



CHARACTERIZATION OF MEDICINAL MUSHROOMS BY HYPHENATED HPTLC

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ISO:9001:2008

... IN PURSUIT OF EXCELLENCE IN MILITARY PHYSIOLOGY

VISION

Be a center of excellence in the field of environmental physiology and human factor research.

MISSION

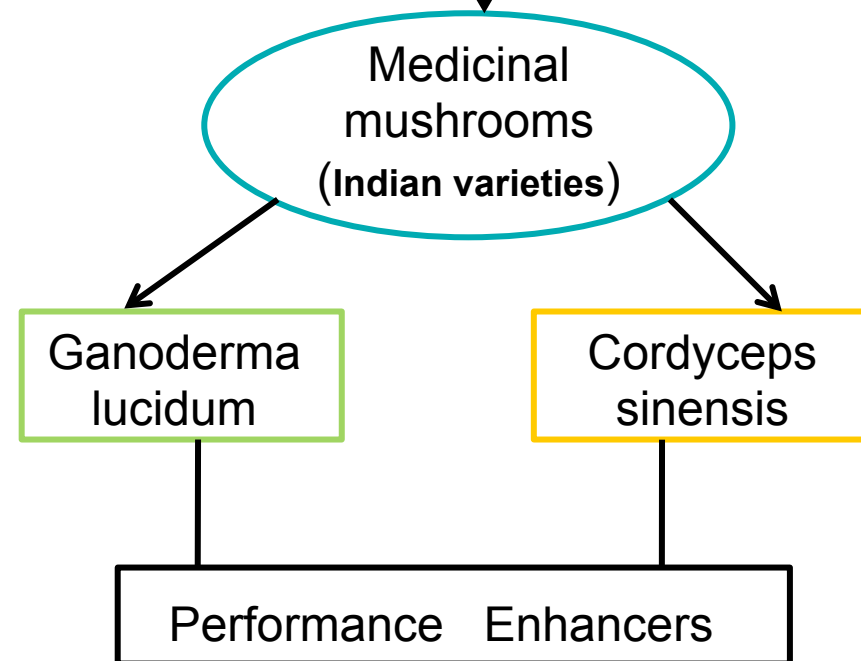
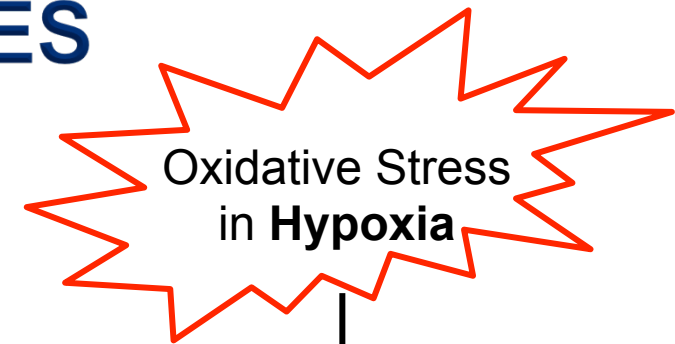
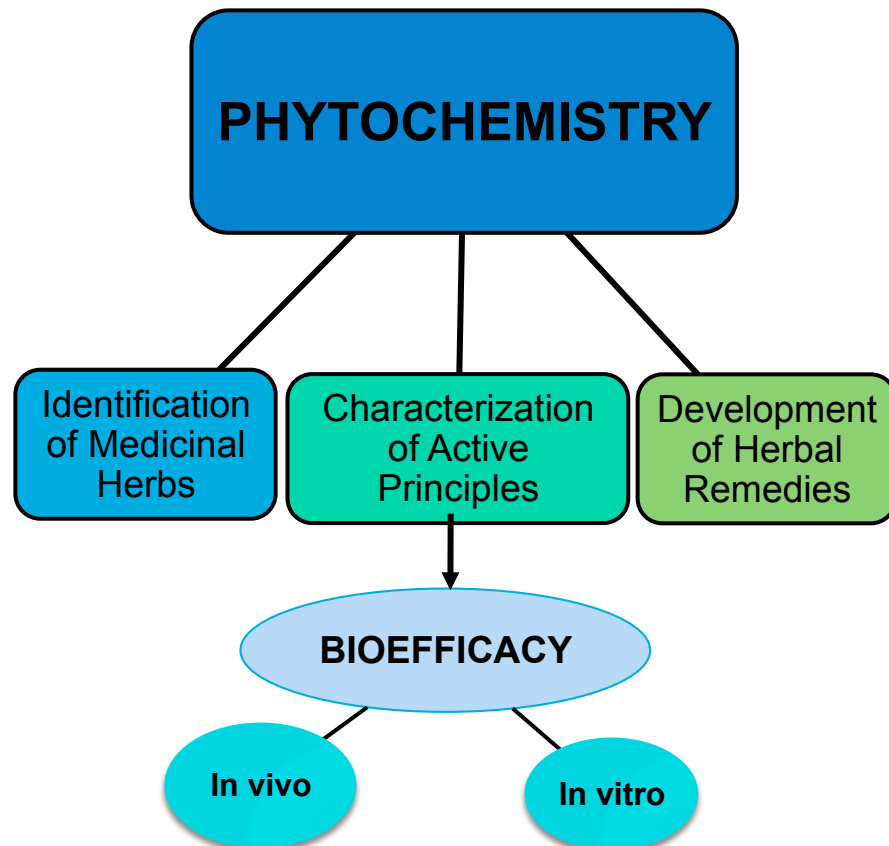
Optimization of human performance in different occupational environment conditions using physiological, biochemical, nutritional and ergonomic approach.

CORE COMPETENCE

Physiological adaptation to environmental stress
Human Engineering for effective Man-Machine Interface

OBJECTIVES

Metabolite profiling of Indian cultured varieties of *Cordyceps sinensis* and *Ganoderma lucidum* using Hyphenated-HPTLC.





MEDICINAL MUSHROOMS



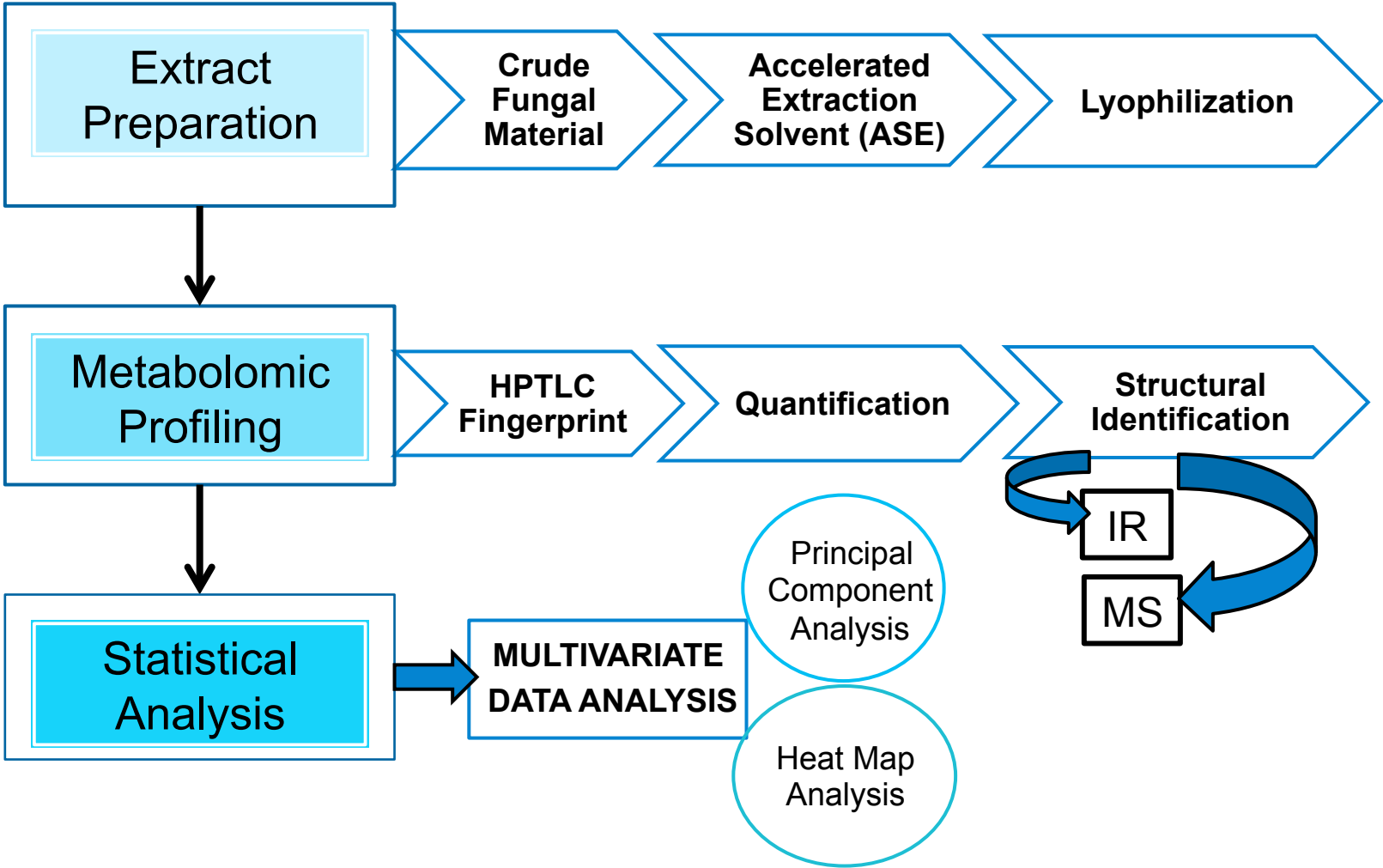
Cordyceps sinensis

- **Catepillar** fungus; **Keera jhar/ Yarsa gumba** (India).
- found throughout the mountain regions of Asia, above 3800-4500 meters.
- **Parasitic** on insect and caterpillar larva (*Hepialis armoricanus*).
- **Active Compounds:** Nucleotides, polysaccharides, sterols, flavonoids, amino acids and proteins etc.
- **Health benefits:** immuno-enhancer, antioxidant, anti-aging, tonic effect, hepatoprotective, anti-metastatic, neuro-cardioprotective.

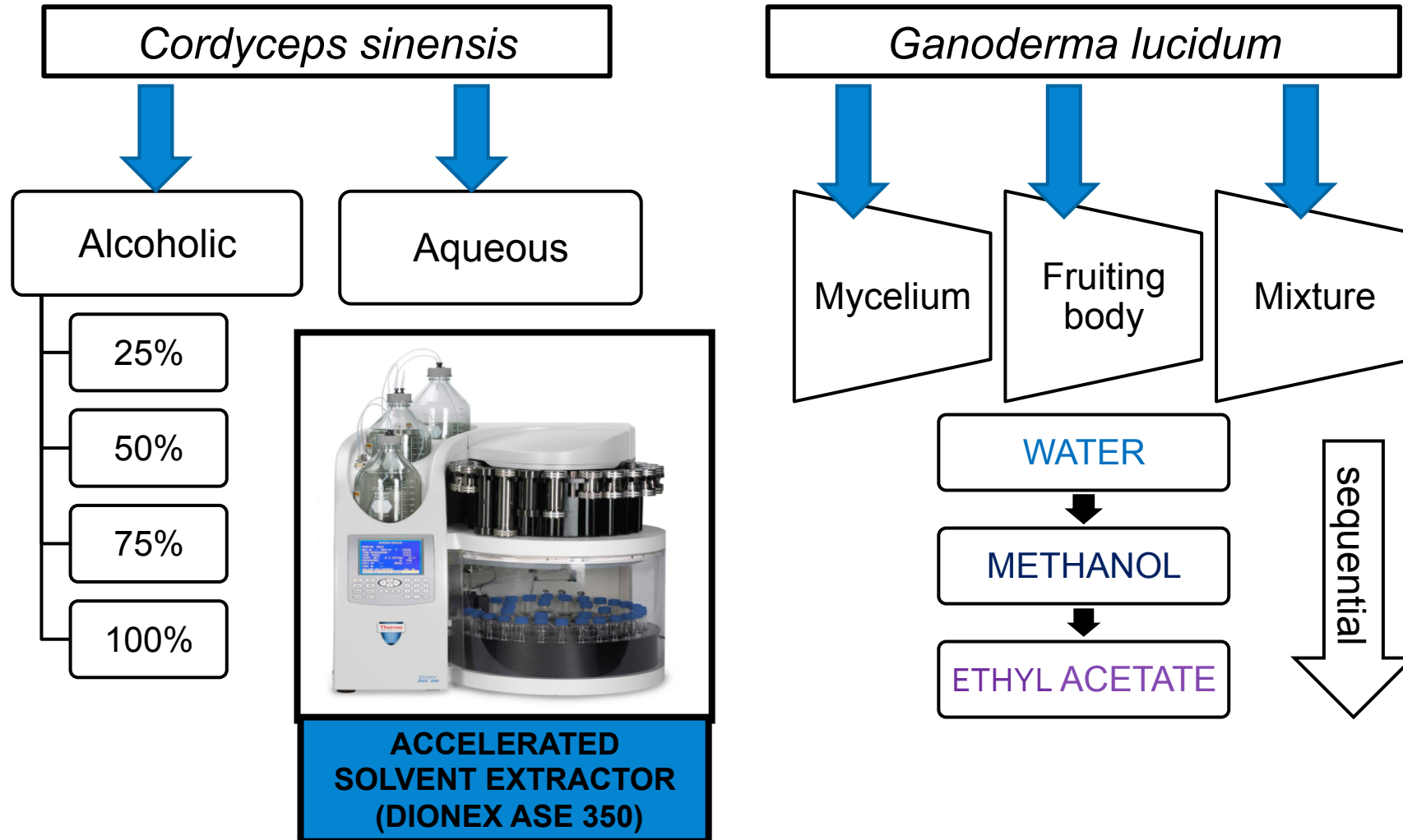
Ganoderma lucidum

- **Mushroom of Immortality, Ling zhi, Reishi Mushroom.**
- found both in tropical and temperate regions of **India**, China, Japan and other Asian countries.
- **Saprophytic**, on the base stumps of wooden logs but can be **parasitic**.
- **Active Compounds:** Polysaccharides, triterpenoids, sterols, adenosine, flavonoids, peptidoglycans, amino acids , proteins etc.
- **Health benefits:** immuno-modulator, antioxidant, adaptogen, hepatoprotective, anti-carcinogenic , anti-tumorogenic, anti-hypertensive.

METHODOLOGY



ACCELERATED SOLVENT EXTRACTION (ASE)



HPTLC ANALYSIS

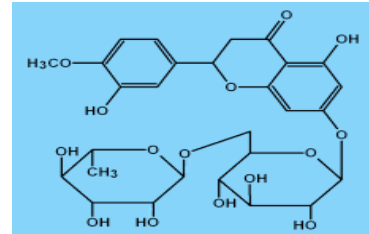
Antioxidants chosen

STATIONARY PHASE:

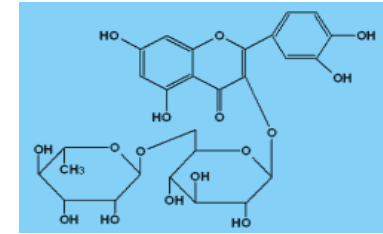
HPTLC Silica gel 60F₂₅₄ plates

MOBILE PHASE:

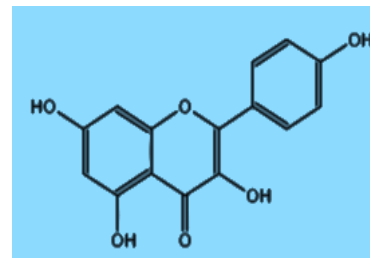
Ethyl acetate: Dichloromethane:
Formic acid: Glacial acetic acid:
Methanol



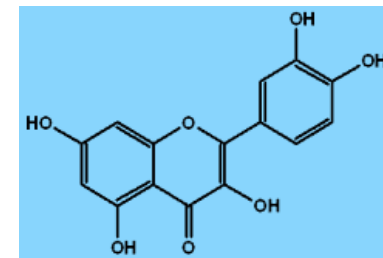
Hesperidin



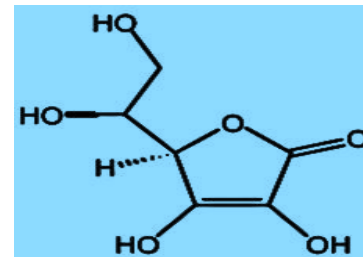
Rutin



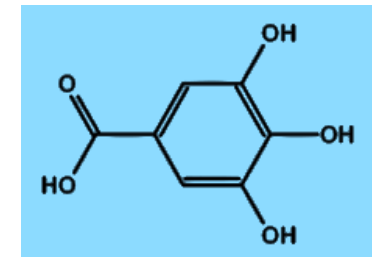
Kaempferol



Quercetin

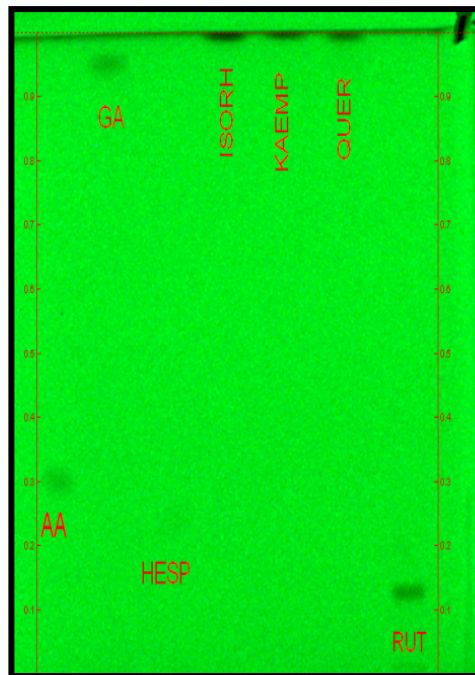


Ascorbic acid



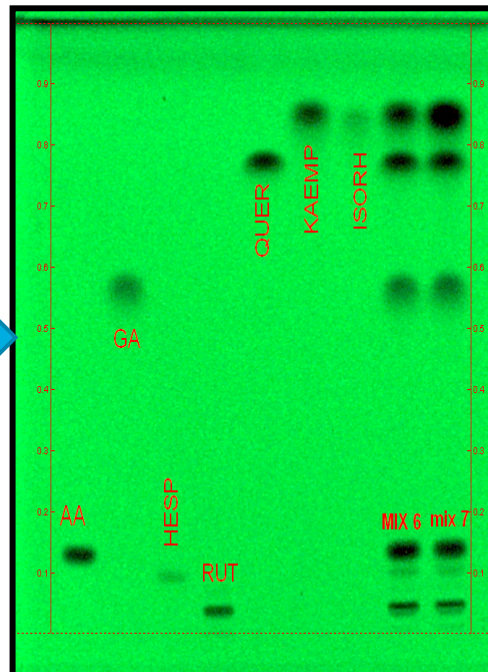
Gallic acid

METHOD DEVELOPMENT



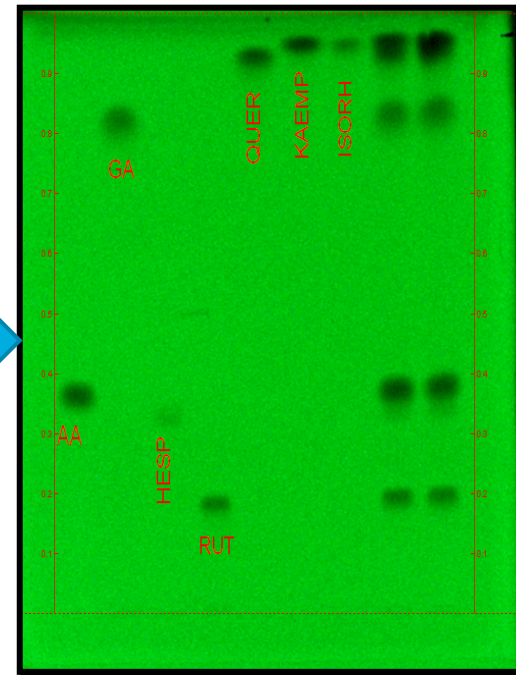
EtAc/DCM/GAA/H₂O
10/2.5/1/0.1/1 (v/v)

1



EtAc/DCM/GAA/FA
5/5/0.5/0.5 (v/v)

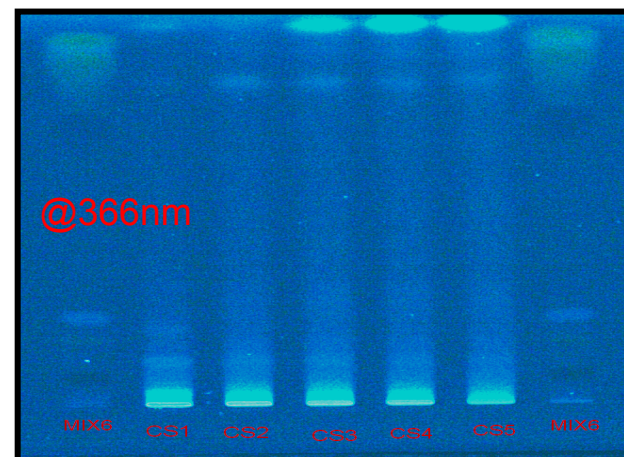
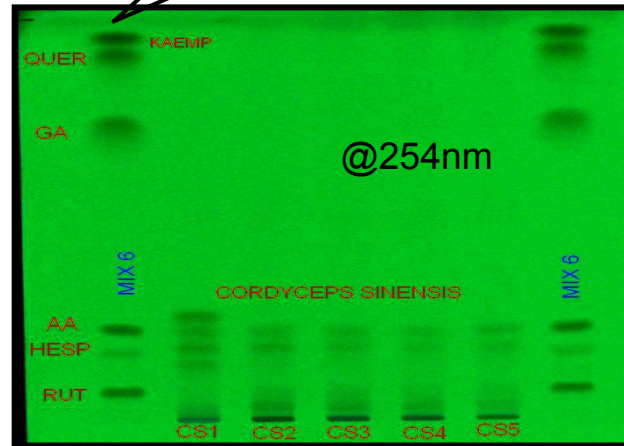
2



EtAc/DCM/GAA/FA/MeOH
5/5/0.5/0.5/1 (v/v)

3

**Cordyceps
sinensis**



QUANTIFICATION OF ANTIOXIDANTS

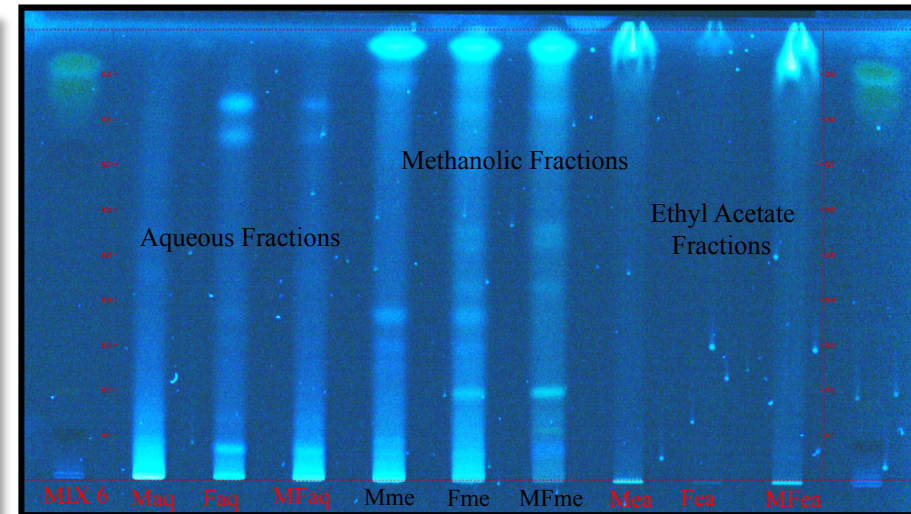
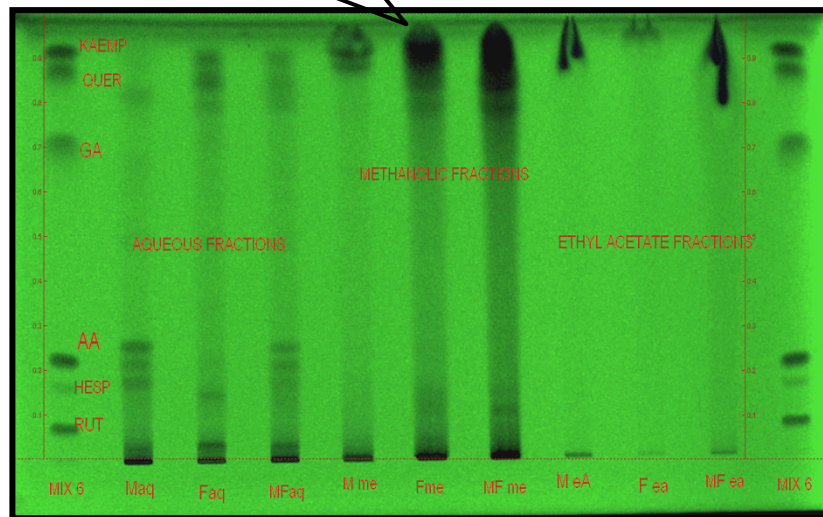
SAMPLE ID (Cordyceps sinensis)	ASCORBIC ACID (ng ascorbic acid / μ g of extract)	HESPERIDIN (ng hesperidine/ μ g of extract)	RUTIN (ng rutin/ μ g of extract)
CS 1 (aqueous)	2.19 \pm 0.014	13.32 \pm 0.101	1.57 \pm .045
CS 2 (25% alc.)	1.94 \pm 0.023	16.13 \pm 0.11	0.65 \pm 0.003
CS 3 (50% alc.)	1.27 \pm 0.051	8.28 \pm 0.026	0.39 \pm .001
CS 4 (75% alc.)	1.71 \pm 0.034	7.50 \pm .019	0.22 \pm 0.006
CS 5 (100% alc.)	1.70 \pm 0.016	3.83 \pm 0.043	0.16 \pm 0.004

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**Ganoderma
lucidum**

QUANTIFICATION OF ANTIOXIDANTS



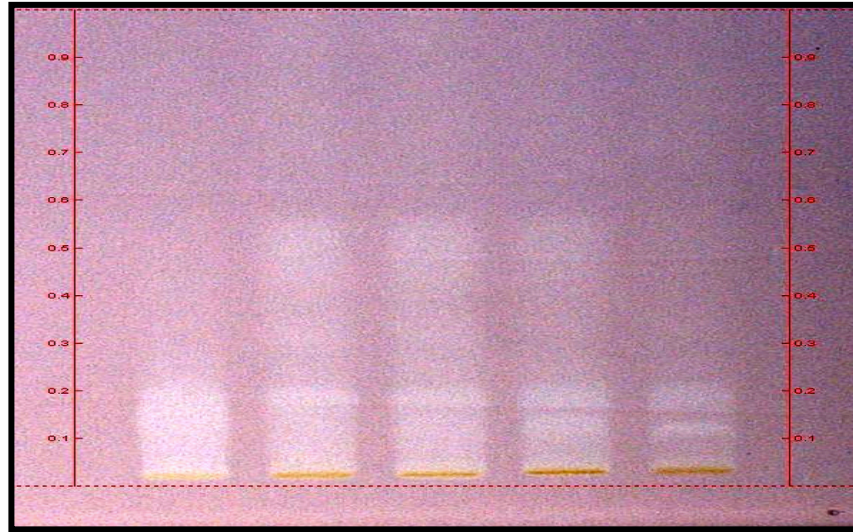
SAMPLE ID (Ganoderma lucidum)	HESPERIDINE (ng hesperidine/ µg of extract)	QUERCETIN (ng quercetin/ µg of extract)	RUTIN (ng rutin/ µg of extract)	GALLIC ACID (ng gallic acid/ µg of extract)
GLM(aq.)	0.628±0.044	1.027±0.052	1.258±0.067	1.089±0.056
GLF (aq.)	3.331±0.018	2.620±0.045	BDL	BDL
GLMF (aq.)	2.465±0.032	1.656±0.021	1.217±0.053	1.099±0.072

*BDL= Below
Detection Level

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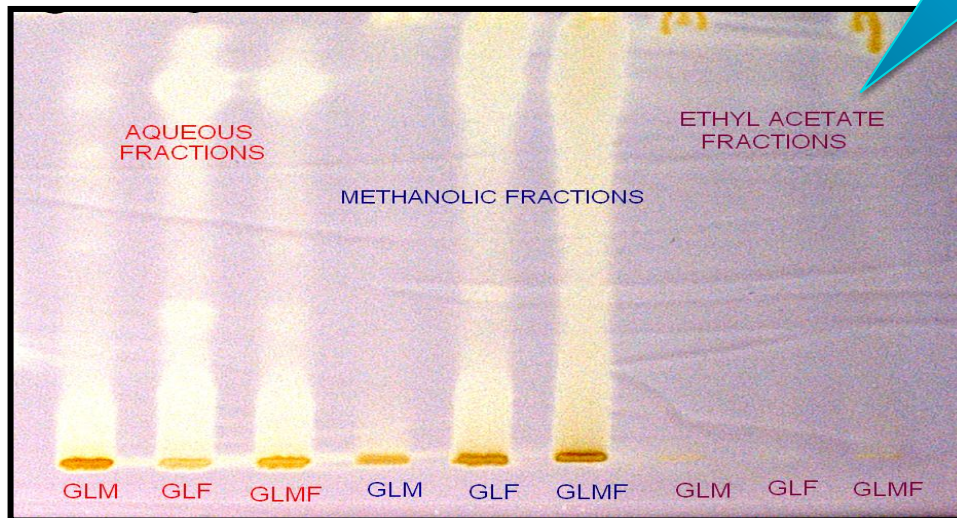
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DPPH DERIVATIZATION FOR ANTIOXIDANTS



**Cordyceps
sinensis**

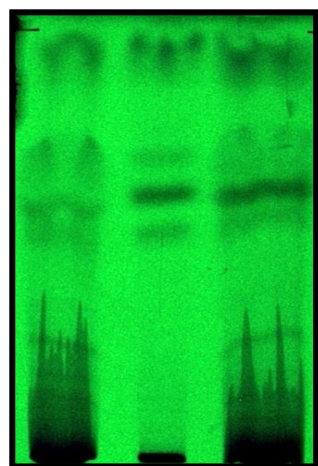
**NO
ANTIOXIDANTS**



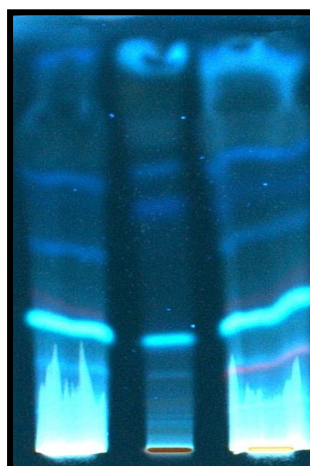
**Ganoderma
lucidum**

GANODERMA LUCIDUM: ETHYL ACETATE EXTRACTS

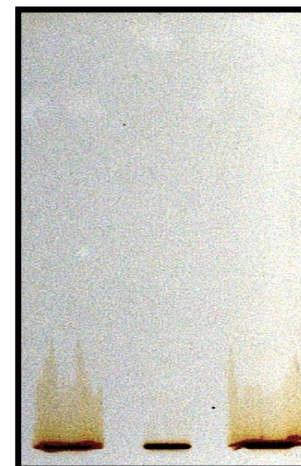
1. at 256nm
2. at 366nm
3. under white light



1



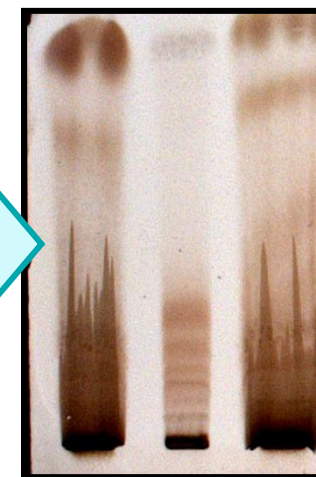
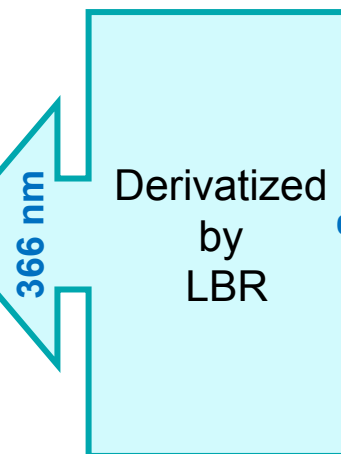
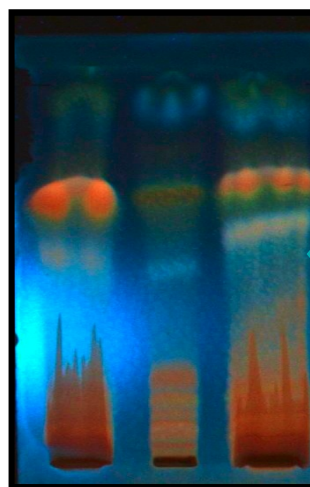
2



3

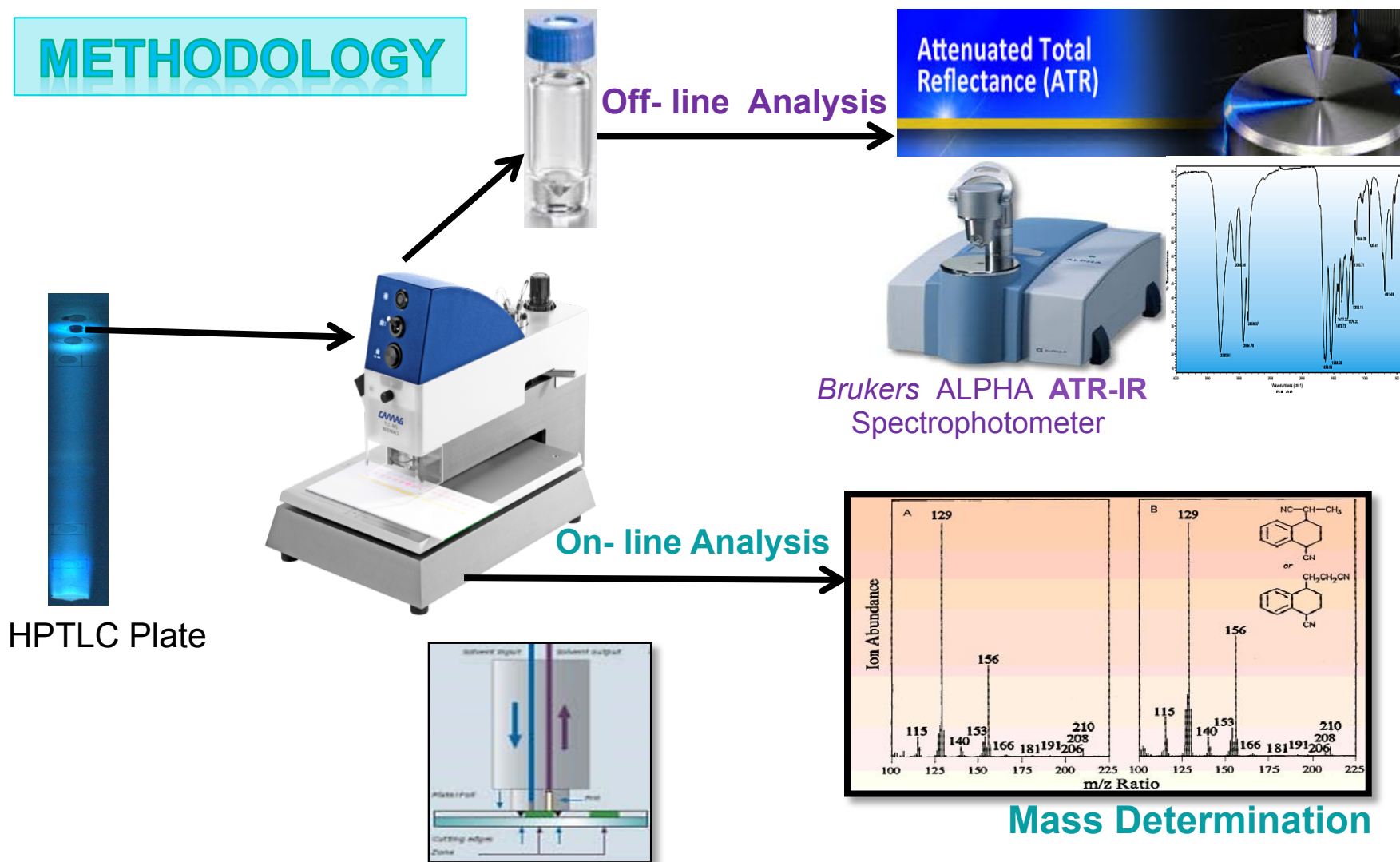
STATIONARY PHASE:
HPTLC Silica gel 60F₂₅₄
plates

MOBILE PHASE:
CHLOROFORM/ TOLUENE
(8:2 v/v)



CONFIRMATION BY IR & MS

METHODOLOGY



HPTLC Plate

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FT-IR: *CORDYCEP SINENSIS*

Example

RUTIN
in CS aq.

S.No	Wave number cm ⁻¹ Sample	Wave number cm ⁻¹ Reference	Functional group assignment
1.	3399.34, 3468.07	3419	Polymeric O-H stretch
2.	1638.43	1656	C=O stretch
3.	1385.59	1410-1310	Phenol or tertiary alcohol, O-H bend
4.	1275.36	1277	C-O stretch
5.	1016.37	1026	CC-C C-C stretch

FT-IR: *GANODERMA LUCIDUM*

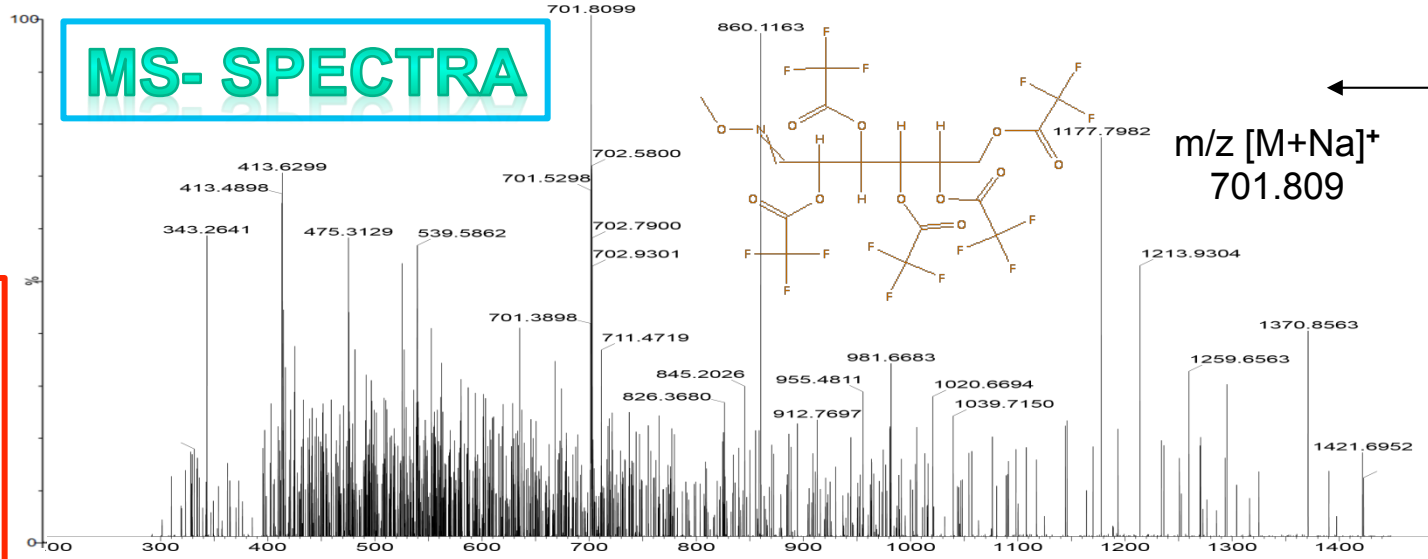
Example

QUERCETIN
in GL aq.

S. No.	Wave number cm ⁻¹ Samples (M/ F/ MF)	Wave number cm ⁻¹ Reference	Functional group assignment
1.	3403.41/ 3410.72/ 3417.20	3419	Polymeric O-H stretch
2.	2954.61/ 2944.01/ 2952.07	2955	C-H group in aromatic ring
3.	1646.70/ 1655.03/ 1648.88	1656	C=O stretch
4.	1410.57/ 1408.72/ 1410.17	1410-1310	Phenol or tertiary alcohol, O-H bend
5.	1110.51/ 1123.55/ 1134.09	1163	C=C stretch
6.	1020.67/ 1021.12/ 1022.56	1026	C-C stretch

Cordyceps sinensis

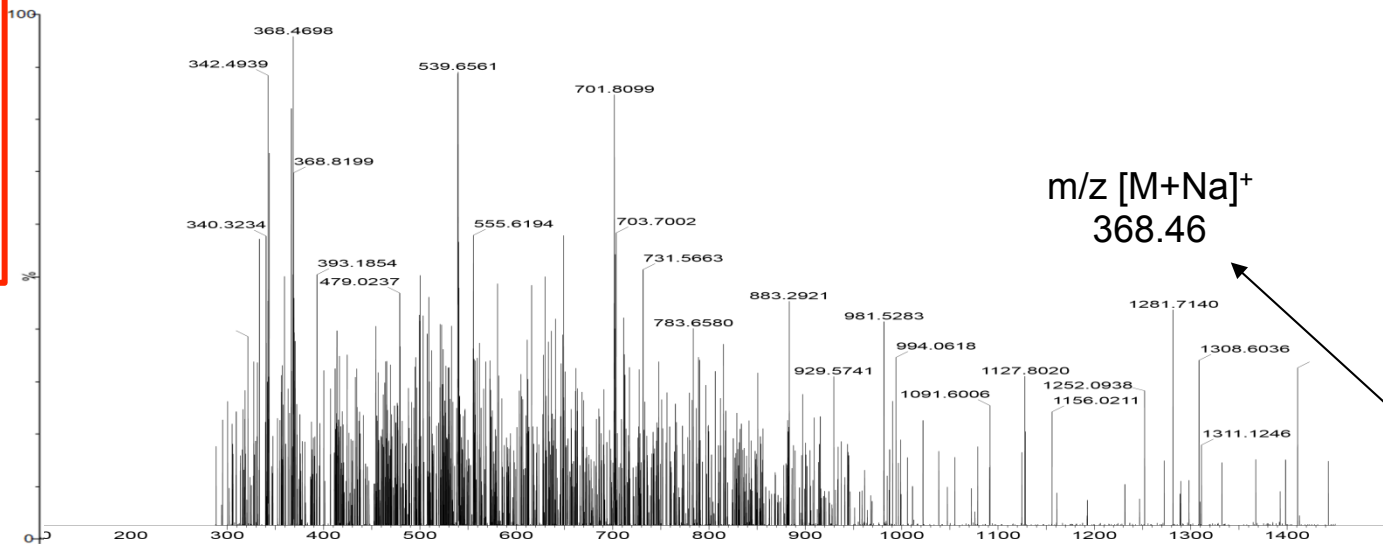
MS- SPECTRA



Rf 0.83

CS 1
(Aq.)

D(+)- Mannose, pentakis(trifluoroacetate),methyloxime

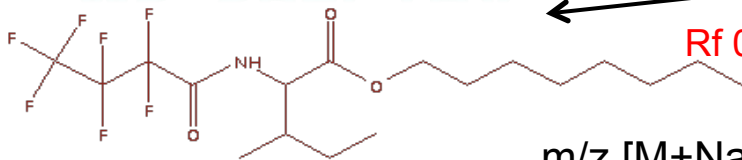


CS 3
(50% Alc.)

Rf 0.19

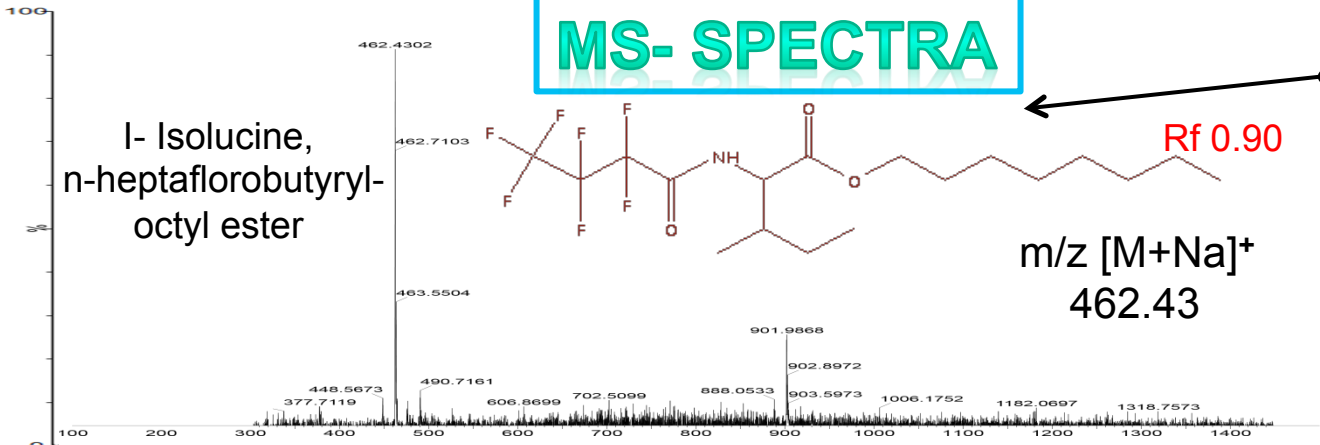
MS- SPECTRA

**L- Isolucine,
n-heptafluorobutyryl-
octyl ester**



Rf 0.90

m/z [M+Na]⁺
462.43

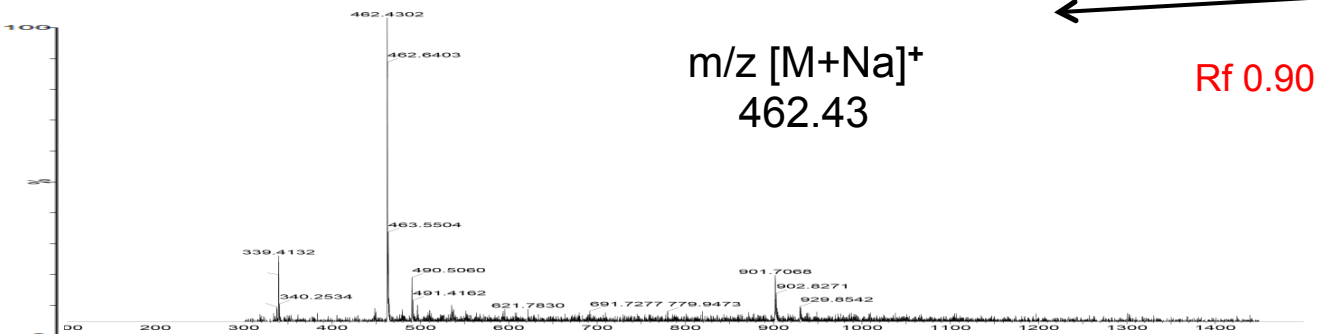


**MEOH
EXTRACTS**

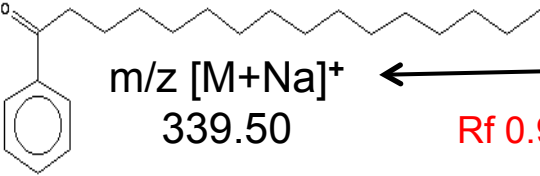
Ganoderma lucidum

m/z [M+Na]⁺
462.43

Rf 0.90

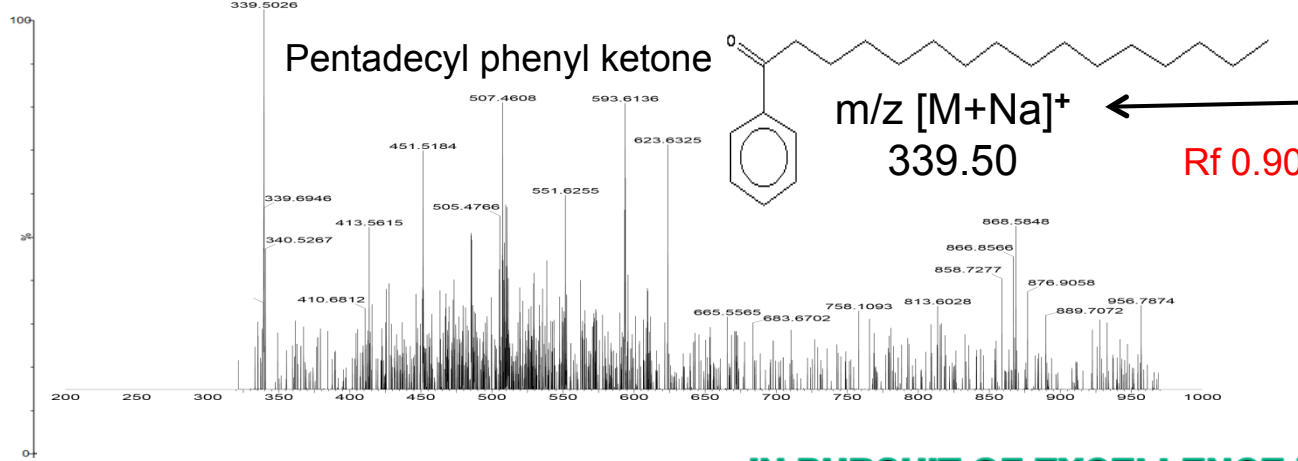


Pentadecyl phenyl ketone



Rf 0.90

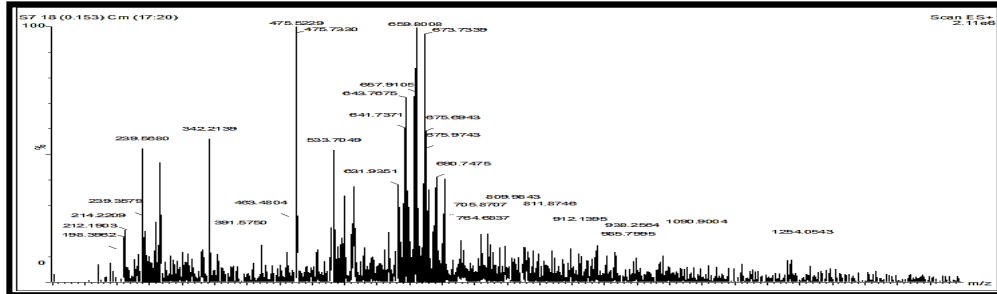
m/z [M+Na]⁺
339.50



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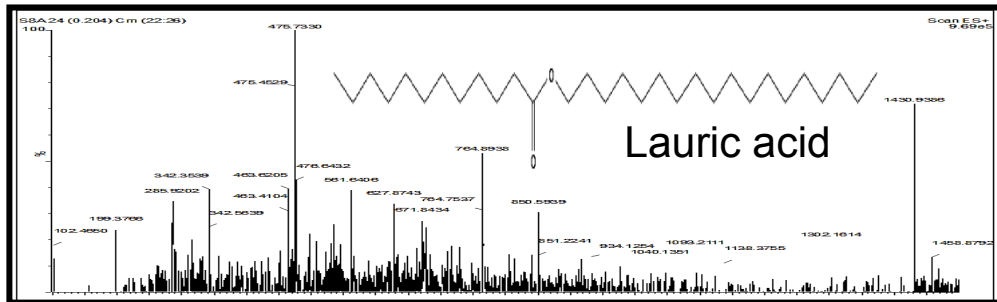
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MS-Spectra of *Ganoderma lucidum* Extracts (ETHYL ACETATE)

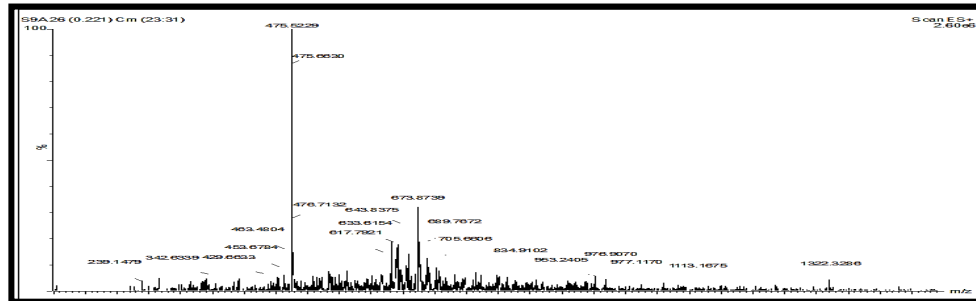


$m/z [M+Na]^+$
475.52

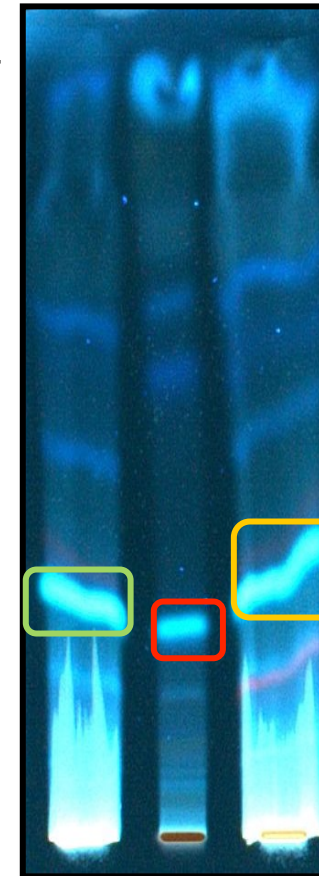
GLM



GLF

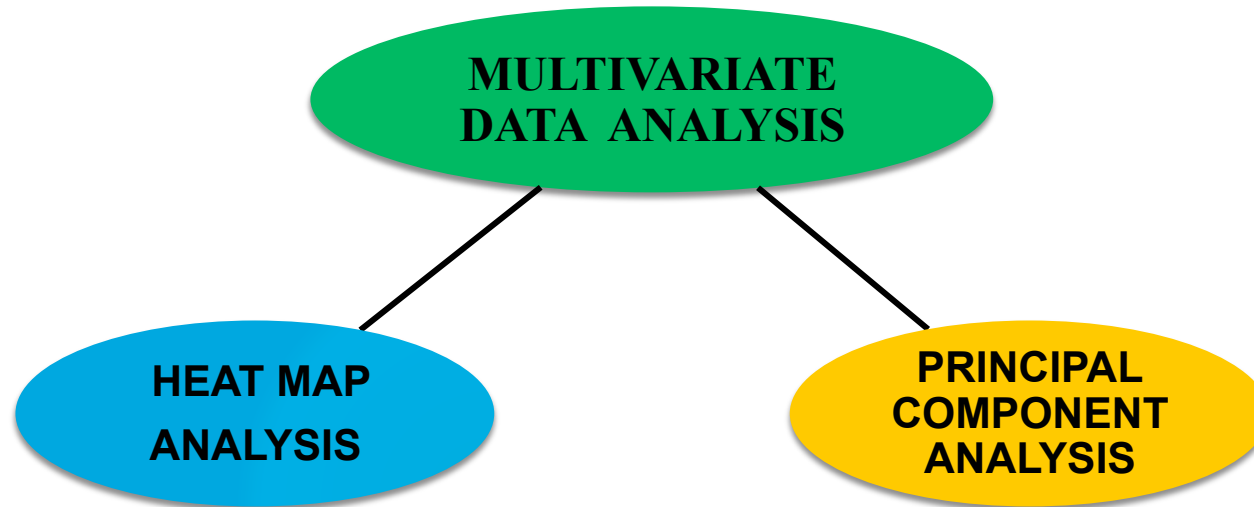


GLMF



GLM GLF GLMF

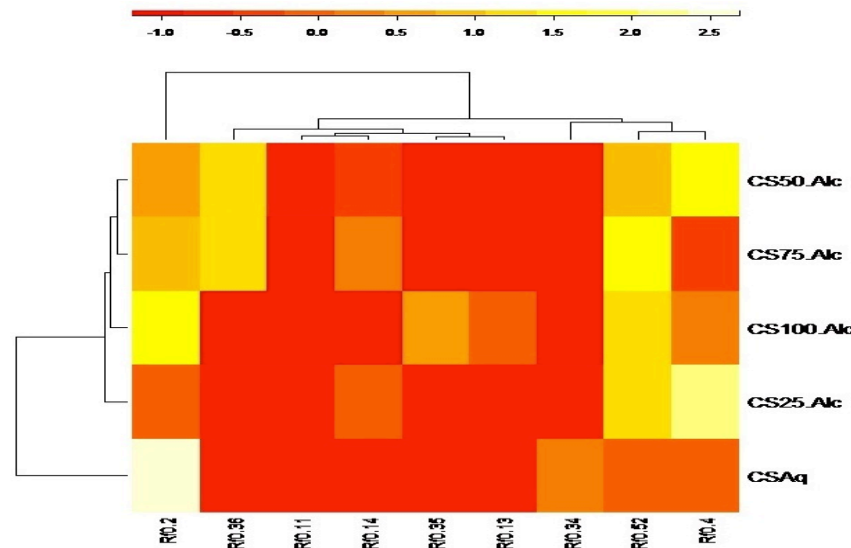
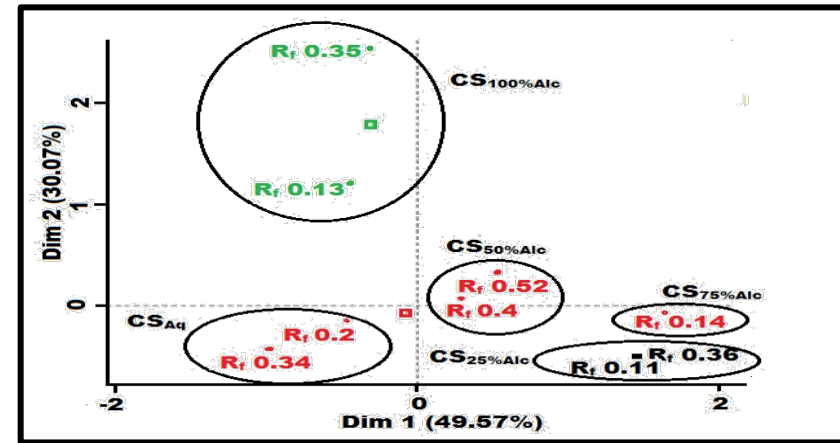
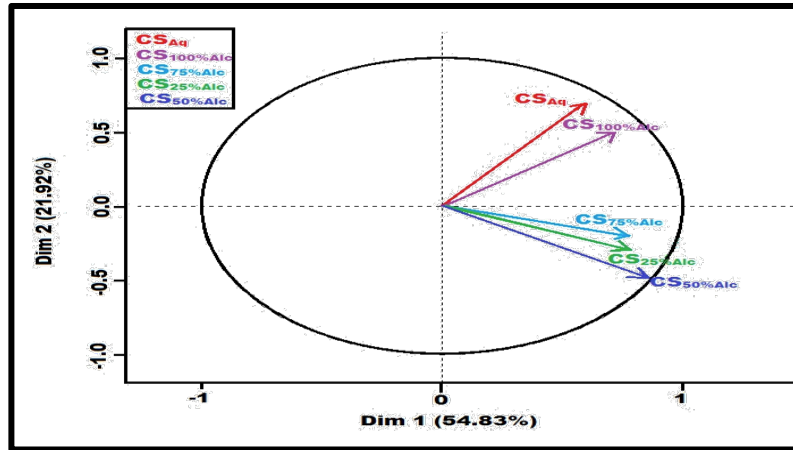
MULTIVARIATE DATA ANALYSIS



- HEAT MAP: 2-D Graphical presentation of data values
- R-programming package "Heatplus"

- Statistical tool for explaining relationships between datasets.
- R-programming package "FactoMineR"

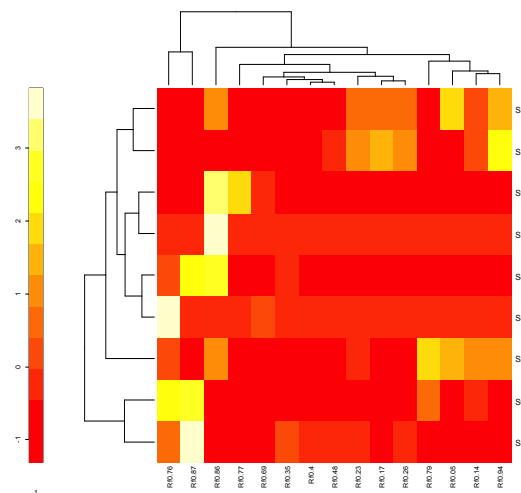
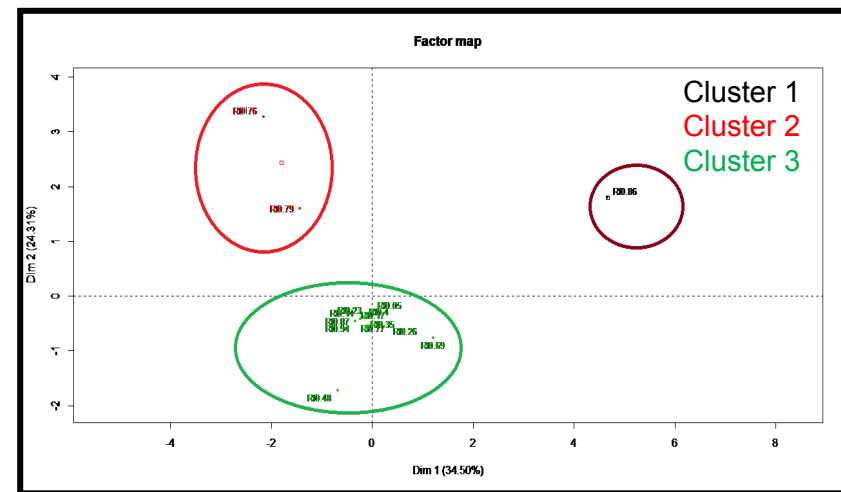
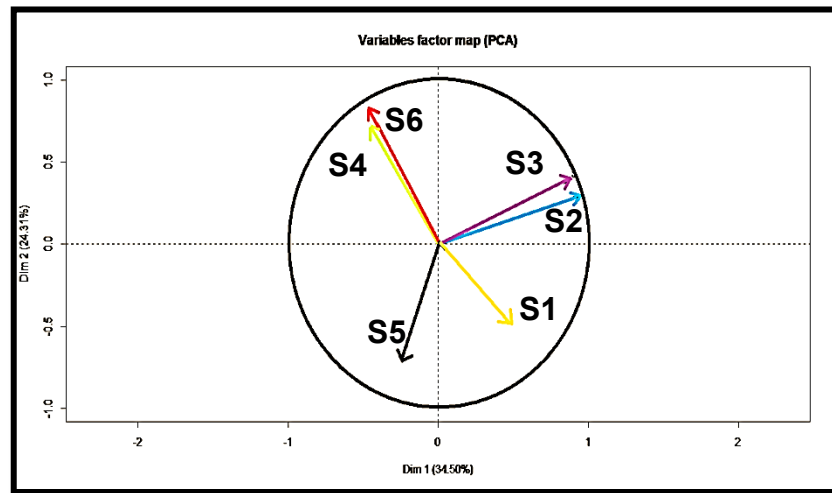
MULTIVARIATE DATA ANALYSIS: *CORDYCEPS SINENSIS*



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MULTIVARIATE DATA ANALYSIS: *GANODERMA LUCIDUM*



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CONCLUSIONS

- Hyphenated-HPTLC: A tool for metabolite profiling of medicinal mushrooms
- Application of chemometrics to HPTLC datasets
- Further confirmation by MS/MS, NMR



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CEFEES (DRDO)

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