *J. Pharm. Pharmacol.*, **10,** 195 (2022).
5. H. E. Garcia-Perdomo, P. B. Solarte, P. P. Espana, *Urol. Colomb.*, **25,** 118 (2016).
6. N. Sharma, B. S. Tanwer, R. Vijayvergia, *Int. J. Pharmtech. Res.*, **3(1),** 110 (2011).
7. M. Akram, M. Idrees, *Int. J. Immunopathol. Pharmacol.,* **33,** (2019).
8. A. Y. Muslumanoglu, M. Binbay, E. Yuruk, T. Akman, A. Tepeler, T. Esen, A. H. Tefekli, *Urol. Res.,* **39,** 309 (2011).
9. E. S. Hyams, B. R. Matlaga, *Transl. Androl. Urol.,* **3(3),** 278 (2014).
10. X. Y. Sun, M. Xu, J. M. O. *ACS Omega,* **2(9),** 6039 (2017).
11. Y. Shen, W. Yue, A. Xie, Z. Lin, F. Huang, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **234,** 35 (2004.
12. J. Cloutier, L. Villa, O. Traxer, M. Daudon, *World J. Urol.,* **33(2**), 157 (2015).
13. F. Tamborino, R. Cicchetti, M. Mascitti, G. Litterio, A. Orsini, S. Ferritti et al., *Int. J. Mol. Sci.,* **25(5)**, 3075 (2024).
14. A. Al-Dubai, E. Akyol, *Bulg. Chem. Commun.,* **55(3),** 278 (2023).
15. T. Jung, W. Kim, C. K. Choi, C.K., *J. Cryst. Growth*, **279,** 154 (2005).
16. J. Yu, H. Tang, B. Cheng, *J. Colloid Interface Sci.*, **288,** 407 (2005).
17. I. Das, S. K. Gupta, S. A. Ansari, V. N. Pandey, R. P. Rastogi, *J. Cryst. Growth.*, **273,** 546 (2004).
18. E. Akyol, K. Ongun, S. Kirboga, M. Öner. *Biointerface Res Appl Chem,* **6**, 1064 (2016).
19. E. Yarner. *World J. Urol.*, **20,** 285 (2002).
20. B. Ballabh, O. P. Chaurasia, Z. Ahmed, S. B. Singh, *J. Ethnopharmacol.*, **118(2),** 331 (2008).
21. H. R. Chitme, S. Alok, S. K. Jain, M. Sabharwal, (2010). *Int. J. Pharm. Sci.,* **1(2),** 24 (2010).
22. M. Petruzzello, Encyclopedia Britannica, 2024.
23. R. D. Cagno, R. F. Surico, G. Minervini, C. G. Rizzello, R. Lovino, M. Servili, A. Taticchi, S. Urbani, M. Gobbetti, *Food Microbiol.,* **28,** 900 (2011).
24. İ. A. Saracuglo, Medicinal Plants and Herbal Health Guide, Gun Ofset, Istanbul, 2009.
25. N. Hooman, F. Mojab, B. Nickavar, P. Pouryousefi-Kermani, *Pak. J. Pharm. Sci.,* **22(4),** 381 (2009).
26. M. Rameshk, *Trad. Integr. Med.,* **9(1),** 74 (2024).
27. M. Maruyama, K. P. Sawada, Y. Tanaka, A. Okada, K. Momma, M. Nakamura, R. Mori et al., *PLoS One*, **18(3),** 1 (2023)