

ICV 5

ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICALS FROM VETIVER *Chrysopogon zizanioides*

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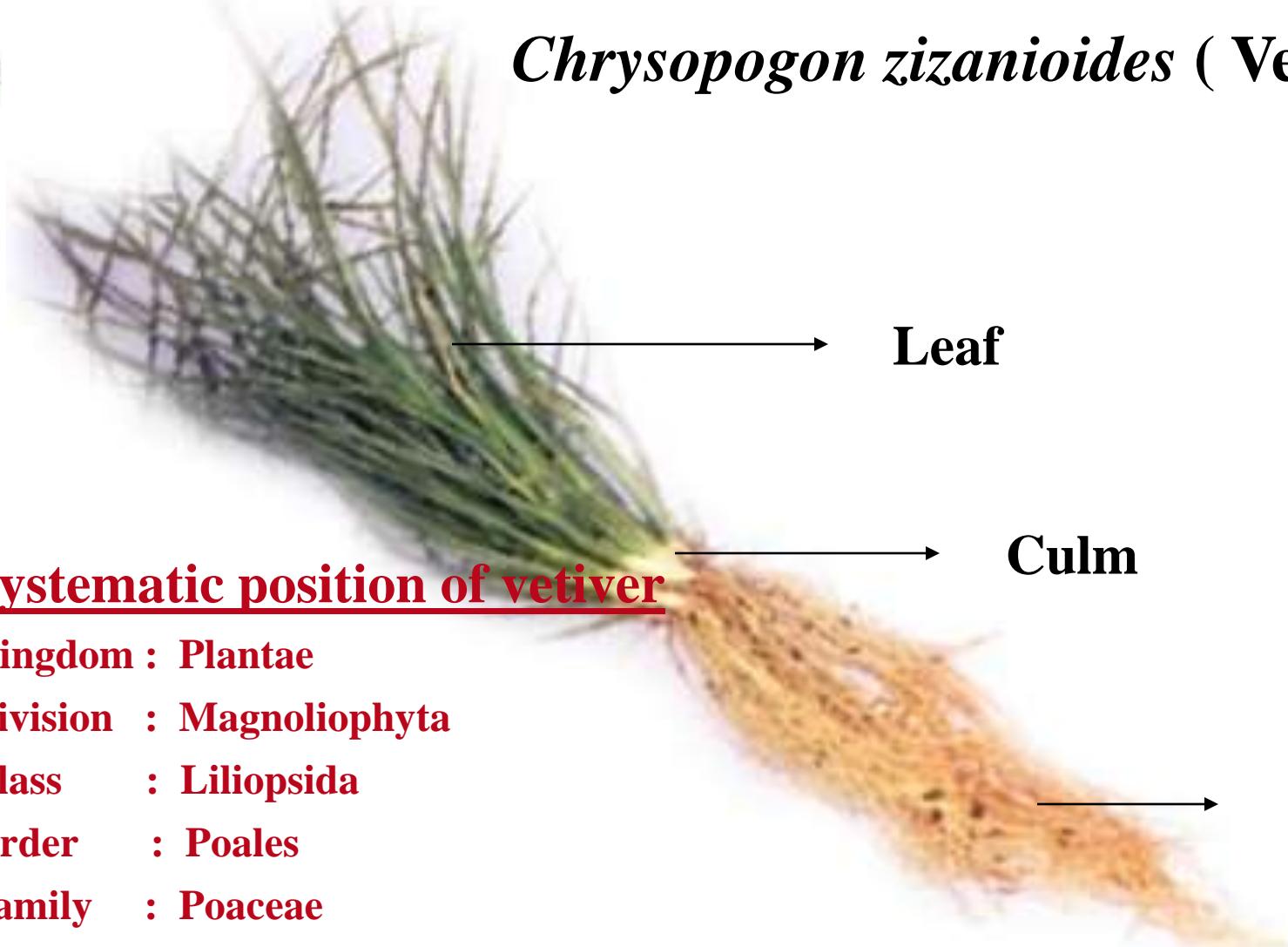
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AND

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Nehru Arts & Science College, Coimbatore, Tamilnadu, India

Chrysopogon zizanioides (Vetiver)



Systematic position of vetiver

Kingdom : Plantae

Division : Magnoliophyta

Class : Liliopsida

Order : Poales

Family : Poaceae

Genus : *Chrysopogon*

Species : *zizanioides*

Binomial name : *Chrysopogon zizanioides* (L.) Roberty

Leaf

Culm

Root

Chrysopogon zizanioides

VETIVER IS A NATIVE GRASS OF INDIA, HAS TRADITIONALLY BEEN IN USE FOR DECADES.

- *Vetiveria zizanioides* - Old name
- Common name vetiver
- Flowering and non-flowering
- Perennial grass - thick fibrous, adventitious roots
- Aromatic
- Partial hydrophyte & Xerophyte
- With stands pH 4 to pH 9
- Temperature – 9 °C to 45°C
- Does not encroach farmland



COLLECTION OF THE PLANT

Foot hill of Maruthamalai, CBE.



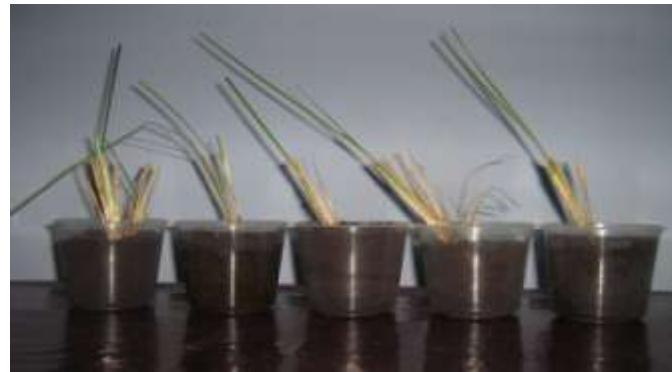
The objective of the study

✓ Biomass production - using

- Vermicompost (VC)
- Coir pith (CP)
- Cow dung (CD)
- Bio inoculants
- Soil types

- ✓ Screening for the bioactive compounds
- ✓ Phytoremediation (contaminated water and soil)
- ✓ Evaluating the economic potential of vetiver

PROPAGATION OF VETIVER CULMS IN THE LAB



IN WOODEN BOXES & SACS





















Propagated in the Department garden.





Lab study – in the experimental tank



VETIVER TO CONTROL SOIL EROSION IN THE UNIVERSITY CAMPUS



SLOPE STABILIZATION



SLOPE STABILIZATION IN THE CAMPUS









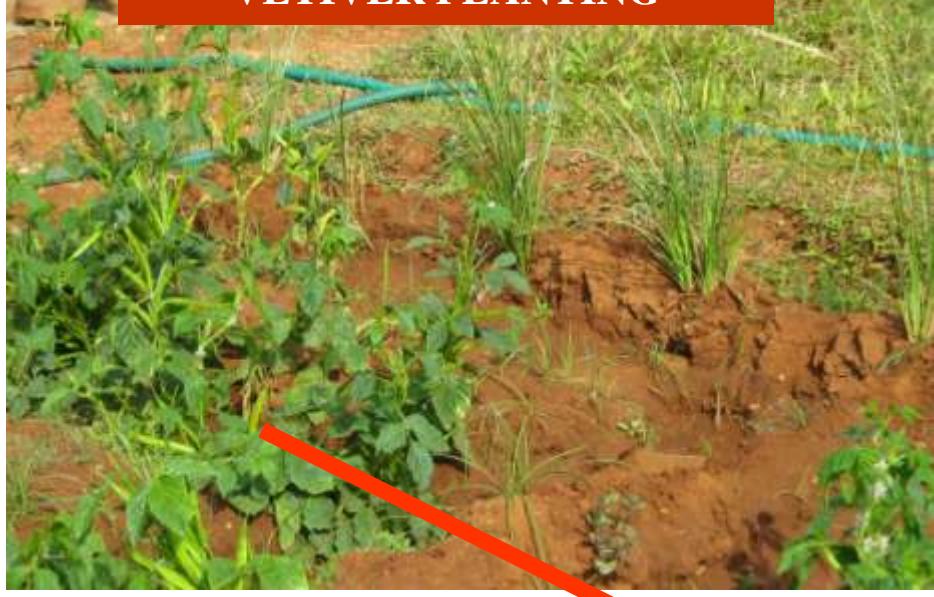
Vetiver as an intervening crop as a pest control – in the Department of Environmental Sciences



PEST ATTACKED PLANTS - BEFORE VETIVER PLANTING



HEALTHY PLANTS - AFTER VETIVER PLANTING



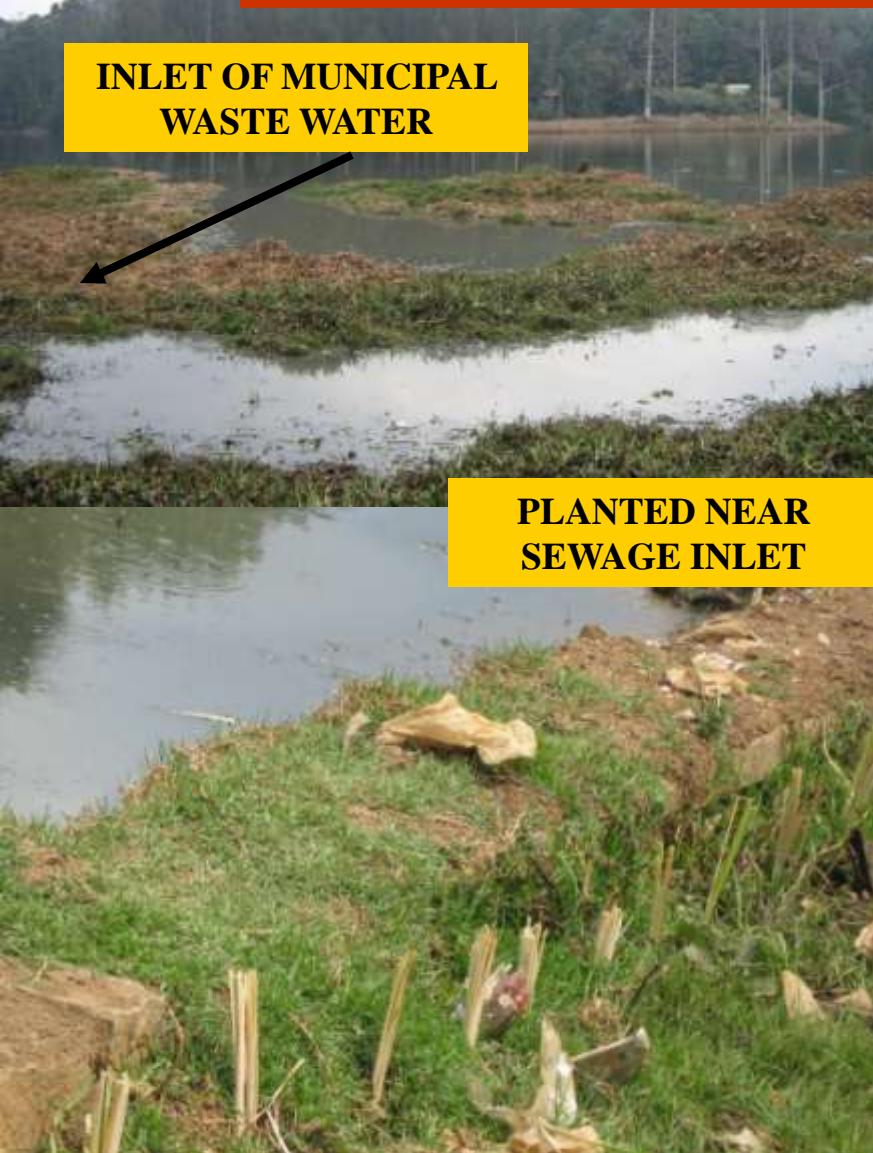
HYDROPONICS SYSTEM OF VETIVER IN OOTY LAKE



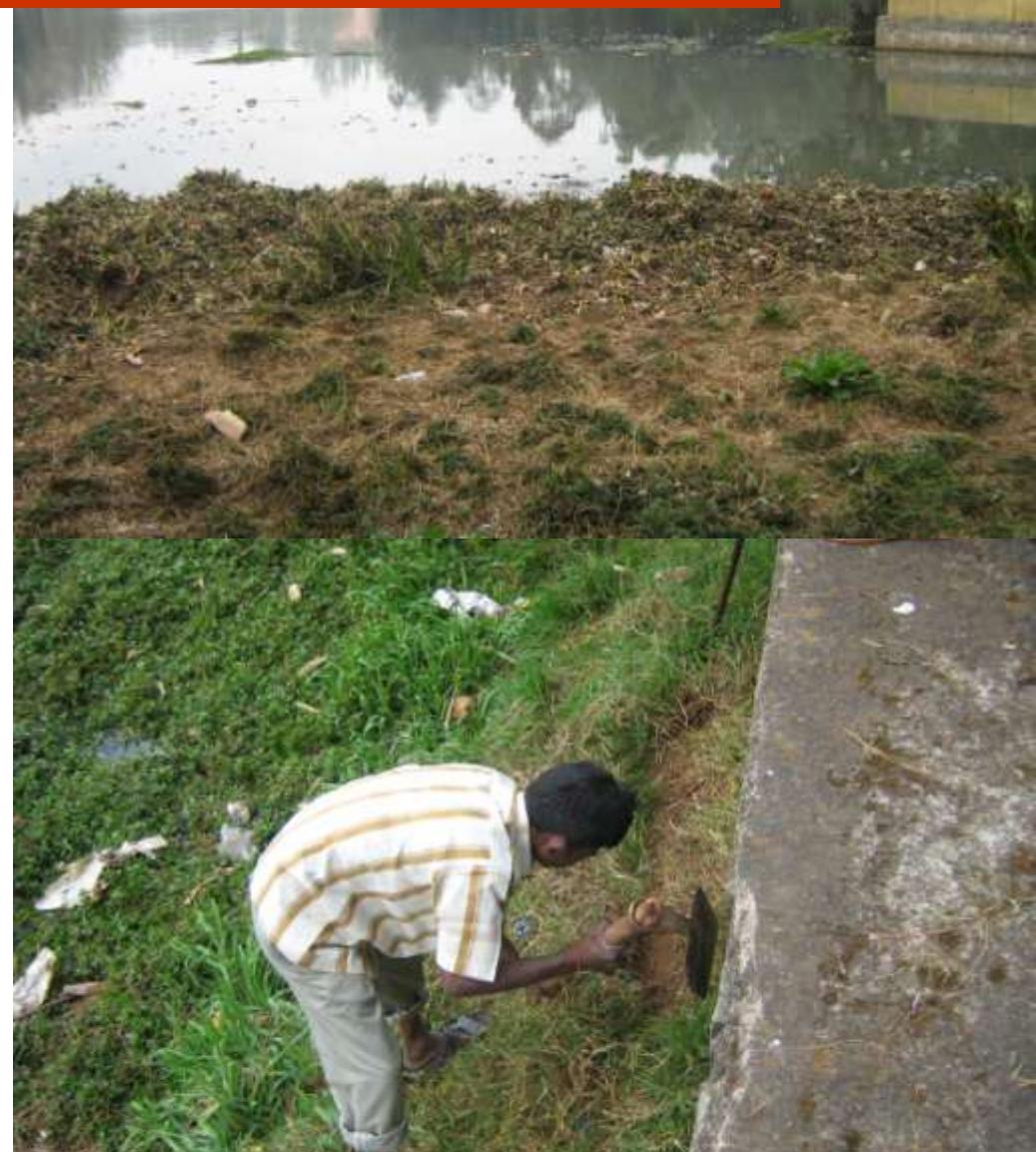


VETIVER FOR MUNICIPAL WASTEWATER TREATMENT

INLET OF MUNICIPAL WASTE WATER



PLANTED NEAR
SEWAGE INLET



WORK SHOP ON VETIVER - 19.06.2008



BOOK RELEASE ON VETIVER



WE HAVE BECOME THE MEMBER OF TVNI

THE VETIVER SYSTEM

INTRODUCING THE VETIVER SYSTEM, VETIVER NETWORKING,
AGRICULTURAL APPLICATIONS, AND FUTURE USES FOR
ENERGY/FUEL AND CARBON SEQUESTRATION



Vetiver Grass - the "Rolls Royce" of Plants

Richard Grimshaw OBE

29.10.2011

THE
RESEARCH
WORK WAS
PUBLISHED
IN THE
FARMER'S
MAGAZINE
WHICH IS
ABOUT THE
GROWTH OF
8 Ft
VETIVER
PLANT





ECONOMIC USES OF

C.zizanioides

- ✓ BIOETHANOL PRODUCTION
- ✓ MUSHROOM CULTIVATION
- ✓ ANTI TERMITE PROPERTY



MUSHROOM CULTIVATION

- ❖ Mushroom production (Chomchalow, 2003).
- ❖ *Pleurotus sp* – chosen for the study.
- ❖ Third largest cultivated mushroom in the world and contribute 16.3 % of the total world mushroom production.
- ❖ Paddy straw – largest used cereal straw for the cultivation of Oyster mushroom, next to that are wheat straw, maize straw, sorgam straw etc.

Since vetiver leaves can be used as an alternate substrate for mushroom culturing the work has been carried out.

ANTITERMITE PROPERTY



- Plants produce many natural compounds that are repellent to many insects

Earlier reports showed that vetiver oil has been tested to control termites but no study has been carried out to test the vetiver roots and leaf powder on tunneling activity, wood consumption and termite mortality.

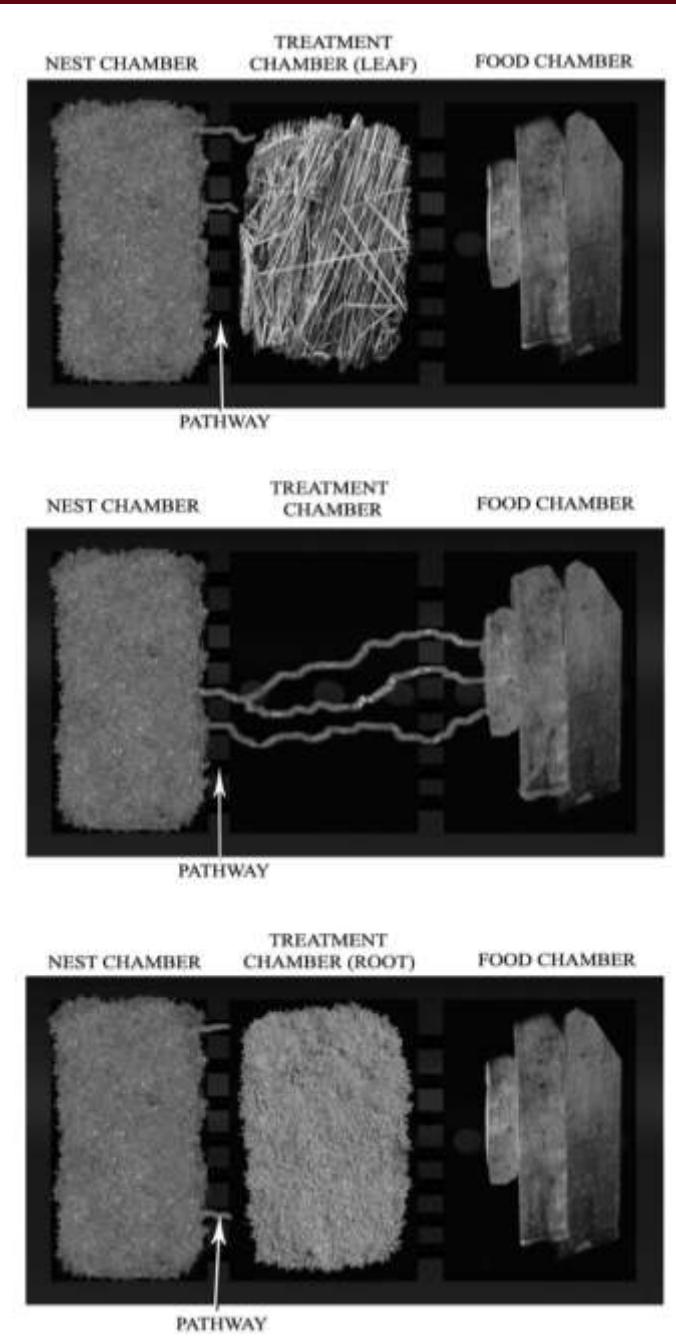
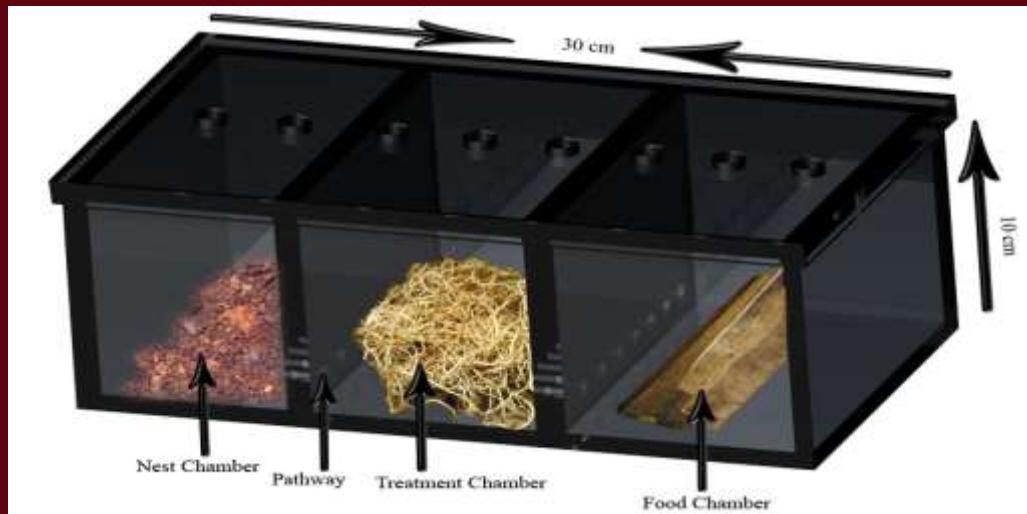


Experimental containers 30cm x 10cm x 10cm size –with 3 compartments.

Chamber 1 - Nest chamber (N) - Sieved soil + 100 termites (Queens, workers & Soldiers)

Chamber 2 – Treatment Chamber (S)

Chamber 3 - Food Chamber (F) – Wood pieces of Ravi tree



The study indicated that the tunneling and consumption of food by the termites was more in control sets, whereas the mortality rate was more in the container with vetiver root powder (VR). Similar observations were made earlier (Maistrello *et al.*, 2000; Maistrello *et al.*, 2001; Zhu *et al.*, 2001a, 2001b, Maistrello *et al.*, 2002; Nix *et al.*, 2003; Ibrahim *et al.*, 2004).

PHYTOREMEDIACTION

Phytoremediation is an emerging technology which uses plants and their associated rhizosphere microorganisms to remove, degrade, or contain chemical contaminants located in the soil, sediments, groundwater, surface water, and even the atmosphere.



MUNICIPAL WASTEWATER TREATMENT



S1 – Control (Tap water + Vetiver)

S2 – Municipal waste water + Vetiver

S3 – Diluted Municipal water 1:1 + Vetiver

The objective of the study

Antibacterial activity

Screening for the bioactive compounds

Phytochemicals

ISOLATION OF BIOACTIVE COMPOUNDS

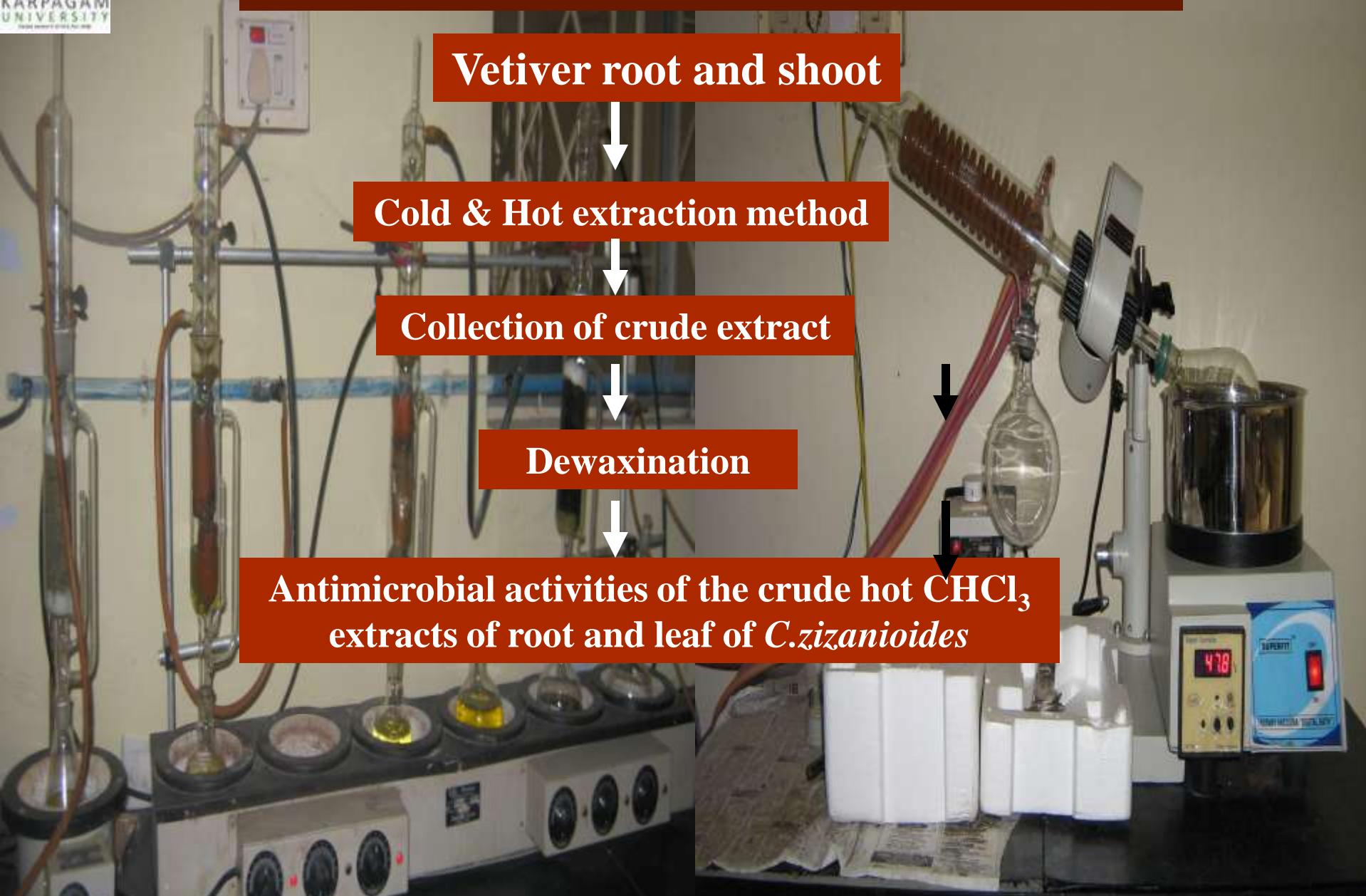
Vetiver root and shoot

Cold & Hot extraction method

Collection of crude extract

Dewaxination

Antimicrobial activities of the crude hot CHCl_3
extracts of root and leaf of *C.zizanioides*



Yield % of crude cold and hot extracts of root and leaf

Sample		Cold				Hot			
		Hexane	CHCl ₃	Ethanol	Water	Hexane	CHCl ₃	Ethanol	Water
E1	Root	1.18	2.28	1.60	1.16	3.2	7.08	3.6	3.4
	Leaf	0.76	1.80	1.52	1.40	2.1	7.04	4.7	4.5
E2	Root	1.01	1.17	1.24	0.98	1.89	3.20	1.29	1.27
	Leaf	0.34	1.08	1.34	1.21	0.76	2.89	1.9	2.2
E3	Root	1.08	2.07	1.49	0.78	2.7	5.28	3.2	3.1
	Leaf	0.70	1.71	1.38	0.99	2.2	5.19	3.1	3.3

The wax content of roots and leaves of *C.zizanioides*

Sample		Wax from cold extract (g)				Wax from hot extract (g)			
		Hexane	CHCl ₃	Ethanol	Water	Hexane	CHCl ₃	Ethanol	Water
E1	Root	0.18	0.29	0.11	0.06	0.23	0.35	0.18	0.12
	Leaf	0.83	1.10	0.68	0.11	0.98	1.37	0.75	0.22
E2	Root	0.23	0.31	0.22	0.20	0.68	0.78	0.52	0.58
	Leaf	0.80	0.9	0.5	0.4	0.93	1.48	0.67	0.62
E3	Root	0.28	0.46	0.36	0.29	0.72	0.78	0.56	0.65
	Leaf	0.82	0.98	0.62	0.53	0.97	1.58	0.68	0.78

E1- Grown in Soil , E2 – Grown in Water, E3- Grown in waste water

Phytochemicals

S.No	Name	Root	Leaf
1	Saponins	+	+
2	Tannins	-	-
3	Triterpenes	+	-
4	Steroids	+	-
5	Flavanoids	+	+
6	Rotenoids	-	+
7	Phenols	+	+

Test Microorganisms

Bacteria

Escherichia coli

Salmonella typhi

Klebsiella pneumoniae

Vibrio cholerae

Pseudomonas aeruginosa

Staphylococcus aureus

Streptococcus fecalis

Enterococcus fecalis

Fungi

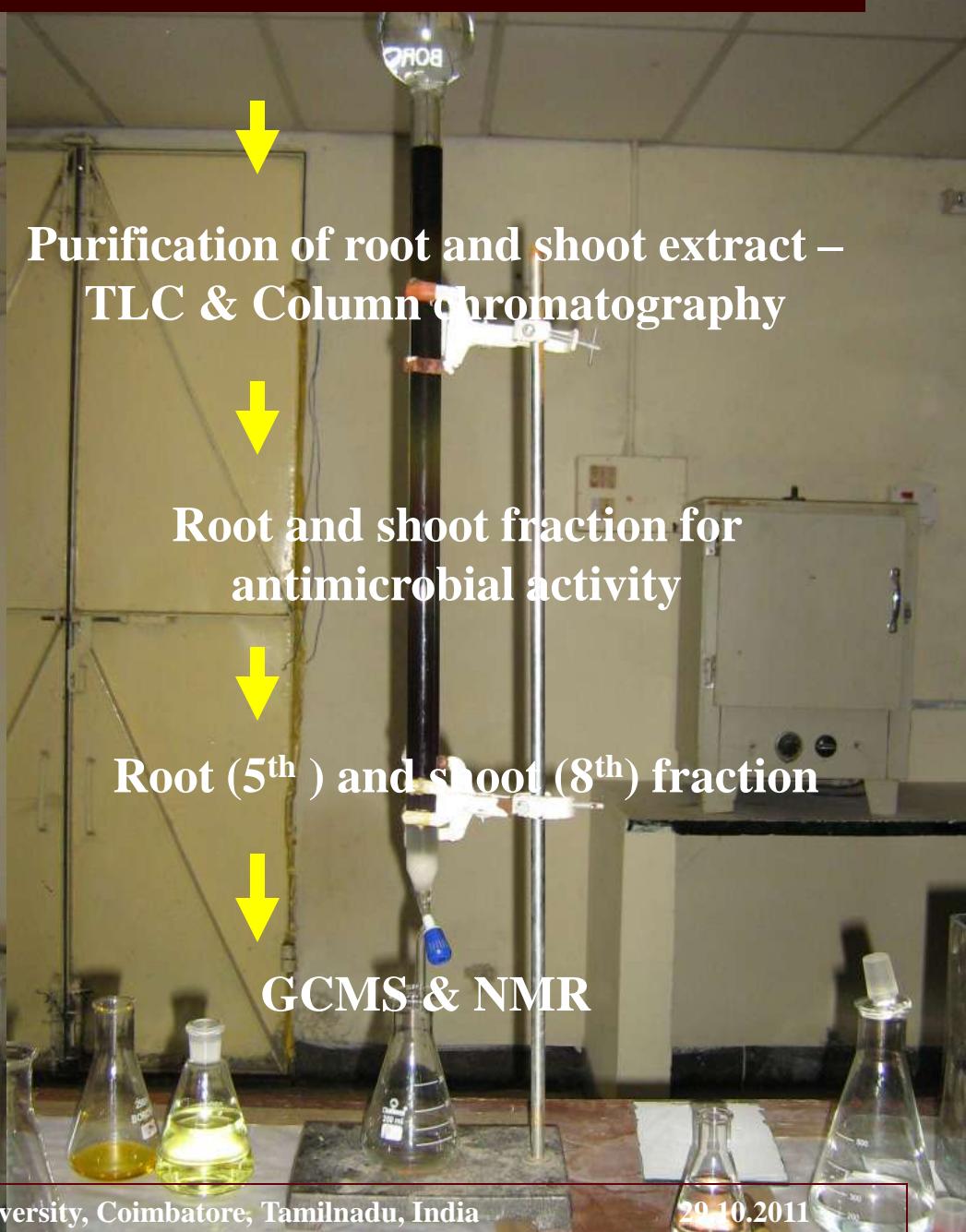
Candida albicans

Cryptococcus neoformens

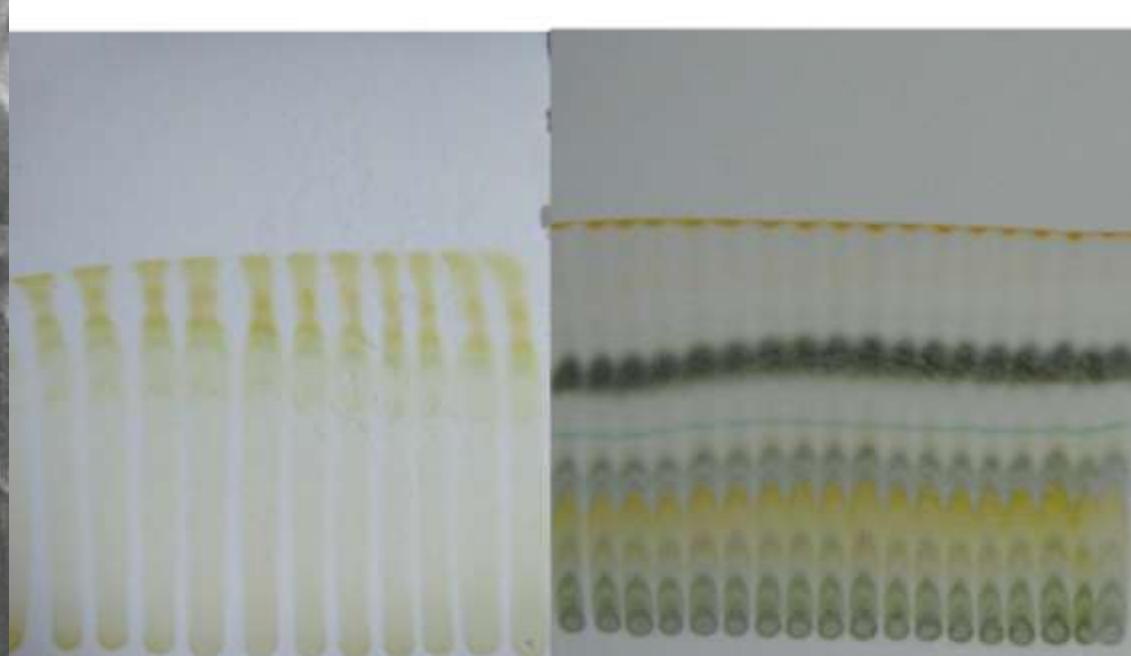
Antimicrobial activity

Bacteria/ Fungi	+ve cont	-ve cont	E1		E2		E3	
			Root	Leaf	Root	Leaf	Root	Leaf
	Zone of inhibition in mm							
<i>E.coli</i>	25	9	20	18	12	10	16	15
<i>K.pneumoniae</i>	24	10	21	17	11	12	20	12
<i>S. typhi</i>	22	11	21	13	11	10	17	12
<i>S. aureus</i>	22	9	24	24	14	16	22	18
<i>V.cholerae</i>	20	NI	5	NI	3	NI	4	NI
<i>S.faecalis</i>	25	12	21	19	11	12	19	15
<i>P.aeruginosa</i>	26	11	25	15	12	11	22	13
<i>E. faecalis</i>	28	10	21	14	16	12	18	12
<i>C. albicans</i>	25	12	28	25.5	13	10	20	17
<i>C.neoformens</i>	23	13	22	20	10	8	18	16

TEST FOR ANTIMICROBIAL ACTIVITY



SEPARATION OF BIOACTIVE COMPOUNDS



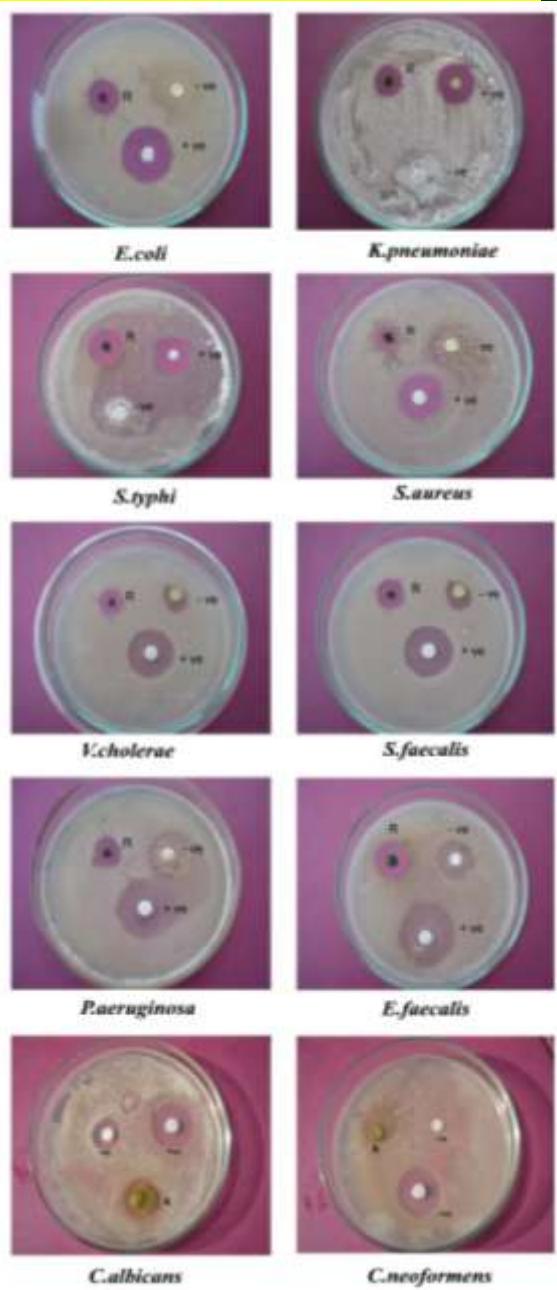
TLC - Root extract

TLC - Leaf extract



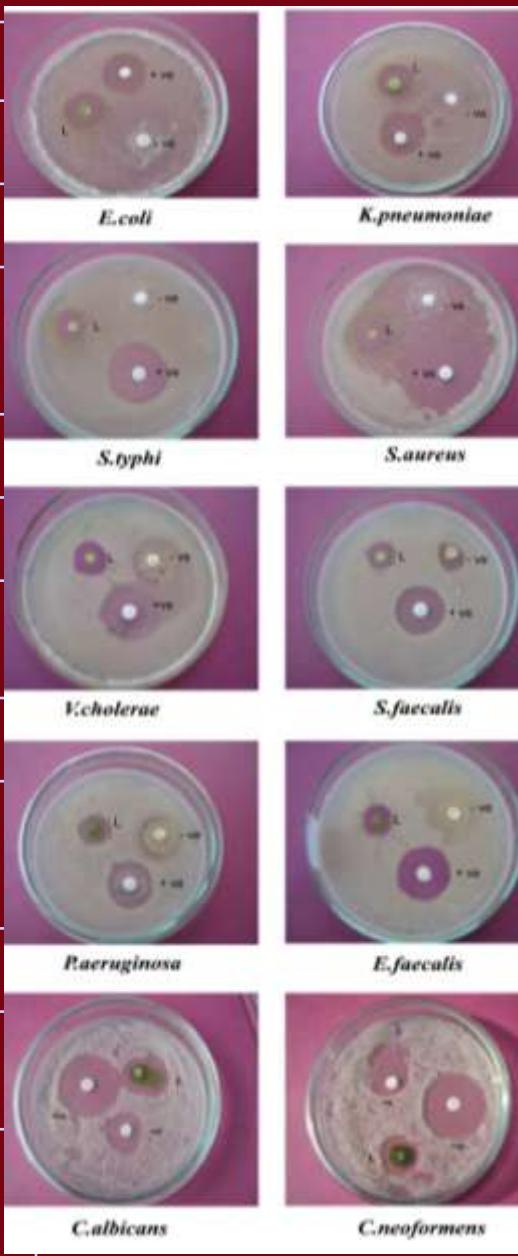
Antimicrobial activity of purified root extracts of *C.zizanioides*

Bacteria / Fungi	Average zone of inhibition (mm)					
	F 1	F 2	F 3	F 4	F 5	F 6
<i>E.coli</i>	20	17	5	21	24	12
<i>K.pneumoniae</i>	16	-	-	-	-	-
<i>S. typhi</i>	-	-	-	-	13	-
<i>S. aureus</i>	-	15	15	30	30	18
<i>V.cholerae</i>	-	-	-	-	-	-
<i>S.faecalis</i>	17	-	-	-	18	-
<i>P.aeruginosa</i>	-	-	-	31	18	-
<i>E. faecalis</i>	-	-	-	25	16	-
<i>C.albicans</i>	15	-	15	33	33	19
<i>C.neoformens</i>	5	5	17	28	28	19

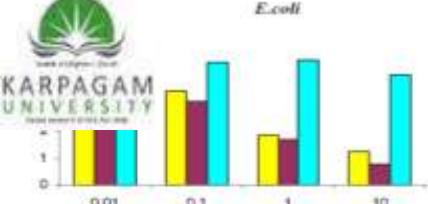


Antimicrobial activities of purified leaf extracts of *C.zizanioides*

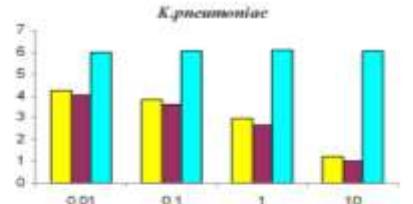
Bacteria / Fungi	Average zone of inhibition (mm)							
	F 1	F 2	F 3	F 4	F 5	F 6	F 7	F 8
<i>E.coli</i>	11	5	10	20	17	14	12	22
<i>K.pneumoniae</i>	13	-	10	21	20	13	12	13
<i>S. typhi</i>	24	7	-	12	21	12	11	16
<i>S. aureus</i>	15	12	8	13	22	14	22	18
<i>V.cholerae</i>	-	-	-	-	-	-	-	-
<i>S.faecalis</i>	20	13	7	17	18	15	19	19
<i>P.aeruginosa</i>	15	20	12	22	17	17	17	17
<i>E. faecalis</i>	17	20	15	23	18	21	18	18
<i>C. albicans</i>	20	27	17	24	33	22	23	34
<i>C.neoformens</i>	20	27	18	25	34	24	26	32



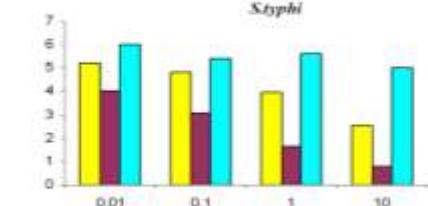
E.coli



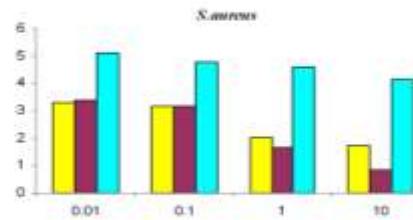
K.pneumoniae



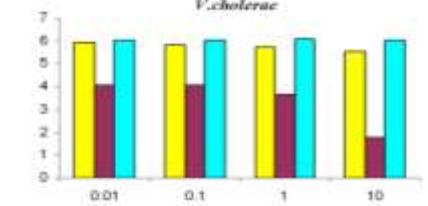
S.typhi



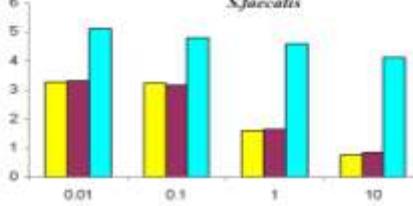
S.aureus



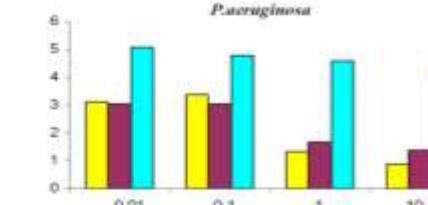
V.cholerae



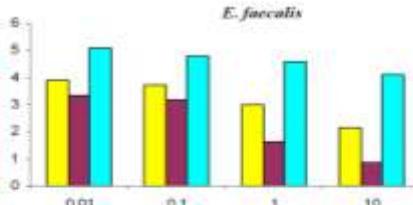
S.faecalis



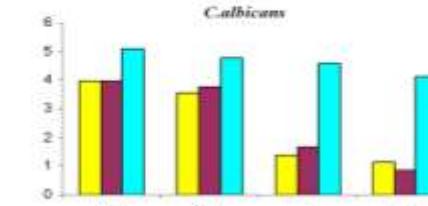
P.aeruginosa



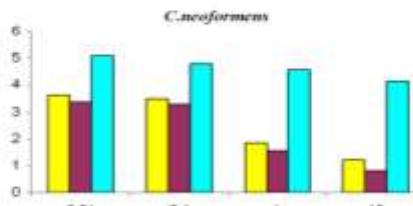
E.faecalis



C.albicans



C.neoformans



MIC of
Root
extract

- Extract (Yellow)
- + ve Control (Maroon)
- ve Control (Cyan)

X axis - Concentration in mg

Y axis - OD value at 655 nm

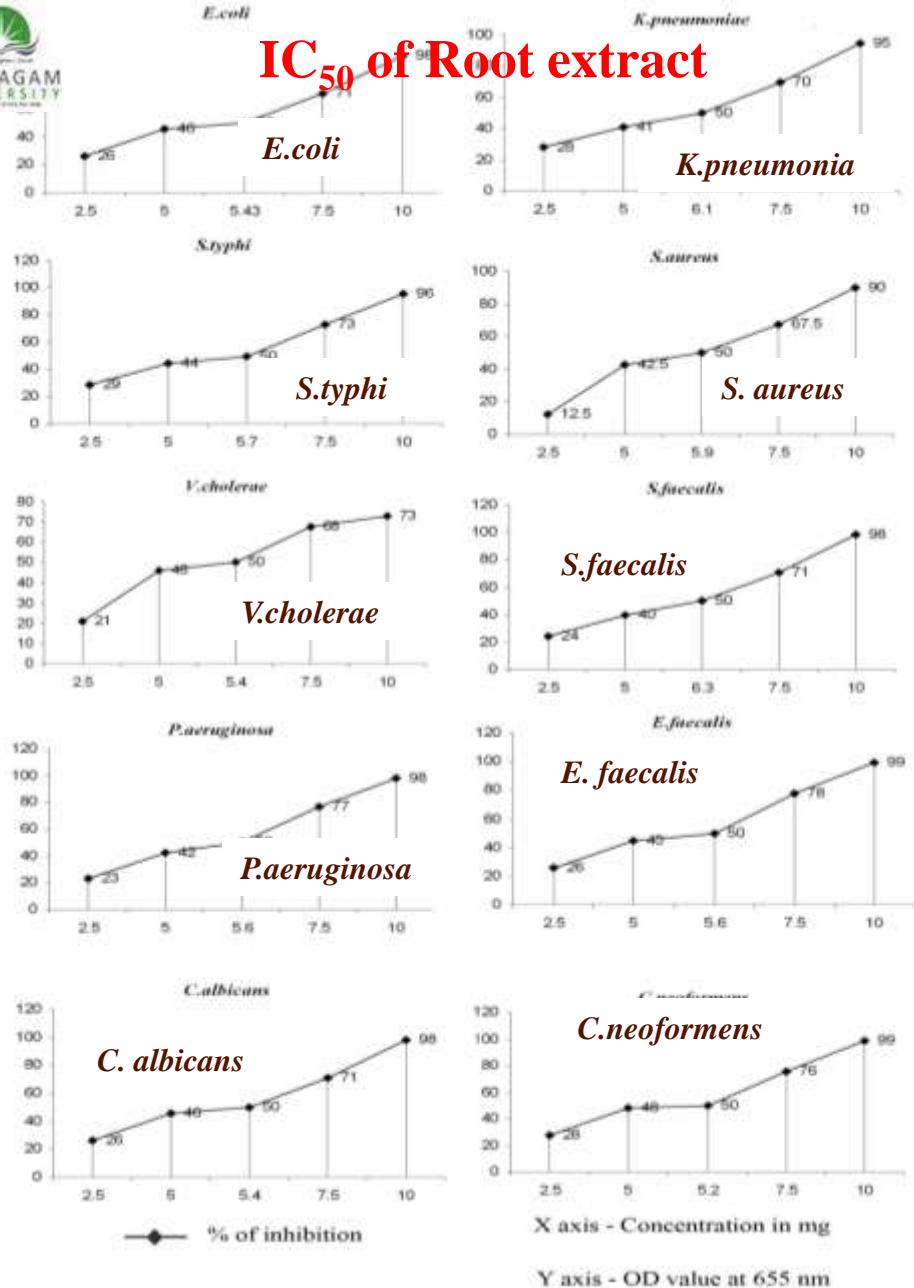
MIC was 10 mg / mL concentration of the extracts

MIC of
Leaf
extracts

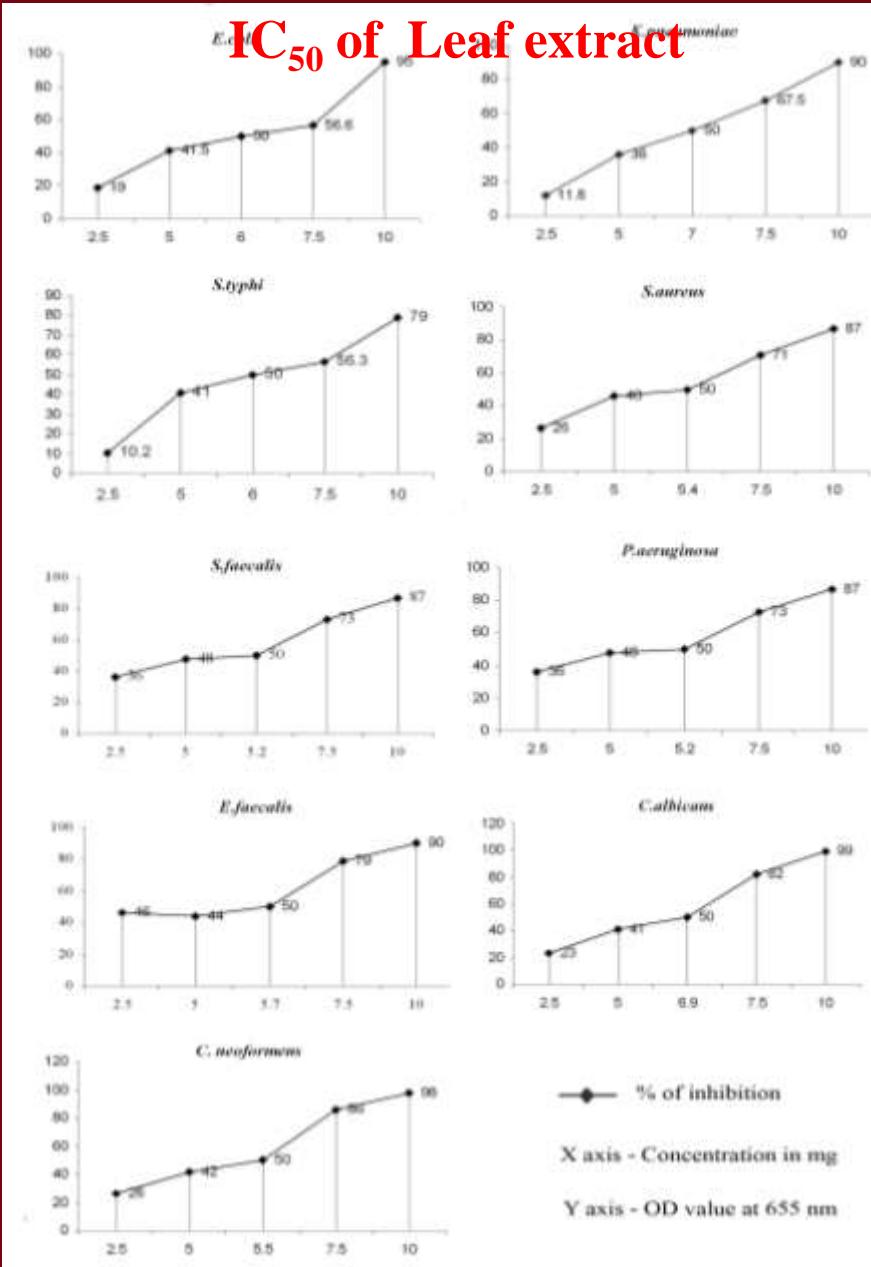
X axis - Concentration in mg

Y axis - OD value at 655 nm

IC₅₀ of Root extract



IC₅₀ of Leaf extract



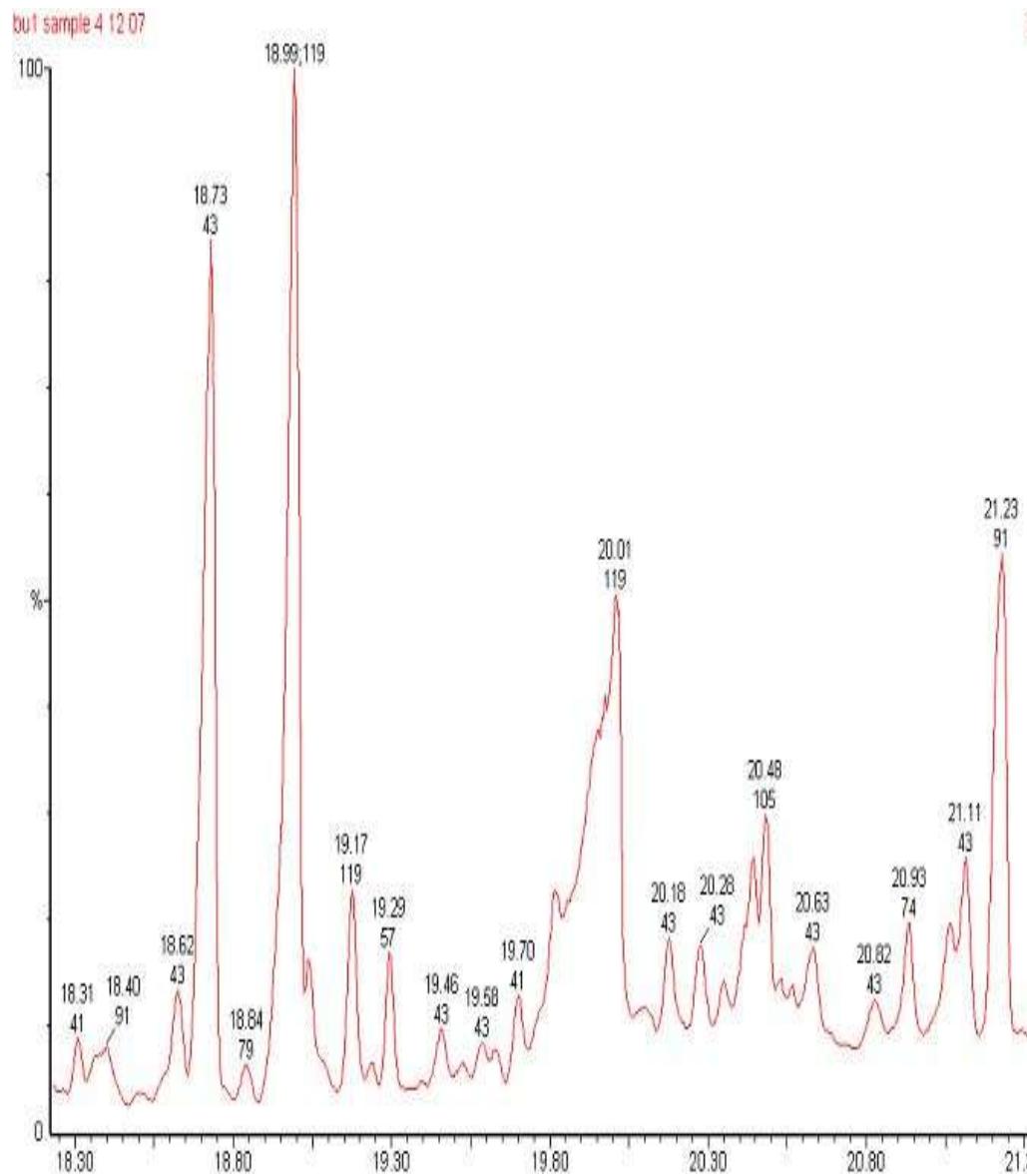
IC₅₀ was 5 mg / mL to 7.5 mg / mL concentration of the extracts

	Compound	Retention time	%peak area
2	3-Isopropyltricyclo[4.3.1.1(2,5)]undec-3-en-10-ol <u>Formula:</u> C14H22O <u>MW:</u> 206	13.06	0.126
3	Tetradecane <u>Formula:</u> C14H30 <u>MW:</u> 198	13.19	0.886
4	Vanillin <u>Formula:</u> C8H8O3 <u>MW:</u> 152	13.28	0.274
5	7-Heptadecene, 17-chloro- <u>Formula:</u> C17H33Cl <u>MW:</u> 272	14.43	0.147
6	(+)-Ledene <u>Formula:</u> C15H24 <u>MW:</u> 204	14.87	0.305
7	7-Oxabicyclo[4.1.0]heptane, 2,2,6-trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene- <u>Formula:</u> C15H22O <u>MW:</u> 218	15.31	0.372
8	Hexadecane <u>Formula:</u> C16H34 <u>MW:</u> 226	16.41	2.859
9	Ledene oxide-(II) <u>Formula:</u> C15H24O <u>MW:</u> 220	17.96	6.505
10	Aromadendrene oxide-(2) <u>Formula:</u> C15H24O <u>MW:</u> 220	18.62	2.567
11	Cedren-13-ol, 8- <u>Formula:</u> C15H24O <u>MW:</u> 220	18.73	19.833
12	Octadecane <u>Formula:</u> C18H38 <u>MW:</u> 254	19.29	3.070
13	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol <u>Formula:</u> C15H24O <u>MW:</u> 220	19.46	1.191
14	Tricyclo[5.1.0.0(2,4)]oct-5-ene-5-propanoic acid, 3,3,8,8-tetramethyl- <u>Formula:</u> C15H22O2 <u>MW:</u> 234	20.01	9.951
15	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl- <u>Formula:</u> C15H24O2 <u>MW:</u> 236	20.18	2.108
16	Cyclopropanebutanoic acid, 2-[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester <u>Formula:</u> C25H42O2 <u>MW:</u> 374	20.93	2.625
17	2(1H)Naphthalenone, 3,5,6,7,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-Synonym:Nootakone <u>Formula:</u> C15H22O <u>MW:</u> 218	21.11	4.049
18	1-Butyn-3-one, 1-(6,6-dimethyl-1,2-epoxycyclohexyl)- <u>Formula:</u> C12H16O2 <u>MW:</u> 192	21.23	11.119
19	Azuleno[6,5-b]furan-2,5-dione, decahydro-4a,8-dimethyl-3-methylene-, [3aR-(3aà,4aá,7aà,8a,9aà)]- <u>Formula:</u> C15H20O3 <u>MW:</u> 248	22.52	0.978
20	Octadecanoic acid, methyl ester <u>Formula:</u> C19H38O2 <u>MW:</u> 298	23.36	0.757
21	Campesterol <u>Formula:</u> C28H48O <u>MW:</u> 400	24.21	2.662
22	Cholesta-22,24-dien-5-ol, 4,4-dimethyl- <u>Formula:</u> C29H48O <u>MW:</u> 412	25.93	2.879
23	9-Octadecenamide, (Z)- <u>Formula:</u> C18H35NO <u>MW:</u> 281	26.14	0.298

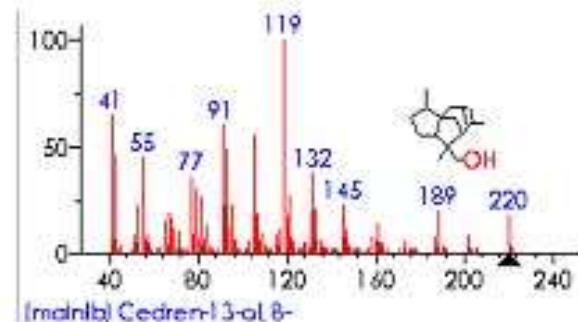
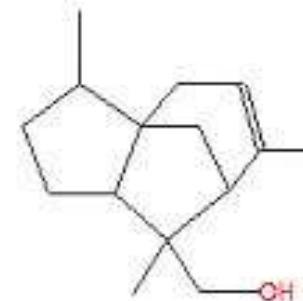
GCMS analysis of the root 5th fraction

S.No	Compound	Retention time (min)	Area %
1	Cedren – 13-ol-8,	18.99	23.105
2	1-Cyclohexanone, 2 methyl-2- (3 methyl –2- oxobutyl)	18.73	19.833
3	1-Butyn-3-one, 1-(6,6-dimethyl-1, 2- epoxycyclohexyl	21.23	11.119
4	Tricyclo(5,1.0.0 (2,4) oct – 5-ene-5- propanoic acid, 3,3,8,8- tetramethyl-	20.01	9.951
5	Ledene oxide – (II)	17.96	6.505

GC analysis of the root 5th fraction



| (M) Cedren-13-ol, 8-
776.785R 23.2P



Name: Cedren-13-ol, 8-

Formula: C₁₅H₂₄O

MW: 220 CAS#: 18319-35-2 NIST#: 141107 ID#: 71326 DB: mainlib

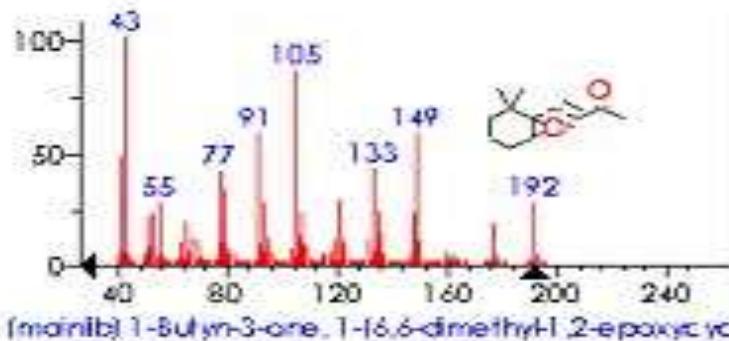
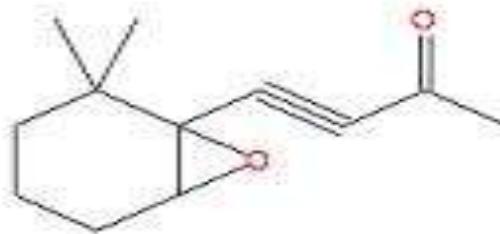
Other DBs: None

Contributor: Mark Whiffen, Florida Museum of Natural History, U.S.A.

10 largest peaks:

119 999		41 639		91 591		105 546		93 463	
43 452		55 446		132 368		77 349		79 294	

IM| 1-Butyn-3-one, 1-(6,6-dimethyl-2-
743 767R 13.1P



Name: 1-Butyn-3-one, 1-(6,6-dimethyl-1,2-epoxycyclohexyl)
Formula: C₁₂H₁₆O₂

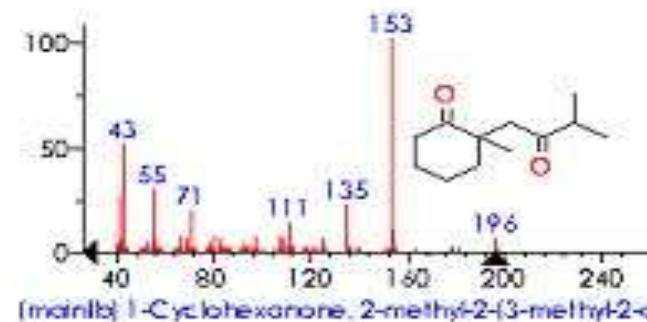
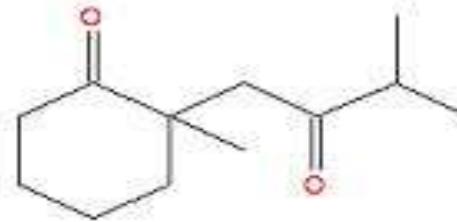
MW: 192 CAS#: N/A NIST#: 196972 ID#: 9655 DB: mainlib
Other DBs: None

Contributor: Chemical Concepts

10 largest peaks:

43 999	105 846	91 576	149 574	41 475
133 414	77 407	39 375	79 325	121 280

I (IM) 1-Cyclohexanone, 2-methyl-2-(3-m..
722 785R 21.1P



Name: 1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl)
Formula: C₁₂H₂₀O₂

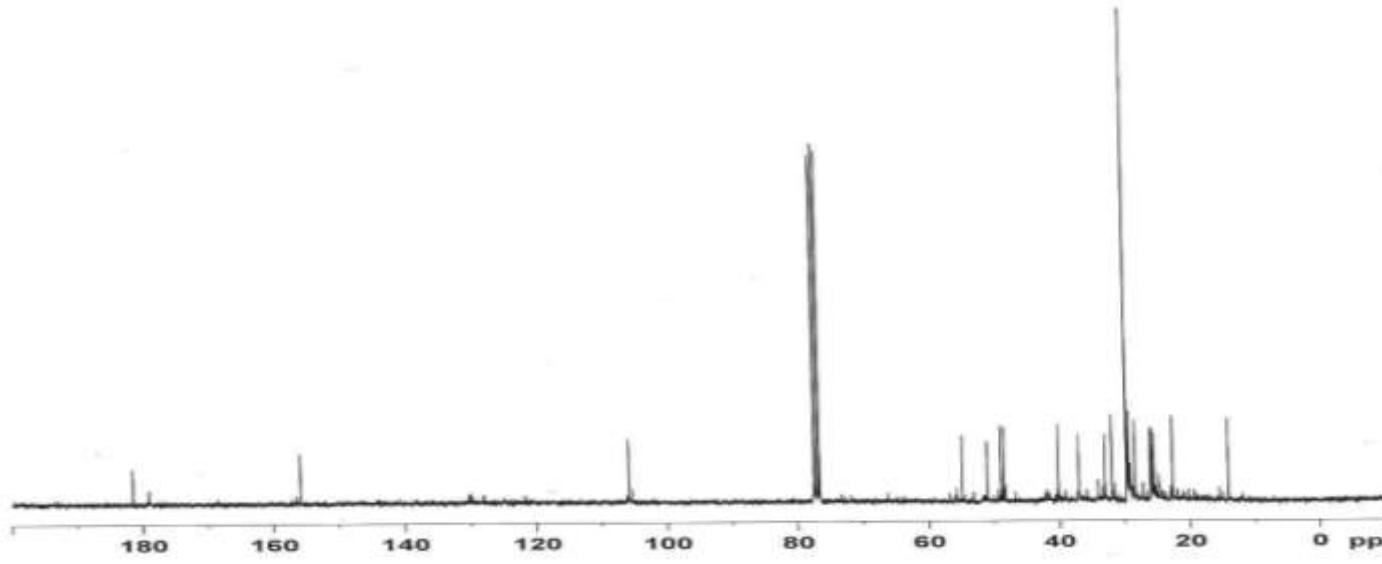
MW: 196 CAS#: N/A NIST#: 196694 ID#: 98988 DB: mainlib
Other DBs: None

Contributor: Chemical Concepts

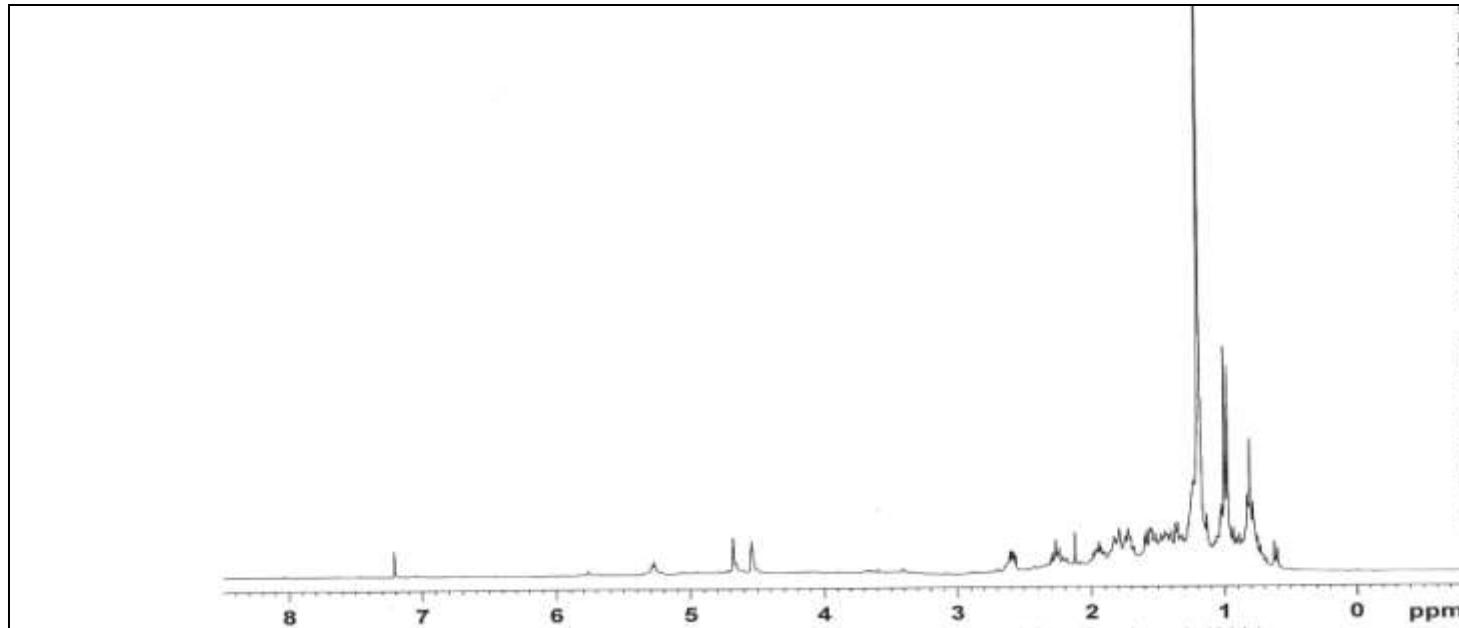
10 largest peaks:

153 999	43 497	55 291	41 254	135 220
71 190	111 131	27 103	39 101	154 98

**Root -
carbon**



**Root -
proton**

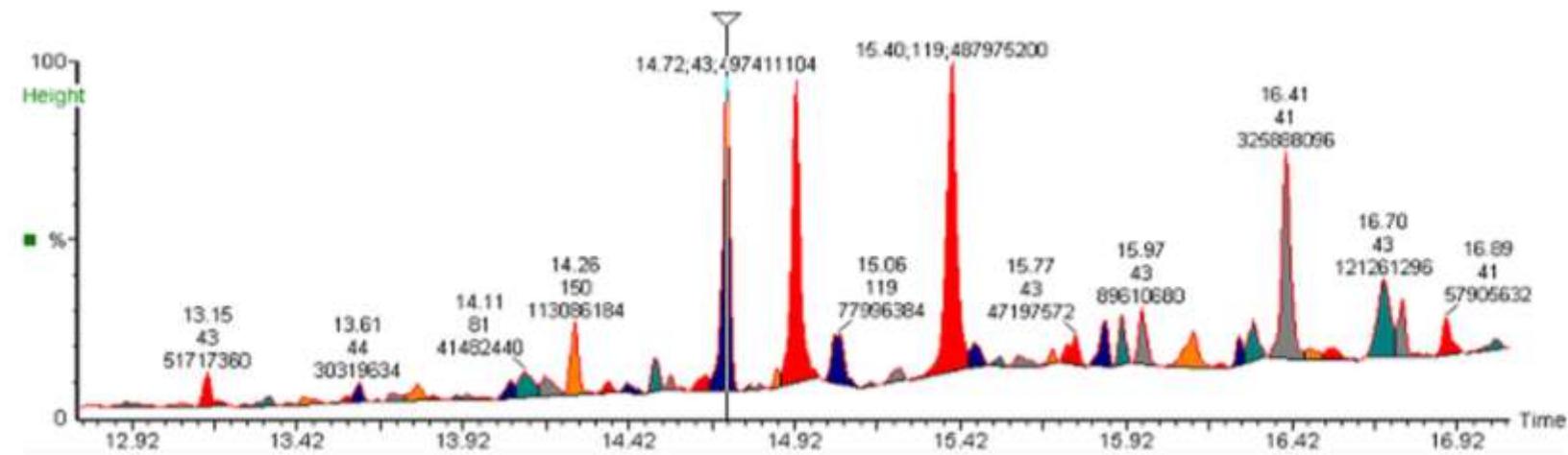
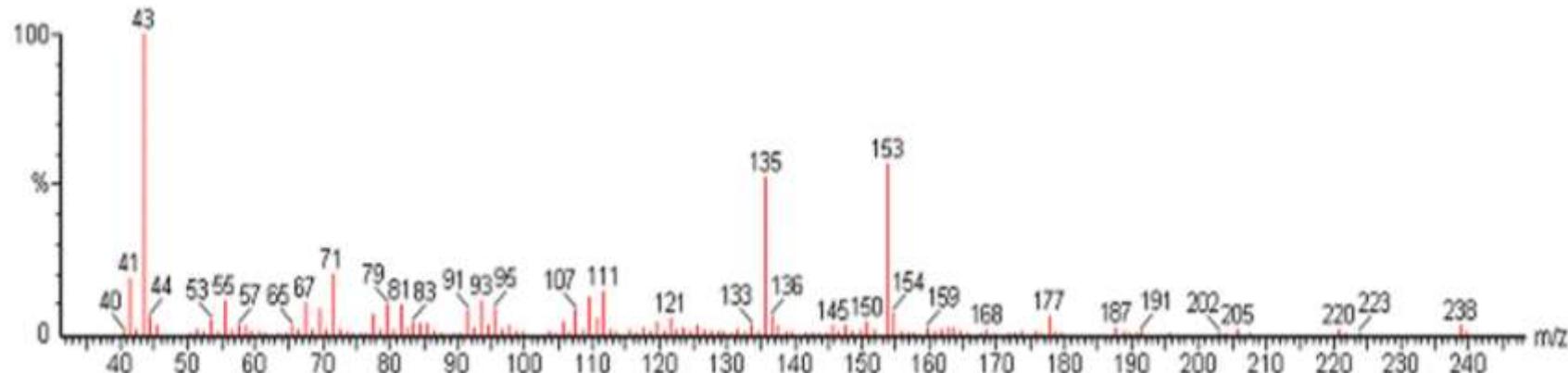


GCMS analysis of the leaf 8th fraction

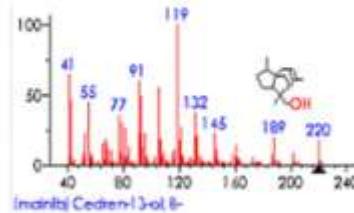
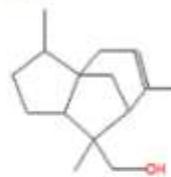
S.No.	Compound	Retention time	%peak area
1	Nonadecane Formula: C19H40 MW: 268	13.15	2.194
2	Tricyclo[5.3.1.1(2,6)]dodecan-11-ol, 11-methyl-12-methylene- Formula: C14H22O MW: 206	13.61	1.286
3	Ledene oxide-(II) Formula: C15H24O MW: 220	14.26	4.798
4	1-Cyclohexanone, 2-methyl-2-(3-methyl-2-oxobutyl) Formula: C12H20O2 MW: 196	14.72	21.105
5	Cedren-13-ol, 8- Formula: C15H24O MW: 220	14.93	20.139
6	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2à,4à,8aá)]- Formula: C15H24 MW: 204	15.06	3.309
7	4,7-Octadecadiynoic acid, methyl ester Formula: C19H30O2 MW: 290	15.40	20.705
8	2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one Formula: C15H22O MW: 218	15.86	3.025
9	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1à,7á,8aà)]- Formula: C15H24 MW: 204	15.91	3..351
10	2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.1.0(1,6)]decan-5-ol Formula: C13H22O2 MW: 210	15.97	3.802
11	4-(3,3-Dimethyl-but-1-ynyl)-4-hydroxy-2,6,6-trimethylcyclohex-2-enone Formula: C15H22O2 MW: 234	16.41	13.827
12	2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl-, [4ar-(4aà,6à,8aá)]- Formula: C15H22O2 MW: 234	16.89	2.456

GCMS analysis of the leaf 8th fraction

S.No	Compound	Retention time (min)	Area %
1	1-Cyclohexanone, 2 methyl-2- (3 methyl – 2- oxobutyl)	14.72	21.105
2	Cedren – 13-ol-8,	14.93	20.139
3	4,7 – Octadecadiynoic acid, methyl ester	15.40	20.705
4	Ledene oxide – (II)	14.26	4.798



| (M) Cedren-13-ol,8-
776781R 21.2P



Name: Cedren-13-ol,8-

Formula: $C_{15}H_{24}O$

MW: 220 CAS#: 16319-35-2 NIST#: 141107 ID#: 71326 DB: mainlib

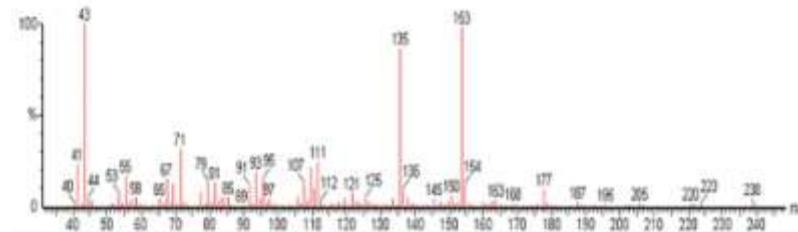
Other DBs: None

Contributor: Mark Whiten, Florida Museum of Natural History, U. o

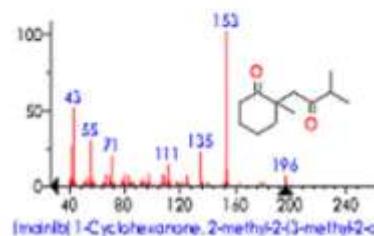
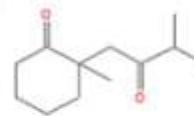
10 largest peaks:

119	999		41	639		91	591		105	546		93	463	
43	452		55	446		132	386		77	349		77	294	

| (M) 4,7-Octadecadiynoic acid, methyl...
741 745R 21.1P



| (M) 1-Cyclohexanone, 2-methyl-2-(3-meth...
729788R 21.1P



Name: 1-Cyclohexanone, 2-methyl-2-(3-methoxybutyl)

Formula: $C_{12}H_{20}O_2$

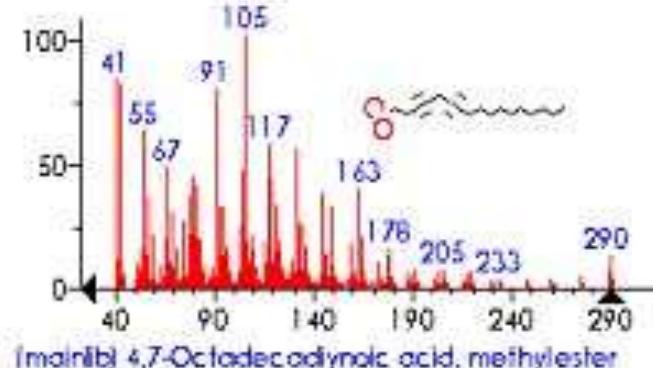
MW: 196 CAS#: N/A NIST#: 196694 ID#: 98988 DB: mainlib

Other DBs: None

Contributor: ChemicalConcepts

10 largest peaks:

153	999		43	497		55	291		41	254		135	220	
71	190		111	131		27	103		39	101		154	98	



Name: 4,7-Octadecadiynoic acid, methylester

Formula: $C_{19}H_{30}O_2$

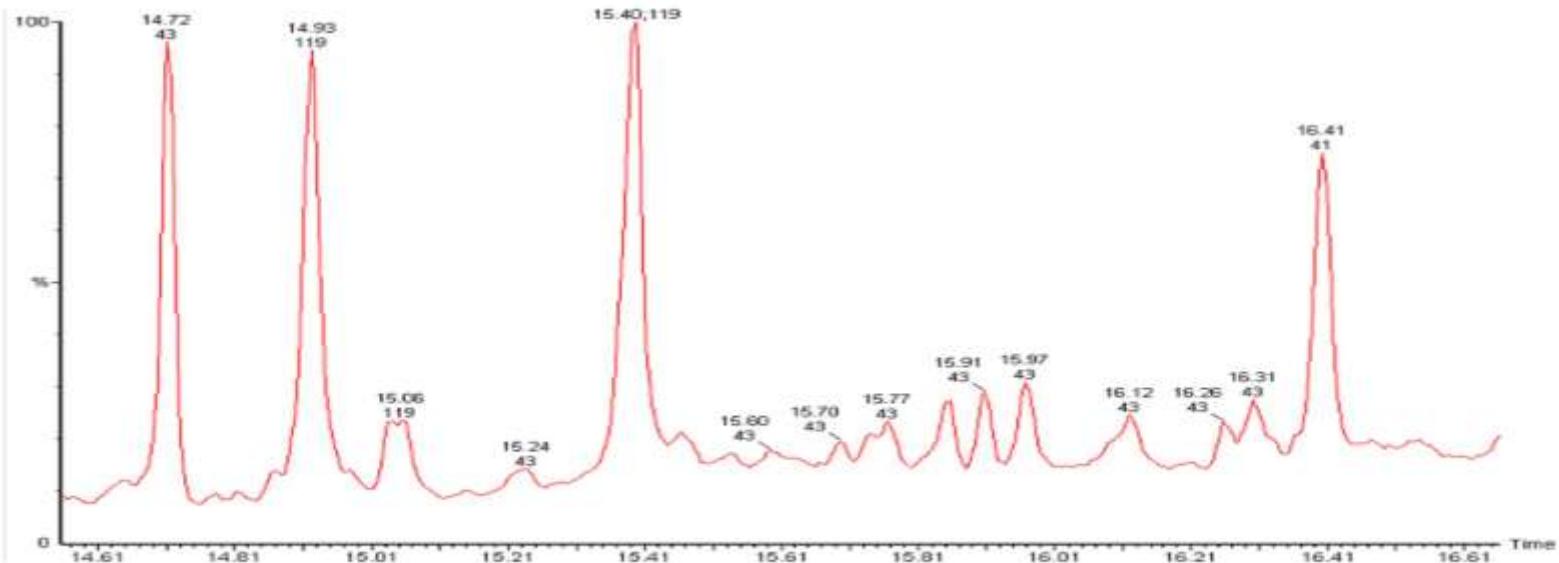
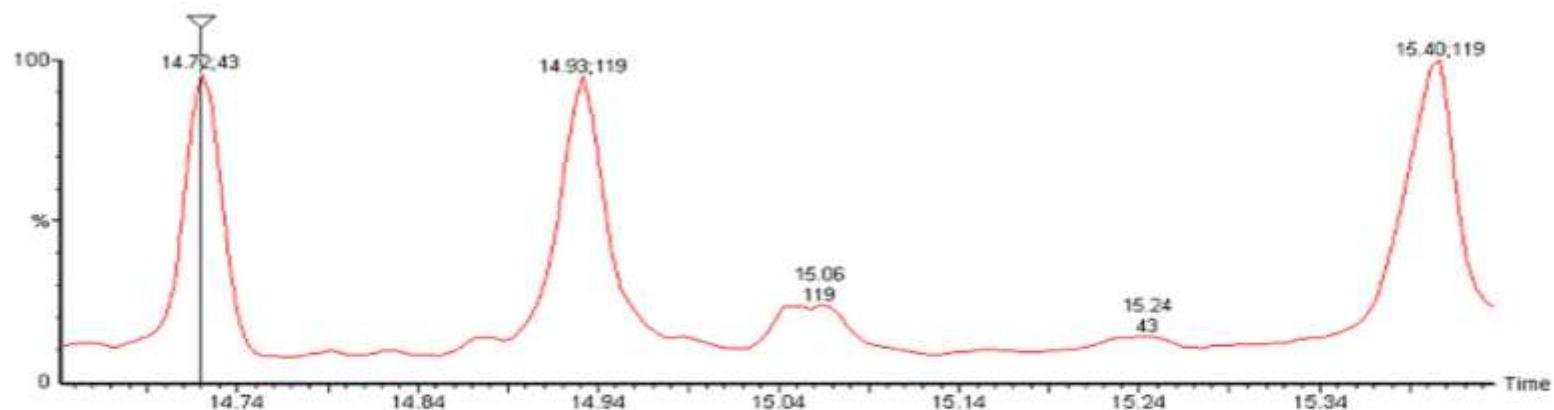
MW: 290 CAS#: 18202-20-5 NIST#: 36078 ID#: 59260 DB: mainlib

Other DBs: None

Contributor: R.T.HOLMAN,UNIVERSITY OF MINNESOTA

10 largest peaks:

105	999		41	620		43	600		91	780		55	620	
117	560		131	550		67	470		104	460		79	440	



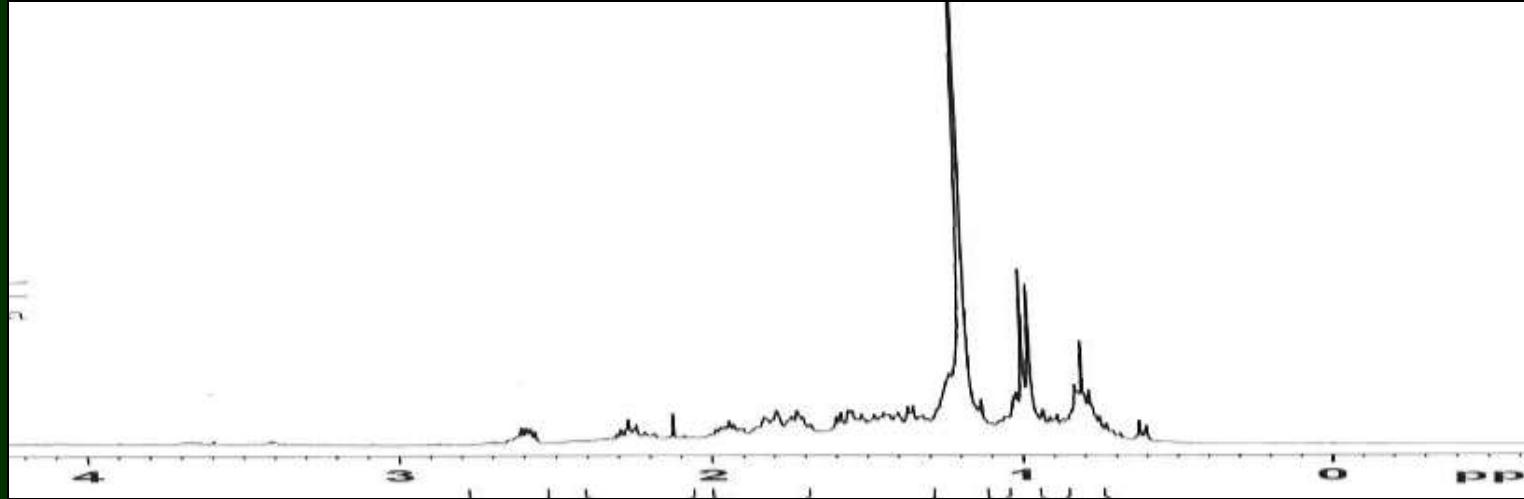
Carbon ^{13}C NMR (CDCl_3) 300 MHz

δ Value ppm	Possible Carbons
0.7 to 0.9	Aliphatic protons
1.2 to 1.4	$\text{R} - \text{CH}_2 - \text{R}$
2.1	OH proton
4.6	Amino or amide Nk2
5.3 and 5.8	Aromatic CH protons
3.5	Methoxy OCH_3
2.3	$\text{CH}-\bullet$
6.4, 6.5, 6.6, 6.7	Aromatic ring protons (H)
9.3, 9.6, 9.7	Amine may be Primary Amines
10.2	CHO
7.5, 7.7	Aromatic Protons

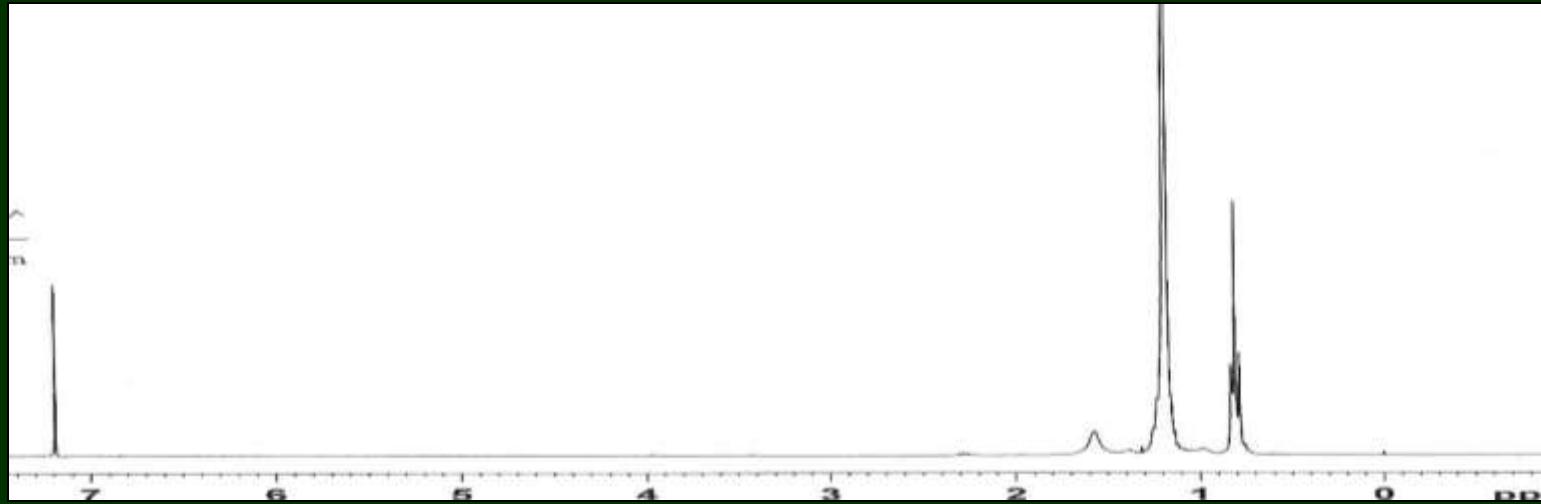
Proton ^1H NMR (CDCl_3) 300 MHz

δ Value ppm	Possible Protons
0.7	$\text{R}-\text{CH}_3$
0.8	Methyl Protons $\text{CH}_2 - \text{CH}_2 - \text{R}$
1.2 to 1.4	$\text{R}-\text{CH}_2-\text{R}$
1.57	$\text{R}-\text{C}=\text{C}=\text{C}-\text{H}$
2.3	$\text{CH} - \text{C}_6\text{H}_4 - \text{phenyl}$
3.5	Methoxy protons

Shoot - carbon



Shoot - proton



VETIVER GRASS THE “ROLLS ROYCE” OF PLANTS

ORIGIN - SOUTH INDIA –APPLY IT !!

THANK YOU