

# Study on in vitro Antinephrolithiasis activity of ethanolic extract of Uncaria gambir Roxb leaves

*by Khoirun Nisyak*

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**Submission date:** 02-Aug-2023 07:06PM (UTC-0700)

**Submission ID:** 2140630694

**File name:** KN\_for\_ISoC\_ITS\_2022.pptx (3.09M)

**Word count:** 418

**Character count:** 2395



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# Study on Antinephrolithiasis Activity of Ethanolic Extract of *Uncaria gambir* Roxb leaves

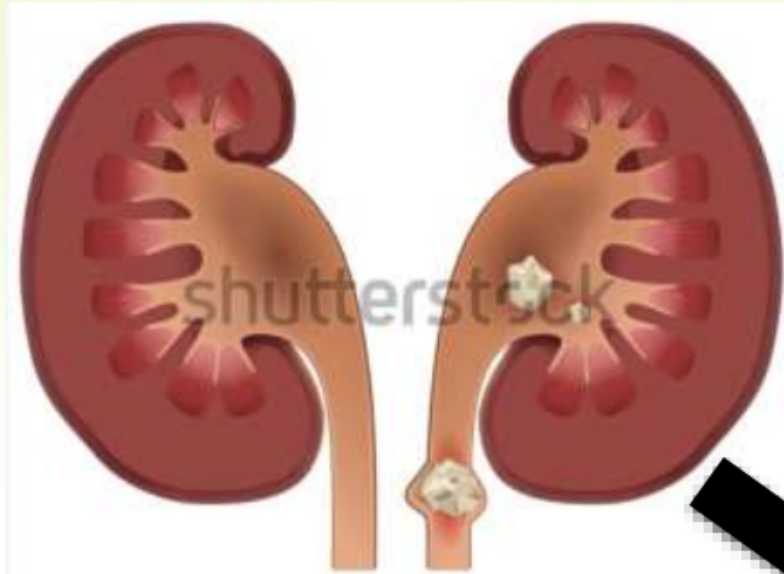
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12 – 13 Oktober 2022  
International Seminar on Chemistry (ISOC V) – Institut Teknologi Sepuluh

# BACKGROUND



renal calculi = crystal concretions formed typically in the kidney

Nephrolithiasis = Kidney Stones

2013

0,6% Indonesia  
0,7% Jawa timur  
0,7% Sidoarjo



Ca-oxalate &  
Ca-phosphate

**SURGICAL  
INTERVENTION**

high costs and results in risks, such as tissue damage, and bacteriuria infection

# GAMBIR

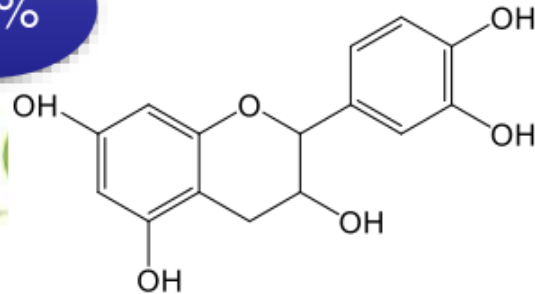
*Uncaria gambir* Roxb.



antioxidant, antidiabetic, antimicrobial,  
anticancer, and anticariogenic

catechins, polyphenols, alkaloids, saponins,  
tannins, epicatechins, and caffeic acid

73%



prevent renal calcium crystallization *in vivo* and *in vitro*

Ca-Catechin Complex

Soluble in  
water



# OBJECTIVE

The purpose of this study was to determine the effect and activity of ethanol extract of gambier leaves on the destruction of kidney stones in vitro.



# METHODS

Extraction

Maceration with EtOH, fractionation with EA and n-hexane

Identification  
of  
chemical  
compounds

Phytochemical screening with TLC and LC-MS

Characterization  
of Kidney stones

Qualitative test & XRF

Kidney stones  
dissolution test

Incubation at 37 C for 5 h, Ca  
level dissolved = AAS

Data  
Analysis

# RESULT AND DISCUSSION



PEMERINTAH PROVINSI JAWA TIMUR  
DINAS KESEHATAN  
UPT LABORATORIUM HERBAL MATERIA MEDICA BATU  
Jalan Labor No.87 Telp. (0343) 993386, e-mail : uktlabherbal@prov.jatim.go.id  
KOTA BATU 65113

Nama : 0701 5413/ 122.7/2020  
Jenis : Daun  
Detail : *Tamarindus indica*

Mencakupi prosedur standar :

Nama : HARVY ANDYAH  
NPM : 1903180030  
Fakultas : FARMASI STIKES BUNDAH SAKIT ANWAR MUDORA

## 1. Perintah dan nama tanaman/garuda

Kepala	Panas (Tanduk)
Bulungan	Tanduk (Tanduk)
Bagian Atas	Spermatophyta (Tanduk)
Daun	Magnoliophyta (Tanduk)
Kulit	Monocotyledon
Batang	Dicotyledon
Daun	Polyploid
Warna	Polyploid
Jenis	Polyploid
Nama Umum	Polyploid
Rasio Determinasi	Polyploid

2. Morfologi  
Habitat: Tamarindus purba, tinggi 5-7 m. Batang: Batang tegak, bulat, perantara  
umputul, warna coklat pucat. Daun: Daun tunggal, berbentuk, bentuk bulat, tepi bergerigi, pangkal  
bulat, ujung membulat, panjang 5-7 cm, lebar 4-7 cm, warna hijau. Bunga: Bunga majemuk, bentuk  
bulat, di ketiak daun, panjang lebih kurang 7 cm, memiliki 7 buah berwujud bulat, warna ungu.  
Buah: Buah berwujud bulat, panjang lebih kurang 1,2 cm, warna hitam.

3. Bagian yang digunakan : Daun

4. Penggunaan : Obat

5. Daftar Pustaka :

- Syamsulhuda, (di) Ageng dan Jufri R. H. (2019). *Tamarindus indica* (Materi Determinasi I).
- Departemen Kesehatan Republik Indonesia. *Buku Panduan dan Pengendalian Kesehatan*.
- Van Soest, (2002). *PCNA: untuk Analisis di Laboratorium*. Pustaka Pustaka, Jakarta.

Dengan cara kerangka determinasi ini bisa jadi untuk mengidentifikasi tumbuhan tersebut.

Batu, 16 November 2020  
UPT LABORATORIUM HERBAL  
MATERIA MEDICA BATU  
FARMASI  
NPM: 1903180030



13,57%

Crude Ethanolic  
Extract

EA

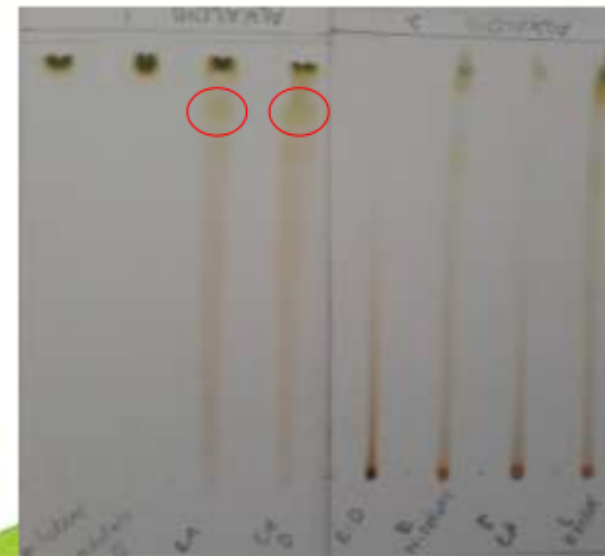
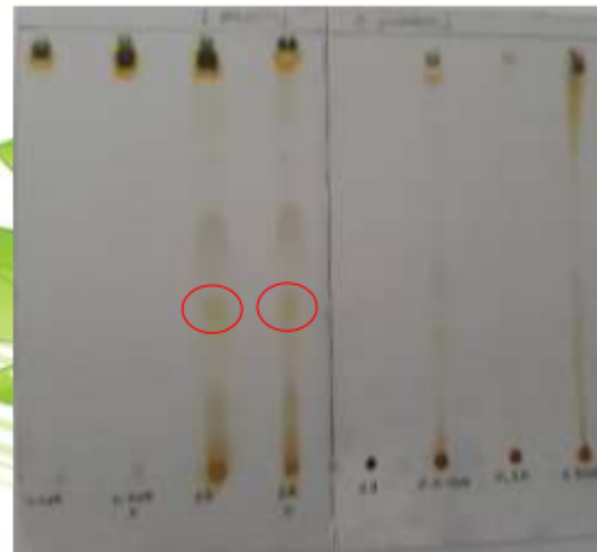
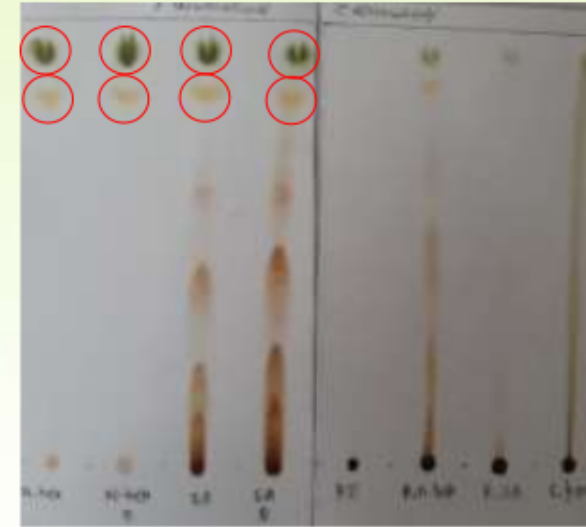
Residue

N-hexane



# Phytochemical Screening of Gambir Leaves Extract

No.	Secondary Metabolite Compound	Results		
		F. EA	F. n-hexane	Residual fraction
1	Alkaloid	+	-	-
2	Flavonoid	+	-	+
3	Tannin	+	-	+
4	Terpenoid	-	-	-
5	Antrakuinon	+	+	-
6	Katekin	+	+	-

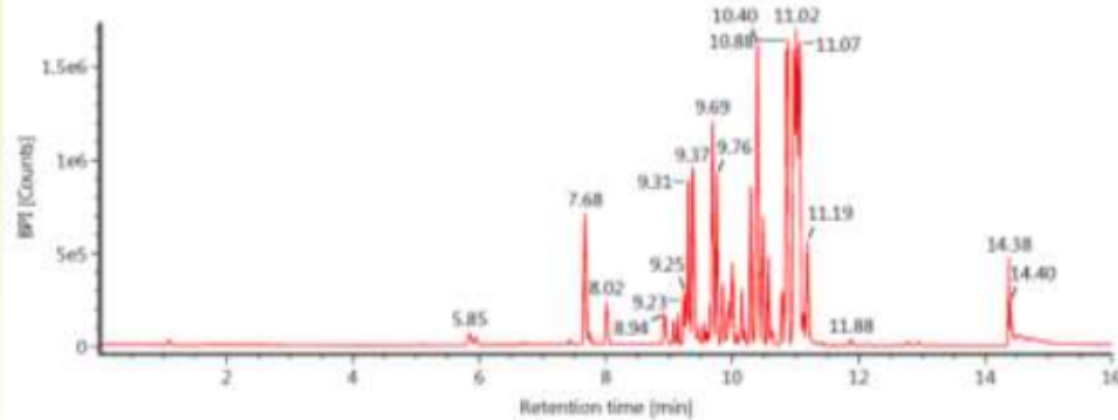




## F. n-hexane

Item name: 210302-0425

Channel name: 1: TOF MS<sup>+</sup> (50-1200) 6eV ESI<sup>+</sup> - Low CE (BPI)

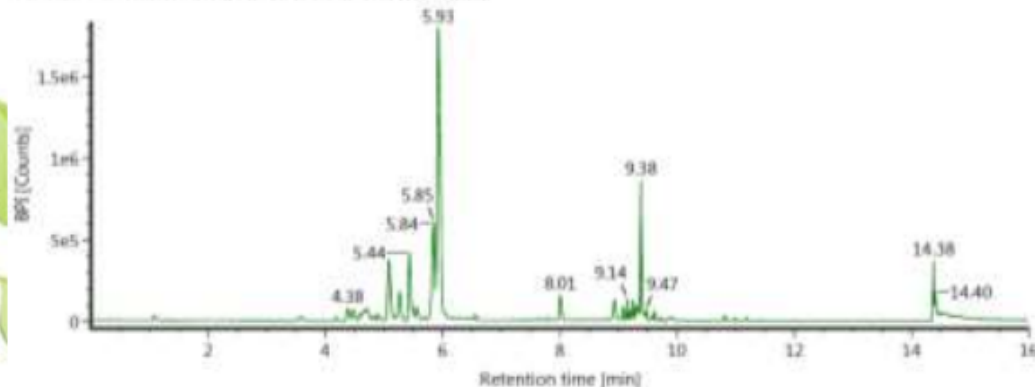


Stigmastan-3,6-dione 5.67%,  $C_{45}H_{84}O_{14}$  (m/z 871) 20.04%,  $C_{45}H_{84}O_{15}$  (m/z 887) 5.98%,  $C_{36}H_{44}O_9$  (m/z 621) 3.95%,  $C_{28}H_{46}O_2$  (m/z 415) 3.3%

## Residual Fraction

Item name: 210302-0427

Channel name: 1: TOF MS<sup>+</sup> (50-1200) 6eV ESI<sup>+</sup> - Low CE (BPI)

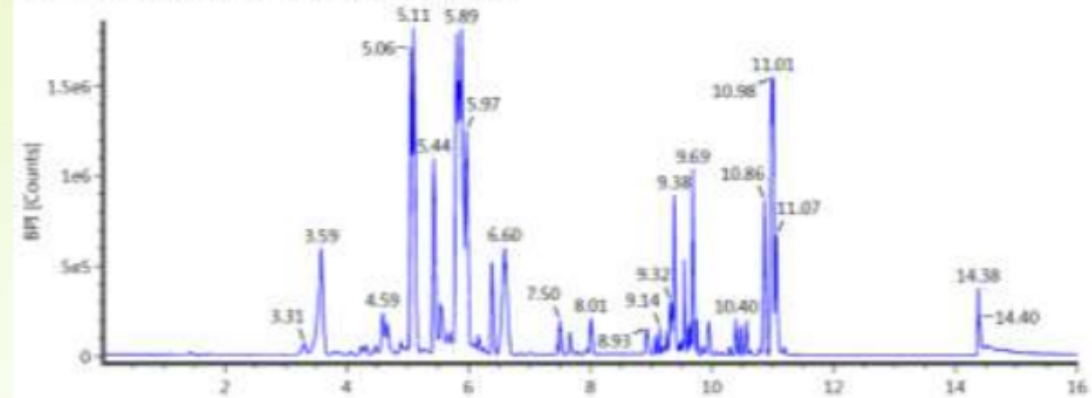


Grosvenorine 0.33%, Procyanidin A2 2%, Quercetin 0.41%,  $C_{23}H_{40}O_{19}$  (m/z 621) 3.12%,  $C_{23}H_{46}O_{20}$  (m/z 643) 2.24%

## F. Ethyl acetate

Item name: 210302-0426

Channel name: 1: TOF MS<sup>+</sup> (50-1200) 6eV ESI<sup>+</sup> - Low CE (BPI)



d-catechin 3.14%, Procyanidin A2 12.23%,  $C_{23}H_{40}O_{19}$  (m/z 621) 17.89%,  $C_{45}H_{84}O_{14}$  (m/z 871) 14.09%,  $C_{23}H_{46}O_{20}$  (m/z 643) 5.87%

# Characterization of Kidney Stones

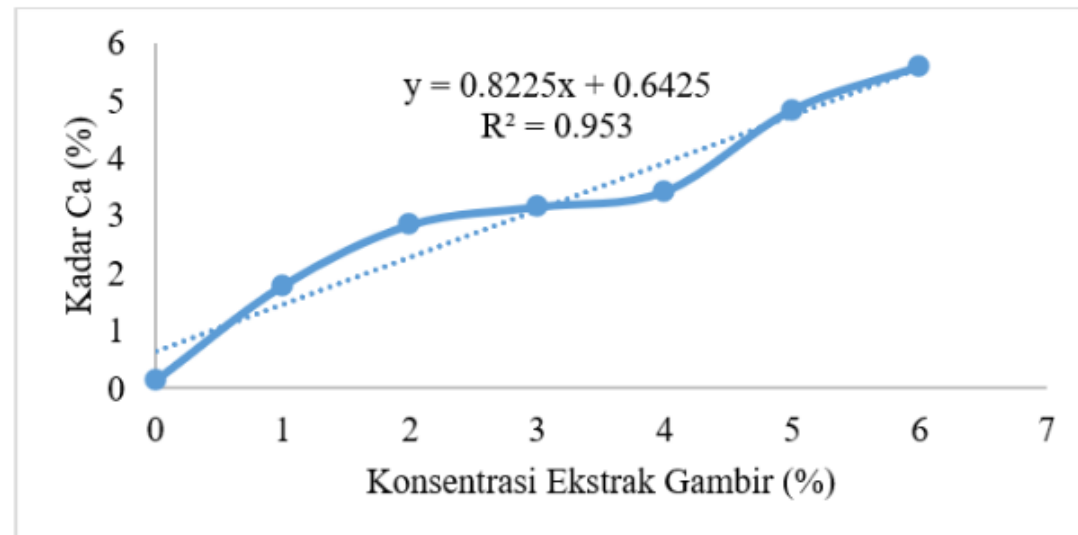


Kidney Stones	
Component	(% weight)
Mg	0,1611
Si	0,201
P	2,7421
S	0,0783
Cl	0,0401
K	0,0512
Ca	16,2657
Zn	0,0221
Sr	0,0221
Ag	0,0378
Balance	80,3784

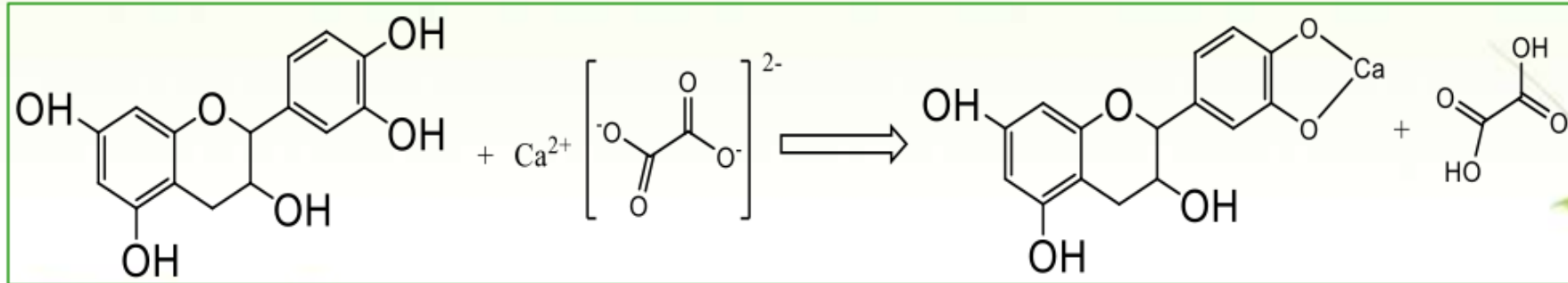
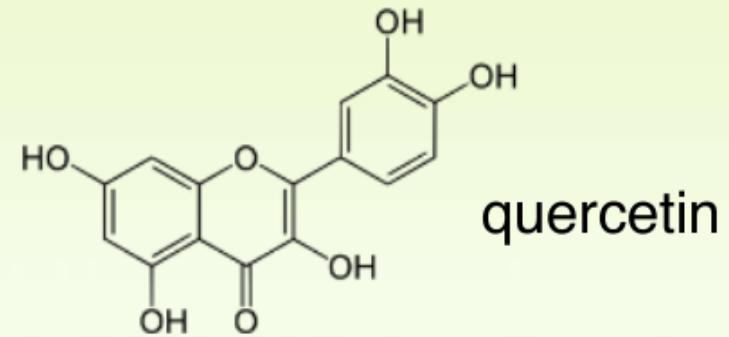
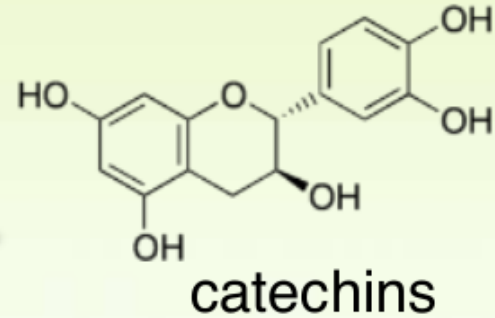
## Kidney stone solubility test with ethanol extract of gambir

No	Sampel	Kadar Ca						Rata-rata		SDV
		Ulangan 1		Ulangan 2		Ulangan 3				
		ppm	%	ppm	%	ppm	%	ppm	%	
1	Aquades	0,89	0,14	0,9	0,14	0,88	0,14	0,89	0,14	0,0082
2	Batugin	16,63	2,56	16,45	2,53	16,5	2,54	16,53	2,54	0,0759
3	EG 1%	11,61	1,78	11,83	1,82	11,42	1,76	11,62	1,79	0,1675
4	EG 2%	18,64	2,86	18,32	2,82	18,5	2,84	18,49	2,84	0,1310
5	EG 3%	20,51	3,15	20,32	3,12	20,62	3,17	20,48	3,15	0,1239
6	EG 4%	22,2	3,41	22,2	3,41	22,4	3,44	22,27	3,42	0,0943
7	EG 5%	31,56	4,85	31,23	4,80	31,66	4,87	31,48	4,84	0,1837
8	EG 6%	36,53	5,61	36,24	5,57	36,4	5,59	36,39	5,59	0,1186

The higher the concentration of gambir leaf extract, the higher the dissolved calcium level of kidney stones



# Plausible Mechanism of Reaction





# Data Analysis

	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Unstandardized Residual	.227	7	.200*	.914	7	.426

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	18.942	1	18.942	101.292	.000 <sup>b</sup>
	Residual	.935	5	.187		
	Total	19.877	6			

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.976 <sup>a</sup>	.953	.944	.43244

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.643	.295		2.180	.081
	Konsentrasi Ekstrak Daun Gambir	.823	.082	.976	10.064	.000

F value > F table => the concentration of gambier leaf extract (X) has an influence on dissolved calcium levels (Y).

# Conclusion

- There is an effect of ethanol extract of gambir leaf as antinephrolithiasis agent, the higher the concentration of ethanol extract gambier leaves, the higher the calcium levels of dissolved kidney stones.
- The presence of catechin compounds in gambier leaves can react with calcium in kidney stones to form a water-soluble Ca-catechin complex.



# THANK YOU



# Study on in vitro Antinephrolithiasis activity of ethanolic extract of Uncaria gambir Roxb leaves

GRADEMARK REPORT

FINAL GRADE

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GENERAL COMMENTS

Instructor

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