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Comprehensive Analysis of *Areca catechu* Flower Extracts: Quantitative Phytochemical Analysis, GC-MS Characterization and *in vitro* Antiurolithiatic Activity

K. Adarsha Govinda[1], Jennifer Fernandes[1,*], S. Anusha[1] and Nishmitha G. DSouza[2]

[1]Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Mangaluru-575018, India
[2]Department of Pharmaceutical Chemistry, Yenepoya Pharmacy College & Research Centre, Yenepoya (Deemed to be University), Deralakatte, Mangaluru-575023, India

*Corresponding author: Fax: +91 824 2203993; Tel: +91 824 2203991; E-mail: fernandesj@nitte.edu.in

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Despite the fact that the root, fruit, and pulp of *Areca catechu* are claimed to possess antiurolithiatic properties, there is no information that has been documented regarding the flowers of the plant. The study was to confirm the active ingredients of *Areca catechu* flower and characterized by the GC-MS technique. The preliminary phytochemical analysis and the assessment of the total content were performed on a different type of extracts of *Areca catechu* flowers. The antiurolithiatic activity of *A. catechu* was determined by nucleation assay, growth assay and titrimetric method. The highest yield was obtained with methanol (7.4%), while chloroform yield was minimal (1.8%). The extracts contained flavonoids, terpenoids, glycosides, steroids and phenolic compounds, while terpenoids, saponins and reducing sugars were absent. The hexane and petroleum ether extracts of *A. catechu* flowers contained 10 chemical constituents. While 13 chemical constituents were analyzed from the ethyl acetate, chloroform, ethanolic and methanolic extracts. It was found that methanolic extract exhibited better action than cystone in nucleation assay and signification inhibitory action in growth assay. The maximum percentage dissolution among the extracts was shown by methanolic extracts (75.35%) at the concentration of 100 mg/mL, whereas cystone showed maximum percentage (86.63%) dissolution at the concentration of 100 mg/mL. Moreover, *A. catechu* extracts also exhibited significant *in vitro* antiurolithiatic activity.

Keywords: ***A. catechu*, Arecaceae, Antiurolithiatic activity, Cystone, GC-MS, Growth assay, Nucleation assay.**

INTRODUCTION

Stone formation is currently the most common and painful urologic condition in the population as a result of food and lifestyle changes. Calculi development is a feature of stone formation or lithiasis. Nephrolithiasis and urolithiasis are two of the primary kinds. Urolithiasis is the term for the development of calculi in the urinary bladder, ureter or any other portion of the urinary system other than the kidney, whereas nephrolithiasis is the development of calculi in kidney [1]. In many cases, kidney stones are very small and may pass out through urine. If a stone obstructs the flow of urine, it can cause extreme pain and require medical treatment [2,3]. With a predicted recurrence rate of less than 50% and a prevalence of over 10%, urolithiasis has a significant impact on the healthcare system. The prevalence of urolithiasis is fairly high worldwide and calcium oxalate (CaOx) stones alone or combined with calcium phosphate make up more than 80% of urinary calculi. Nephrolithiasis is more common in males (12%) than in women (6%) and is more common in both sexes between the ages of 20 and 40, according to epidemiological research [4].

Since there are currently no effective treatments in the contemporary medicine that can dissolve the stone, doctors continue to rely on alternative medical systems for more effective alleviation [5]. The surgical removal of stones is the major component of the other medical therapy of urolithiasis. The avoidance of recurrence of the stone is not guaranteed by procedures like extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy (PCNL). Additionally, they result in adverse effects include haemorrhage, hypertension, tubular necrosis and ensuing renal fibrosis [6]. Most of the treatments used in ancient medical systems were from plants

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and were shown to be effective for a variety of illness problems. The study of employing herbal medicines to cure various illnesses is known as herbal medicine, commonly referred to as phytotherapy. As more individuals become aware of the side effects of the synthetic medications, natural remedies for illnesses are being developed at a faster rate [7].

Areca catechu L., often known as the areca palm, betel nut palm or betel tree, belongs to the Arecaceae family. These are widely farmed and produced throughout the year in India and many other Asian nations. Because its fruits are frequently eaten with betel leaf and lime, the betel nut palm or betel palm is so named. The fruits of the palm tree have long been used for digestive help, urinary issues, worm removal from the intestines, stimulating effects and breath freshening effects [8]. A number of medicinal plant extracts, including *Herniaria hirsute* [9], *Tribulus terrestris* [10], *Bergenia ciliate* [11], *Piper nigrum* [12], *Dolichos biflorus* [13], *Bergenia ligulate* [14] and *Plantago major* [15], have been documented for the *in vitro* anti-crystallization actions in the literature. However, in literature no information about the *A. catechu* flowers, despite the fact that its root, fruit and pulp [16] have been claimed to have anti-urolithiatic properties. The current study is set up to verify the active components of the flowers. In light of this, the flowers of the plant were chosen for the purpose of conducting research on the antiurolithiatic function of the plant.

EXPERIMENTAL

The flowers of *A. catechu* were collected in and around Puttur, India, in the month of March - July 2022. The collected plant was authenticated by Dr. Krishna Kumar, Professor and Chairman, Department of Applied Botany, Mangalore University, Mangala Gangotri, India. The herbarium was maintained for the plant under herbarium number 20PC001R.

Extraction: Collected flowers were cleaned to remove any impurities like mud or dust and these flowers were subjected to drying under shade at room temperature. After complete drying flowers were powdered using a household mixer and was stored in an airtight container. Extraction was carried out by cold maceration using seven different solvents (water, hexane, ethyl acetate, chloroform, methanol, ethanol and petroleum ether), which were selected on basis of polarity. Initially, seven maceration chambers were cleaned and dried followed by rinsing with respective solvent systems. The powdered extracts (50 g) were weighed and added to seven different chambers and respective solvents were added to each maceration assembly till powder gets immersed in the solvent system. The methanolic extract was prepared in eight batches of 50 g and chloroform extract was prepared in fourteen batches of 50 g. Maceration chambers were sealed to prevent the entry of contaminants and protected from light. Maceration was carried out for a week with occasional shaking and the whole process was carried out at room temperature. Further, the contents of maceration were filtered separately and filtrates were evaporated to dryness to obtain the crude extracts. All the extracts were preserved in desiccators to prevent degradation due to moisture.

Preliminary phytochemical screening (qualitative analysis): The preliminary phytochemical study was carried for all seven extracts of flowers of *A. catechu* L., to determine and characterize the different functional groups present in it. The chemical tests were carried out as per the standard methods [17,18].

Quantitative analysis

Determination of total phenolic content: Total phenolic content present in different extracts of *A. catechu* was determined using Folin-Ciocalteu (FC) reagent. Gallic acid was used as the standard. Initially, 1 mg of each plant extracts were dissolved in 1mL of respective solvent systems (1000 µg/mL), from the above solution pipette out 20, 40, 60, 80 and 100 µL and make up the volume to 1 mL with respective solvents (20, 40, 60, 80, 100 µg/mL). To 1 mL of each solution 2 mL 7% Na_2CO_3 was added was added and kept aside for 2 min. further 0.2 mL Folin–Ciocalteu reagent (1:2 ratio of FC:water) was added and incubated in dark for 30 min at room temperature. Each resultant mixtures (200 µL) were pipetted onto 96-well microplates and the absorbance was measured at 630 nm using microplate reader. For the standard plant extracts were replaced with gallic acid (dissolved in ethanol). Total phenolic compounds were expressed as mg/g gallic acid equivalent (GAE) of dry extract [19].

Determination of total flavonoid content: The total flavonoid content of each extract was carried out using the aluminium chloride colorimetric method. The calibration curve was prepared by using quercetin. Each plant extracts (1 mg) were dissolved in 1mL of respective solvent systems (1000 µg/mL), from the above solution pipette out 20, 40, 60, 80 and 100 µL and make up the volume to 1 mL with respective solvents (20, 40, 60, 80,100 µg/mL). To each of extract solutions 4 mL distilled water was added followed by addition of 0.3 mL $NaNO_2$, after 5 min 0.3 mL of 10% $AlCl_3$ was added and then 2 mL of 1 M NaOH was added and made up the volume up to 10 mL using distilled water. The reaction mixture was incubated at room temperature for 30 min. These mixtures (200 µL) were placed in 96-well microplates, then absorbance was measured at 510 nm using a microplate reader. For the standard quercetin was used in the place of extract. The flavonoid content was expressed as mg/g of quercetin equivalent (QE) of dry extract [20].

Determination of total alkaloid content: Total alkaloid content was determined by using atropine as standard. All the seven different plant extracts (1 mg) were dissolved in 1 mL DMSO, from this solution 20, 40, 60, 80 and 100 µL were pipetted onto a test tube and volume was made up to 1 mL with DMSO. Resulting 1 mL of this solution was taken in a separating funnel and to this 5 mL of bromocresol green (BCG) solution (69.8 mg BCG was heated along with 3 mL of 2 N NaOH and 5 mL water, further volume was made up to 1000 mL with water) along with 5 mL of phosphate buffer (2 M sodium phosphate was mixed with 0.2 M citric acid in 1 L water, pH was maintained at 4.7). To the above mixture 10 mL of chloroform was added and shaken well. The chloroform layer was collected in a 10 mL volumetric flask and volume was made up to 10 mL with chloroform. Placed 200 µL of collected chloroform layer in 96-well plates and absorbance of the complex was measured at 470 nm using microplate reader [21].

GC-MS analysis: Hexane, petroleum ether, ethyl acetate, chloroform, ethanol and methanolic extracts of *A. catechu*

flowers were performed for GC-MS analysis. The GC-MS was carried out using Shimadzu model QP2020 with autosampler AOI 2oi, mass column used was SH Rxi 5Sil. The length of the column used was 20 m with the diameter being 0.25 mm. Molecules were ionized on basis of electron ionization with energy 70 eV. Carrier gas used was helium and the flow rate was maintained at 1.2 mL/min. The injection volume was 0.5 µL and the injection temperature was maintained at 300 ºC, whereas ion source temperature was 220 ºC.

Screening of antiurolithiatic activity by *in vitro* methods

Nucleation assay: Calcium chloride (5 mmol/L) and 7.5 mmol/L of sodium oxalate solutions were prepared using a buffer (0.05 mol/L Tris and 0.15 mol/L NaCl at pH 6.5); prepared solutions were filtered in order to remove any particulate matter if present. A 100 µL of extract (20, 40, 60, 80 and 100 mg/mL) was mixed with 950 µL of calcium chloride (blank was prepared in absence of extract). To this mixture, 950 µL of sodium oxalate was added and mixed. Absorbance of resultant solution was measured using UV-vis spectrophotometer at 620 nm. The percentage inhibition of nucleation was calculated using the following formula:

$$\text{Inhibition of nucleation (\%)} = \frac{C-S}{C} \times 100$$

where C is the turbidity without plant extract and S is turbidity with plant extract [22].

Growth assay: A 4 mmol/L of sodium oxalate and calcium chloride was prepared. A 10 mmol/L of tris buffer as well as 10 mmol/L of sodium chloride were also prepared. A 1 mL each of prepared calcium chloride and sodium oxalate were mixed with 1.5 mL of NaCl, this mixture was buffered to pH 7.2 using tris buffer. To this mixture, 30 µL of calcium oxalate monohydrate crystal slurry was added followed by addition of extract (20, 40, 60, 80 and 100 µg/mL). This solution was left for 5 min and then the absorbance was measured at 214 nm. The inhibitory capacity of extract was calculated as follows:

$$\text{Relative inhibitory activity (\%)} = \frac{C-S}{C} \times 100$$

where C = the rate of reduction of free oxalate without any extract and S = the rate of reduction of free oxalate in the presence of drug extract [23].

Titrimetric assay: Titrimetric assay was performed in order to examine the dissolution percentage of calcium oxalate crystals. It involves three steps:

a) Preparation of calcium oxalate crystals by homogenous precipitation method: In separate 250 mL beakers, 4.41 g of calcium chloride dihydrate dissolved in distilled water was mixed with 4.02 g of sodium oxalate dissolved in 2 N H_2SO_4, which resulted in the precipitation of calcium oxalate crystals. Ammonia solution was added to remove excess H_2SO_4 and then the precipitate was filtered and dried at 60 ºC for 4 h.

b) Preparation of egg semipermeable membrane: Eggs were punctured and its contents were removed completely. The, obtained egg shells were washed with distilled water. The decalcification of egg was carried out by placing egg shells in a beaker containing 2 M HCl overnight. Excess acids were removed by rinsing shells with ammonia followed by washing with water and stored in refrigerator at pH 7-7.4.

c) Evaluation of antiurolithiatic activity by titrimetric method: The percentage dissolution of calcium oxalate was carried out by taking 1 mg of calcium oxalate, 10 mg of flower extracts of different concentrations (20, 40, 60, 80, 100 mg/mL) and 10 mg of standard drug cystone were packed together in separate egg semipermeable membrane. These were allowed to immerse in a separate conical flask containing 100 mL of 0.1 M tris buffer solution. The above conical flasks were kept for incubation at 37 ºC for 4 h. Then, the contents of semipermeable membranes were removed and transferred to a small beaker containing 2 mL of 1 N H_2SO_4. Finally, the resulting solution was titrated with 0.9494 N $KMnO_4$ solution until a light pink colour is obtained (each mL of 0.9494 N $KMnO_4$ equivalents to 0.1898 mg of calcium oxalate). The total amount of calcium oxalate dissolved by the different extracts were calculated by subtracting the amount of remaining undissolved calcium oxalate from the total amount used in the experiment [23].

RESULTS AND DISCUSSION

Extraction yield: Maceration was carried out using seven different solvents for extraction of the phytoconstituents. Out of all the solvent extracts, the methanolic extract yield was found to be maximum. The percentage yields of the crude extracts obtained from different solvents are shown in Table-1. The final extract obtained was highest (7.4%) when methanol was used for maceration whereas, with chloroform the yield was minimal (1.8%). The extract yield varied with different solvent systems, possibly due to solvent polarity, penetrating power, and phytoconstituent solubilization.

TABLE-1
YIELD OF VARIOUS CRUDE EXTRACTS FROM MACERATION

Solvents	Initial weight (g)	Final weight (g)	Yield (%)
Hexane	50	1.2	2.4
Pet. ether	50	1.7	3.4
Ethyl acetate	50	1.7	3.4
Chloroform	700	12.6	1.8
Ethanol	50	2.3	4.6
Methanol	400	29.6	7.4
Water	50	1.5	3.0

Preliminary phytochemical investigation: The preliminary phytochemical analysis of *A. catechu* flower extracts showed the presence of flavonoids, terpenoids, glycosides, saponins, steroids, phenolic compounds and tannins. The results of the preliminary phytochemical tests are shown in Table-2. Out of all phytoconstituents flavonoids, glycosides, steroids, tannins and phenolic compounds were present in all the solvent extracts whereas reducing sugars were absent in petroleum ether. Terpenoids were absent in ethanol, methanol and water, while saponins were absent in petroleum ether, ethyl acetate, ethanol and methanol. Presence of all these active constituents might be responsible for *A. catechu* flower extracts pharmacological activity.

TABLE-2
RESULTS OF THE PRELIMINARY PHYTOCHEMICAL TEST

Phytoconstituents	Solvent systems						
	Hexane	Pet. ether	Ethyl acetate	Chloroform	Ethanol	Methanol	Water
Alkaloids	-	-	-	-	-	-	-
Reducing sugars	+	-	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	-	-	-
Glycosides	+	+	+	+	+	+	+
Protein	-	-	-	-	-	-	-
Saponins	+	-	-	+	-	-	+
Steroids	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+	+

(+) indicates presence of phytoconstituents and (–) indicates absence of phytoconstituents.

Determination of total contents: Total flavonoid, phenolic and alkaloid contents were estimated by using quercetin, gallic acid and atropine as standard, ethyl acetate extracts were found to possess maximum flavonoid and alkaloid content whereas, methanolic extract was found to contain maximum phenolic content. The results of the total contents present in the different extracts are shown in Table-3.

TABLE-3
TOTAL CONTENT ESTIMATION

Solvent system	Total flavonoid content mg of QE/g	Total phenolic content mg of GAE/g	Total alkaloid content mg of AE/g
Hexane	32.403 ± 0.748	46.697 ± 0.453	15.157 ± 0.940
Pet. ether	23.830 ± 1.091	46.747 ± 1.390	29.780 ± 0.788
Ethyl acetate	56.043 ± 1.365	55.337 ± 1.089	35.493 ± 0.611
Chloroform	19.757 ± 0.873	75.417 ± 1.440	31.367 ± 1.124
Ethanol	36.337 ± 1.784	108.097 ± 0.854	25.643 ± 0.751
Methanol	41.837 ± 1.020	113.950 ± 1.492	24.363 ± 0.828
Aqueous	54.417 ± 1.344	1.007 ± 0.112	22.263 ± 0.831

All values are mean ± SD, n = 3.

GC-MS: Overall, 22 compounds were found to be present in seven different solvent extracts of *A. catechu* flowers. Most of the reported compounds were found to possess the pharmacological activities. The details of the isolated main constituents using different solvents are given in Table-4, while the GC-MS spectra are shown in Fig. 1.

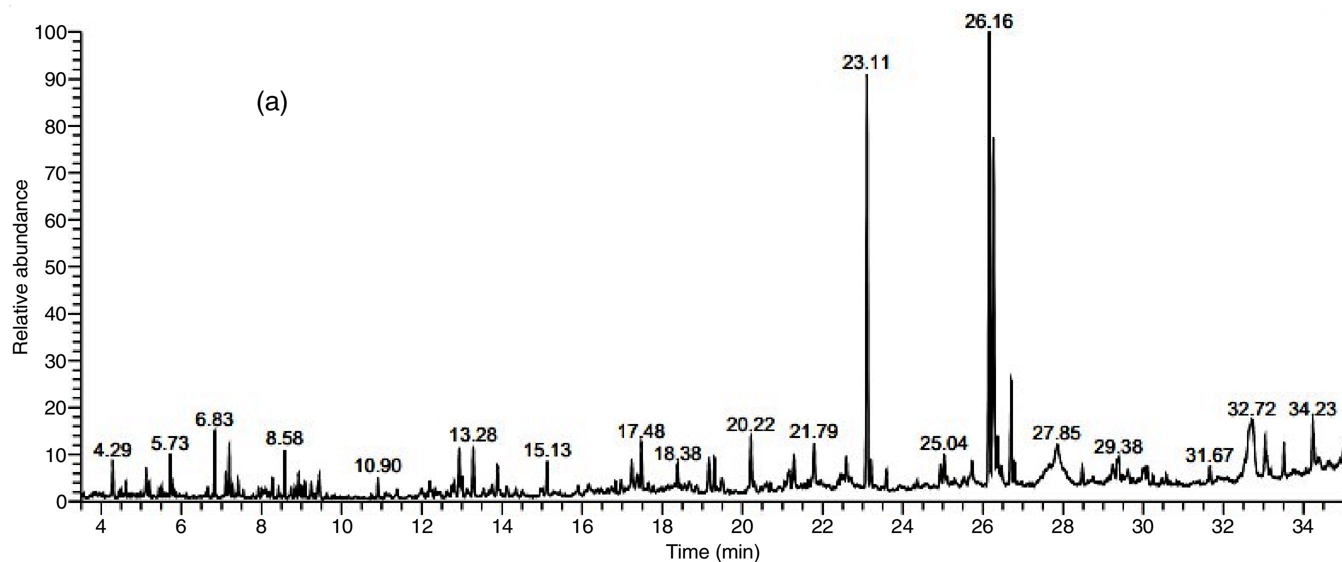
***In vitro* antiurolithiatic activity:** The methanolic extract inhibited nucleation and growth of crystals to the highest extent in comparison to all other extracts and it was also found out that methanolic extract showed maximum percentage dissolution of calcium oxalate crystals.

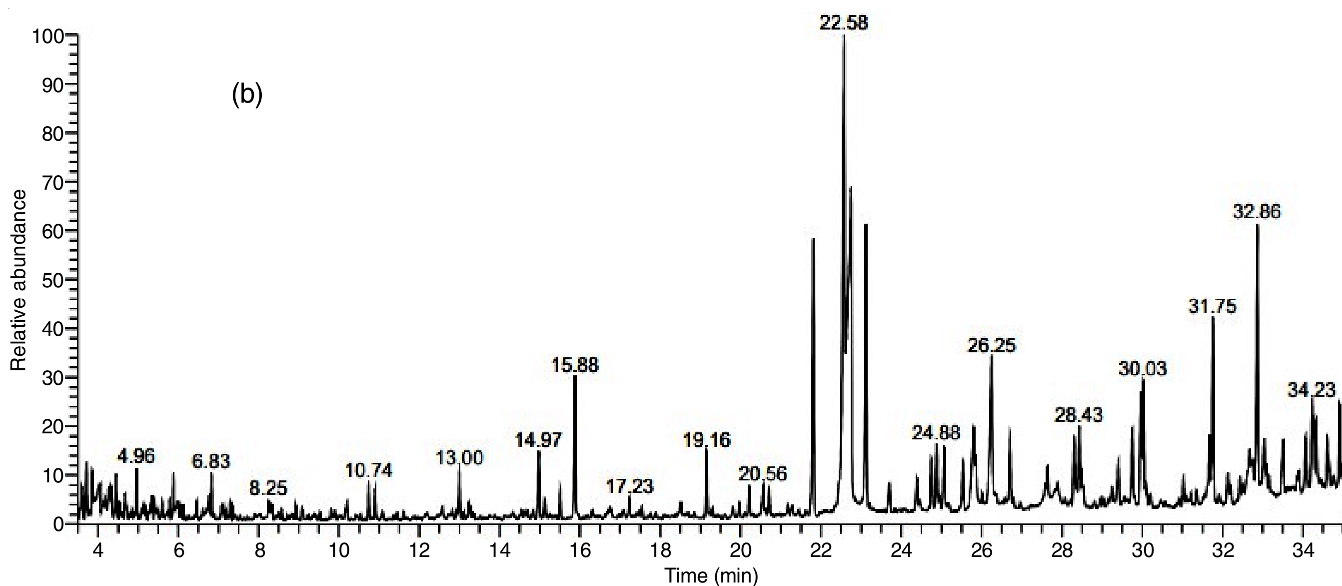
Nucleation assay: The results of effect of various *A. catechu* flower extracts on the inhibition of nucleation are shown in Table-5. The methanolic extract *A. catechu* showed the highest inhibition (61.17 ± 2.28) along with IC_{50} value of 61.79 at the concentration of 100 µg/mL, when compared to cystone standard solution (IC_{50} value of 76.83), which exhibited the IC_{50} value of 76.83 in the nucleation of calcium oxalate salts.

TABLE-4
PHYTOCONSTITUENTS ISOLATED IN DIFFERENT SOLVENTS OF *Areca catechu* FLOWER EXTRACTS BY GC-MS ANALYSIS

Compound name	Formula	m.w.	Retention time	Area (%)
Hexane extract				
cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	26.26	9.35
Hexacosanal	$C_{26}H_{52}O$	380.7	35.65	4.34
Glycodeoxycholic acid	$C_{26}H_{43}NO_5$	449.6	36.68	2.73
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.6	32.70	1.82
Stigmasterol	$C_{29}H_{48}O$	412.7	27.85	1.64
1-Heptatriacotanol	$C_{37}H_{76}O$	537.0	27.85	1.64
2,4-Di-*tert*-butylphenol	$C_{14}H_{22}O$	206.3	13.28	1.18
Pentadecane	$C_{15}H_{32}$	212.4	8.58	0.85
Cholest-4-en-3- one	$C_{27}H_{44}O$	384.6	34.37	0.85
17-Pentatriacontene	$C_{35}H_{70}$	490.9	29.24	0.81
Petroleum ether extract				
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	22.75	10.93
Eicosanoic acid	$C_{20}H_{40}O_2$	312.5	26.25	3.96
cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	25.81	2.35
Oleic acid	$C_{18}H_{34}O_2$	282.5	25.81	2.35
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.6	35.26	2.23
Hexacosanal	$C_{26}H_{52}O$	380.7	35.65	2.01
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	22.75	1.99
1-Heptatriacotanol	$C_{37}H_{76}O$	537.0	32.68	1.76
17-Pentatriacontene	$C_{35}H_{70}$	490.9	33.03	1.36
Stigmasterol	$C_{29}H_{48}O$	412.7	27.64	0.82

Ethyl acetate extract				
Eicosanoic acid	$C_{20}H_{40}O_2$	312.5	24.35	1.39
17-Pentatriacontene	$C_{35}H_{70}$	490.9	34.23	1.38
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.6	35.26	1.07
cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	25.72	0.93
Oleic acid	$C_{18}H_{34}O_2$	282.5	25.72	0.93
Rhodopin	$C_{40}H_{58}O$	554.9	36.04	0.52
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	18.49	0.47
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	18.49	0.47
2,4-Di-*tert*-butylphenol	$C_{14}H_{22}O$	206.3	13.29	0.45
1-Heptatriacotanol	$C_{37}H_{76}O$	537.0	19.17	0.45
Chloroform extract				
Eicosanoic acid	$C_{20}H_{40}O_2$	312.5	26.14	3.26
cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	25.71	1.61
2,4-Di-*tert*-butylphenol	$C_{14}H_{22}O$	206.3	13.28	1.30
1-Heptatriacotanol	$C_{37}H_{76}O$	537.0	29.39	0.96
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.6	29.39	0.96
17-Pentatriacontene	$C_{35}H_{70}$	490.9	30.02	0.69
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	14.27	0.63
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	14.27	0.63
Ethanolic extract				
cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	25.87	4.31
Oleic acid	$C_{18}H_{34}O_2$	282.5	25.87	4.31
Eicosanoic acid	$C_{20}H_{40}O_2$	312.5	24.40	1.31
Rhodopin	$C_{40}H_{58}O$	554.9	37.56	0.88
Astaxanthin	$C_{40}H_{52}O_4$	596.8	38.47	(0.65
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	18.52	0.39
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	18.52	0.39
Methanolic extract				
Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	22.62	22.44
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	22.62	22.44
cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	25.82	8.55
Oleic acid	$C_{18}H_{34}O_2$	282.5	25.82	8.55
3-Hydroxy-4-methoxycinnamic acid	$C_{10}H_{10}O_4$	194.1	20.76	0.88
trans-Ferulic acid	$C_{10}H_{10}O_4$	194.1	20.76	0.88
1-Heptatriacotanol	$C_{37}H_{76}O$	537.0	29.40	0.75
Niacin	$C_6H_5NO_2$	123.1	07.75	0.75
Rhodopin	$C_{40}H_{58}O$	554.9	35.26	0.68
2,4-Di-*tert*-butylphenol	$C_{14}H_{22}O$	206.3	13.30	0.57
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.6	13.80	0.53
Ecgonine	$C_9H_{15}NO_3$	185.2	10.45	0.50
Paromomycin	$C_{23}H_{47}N_5O_{18}S$	615.6	10.16	0.48
Arecaidine	$C_7H_{11}NO_2$	141.1	10.16	0.48




(b)
Relative abundance
Time (min)
22.58
32.86
31.75
26.25
30.03
15.88
34.23
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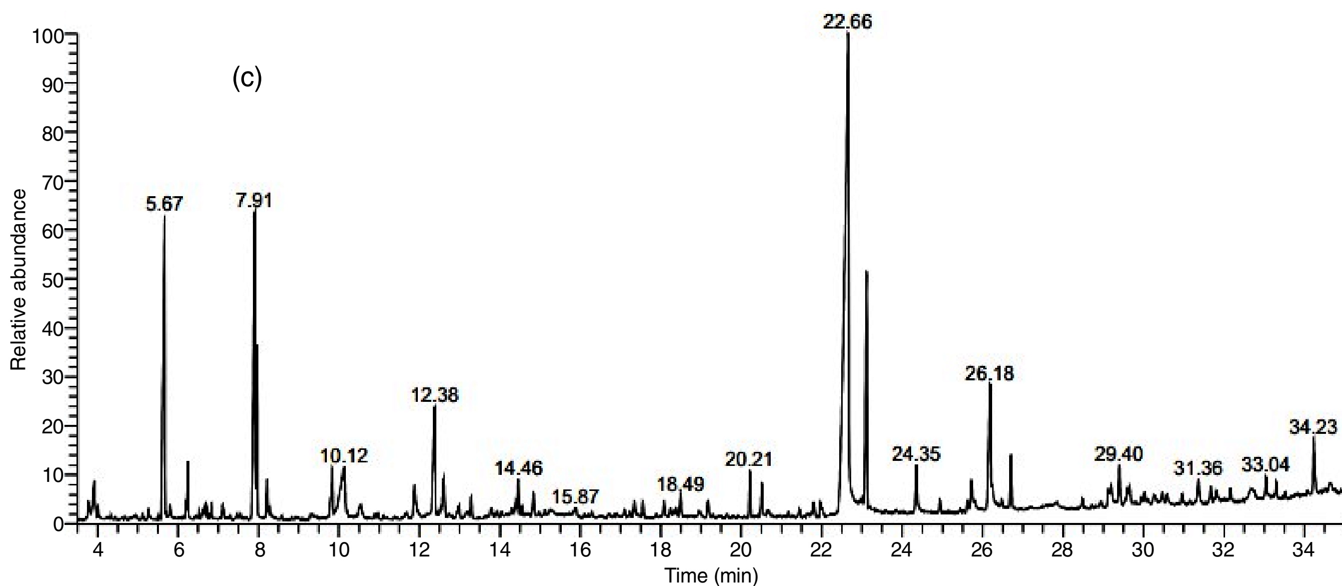
24.88
14.97
19.16
4.96
6.83
13.00
10.74
20.56
17.23
8.25


(c)
Relative abundance
Time (min)
22.66
5.67
7.91
26.18
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10.12
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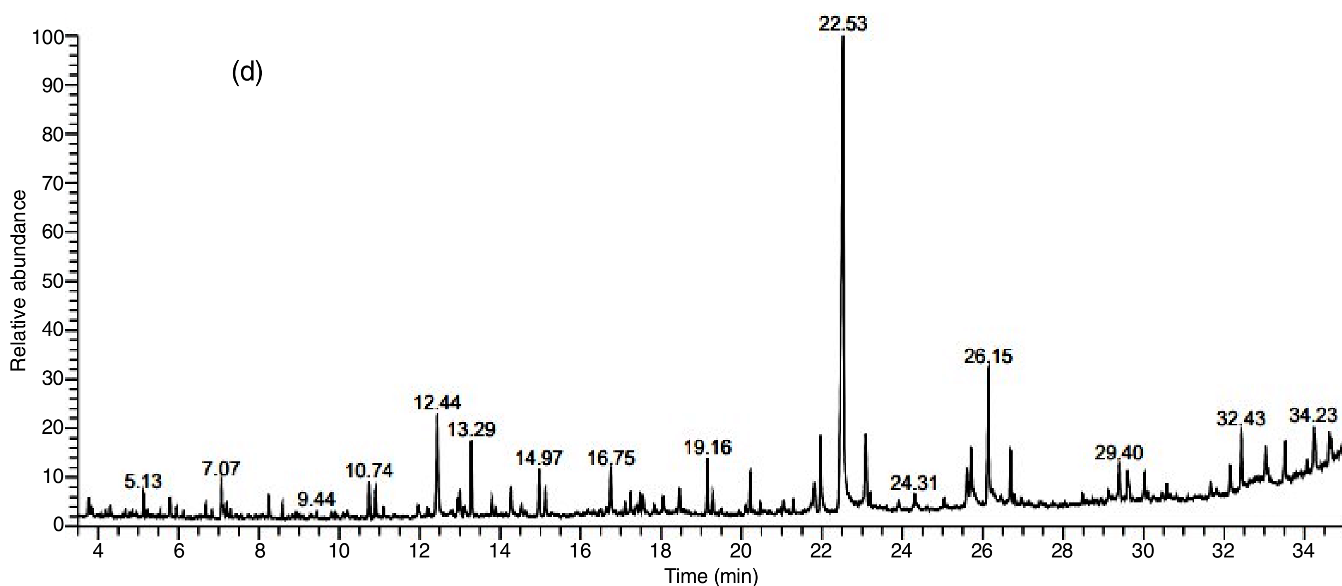
20.21
24.35
29.40
31.36
33.04
18.49
15.87


(d)
Relative abundance
Time (min)
22.53
26.15
12.44
13.29
32.43
34.23
14.97
16.75
19.16
29.40
5.13
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10.74
24.31
9.44

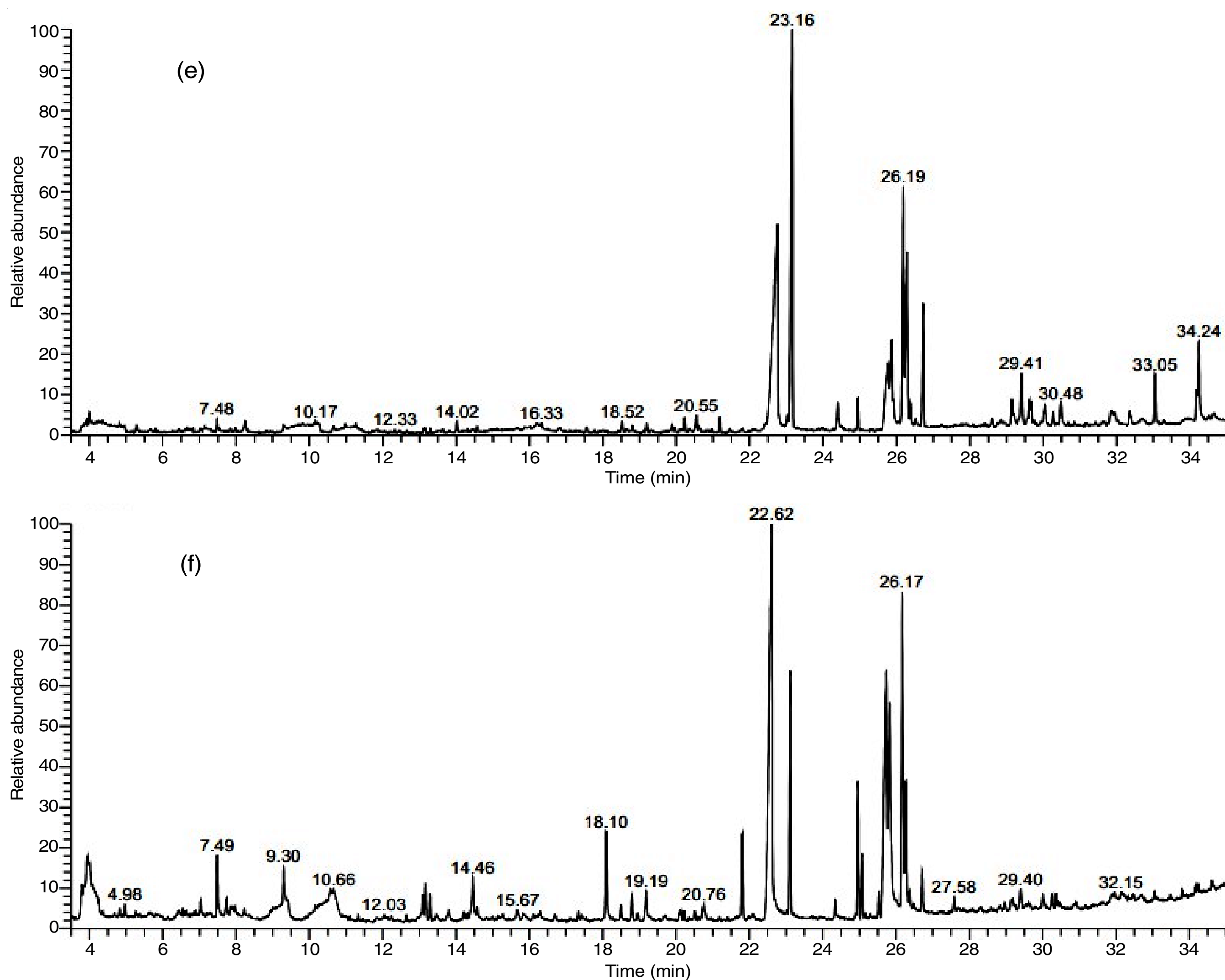

Fig. 1. GC-MS chromatogram of (a) hexane, (b) petroleum ether, (c) ethyl acetate, (d) chloroform, (e) ethanolic and (f) methanolic extract of *Areca catechu* flowers

TABLE-5
EFFECT OF VARIOUS *Areca catechu* FLOWER EXTRACTS ON INHIBITION OF NUCLEATION

Conc. (µg/mL)	Cystone		HEAC		PEEAC		EAEAC	
	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value
20	13.057 ± 0.04		31.38 ± 0.57		9.95 ± 0.16		4.69 ± 0.10	
40	17.68 ± 0.15		36.75 ± 0.89		23.57 ± 0.73		10.41 ± 0.47	
60	26.786 ± 0.17	76.83	45.87 ± 1.13	79.80	34.71 ± 2.05	79.21	11.29 ± 0.68	114.24
80	38.687 ± 0.23		46.14 ± 0.10		37.67 ± 0.46		19.83 ± 0.45	
100	55.774 ± 0.20		48.48 ± 0.85		51.06 ± 0.91		43.84 ± 1.15	
Conc. (µg/mL)	CEAC		EEAC		MEAC		AEAC	
	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value
20	3.52 ± 0.05		7.79 ± 0.16		17.14 ± 1.08		0.24 ± 0.07	
40	4.9 ± 0.29		9.42 ± 0.23		32.82 ± 0.77		3.87 ± 0.28	
60	7.79 ± 0.17	544.29	13.13 ± 0.22	328.38	40.80 ± 0.85	61.79	4.29 ± 0.30	249.82
80	8.37 ± 0.48		16.7 ± 0.44		47.80 ± 0.85		6.23 ± 0.11	
100	10.33 ± 0.33		17.14 ± 0.08		61.17 ± 2.28		10.27 ± 0.42	

All values are expressed in terms of ± SD and are found to be significant when compared to control $p < 0.05$.

Growth assay: The results of effect of various *A. catechu* flower extracts on inhibition of crystal growth are shown in Table-6. Cystone standard solution exhibited the least IC_{50} value of 84.21 and showed stronger inhibition activity than the extract of *A. catechu* in the nucleation of calcium oxalate salts, whereas the methanolic extract showed the maximum inhibition of 49.43 ± 0.93 at the concentration of 100 µg/mL with the IC_{50} values of 90.00.

TABLE-6
EFFECT OF VARIOUS *Areca catechu* FLOWER EXTRACTS ON INHIBITION OF CRYSTAL GROWTH

Conc. (µg/mL)	Cystone		HEAC		PEEAC		EAEAC	
	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value
20	15.687 ± 0.12		8.51 ± 0.53		19.29 ± 0.40		10.43 ± 0.69	
40	18.787 ± 0.10		9.3 ± 0.11		24.30 ± 1.40		12.32 ± 0.36	
60	29.788 ± 0.34	84.21	13.03 ± 0.17	367.89	25.52 ± 0.58	123.38	14.88 ± 0.36	299.09
80	36.576 ± 0.07		14.94 ± 0.85		35.10 ± 1.51		18.63 ± 1.00	
100	51.132 ± 0.26		17.02 ± 0.96		39.66 ± 0.89		21.34 ± 0.71	
Conc. (µg/mL)	CEAC		EEAC		MEAC		AEAC	
	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value	Inhibition (%)	IC_{50} value
20	3.80 ± 0.10		5.87 ± 0.79		27.82 ± 1.80		2.53 ± 0.20	
40	13.77 ± 1.13		17.52 ± 0.85		34.54 ± 1.09		13.42 ± 2.39	
60	13.35 ± 2.15	122.04	19.17 ± 0.41	151.05	37.79 ± 1.97	90.00	19.59 ± 0.45	172.83
80	26.81 ± 0.40		23.92 ± 0.22		40.72 ± 2.24		20.56 ± 0.16	
100	36.19 ± 0.65		30.67 ± 0.50		49.43 ± 0.93		25.42 ± 0.96	

All values are expressed in terms of ± SD and are found to be significant when compared to control $p < 0.05$.

Titrimetric method: The results of effect of various solvent extracts of *A. catechu* flowers on dissolution of crystals are shown in Table-7. It was observed that the maximum percentage dissolution among the extracts was shown by methanolic extracts

TABLE-7
EFFECT OF VARIOUS SOLVENT EXTRACTS OF *Areca catechu* FLOWERS ON THE DISSOLUTION OF CRYSTALS

Group	Concentration (mg/mL)	Vol. of std $KMnO_4$ (mL)	Weight of calcium estimated (mg)	Weight of calcium reduced	Dissolution (%)
Control	–	1.5	0.284	–	–
Cystone	20	0.9	0.171	0.113	39.45
	40	0.5	0.095	0.189	66.58
	60	0.4	0.076	0.208	73.27
	80	0.3	0.057	0.227	79.95
	100	0.2	0.038	0.246	86.63
HEAC	20	1.7	0.256	0.028	9.85
	40	1.5	0.244	0.040	14.08
	60	1.2	0.174	0.110	38.73
	80	1.2	0.140	0.144	50.70
	100	0.9	0.119	0.165	58.09
PEEAC	20	1.4	0.224	0.060	21.12
	40	1.4	0.201	0.083	29.22
	60	1.2	0.179	0.105	36.97
	80	0.9	0.176	0.108	38.02
	100	0.8	0.143	0.141	49.64
EAEAC	20	1.3	0.198	0.086	30.28
	40	1.4	0.171	0.113	39.78
	60	1.1	0.147	0.137	48.23
	80	0.9	0.125	0.159	55.98
	100	0.8	0.103	0.181	63.73
CEAC	20	1.5	0.201	0.083	29.22
	40	1.4	0.155	0.129	45.42
	60	1.2	0.132	0.152	53.52
	80	1.0	0.117	0.177	62.32
	100	0.7	0.098	0.186	65.49
EEAC	20	1.2	0.189	0.095	33.45
	40	1.0	0.118	0.166	58.45
	60	0.9	0.097	0.187	65.84
	80	0.8	0.078	0.206	72.53
	100	0.7	0.070	0.214	75.35
MEAC	20	1.2	0.184	0.100	35.21
	40	1.1	0.156	0.128	45.07
	60	0.8	0.122	0.162	57.04
	80	0.8	0.091	0.193	67.95
	100	0.6	0.066	0.218	76.76
AEAC	20	1.8	0.245	0.039	13.73
	40	1.5	0.221	0.063	22.18
	60	1.4	0.207	0.081	28.52
	80	1.1	0.198	0.086	30.28
	100	1.0	0.170	0.114	40.14

(75.35%) at the concentration of 100 mg/mL. Cystone showed maximum percentage (86.63%) dissolution at the concentration of 100 mg/mL.

Conclusion

Based on the different solvent extracts, the current study shows that the methanolic extract of *A. catechu* flower have potent antiurolithiatic activity. Additionally, the effect of the methanolic extracts on calcium oxalate crystallization showed that the percentage of nucleation inhibition was the highest inhibition (61.17 ± 2.28) along with IC_{50} value of 61.79 at the concentration of 100 µg/mL. The identified phytochemicals in the *A. catechu* flower were especially dominated by phenolic compounds, which could be responsible for the antiurolithiatic activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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