Research and Development of Natural Product Chemistry in Sri Lanka

Lalith Jayasinghe

Senior Research Professor

National Institute of Fundamental Studies
Sri Lanka


Iceland
Sweden
Norway
Russia
United Kingdom
Canada
Poland
Germany
Ukraine
Kazakhstan
Mongolia
France
Italy
Spain
Turkey
China
Japan
South Korea
United States
North Atlantic Ocean
Afghanistan
Iraq
Iran
Pakistan
Algeria
Libya
Egypt
Saudi Arabia
India
Thailand
Mexico
Mali
Niger
Sudan
Chad
Nigeria
Ethiopia
Venezuela
Colombia
Kenya
DR Congo
Indonesia
Papua New Guinea
Tanzania
Brazil
Peru
Angola
Bolivia
Namibia
Madagascar
Indian Ocean
Botswana
Chile
South Atlantic Ocean
Australia
South Africa
Argentina

Sri Lanka is an island - 65,810 km^2

Highest mountain ~ 2524 m

Population ~ 22M

Average temperature in Colombo

Daytime 30^o Nights 27^o

Occasional Rain

Flora

~ 3500 plant species

800 – endemic

750 – traditional medicine

Relatively very low number of plants have been chemically and biologically investigated

- **Capital**
  Sri Jayawardanepura
  Colombo (Commercial capital)

- **Literacy Rate** - 92%

- **Languages**
  Sinhala, Tamil, English

- **Religions**
  Buddhism (70%) , Hinduism (15%),
  Christianity (8%), Islam (7%)

Sri Lanaka possesses a written history of over 2,500 years and an unwritten history of over 125,000 years attested to by archaeological ruins and other evidence. (**Wikipedia, encyclopedia)**

Ruined Cities

Anuradhapura

Polonnaruwa

Yapahuwa

Sigiriya – Rock Palace

Colombo

Kandy city

Hill capital – 500 m above sea level

Kandy - UNESCO declared as a world heritage city (1988)


Tea


Coconut


Rubber


Coffee


Spices

Sri Lanka – beautiful beaches


Gem


Tourism

One of 25 World's Biodiversity Hot Spots.


nature in stock

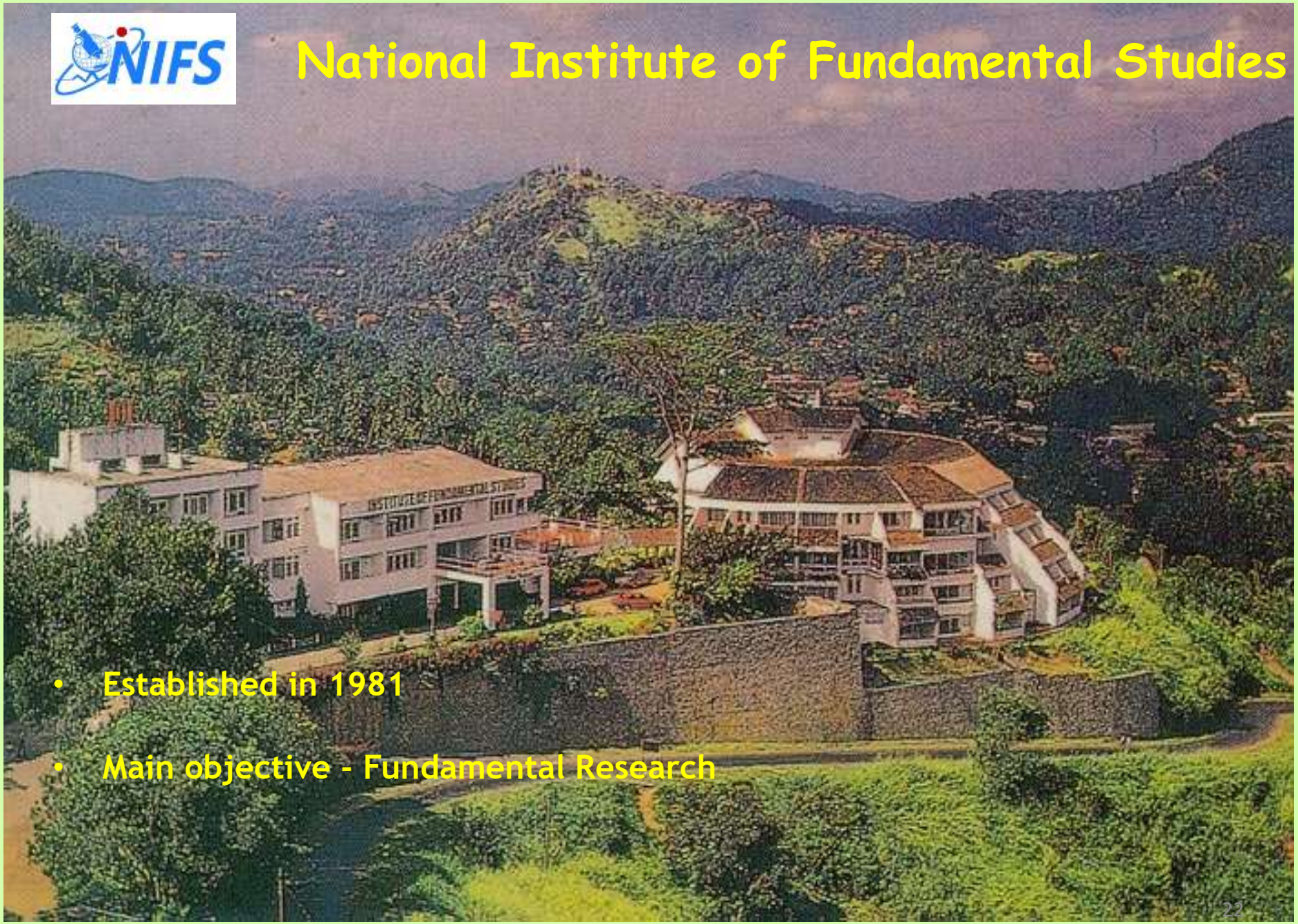
Hill country - Tea - 700 - 1800 m above s.l.


Sri Lanka - an agricultural country
Paddy – largest agricultural crop
Self-sufficient with rice


Flora
~ 3300 plant species
800 – endemic
750 – traditional medicine

Tropical rain forest

National Institute of Fundamental Studies



- Established in 1981
- Main objective - Fundamental Research

National Institute of Fundamental Studies

- Established in 1981

- The only national institute which, by its Act, has the main objective as to engage in **Fundamental Research**

Vision

To be a world renowned Centre of excellence for research in fundamental studies

Mission

Initiate, promote and engage in advanced research in fundamental studies for the enhancement of scientific knowledge, human resources and national development

NIFS Staff

- 16 Senior Scientists / 04 Visiting Scientists
- 81 Research Students (12 PhD, 69 MPhil candidates)
- > 50 M.Sc. Students/ UG Students annually
- > 30 Pre-University Students & Volunteers annually
- Non-research staff – 60

Research Projects

1. Chemical & Environmental Systems Modeling
2. Microbial Biotechnology
3. Condensed Matter Physics & Solid State Chemistry
4. Photochemistry
5. Nanotechnology/ Physics of Materials
6. Biofuel
7. Artificial Intelligence and Applied Electronics
8. Ecology & Environmental Biology
9. Primate Biology
10. **Natural Products**
11. Functional Food Product Development
12. Plant Biology
13. Natural Resources and Renewable Energy
14. Cell Biology
15. Nutritional Biochemistry

Funding:

National Institute of Fundamental Studies (NIFS)

National Research Council (NRC) & National Science Foundation (NSF)

I completed my Ph.D. in 1992 Oct.

Ph.D. in Natural Product Chemistry - 1992

University of Peradeniya, Sri Lanka.

Thesis Title : Chemistry and Bioactivity of Some Sri Lankan Menisperaceae and Lauraceae

Supervisor : **Prof. G.P. Wannigama**

Diploma in Natural Product Chemistry - 1994

Tokyo Institute of Technology, Japan.

Diploma Thesis :

Biologically Active Saponins from *Pometia eximia*

Supervisor : **Prof. Yoshinori Fujimoto**

UNESCO-MOMBUSHO Program, 1 Year at TIT

My connection with Japan

I was awarded UNESCO-MOMBUSHO Research Fellowship

- 1 year (1993/94), Tokyo Institute of Technology
- Diploma on Natural Product Chemistry
- My Supervisor **Prof. Yoshinori Fujimoto**

Since then I am keeping very close contacts with Prof. YF.

Joint activities with Prof. Fujimoto & Prof. Araya

	Publications	Commun.	Book Chap.	Visits to SL
Prof. Y. Fujimoto	44	66	2	4
Prof. H. Araya	9	25	-	1

2-Ph.D 17-M.Phil. 1 -MSc.

Natural Products Research Project

National Institute of Fundamental Studies

Theme

Search for bioactive compounds from natural sources as potential resources for control of human and plant diseases

Our Team - 2019

Senior Scientists	- 2	Research Assistants	- 7
Technical Officers	- 1	Undergraduate Trainees	- 3

Collaborators (Universities)

Current Research

- Plant Secondary Metabolites

  -Chemistry & Bioactivity of Medicinal Plants/Edible Fruits/Seeds/Spices etc.
- Fungal Metabolites, Isolation and Bioactivity
- Study of Phenolic Profile of some Fruits, Spices, Medicinal Plants by LC/MS
- Microbial Transformation of Organic compounds
- Cause and control of postharvest diseases and disorders of edible fruits

Bioprospecting for Drug Leads from Sri Lankan Medicinal Plants and Associated Fungi

Bioprospecting

Search for new **chemicals in living things** that will have some medical or commercial use.

(Food production, New drugs, Pest control, ….etc)

Natural Products - Chemicals in living things

Natural Products are chemical compounds produced by plants, fungi, marine organisms, etc. These compounds can be used to improve the quality of human life.

The use of natural products in the management and treatment of diseases in humans and plants is culturally more acceptable and offer less risk than use of synthetic compounds.

Synthetic compounds are often toxicologically and environmentally undesired.

- The continuous use of synthetic pesticides .. ..

- Problems - Physiological resistance,
  Environmental problems,
  High operational cost,
  Toxic to non-target organisms etc.

- Identification of environmentally friendly natural bioactive compounds from natural sources has come to play a prominent role.

For thousand of years, natural products have played an important role throughout the world in treating and preventing human diseases.

Natural products and their derivatives

- historically invaluable as a source of therapeutic agents.
- the most successful source of drug leads.
- continue to provide greater structural diversity
- offer opportunities for finding novel low MW lead structures

Plant & Microorganisms produce variety of metabolites and **some of them are capable of locking a specific disease mechanism.**

~80% are of the world population still depend on plant based traditional medicine.

>60% of approved drugs or pre-new drug application candidates are of natural origin.

Some important drugs from plants

- **Artimisinin** from *Artemesia annua*
- **Atropine** from *Atropa belladona*
- **Digoxin** from *Digitalis* spp.,
- **Morphine & Codeine** from *Papaver somniferum*
- **Quinine & Quinidine** from *Cinchona* spp.,
- **Taxol** from *Taxus brevifolia*
- **Vincristrine & Vinblastine** from *Catharanthus roseus*,

Microorganisms are also ample source of structurally diverse bioactive substances and have provided important contributions to the discovery of antibacterial agents including penicillins, cephalosporins, amynoglycosides, tetracyclines, and polyketides.

Current therapeutic applications of metabolites from microorganisms have expanded in to:

- Anticancer agents (eg., pentostatin, peplomycin, epirubicin)
- Antidiabetic agent (eg., acarbose)
- Anthelmintic agents (eg., ivermectin)
- Cholesterol-lowering agents (eg., lovastatin and mevastatin)
- Immunosuppressive agents (eg., cyclosporins, rapamycin)

Food industry

Textile industry

Natural Products from plant & microbial origin

Agriculture

Cosmetics

Pharmaceutical industry

History of Plant & Microorganism Derived Drugs

1826	- Morphine	-	analgesic
1899	- Aspirin	-	analgesic
1941	- Penicillin	-	antimicrobial
1964	- Cephalosporin	-	antibacterial
1983	- Cyclosporin A	-	immunosuppressant
1987	- Artimisinin	-	antimalaria
1987	- Lovastatin	-	antihyperlipidermic
1994	- Fluvastatin	-	antihyperlipidermic
1990	- Acarbose	-	antidiabetic
1993	- Taxol	-	anticancer
1996	- Miglitol	-	antidiabetic
1999	- Orlistat	-	antiobesity

To develop a new drug .....

Cost = ~$ 2 billion

Duration = ~20 years R&D

>800 Scientists with multi-disciplinary expertise

Screening ~ 100,000 compounds

Pioneers of Natural Products Research in Sri Lanka

Prof . J.P.C. Chandrasena

Prof. M.U. Sultanbawa

Dr. L.B. De Silva

Dr. R.O.B. Wijesekera

Prof. G.P. Wannigama

Criteria for the selection of plants

Medicinal plants

Plants related to medicinal plants

Plants used as food

Steps Involved in Natural Product Research

Collection and identification of natural sources
plants, fungi, microorganisms etc.

Extraction - with organic solvents / water

Bioassays

Activity guided fractionation - chromatography

Isolation of pure compounds - MPLC, VLC, Chromatron, GC, HPLC etc

Identification - Physical methods – UV, IR, NMR, MS etc
Chemical methods

Activity enhancement - Partial synthesis

Bench-top Bioassays

Simple, Inexpensive, Short time, Broad spectrum of information, Reproducibility

- Antioxidant
- Antifungal
- Brine shrimp toxicity
- Phytotoxicity
- Mosquito larvicidal activity
- Enzyme inhibitory

  (lipase, α-amylase, glucosidase, collagenase, urease, chymotripsin, Xanthine oxidase)

Hemolytic activity

Total polyphenolic content

TLC Bioautography Methods

TLC bioautography methods are important in the search for bioactive compounds, based on localization of active compounds on TLC chromatogram.

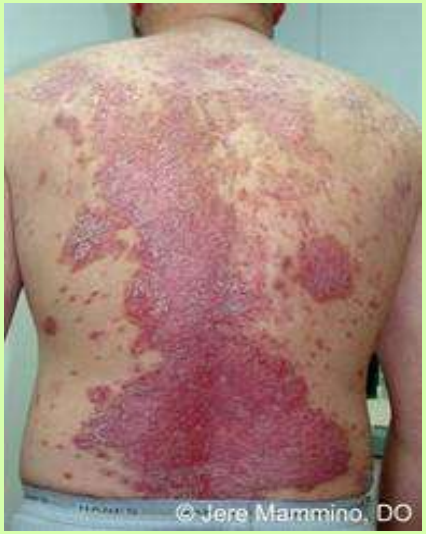
This method can be used to screen especially antifungal, antibacterial, antioxidant & some enzyme inhibitory activity etc -qualitatively.

Simplicity, speed and also low cost are the advantages of this method.

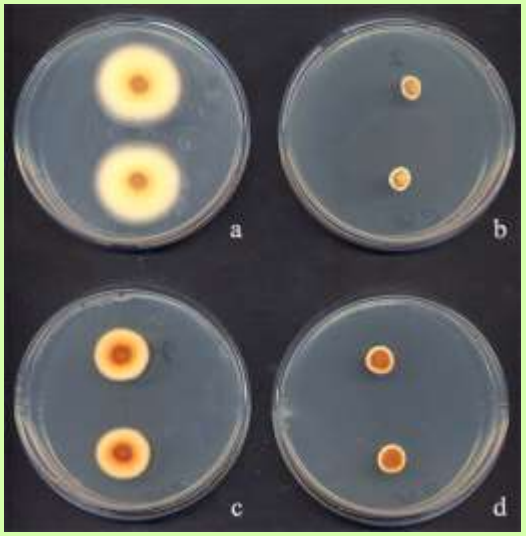
Antifungal Activity

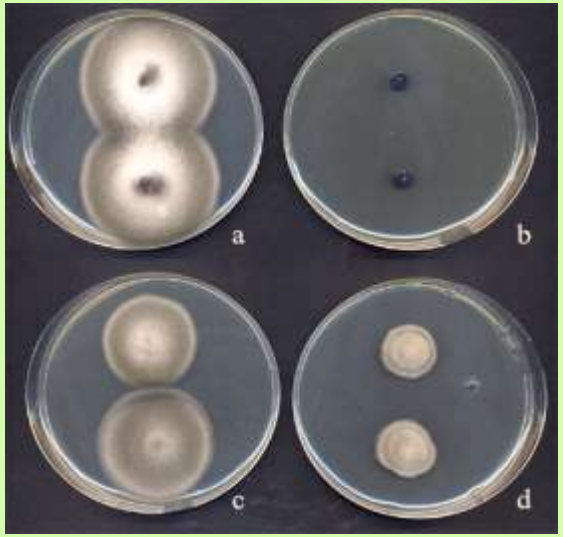
- Fungi cause great losses in agriculture, food industry and health problems.

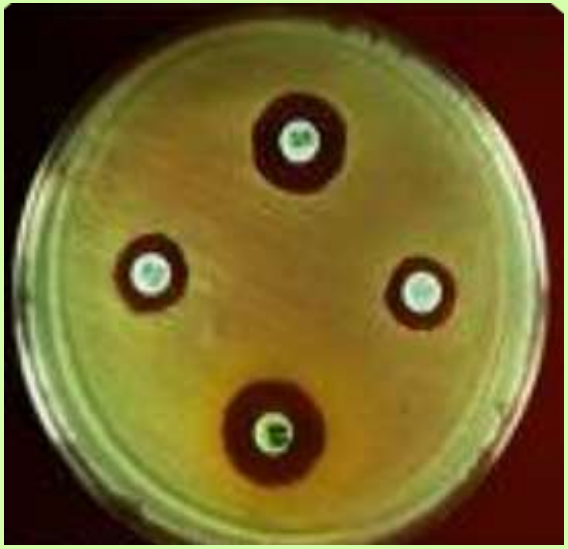
- Great demand for novel antifungal belonging to a wide range of structural classes, selectively acting on new targets with fewer side effects.

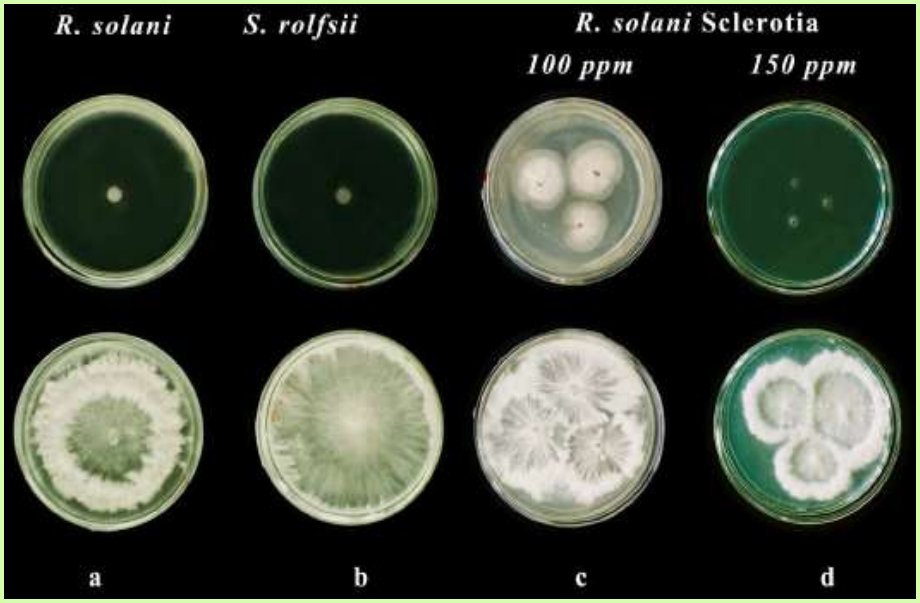


TLC Bioautography Method









Disk diffusion method

Inhibition of the radial growth

Antioxidants

Research on free radical science.

Alzhimer's disease, rheumatoid arthritis, cardiovascular disease, cataracts, diabetes, hypertension and aging itself all, may be in part, caused by a phenomenon known as oxidative or free radical damage.

Antioxidants - prevent, stop, or reduce oxidative damage.

TLC Bioautography method
Spectrophotometry method

Brine Shrimp Assay

A useful tool for preliminary assessment of toxicity.

Detection of fungal / Plant / Heavy metal / cyanobacteria Pesticides, Dental materials etc

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Phytotoxicity

Lettuce seed germination test

Selective activity – monocot / dicot ;

Important in agriculture, maintain home gardens, golf yards etc.,

Natural herbicides, weedicides etc.

…. can be used to reduce or replace the synthetic herbicides and weedicides

- Enzyme inhibitors are important in the field of drug research.
- Specific enzyme inhibitors are biochemical tools that have potential utility in the treatment of diseases.

Enzyme	Drug Target
α- amylase	Diabetes II, cardiovascular diseases
Collagenase	Arthritis, cartilage degradation
α- glucosidase	Diabetes II, cardiovascular diseases
Lipase	Obesity, cardiovascular diseases
Urase	Gastric and peptic ulcers, intestine cancer
Chymotripsin	Hepatitis induced liver injury, cirrhosis, liver cancer
Xanthine oxidase	Aging, Inflammation, atherosclerosis, cancer,
AchE	Alzheimer‘s disease
Tyrosinase	Skin cancer

Diabetes mellitus (Type II) - No known permanent cure and is highly prevalent worldwide. In Sri Lanka Traditional and Ayurvedic physicians treats diabetes mellitus very effectively by using **various parts of the several medicinal plants**.

"**A Review on Herbs used in treatment of diabetes mellitus by Sri Lankan Ayurvedic and Traditional Physicians**" - > 125 plant species (Ediriweera & Ratnasooriya, 2009)

Flowers – *Butea monosperma*

Fruits – *Momordica vasica*

Stems – *Tinospora cordifolia*

Root Bark – *Salacia reticulate*

Aerial Roots – *Ficus benghalensis*

Bulb – *Allium sativum*

Entire Plant – *Scoparia dulcis*

Leaves – *Adathoda vasica*

Seeds – *Syzygium cumini*

Stem Bark – *Ficus religosa*

Roots – *Oryza sativa*

Rhizome – *Alpinia galanga*

Creeper – *Passiflora foetida*

α-Amylase Inhibitor from the Leaves of *Syzygium cumini*

- Pancreatic α-amylase and α-glucosidase inhibitors serve as important strategies in the management of blood glucose.
- Even though *Syzygium cumini* (L.) (Myrtaceae) (SC) is used extensively to treat diabetes; scientific evidence on antidiabetic effects of SC leaves is rare.

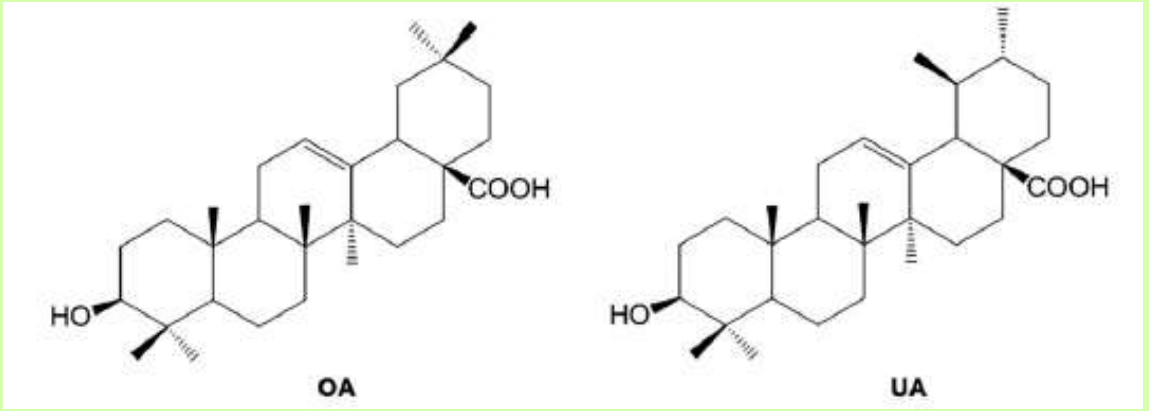
An active fraction obtained with chromatographic separation of the extract inhibited pancreatic α-amylase with an IC50 of 39.9 μg/mL and α-glucosidase with an IC50 of 28.2 μg/mL.

The active fraction was determined to be a 3:1 mixture of ursolic acid and oleanolic acid.

Pure ursolic acid and oleanolic acid showed IC50 values of 6.7 and 57.4 μg/mL, respectively, against α-amylase and 3.1 and 44.1 μg/mL respectively, against α-glucosidase.

This is the first report validating the use of SC leaves in antidiabetic therapy. *Pharmaceutical Biology*, 2017

α-Amylase Inhibitor from the Leaves of *Syzygium cumini*



3:1 mixture of UA & OA

PHARMACEUTICAL BIOLOGY, 2016
VOL. 55, NO. 1, 206–211
http://dx.doi.org/10.1080/13880209.2016.1257031

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS

Bioassay-guided fractionation and identification of α-amylase inhibitors from *Syzygium cumini* leaves

Jeyakumaran Poongunran[a], Handunge Kumudu Irani Perera[b], Lalith Jayasinghe[c], Irushika Thushari Fernando[b], Ramaiah Sivakanesan[b], Hiroshi Araya[d] and Yoshinori Fujimoto[c,d]

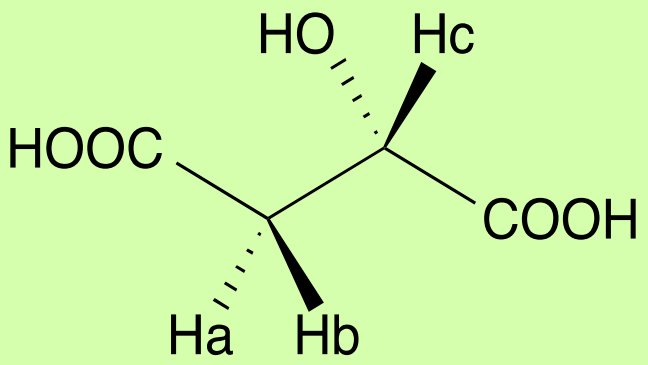
J Food Sci Technol (December 2015) 52(12):8383–8388
DOI 10.1007/s13197-015-1937-6

ORIGINAL ARTICLE

Antioxidant property and α -glucosidase, α -amylase and lipase inhibiting activities of *Flacourtia inermis* fruits: characterization of malic acid as an inhibitor of the enzymes

A. G. A. W. Alakolanga [1] · N. Savitri Kumar [1] · Lalith Jayasinghe [1] · Yoshinori Fujimoto [2]

The EtOAc and MeOH extracts - α-glucosidase, α-amylase, lipase enzymes .
The active principle - (*S*)-malic acid.



(*S*)-malic acid.

F. inermis fruits have the potential to be used in health foods and in nutritional supplements.

South African Journal of Botany 121 (2019) 418–421

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Isolation, identification and characterization of pancreatic lipase inhibitors from *Trigonella foenum-graecum* seeds

W.I.T. Fernando [a], A.M.K.C. Attanayake [a], H.K.I. Perera [a], R. Sivakanesan [a], L. Jayasinghe [b,*], H. Araya [c], Y. Fujimoto [b,c]

Lipase inhibitor --- Control Obesity (Major Risk factor for DM Type II

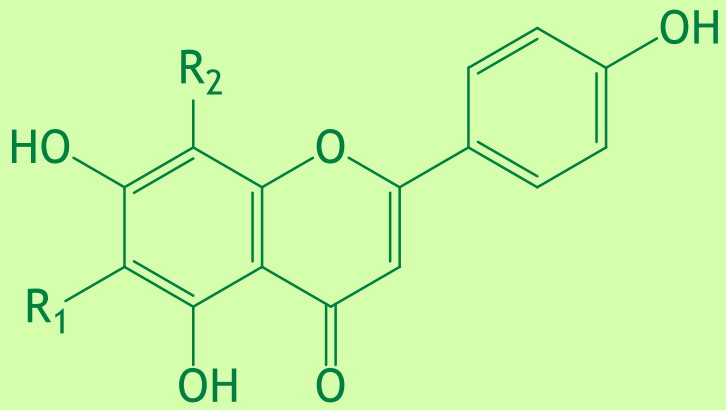
Trigonella foenum-graecum seeds

Three flavone C-glycosides with Lipase inhibitory activity

(1) Vicenin 1

(2) Isoschaftoside

(3) Schaftoside



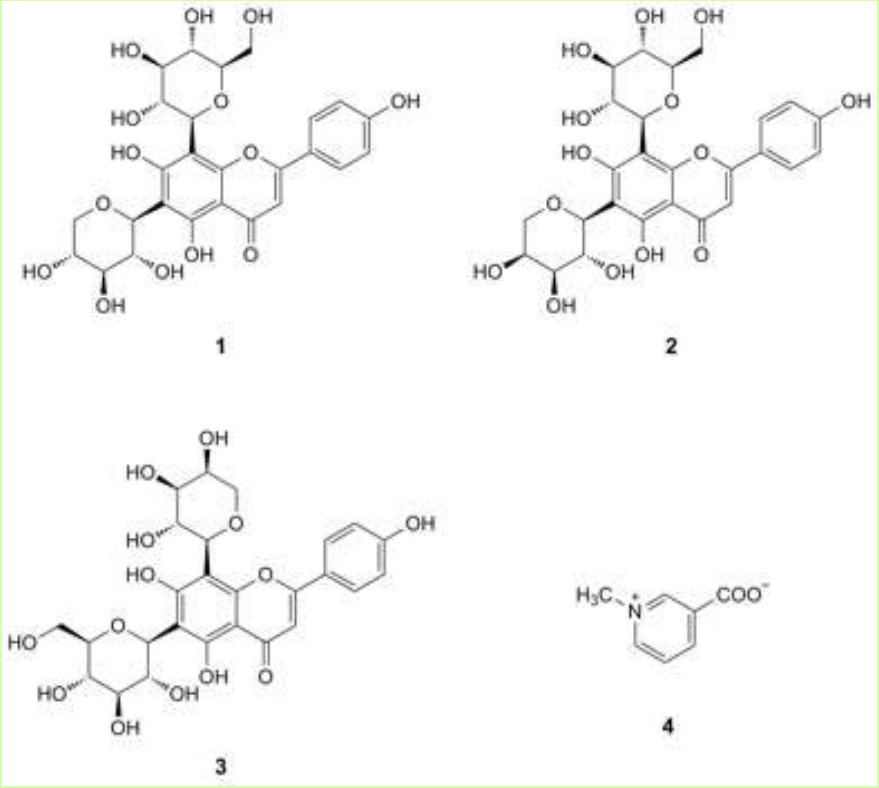
Three Lipase Inhibitors

Flavonoid C-glycosides

Cpd	R_1	R_2	
1	xyl	glc	Vicenin 1
2	ara	glc	Isoschaftoside
3	glc	ara	Schaftoside

1st Report of Lipase inhibitors from *Tigonella*

1 & **3** identified as lipase inhibitors from *Trigonella* for the 1st time



This study led to the isolation of vicenin-1 (**1**), isoschaftoside (**2**) and schaftoside (**3**) along with trigonelline (**4**) from the methanol extract of *T. foenum-graecum*.

These compounds showed percentage lipase enzyme inhibition **60.3% (1)**, 33.8% (**2**) and **95.5% (3)** at the concentration of 250 µg/ml and IC_{50} values of the inhibition were **207 µg/ml (1), 330 µg/ml (2)** and **130 µg/ml (3).**

- This is the first report of the isolation of lipase inhibitors from *T. foenum-graecum* seeds and these results proved the potential of *T. foenum-graecum* as a natural lipase inhibitor, which helps to control the obesity which is a risk factor of cardiovascular diseases.

Acetylcholinesterase & α-glucosidase inhibitory activity
Myristica fragrans (Mace)

Mace *(Myristica fragrans)*

Family - Myristicaceae

Native - Indonasia

Grown in up countries of Sri Lanka

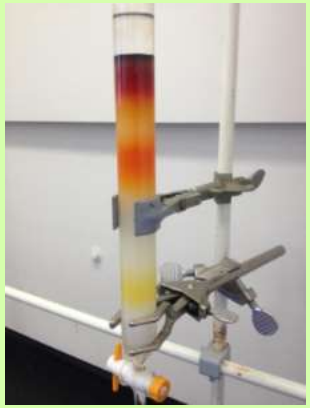
Two parts in fruit- Seed (nutmeg)

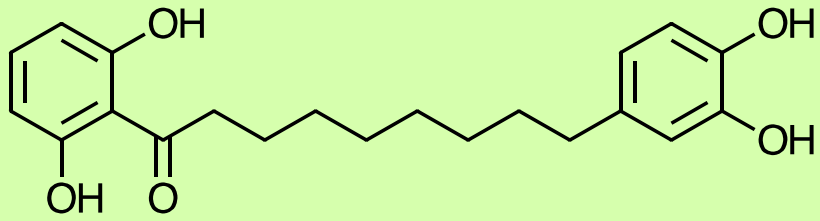
Fleshly aril (mace)

- Acetylcholinesterase AChE is one of the essential enzymes for nerve response.
  Inhibition of AChE - Alzheimer's Disease
- α-Glucosidase is one of the essential enzyme in sugar metabolism
  Inhibition of α-glucosidase- Diabetes

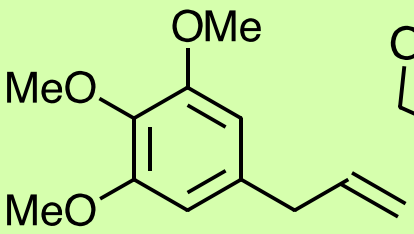
Activity guided fractionation of mace extracts

- Mace showed highest AChE inhibitory activity
- Isolated compounds were investigated for
  - AChE inhibitory
  - α-Glucosidase inhibitory
  - Antioxidant activity

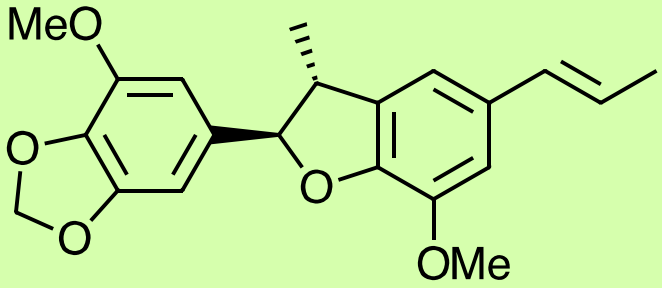




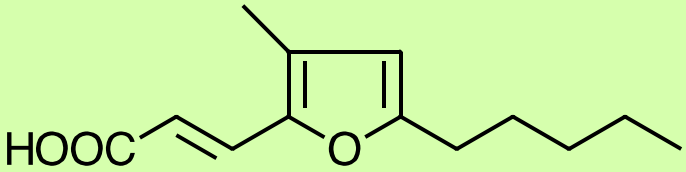
malabaricone-C (1)



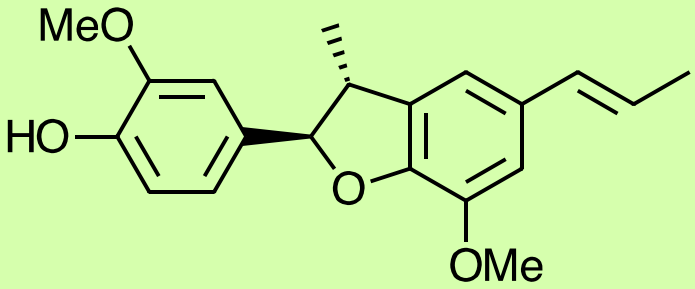
elemicin (4)



maceneolignan B (5)



3'-methyl-5'-pentyl-furylarylic acid (2)



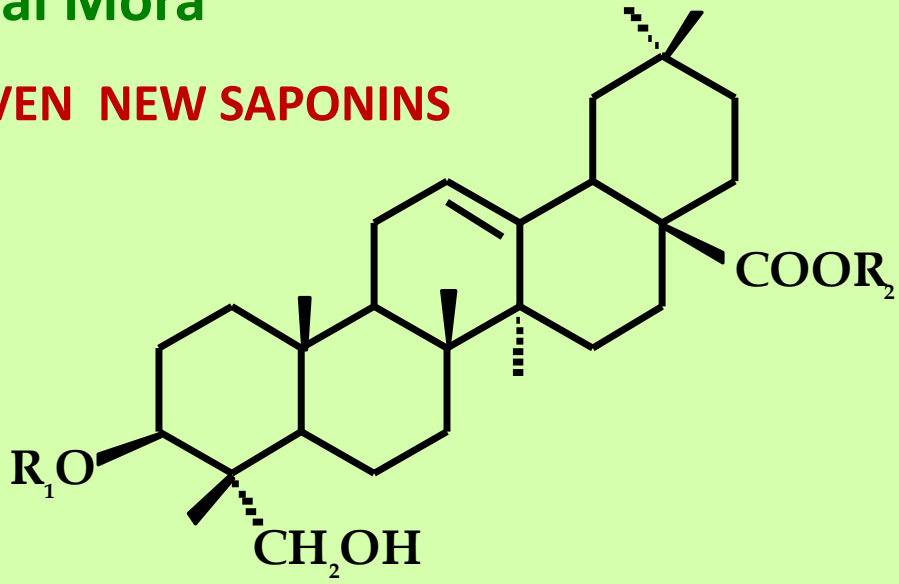
Dehydrodiidoeugenol (3)

At 100 ppm	AChE activity (%)	Antioxidant activity (%)	α-glucosidase inhibitory activity(%)
1	100	96	NA
2	14	28	90.63
3	48	73	NA
4	20	NA	NA
5	NA	NA	30

HEDERAGENIN GLYCOSIDES FROM *POMETIA EXIMIA*

Gal Mora

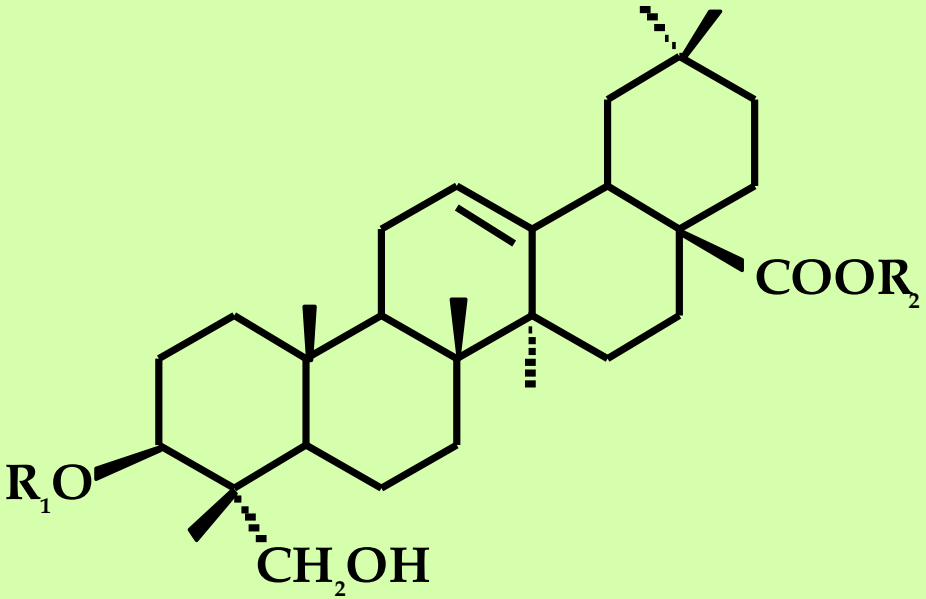
SEVEN NEW SAPONINS



R_1	R_2
H	H
ara-	H
xyl-^{3}ara-	H

R_1	R_2
H	api-^{3}glc-
rha-^{2}xyl- ^{3}| ara(*f*)	H
rha-^{2}glc- ^{3}| api	H
rha-^{2}ara*- ^{3}| ara(*f*)	H
rha-^{2}ara- ^{3}| xyl	H
rha-^{2}glc- ^{3}| xyl	H
rha-^{2}glc- ^{3}| gal	H

Molluscicidal activity



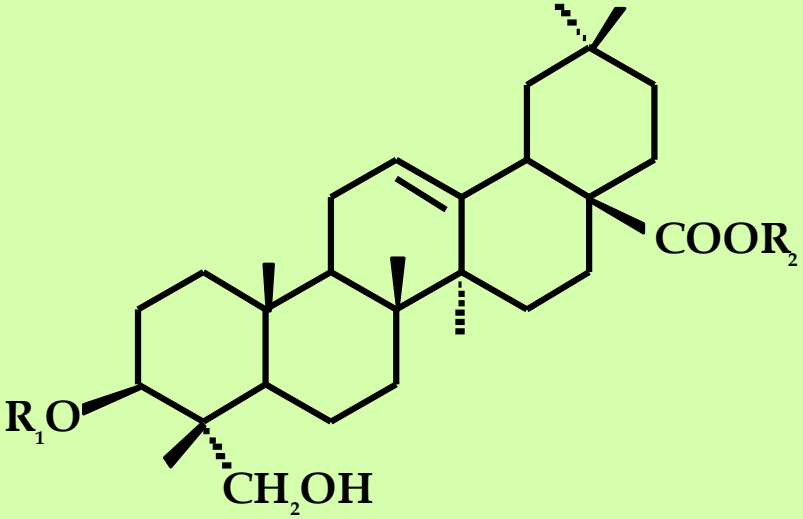
R_1	R_2	activity
ara	H	40 ppm
xyl-^{3}ara-	H	40 ppm
rha-^{2}xyl (-^{3}ara(*f*))	H	10 ppm
rha-^{2}ara* (-^{3}ara(*f*))	H	2.5 ppm
rha-^{2}ara (-^{3}xyl)	H	40 ppm

SAR

presence of arabinose

absence of glucose

Insecticidal activity



Brown rice planthopper *Nilaparvata lugens*

R_1	R_2
rha-^{2}ara*- $\vert^3$ ara(*f*)	H

200ppm - 100% mortality

Fruits play a prominent role in the human diet in worldwide.

Most of the studies on edible fruits are limited to their nutritional properties.

Fruits play a preventative role in many age-related diseases

- Cancer
- Cardiovascular diseases
- Hypertension
- Skin wrinkling
- Diabet
- Arthritis
- Stroke
- Cataracts

Edible fruits are a potential source to identify environmental friendly bioactive compounds since their safety and toxicological issues are remarkably less than other natural sources.

Fruits play a preventative role in many age-related diseases

Cancer
Cardiovascular diseases
Hypertension
Skin wrinkling

Diabetes
Arthritis
Stroke
Cataracts

A. Carambola

A. altilis

A. nobilis

A. marmelos

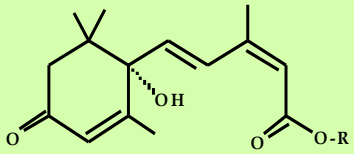
F. Indica

F. inermis

D. glaucescens

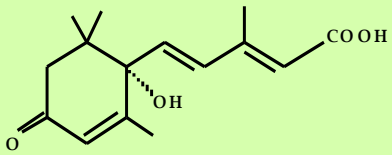
various classes of compounds including some novel natural products.
significant antioxidant, antifungal, cytotoxic, phytotoxic activities

Phytotoxic Constituents of the Fruits of Averrhoa carambola



1 R = H

5 R = glc



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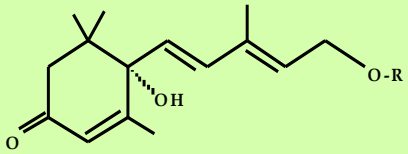
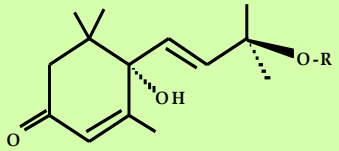


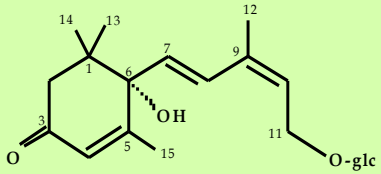
3 R = H

6 R = glc



4 R = H

7 R = glc



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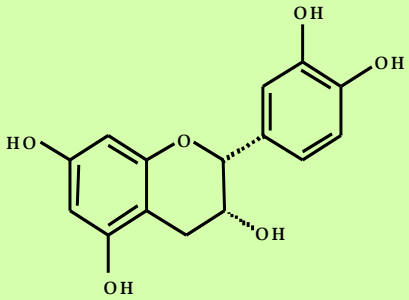


9

Phytotoxicity

Cpd	IC_{50} (µg/ml)
± Abscisic acid	5
3	80
4	10
5	5
6	80
7	-
8	10
9	-

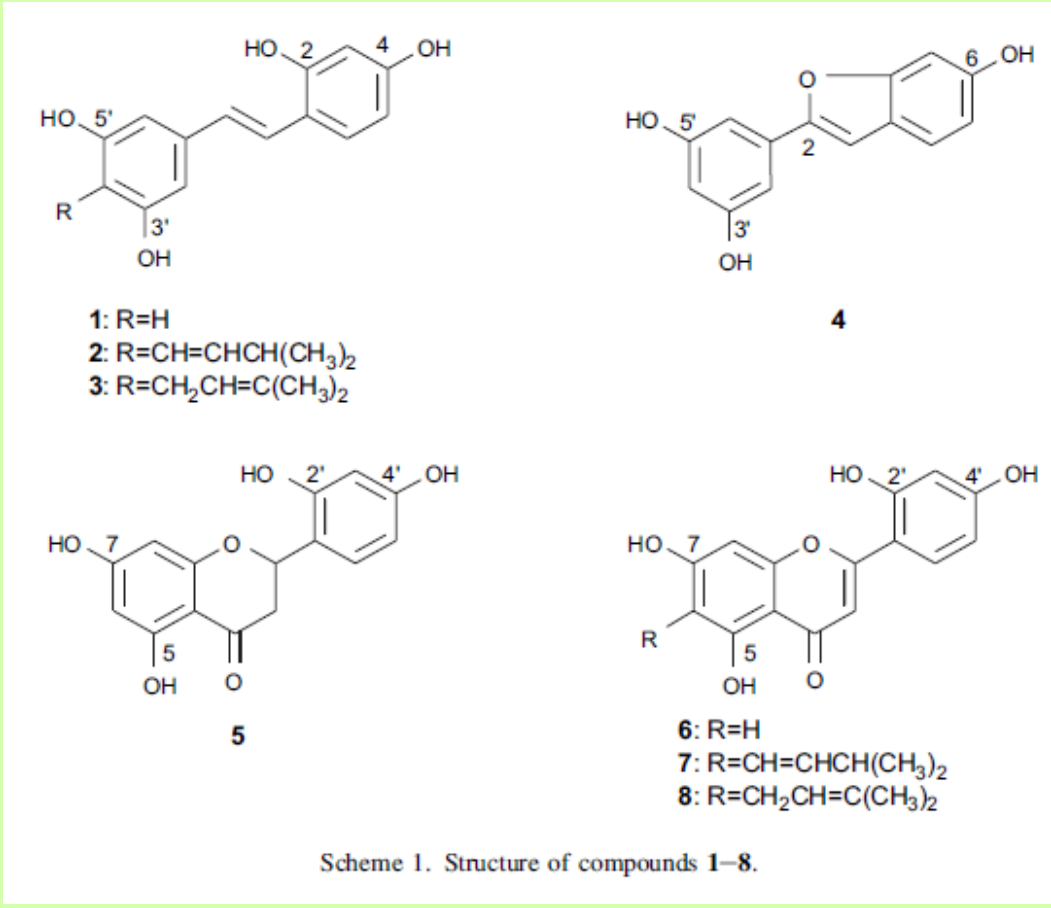
7 , 8 – 1st report

9 - High AO activity

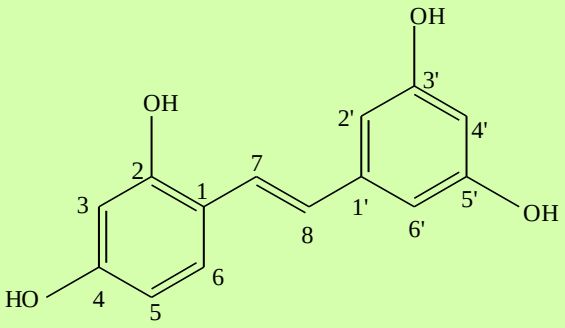
Fruits of *Artocarpus altilis* - Moraceae

Antifungal, antioxidant, phytotoxic, cytotoxic

Taxonomic markers – Moraceae



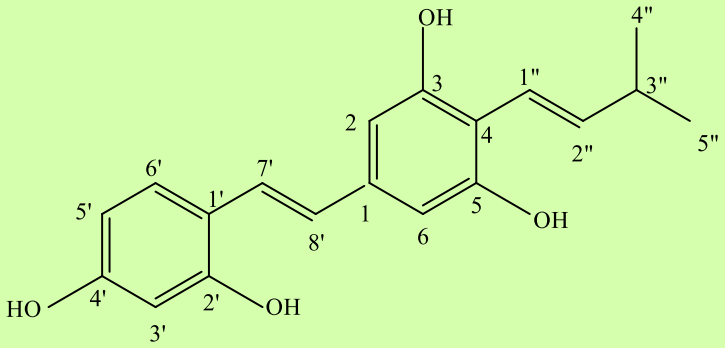
Scheme 1. Structure of compounds **1**–**8**.



(*E*)-2,3′,4,5′-Stilbenetetraol (1)

Antioxidant activity: IC_{50}= 4 ppm

- First report from fruits of *A. altilis*

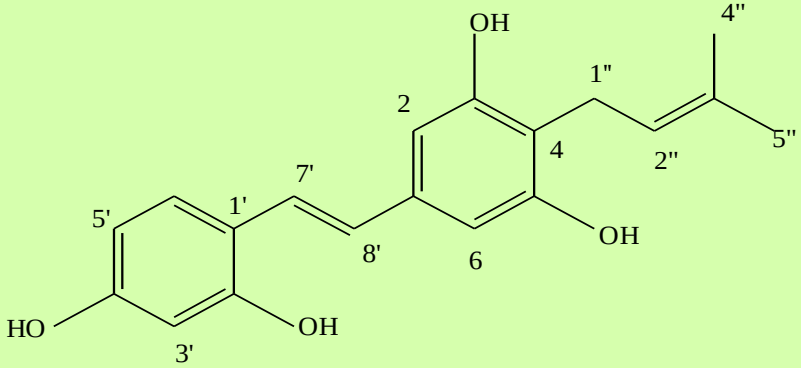


4-[3-Methyl-1-(*E*)-butenyl]-(*E*)- 2′,3,4′,5-stilbenetetraol (2)

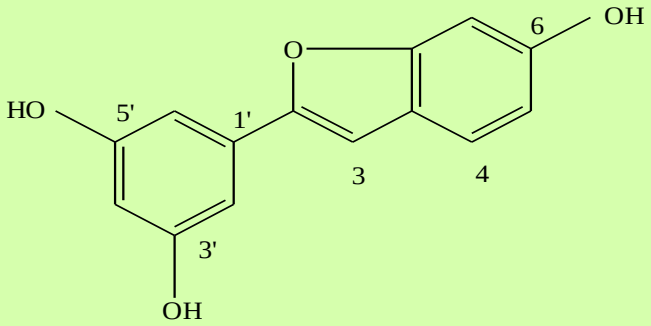
Antifungal activity

Antioxidant activity: IC_{50}= 10 ppm

First report from *A. altilis*



(3-Methyl-2-butenyl)-(*E*)-2′,3,4′,5- stilbenetetrol (3)



3′,5′,6-Trihydroxy-2-phenylbenzofuran (4)

- Antifungal activity
- Antioxidant activity: IC_{50}= 4 ppm
- Phytotoxicity
- Cytotoxicity: LC_{50}= 55 ppm
- First report from fruits of *A. altilis*

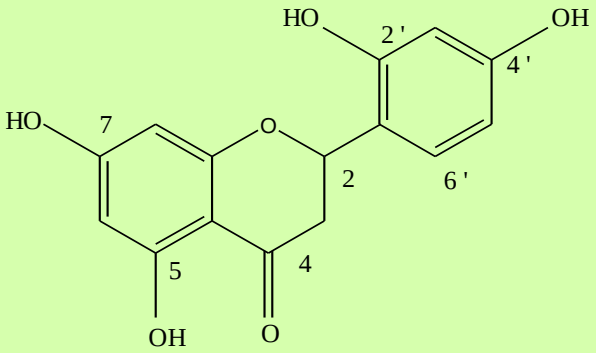
Highest antifungal activity against 5 fungal cultures

Antioxidant activity: IC_{50}= 2 ppm

100% inhibition growth & germination of lettuce seeds

Cytotoxicity: LC50= 20 ppm

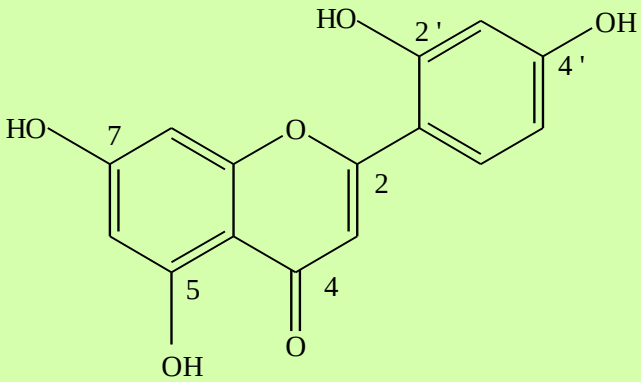
First report from *A. altilis*



2’,4’,5,7-Tetrahydroxylflavanone (5)

Cytotoxicity: LC_{50}= 80 ppm

First report from fruits of *A. altilis*

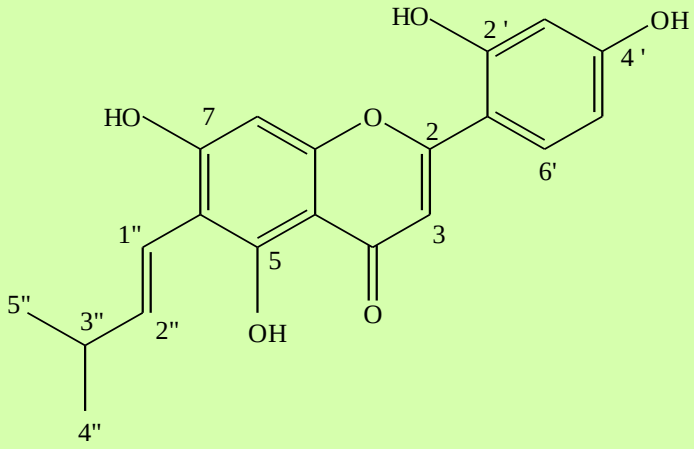


2’,4’,5,7-Tetrahydroxyflavone (6)

Root growth promoting activity

Cytotoxicity: LC_{50}= 325 ppm

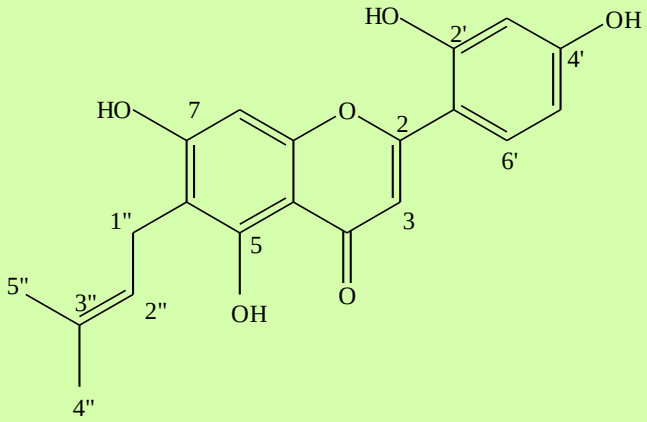
First report from of *A. altilis*



2’,4’,5,7-Tetrahydroxy-6-[3-methyl-1-(*E*)-butenyl]flavone (7)

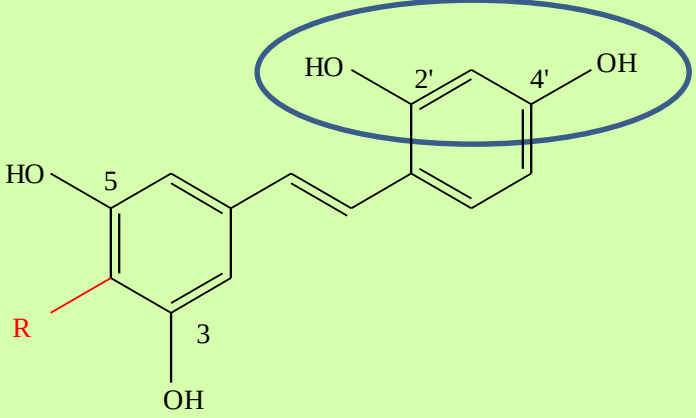
Moderate antifungal against *Alternaria* sp.,
C. cladosporioides, *Fusarium* sp. *Rhizoctonia* sp.

First report from fruits of *A. altilis*

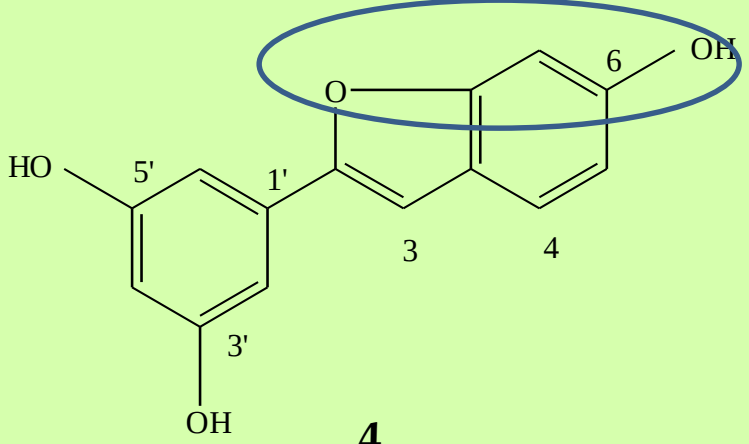


2’,4’,5,7-Tetrahydroxy-6-(3-methyl-2-butenyl)flavone (8)

First report from fruits of *A. altilis*



1. R= H
2. R= CH=CHCH$(CH_3)_2$
3. R= $CH_2CH=C(CH_3)_2$



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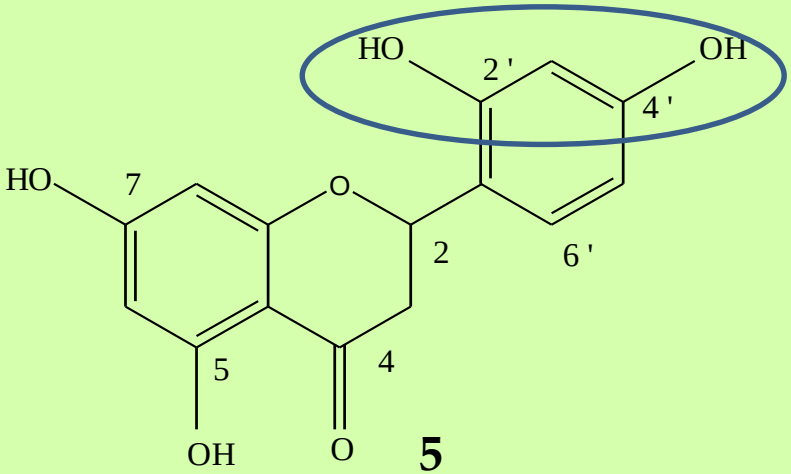

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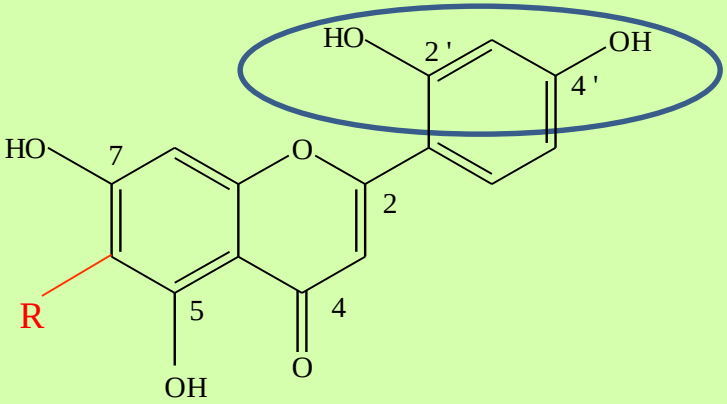


6. R= H
7. R= CH=CHCH$(CH_3)_2$
8. R= $CH_2CH=C(CH_3)_2$

Fruits of *Artocarpus altilis* contains characteristic phenols with -2,4 dioxygenation pattern and prenyl substitution

Compounds 1-8 → taxonomic markers for genus *Artocarpus*

Geranylated phenolic constituents from the fruits of *Artocarpus nobilis*

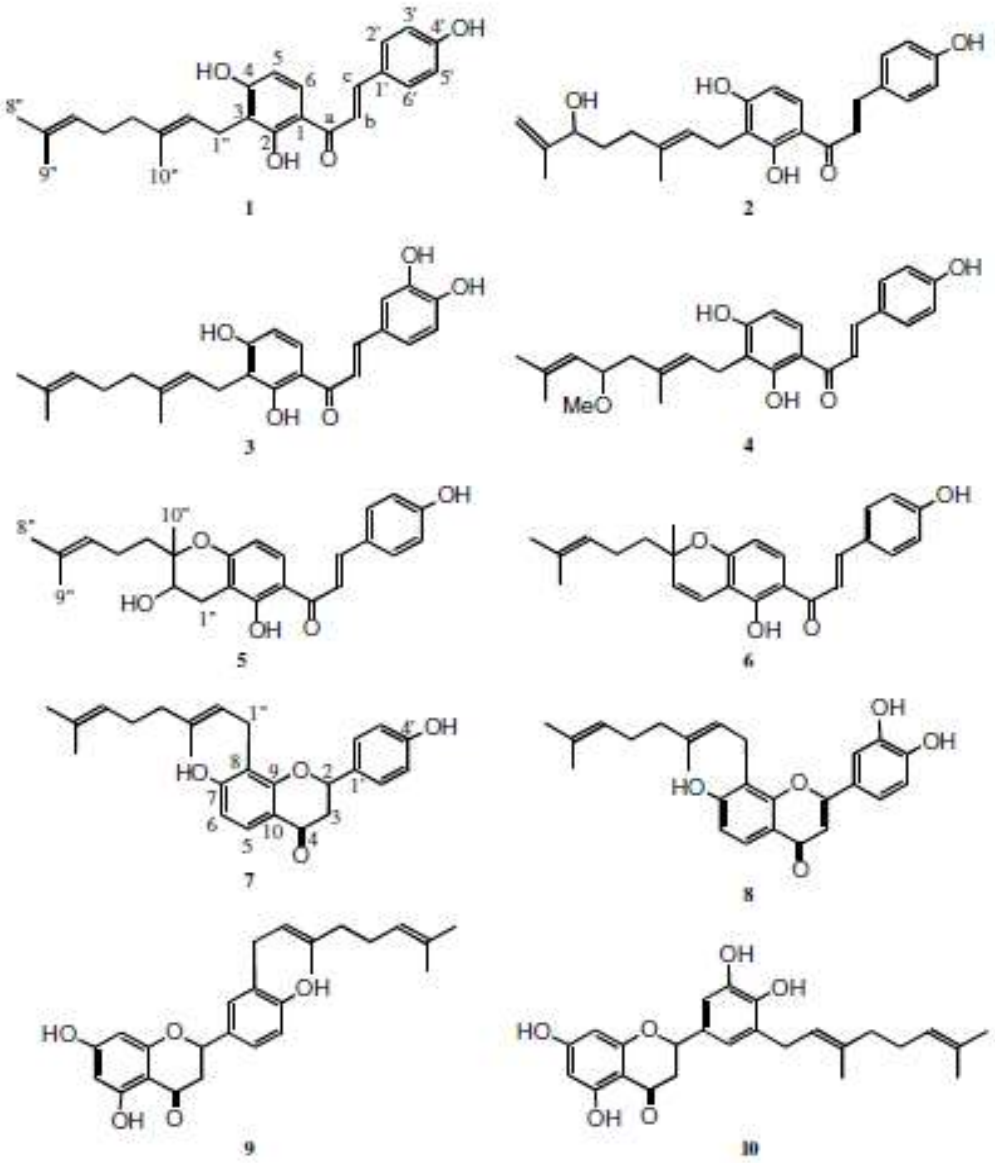


Fig. 1. Structures of compounds 1–10.

Four new geranylated phenolic metabolites – **4**, **5**, **8**, **9**

Antioxidant activity

All these compounds showed (**+**) response for **TLC bioautography** (1μg/spot)

- **3**, **8**, **10** showed (**+++**) response even at 0.1μg/spot

Spectrophotometry method IC_{50} – **3** (5 μg), **8** (6.3 μg), **10** (4.4 μg),

α-tocopherol (13.8 μg)

Antifungal constituents of the stem bark of *Bridelia retusa*

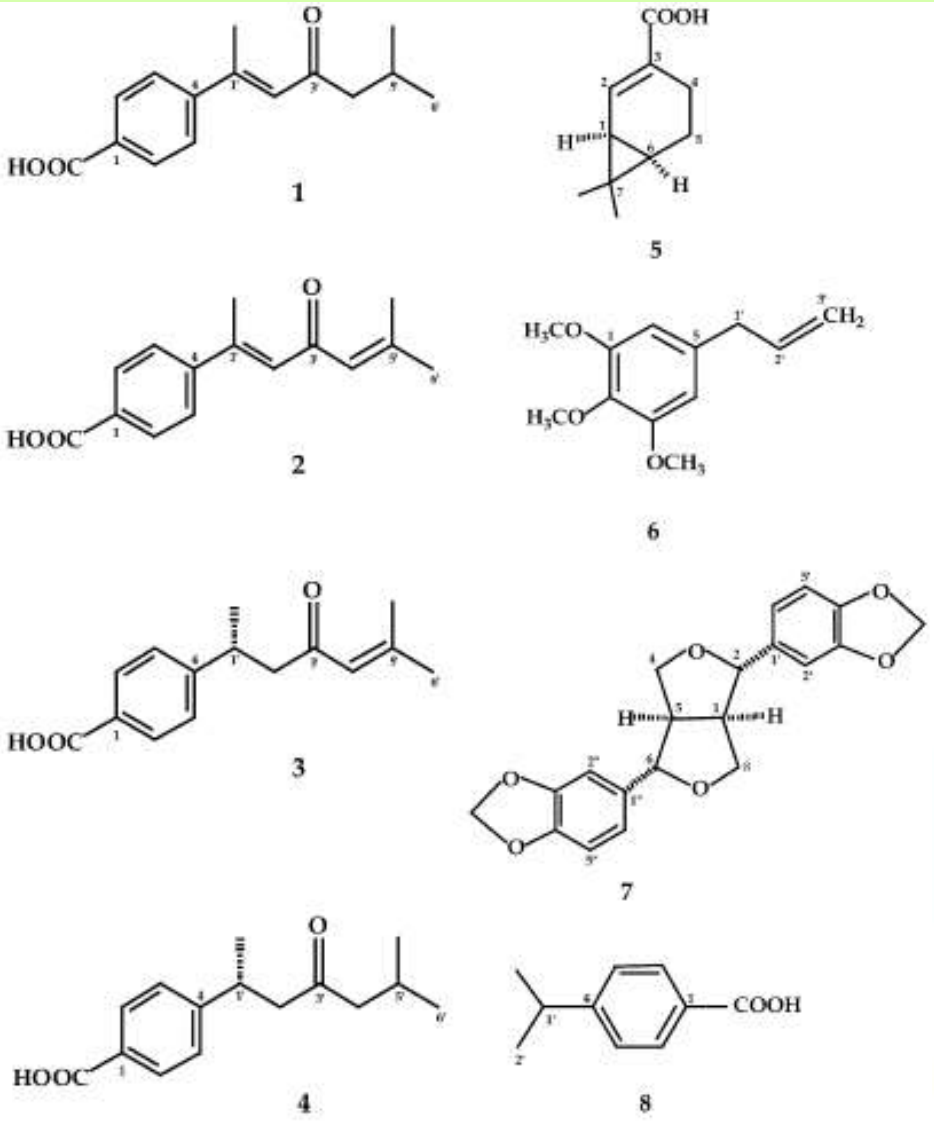


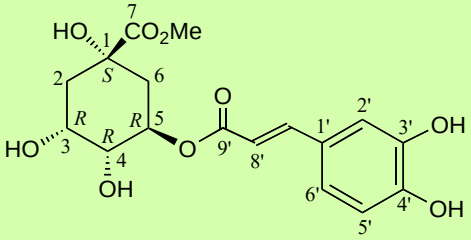
Fig. 1. Compounds **1–8**.

Table 1
Fungitoxic activity of the compound **1–8**

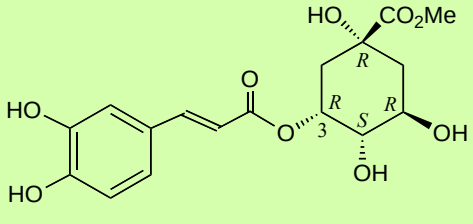
Compound no.	Minimum amount for activity (μg)
1	50
2	25
3	5
4	25
5	10
6	No activity
7	25
8	10

The table shows the minimum amount of compounds needed to inhibit the growth of *Cladosporium cladosporioides* on TLC plate.

Fruits of *Flacourtia inermis*



1



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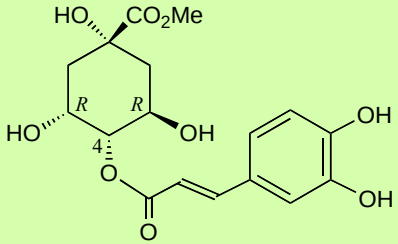

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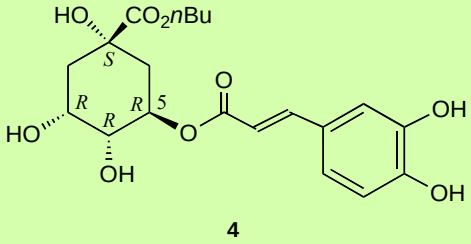

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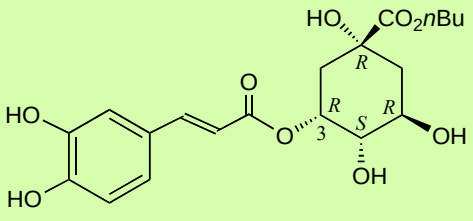

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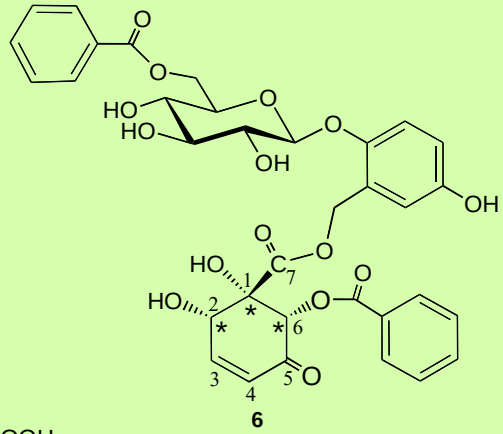

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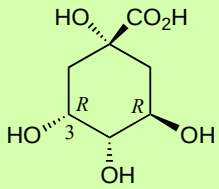

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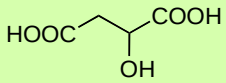


8

Fruits are edible & very popular
No previous chemical investigations

a rare phenolic glucoside, three isomers of chlorogenic acid methyl ester, two isomers of chlorogenic acid *n*-butyl ester, quinic acid, malic acid.

Antioxidant activity (μg/ml)

1 - 5.1		ascorbic acid	- 3.1
2 - 3.1		BHA	- 3.5
3 - 25			
4 - 4			
5 - 4.4			
6 - 12			
7 - >125			
8 - >125			

Why fungal metabolites?

1. Some landmark medicines produced by microorganisms

   Antibacterial: Erythromycin, Vancomycin, Penicillin;

   Antifungals: Amphotericin B,

   Anticancer: Doxorubicin, Mytomycin C, Taxol;

   Cholesterol lowering statins: Lovastatin

   Immunosuppressant: Rapamycin

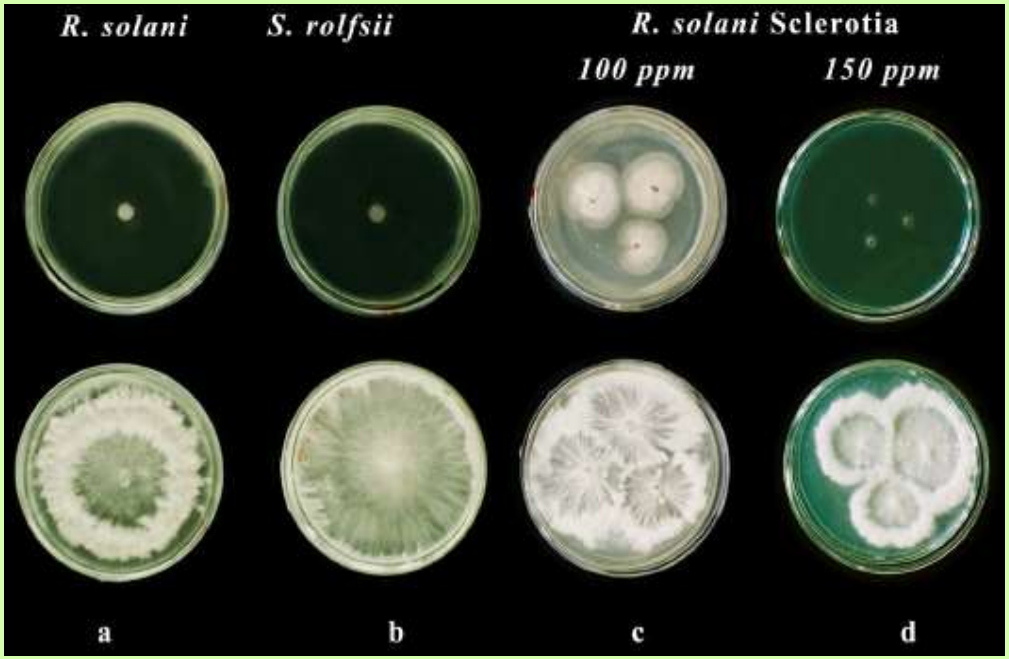
2. A very little of research work on Endophytes in Sri Lankan Plants

   Camptothecin, Huperzine A, Podophyllotoxin, Taxol, Vinblastine Vincristine

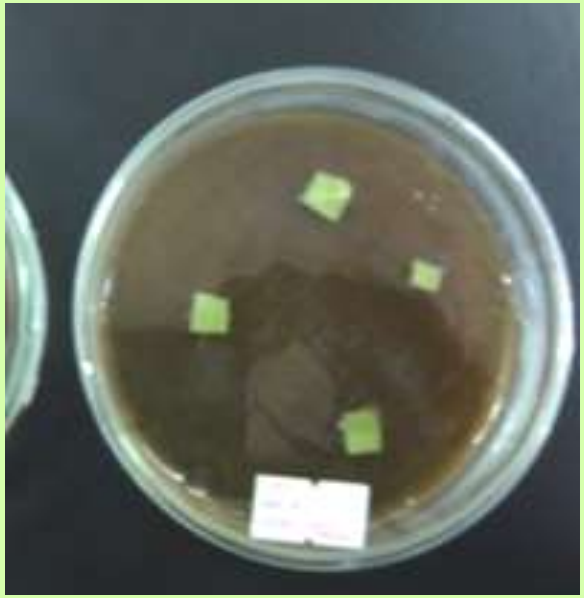
3. A very little of research work on Endophytes in Sri Lankan Plants

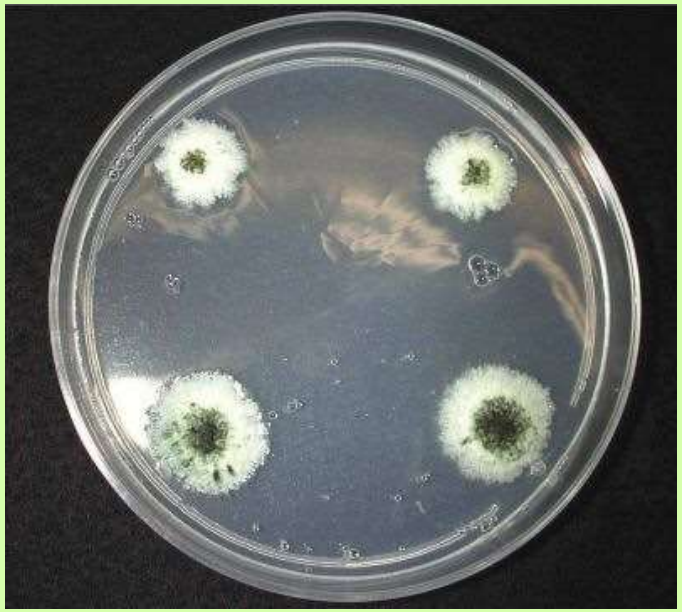
Fungal Metabolites - Isolation and Bioactivity

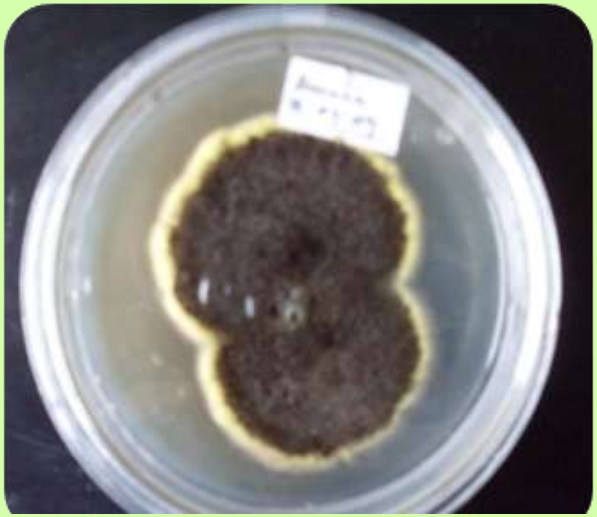
- Epiphytic fungi, Endophytic fungi



Isolation of endophytic fungi







Host plants & their endophytic fungi identified by molecular means

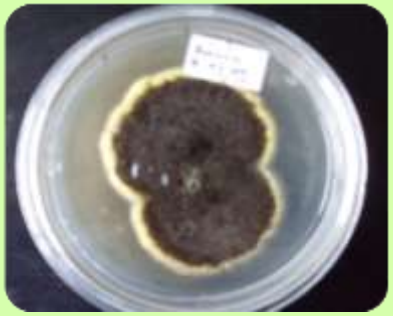
Host Plant	Endophytic fungi
Artocarpus altilis (Del)	*Phoma macrostoma*
Camellia sinensis (Tea)	*Pestalotiopsis camelliae, Phoma multirostrata Fusarium ambrosium, Guignardia mangiferae*
Coccinia grandis (Kowakka)	*Nigrospora oryzae*
Costus speciosus (Thebu)	*Bipolaris sorokiniana*
Garcinia mangostana (Mangostin)	*Penicillium citrinum*
Manilkara zapota (Sapodilla)	*Pestalotiopsis microspora*
Manihot esculanta (Casava)	*Sarocladium hominis*
Musa sp. (Banana)	*Aspergillus awamori*
Passiflora edulis (Passion fruit)	*Phialemonium curvatum, Nigrospora oryzae*
Piper betel (Bulath)	*Colletotrichum capsici, Glomerella magna*
Piper nigrum (Gammiris)	*Xylaria berteri, Colletotrichum siamense*
Phyllanthus acidus (Nelli)	*Daldinia eschscholtzii*
Pouteria campechiana (Lavulu)	*Talaromyces purpurogenus*
Syzygium samarangense (Jambu)	*Pestalotiopsis mangifeae*

Fungi pure cultures grow in different medium

Solid
(Rice/Potato)

Liquid
(Potato Dextrose Broth- PDB)

Semi solid
(Potato Dextrose Agar – PDA

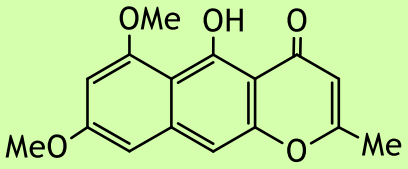


1. Extraction
2. Bioassays
3. Chemical investigation

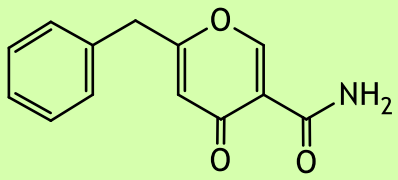
Endophytes – Edible fruits

Polyketides from an endophyte *Fusarium chlamydosporum* from *Carica papaya*

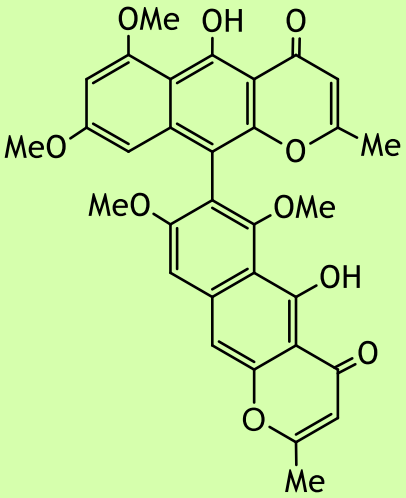
	AO	AF	Phytotoxicity	Brine shrimp	α-amylase
TCP extract	+++	+++	+++	++	+



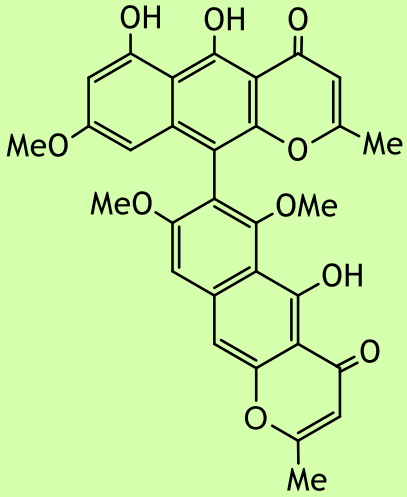
Rubrafusarin B (1)



Carbonarone A (2)

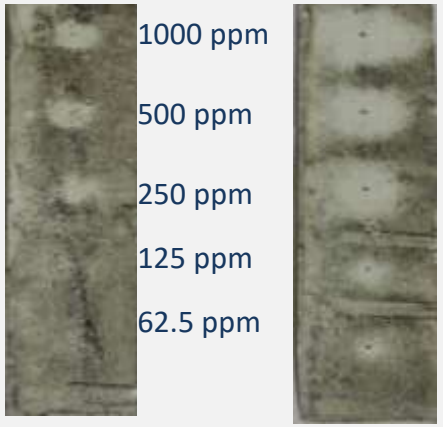


Aurasperone A (3)



Aurasperone D (4)

Antifungal activity of 2



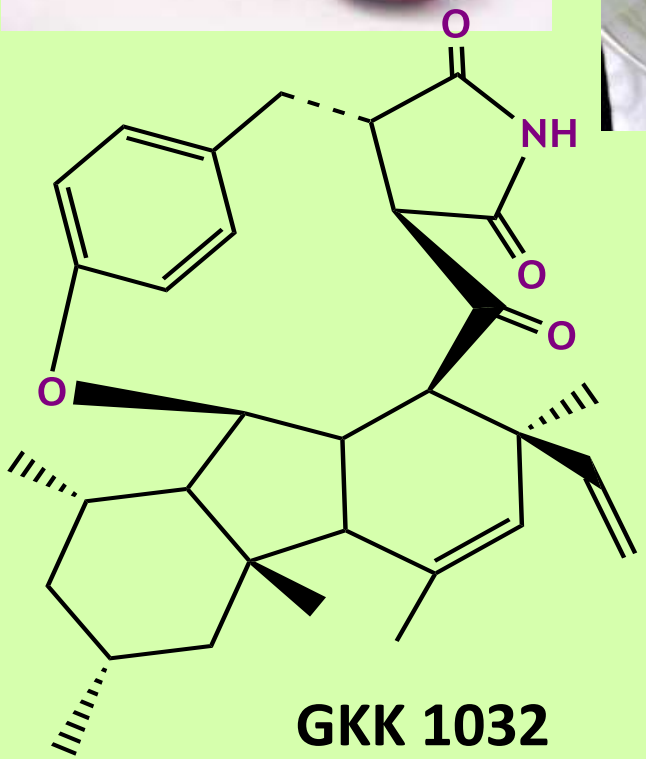
1, **3**, **4** - Moderate Antioxidant, BS toxic, Phytotoxicity (shoot inhib.)

2 - BS toxicity (IC_{50} - 97 ppm),
Phytotoxicity activity {IC_{50} – 31ppm, 37ppm (shoot and inhibition)}
Antifungal activity at 62.5 ppm/spot

Antitumor antibiotics GKK1032B production by *Penicillium citrinum*, an endophytic fungus isolated from *Garcinia mangostana* fruits

Crude extracts

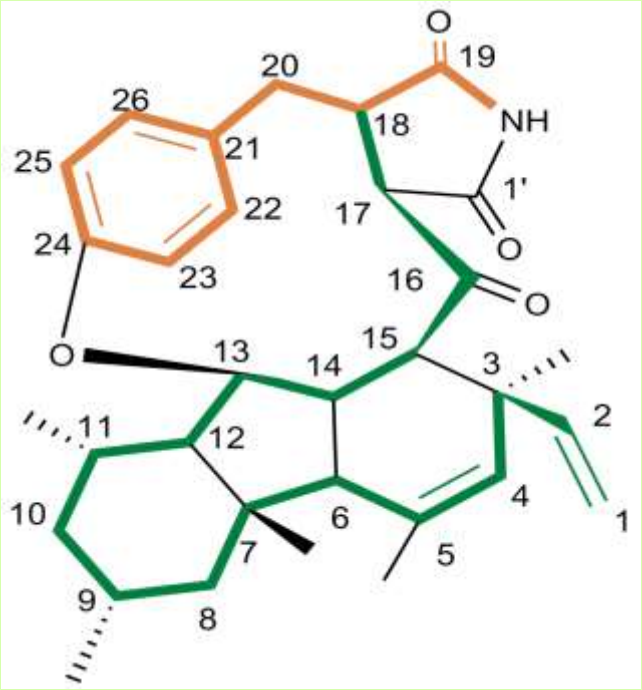
Antioxidant	+++
α-amylase	+++
Brine shrimp	+++



GKK 1032

Peptide-polyketide hybrid compound

GKK1032B

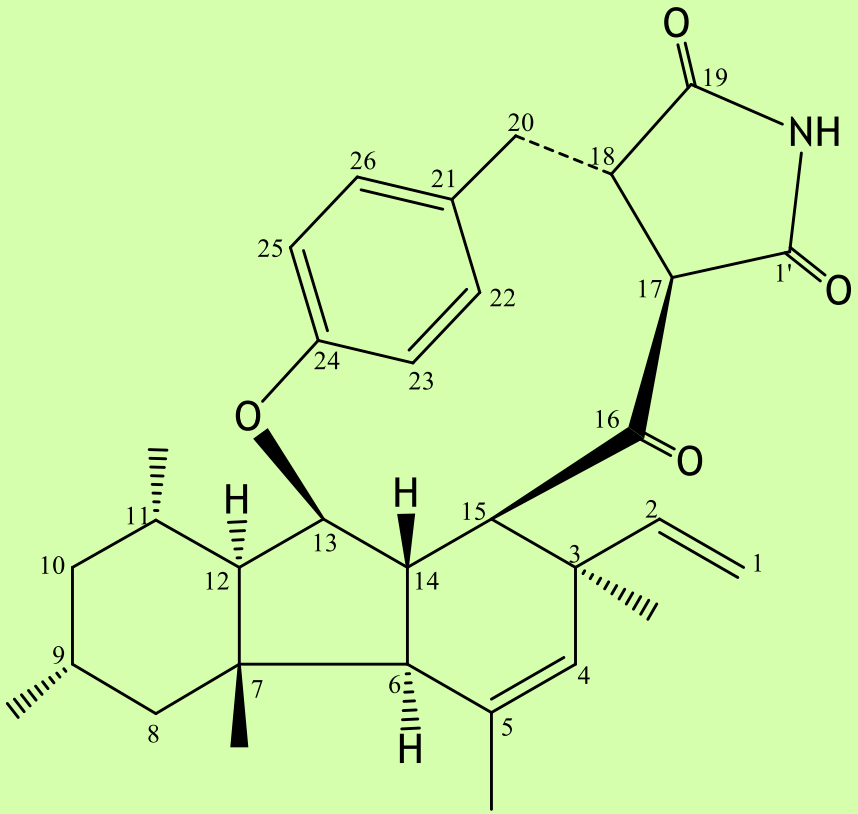


- Anticancer, antitumor, cytotoxic, antibiotic activities
- Peptide polyketide hybrid compound
- Unique features of GKK1032 family;
  - ether containing macrocyclic ring
  - *P*-substituted phenyl group
  - Succinimide moieties

•Biosynthetic pathway was studied by Oikawa in 2003.

•Originated by mixed alkaloid, amino acid-polyketide pathway

- amino acid – L-tyrosine
- Backbone of the compound is from reduced polyketide pathway
- 5, Me groups are from L-methionine



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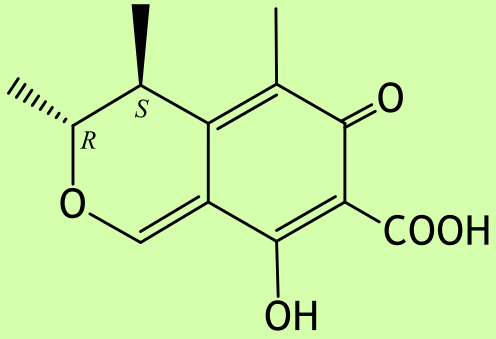


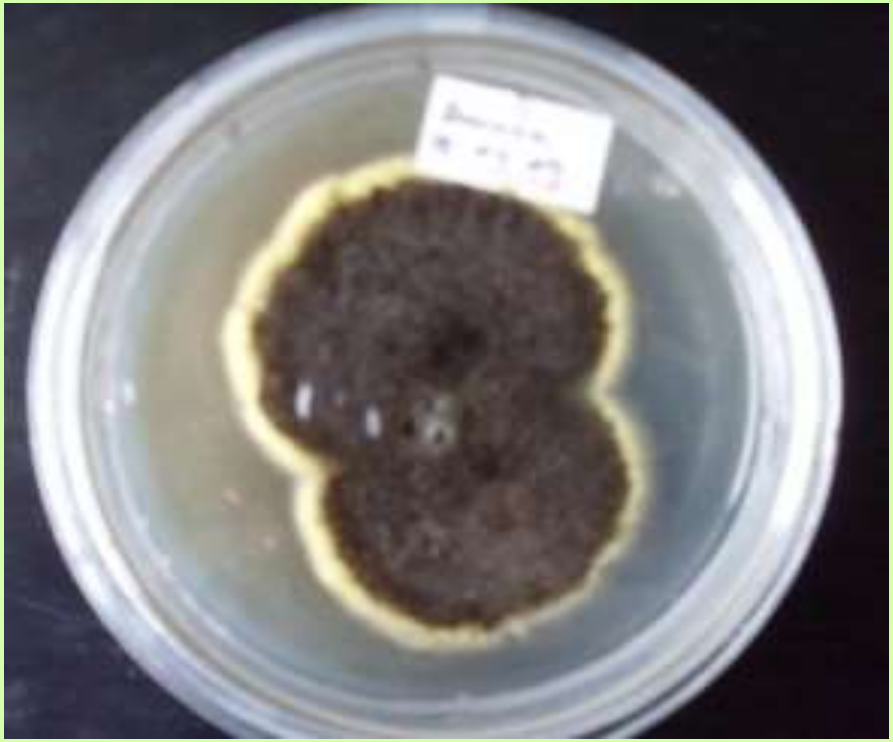
2

Highlights :

High yield of citrinin 2.3g/8 L

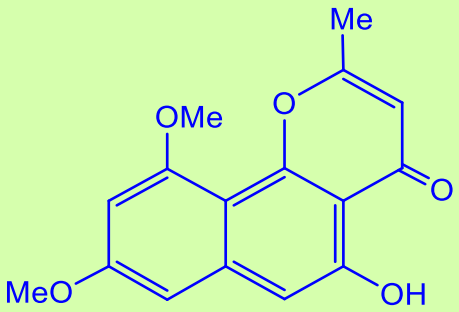
This study established that *P. citrinum* is the producer of GKK1032B for the first time.

Polyketides and a Cephem derivative from *Aspergillus awamori* associated with *Musa* sp.



B.S toxic, Phytotoxic, Antifungal

7 - Compounds



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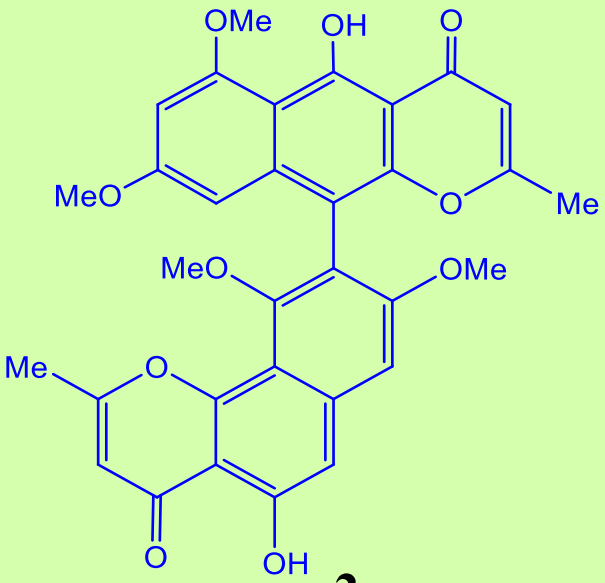

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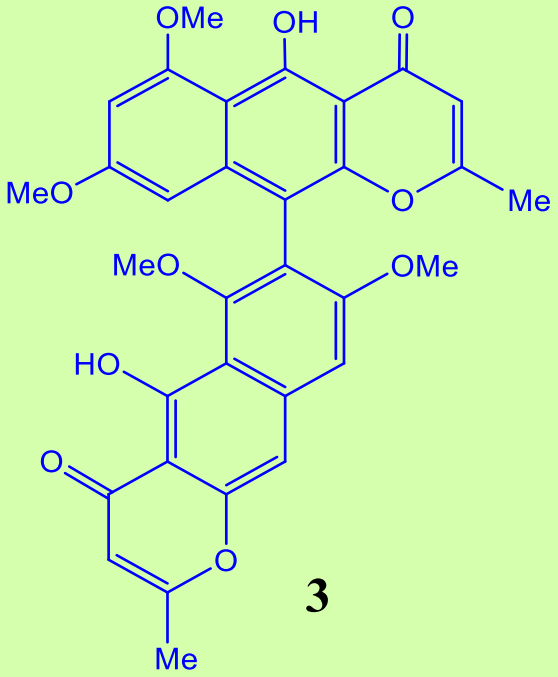

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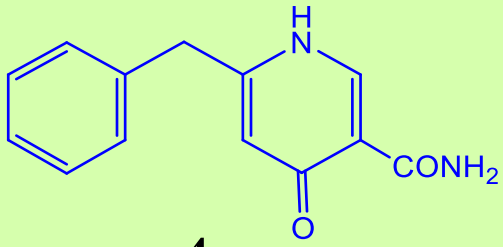

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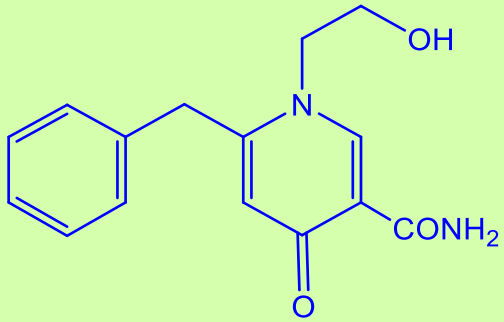

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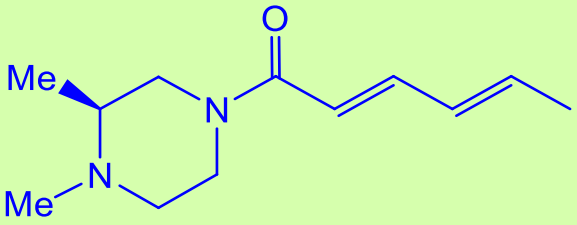
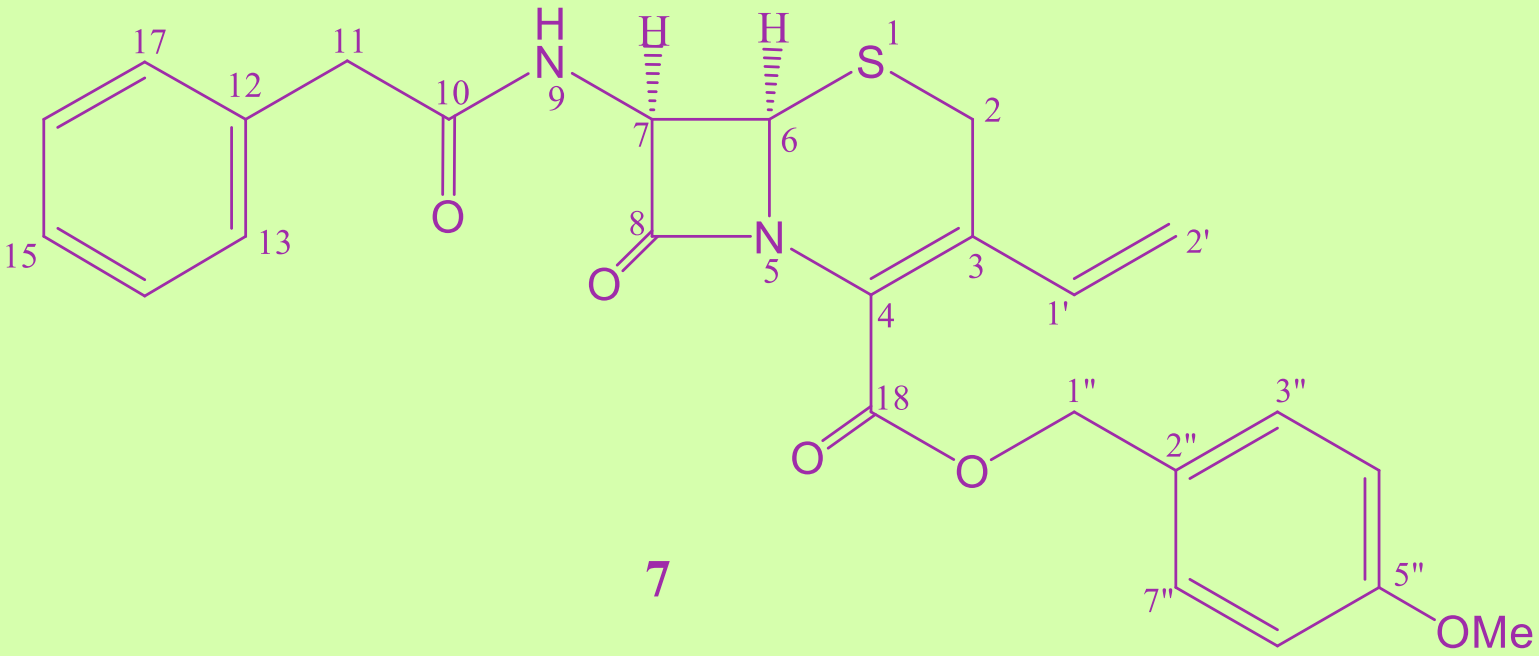


6

1 Flavasperone
2 Aurasperone A
3 Fonsecinone A
4 Aspernigrin A
5 Pestelamide C
6 Nigragillin

Only aurasperone A (**2**) and foncesinone A (**3**) - highly toxic to B.S.
pestalamide C (**5**) was moderately toxic.



7

A 3-vinyl cephem derivative, useful intermediate in the synthesis of cephem antibiotics, from *Aspergillus awamori* associated with banana fruit,

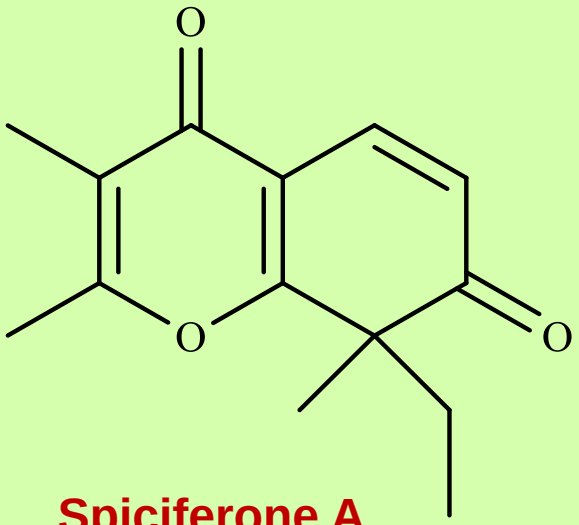
Natural Product Communications, 10, 1663-1666. (2015)

Spiciferone A, from *Phoma macrostoma* from *Artocarpus altilis* fruits

A. altils fruit

Top view

Bottom view



Spiciferone A

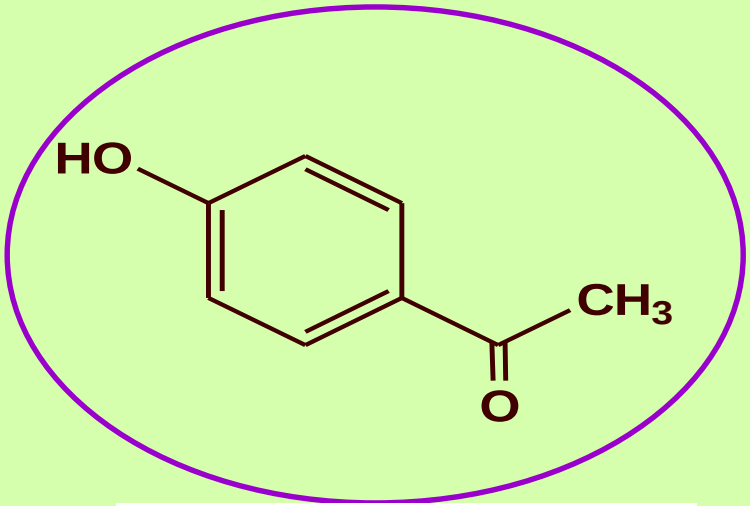
Weak antioxidant, α-amylase and Brine shrimp toxicity

Phoma macrostoma first time *Artocarpus altilis;* spiciferone A first time from *Phoma macrostoma*

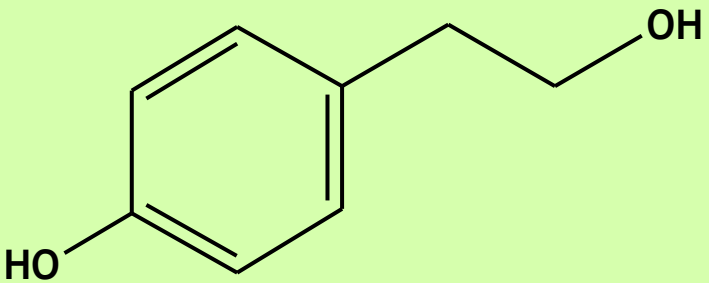
Bioactive endophytic fungi *Talaromyces purpurogenus* from the seeds of *Pouteria campechiana*

Penicillium purpurogenum

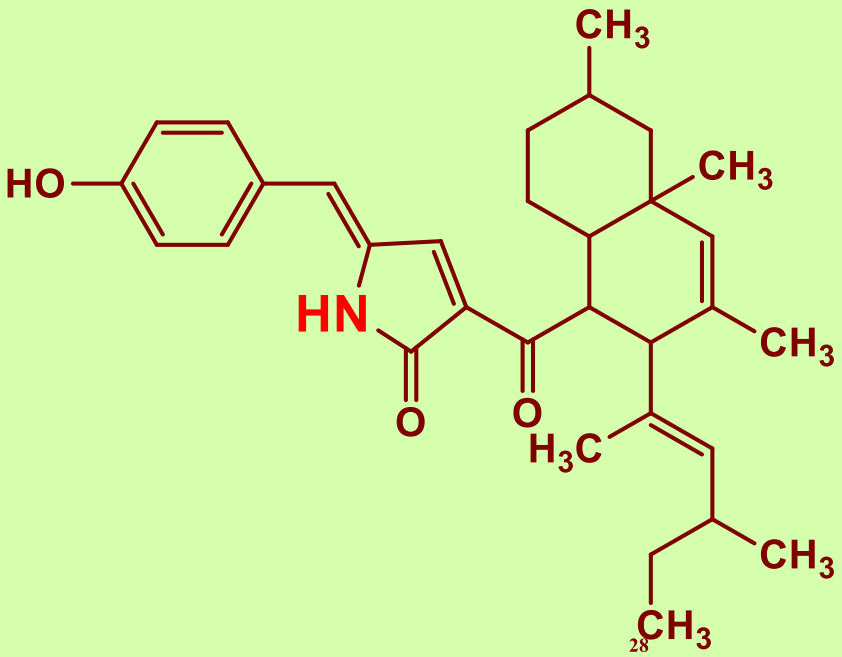
- Antioxidant
- Phytotoxic
- Toxic to brine shrimps

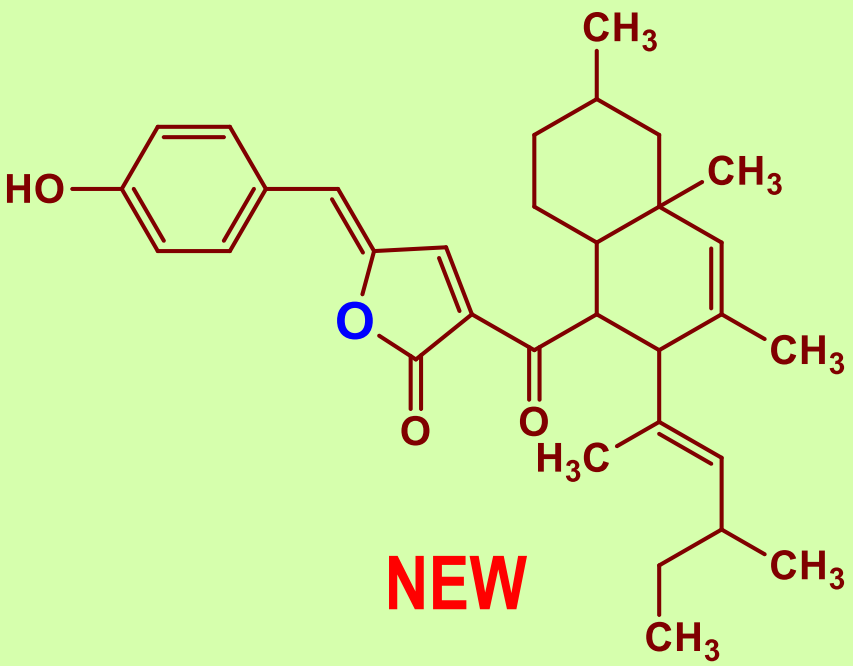


4-hydroxyacetophenone



Tyrosol





GPSF–2: Taloroconvolutin A

Phytotoxic – IC_{50} ~40 ppm

BS toxic – LD_{50} 3.5ppm

GPSF-7: HRMS at *m/z* 489.3007 (calcd for $C_{32}H_{41}O_4$ – 489.3005)

Taloroconvolutin A - *Talaromyces convolutes*

Hydroxyl derivative - *Penicillium rubrum*

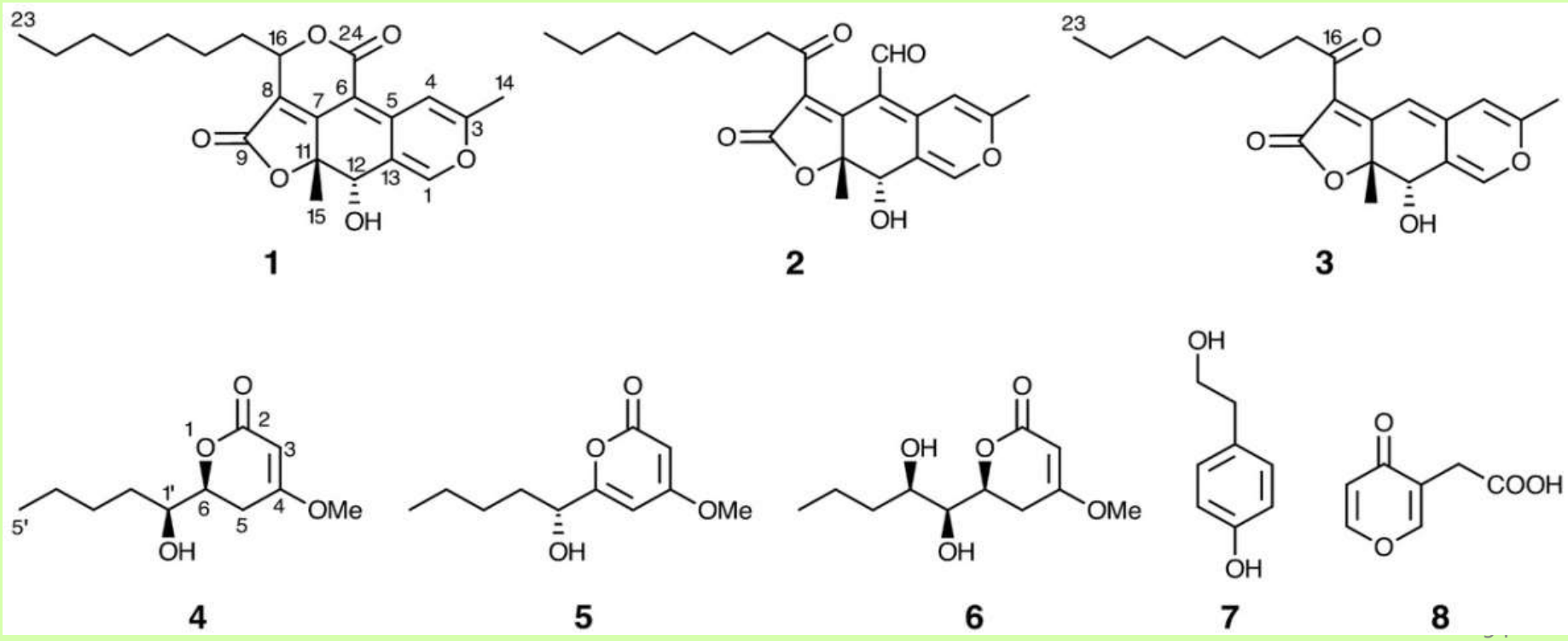
Pestalotiopsis microspore from the fruits of *Manilkara zapota*

Azaphilonoid

Pyran-2-one metabolites

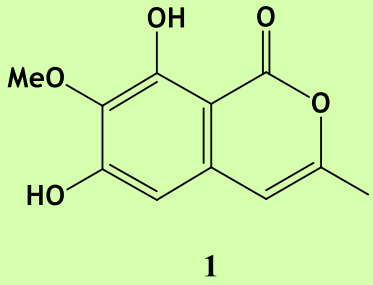
Tyrosol

Xylaric acid



Antifungal dihydroisocoumarins from *Biscogniauxia capnodes* from *Averrhoa carambola*

Crude extracts - Good antioxidant,
Phytotoxic activity
Moderate antifungal *C. cladosporioides*
Low B.S toxicity



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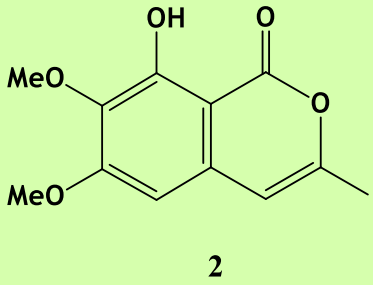
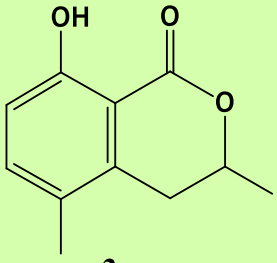


2

Two isocouamrins

reticulol (**1**),
6-*O*-methyl-reticulol (**2**)



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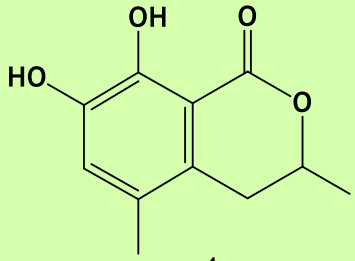


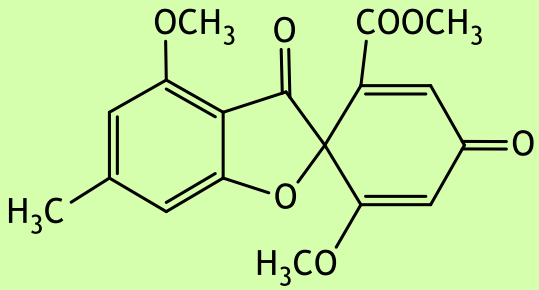
4

Two dihydroisocoumarins

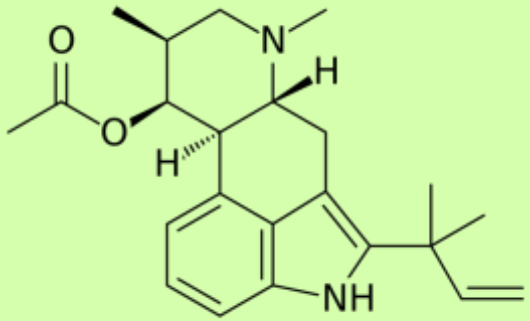
5-methylmellein (**3**),
7-hydroxy-5-methylmellein (**4**)
Antifungal activity

Alkaloids from *Aspergillus fumigatus* from *Solanum insanum*

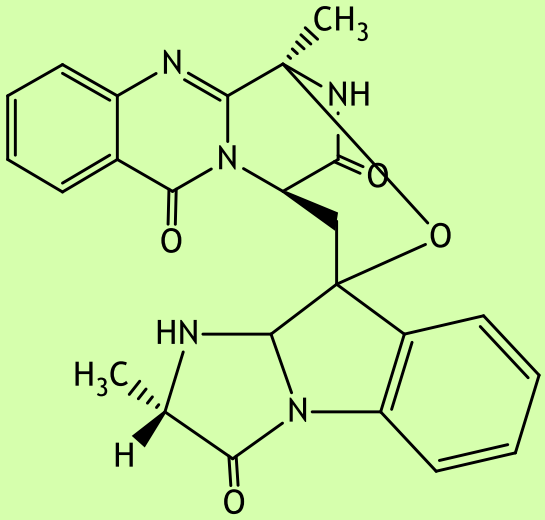
Solanum insanum



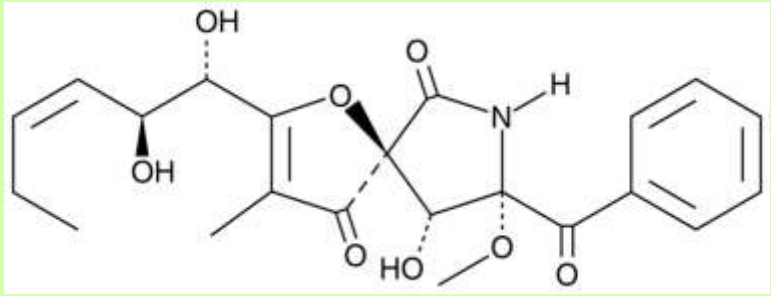
Trypacidine (1)



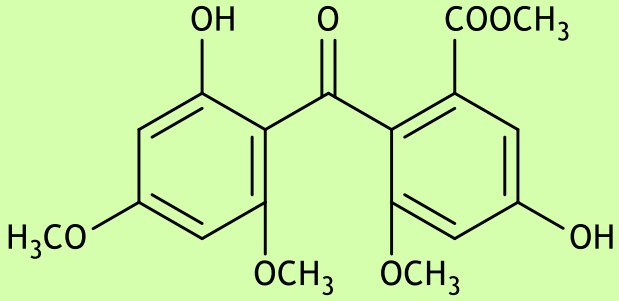
Fumigaclavine C (2)
Ergoline alkaloid



Fumiquinazoline C (3)



Pseurotin A (5)

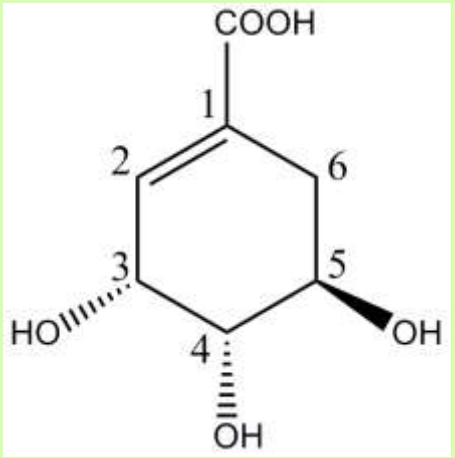


Monomethylsulochrin (4)

Antifungal - **2**, **3**
B.S. Toxic - **2**, **4**

Shikimic Acid Production by *Fusarium decemcellulare*, An Endophytic Fungus Isolated from *Flacourtia inermis* Fruits

Flacourtia inermis

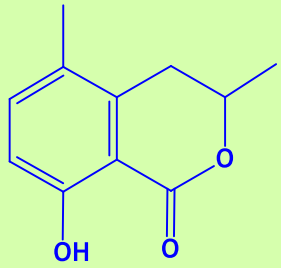


Shikimic acid

•SA is responsible in the biosynthesis of aromatic amino acids (L-phenylalanine, L-tyrosine, L-tryptophan), lignin, flavanoids, tannins, folic acid, vitamins and most of the alkaloids present in plants and microorganisms.

A series of isocoumarins from an endophyte

Biscogniauxia capnodes from *Phyllanthus acidus*



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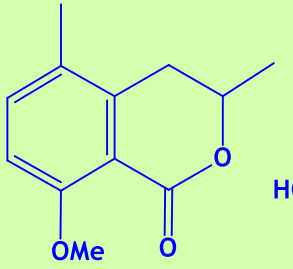

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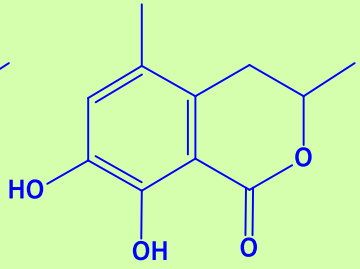

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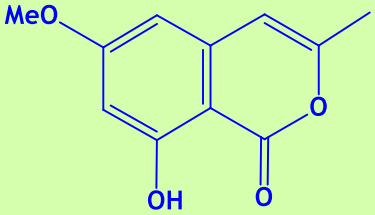

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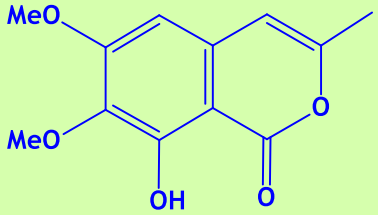

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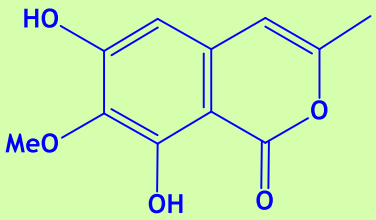

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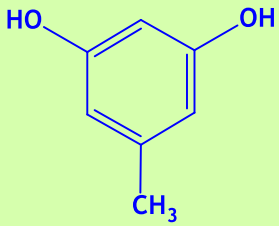


7

8-hydroxy-3,5-dimethylisochroman-1-one (**1**)

8-methoxy-3,5-dimethylisochroman-1-one (**2**)

7,8-dihydroxy-3,5-dimethylisochroman-1-one (**3**)

8-hydroxy-6-methoxy-3-methyl-1*H*-isochromen-1-one (**4**)

8-hydroxy-6,7-dimethoxy-3-methyl-1*H*-isochromen-1-one (**5**)

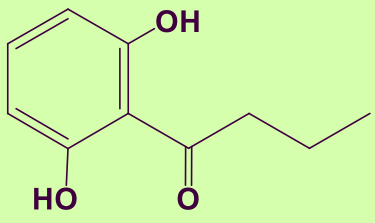
6,8-dihydroxy-7-methoxy-3-methyl-1*H*-isochromen-1-one (**6**)

5-methylbenzene-1,3-diol (**7**)

1 2 3 4 5 6 7

Phenolic metabolites from *Daldinia eschscholtzii* from the fruits of *Phyllanthus acidus*

Daldinia eschscholtzii



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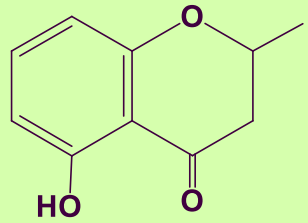

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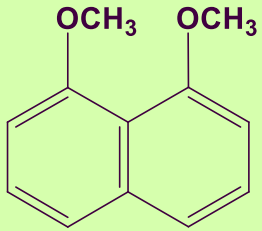

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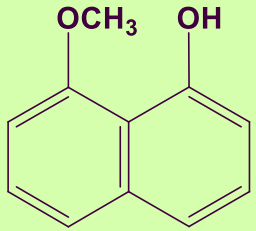


4

Extracts –

Antioxidant (DPPH) &

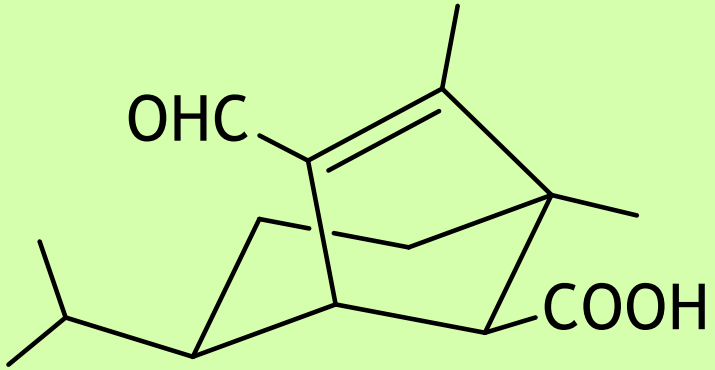
Brine shrimp lethality

Endophytes – Medicinal Plants / Herbs

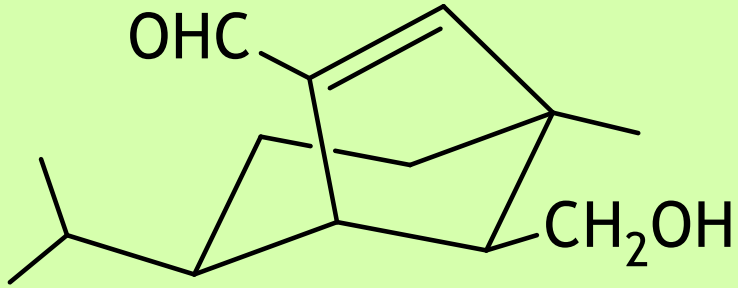
Two rare Sesquiterpenoids from an endophyte *Bipolaris sorokiniana* from the leaves of ***Costus speciosus***

Antidiabetic properties

	Antifungal	Antioxidant	Phytotoxic	Brine shrimp	α- amylase
EtOAc extract	+++	+++	+++	+++	+
MeOH extract	+++	++	++	++	+



Helminthosporal acid



Helminthosporol

Both Compounds: Antifungal *C. cladosporioides* **+++**
BS Toxic

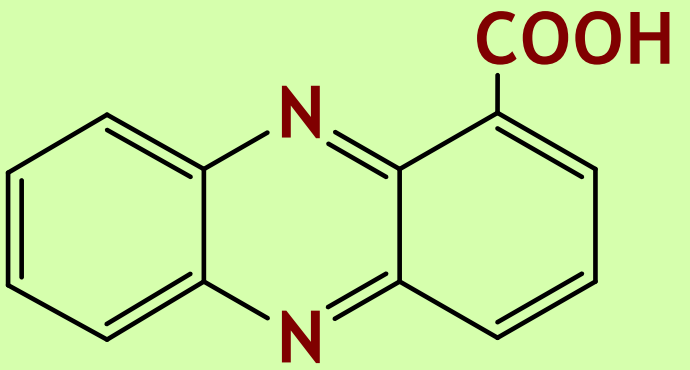
Helminthosporol have been reported from the same fungus which shows pathogenic effects on cereals and grasses.

This is the first report on isolation of *B. sorokiana* from *C. speciosus* and production of a rare sesquiterpene helminthosporal acid from *B. sorokiniana.*

Phenazine derivatives from an endohytic fungus *Nigrospora oryzae* from the leaves of *Coccinia grandis* (Kowakka)

Antidiabetic properties

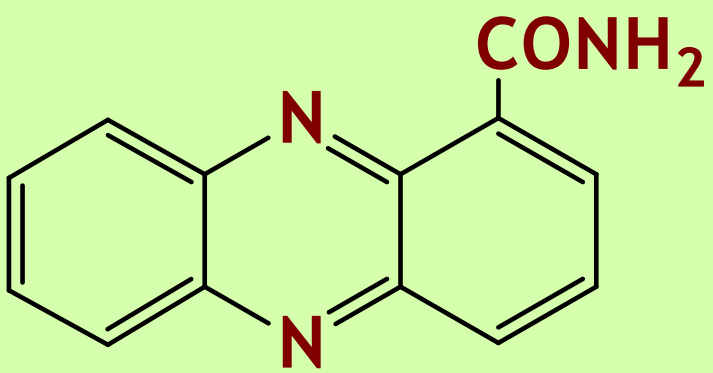
	Antifungal	Antioxidant	Phytotoxic	Brine shrimp	α- amylase
EtOAc extract	+++	++	+++	+++	+
MeOH extract	+++	+	++	+	+



1

Phenazine-1-carboxlic acid

Phytotoxicity IC_{50} 121 ppm



High Yield (1.0 g/4 L)

2

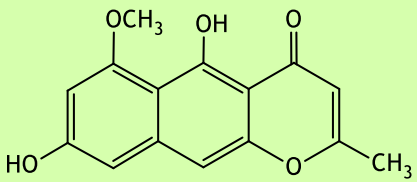
Phenazine-1-carboxamide

Antifungal **+++** (4 μg/spot)
B.S. toxic **+++** (IC_{50} 98 ppm)
Phytotoxicity (IC_{50} 99 ppm)

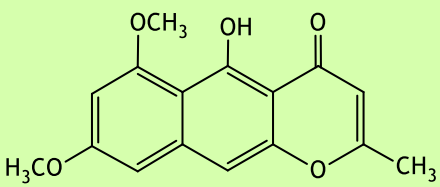
Highlights

- Endophytic fungus *Nigrospora oryzae* from *Coccinia grandis.*
- First report of the isolation of phenazines from a fungal source. Thus far, bacteria have been the only known source of natural phenazines

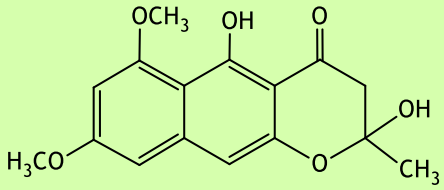
Phytotoxic Polyketides from an endohyti fungi isolated from the leaves of *Becella alba* (Nivithi)



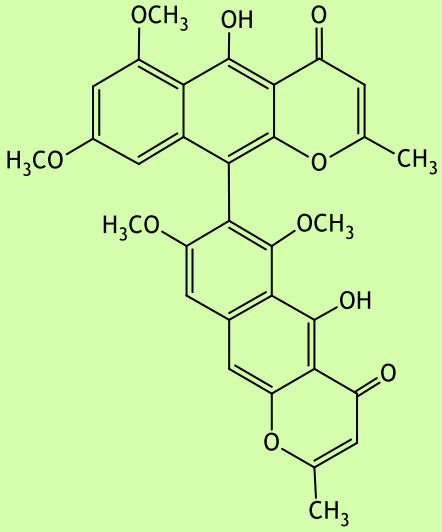
TMC 256A1(**1**)



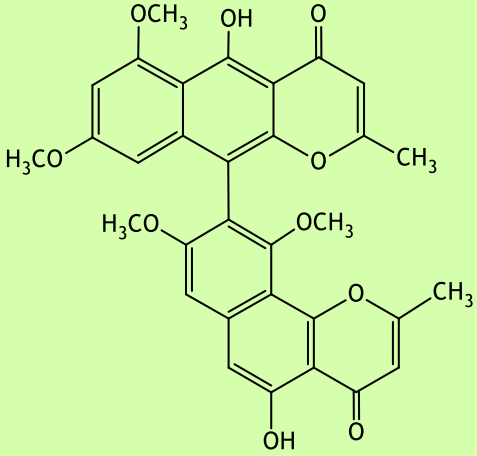
Rubrofusarin B (**2**)



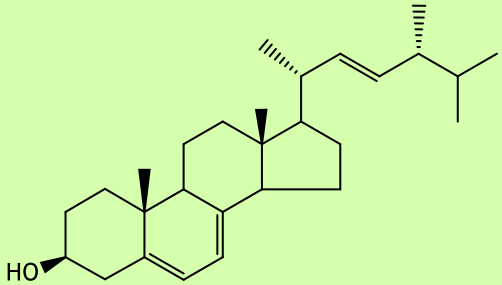
Foncesin B (**3**)



Aurasperone A (**4**)



Fonsecinone A (**5**)



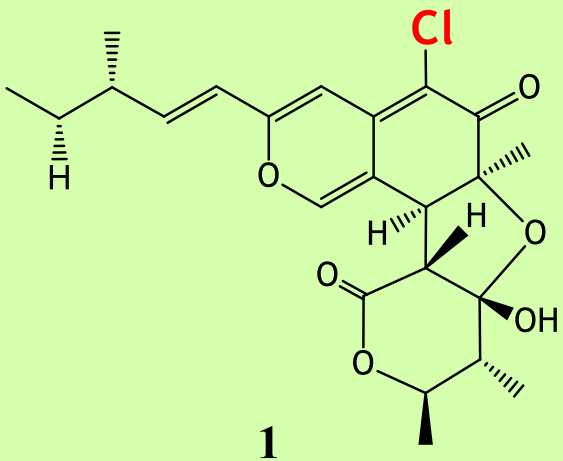
Ergosterol (**6**)

Phytotoxicity - Lettuce seed germination bioassay.

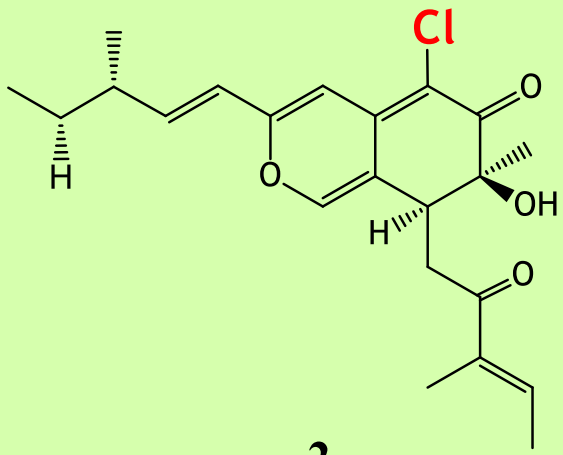
Root growth inhibition IC_{50} - **1** (45.4 ppm), **2** (49.7 ppm), **3** (47.8 ppm)
shoot growth inhibition IC_{50} - **1** (49.7ppm), **2** (48.7 ppm), **3** (48.2 ppm)

Chaetomugilins from an endophytic fungi *Chaetomium globosum* from *Amarnthus viridis* (Kurathampala)

chlorine-containing azaphilone derivatives



1



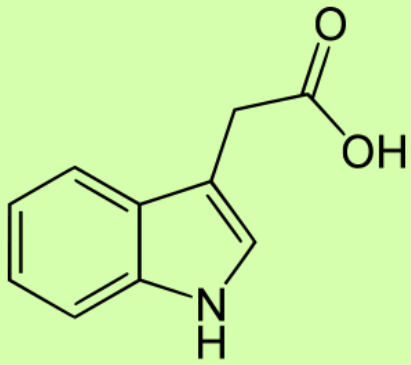
2

Compounds **1** and **2** inhibited (100%) seed germination at 100 ppm.
IC_{50} for radicle growth inhibition of **1** and **2** were 24.2 and 22.6 ppm, respectively, while IC_{50} values of percentage shoot growth inhibition were 27.8 and 21.9 ppm, respectively.

Highlights - Phytotoxicity of chaetomugilin-type cpds is reported for the 1st time.

Indole-3-acetic acid from an endophytic fungus *Colletotrichum siamense* from *Piper nigrum* L. Leaves

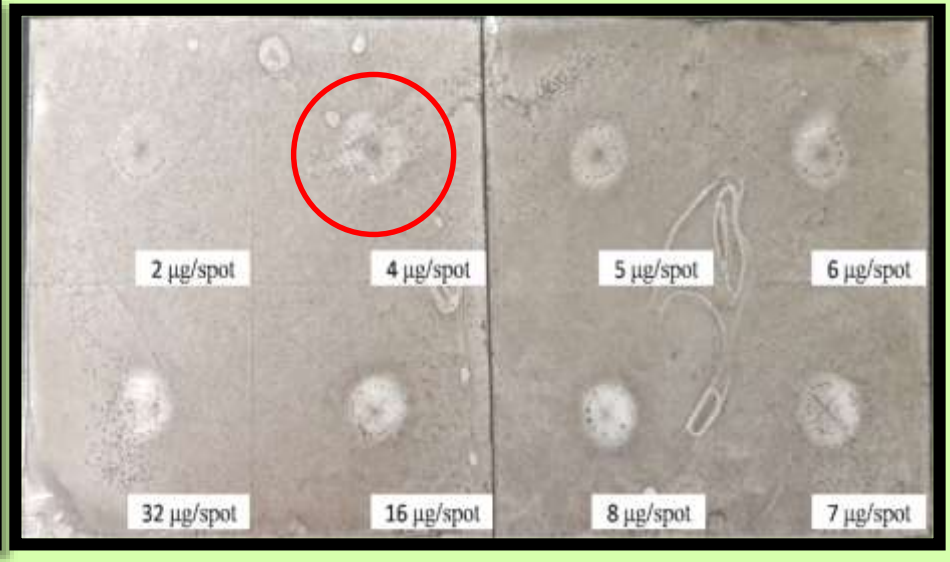
Piper nigrum



indole-3-acetic acid

Antifungal activity *C. cladosporiodies* **+++**

IAA - naturally occurring plant hormone



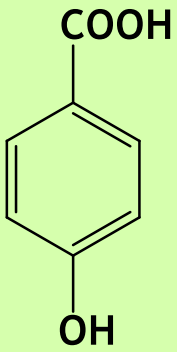
Benlate (Positive control)

IAA

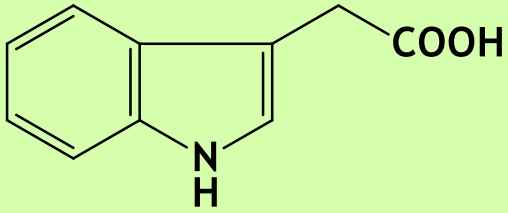
MIC - 4µg/spot.

(IAA required to inhibit the growth of *C. cladosporioides)*

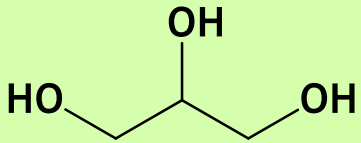
Phialemonium curvatum from Passiflora edulis

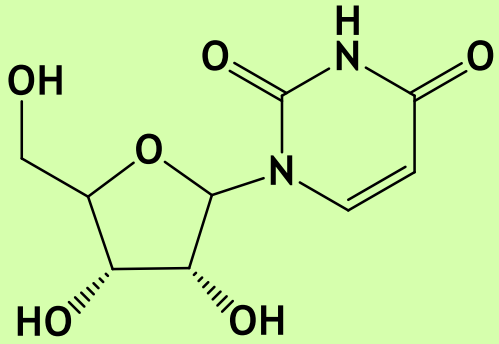


4-Hydroxy BA

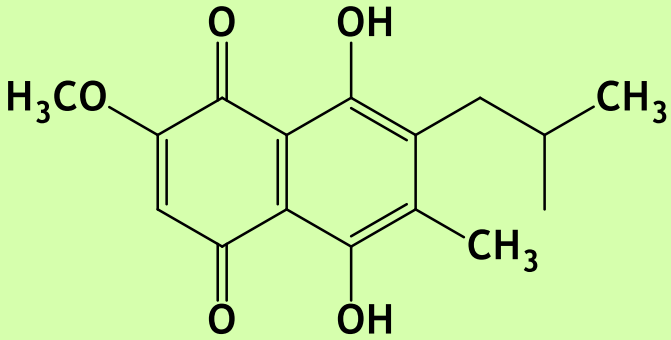


3-IAA





Uridine



Solaniol

Tea shot-hole borer beetle (TSHB) infestation of tea

Three living organisms involved:-

Tea plant - *Camellia sinensis*

Ambrosia fungus

- *Monacrosporium ambrosium*

Shot-hole borer beetle

- *Xyleborus fornicatus*

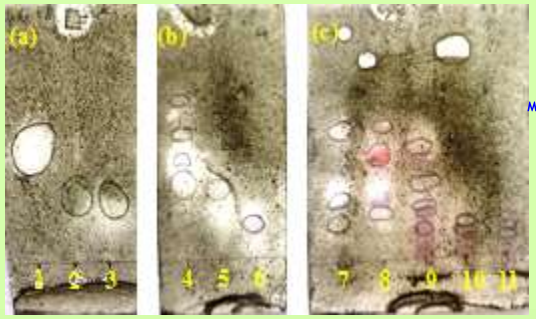
Beetle gallery with fungus

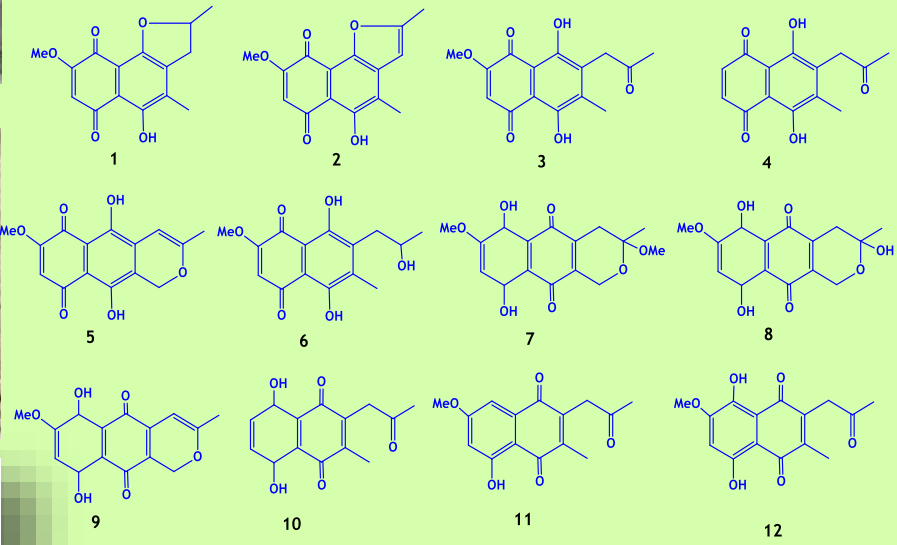
Ambrosia fungus in liquid medium

- Role of Naphthoquinone Metabolites Produced by *Monacrosporium ambrosium*, Ecosymbiote of Shot-Hole Borer Beetle (*Xyleborus fornicatus*) that Infests Tea (*Camellia sinensis*) Stems.

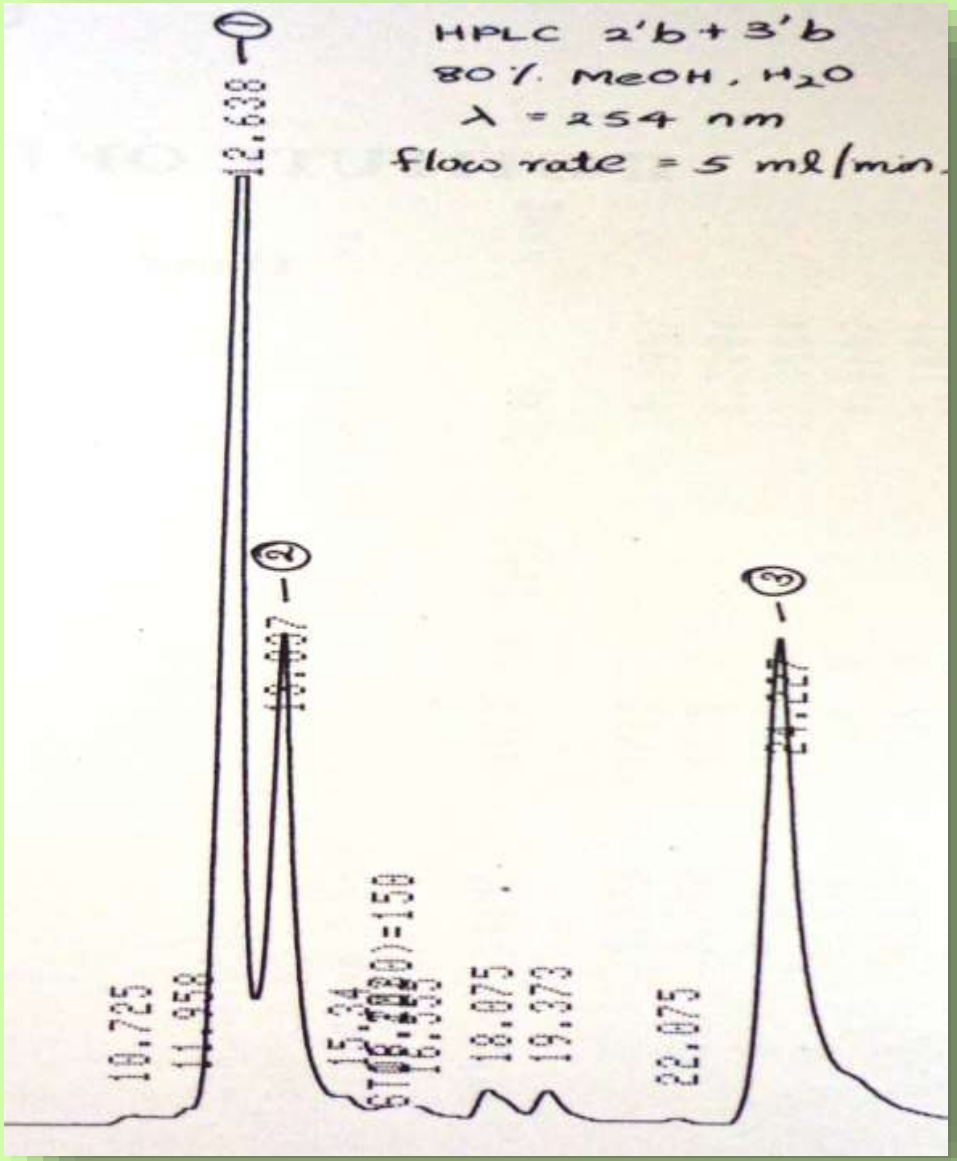
Naphthoquinones *Fusarium ambrosium* fungal symbiote of shot-hole borer beetle of Tea

Fusarium ambrosium (Syn. *Monacrosporium ambrosium*)





TLC of the extract
50% CH_2Cl_2 / Hexane

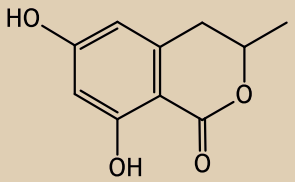


HPLC of the extract
20% H_2O/MeOH
254 nm, 5 ml/min

After separation by HPLC

Ms. - A possible role for Napthoquinone Metabolites Produced by *Monocrosporium ambrosium* in Shot hole Borer Beetle (*Xyleborus fornicates*) Infestation of Tea (*Camellia sinensis*)

TLC of separated components
Triple elution with 10% EtOAc/hexane



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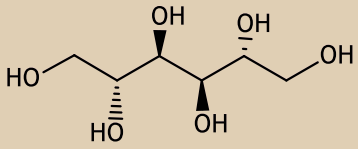

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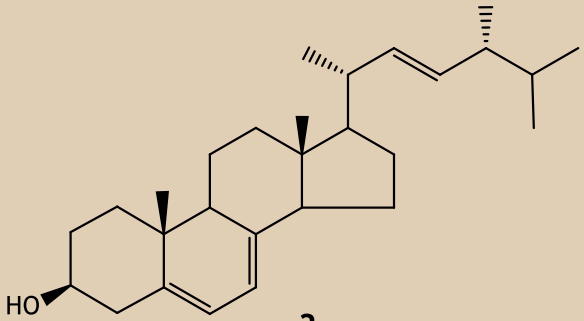

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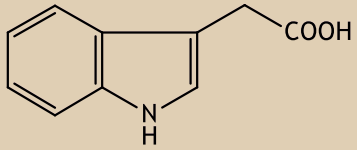

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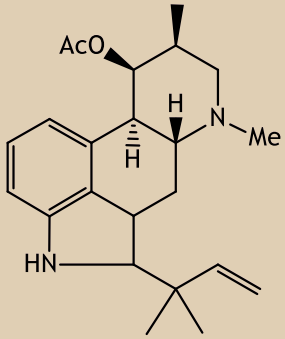

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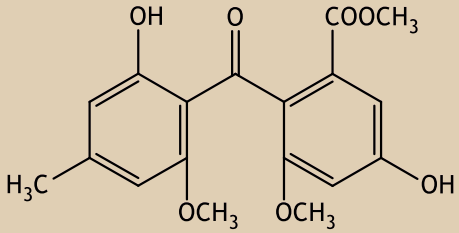

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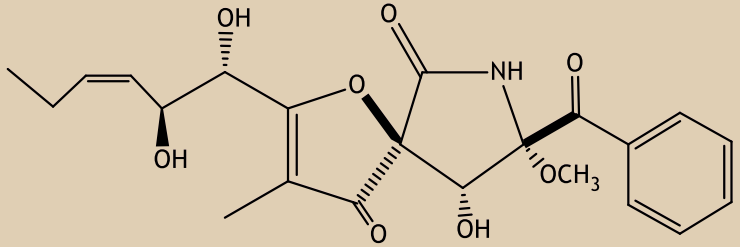

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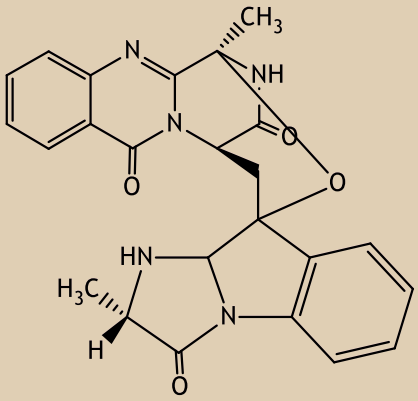

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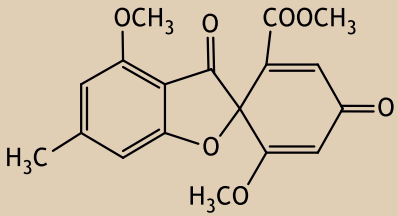

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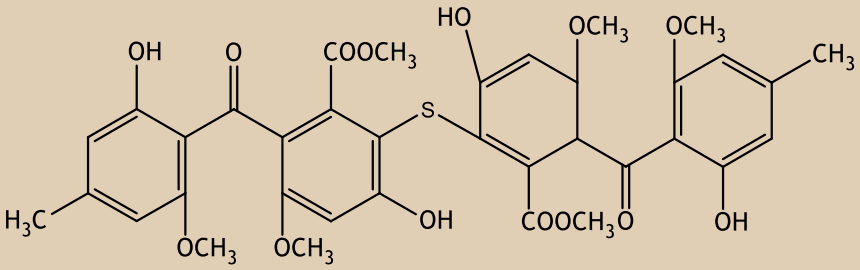

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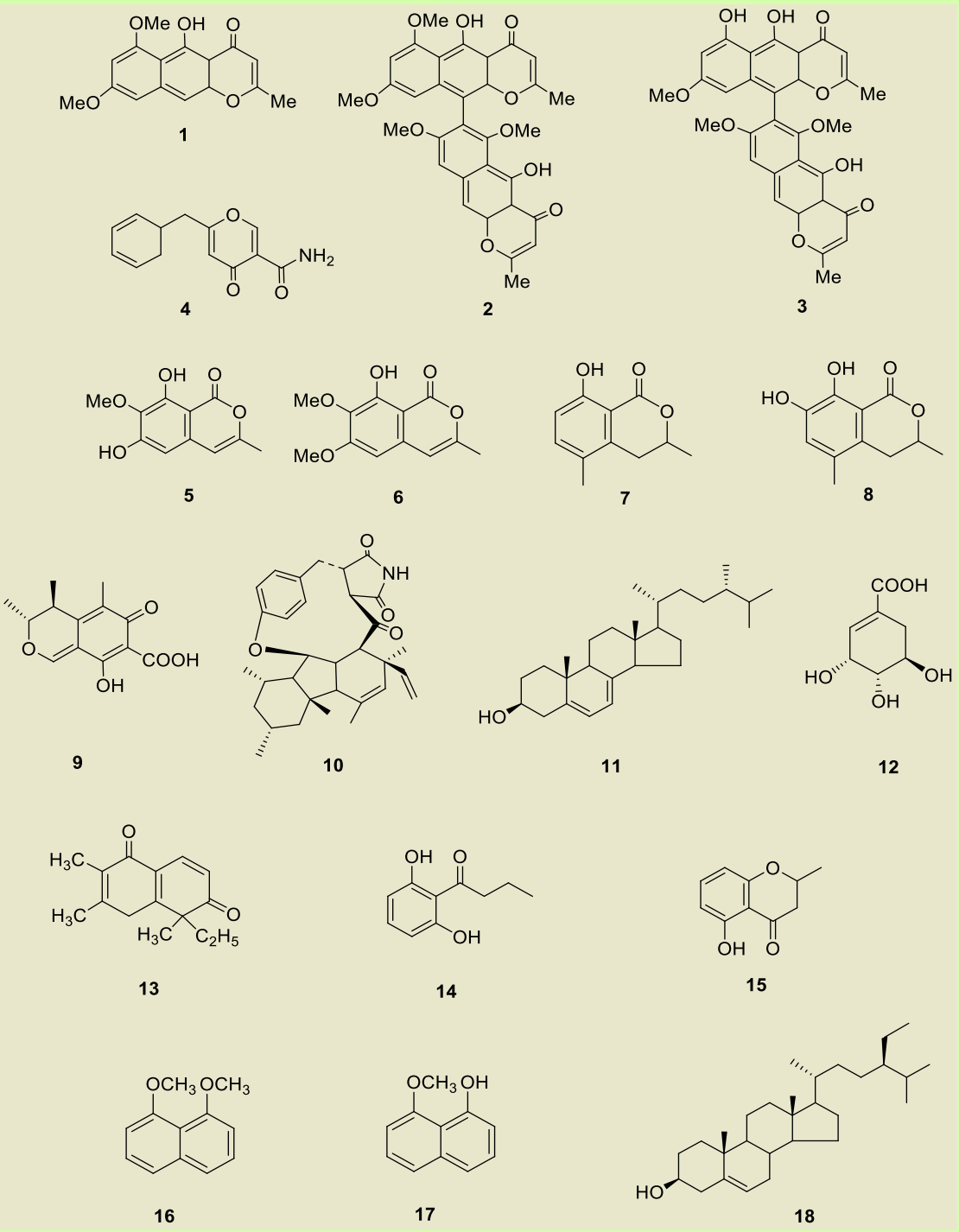


Future directions:

Large no. of cpds from microbial sources.

Synthetic derivatives

Enzyme bioassays –
Drug targets

Microorganism - Advantages

Large number of varieties

Genetic manipulation

Bio control

Large scale production

No environmental problems

Manipulation culture conditions

Co-culturing

- Variety of natural products

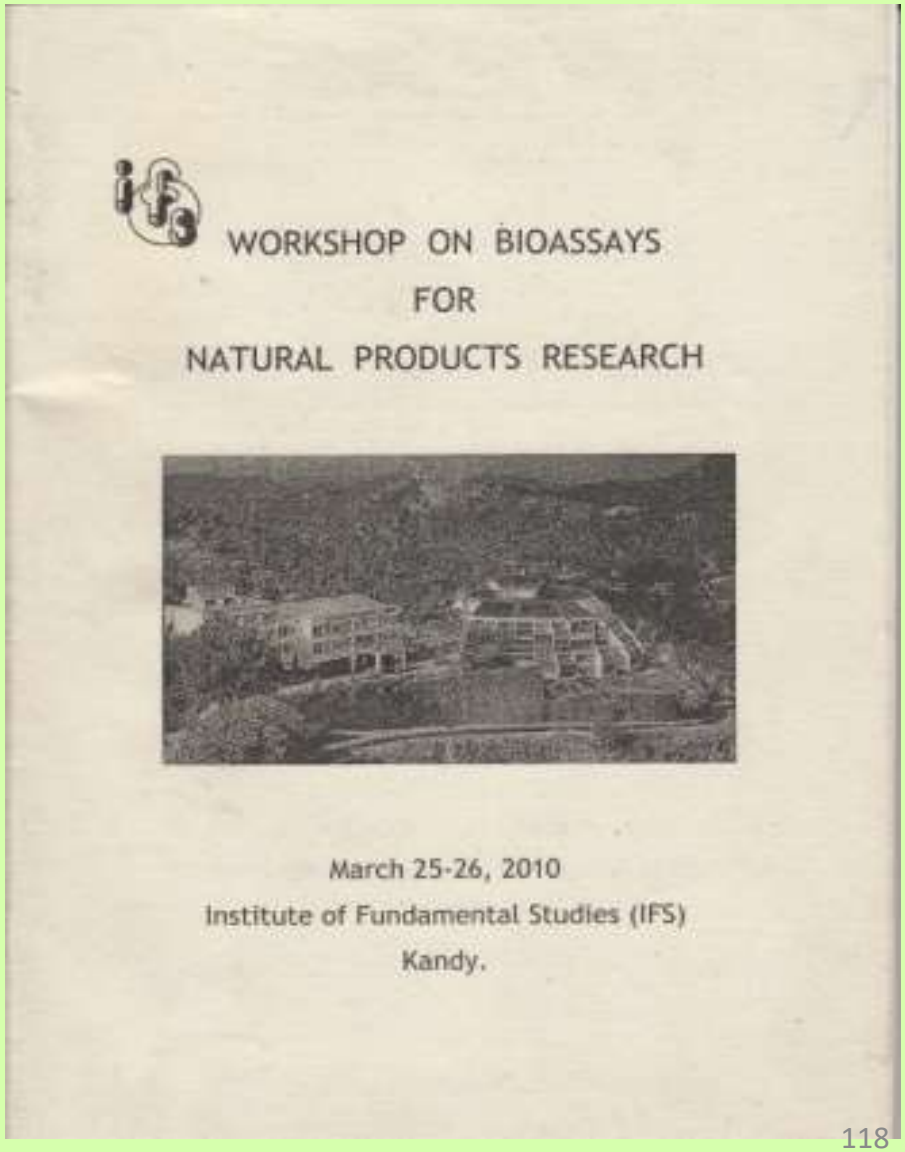
Opportunities

Constraints

2010

Workshop on Bioassays
for Natural Product Research

~ 50 participants

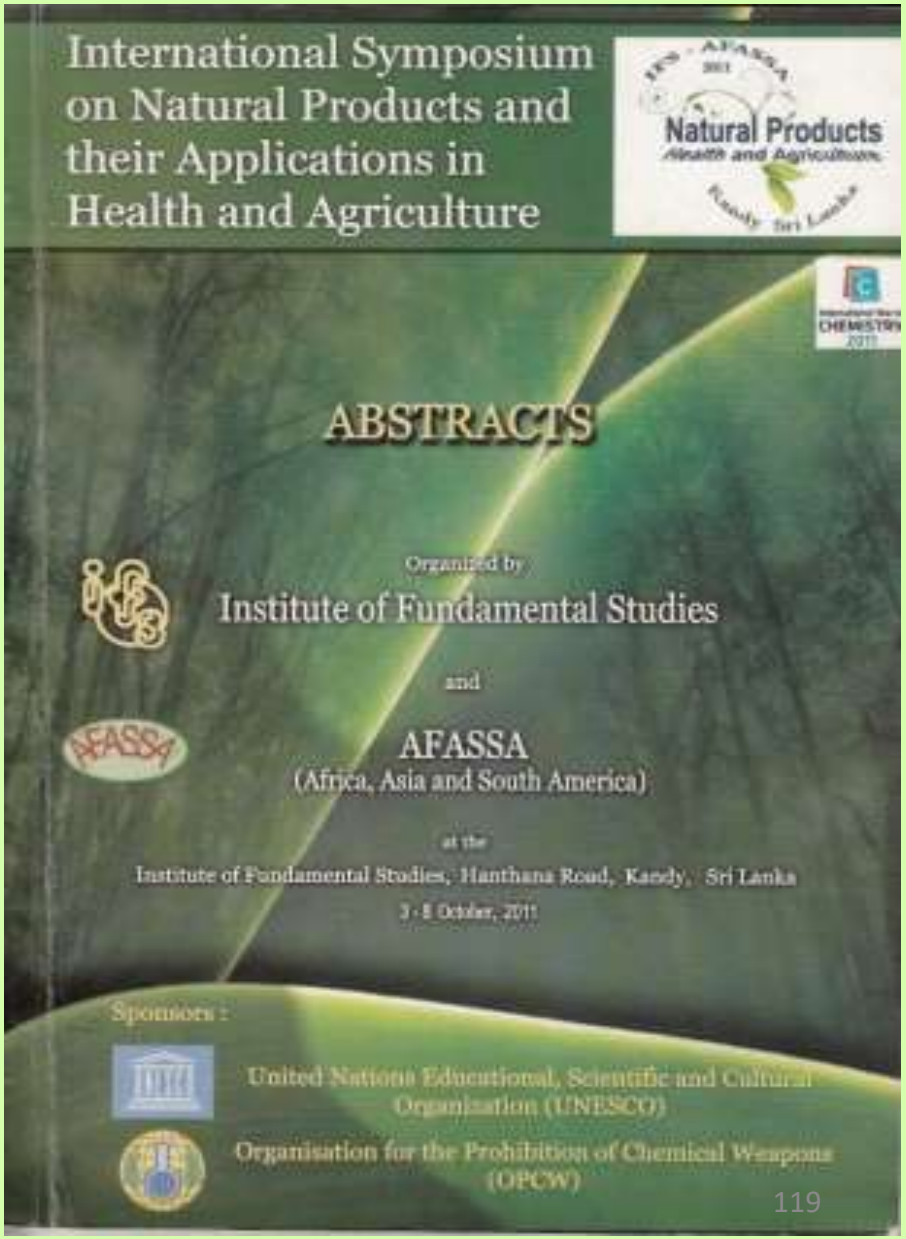


2011

International Symposium on Natural Products and their Applications in Health and Agriculture

38- Foreign participants

75- Sri Lankan participants



2011

IFS – AFASSA International Symposium on Natural Products and their Applications in Health and Agriculture
Institute of Fundamental Studies, Kandy, Sri Lanka 3 – 8 October, 2011

STUDIO LAXMA

1st Row (L. to R.) M.C. Rajapakse, S. Tasleem (Pakistan), D. Arbain (Indonesia), J.O. Midiwo (Kenya), Y. Fujimoto (Japan), M. Mosihuzzaman (Bangladesh), L. Mirossay (Slovak Republic), S. Dallavalle (Italy), S. Sotheeswaran, L. Jayasinghe, N.S. Kumar C.B. Dissanayake, Atta-ur-Rahman (Pakistan), A.A. Bekhit (Egypt), N. Kuhnert (Germany), M.I. Choudhary (Pakistan), V. Kumar, J.M. Rao (India), S.P. Gunasekera (USA), A. Wickramasinghe, A.A.L. Gunatilaka (USA), R. Weerasooriya, M.A.K.L. Dissanayake, C.T.K. Tilakaratne, G. Mojzisova (Slovak Republic)

2nd Row (L. to R.) S. Sotheeswaran, M.K. Mugisha (Uganda), B. Ali (Pakistan), H. Rashid (Pakistan), L.O. Kerubo (Kenya), P.S. Warakagoda, R. Samarasekara, U.G. Chandrika, N. Salim, M.A. Hettiarachchi, H.P.D.S. Jinasena, P.A. Paranagama, H.N.B. Mendis, P. Senanayake, C.G. Rajapakse, S. Derese (Kenya), R. Sivakanesan, S. Devarajan, V.M. Thadhani, H.K.I. Perera, P.A.S. Wickramarachchi, H.M.D.K. Kanatiwela, W.I.T. Fernando, G.G.E.H. De Silva, A.M.D.A. Siriwardane, H.M.S.K.H. Bandara, K.G.E. Padmathilake, A.G.A.W. Alakolanga, P.S.S. Samarakkody, D.N. Magana-Arachchi, L.C.P.T. Liyanaarachchie

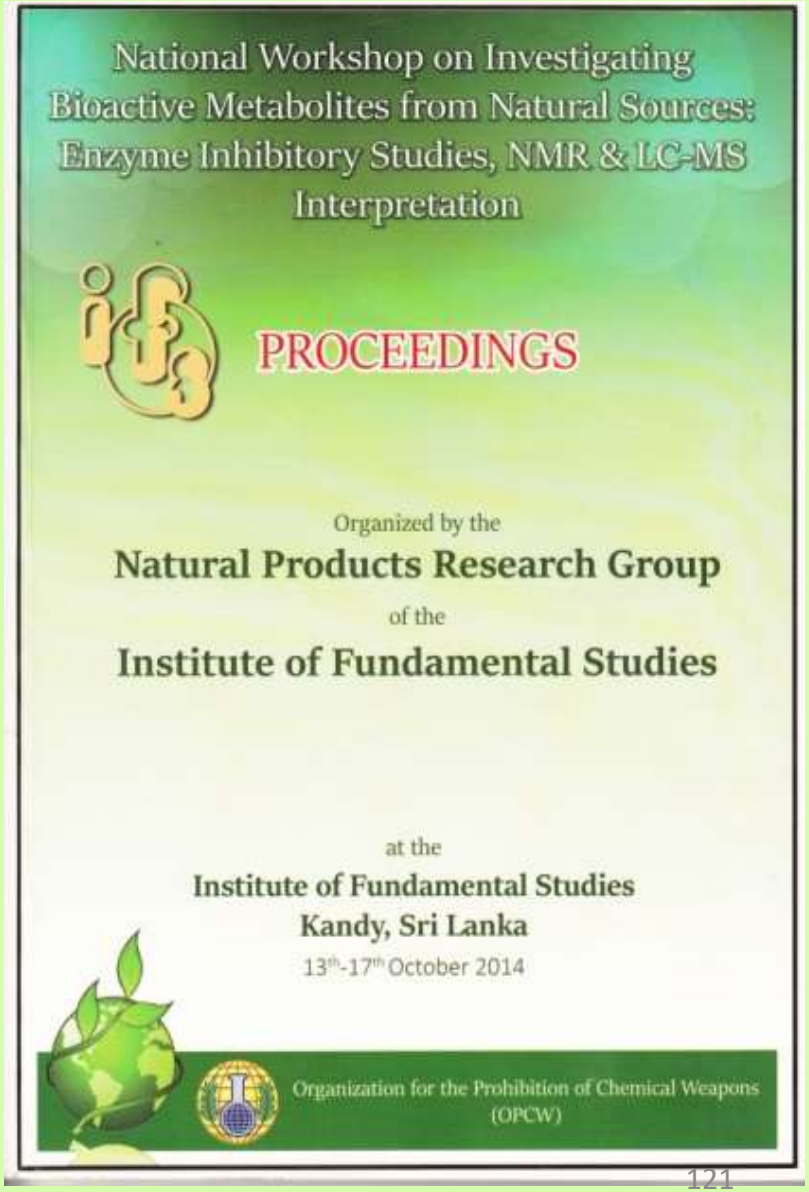
3rd Row (L. to R.) M. Gunasekera, I. Sajid (Pakistan), O.S. Perera, N.H.N. Priyanwada, A.M. Abeysekara, R.P. Wanigathunge, H.M.L.I. Herath, H.M. Liyanage, D. Bopitiya, N. Syafni (Indonesia), K.M.D. Gunathilake, A.A.K. Karunathilake, K.A.N. Rajan, B. Choudhary (Pakistan), D. Nanayakkara, T. Sothyrupan, R.M.C.J. Bandara, D.A.S. Siriwardhana, N.V.A. Kumar (India), T.M.S.G. Tennakoon, D.S. Jayaweera, N. Govindappa (india), J. Wijayabandara, M.A. Siriwardhene, D.B.M. Wickramaratne, K.M.S. Wimalasiri, R.R. Ratnayake, D. Niyangoda, R. Liyanage, T. Thileepan, J. Mojzis (Slovak Republic)

studiolaxman@gmail.com

2014

National Workshop on Investigating Bioactive Metabolites from Natural Sources: Enzyme Inhibitory Studies, NMR & LC-MS Interpretation

4 – International Resource Persons,
60 - Participants



2014

National Workshop on Investigating Bioactive Metabolites from Natural Sources:
Enzyme Inhibitory Studies, NMR & LC-MS Interpretation.
Institute of Fundamental Studies, Kandy. 13th – 17th October - 2014

STUDIO LAXMAN KANDY

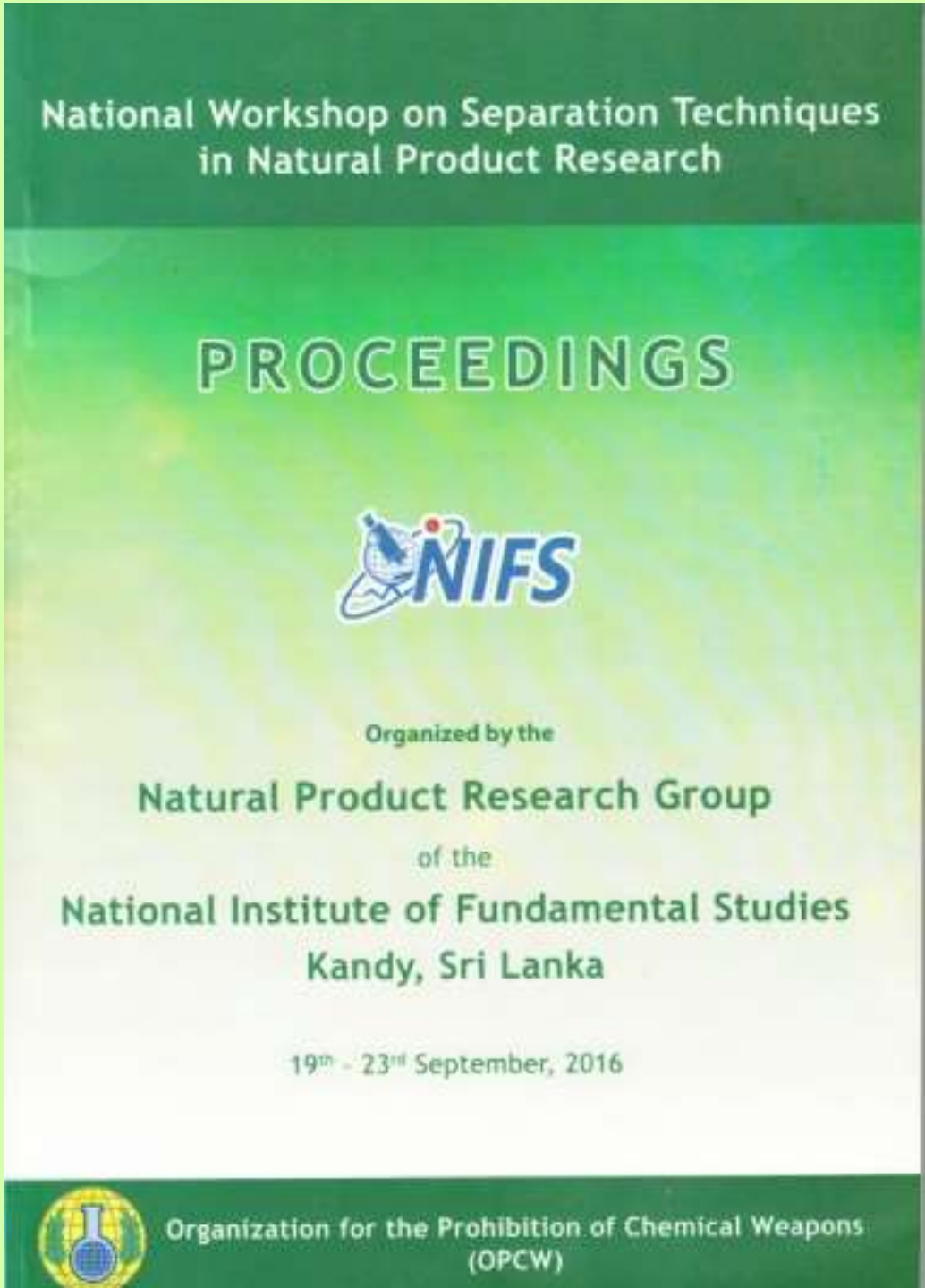
4 – International Resource Persons, 60 - Participants

2016

National Workshop on Separation Techniques in Natural Product Research

19th – 23rd September

- 4 - International Resource Persons
- 5 day workshop
- 70 participants
- Field visits



National Workshop on Separation Techniques in Natural Product Research

National Institute of Fundamental Studies, Kandy, Sri Lanka

19th – 23rd September, 2016

STUDIO LAXMAN KANDY

1st Raw Seated (Left to Right)

D.S. Jayaweera, S. Samarakkody, K. Tilakaratne, D.S. Wijesundara, S. Wimalasiri, N. H. Ismail (Malaysia), B.M.R. Bandara, L. Jayasinghe, S.H.P.P. Karunaratne, Y. Fujimoto (Japan), N.S. Kumar, A. Wickramasinghe, H. Araya (Japan), N. Kuhnert (Germany), R. Shanthini, D.N. Karunaratne, P.S.B. Wanduragala

2nd Raw (Left to Right)

M. Karunarathna, T. Dharushana, K. Ratnayake, A.U. Karunarathna, D. Niyangoda, J. Rajapakse, N.K. Narayana, D. Dissanayake, C.L. Kehelpannala, A. Samarasinghe, J. Jayasundara, S. Thilakarathne, S. Rajapakse, N.C. Bandara, S. Fernando, W.K. Balasooriya, S.D. Hapuarachchi, S. Bogahawatta, H. Samarasinghe, T. Manthrirathna, S. Sinthujah, T. Atugoda, A. Alakolanga, P.K.V. Ranji, C. Liyanaarachchi

3rd Raw (Left to Right)

N.Y. Hirimuthugoda, S. Sathya, W.I.T. Fernando, S. Premina, S. Bandara, V.J. Weerasinghe, C.B. Gunawardhana, N. Jayalath Manike, D.L. Ranathunge, C. Suraweera, A.N. Kodithuwakku, M.V.K. Munasinghe, K.D.K.P. Kumari, G.U. Jayaweera, K. Karunaratne, M.N. Napagoda, D.G.H. Shamika, N.R. Amarasinghe, D. Premasiri, R. Liyanage, D.M. Magana-Arachchi, M. Vithanage, D. Pathirana

4th Raw (Left to Right)

P.H.P. Fernando, S. Lamahewage, J. Wijesinghe, E.D.N.S. Abeyrathne, M.A. Wijesekara, G.M. Wimalasena, P. Ragutharan, E. Ranasinghe, S. Fonseka, K.L.S. Silva,

ASIAN NETWORK OF RESEARCH ON ANTIDIABETIC PLANTS (ANRAP)

1st Sri Lankan ANRAP Regional Seminar (ANRAPSL1) on
"Herbal Approaches in Combating Diabetes and Common Tropical Diseases"
17 - 19 January 2018

National Institute of Fundamental Studies, (NIFS) Kandy, Sri Lanka.

Chemistry for the Modern World

EuAsC$_2$S-16

16th Eurasia Conference on Chemical Sciences

Venue: MAS Athena, Thulhiriya, Sri Lanka

.

23rd – 25th September, 2020

Organized by

National Institute of Fundamental Studies (NIFS), Kandy, Sri Lanka

Natural Products Research Group – 2017/19

Acknowledgements

NIFS

NSF / Sri Lanka

NRC / Sri Lanka

Prof. Y. Fujimoto, Prof. H. Araya - Meiji University, Japan

Prof. N. Kuhnert - Germany

Natural Products Research Group Members


Thank
you!!