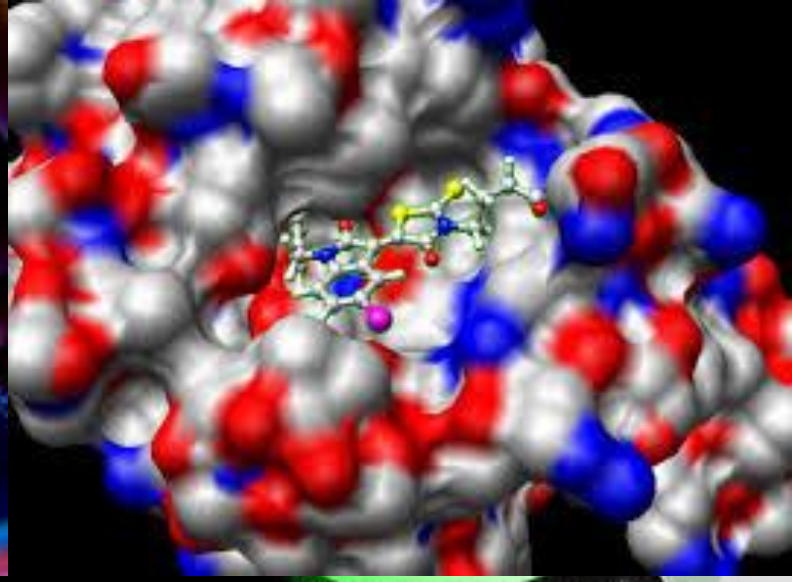
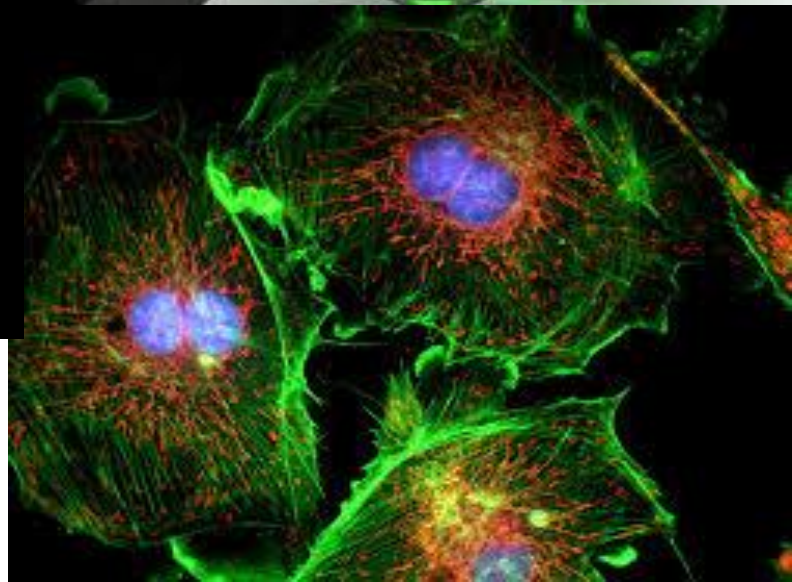
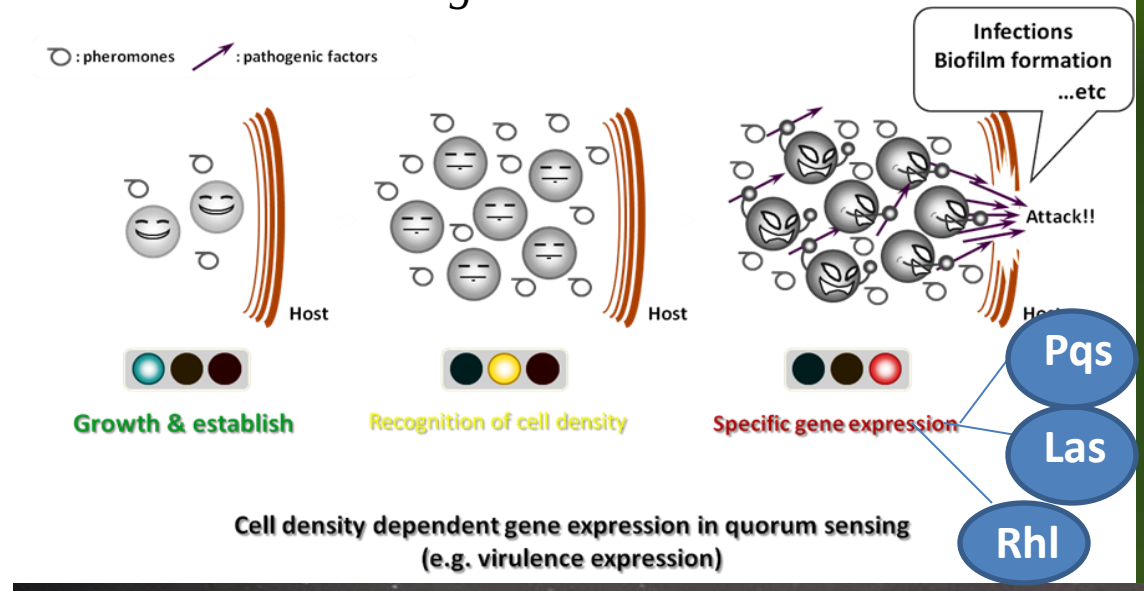


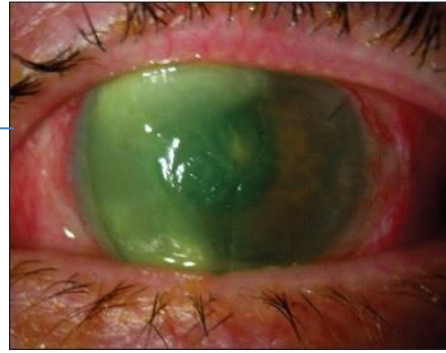
ROLE OF ANALYTICAL, *IN SILICO* AND IMAGING TOOLS IN CHARACTERISATION AND ACTIVITY STUDIES OF AJOENE FROM GARLIC BULBS AGAINST BIOFILM OF *Pseudomonas aeruginosa*

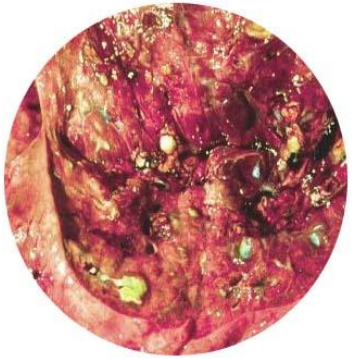


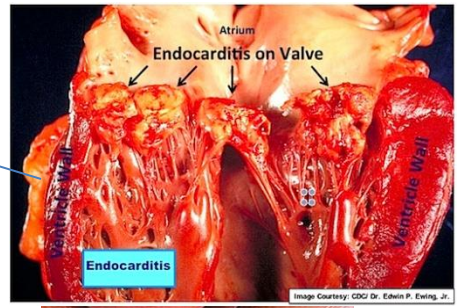
ANITHA VADEKEETIL

Pseudomonas aeruginosa








Atrium
Endocarditis on Valve
Ventricle Wall
Ventricle Wall
Endocarditis
Image Courtesy: CDC/ Dr. Edwin P. Ewing, Jr.

Garlic

Regulate Blood Sugar

Helps High Blood Pressure

Fights Off Flu

Reduces Cardiovascular Disease

Treats Skin Infections

Fights infections

Boosts Immune System

Lower Cholesterol

Kills Fungus

High In Antioxidants

Helps Acne

Prevents Food Poisoning

Reduces Colds

Prevents Cancer

Anti-Inflammatory

Treats Hyperthyroid

Source of Vitamin B6

Reduces Risk Of Osteoarthritis

Heals Cold Sores

Natural Antibiotic

Kills Bacteria

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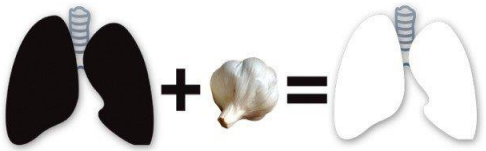
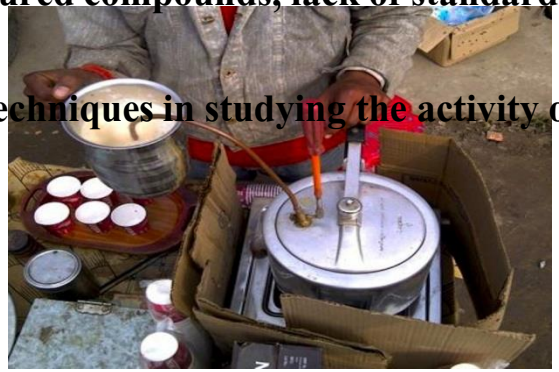


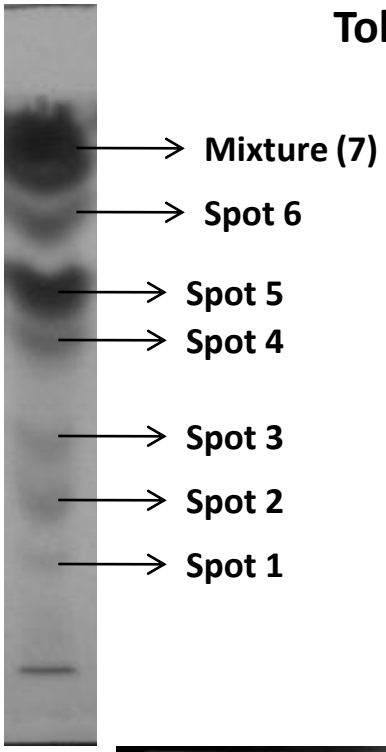
❖**However, the occurence of similarly structured compounds, lack of standards, limits the separation of active constituents.**

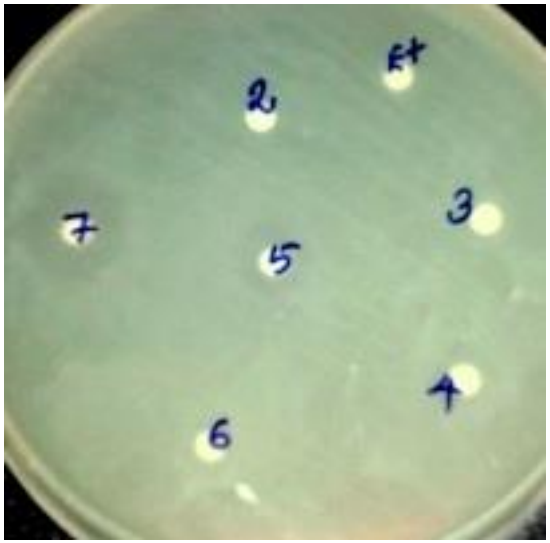
❖ **In addition to that, appropriate usage of techniques in studying the activity of active constituents against biofilm is required**



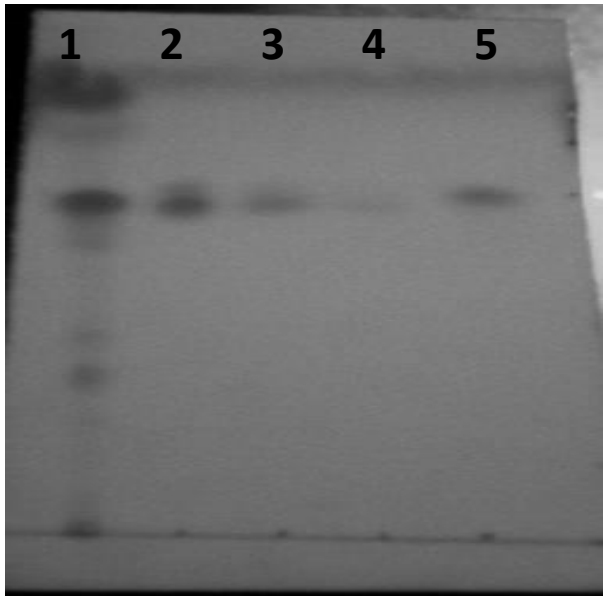
1. Analytical tools — TLC, HPTLC, HPLC, NMR, MS
2. Imaging tools — CLSM, SEM
3. *In silico* tools — Molecular docking

Separation and Identification of ajoene as a QSI from Toluene Garlic Extract (TGE)





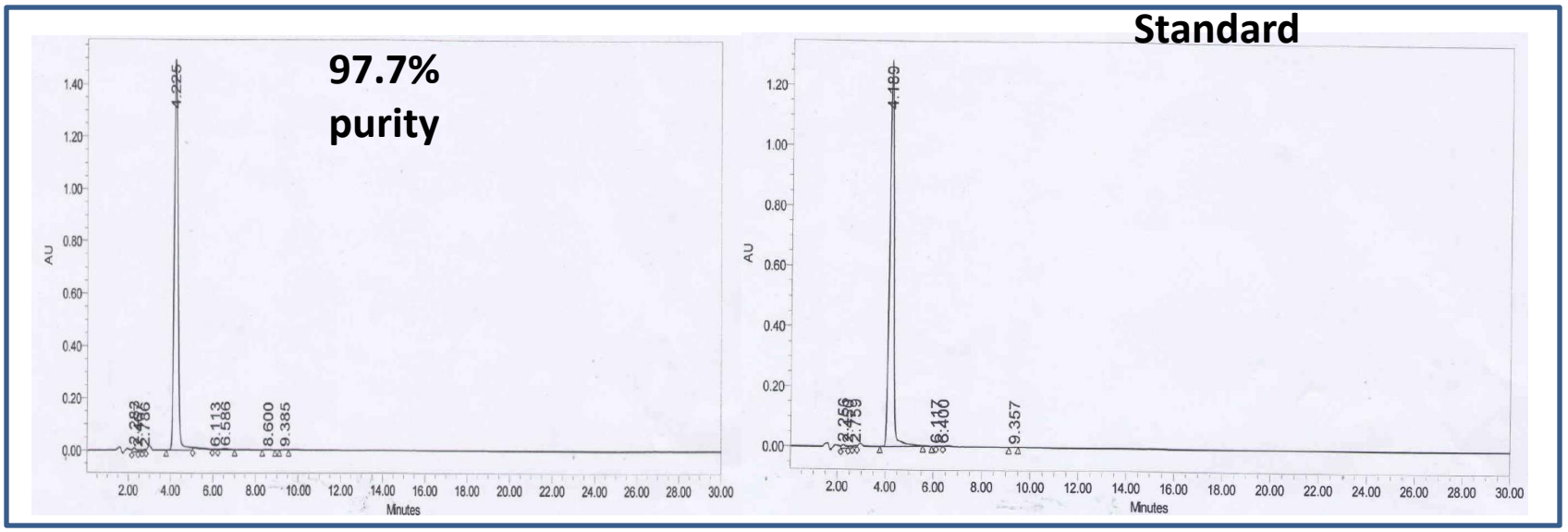
TLC SPOTS (2 mg/10 µl EtoAc)	DIAMETER (cm)
2	NIL
3	NIL
4	0.7
5	1.0
6	0.6
7	1.2
EtoAc (10 µl)	NIL

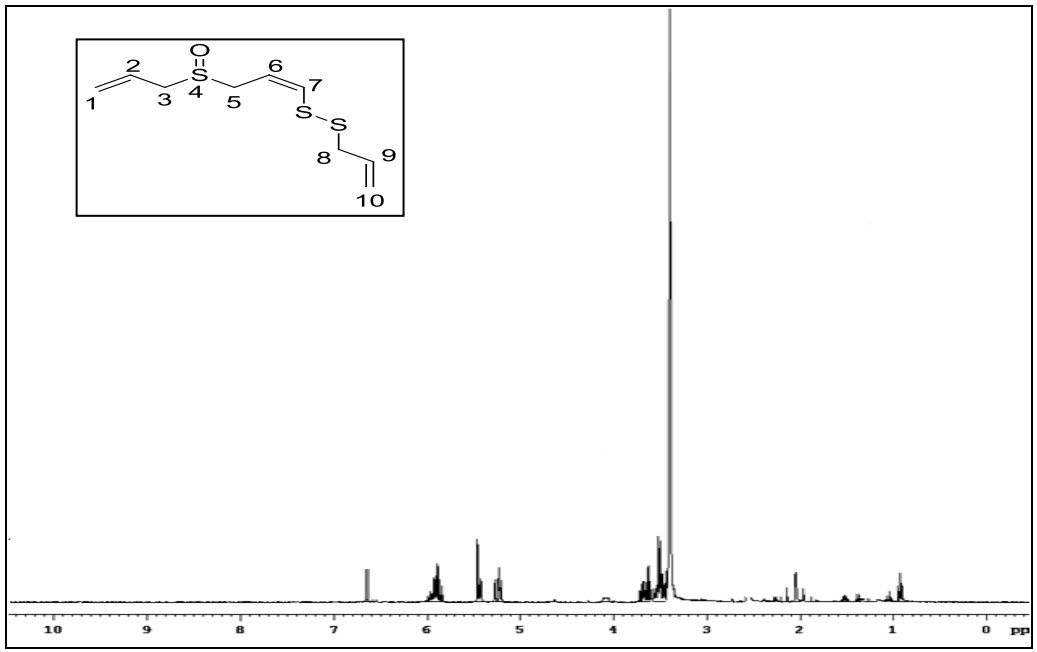


Lane	Sample	Volume
1	TGE (50mg/ml)	10 µl
2	Standard ajoene (5mg/ml)	5 µl
3	Standard ajoene (1 mg/ml)	5 µl
4	Standard ajoene (250 µg/ml)	5 µl
5	Spot 5 (5 mg/ml)	5 µl

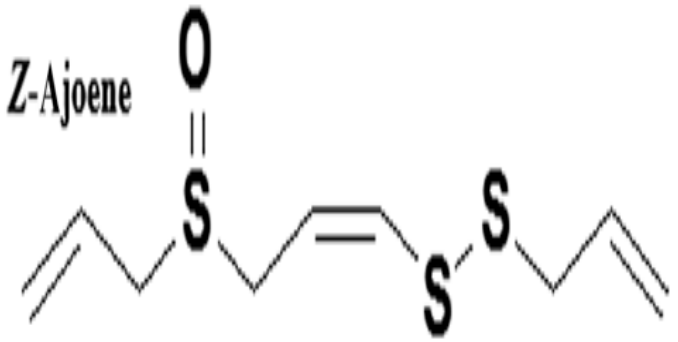
Characterization of Ajoene

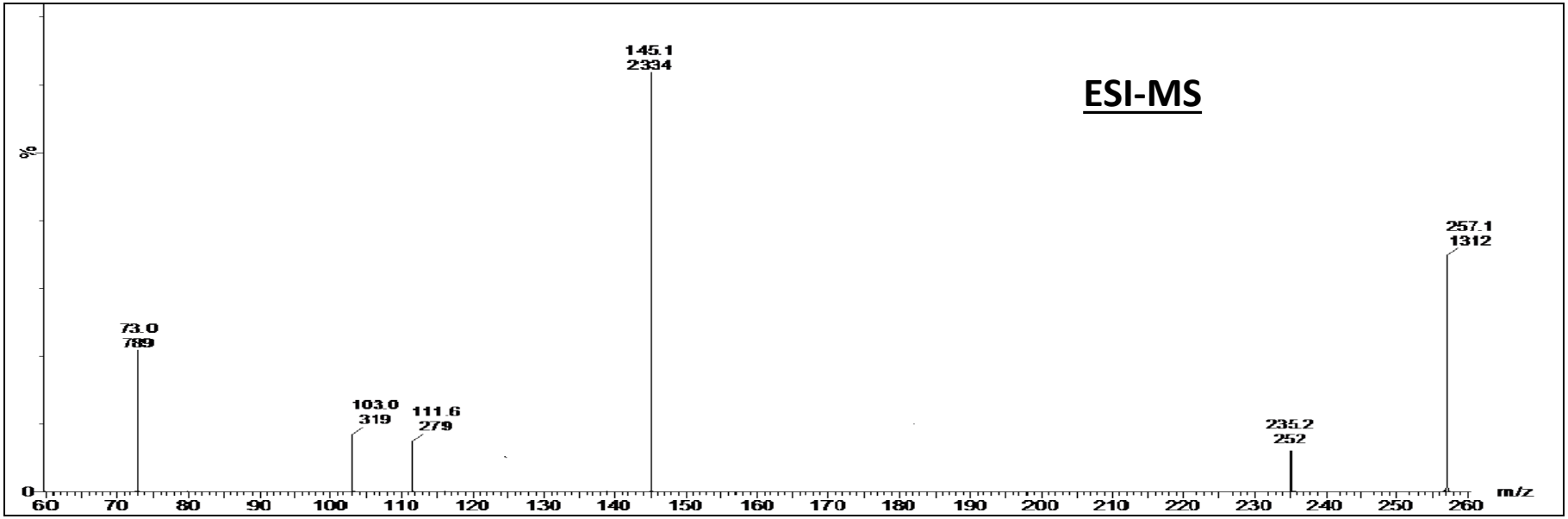
HPLC

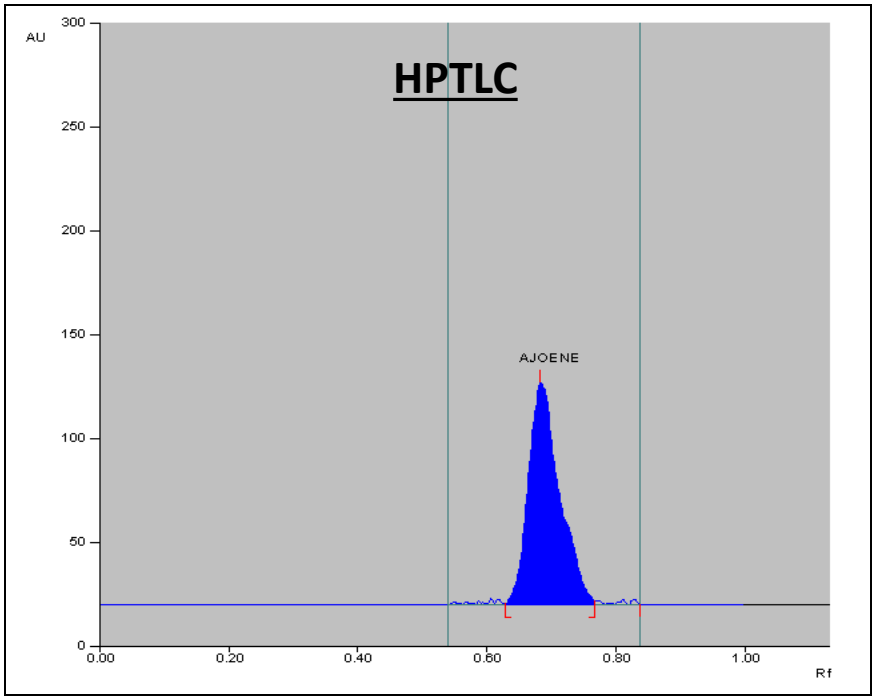


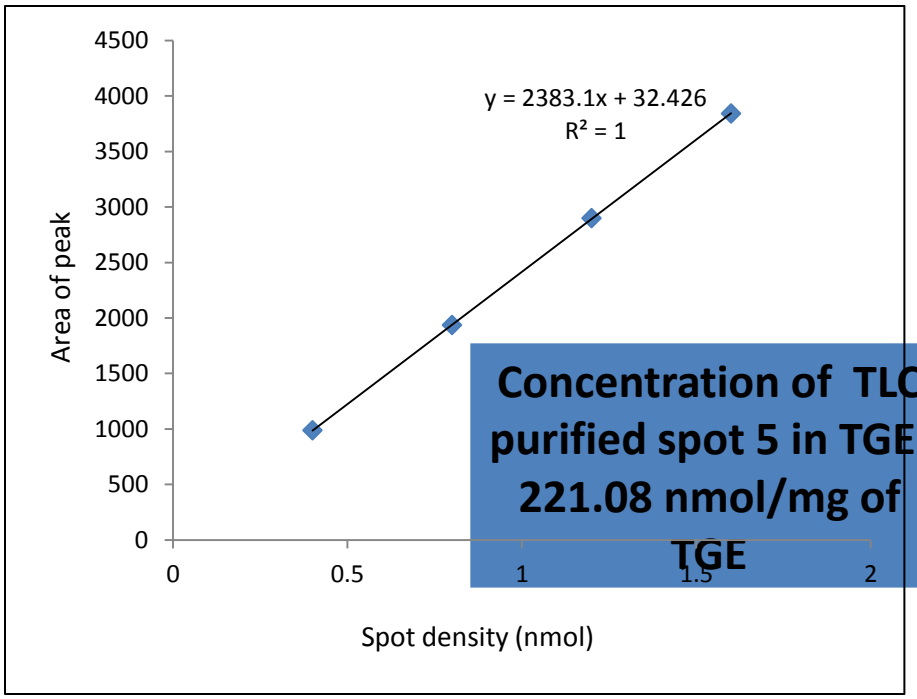


NMR




ESI-MS
145.1
2394
257.1
1312
73.0
789
103.0
319
111.6
279
235.2
252
%
0
m/z
60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260


HPTLC
AU
AJOENE
300
250
200
150
100
50
0
0.00 0.20 0.40 0.60 0.80 1.00
Rf


y = 2383.1x + 32.426
R² = 1
Area of peak
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0 0.5 1 1.5 2
Spot density (nmol)
Concentration of TLC purified spot 5 in TGE: 221.08 nmol/mg of TGE

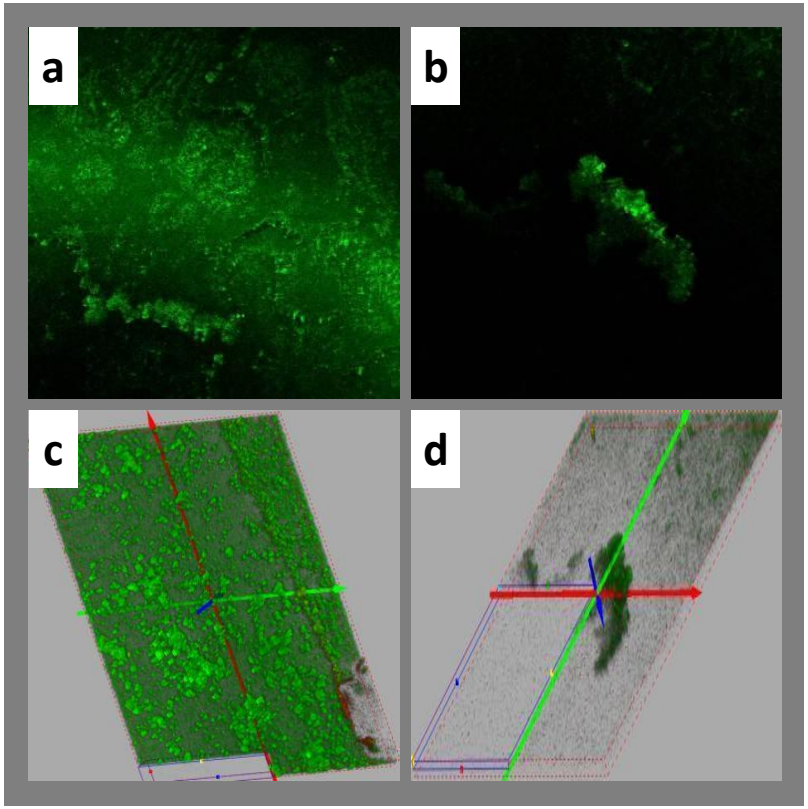
STABILITY OF NATURAL AJOENE

TLC-densitometric analysis showing effect of physical conditions on the amount of natural ajoene in toluene extract of garlic

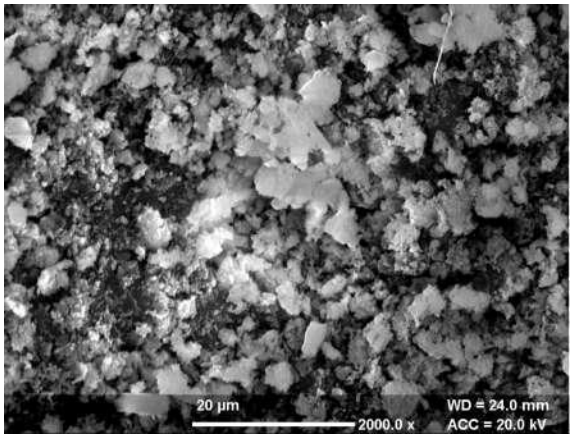
Sr. No.	Physical conditions	Amount of ajoene (nmol/mg of TGE)
1.	4°C, pH 7.0	221.06
2.	37°C, pH 2.0	144.19
3.	37°C,pH 4.0	138.3
4.	37°C, pH 9.0	48.852
5.	37°C,pH 7.0	28.516

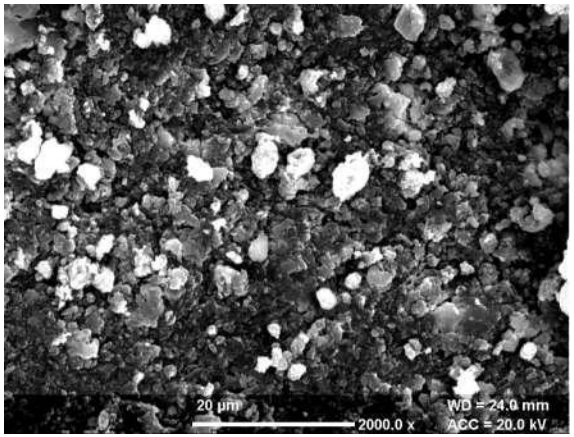
Hence the analytical tools helped to completely characterise the TLC purified spot 5 as Ajoene

CLSM


a
b
c
d

SEM

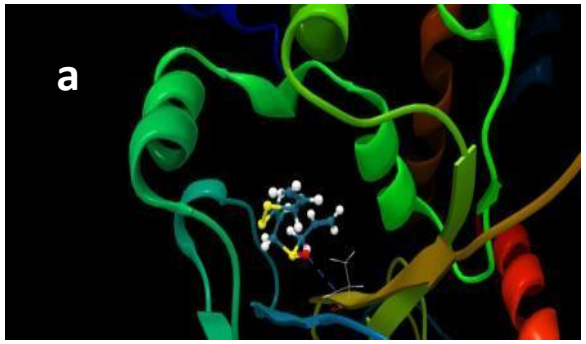

20 µm
2000.0 x
WD = 24.0 mm
ACC = 20.0 kV

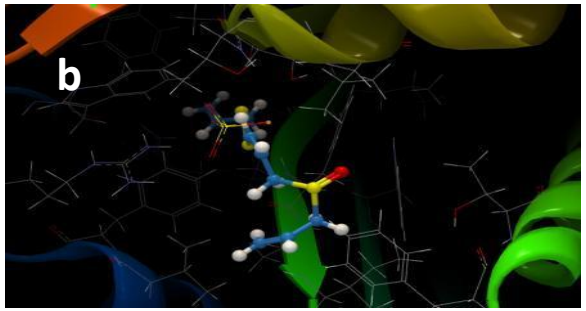

20 µm
2000.0 x
WD = 24.0 mm
ACC = 20.0 kV

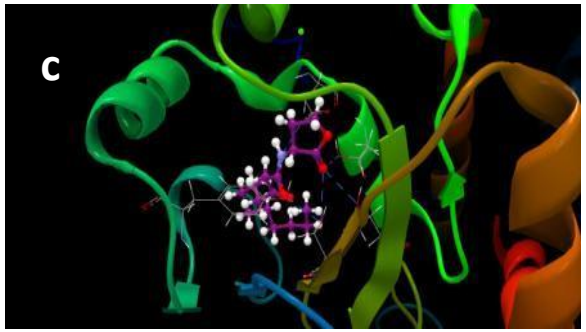
MOLECULAR DOCKING

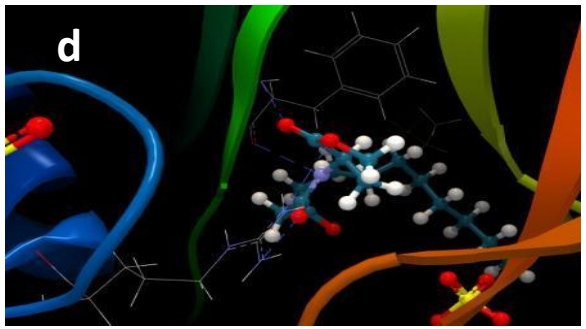
Ligand	Target	Docking Score
Ajoene	a) LasR	-57.08
	b) LasI	-52.5
	PqsR	-53.5
	PqsD	-45.6
	RhlR	-53.4
C12	c) LasR	-80.4
C12	d) LasI	-57.7
C4	RhlR	-41.9
PQS	PqsR	-62.8
	PqsD	-50.05

CLC Drug discovery work bench 1.5.1









Conclusion

The QSI from TGE is identified and characterised as Ajoene using the simplest methods

Ajoene distorts the biofilm architecture drastically using its QSI mechanism

The QSI mechanism associated with ajoene might be due to its interaction predominantly with LasI synthase and Rhl R receptor

Thank you for your attention !

Questions ?

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anicpeace@gmail.com