Egypt. J. Chem. **Vol. 68**, No. 2 pp.1 – 13 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effect of Some Algerian Saharan Medicinal Plants on Brushite Crystallization in Artificial Urine

Nacira Benahmed[a*], Abdelkrim Cheriti[a]

[a] *Phytochemistry and Organic Synthesis Laboratory (POSL).*
Tahri Mohamed University, P.O. BOX 417 Bechar- 08000, Algeria.

Abstract

Brushite (DCPD) is considered one of the most important types of phosphate kidney stones, it easily forms large, well-developed crystals well suited for morphological studies. The extracts effect of medicinal plants on brushite crystallization was studied *in vitro* for nine species used in Algerian Sahara traditional pharmacopoeia, which are: *Salsola sp, Launaea arborescens, Panicum turgidium, Artemisia campestris, Zea mays L, Zizyphus lotus, Cynodon dactylon L, Zilla macroptera* and *Tamarix galica*.

Experiments were carried out at 37°C with acidic pH (6.5). The initial pH, temperature, agitation and concentration of constituents were kept constant during a reaction time of 6 h. The effect of aqueous and organic extracts of the selected plants on the morphology of brushite crystals was studied by optical polarizing microscopy, image analysis, micrometric measurement, FTIR spectroscopic analysis.

Most extracts have significant inhibitory effects. Total inhibition of DCPD crystallization presented by nine extracts of six plants: methanolic extract of *Salsola sp, Zilla macroptera and Tamarix galica*, ethanolic extract of *Zizyphus lotus* and *Artemisia campestris*, Acetonic extract of *Zizyphus lotus* and *Salsola sp*, chloroforme extract of *Zizyphus lotus* and *Zilla macroptera*. Total inhibition of crystalline aggregation observed for acetonic extract of *Zea mays L* and methanolic extract of *Zizyphus lotus*. While the other extracts slow the kinetics of DCPD crystallization up to 240 minutes. In addition, the most extracts give a considerable inhibition of the crystals size, in which, the mean crystal size decreased from 6 µm to 1-3 µm. Even inhibitory effect for the aggregation phase noted for all plant extracts especially, *Cynodon dactylon L* and *Zea mays L* presented a very important inhibition, in which, the mean aggregate size decreased from 55 µm to 5-7 µm.

The selected plant extracts showed a significant inhibitory effect on brushite crystallization presented as total inhibition of all crystallization phases. For the other extracts, the effect manifested in the decrease of the size and the number of crystals and aggregates brushite.

Keywords :Urolithiasis, Brushite cristallization, medicinal plants, cristallization inhibitors, Algerian Saha.

1. Introduction

The urolithiasis constitutes a major problem to public health, it is a disease known since ancient times, resulting in the presence of stones in the kidneys or urinary tract, and until today no efficient therapeutical treatments have not yet been developed [1, 2, 3]. For this reason, it must be managed at different levels and it still requires research that leads to rational treatment and cessation of recurrence.

The urinary stones (calculi) consist mainly of crystalline components, mainly calcium, oxalates and phosphates. Calcium and/or magnesium phosphates are reported to be components of about 25% of the urinary calculi [4, 5].

Nucleation of a solid phase in human urine can be provoked by slight changes in the urine composition [6-8]. Crystal growth and agglomeration may be due to supersaturation with respect to stone forming constituents or the presence of various inhibitory or stimulatory biomolecules or even pH [9]. When the urine becomes supersaturated with insoluble material, because excretion rates are excessive and/or because water conservation is extreme, crystals are formed and may grow and aggregate to form a stone [10, 11]. It is reasonable to assume that the formation of calcium phosphate stones requires a supersaturated state of urine with respect to brushite ($CaHPO_4\_2H_2O$) [2].

Urine is also containing numerous substances, which act as natural crystallization inhibitors [12]. The crystallization inhibiting capacity of urine does not allow urolithiasis to happen in most of the individuals, whereas this natural inhibition is impaired in stone formers [13]. The role of crystallization inhibitors is to prevent, slow or reduce one or another phase of given species crystallization. These are substances that are able to object, either by a specific mechanism for the substance or by a more general mode of action, to any stage of crystallization. They act by occupying at the level of the surface of the crystal lattice, the growth sites, so as to block them [14]. Due to its large surface area and diverse climate, Algeria has on the one hand a varied flora, thus a source of rich and abundant medical material. On the other hand, by its history, and its strategic position, Algeria has benefited from different cultures: Berber, Greco-Roman and Islamic. Local medicinal pharmacopoeia constitutes an important source of remedies for primary health care [15]. As part of our investigation into medicinal plants growing in

*Corresponding author e-mail: nacira10dz@yahoo.fr.; (Nacira Benahmed).
Receive Date: 09 May 2022, Revise Date: 26 July 2022, Accept Date: 25 June 2024
DOI: 10.21608/EJCHEM.2024.137538.6055

Algerian Sahara [15, 16-19]. The work carried out is dedicated to the *in vitro* study of aqueous and organic extracts effect of nine medicinal plants used in Algerian Sahara traditional pharmacopoeia on the growth and inhibition of brushite. These plants selected from an ethnopharmgical investigation: *zizyphus lotus, Launaea arborescens, Panicum turgidium, Artemisia campestris L, Salsola sp, Zea mays L, Cynodon dactylon L, Zilla macroptera, Tamarix galica* [19]. The common names, families, and the part used are manipulated in Table 1.

2. Materials and methods

2.1. Brushite precipitation from artificial urine

The model of DCPD crystallization incorporated into our work is based on artificial urine [20, 21]. Two sets of experiments were conducted to study the DCPD crystallization in aqueous solutions. The first without inhibitors and the second with inhibitors to assess the inhibiting effect of the studied extracts.

a) Study in absence of inhibitors:
The artificial urine [20, 21] was prepared by mixing together equal volumes of two solutions, A and B respectively. These solutions were prepared by dissolving in deionized water. Solution A contains a chemical composition as follows: 11.02 g/l $Na_2SO_4.10H_2O$,1.46 g/l $MgSO_4.7H_2O$, 4.64 g/l NH_4Cl, 12.13 g/l KCl and 0.24 g/l Ca^{2+}, and solution B: 2.65 g/l $NaH_2PO_4.2H_2O$, 18.82 g/l $Na_2HPO_4.12H_2O$, 13.05 g/l NaCl, 1 g/l $Na_3C_6H_5O_7.2H_2O$ and 0.05 g/l $Na_2C_2O_4$. Ca^{2+} is added as a standard solution prepared from $CaCO_3$ dissolved in HCl. They have thermostated at physiological temperature 37 °C using a water bath mounted. The values of initial pH of the artificial urine (6.5) were adjusted to a required value by adding either HCI or NaOH solution, and kept constant during the reaction time. The agitation is provided by using a magnetic stirrer with a constant speed (500 rpm).

b) study in presence of inhibitors (plant extracts)
It concerns the study of the effect of plant extracts on nucleation and aggregation of DCPD formed in artificial urine at pH = 6.5. The model, the conditions and the analysis methods used to study the DCPD crystallization in presence of inhibitors adopted in our work is the same as, the one without inhibitors. The study followed by mixing the two volumes equivalent of solutions A and B, containing the body of inhibitor at pH 6.5 to 37°C agitated magnetically at a constant speed. We have determined the mean sizes and the numbers of DCPD crystals and aggregates in the presence of plant extracts and compared with the results in absence of these extracts to assess their effect. All experiments are corresponding to a physiological concentration of 1 g/l of extract for each sample. Note that we have maintained the experimental parameters constant during each series (temperature, agitation and concentration of constituents).

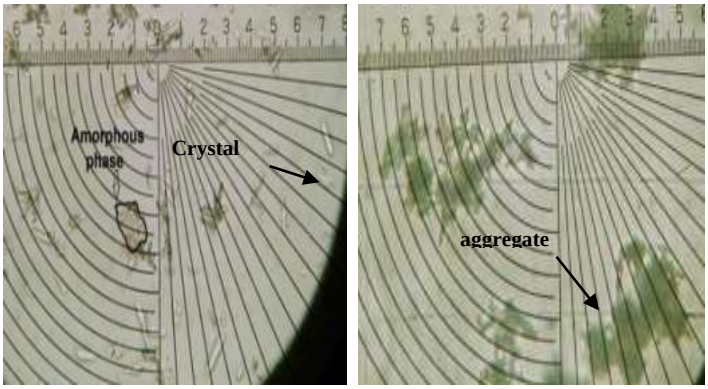
2.2. Optical microscopy
The kinetics are followed for 6 hours at pH 6.5 immediately after mixing the solutions A and B. The evolution of the mean sizes and the numbers of DCPD crystals and aggregates as a function of time is followed using optical microscopy (Optika N-400POL Polarizing microscope with digital image acquisition) equipped with micrometer. Crystals were identified with x 40 magnifying lens. The microscopic analysis was carried out by taking samples in the form of a drop of the reaction mixture using a Pasteur pipette, placed on a cell mallasez every five minutes and analyzed under a polarizing microscope. The sizes were measured with the micrometer. The agglomeration of two or more individual crystals is considered as an aggregate Fig.1. At the end of the experiment, the total volume is collected and filtered. The precipitate is dried at room temperature for 24 hours and subjected to a Fourier-Transform Infrared Spectroscopy (FTIR) analysis.

2.3. FTIR spectroscopic Analysis
To determine the crystalline phases formed by crystallization in presence and in absence of inhibitor, Infrared Spectroscopy Fourier Transform was used. All powder samples were characterized by using a Fourier-transform infrared spectroscopy (Termo Nicolet Avatar 320FT-IR). Analysis were performed after mixing 1 mg of sample powders with 300 mg of KBr powder, followed by compacting those into a thin pellet in a stainless steel die of 1 cm inner diameter. FTIR data were recorded over the range of 4000 to 400 cm^{-1}.

2.4. Extract preparation
The plants were collected from Bechar region (Western South of Algeria). Plant materials were air-dried and protected from light. Plant materials were prepared from a reflux extractor for two hours by taking 30 grams of dried plant material and cut with 200 ml of solvents of different polarity (Methanol (MeOH), Ethanol (EtOH), Acetone (Ac), Chloroform (Ch), Hexane (Hex) and H_2O (Aq)). Each extract is subsequently filtered and evaporated to determine the mass and the extraction yield Table 2.

3. Results and discussion

3.1. Crystallization kinetics in absence of inhibitors
The characterization of the precipitate is defined by polarized light optical microscopy (Fig.1). The results concerning the evolution of the mean crystals and aggregates size of DCPD are represented in Fig. 2. The only crystalline phase formed at pH=6.5 is brushite (DCPD). The granules identified by optical



(a) (b)

Fig. 1. Optical micrographs showing typical morphologies of DCPD crystals and aggregates

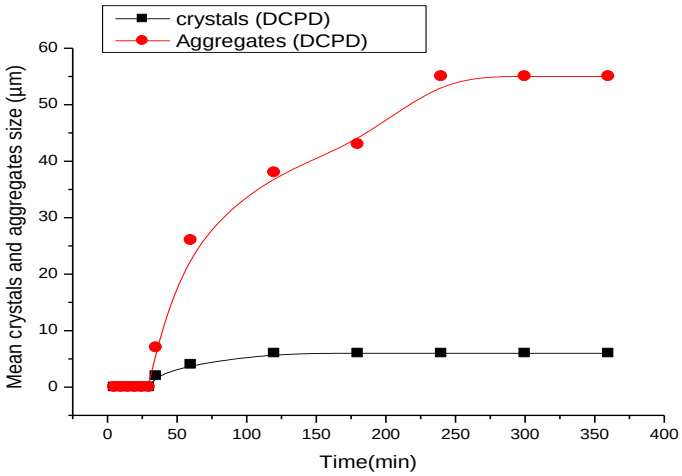


Fig. 2. Evolution of the mean crystals and aggregates size of DCPD at pH=6.5 in absence of inhibitors

microscope as amorphous phase Fig. 1(a) appeared instantly after five minutes. It is easily obtained under these conditions, which is commonly denoted as ACP (amorphous calcium phosphate) and usually converted into DCPD.

After six hours, all the granules are transformed into brushite Fig. 1(b). This will be proved by FTIR analysis. We also note that the brushite crystals appeared after 35 minutes (Fig. 2). Beyond T (time) = 120 minutes, the mean size of DCPD crystals remains constant (Sc = 6 µm) and their number decreased after 60 minutes because of the transformation crystal in aggregates. The mean size of the latter increases and stabilizes at approximately 55 µm after 240 minutes (4 hours), their number oscillates before reaching a stable value 250/mm^3.

Table 1 Classification of plants used

Species	***Salsola Sp***	***Launaea arborescens***	***Panicum turgidium***	***Artemisia campestris L***	***Zea mays L***	***Zizyphus lotus***	***Cynodon dactylon L***	***Zilla macroptera***	***Tamarix galica***
Botanical Family	Chenopodiaceae	Asteraceae	Poaceae	Asteraceae	Poaceae	Rhamnaces	Poaceae	Brassicaceae	Tamaricacées
Local Name	Cherira	Oum lbina	Nadkhir	Allal	Dra	Nbeg	Nedjm	Boukhelala	Fersik
studied	Air part	Air part	Air part	Air part	Leaf	Fruits	Root	Air part	Air part
abbreviation	Sp	Lb	Pt	Ar	Zm	Zl	Cd	Zc	Tg

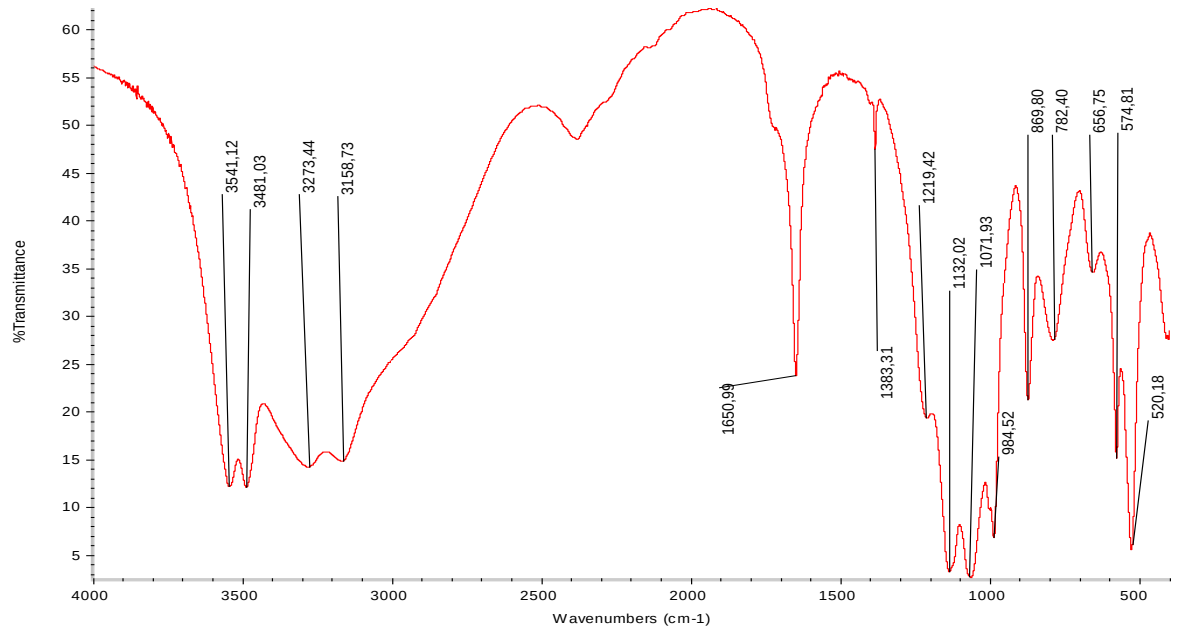


Fig. 3. FTIR spectrum of precipitate at pH=6.5

Fig.3 shows the FTIR spectra of precipitates at pH 6.5 after filtration. The spectra obtained at this pH were compared with those published for DCPD [22-24]. We determined that they are essentially identical. The IR bands shown in Fig. 3 were observed in the FTIR spectrum of precipitates at pH 6.5. They indicated that the absorption bands of this synthetic phase are comparable to the bands typical for natural calcium hydrogen phosphate dihydrate (DCPD).

The presence of water of crystallization was referenced from absorptions at 3541.12, 3481.03, 3373.44 and 3158.73 cm^{-1}, which are due to intermolecular and weakly H bonded OH [3]. The symmetric bending of H-O-H of water molecule gives rise to absorption at 1640 cm^{-1}, while P=O associated stretching vibrations were observed at three different wavenumbers (1219.42, 1132.02 and 1071.93cm^{-1})[25]. Likewise, the P-O-(H) asymmetric stretching vibrations were observed at 984.52, 869.9 and 782.4 cm^{-1}. The strong absorptions at 574.81 and 620.18 cm^{-1} are due to acid phosphates P=O (O-H) bond vibrations.

3.2. Study in presence of plant extracts:

The choice of inhibitors derived from ethnoph- armgical investigation [19] to study their inhibiting effect on phosphate lithiasis. In the Sahara of Algeria, exactly in the region of Bechar (Western South of Algeria), most patients use medicinal plants as an alternative remedy to treat various types of illness including urolithiasis. As for monitoring the study we selected a plant whose therapeutic reputation is very important for the local population. The second table represented the mass (m) and the extraction yield (yd), show that, water and methanol are generally the most extractive solvents which give higher yields up to (30%), whereas extraction with hexane and chloroform gives a low yield. Ethanol and acetone give average yields of extraction. *Artemisia campesiris L*, *Tamarix galica* and *Zizyphus lotus* are the plants that give the best extraction yield compared to other plants. On the other hand, the extraction of *Salsola sp* and *Zilla macroptera* gives an average yield. *Cynodon dactylon L* and *Zea mays L* give a low yield.

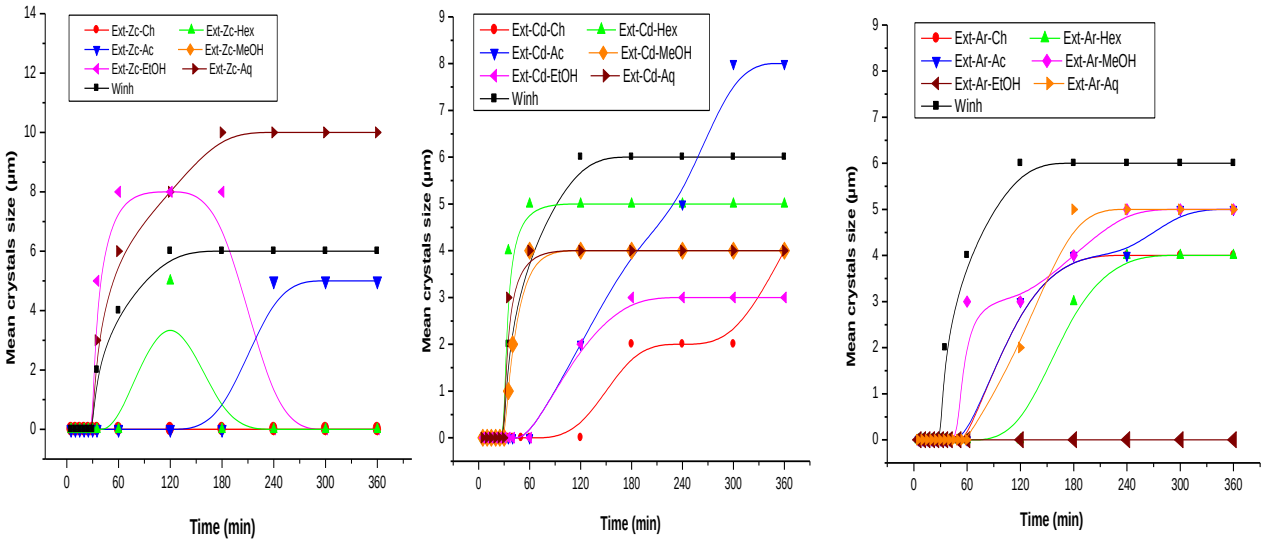
3.2.1. Effect of plants extracts on brushite cristallisation:

The results obtained, in the presence of extract (Ext) are presented in the following Fig. 4 - (A), (B), (C), (D), (E), (F), (G), (H), (I) and Fig. 5-(A), (B), (C), (D), (E), (F), (G), (H), (I). They presented the effect of different plant extracts on the mean size of DCPD crystals and aggregates, respectively, as a function of time compared with the results without inhibitors (Winh).

Table 2. The masses and the extraction yields

Species / Extract	*Salsola sp*		*Launaea arborescens*		*Panicum turgidium*		*Artemisia Campestris L*		*Zea mays L*		*Zizyphus lotus*		*Cynodon dactylon L*		*Zilla macroptera*		*Tamarix galica*	
	m	yd	m	yd	M	yd	m	yd	m	yd	m	yd	m	yd	m	yd	m	yd
	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)
Methanolic	**1**	**3,33**	**2,6**	**8.3**	**1,8**	**6**	**8,3**	**27,66**	**2,4**	**8**	**3,4**	**11,33**	**2**	**6,67**	**2,7**	**8,99**	**3,1**	**10,33**
Ethanolic	**1,1**	**3,66**	**2,4**	**7.2**	**3,8**	**12,66**	**7,2**	**24**	**2,5**	**8,33**	**1,9**	**6,33**	**2,2**	**7,33**	**2,1**	**6,99**	**1,6**	**5,33**
Acetone	**1**	**3,33**	**1,3**	**3.2**	**1,6**	**5,33**	**3,2**	**10,66**	**0,2**	**0,67**	**1**	**3,33**	**0,2**	**0,67**	**3**	**9,99**	**2,3**	**7,66**
Chloroform	**0,5**	**1,66**	**2,3**	**3 ,7**	**1**	**3,33**	**3,7**	**12,33**	**0,2**	**0,67**	**0,5**	**1,66**	**0,2**	**0,67**	**1**	**3,33**	**1,1**	**3,66**
Hexane	**0,5**	**1,66**	**1,6**	**0 ,5**	**0,9**	**3**	**0,5**	**1,66**	**0,2**	**0,67**	**0,6**	**2**	**0,3**	**1**	**0,5**	**1,66**	**1,8**	**5,99**
Aqueous	**4,2**	**14**	**4,2**	**7,87**	**4,15**	**13,83**	**7,87**	**26,33**	**2,3**	**1**	**9**	**30**	**3,27**	**10,9**	**3,8**	**12 ,6**	**6.8**	**22,6**

m: mass; yd: the extraction yield



(A) (B) (C)

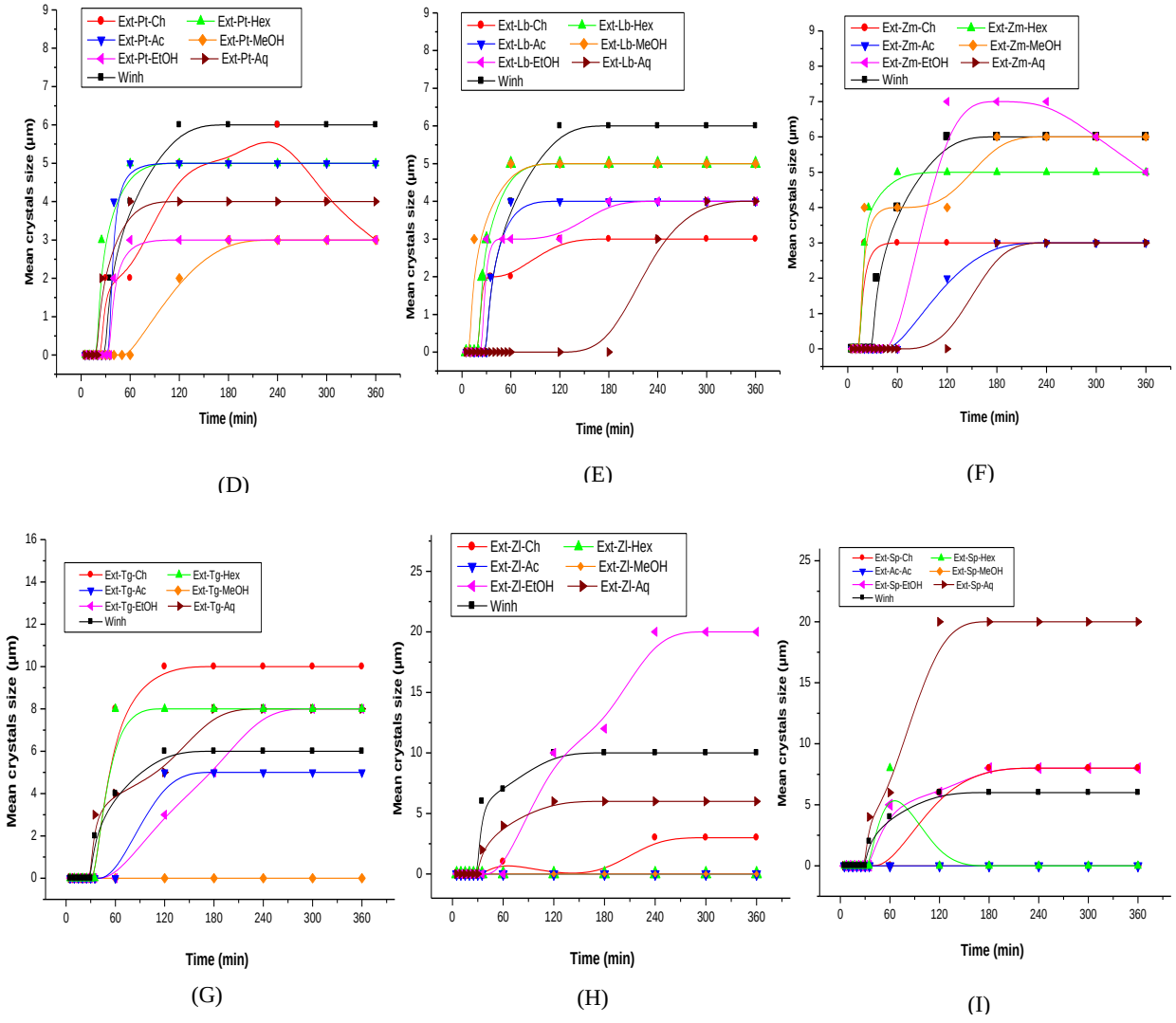
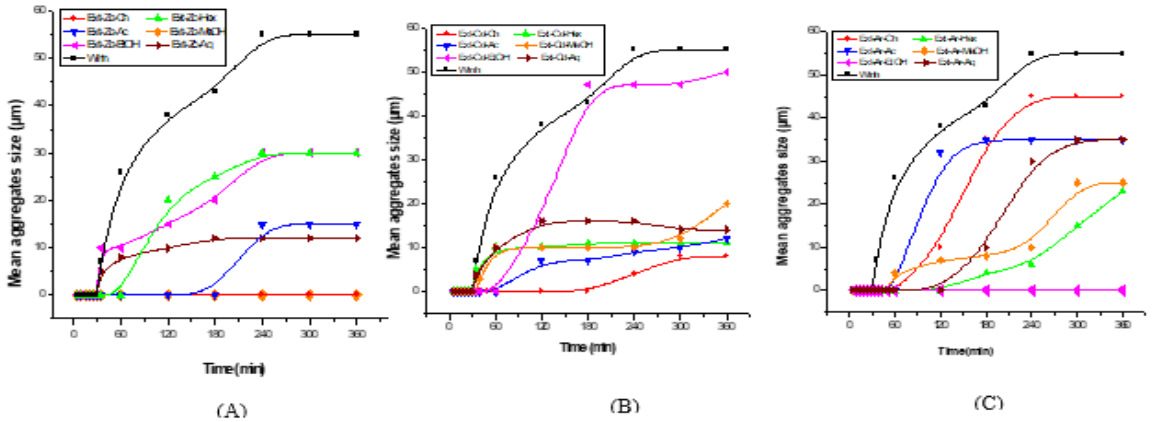


Fig. 4: Evolution the mean size of DCPD crystals at pH=6,5 in presence of extracts : (A) : extracts of *Zilla macroptera*(Zc), (B): extracts of *Cynodon dactylon L* (Cd), (C): extracts of *Artemisia campestris L* (Ar), (D): extracts of *Panicum turgidium* (Pt), (E): extracts of *Launaea arborescens* (Lb), (F) :extracts of *Zea mays L(Zm)*, (G) : extract of *Tamarix galica* (Tg), (H) : Extract of *Zizyphus lotus*(Zl),



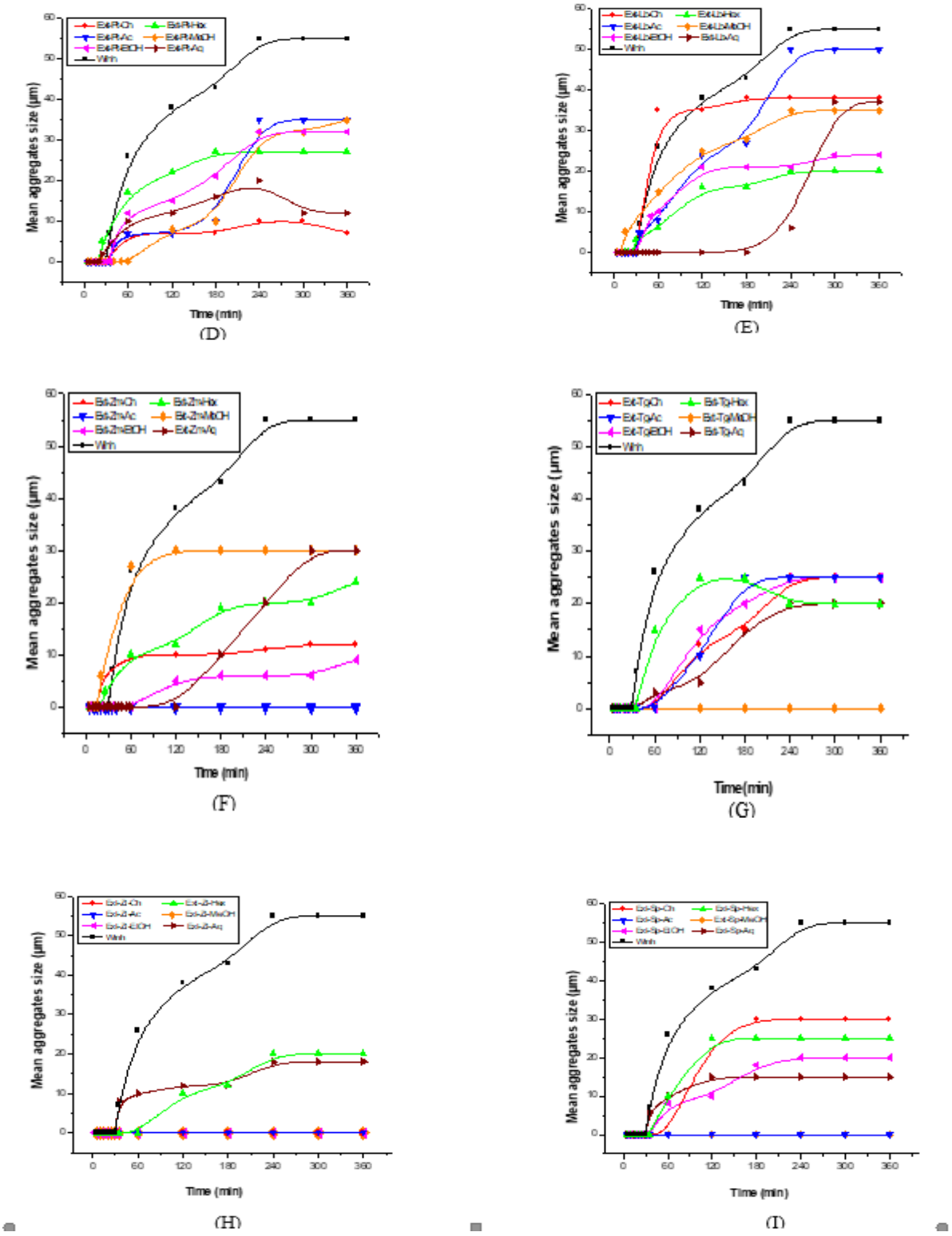
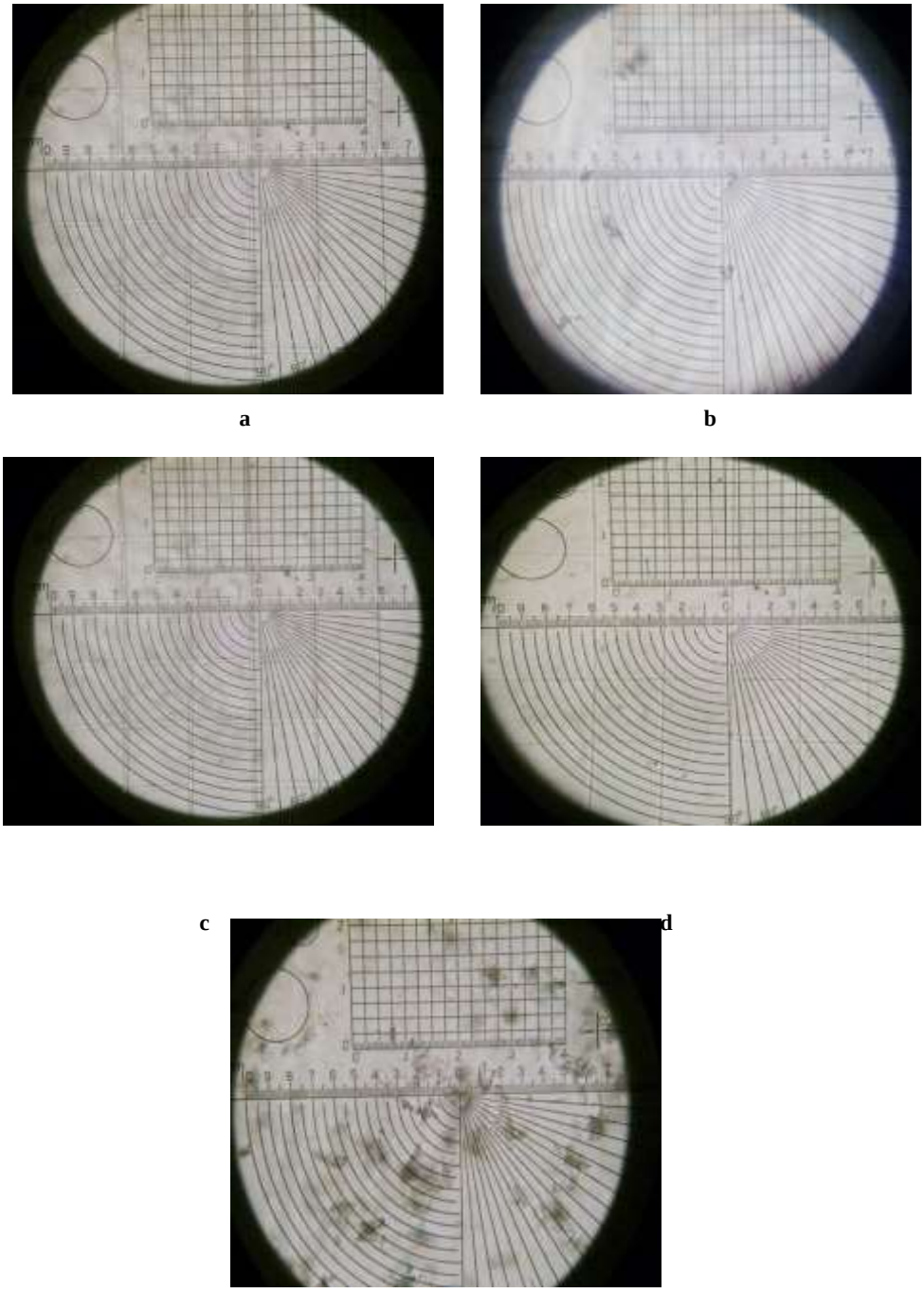


Fig. 5: Evolution the mean size of DCPD aggregates at pH=6,5 in presence of extracts: (A) : extracts of *Zilla macroptera*(Zc), (B): extracts of *Cynodon dactylon L*(Cd), (C): extracts of *Artemisia campestris L* (Ar), (D): extracts of *Panicum turgidium* (Pt), (E): extracts of *Launaea arborescens* (Lb), (F): extracts of *Zea mays L(Zm)*, (G) : extract of *Tamarix galica* (Tg), (H) : Extract of *Zizyphus lotus*(Zl), (I) : Extract of *Salsola sp* (Sp).



a **b**

c **d**

e

Fig. 6 : some Comparative view at pH = 6.5

a) Zizyphus lotus **Acetone extract** **b)** ***Tamarix galica*** **methanolic extract**
c) Zilla macroptera **chloroform extract** **d)** ***Salsola sp*** **Acétone extract**
e) without inhibitors

3.3. Comparative discussion of the results:

According to the DCPD (pH 6.5) crystallization results obtained in the presence of the extracts, we proposed a classification of the effect of these extracts on the time of appearance of the first crystals, the size of crystals, the size of aggregates and the number of their relative to crystallization without inhibitor presented in the following tables 3, 4, 5, 6, 7, 8 respectively, Fig. 6.

a) *The effect on the time of appearance of crystals and aggregates:*

Table 3: Effect of the extracts on the time of appearance of the crystals. of the crystals.

Solvent / Plant	Ch	Hex	Ac	MeOH	EtOH	Aq
Panicum turgidium	–	–	+	+ +	+	–
Cynodon dactylon L	+ +	**0**	+ +	**0**	+ +	0
Launaea arborescens	–	–	**0**	–	–	+++
Zea mays L	–	–	+ +	–	+ +	+++
Artemisia campestris L	+ +	+ + +	+ +	+	∞	+ +
Zizyphus lotus	∞	++	∞	+	∞	0
Salsola sp	++	+	∞	∞	+	0
Zilla macroptera	∞	++	+++	∞	0	0
Tamarix galica	+	+	++	∞	++	0

+	: Slight increase of the time of appearance of crystals (between 35 and 60 min).
+ +	:Average increase of the time of appearance of crystals (between 60 and 180 minutes) .
++ +	: Strong increase of the time of appearance of crystals (180 to 300 min).
∞	: Total inhibition (absence of crystals)
–	: Decrease of the time of appearance of crystals (between 5 and 35 min).
0	: No effect (neither increase nor decrease)

Table 4: Effect of extracts on the time of appearance of the aggregates.

Solvent / Plant	Ch	Hex	Ac	MeOH	EtOH	Aq
Panicum turgidium	+	–	+	+ +	+	–
Cynodon dactylon L	+++	**0**	+ +	+	+ +	**0**
Launaea arborescens	**0**	–	**0**	–	+	+++
Zea mays L	–	–	∞	–	+ +	+++
Artemisia campestris L	+ +	+ + +	+ +	+	∞	+++
Zizyphus lotus	∞	+ +	∞	∞	∞	**0**
Salsola sp	++	+	∞	∞	+	**0**
Zilla macroptera	∞	+ +	+++	∞	**0**	**0**
Tamarix galica	++	+	++	∞	++	+

+	Slight increase of the time of appearance of aggregates (between 35 and 60 min).
+ +	Average increase of the time of appearance of aggregates(between 60 and 180 minutes).
++ +	Strong increase of the time of appearance of aggregates (180 to 300 min).
∞	Total inhibition (absence of aggregates)
–	Decrease of the time of appearance of aggregates (between 5 and 35 min).
0	: No effect (neither increase nor decrease)

The plant *Zizyphus lotus* gave the best result; it represented three total inhibition of DCPD (total absence of crystals and aggregates) by three extracts:
Ext-Zl-EtOH, Ext-Zl-Ch and Ext-Zl-Ac, as well as, *Salsola Sp* and *Zilla macroptera* have made two total inhibitions of DCPD by two extracts for each one of them represented in: Ext-Sp-MeOH, Ext-Sp-Ac and Ext-Zc-MeOH, Ext-Zc-Ch, in addition, the ethanolic extract of *Artemisia campesiris L*, the methanolic extract of the plant *Tamarix galica* were presented one total inhibition of DCPD (Table 3 and 4). Total inhibition of the crystalline aggregation phase is also observed for the *Zea mays L* acetone extract and for the *Zizyphus lotus* methanolic, acetone, chloroform, and ethanol extracts. (Table 4).
Other extracts like Ext-*Zm*-Aq, Ext-*Ar*-Hex and Ext-*Zc*-Ac have an effect on the kinetics of appearance of the first crystals and aggregates, by a delay remarkable up to 240 minutes (Table 3 and 4).
The extracts of the plant *Launaea arborescens* do not have an inhibitory effect on the crystallization time of DCPD, with the exception of the aqueous extract; we have noticed a sharp increase in the time of appearance of crystals and aggreagates. But chloroform and hexane extracts of *Panicum turgidium, Launaea arborescens* and *Zea mays L* favor the appearance of crystals (Table 3), on the other hand, the methanolic and hexane extract of the last two plants who have mentioned previously favor the appearance of aggregates (Table 4)

b) The effect on the mean crystals and aggregates size:

Table 5: effect of the extract on mean crystals size

Solvent / Plant	Ch	Hex	Ac	MeOH	EtOH	Aq
Panicum turgidium	+	+	+	+ +	+ +	+
Cynodon dactylon L	++	+	–	+	+ +	+
Launaea arborescens	++	+	+	+	+	+
Zea mays L	++	+	++	+	–	+ +
Artemisia campestris L	+	+	+	+	∞	+
Zizyphus lotus	∞	+	∞	++	∞	+
Salsola sp	+	+	∞	∞	+	–
Zilla macroptera	∞	++	++	∞	+	+
Tamarix galica	+	+	++	∞	+	+

\+ : Slight decrease in crystal size (between 4 to 6 µm)
\+ + : Strong decrease in crystal size (between 1 to 3 µm).
∞ : Total absence of crystals.
– : Increase in crystal size (greater than 6 µm).

Table 6: effect of extract on the mean aggregates

Solvent / Plant	Ch	Hex	Ac	MeOH	EtOH	Aq
Panicum turgidium	+++	+ +	+ +	+ +	+ +	+++
Cynodon dactylon L	+++	+++	+++	+ + +	+	+++
Launaea Arborescens	+ +	+++	+	+ +	+ +	+ +
Zea mays L	+++	+++	∞	+ +	+ + +	+ +
Artemisia campestris L	+	+++	+ +	+ +	∞	+ +
Zizyphus lotus	∞	++	∞	∞	∞	+++
Salsola sp	++	++	∞	∞	++	+++
Zilla macroptera	∞	++	+++	∞	++	+++
Tamarix galica	++	++	++	∞	++	++

\+ : Small decrease in aggregate size (between 40 to 55 µm)
++ : Average decrease in size of aggregates (between 20 to 39 µm)
+++ : Strong decrease in the size of aggregates (between 1 to 19 µm).
∞ : Total inhibition (absence of crystals).
– : Increased crystal size (greater than 55 µm).

Most extracts of the selected plants gave an inhibition of crystals size (Table 5), this inhibition effect is considerable (decrease in crystal size from 6 µm to 1-3 µm) for the chloroform extract of *Cynodon dactylon L, Launaea Arborescens* and *Zea mays L*; the methanolic extract of *Panicum turgidium* and *Zizyphus lotus*; the acetone extract of *Zilla macroptera, Zea mays L* and *Tamarix galica*; the ethanolic extract of *Panicum turgidium* and *Cynodon dactylon L* and the aqueous extract of *Zea mays L* (Table 5). We observed that the plant *Zizyphus lotus* presented a sharp decrease in the size of the crystals and aggregates compared to other plants. On the other hand, we noticed an increase of the size of crystals for three extracts: Ext-Zm-EtOH, Ext-Cd-Ac, and Ext - Sp-Aq (Table 5). While the aggregates size decreased for all the extracts used, which showed that these plants have a significant inhibitory effect on the aggregation phase. This effect is considerable mainly for plants: *Cynodon dactylon L* and *Zilla macroptera* (aggregate size decreased from 55 µm to 5-7 µm) (Table 6).

c) The effect on the crystals and aggregates number:

The inhibition results of the crystals and aggregates number are less significant compared to the inhibition results of the crystals and aggregates size, as well as, the time of their appearance. The instability of the number poses difficulties to specify the effect of the extract on the inhibition of crystals and aggregates.

Table 7: effect of extracts on the crystals number.

Solvent / Plant	Ch	Hex	Ac	MeOH	EtOH	Aq
Panicum turgidium	+	–	+	+	+	–
Cynodon dactylon L	+	+	+	–	–	–
Launaea arborescens	+	–	+	–	–	+
Zea mays L	+	–	∞	–	+	–
Artemisia campestris L	–	+	+	–	∞	+
Zizyphus lotus	∞	+	∞	+	∞	+
Salsola sp	+	+	∞	∞	+	+
Zilla macroptera	∞	+	+	∞	+	+
Tamarix galica	+	+	+	∞	+	+

\+ : Decrease in the number of aggregates

∞ : Total absence of aggregates.

– : Increase in the number of aggregates.

Table 8: effect of the extracts on aggregates number.

Solvent / Plant	Ch	Hex	Ac	MeOH	EtOH	Aq
Panicum turgidium	+	–	+	+	–	–
Cynodon dactylon L	–	+	–	–	–	–
Launaea arborescens	+	–	+	–	+	–
Zea mays L	–	–	+	–	–	–
Artemisia campestris L	–	–	+	–	∞	–
Zizyphus lotus	∞	+	∞	+	∞	_
Salsola sp	+	+	∞	∞	+	0
Zilla macroptera	∞	+	+	∞	+	+
Tamarix galica	+	+	+	∞	+	_

\+ : Decrease in the number of crystals

∞ : Total absence of crystals.

– : Increase in the number of crystals.

0 : No effect (neither increase nor decrease)

According to the results, some plants totally inhibited the number of crystals and aggregates but others decrease the number of crystals and aggregates such as *Panicum turgidium*, *Salsola sp*, *Zilla macroptera* and *tamarix galica* at the rate of five extracts out of six for each one of them. The reduction is very important in presence of Ext-Pt-Ch.: from 78-21 crystals / mm^3 to 4-2 crystals / mm^3 and from 250 agg / mm^3 to 3 agg/ mm^3. We have found that acetone, chloroform and ethanol extracts for the majority of the plants used, generally give a decrease in the number of crystals and aggregates. On the other hand, the aqueous and hexane extracts of these plants cause an increase in this number.

4. Conclusion

In conclusion, the results obtained showed that these selected medicinal plants prove their therapeutic interest for the DCPD inhibition and confirm the results of the ethnopharmacological survey, in which, most extracts have a significant inhibitory effect on nucleation and aggregation phase of DCPD. Among the plants chosen for this study, it was identified that, six showed a total inhibition of crystallization phases of DCPD (total absence of crystals and aggregates) represented in nine different extracts. In addition, others plant extracts inhibited crystal aggregation. This could be beneficial in the prevention of stone formation. While other extracts retard the kinetics of crystallization up to 240 minutes. Some extracts decreased the crystal size. Even the inhibitory effect of the aggregation phase observed for all plant extracts, in the result, it shows that the formed crystals can be easily removed by natural means (plant extracts) through urinating by the urinary tract. The extracts presented a strong reduction of the number of crystals and aggregates.

In the light of these studies, these plants can be regarded as a promising candidate from natural sources of antilithiatic activity with high value.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

This work is self-funded

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