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The Anti-urolithiatic effect of the roots of *Saussurea costus* (falc) Lipsch agonist ethylene glycol and magnesium oxide induced urolithiasis in rats

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ABSTRACT

Phytotherapy, which involves the use of plant extracts and natural compounds for medicinal purposes, is indeed a promising alternative for managing urinary lithiasis. Many plants have been studied for their potential to prevent and treat kidney stones, and they may offer a more natural and potentially less harmful approach compared to conventional treatments. Additionally, phytotherapy may be more cost-effective. The aim of the present study was to investigate the antilithic potential of extracts and essential oils of *Saussurea costus* (Falc) Lipsch in two *in vivo* models, one on ethylene glycol-induced calcium oxalate crystal formation and the other to assess the effects of these extracts on magnesium oxide-induced struvite crystal formation. The experiment involved the administration of different doses of aqueous and ethanolic extracts of *S. costus* (200 and 400 mg/kg) and essential oils (25 and 50 mg/kg) to male *Wistar* rats, followed by the evaluation of various physiological, biochemical and histopathological parameters. The results demonstrated that the administration of *S. costus* essential oils and extracts had significant effects on the rats, influencing body weight, urine volume, crystal deposition, cytobacteriological examination of urine, and serum biochemical parameters. Histopathological examinations revealed varying impacts on the kidneys and livers of the treated rats. The findings suggest that *S. costus* extracts and essential oils may hold promise in inhibiting calcium oxalate crystal formation *in vivo* and influencing various physiological and biochemical parameters in rats. Overall, the 200 mg/kg ethanolic extract of *S. costus* demonstrated antilithiatic efficacy, did not exhibit signs of toxicity and reduced the number of crystals in the kidneys. Furthermore, the study did not find a significant effect on reducing struvite crystals.

1. Introduction

Urinary lithiasis, commonly known as kidney stones (Chandrajith et al., 2006), is the formation of stones in the kidneys or urinary tract, leading to severe pain and potential damage (Bahmani et al., 2016). This disease arises from an imbalance in urinary biochemicals, between substances that inhibit (e.g., citrate, aspartate, calprotectin) and those that promote the formation of stones (e.g., calcium, urate, cystine.) (Basavaraj et al., 2007; Freitas et al., 2002; Kachkoul et al., 2023b). Stone formation, known as lithogenesis (Słojewski, 2011), encompasses all the biological and physic-chemical processes that occur when urine becomes concentrated, leading to the formation of mineral deposits inside the kidneys (Aggarwal et al., 2013), where minerals can crystallize and agglomerate. The etiopathogenesis of this disease is multifactorial, involving anatomical, environmental, genetic and nutritional factors (Moe, 2006). Urinary calculi come in different types (Alelign and Petros,

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Table 1
Chemical compounds identified in essential oils of *S. costus*.

Peak	Name	R.Time	I.Time	F.Time	Area	Area%	Similarity
1	Beta. –Myrcene	8.143	8.115	8.180	22,488	0.01	86
2	Cyclohexane, 1-methylene-4-(1-methylethenyl) -	8.510	8.470	8.560	44,128	0.03	87
3	3-Carene	8.673	8.640	8.720	38,609	0.02	93
4	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	8.841	8.795	8.890	67,167	0.04	85
5	o-Cymene	9.059	9.020	9.110	87,601	0.06	94
6	D-Limonene	9.171	9.120	9.220	669,284	0.43	96
7	Eucalyptol	9.253	9.220	9.315	249,554	0.16	95
8	Gamma.-Terpinene	9.994	9.940	10.040	55,312	0.04	88
9	2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-	10,373	10.325	10.420	59,883	0.04	93
10	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	10.804	10.765	10.885	119,557	0.08	81
11	Linalool	11.084	11.030	11.155	1,046,483	0.66	96
12	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-	12.317	12.275	12.380	54,091	0.03	89
13	Endo-Borneol	12.916	12.870	12.380	53,387	0.03	94
14	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,	13.198	13.150	13.265	643,957	0.41	95
15	L-.alpha.-Terpineol	13.545	13.495	13.610	1,231,256	0.78	95
16	Estragole	13.749	13.685	13.815	8,315,212	5.28	95
17	5-(2H-1,3-Benzodioxol-5-ylmethyl)-3-(3-methoxyphenyl)-4,5-dihydro-1,2-oxazole	15.205	15.095	15.285	1,992,986	1.27	83
18	Anethole	16.079	15.900	16.215	141,704,677	90.05	95
19	Phenol, 2-methyl-5-(1-methylethyl)-	16.350	16.285	16.420	131,387	0.08	92
20	Benzoic acid, 4-methoxy-, methyl ester	18.141	18.100	18.185	78,181	0.05	95
21	2-Propanone, 1-(4-methoxyphenyl)-	18.366	18.330	18.430	213,729	0.14	96
22	1-(3-Methyl-2-butenoxy)-4-(1-propenyl)benzene	24.743	24.680	24.820	489,747	0.31	97

R. Time: Retention time, I. Time: initial time, F. Time: final time.

Table 2
Changes in body weight, water intake, 24-hour urine volume and pH during induction and calcium oxalate treatment for different study groups.

Groups	Change in weight (%)		Quantity consumed in ml/24 h		
	Initial weight (g)	Day 28	Water consumption	Urine volume	Urine pH
Group I (Normal)	156 ± 5.29	40 % ± 0.06	15.5 ± 1.29	18.37 ± 0.43	6.28 ± 0.53
Group II (Negative)	203 ± 15.72	28 % ± 0.09	14.46 ± 1.23	9.625 ± 0.48 ^{a*}	6.38 ± 0.63
Group III (Positive)	180 ± 18.25	32 % ± 0.07	13.13 ± 5.48	12.5 ± 0.71 $^{a\#}$	6.58 ± 0.14
Group IV (EESC 400 mg/kg)	171.3 ± 21.50	32 % ± 0.06	22.84 ± 5.15	20.53 ± 6.37$^{b\#}$	6.62 ± 1.18
Group V (EESC 200 mg/kg)	188.3 ± 9.71	22 % ± 0.04	21.63 ± 5.85	13.33 ± 3.67 ^{a*}	6.57 ± 0.95
Group VI (AESC 400 mg/kg)	199.4 ± 3.79	26 % ± 0.09	13.25 ± 4.72	11.38 ± 0.6 ^{ab*}	5.99 ± 0.63
Group VII (AESC 400 mg/kg)	190.3 ± 5.51	29 % ± 0.11	21.93 ± 5.24	17.04 ± 1.01^{b*}	6.57 ± 0.22
Group VIII (SCEO 50 µl/kg)	152.3 ± 4.93	56 % ± 0.29	19.25 ± 0.87	15.6 ± 1.34^{b*}	6.56 ± 0.05
Group IX (SCEO 25 µl/kg)	162 ± 6.08	24 % ± 0.01	19.87 ± 6.91	16.55 ± 1.45^{b*}	6.31 ± 0.14

a Comparisons with Normal Group, b Comparisons with negative Group, * Statistically significant at $p < 0.05$, # Statistically significant at $p < 0.01$, EESC: Ethanolic extract of *S. costus*. AESC: Aqueous extract of *S. costus*. SCEO: *S. costus* essential oils.

2018), such as calcium oxalate and struvite calculi, which are characterized by their chemical composition and crystalline structure, and can be identified by disease-specific morpho-constitutional analysis (Cloutier et al., 2015).

Calcium oxalate, which accounts for over 80 % of urinary calculi (Cho, 2023), is the most common type nationwide (Habbani et al., 2023; Bouatia et al., 2015). It can be classified into two types: calcium oxalate monohydrate (Whewellite) and calcium oxalate dihydrate (Weddellite) (Conti et al., 2014). The mechanism is linked to the calcium content of the intestine, which is an important determinant of calcium absorption. Thus, any decrease in calcium in the intestinal lumen raises the amount of free absorbable oxalate (Asplin, 2016). Struvite calculi are classified as infectious lithiasis (Flannigan et al., 2014), because their presence implies the intervention of a ureolytic germ capable of raising urine alkalinity sufficiently to cause the simultaneous precipitation of ammonium and magnesium phosphates (Arias et al., 2017; Erdmann and Strieth, 2023). The treatment of these two types of stones relies on conventional techniques such as extracorporeal lithotripsy and percutaneous nephrolithotomy, as well as on costly drugs that can cause side effects (Mohamed et al., 2023). This justifies the need for research into new antilithiatic compounds. Moreover, the use of phytotherapy is widely recognized in the traditional medicine of many countries for the treatment and prevention of urinary lithiasis (Nissanka et al., 2023). Numerous botanical studies have reported the use of several plants for this purpose (Nayab et al., 2023). As a result, there is growing interest in herbal medicine as a promising alternative against kidney stones. It is therefore essential to take advantage of this natural heritage to experimentally verify the efficacy of this plant.

In this study, our focus is on evaluating the antilithiatic efficacy of essential oils, aqueous and ethanolic extracts of *S. costus* using an *in vivo* model. We started by extracting the aqueous and ethanolic extracts using a Soxhlet, and the essential oils using a Clevenger. The plant compounds were then characterized using gas chromatography-mass spectrometry (GC–MS). Subsequently, we conducted a pharmacological study on animals to assess the antilithiatic activity. The first model involved rats subjected to ethylene glycol (calcium oxalate)-induced urolithiasis, while the second model used rats subjected to magnesium oxide (struvite)-induced urolithiasis. The extracts and essential oils were administered to treat the rats. In these models, we performed a cyto-bacteriological examination of the urine, used urine strips for biochemical analyses and conducted a histopathological study of the kidneys and liver. This approach enabled us to assess the safety and reliability of the doses of oils and extracts used to treat lithiasis, as well as to identify the specific crystals on which this plant has a significant effect at the scale of the living organism, allowing us to develop a Phytodrug based on this plant in future research.

2. Materials and methods

2.1. Plant collection and identification

The roots of *S. costus* were gathered in autumn in Jammu, India

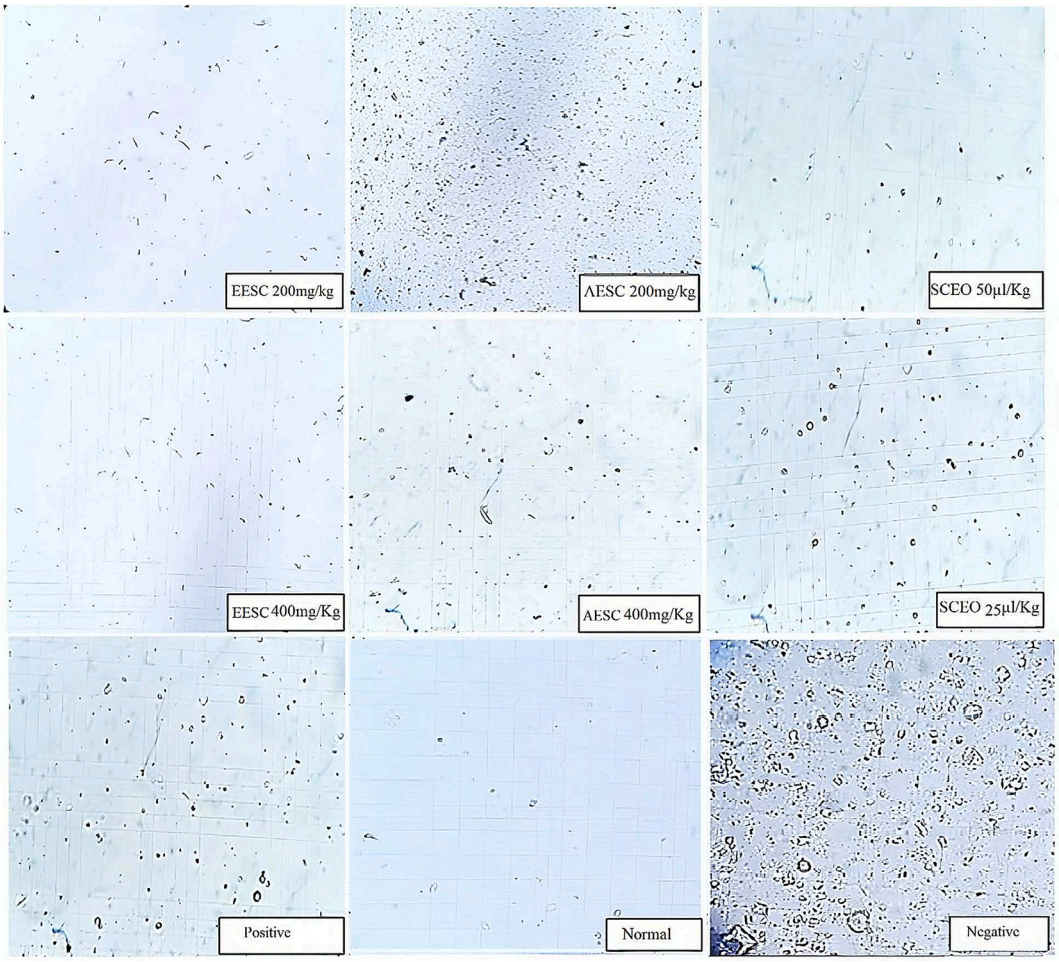

Fig. 1. Crystals and aggregates for groups treated with extracts (EESC 200 mg/kg, EESC 400 mg/kg, AESC 200 mg/kg, AESC 400 mg/kg) and essential oils (50 µl/kg and 25 µl/kg) and untreated groups (Normal and negative control) (x 400).

Table 3
Crystals and aggregates for the different groups of *Wistar* rats studied.

Groups	Number of crystals /mm^3	Aggregates
Group I (Normal)	>162.5 ± 75	–
Group II (Negative)	>987.5 ± 25 $^{a\#}$	+
Group III (Positive)	575 ± 95.74 $^{b*a\#}$	+
Group IV (EESC 400 mg/kg)	>337.5 ± 110.87 $^{b\#}$	–
Group V (EESC 200 mg/kg)	>375 ± 144.34 $^{b\#}$	+
Group VI (AESC 400 mg/kg)	950 ± 57.74 $^{a\#}$	–
Group VII (AESC 400 mg/kg)	775 ± 5 0 $^{a\#}$	+
Group VIII (SCEO 50 µl/kg)	300 ± 158.43 $^{b\#}$	–
Group IX (SCEO 25 µl/kg)	>350.5 ± 182.57 $^{b\#}$	+

+ Presence, -Absence, a Comparisons with Normal Group, b Comparisons with negative Groupe, * Statistically significant at $p < 0.05$, # Statistically significant at $p < 0.01$, EESC: Ethanolic extract of *S. costus*. AESC: Aqueous extract of *S. costus*. SCEO: *S. costus* essential oils.

(32.73° N latitude and 74.87° W longitude), and subsequently exported to Morocco in March 2021. The plant was identified at the Faculty of Medicine, Pharmacy and Dentistry, Sidi Mohammed Ben Abdellah University, Fez, Morocco, and assigned the reference specimen No. LERH-SC/15-03-21.

2.2. Preparation of plant material

2.2.1. Soxhlet extraction

Extraction was performed using the Soxhlet extractor. 20 g of *S. costus* powder was placed in a cellulose cartridge, which was then inserted into an extractor connected to a flask containing 150 ml of solvents, either 70 % ethanol (ethanolic extract) or distilled water (aqueous extract), for 5 h. When the extracting solvent became increasingly clear, extraction was finished, and the resulting solution was condensed using a vacuum rotary evaporator, then dried and stored (Jouda et al., n.d.).

2.2.2. Hydrodistillation by Clevenger

The plant's essential oils were extracted by hydrodistillation in a Clevenger. 1000 g of *S. costus* powder was placed into a 2 L flask, soaked with distilled water, and heated to boiling for 5 h. The oil-laden vapor condenses through a condenser and fall into a separating funnel. The separated oil was then dehydrated with sodium sulphate and stored at 4 °C (Negi, 2013).

2.3. Characterization of plant chemical compounds

2.3.1. Gas chromatography-mass spectrometry (GC–MS) analysis

Gas chromatography coupled with a mass spectrometer GCMS-TQ8040 SHIMADZU, JAPAN. Rtx®-5MS fused-bond column (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness, Restek, PA, USA) was utilized. The initial temperature was 50 °C and ramped up to 300 °C at a rate of 5 °C/min, followed by an isothermal hold at 300 °C for 3 min. The injector temperature was maintained at 250 °C and the helium with a flow rate of 1.5 ml/min served as carrier gas. The mass spectra conditions were as follows: Ion source temperature at 200 °C,

Table 4
Cytobacteriological examination and detection of substances in the urine of different groups of *Wistar* rats studied.

Groups	Urinary cytology				
	Red blood cells	Leukocytes	Epithelial cells	Bacteria	Yeast
Group I (Normal)	–	–	–	–	–
Group II (Negative)	–	–	+	+	–
Group III (Positive)	–	–	–	–	–
Group IV (EESC 400 mg/kg)	–	–	–	–	–
Group V (EESC 200 mg/kg)	–	–	–	–	–
Group VI (AESC 400 mg/kg)	–	–	–	–	–
Group VII (AESC 400 mg/kg)	–	–	–	–	–
Group VIII (SCEO 50 µl/kg)	–	–	–	–	–
Group IX (SCEO 25 µl/kg)	–	–	–	–	–

+Presence, -Absence, EESC: Ethanolic extract of *S. costus*. AESC: Aqueous extract of *S. costus*. SCEO: *S. costus* essential oils.

Interface temperature at 280 °C, and a mass range of 50–500 *m/z*. The samples were diluted to 1 % v/v and injected in split mode. The compounds were identified using the NIST 2017 database, and the retention indices (RIs) of the isolated components were calculated against a set of standard n-alkanes that were analyzed separately temperature at under the same chromatographic conditions (Benkaci-Ali et al., 2006).

2.4. Study of antilithiatic activity in the animal model

This study was carried out using ethanolic and aqueous extracts of the *S. costus* plant. In our previous research, these extracts revealed a better effect in the *in vitro* calcium oxalate crystallization inhibition test (Mammate et al., 2022); they also have a significant effect in inhibiting struvite crystals *in vitro* (Mammate et al., 2023). Additionally, the study tested the impact of *S. costus* essential oils. The selection of doses was based on toxicological studies to ensure the safety and efficacy (Abdelwahab et al., 2021; Abd El-Rahman et al., 2020; Mohamed Saleem et al., 2013).

2.4.1. Animal selection

Male *Wister* rats weighing 150 and 200 g, of an age corresponding to 8–9 weeks, were used in this study. The animals were kept in polypropylene cages, supplied with drinking water and fed standardized pet food (100 kg contains 400 µl vitamin A, 60 µl vitamin D3, 2.5 mg vitamin E, 0.8 kg calcium, 0.5 kg phosphorus, 0.0 kg protein, 0.0 kg lipids and 1 kg carbohydrates). In order to minimize stress on the animals, they were placed in consistent conditions. Rats were used after being acclimatized under controlled conditions of 25 ± 2 °C temperature and 12-hour light/dark cycles for 7 days. The animal care and experimental protocols followed the guidelines of the Canadian Council on Animal Care (Cross et al., 1993).

2.4.2. Ethylene glycol-induced urolithiasic (calcium oxalate) model in rats

The ethylene glycol-induced hyperoxaluria model to evaluate the antilithiatic activity of extracts and essential oils of the *S. costus* plant in the elimination of CaOx crystal deposition in *Wistar* rats. This model was carried out on nine groups of six male *Wistar* rats randomly selected to receive different treatments. The animals were exposed to a solution containing 0.75 % (v/v) ethylene glycol (EG) and 1 % (w/v) ammonium chloride for 3 days (Kachkoul et al., 2023). Then, on day 4, they were subjected to ethylene glycol only, without ammonium chloride, for a period of 11 days. The different study groups then received oral treatment via gastric tube from days 15 to 28 (Karadi et al., 2006).

Group I is the normal group that received distilled water (DW).

Group II the negative control received (0.75 % $C_2H_4(OH)_2$ +1% NH_4Cl + distilled water).

Group III the positive control received Cystone (750 mg/kg body weight).

Group IV received 400 mg/kg body weight of ethanolic extract of *S. costus* (EESC).

Group V received 200 mg/kg body weight of ethanolic extract of *S. costus* (EESC).

Group VI received 400 mg/kg body weight of aqueous extract of *S. costus* (AESC).

Group VII received 200 mg/kg body weight of aqueous extract of *S. costus* (AESC).

Group VIII received 50 µl/kg body weight of *S. costus* essential oils (SCEO).

Group IX received 25 µl/kg body weight of *S. costus* essential oils (SCEO).

2.4.3. Magnesium oxide-induced urolithiasis (Struvite) model in rats

The aim of this animal model is to induce urinary ammonium, phosphate and magnesium crystals (struvite) in rats in order to assess the antilithiatic activity of extracts and essential oils of the plant studied. This model was carried out on 9 groups containing six male *Wistar* rats randomized to receive different treatments. The animals received 0.4 % magnesium oxide (MgO) for 15 days. The different study groups then received oral treatment by gastric gavage from days 15 to 30 (Kaleeswaran et al., 2019).

Group I: The normal group received distilled water (DW).

Group II: The group received the standard drug Cystone (500 mg/kg body weight).

Group III: The negative group received magnesium oxide (0.4 %) in water.

Group IV: Rats treated with EESC (400 mg/kg body weight).

Group V: Rats treated with EESC (200 mg/kg body weight).

Group VI: Rats treated with AESC (400 mg/kg body weight).

Group VII: Rats treated with AESC (200 mg/kg body weight).

Group VIII: Rats treated with 50 µl/kg body weight SCEO essential oils.

Group IX: Rats treated with 25 µl/kg body weight of SCEO essential oils.

2.4.4. Urine sample collection and analysis

24-hour urine samples were collected from rats housed in individual metabolic cages at the end of the calcium oxalate and struvite assays. Urine was collected in a 50 ml tube for microscopic study of crystal deposition and cytobacteriological examination of the urine, and 0.5 ml of the urine sample was placed in a Malassez slide, from which the type and number of crystals were identified by polarized light microscopy (Chakit et al., 2022). The remaining urine was placed in Eppendorf tubes, to which a drop of hydrochloric acid was added and kept at 4 °C (Rashid et al., 2023).

2.4.5. Cytobacteriological examination of urine

Cytobacteriological examination of urine is a qualitative technique used to detect urinary tract infections. The sample is taken by centrifuging rat urine to remove sediment. Next, a quantity of urine is collected using a sterile swab and inoculated into a Petri dish containing Tryptone Soya Agar (TSA). The dish is incubated (at 37 °C for 24 h). The next step is to examine the bacterial colonies that have developed on the

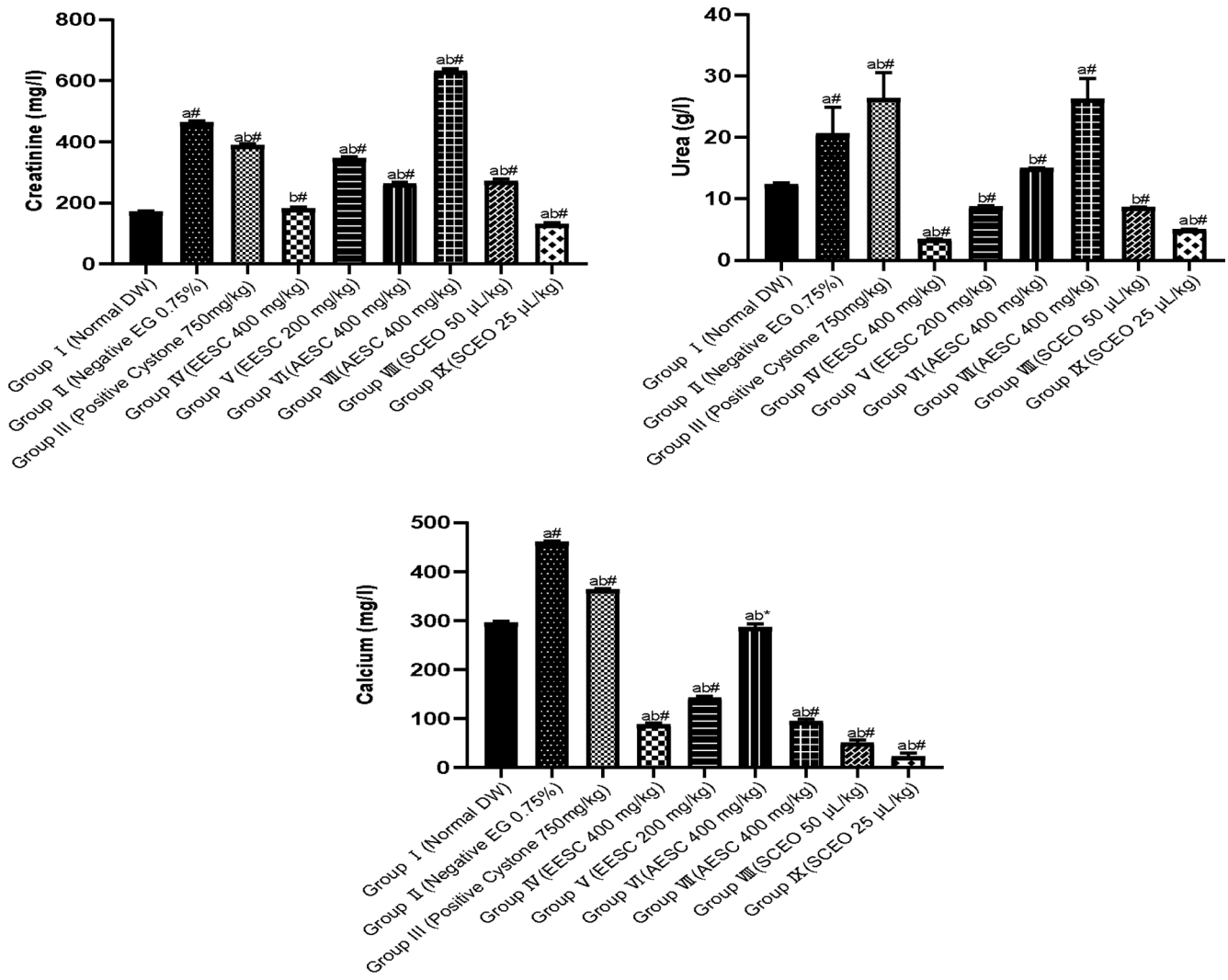

Fig. 2. Effect of *S. costus* root extracts (EESC: Ethanolic extract of *S. costus*. AESC: Aqueous extract of *S. costus*) and essential oils (SCEO: *S. costus* essential oils) on urinary parameters in control and experimental animals. a Comparisons are made with the Normal group. b Comparisons with negative group; * Statistically significant at $p < 0.05$;# Statistically significant at $p < 0.01$.

plate. Gram-positive bacteria appear purple (spherical cocci), while Gram-negative bacteria appear pink (rod-shaped bacilli) (Tripathi and Sapra, 2022). Biochemical tests such as the oxidase test and the citrate test are then used to identify Gram-negative bacteria, while the catalase test is used to identify Gram-positive bacteria. Finally, the API Gallery (BioMérieux, France) is used for precise bacterial identification.

2.4.6. Collection and analysis of serum samples

The animals were fasted for 16 h, then sacrificed according to the euthanasia method (Charbonneau et al., 2010) under anesthesia, and blood was collected by cardiac puncture (terminal technique) (Bhoopalan et al., 2023) or analysis of serum samples. The serum was separated from the blood by centrifugation at 10,000 rpm for 10 min, and standard biochemical kits were used to assay creatinine, uric acid, phosphorus and calcium. The kidneys and liver were then removed for histopathological evaluation (Patel and Acharya, 2020).

2.5. Histological study

Isolated kidneys and livers were cleaned of fatty tissue and preserved in 10 % neutral formalin. The isolated organs were then embedded in a series of alcohol, toluene baths and then placed in kerosene. Kerosene blocks are cut to 5 µm-thick sections by a microtome. Sections are collected on glass slides and allowed to dry (40–45 °C, 1 h). Eosin, toluene, hematoxylin and saffron were used to stain them. Finally, histological slides were examined under a light microscope to detect histopathological alterations in kidney tissue architecture (Guan et al., 2013). The slides were read by Pr. Hinde EL FATEMI, Departments of Pathology, University Hospital Hassan II, Fez, Morocco.

2.6. Data analysis

Results were expressed as mean ± standard deviation. Statistical comparisons were made using a one-way ANOVA employed by (GraphPad Prism 8). A significance level of $p < 0.05$ was considered statistically significant, while $p < 0.01$ was considered highly significant.

3. Results

3.1. GC–MS analysis

The composition of *S. costus* essential oils were analyzed using gas chromatography-mass spectrometry (GC–MS), which allows for the identification of volatile constituents by separating them. GC–MS analysis identified 22 different compounds in essential oils of *S. costus*,

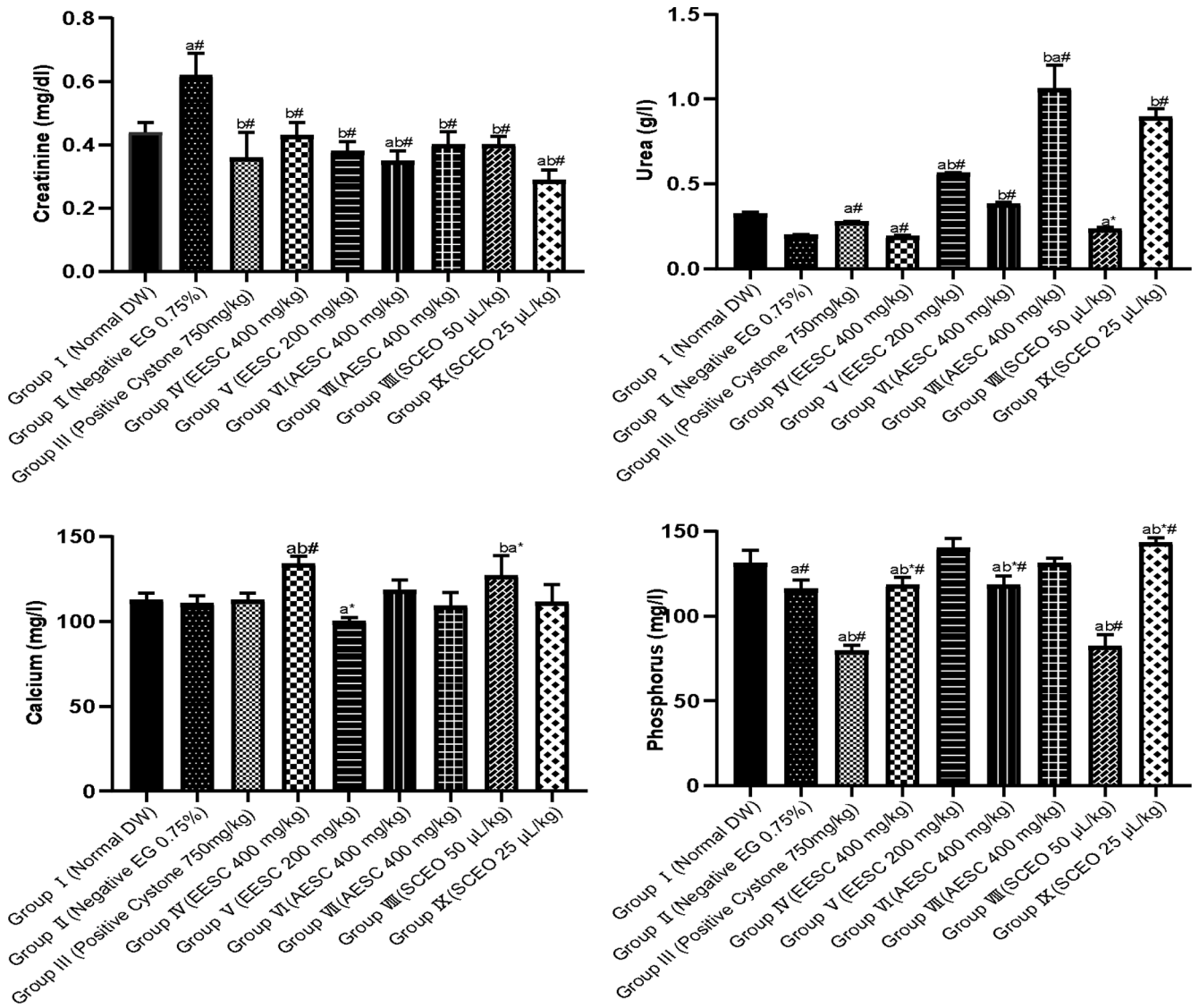

Fig. 3. Effect of *S. costus* root extracts (EESC: Ethanolic extract of *S. costus*. AESC: Aqueous extract of *S. costus*) and essential oils (SCEO: *S. costus* essential oils) on serum parameters of control and experimental animals. a Comparisons are made with the Normal group. b Comparisons with negative group; * Statistically significant at $p < 0.05$;# Statistically significant at $p < 0.01$.

including linalool (a monoterpene), L-α-terpineol (a monoterpene), estragol (a phenylpropanoid), phenol, 2-methyl-5-(1-methylethyl) (a phenolic compound) and other detected compounds, as shown in Table 1. Furthermore, analysis of the compounds in the aqueous and ethanolic extracts of *S. costus* by gas chromatography coupled to a mass spectrophotometer revealed that they were composed of several elements such as (caryophyllene oxide, 2-(hydroxymethyl)-2-nitro, quercetin, dehydrocostus lactone, etc.) for the ethanolic extract and (alpha guaiene, 2(3H)-benzofuranone, cyclodecacyclotetradecene,ect.) for the ethanolic extract and (alpha guaiene, 2(3H)-benzofuranone, cyclodecacyclotetradecene,ect.) for the aqueous extract, as demonstrated in our previous work (Mammate et al., 2022).

3.2. Study of antilithiatic activity in animal models

3.2.1. Ethylene glycol-induced urolithiasis (calcium oxalate) model in rats

3.2.1.1. The effect of S. costus extracts and essential oils on body weight variation. Table 2 illustrates the changes in rat body weight observed over the course of the 28-day experiment, from day 1 to day 28. All groups showed an increase in weight. The Negative Control group (Group II) and the Positive Control group (Group III) exhibited less weight gain compared to the Normal group (Group I). Among the treated groups, rats receiving *S. costus* ethanolic extract at a dose of 400 mg/kg and *S. costus* essential oils at a dose of 50 µl/kg displayed weight increases of 32 % ± 0.06 and 56 % ± 0.29, respectively. In contrast, the two groups treated with *S. costus* essential oils at 25 µl/kg and ethanolic extract at 200 mg/kg showed lower weight gain compared to all other groups, with respective weight increases of 24 % ± 0.01 and 22 % ± 0.04.

3.2.1.2. Water consumption, volume and pH over 24 h. The results presented in Table 2 show an increase in water consumption for the two groups receiving *S. costus* ethanolic extract at 400 mg/kg and 200 mg/kg, with mean volumes of 22.84 ± 5.15 and 21.63 ± 5.85, respectively, compared with the other study groups. Furthermore, the 24-hour urine volume values in Table 2 demonstrate a close correlation with the amount of water consumed, showing a difference of 3 ml. Indeed, a significant difference is observed between the group receiving ethanolic extract of *S. costus* at 200 mg/kg, with a mean volume of 11.38 ± 0.6 (<0.05), and the I (Normal) and II (Negative Control) groups, with respective volumes of 18.37 ± 0.43 and 9.625 ± 0.48. Furthermore,

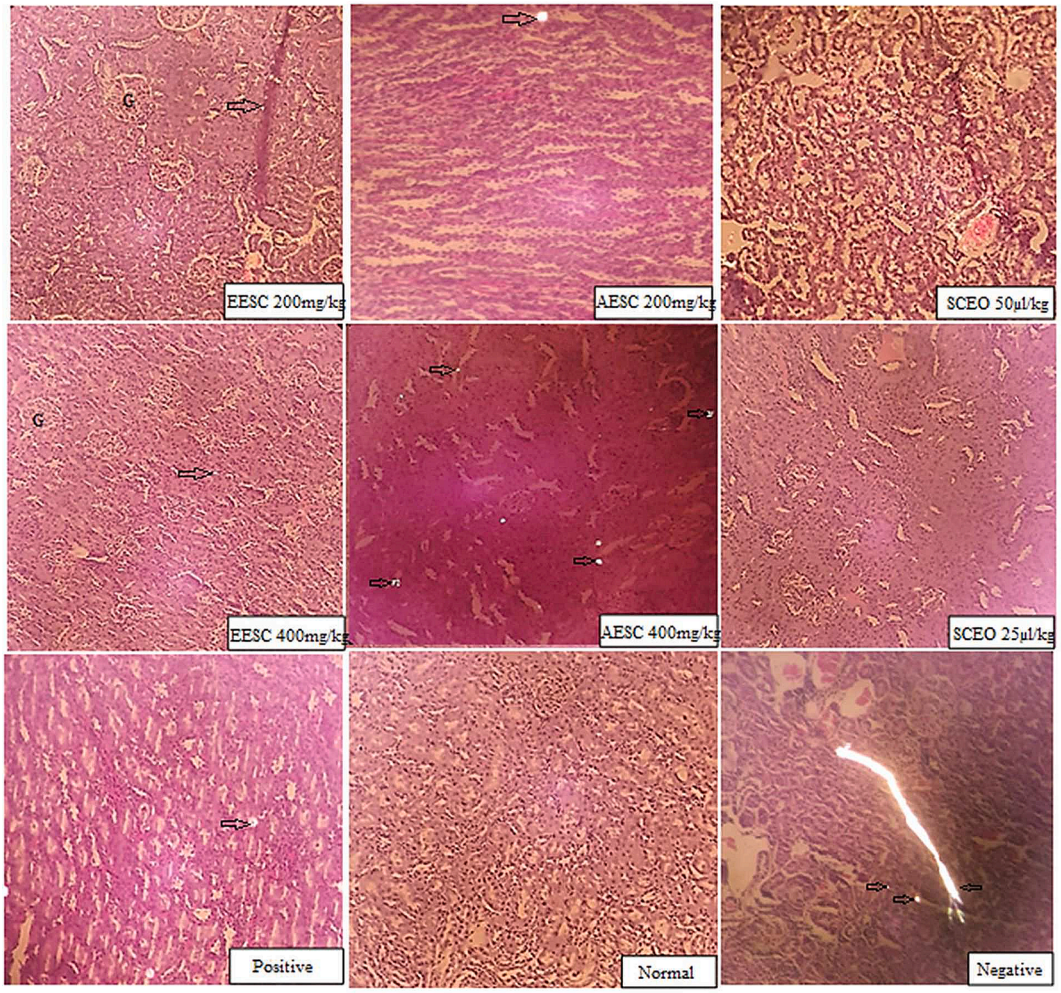

Fig. 4. Histology of kidneys from groups treated (Normal and negative) with extracts (EESC 200 mg/kg, EESC 400 mg/kg, AESC 200 mg/kg, AESC 400 mg/kg) and essential oils (50 µl/kg and 25 µl/kg). Black arrow: calcium oxalate crystals and aggregation; G: tubular glomerulus; H&E staining (x400).

with regard to pH, none of the groups studied showed any significant influence compared with the group I (Normal).

3.2.1.3. Microscopic observation. According to the microscopic observations presented in Fig. 1, calcium oxalate crystals were detected in the urine of all rat groups. However, rats in group II (Negative Control), exposed to ethylene glycol for 14 days, showed a higher number of large crystals compared with the group I (Normal), which had only received distilled water. The latter showed the lowest number of calcium oxalate crystals. Crystallization was performed at the end of the experiment for all groups of rats, except for the EG group (exposed to ethylene glycol) where crystallization was performed on day 14.

Table 3 shows crystal count data, demonstrating that *S. costus* essential oils, administered at a dose of 50 µl/kg, resulted in an observed crystal count of $300 \pm 158.43/mm^3$ ($p < 0.01$). In contrast, essential oils administered at a dose of 25 µl/kg showed an observed crystal count greater than $350.5 \pm 182.57/mm^3$ ($p < 0.01$), and ethanolic extract of *Saussurea costus*, administered at a dose of 400 mg/kg, showed an observed crystal count greater than $337.5 \pm 110.87/mm^3$ ($p < 0.01$).

3.2.1.4. Cytobacteriological examination of urine and detection of substances in urine. Based on the negative urine cytology results presented in Table 4, urine samples from rats treated with *S. costus* extract and essential oils, as well as untreated rats, did not show any abnormal cells or bacteria. Consequently, it is inferred that a urine cytobacteriological examination is unnecessary, as the urine cytology results suggest the absence of abnormalities.

3.2.1.5. Urinary parameters of rats treated and untreated with S. costus extracts and essential oils. The results depicted in Fig. 2 indicate that all groups exhibited higher creatinine concentrations compared to group I (Normal), except for group IV treated with 400 mg/kg ethanolic extract of *S. costus*, which showed a concentration of 181.9 ± 5.54 ($p < 0.01$), and was statistically similar to group I (Normal). Urea and calcium concentrations in ethanolic extracts of 200 mg/kg (8.78 ± 0.106; 142.7 ± 2.363 ($p < 0.01$)) and 400 mg/kg (3.47 ± 0.046; 88.4 ± 2.278 ($p < 0.01$)) were lower than those in group I (Normal). Furthermore, administration of essential oils at a dose of 25 mg/kg reduced creatinine, urea and calcium concentrations.

3.2.1.6. Biochemical blood parameters of rats treated and untreated with S. costus extracts and essential oils. The experiment demonstrated that rats treated with various *S. costus* compounds had lower creatinine levels than the normal group. Essential oils doses (25 µl/kg), aqueous extract (400 mg/kg) and ethanolic extract (200 mg/kg) resulted in reduced creatinine concentration, yielding concentrations of 0.29 ± 0.030 mg/dl ($p < 0.01$), 0.35 ± 0.030 mg/dl ($p < 0.01$), and 0.38 ± 0.029 mg/dl ($p < 0.01$) respectively, which were lower than that of the negative control group (0.61 ± 0.070 mg/dl). In addition, urea, calcium and phosphorus concentrations for all treated groups were similar to those of the normal group, in contrast to the negative control group.

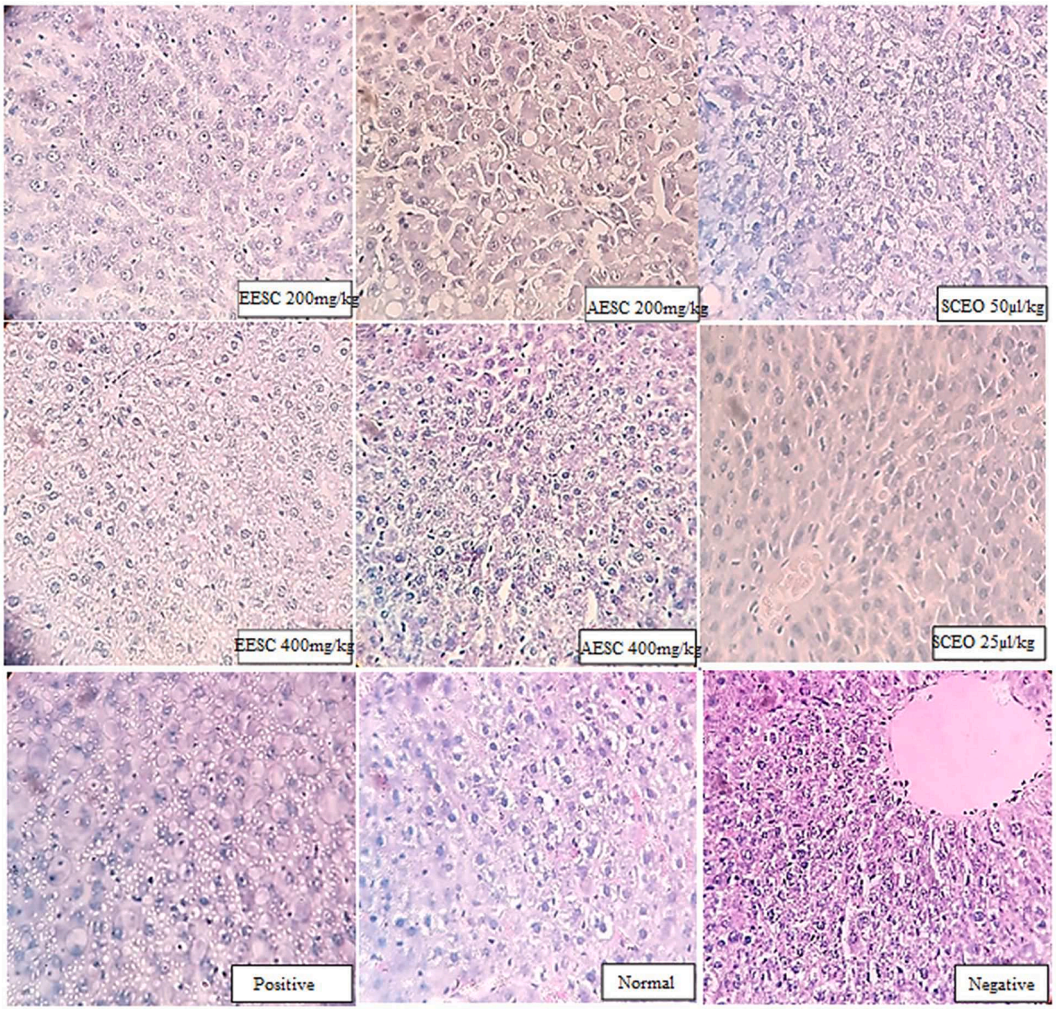

Fig. 5. Histology of livers from groups treated (Normal and EG0.75 %) with extracts (EESC 200 mg/kg, EESC 400 mg/kg, AESC 200 mg/kg, AESC 400 mg/kg) and essential oils (50 µl/kg and 25 µl/kg); H&E staining (x400).

3.2.1.7. Histological study. This study analyzed the effect of various *S. costus* extracts on rat kidney and liver through histopathological examination, as shown in Figs. 4 and 5. Doses of 200 and 400 mg/kg of aqueous and ethanolic extracts showed no toxicity and smaller calcium oxalate crystals. However, essential oils doses of 50 and 25 µl/kg caused foci of acute tubular necrosis without crystals. The negative control group showed irregular crystals and foci of acute tubular necrosis. In the liver, 200 and 400 mg/kg extracts caused no toxicity, while the doses of the essential oil produced Centrilobular necrosis, similar to the negative control group with ethylene glycol.

3.2.2. Magnesium oxide induced urolithiasis (Struvite) model in rats

3.2.2.1. Effect of S. costus extracts and essential oils on body weight variation. Throughout the 30-day experiment, changes in rat body weight were monitored and are summarized in Table 5. All groups exhibited weight gain during this period. Notably, the Negative Control (Group II) and the Positive Control (Group III) showed a greater increase in weight compared to the Normal group (Group I). Among the treated groups, those administered with 400 mg/kg ethanolic extract, 400 mg/kg aqueous extract and 25 µl/kg *S. costus* essential oils demonstrated weight gains of 35 % ± 0.11, 23 % ± 0.08 and 22 % ± 0.05 respectively. In contrast, the group receiving 200 mg/kg aqueous extract showed lower weight gain, with a percentage of 18 % ± 0.05, compared to all other groups.

3.2.2.2. Water consumption, volume and pH over 24 h. According to the data in Table 5, the groups treated with extracts and essential oils of the *S. costus* plant showed closer water consumption than the I (Normal) group. These results revealed an increase in water consumption for both groups administered with ethanolic extracts at 400 mg/kg, with a volume of 18.58 ± 2.21, and at 200 mg/kg with a volume of 18.63 ± 0.48. Furthermore, with regard to 24-hour urine volume, the values in Table 5 showed a close correlation with the amount of water consumed, with a difference of 4 ml. The group treated with the 50 µl/kg dose of essential oils displayed a lower urinary volume of 12.87 ± 1.38 compared to all other groups. In terms of pH, none of the studied groups showed any significant deviation compared to the normal group.

3.2.2.3. Microscopic observation. Ammonium and magnesium phosphate crystals were observed in the urine of all groups of rats, with a higher number of large crystals in the magnesium oxide-exposed group than in the control group. The normal group had the fewest calcium oxalate crystals, the basis of struvite crystals. However, the groups treated with aqueous and ethanolic extract of *S. costus* at specific doses showed a significant reduction in crystal size compared with the other groups, as shown in Fig. 6.

Based on the information provided, it can be concluded that the aqueous and ethanolic extracts of the studied plant demonstrated significant effects on the inhibition of struvite crystallization *in vivo*. Specifically, at doses of 400 mg/kg and 200 mg/kg respectively, these

Table 5
Variations in body weight, water intake, 24-hour urine volume and pH, and struvite crystals and aggregates and their numbers for different groups of *Wistar* rats.

Groups	Change in weight (%)		Quantity consumed in ml 24 h		
	Initial weight (g)	Day 28	Water consumption	Urine volume	Urine pH
Group I (Normal)	137.33 ± 16.16	49 % ± 0.08	17.88 ± 0.85	14.35 ± 2.95	6.37 ± 0.07
Group II (Negative)	169.67 ± 43.36	48 % ± 0.51	17.96 ± 2.42	13.18 ± 4.81	9.11 ± 0.51
Group III (Positive)	156 ± 35.68	52 % ± 0.55	17.13 ± 07.5	14.38 ± 2.43	7.48 ± 0.97
Group IV (EESC 400 mg/kg)	158 ± 7	35 % ± 0.11	18.58 ± 2.21	13.12 ± 3.14	7.07 ± 0.43
Group V (EESC 200 mg/kg)	182.33 ± 22.75	20 % ± 0.14	18.63 ± 0.48	13.87 ± 0.75	7.32 ± 0.01
Group VI (AESC 400 mg/kg)	196.67 ± 2.88	23 % ± 0.08	17.87 ± 2.29	13 ± 1.58	8.89 ± 0.02
Group VII (AESC 400 mg/kg)	194.33 ± 7.02	18 % ± 0.05	17.05 ± 1.65	13.44 ± 3.39	8.39 ± 0.05
Group VIII (SCEO 50 µl/kg)	178 ± 17.35	20 % ± 0.11	17.45 ± 1.38	12.87 ± 1.38	7.53 ± 0.03
Group IX (SCEO 25 µl/kg)	165.33 ± 5.51	22 % ± 0.05	17.75 ± 2.81	13.25 ± 1.70	7.25 ± 0.02

EESC: Ethanolic extract of *S. costus*. AESC: Aqueous extract of *S. costus*. SCEO: *S. costus* essential oils.

extracts significantly reduced the amount of crystals. The first extract showed a reduction of 93 ± 91.95 ($p < 0.01$) compared to the normal group, while the second extract showed a reduction of over 537 ± 335.09 ($p < 0.05$) (Table 6). Microscopic observations of the crystals also confirmed these results (Fig. 6).

3.2.2.4. Cytobacteriological examination of the urine. Table 7 displays negative urine cytology results for urine samples from treated rats, while the negative control group (group II) shows the presence of epithelial cells and bacilli. Subsequently, cytobacteriological examination of the urine was carried out to identify the types of bacteria (Fig. 7).

The urinary cytobacteriological examination reveals the presence of four bacterial strains in the urine of the group II, induced by magnesium oxide (Fig. 7). The strains identified are *Staphylococcus coagulase-negative*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Acinetobacter baumanni/calcoaceticus*, with the last three strains having urease activity.

3.2.2.5. Urinary parameters of rats treated and untreated with S. costus extracts and essential oils. The results of the study show that aqueous, ethanolic and essential oils extracts of the plant studied reduced creatinine and urea levels in the treated groups (Fig. 8). The group treated with 25 µl/kg *S. costus* essential oils had the lowest concentrations of creatinine (143.8 ± 9.65) and urea (5.60 ± 1.34) compared with the control group II ($p < 0.01$), which had higher concentrations of these markers (creatinine: 775.8 ± 8.47, urea: 18.59 ± 6.22). However, all treated groups had higher magnesium concentrations than group II.

3.2.2.6. Biochemical blood parameters of rats treated and untreated with S. costus extracts and essential oils. Fig. 9 shows that biochemical analyses of sera from treated groups indicate lower concentrations than those from group II (negative control). Furthermore, serum concentrations in all groups treated with extracts and essential oils of the studied plant are nearly comparable to those in group I (normal). The 50 µl/kg essential oils group showed lower concentrations of creatinine (0.20 mg/dl ± 0.06 ($p < 0.01$)), urea (0.177 g/l ± 0.006 ($p < 0.01$)), phosphate (29.80 ± 6.83 mg/l) and magnesium (74.6 mg/l ± 4.62 ($p < 0.01$)) than group I (normal).

3.2.2.7. Histological studies. In this experiment, we investigated the effects of aqueous, ethanolic and oil extracts of *S. costus* on the kidneys and livers of rats. Histopathological examinations revealed that all groups treated with 50 and 25 µm/kg oils, as well as aqueous and ethanolic extracts of *S. costus* at doses of 200 and 400 mg/kg, exhibited the presence of ammonium and magnesium phosphate crystals, as well as acute congestion of glomerular vessels in Figs. 10 and 11. The 50 and 25 µm/kg oils also showed foci of necrosis, except for the normal control group. Liver samples from groups treated with aqueous and ethanolic extracts of *S. costus*, as well as the group treated with cystone, displayed normal characteristics, with no signs of toxicity, similar to the normal control group. Conversely, the groups treated with *S. costus* essential oils at 50 and 25 mg/kg showed hepatic steatosis, similar to the negative control group.

4. Discussion

In this research, we first carried out aqueous and ethanolic extraction of *S. costus* roots using the Soxhlet method, giving yields of 27 % and 35 % (w/w) respectively. In our recent work (Mammate et al., 2022), these extracts were identified by GC–MS, which showed the presence of compounds such as quercetin, cyclocycline tetradecin and costus; 9,12,15-octadecatriene. We also extracted *S. costus* essential oils by the Clevenger method, obtaining a yield of 0.86 % (v/v). These results are similar to those obtained by (Liu et al., 2012), who obtained a yield of 0.89 % in their study. GC–MS revealed the presence of 22 compounds in the essential oils, which similar slightly from a study by (Lammari et al., 2021), which counted a total of 21 components in the essential oil of the same plant, including phenolic compounds, sesquiterpenoids and monoterpenoids such as linalool, limonin, L-α-terpineol and estragole. These compounds are also identified in the work of (Liu et al., 2012), as well as in that of (Andola et al., 2013). These results demonstrate the richness of the aqueous and ethanolic extracts and essential oils of the plant studied in this experiment in terms of secondary metabolites, which could be responsible for the anti-lithiasis effects in the *in vitro* model. However, confirmation of these results depends on evaluating the effects of this plant in an *in vivo* model. In this study, we used two *in vivo* models. The first model studied the effect of extracts and essential of this plant on calcium oxalate kidney stones induced in male *Wistar* rats. This was achieved using 0.75 % ethylene glycol and 1 % ammonium chloride, which induced hyperoxaluria and renal retention in the rats. Table 2 shows the evolution of rat body weight, with all groups showing an increase in weight, but groups II and III gaining less weight than group I. Among the treated groups, rats receiving ethanolic extract of *S. costus* at a dose of 400 mg/kg and essential oils at a dose of 50 µl/kg showed weight increases of 32 % ± 0.06 and 56 % ± 0.29 respectively. In contrast, rats treated with essential oils at 25 µl/kg and ethanolic extract at 200 mg/kg showed lower weight gain than the other groups, with respective percentages of 24 % ± 0.01 and 22 % ± 0.04. These results are not compatible with the work of (Sadki and Atmani, 2017), whose study shows that the ethylene glycol-induced group gained more weight than the group treated with the aqueous extract of *E. multiflora*. Fig. 1 illustrates the presence of calcium oxalate crystals in the urine of all rat groups. However, the group II, exposed to ethylene glycol for 14 days, showed a greater number of large crystals, followed by the group III. These results corroborate earlier work by (Karadi et al., 2006; Kachkoul et al., 2023a). Furthermore, this figure clearly demonstrates that the administration of 50 µl/kg essential oils and 400 mg/kg ethanolic

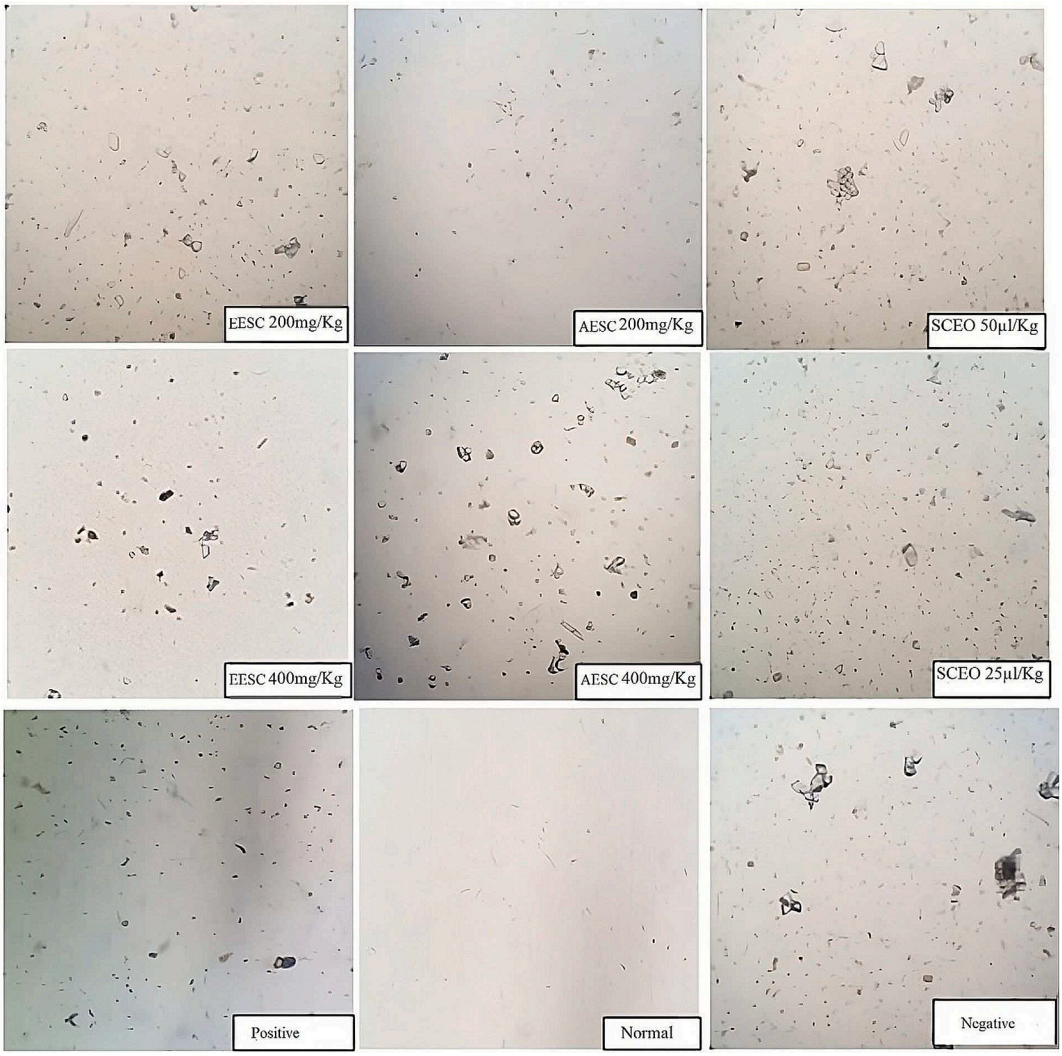

Fig. 6. Crystals and aggregates for groups treated with extracts (EESC 200 mg/kg, EESC 400 mg/kg, AESC 200 mg/kg, AESC 400 mg/kg) and essential oils (50 µl/kg and 25 µl/kg) and untreated (normal and negative control) (x400).

Table 6
Crystals and aggregates for the different groups of *Wistar* rats studied.

Groups	Number of crystals /mm^3	Aggregates
Group I (Normal)	>220 ± 91.77	–
Group II (Negative)	>998 ± 2.5	+
Group III (Positive)	>707 ± 35.94 $^{a\#b*}$	+
Group IV (EESC 400 mg/kg)	>612 ± 51.88 ^{a*b*}	+
Group V (EESC 200 mg/kg)	>93 ± 91.95$^{a\#}$	+
Group VI (AESC 400 mg/kg)	>537 ± 335.09 ^{a*b*}	+
Group VII (AESC 400 mg/kg)	>503 ± 140.44 ^{a*b*}	–
Group VIII (SCEO 50 µl/kg)	>921 ± 147.67$^{a\#}$	+
Group IX (SCEO 25 µl/kg)	>947 ± 105^{a*}	+

+Presence, −Absence, a Comparisons with Normal Group, b Comparisons with EG Group, * Statistically significant à $p < 0.05$, # Statistically significant à $p < 0.01$, EESC: Ethanolic extract of *S. costus*. AESC: Aqueous extract of *S. costus*. SCEO: *S. costus* essential oils.

extract of *S. costus* has a significant effect on the elimination and reduction of crystal size, while the group treated with 400 mg/kg ethanolic extract achieves better results than the other groups. The data in Table 3 indicate that the essential oils of the plant studied, administered at a dose of 50 µl/kg, reduced the number of crystals observed to 300 ± 158.43/mm^3 ($p < 0.01$). In contrast, administration of ethanolic extract at a dose of 400 mg/kg led to a higher number of crystals observed, with 337.5 ± 110.87/mm^3 ($p < 0.01$). All these doses have a significant effect on the inhibition of calcium oxalate crystallization *in vivo*. These results suggest that the use of the plant studied may be a promising option for inhibiting calcium oxalate crystal formation *in vivo*.

The uric biochemical parameter results in Fig. 2 show that all groups have higher creatinine concentrations than the I group, suggesting a possible impairment of glomerular filtration. With the exception of the IV group treated with 400 mg/kg ethanolic extract of *S. costus* and having a concentration of 181.9 ± 5.54 ($p < 0.01$) similar to the group I, urea and calcium concentrations in ethanolic extracts (3.47 ± 0.046; 88.4 ± 2.278, $p < 0.01$) were lower than those of the group I. In addition, the administration of 25 mg/kg of essential oils decreased creatinine, urea and calcium concentrations. These results are not similar to those of work by (Karadi et al., 2006) and by (Bagheri et al., 2018), because in his experiment, the normal group had a lower value than the treated group. With regard to serum biochemical parameters, Fig. 3 reveals that treated rats show serum creatinine levels similar to those of the I group (Normal). However, the administration of 25 µl/kg essential oils and 400 mg/kg aqueous extract resulted in a decrease in creatinine concentration with 0.29 ± 0.030 mg/dl ($p < 0.01$) and 0.35 ± 0.030 mg/dl ($p < 0.01$) respectively, compared with those observed in the group II, which had a creatinine concentration of 0.61 ± 0.070 mg/dl. In addition, urea, calcium and phosphorus concentrations for all treated groups were close to those observed in the group I. These results are

Table 7
Cytobacteriological examination and detection of certain substances in the administration of the different groups of *Wister* rats studied.

Groupes	Urinary cytology				
	Red blood cells	Leukocytes	Epithelial cells	Bacteria	Yeast
Groupe I (Normal)	–	–	–	–	–
Groupe II (Negative)	–	–	+	+	–
Groupe III (Positive)	–	–	–	–	–
Groupe IV (EESC 400 mg/kg)	–	–	–	–	–
Groupe V (EESC 200 mg/kg)	–	–	–	–	–
Groupe VI (AESC 400 mg/kg)	–	–	–	–	–
Groupe VII (AESC 400 mg/kg)	–	–	–	–	–
Groupe VIII (SCEO 50 µl/kg)	–	–	–	–	–
Groupe IX (SCEO 25 µl/kg)	–	–	–	–	–

+Presence, -Absence, EESC: Ethanolic extract of *S. costus*. AESC: Aqueous extract of *S. costus*. SCEO: *S. costus* essential oils.

similar to those of (Karadi et al., 2006; Kumar et al., 2016; Sayed, 2023) even if there is a difference in the plants used, but the results for the control group remain almost the same.

Analyze the impact of various extracts and essential oils of *S. costus* on rat kidneys and liver, based on histopathological examinations. The results of the study, shown in Fig. 4, indicate that groups of rats given aqueous and ethanolic extracts of the plant studied at doses of 200 and 400 mg/kg showed no signs of toxicity. These groups of rats show smaller calcium oxalate crystals than the III group treated with cystone (750 mg/kg), present inside the tubules, along the nephron and at the papillary level. In contrast, groups of rats given essential oils doses of 50 and 25 µl/kg showed no crystals, but did reveals foci of acute glomerular necrosis. Group II showed large, irregular crystals and foci of acute glomerular necrosis. These results are quite similar to those of (Aburjai and Al-Mamoori, 2020) and (Liu et al., 2012). The histopathological study also showed the presence of crystals with signs of inflammation. Furthermore, the histological analyses of liver samples presented in Fig. 5 show that the groups of rats treated with the aqueous and ethanolic extracts of *S. costus* at doses of 200 and 400 mg/kg, as well as group III present a normal appearance and no signs of toxicity, which is similar to group I. In contrast, the groups of rats treated with essential oils at doses of 50 and 25 mg/kg show the presence of hepatic steatosis and these results are similar to those observed in the group II that received ethylene glycol. Thus, these results are in agreement with the findings of (Bagheri et al., 2018) which showed that the ethylene glycol-induced group causes liver damage.

The second model was used to study the effect of the plant on another type of crystal called struvite, induced in rats by the administration of 0.4 % magnesium oxide (MgO). This leads to the formation of stones are composed of magnesium ammonium phosphate. Ammonium-magnesium phosphate (struvite) crystals were observed in the urine of all groups of rats, as shown in Fig. 6. However, group II, which was exposed to 0.4 % magnesium oxide for 15 days, showed a greater number of large crystals than group I. These results are similar to those obtained by (Kaleeswaran et al., 2019), in their experiment. Nevertheless, Fig. 6 clearly demonstrates that the group treated with a 200 mg/kg dose of aqueous extract and a 400 mg/kg dose of ethanolic extract of *S. costus* had an effect on crystal size reduction compared with the other groups. Table 6 presents data on the quantity of crystals, demonstrating the significant effect of the administration of aqueous and ethanolic extracts of the plant studied at doses of 400 mg/kg and 200 mg/kg respectively on the inhibition of struvite crystallization *in vivo*. For the first extract, the amount of crystals was 93 ± 91.95 ($p < 0.01$) higher, while for the second extract it was 537 ± 335.09 ($p < 0.05$) higher, with the presence of aggregates in all groups except group I. These results are similar to those obtained by (Kaleeswaran et al., 2019). The data presented in Table 7 reveal that urine samples from the treated rat groups all gave negative results for urinary cytology, unlike group II where the presence of epithelial cells and bacteria was observed. On this basis, we carried out a cytobacteriological examination of the urine in order to identify the types of bacteria, Fig. 7 reveal the presence of four bacterial strains in the urine of animals in the group II. The strains identified were *Staphylococcus* coagulase-negative, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Acinetobacter baumanni/calcoaceticus*, the latter three having ureasic activity. This result is important because it suggests that animals can host these urease bacteria when induced with struvite. This conclusion is supported by research (Pang et al., 2015) showing that struvite promotes biofilm formation, creating an environment conducive to the growth of bacteria, particularly those producing urease. In addition, the results of the urinary biochemical parameters in Fig. 8 show that the groups treated with the aqueous, ethanolic and essential oils extracts of the plant studied had lower creatinine and urea concentrations. In particular, the group treated with essential oils at a dose of 25 µl/kg showed a creatinine concentration of 143.8 ± 9.65 ($p < 0.01$) and a urea concentration of 5.60 ± 1.34 ($p < 0.01$), both lower than group II with a creatinine concentration of 775.8 ± 8.47 and a urea concentration of 18.59 ± 6.22. However, all groups treated with *S. costus* extracts and essential oils had higher magnesium concentrations than group II. These results are similar to those obtained by B. Kaleeswaran and al (Kaleeswaran et al., 2019) in their experiment, which showed variations in urinary biochemical parameters between normal-negative and treated groups. These results suggest that this increase could be due to the presence of mineral components, notably magnesium, in the plant studied. On the other hand, Fig. 9 reveals that

Fig. 7. Petri dishes containing the different bacteria identified, A (*Acinetobacter baumannii/calcoaceticus*), B (*Klebsiella pneumoniae*), C (*Staphylococcus aureus*) and D (*Staphylococcus* sp.).

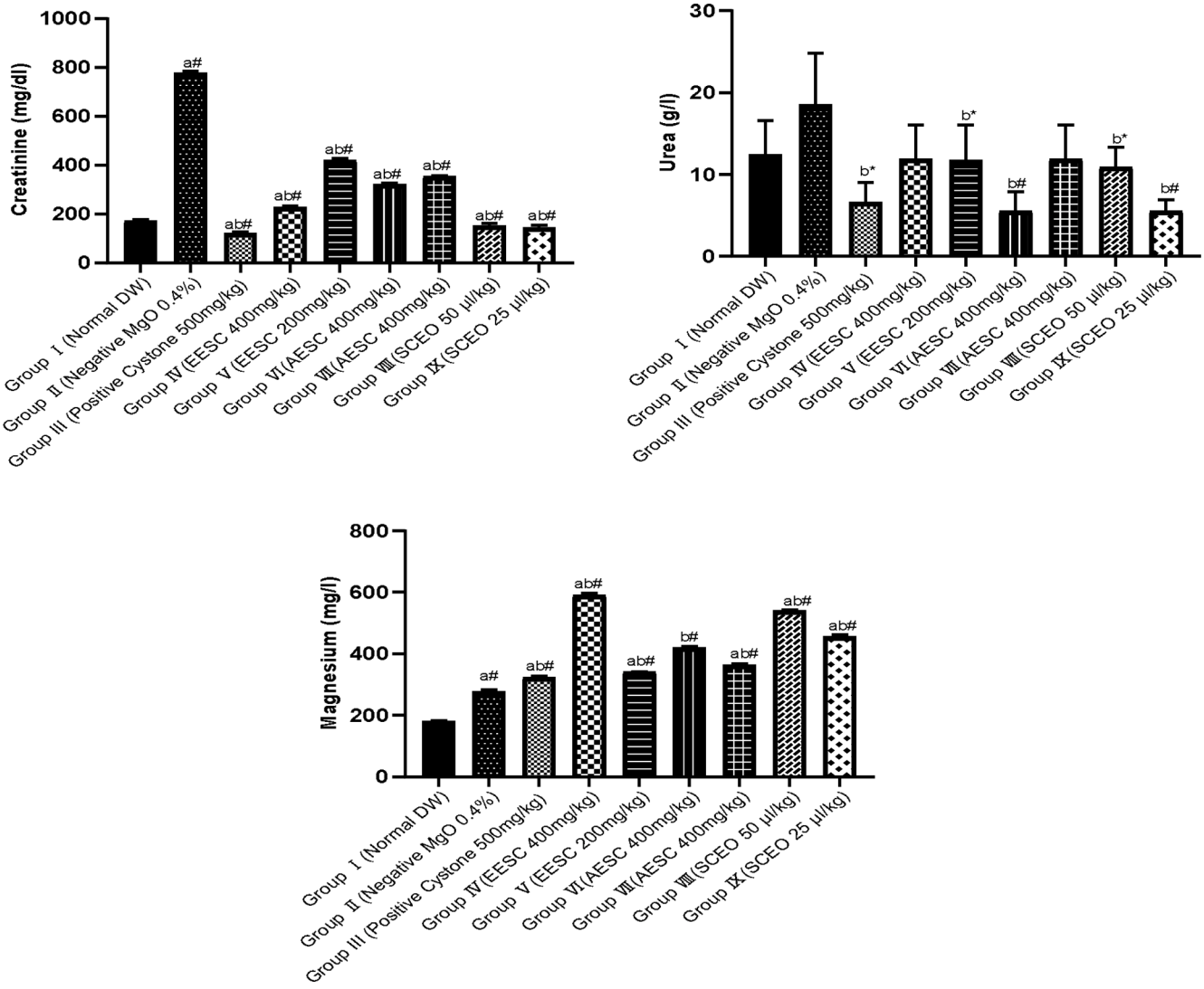

Fig. 8. Effect of *S. costus* root extracts and essential oils on urinary parameters in control and treated animals. a Comparisons are made with the Normal group. b Comparisons with negative group; * Statistically significant at $p < 0.05$;# Statistically significant at $p < 0.01$.

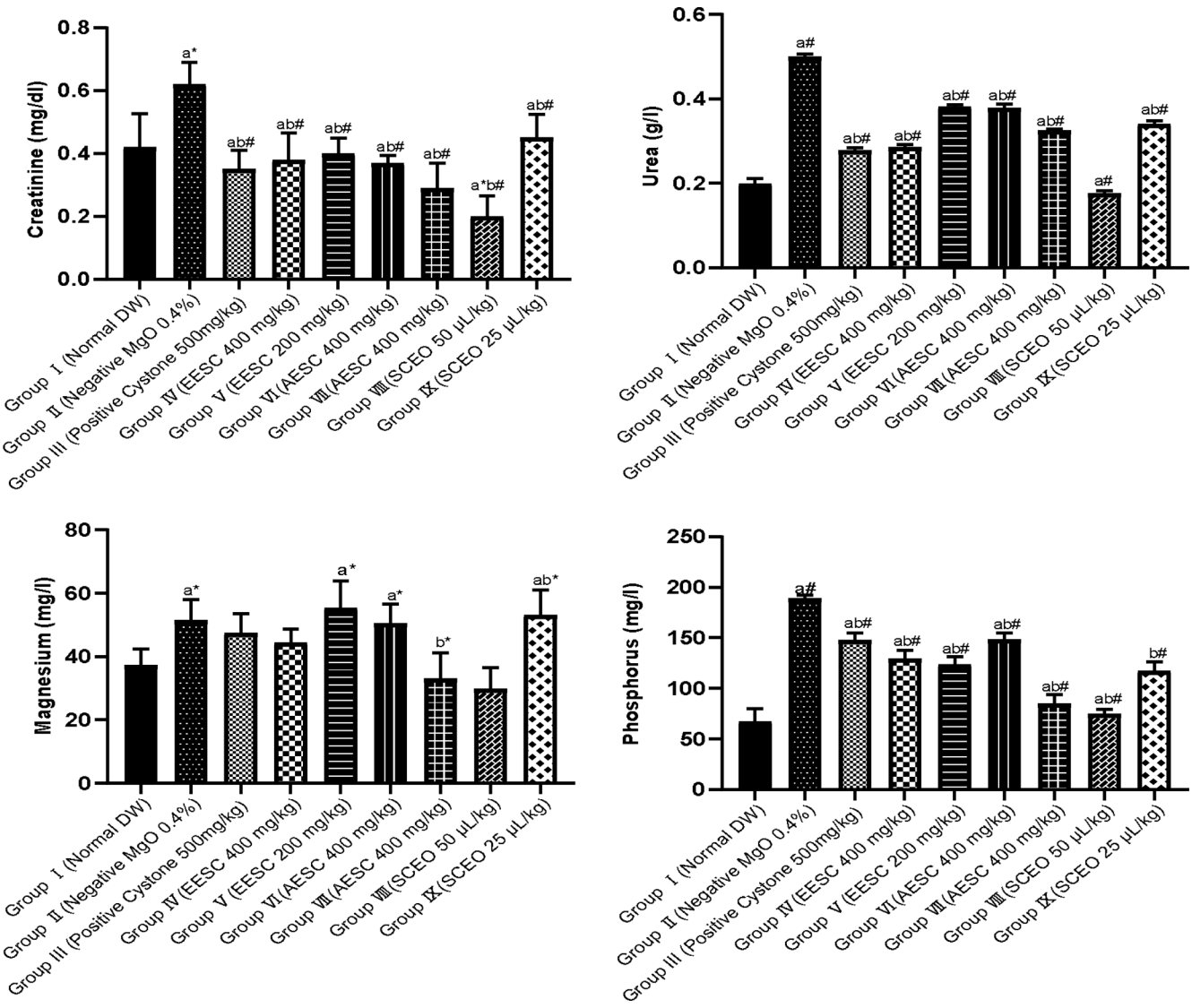

Fig. 9. Effect of extracts and essential oils of *S. costus* root on serum parameters in control and experimental animals.

serum biochemical analyses of all treated groups show concentrations lower than those of group II, and all groups treated with extracts and essential oils at different doses of the plant studied show serum concentrations almost similar to those of group I (Normal). The group treated with 50 µl/kg essential oils showed lower concentrations of creatinine (0.20 mg/dl ± 0.06 ($p < 0.01$)), urea (0.177 g/l ± 0.006 ($p < 0.01$)), phosphate (29.80 ± 6.83 mg/l) and magnesium (74.6 mg/l ± 4.62 ($p < 0.01$)) than group I. This observation suggests that this plant could have an effect on reducing these biochemical parameters. These results are similar to those obtained by B. Kaleeswaran and al (Kaleeswaran et al., 2019) in their experiment, which showed variations in serum biochemical parameters between the normal-negative group and the treated group. However, use of the essential oils at this dose of 50 µl/kg may have adverse effects on the kidneys. A drop in creatinine and urea levels compared with normal values may indicate renal failure, as the kidneys also play a crucial role in the regulation of phosphate and magnesium. A drop in these levels may indicate a malfunction in the kidneys' ability to maintain the body's electrolyte balance.

The results of the study based on histopathological examinations, presented in Fig. 10, show that the kidneys of all groups treated with 50 and 25 µm/kg oils and aqueous and ethanolic extracts of *S. costus* at doses of 200 and 400 mg/kg, as well as the untreated rat groups that received only magnesium oxide, all showed the presence of ammonium and magnesium phosphate crystals, with the presence of acute glomerular vessel congestion in all experimental groups. However, for the 50 and 25 µm/kg oils, foci of necrosis were also observed, with the exception of group I. Histological analyses of the liver samples shown in Fig. 11 show that the groups of rats treated with the 200 and 400 mg/kg aqueous and ethanolic extracts, as well as the group treated with cystone (750 mg/kg), were normal and showed no signs of toxicity. In contrast, the groups of rats treated with essential oils at 50 and 25 mg/kg showed discrete centro-lobular necrosis. These results are similar to those obtained by B. Kaleeswaran and al (Kaleeswaran et al., 2019) which shows that crystallization has occurred in the negative group, leading to some destruction of the glomerulus and tubular dilatation.

5. Conclusion

The novelty of the study lies in its investigation of the antilithiatic potential of *Saussurea costus* (Falc) Lipsch plant extracts and essential oils in two rat models, one induced by renal calcium oxalate crystals and the other by ammonium, phosphate and magnesium crystals. The study explored the effects of different doses of *S. costus* extracts and essential oils on various physiological, biochemical, and histopathological parameters, providing valuable insights into the potential therapeutic effects of *S. costus* in managing urinary lithiasis. However, the study has

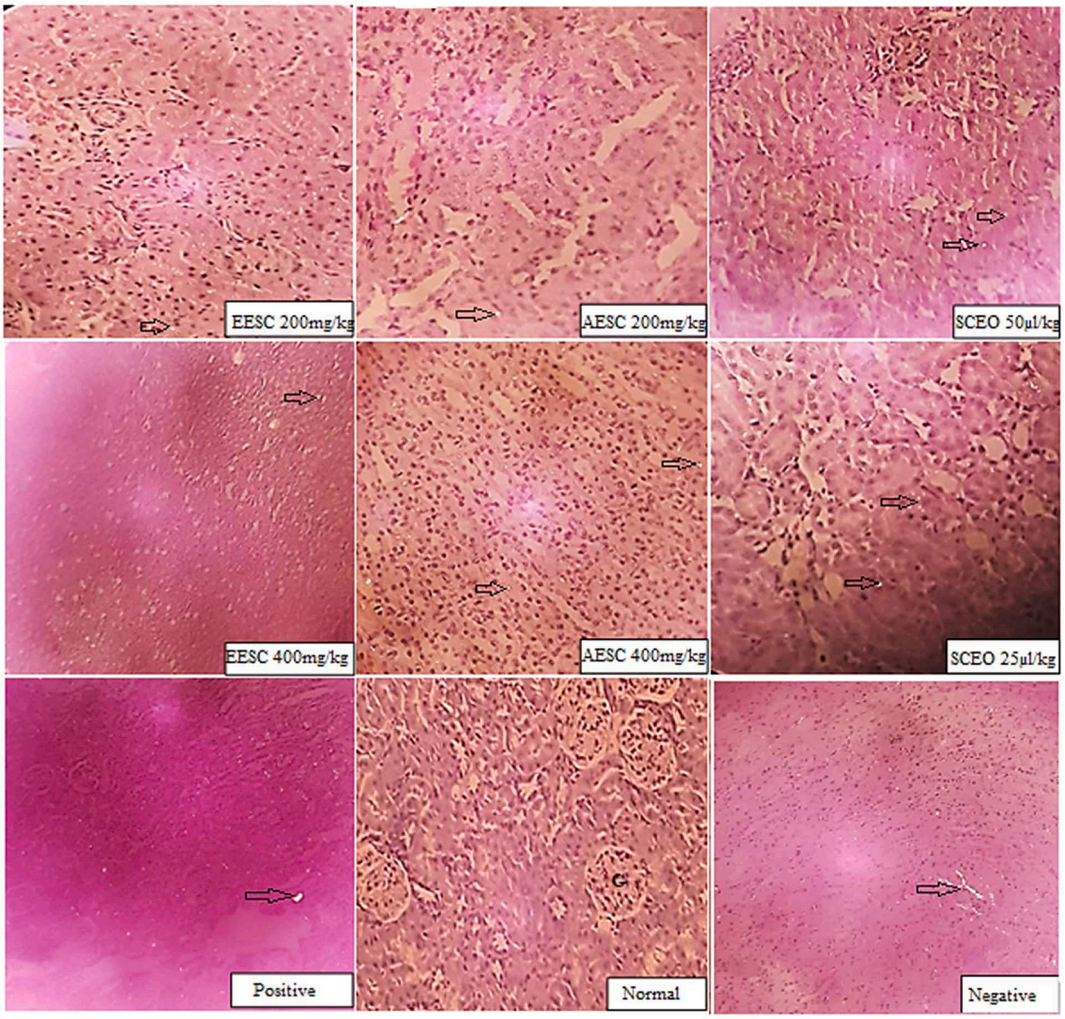

Fig. 10. Histology of kidneys from groups treated with extracts (EESC 200 mg/kg, EESC 400 mg/kg, AESC 200 mg/kg, AESC 400 mg/kg) and essential oils (50 µl/kg and 25 µl/kg) and untreated groups (Normal and negative control). Black arrow: struvite crystals and aggregation; G: tubular glomerulus; H&E staining (x400).

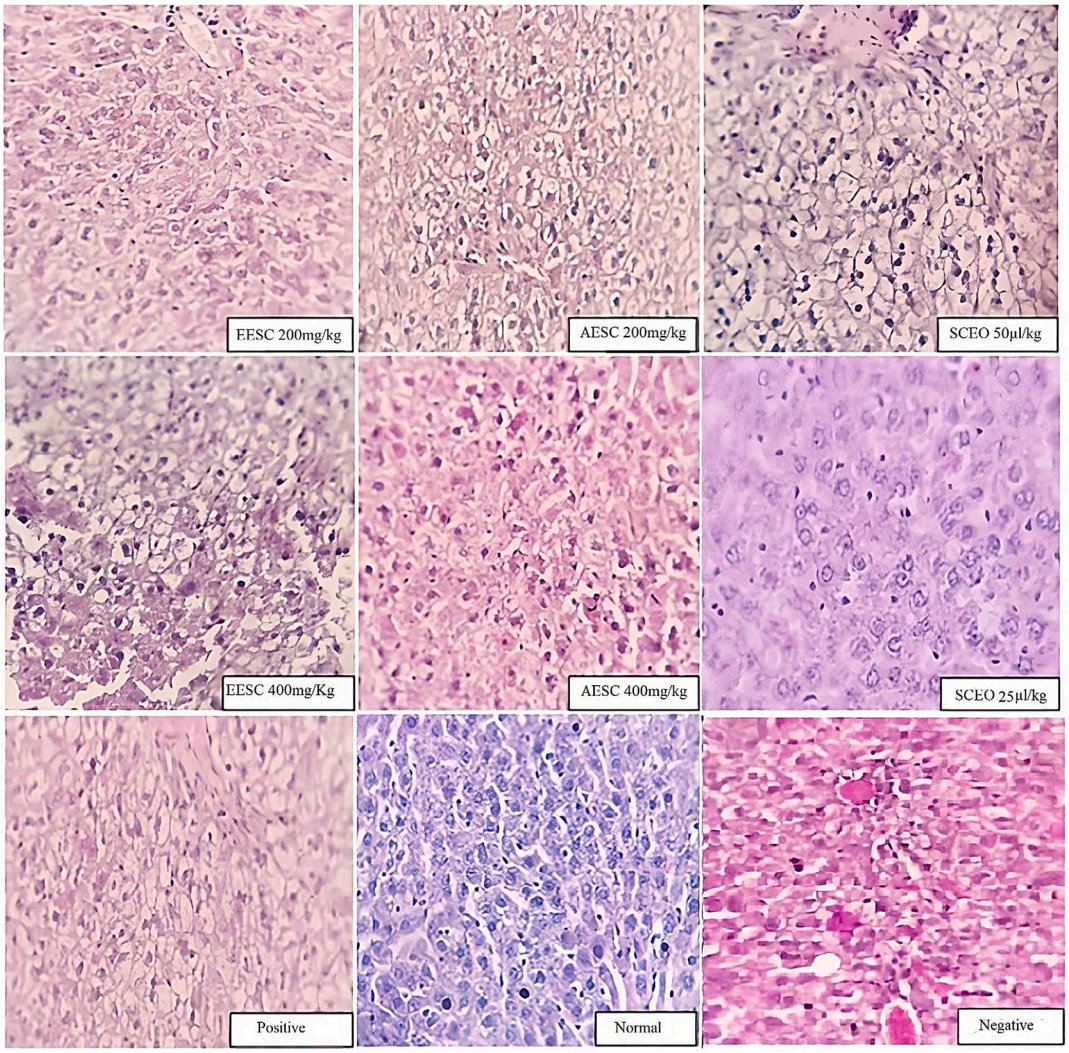

Fig 11. Histology of livers from groups treated with extracts (EESC 200 mg/kg, EESC 400 mg/kg, AESC 200 mg/kg, AESC 400 mg/kg) and essential oils (50 µl/kg and 25 µl/kg) and control groups (Normal, Negative and positive); H&E staining (×400).

certain limitations. Firstly, the research was conducted on a rat model, and the translation of these findings to human clinical applications require further investigation. Additionally, the study primarily focused on the effects of *S. costus* on calcium oxalate and struvite crystal formation, and further research is needed to understand its impact on other types of urinary stones. Moreover, the histopathological examinations revealed varying impacts on the kidneys and livers of the treated rats, but the long-term effects and potential adverse reactions of *S. costus* extracts and essential oils require comprehensive evaluation. In conclusion, while the study provides valuable insights into the potential therapeutic effects of *S. costus* in managing urinary lithiasis, further research is essential to elucidate the mechanisms underlying the observed effects, assess long-term safety, and explore the clinical implications of *S. costus* in the treatment of urinary stone formation.

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Naima Mammate: Writing – original draft, Formal analysis, Methodology, Conceptualization. **Fatima Ezzahra El Oumari:** Writing – original draft, Formal analysis, Methodology. **Hamada Imtara:** Writing – review & editing, Resources, Conceptualization. **Salim Belchkar:** Methodology. **Ramzi A. Mothana:** Resources, Writing – review & editing. **Hinde E.L. Fatemi:** Formal analysis. **Mohammed Danouche:** Formal analysis. **Sara Er-rahmani:** Resources. **Nabil Boucetta:** Writing – review & editing. **Omar M. Noman:** Writing – review & editing, Resources. **Mahmoud Tarayrah:** Writing – review & editing, Resources. **Tarik Sqalli Houssaini:** Supervision, Formal analysis, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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