

Biofunctional Molecules from Several Egyptian Herbal Medicines

– Black Cumin, Colocynth, Black Pepper –

Hisashi Matsuda and Masayuki Yoshikawa

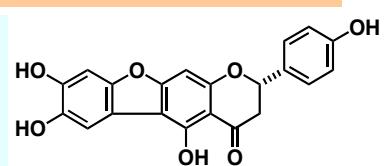
Department of Pharmacognosy,
Kyoto Pharmaceutical University



Search for Biofunctional Molecules from Other Egyptian Herbal Medicines - 1

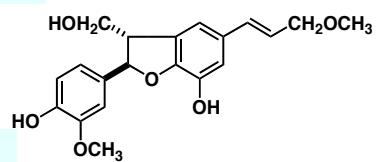
1. St. Mary's Flower (the whole plant of *Anastatica hierochuntica*)

Hepatoprotective activity ... Flavonolignans
Antiinflammatory activity
(NO production-inhibitory activity) ... Neolignans
Anti-melanogenesis activity ... Flavonoids, Neolignans



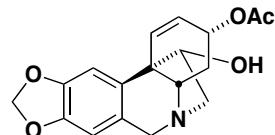
2. *Crinum yemense* (C. album), bulbs

Antiinflammatory activity ... Amaryllidaceae alkaloids
(NO production-inhibitory activity)



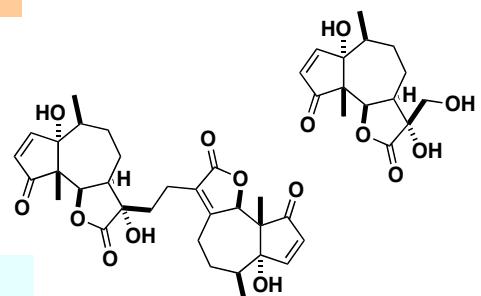
3. *Dichrocephala integrifolia*, aerial parts

Antiinflammatory activity ... Sesquiterpenes
(NO production-inhibitory activity)



4. *Boswellia carterri*, resin

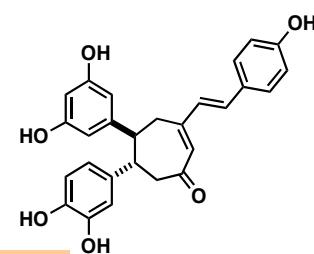
Differentiation-inducing activity ... Triterpenes



Search for Biofunctional Molecules from Other Egyptian Herbal Medicines - 2

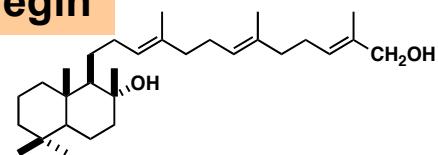
5. *Cyperus longus*, whole plants

Antiinflammatory activity ... Stilbene dimers
(NO production-inhibitory activity)
Hepatoprotective activity ... Sesquiterpenes



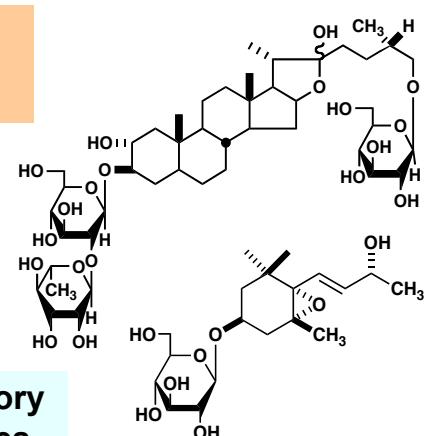
6. Guggul-gum (*Balsamodendron mukul*), resin

Antiinflammatory activity ... triterpenenes



7. Fenugreek (*Trigonella foenum-graecum*), seeds

Immunological adjuvant activity
... triterpenene glycosides



8. Moroheiya (*Corchorus olitorius*), leaves, seeds

Anti-allergy, Antiinflammation (NO production-inhibitory activity)
... fatty acids, ionone glucosides

Black Cumin

كمون أسود *kamūn esmid*

Origin

the seeds of *Nigella sativa* (Ranunculaceae)

Distribution

Egypt, southwestern Asia, Mediterranean areas

Constituents

essential oils, fatty acids, saponins, alkaloids

Application

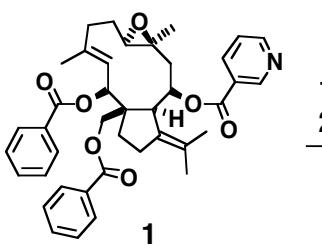
traditional Egyptian medicine for
influenza, asthma, conjunctivitis, etc.

Pharmacology

antitumor, antiinflammation,
antihypertension, hypoglycemia

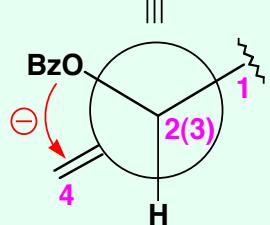
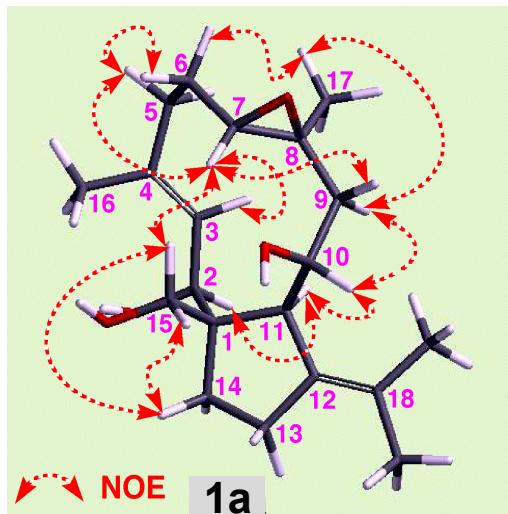
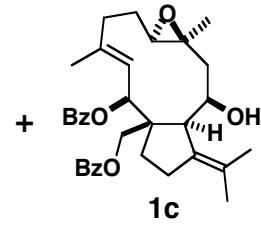
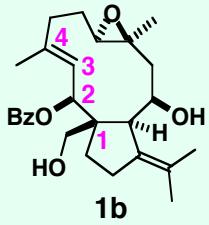


Absolute Stereostructure of Nigellamine A₁ (1)



1) 0.1% NaOMe-MeOH, 0°C
2) HPLC separation

Bz: benzoyl
Nic: nicotinoyl



A white powder

$[\alpha]_D^{24} +36.3^\circ$ ($c=0.4$, CHCl₃)

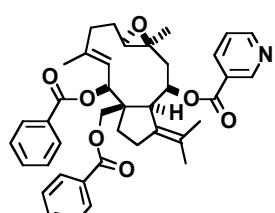
High resolution Pos. FAB-MS

Calcd for C₂₇H₃₇O₅ (M+H)⁺: 441.2641

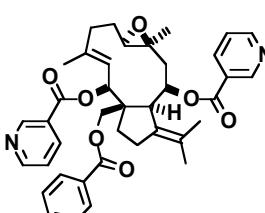
Found : 441.2650

CD (MeOH, nm, $\Delta\epsilon$): 246 (-1.31)

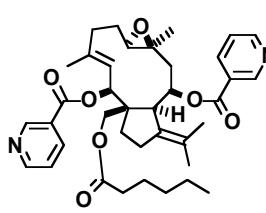
New Dolabellane-type Diterpenes Isolated from *N. sativa*



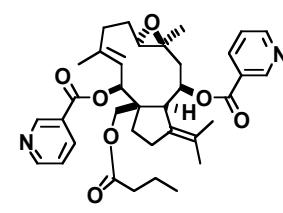
nigellamine A₁ (1)



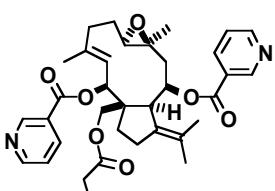
nigellamine A₂ (2)



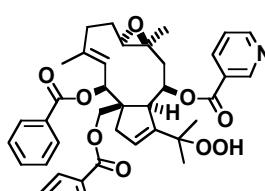
nigellamine A₃ (3)



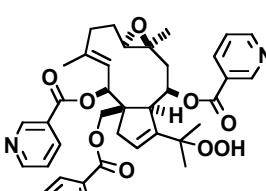
nigellamine A₄ (4)



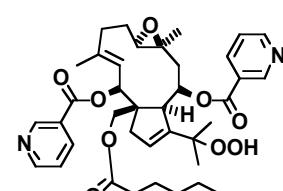
nigellamine A₅ (5)



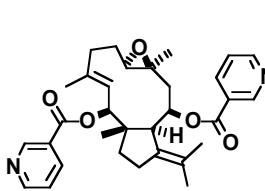
nigellamine B₁ (6)



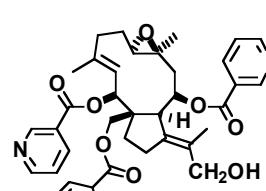
nigellamine B₂ (7)



nigellamine B₃ (8)



nigellamine C (9)

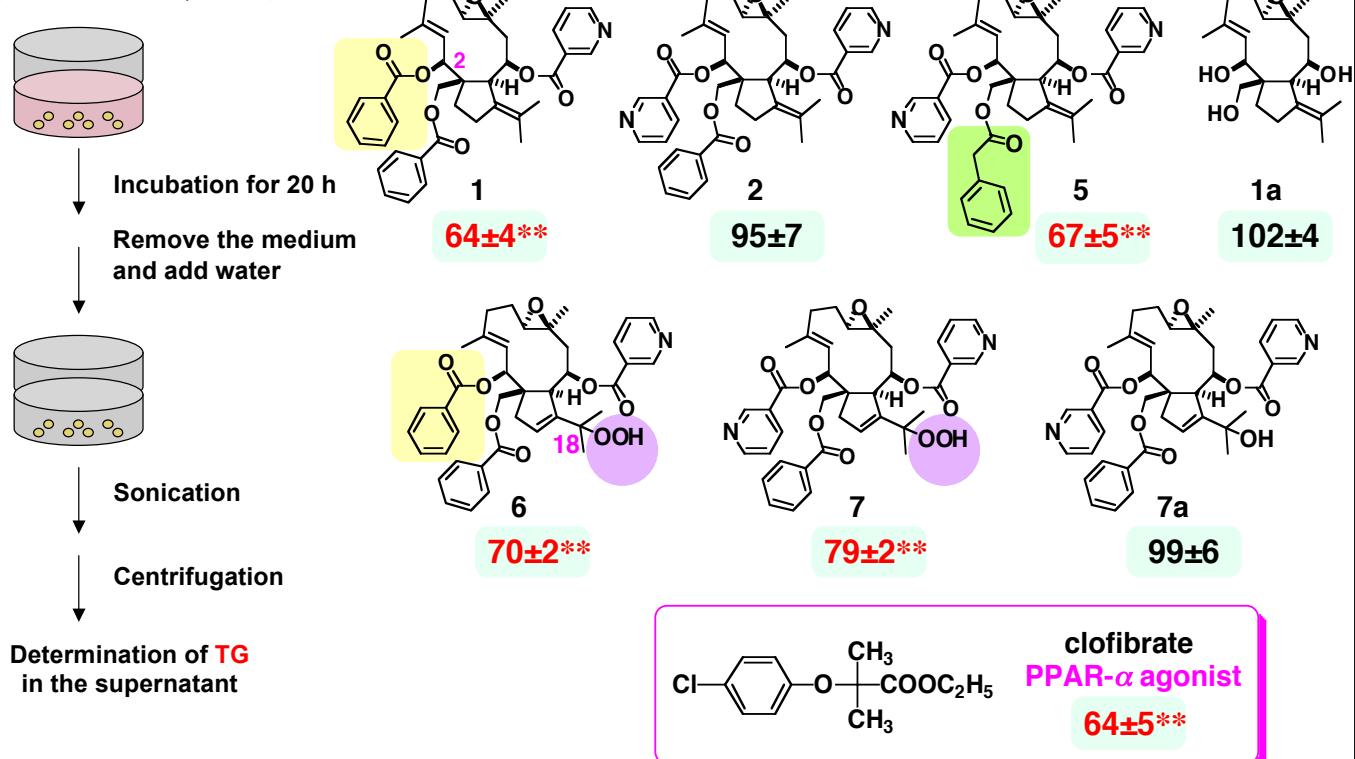


nigellamine D (10)

Promotion of Triglyceride (TG) Metabolism in Primary-Cultured Mouse Hepatocytes

Mouse hepatocytes + Test Sample
in William's E medium with 10% FCS
(8×10^4 cells/200 μ L/well)

TG: % of control at 0.1 μ M
 $N=4, p<0.01$



Colocynth حنطلي hanzal

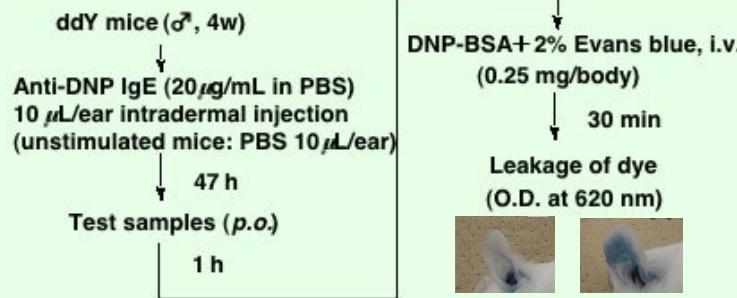
Inhibitory Effects of MeOH Ext. and Its Fractions on Ear PCA Reaction in Mice

Source :

Citrullus colocynthis (L.) SCHRAD.
(Cucurbitaceae), fruit



The fruit of *C. colocynthis*



Distribution :

Africa (Egypt), Arabia, India

Application :

Catharsis, Antidiabetic

Constituents :

Flavonoid glycosides
Triterpenoid saponins
Fatty acids



Citrullus colocynthis (fruit, Egypt)

MeOH, Δ

* SiO₂ column
ODS column (MeOH-H₂O)
HPLC (MeOH-H₂O)

MeOH ext. (13.9%)

EtOAc/H₂O

EtOAc fraction (6.3%)

n-BuOH fraction (2.8%)

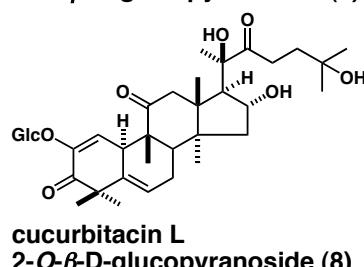
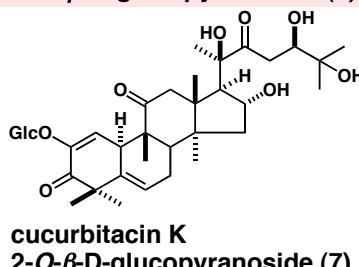
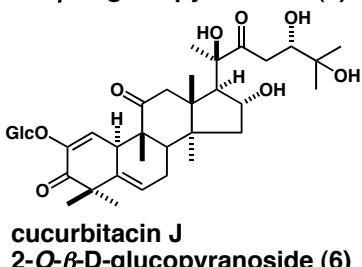
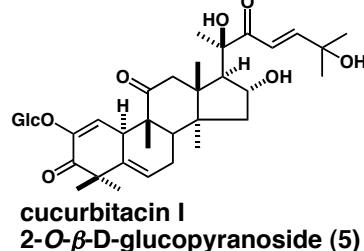
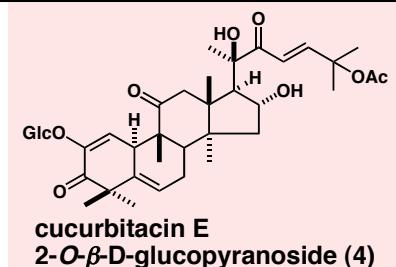
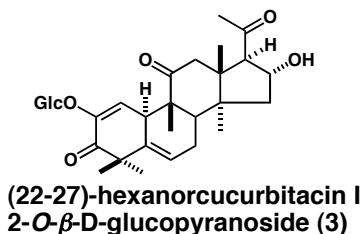
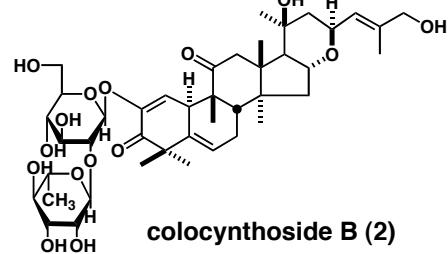
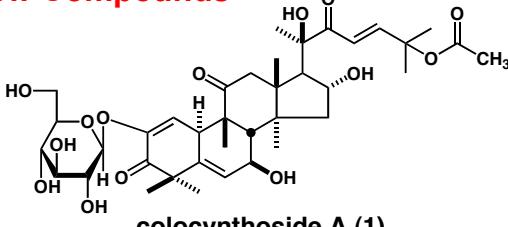
H₂O fraction (4.8%)

cucurbitacin E
2-O-β-D-glucopyranoside (4, 3.08%)
3',4-Dihydroxy-3-methoxypropophenone (0.0008%)

colocynthoside A (1, 0.0036%)
colocynthoside B (2, 0.0095%)
(22,27)-hexanorcucurbitacin I 2-O-β-D-glucopyranoside (3, 0.0020%)
cucurbitacin E 2-O-β-D-glucopyranoside (4, 0.0077%)
cucurbitacin I 2-O-β-D-glucopyranoside (5, 0.150%)
cucurbitacin J 2-O-β-D-glucopyranoside (6, 0.0015%)
cucurbitacin K 2-O-β-D-glucopyranoside (7, 0.00073%)
cucurbitacin L 2-O-β-D-glucopyranoside (8, 0.032%)
isoorientin 3'-methyl ether (0.0032%)
isovitexin (0.039%)
isosaponarin (0.00051%)
gastrodin (0.015%)
benzyl β-D-glucopyranoside (0.00057%)
4-(β-D-glucopyranosyloxy)-benzaldehyde (0.00094%)
4-hydroxybenzyl-β-D-glucopyranoside (0.00094%)

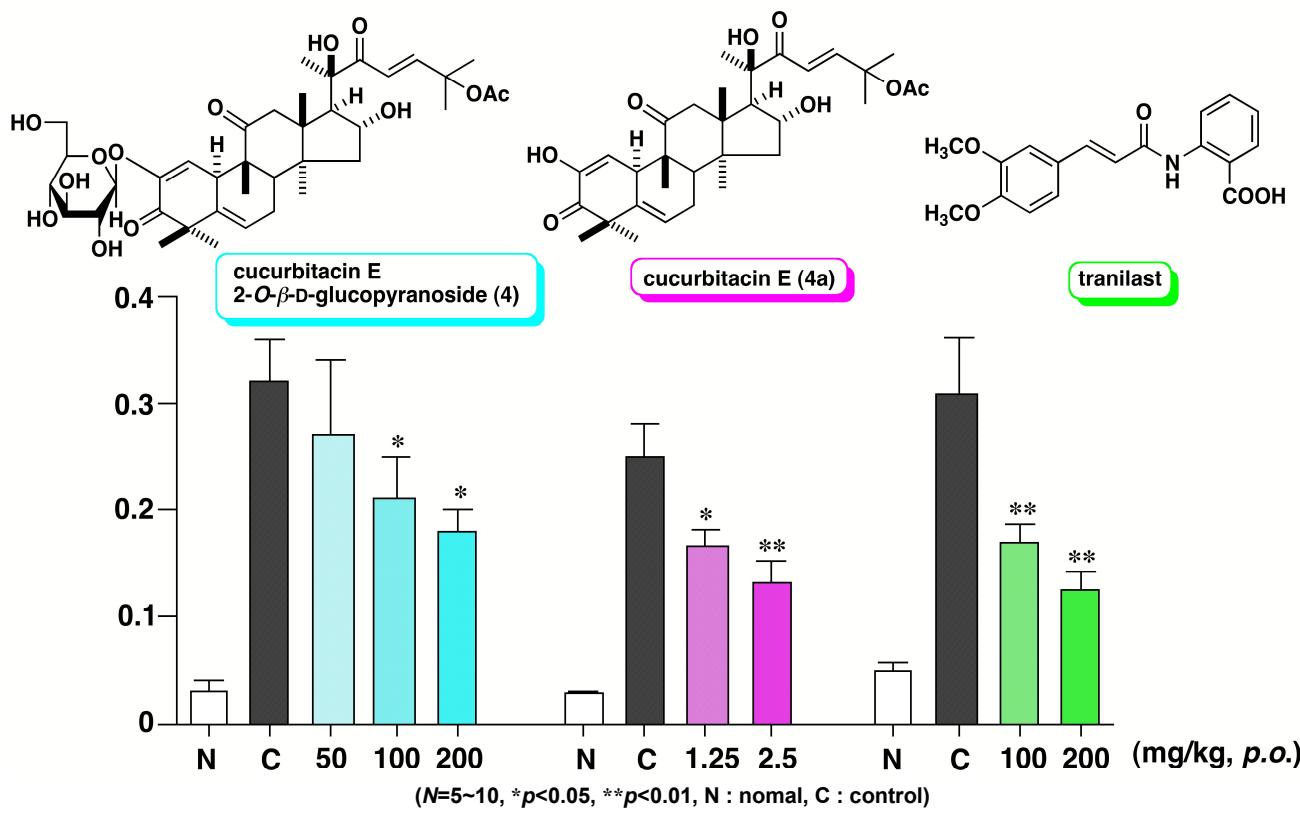
New and Known Constituents of *C. colocynthis* L.

New Compounds



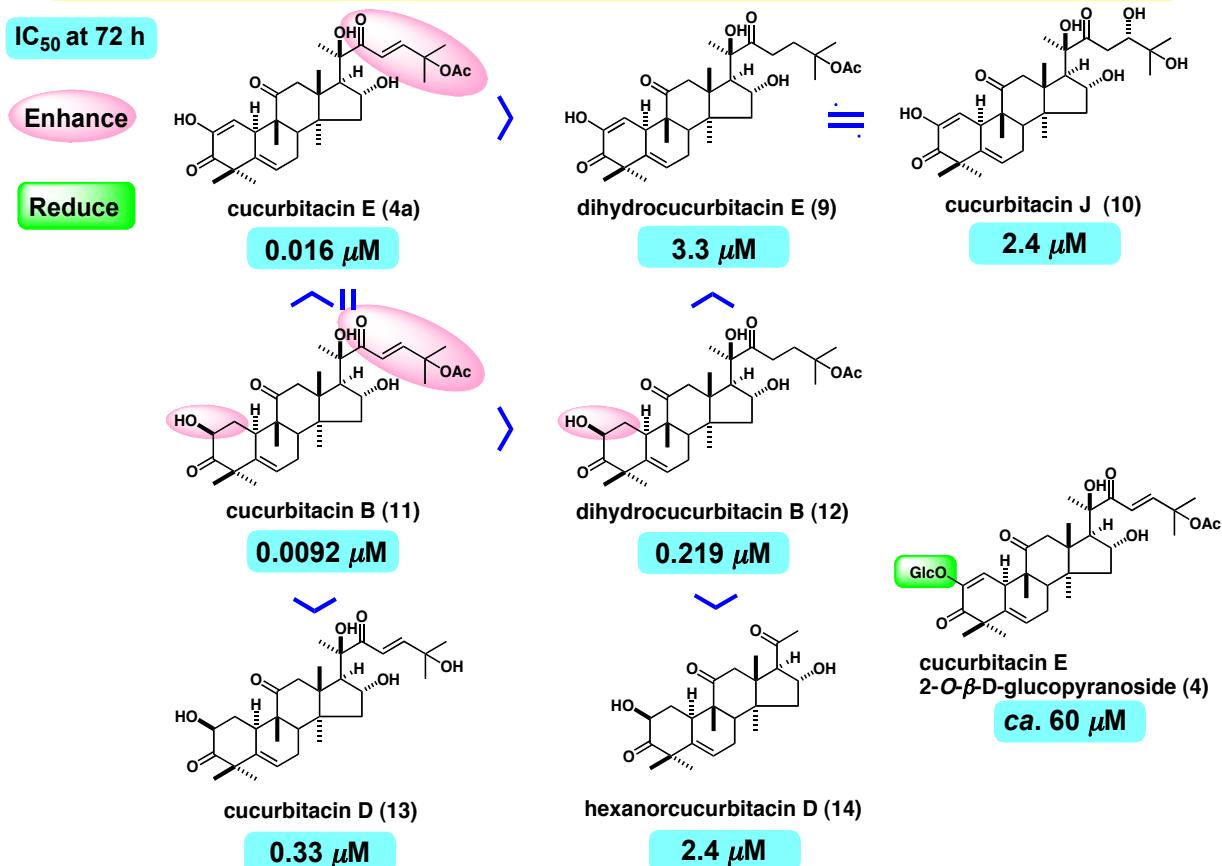
Glc = β - D-glucopyranose

Inhibitory Effects of Cucurbitacin E 2-O- β -D-glucopyranoside on Ear PCA Reaction in Mice



Chem. Pharm. Bull., 55, 428–434 (2004).

Inhibitory Effects of Cucurbitacin E and Related Compounds on Proliferation in Human Leukemia U937 Cells (WST-1 Assay)

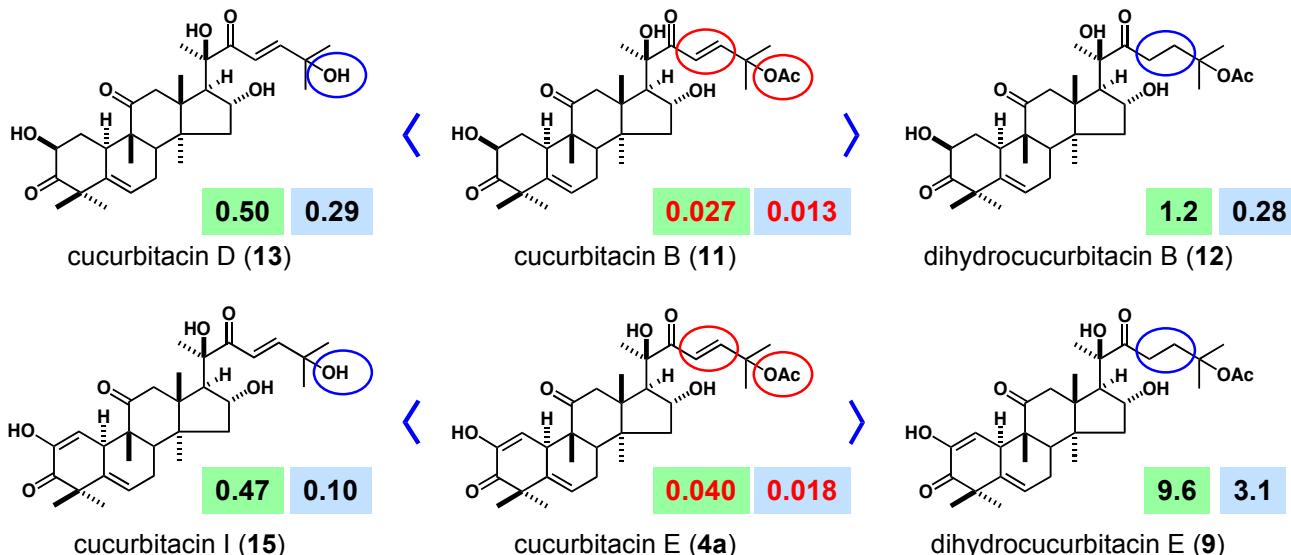


Structure-Activity Relationships of Inhibitory Effects of Cucurbitacins on Proliferation in HT1080 and HL60 Cells

IC₅₀ in HT1080 cell (μM)
IC₅₀ in HL60 cell (μM)
: enhance : reduce

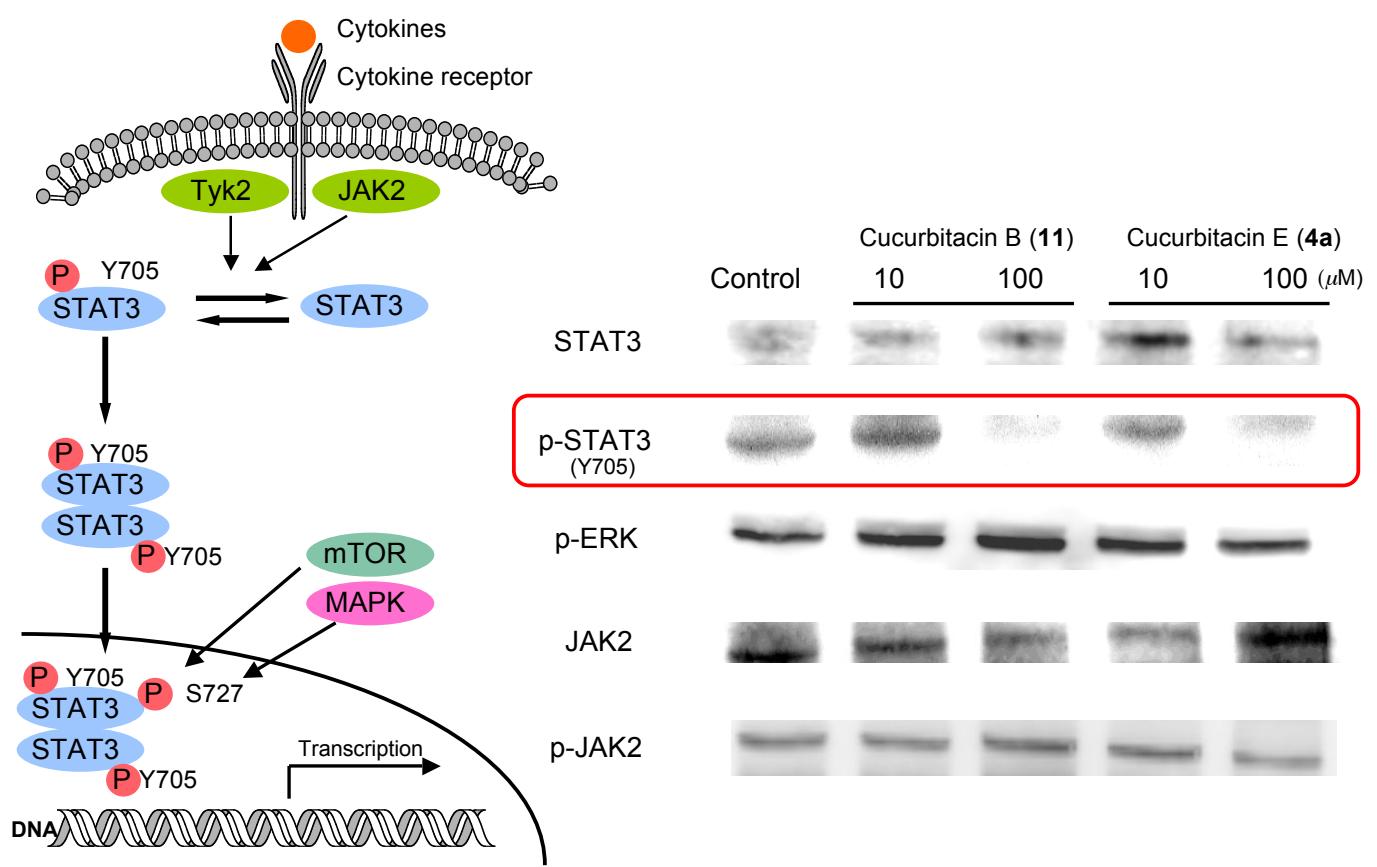
HT1080 : Human fibrosarcoma cell line

HL60 : Human leukemic cell line
(anaplastic)



Chem. Pharm. Bull., **58**, 747–751 (2010); *Bioorg. Med. Chem. Lett.*, **20**, 2994–2997 (2010).

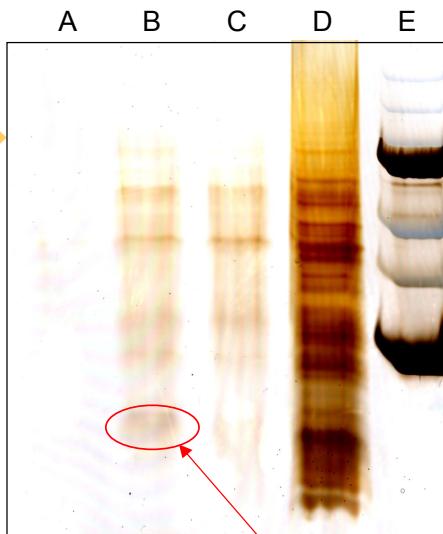
JAK/STAT3 Signaling Pathway and Effects of Cucurbitacins B (11) and E (4a) on Activation of STAT3 in U937 Cells



Isolation of Cucurbitacin E-Selective Binding Protein in U937



SDS-PAGE



Isolation using
HiTrap Streptavidin HP (GE
Healthcare)

excised from the gel

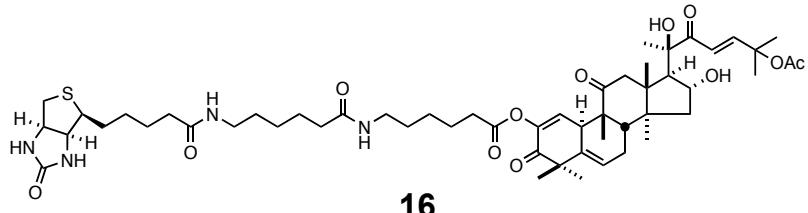
in-gel digestion

tryptic peptides

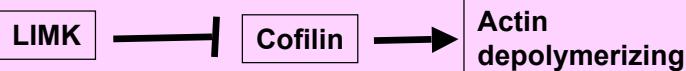
LC-MS/MS
analysis

Scaffold analysis
(NextGen Sciences)

cofilin



Effects of Cucurbitacin E on p-Cofilin and p-LIMK1/2 Levels in U937 Cells and on Fibrous-/Globular-Actin Ratio in HT1080 Cells



Cofilin promotes the regeneration of actin filaments by cleaving preexisting filaments. The cleaving activity of cofilin is suppressed by its phosphorylation by LIM kinase (LIMK).

p-cofilin

p-LIMK1
p-LIMK2

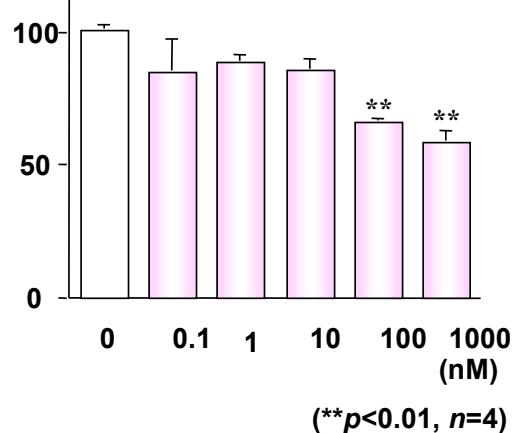
β -actin

0 0.1 1 10 100

Cucurbitacin E (4a)

Fibrous-/Globular-actin Ratio in HT1080 Cells

F/G- ratio

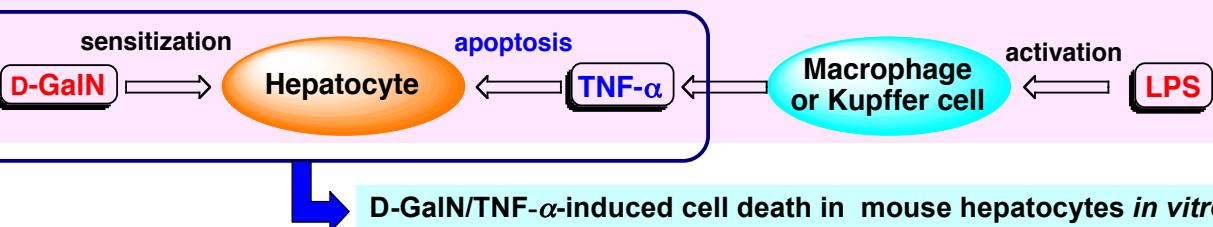
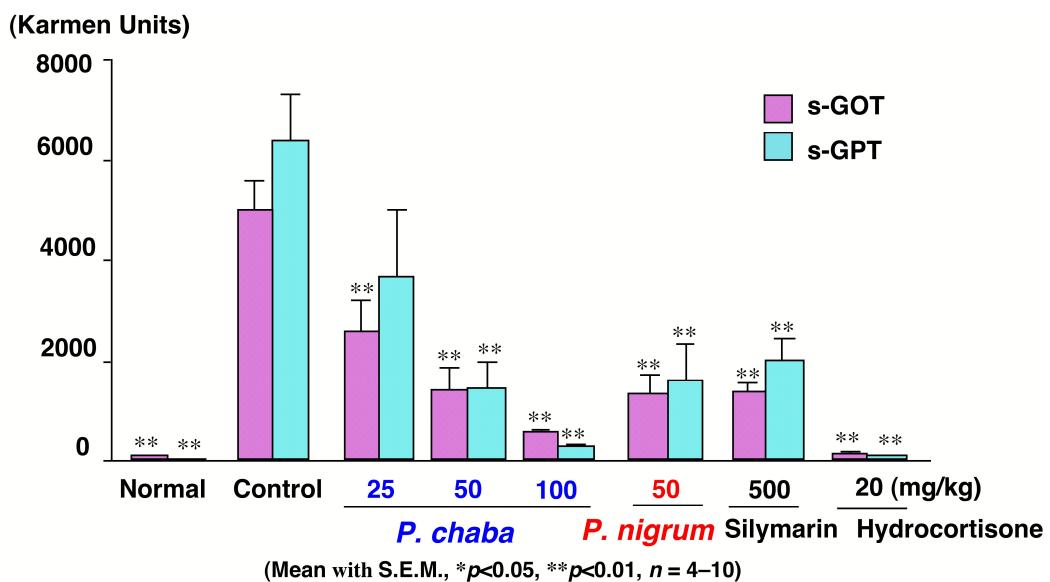


Black Pepper فلفل felfel

Dee Plee

Origin	the fruit of <i>Piper nigrum</i> L. (Piperaceae)	the fruit of <i>Piper chaba</i> HUNTER (Piperaceae)
Distribution	India, southeast Asia, etc.	India, Malaysia, Thailand, etc.
Constituents	piperine, cahvicine, etc.	piperine, etc.
Application	spice carminative, stomachic, tonic, antibacterial, insecticide, etc.	spice carminative, stomachic, expectorant, tonic, antifungal, and counterirritant , etc.
Pharmacology	anti-inflammation, gastroprotective effects	antimalarial activity, gastroprotective effects
		

Effects of 80% aq. Acetone Ext. of *P. chaba* and *P. nigrum* on D-GalN/LPS-Induced Liver Injury in Mice



Dried Fruit of *Piper chaba*

- a) SiO₂ column (Hexane-AcOEt,CH₃Cl-MeOH-H₂O)
 b) ODS column (MeOH-H₂O)
 c) HPLC (MeOH-H₂O)
 d) HPLC (CH₃CN-H₂O)
 e) crystallization

80% aq. acetone, r.t.
 80% aq. acetone ext. (19.7%)
 EtOAc/H₂O

AcOEt-soluble fraction (9.7%)

H₂O-soluble fraction (10.0%)

↓ a), b), c), d), e)

piperchabamide A (**1**, 0.0029%)
 piperchabamide B (**2**, 0.0041%)
 piperchabamide C (**3**, 0.0032%)
 piperchabamide D (**4**, 0.0037%)
 piperchabamide E (**5**, 0.0083%)
 piperchabamide F (**6**, 0.00071%)
 piperonal (7, 0.0047%)
 methyl piperate (8, 0.090%)
 piperine (**9**, 2.9%)
 piperanine (**10**, 0.41%)
 piperoleine B (**11**, 0.0082%)
 pipernonaline (**12**, 0.41%)
 dehydropipernonaline (**13**, 0.079%)
 piperundecalidine (0.017%)
 fragaramide (0.00060%)
 piperlonguminine (**14**, 0.19%)
 dihydropiperlonguminine (**15**, 0.015%)
 retrofractamide A (**16**, 0.0075%)
 retrofractamide B (**17**, 0.049%)
 retrofractamide C (0.036%)
 guineensine (**18**, 0.087%)

dihydrogineensine (**19**, 0.0015%)
 brachystamide B (**24**, 0.0083%)
 N-isobutyl-(2E,4E)-deca-2,4-dienamide (pellitorine, **20**, 0.018%)
 N-isobutyl-(2E,4E)-dodeca-2,4-dienamide (**21**, 0.0034%)
 N-isobutyl-(2E,4E)-tetradeca-2,4-dienamide (0.0044%)
 N-isobutyl-(2E,4E)-octadeca-2,4-dienamide (**22**, 0.139%)
 N-isobutyl-(2E,4E)-hexadeca-2,4-dienamide (0.011%)
 N-isobutyl-(2E,4E,8Z)-tetradeca-2,4,8-trienamide (0.0017%)
 N-isobutyl-(2E,4E,12Z)-octadeca-2,4,12-trienamide (0.0057%)
 N-isobutyl-(2E,4E,14Z)-eicosa-2,4,14-trienamide (**23**, 0.18%)
 2,4-pentadienamide (0.0011%)
 3,4-dihydroxybisabola-1,10-diene (0.0081%)
 1-hydroxybisabola-2,10-dien-4-one (0.00065%)
 1,4-dihydroxybisaboladiene (0.0079%)
 benzenepropanoic acid (0.13%)
 2-propenal (0.0031%)
 1-cinnamoylpiperide (0.0016%)
 ilepcimide (0.0048%)
 isopiperine (0.040%)
 isochavicine (0.039%)
 chavicine (0.0042%)

Effects of the Aq. 80% Acetone Extract and Constituents of the Fruit of *P. chaba* on D-GalN/TNF- α -Induced Cell Death in Primary Cultured Mouse Hepatocytes – 1

Methods

Williams' E medium (10% FCS)

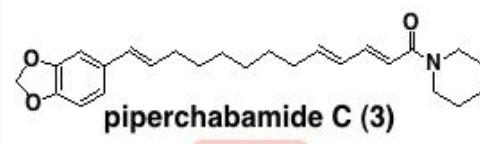
(D-GalN (1 mM)
 TNF- α (20 ng/ml))
 Test sample

Incubation for 20 h
 37°C, 5% CO₂

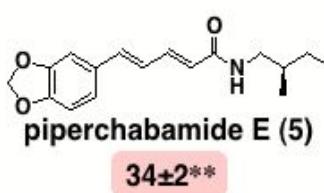
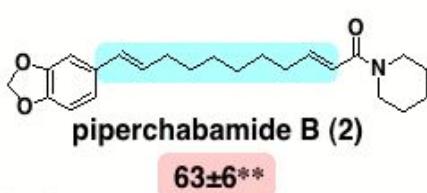
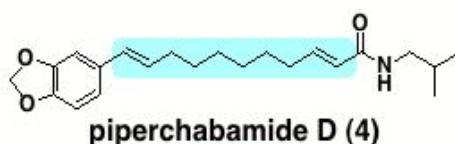
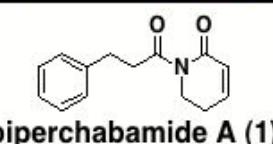
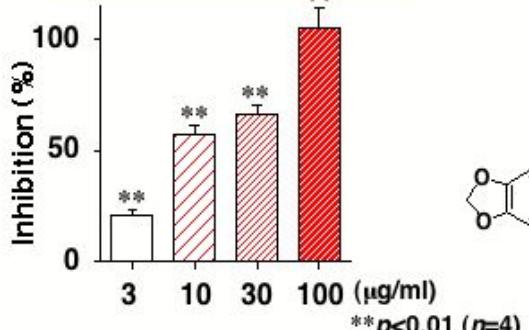
MTT assay

MTT solution
 Incubation for 4 h

Inhibition (%) at 3 μ M

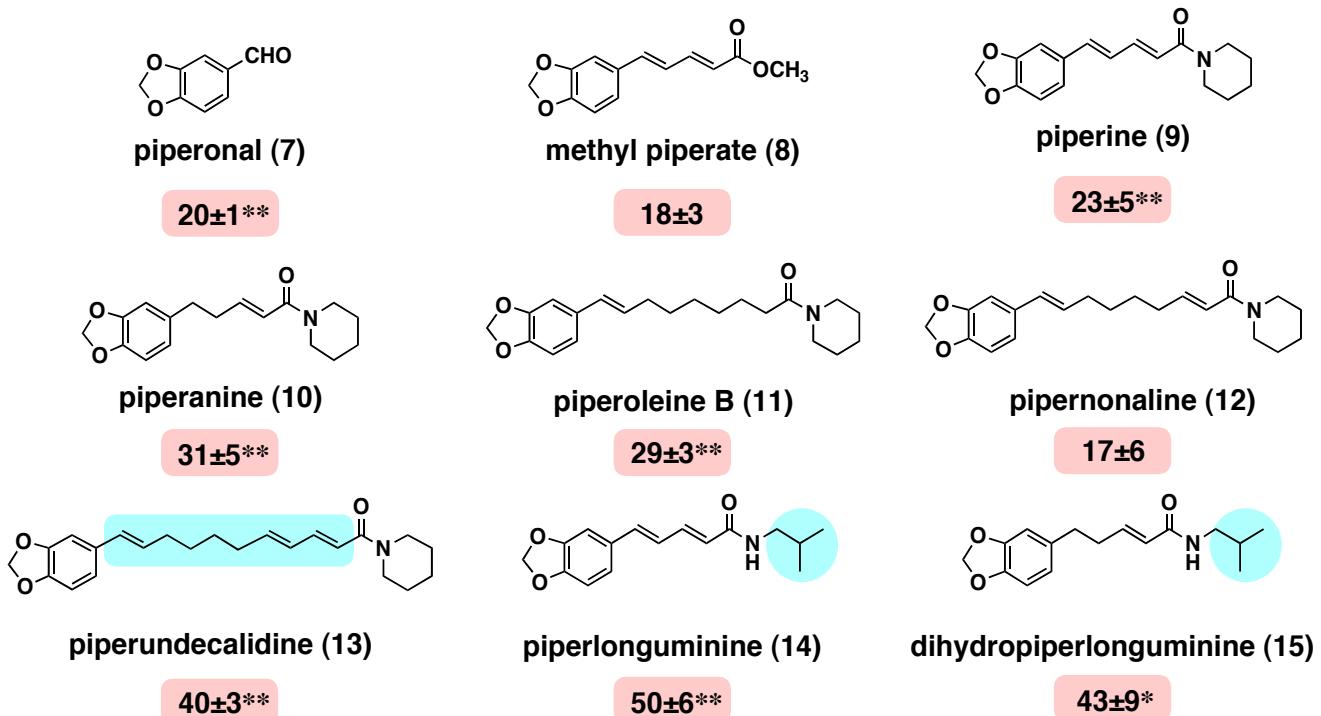


the Aq. 80% Acetone Ext.



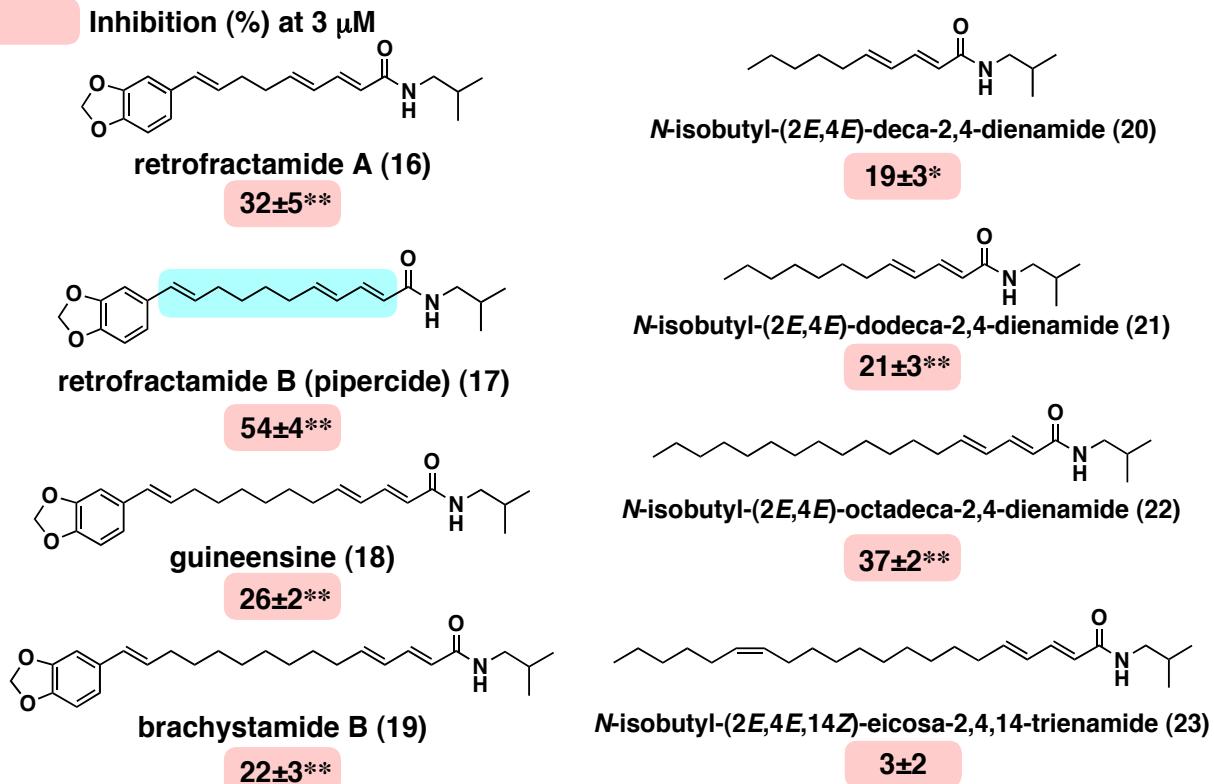
Effects of Constituents of the Fruit of *P. chaba* on D-GalN/TNF- α -Induced Cell Death in Primary Cultured Mouse Hepatocytes – 2

Inhibition (%) at 3 μ M

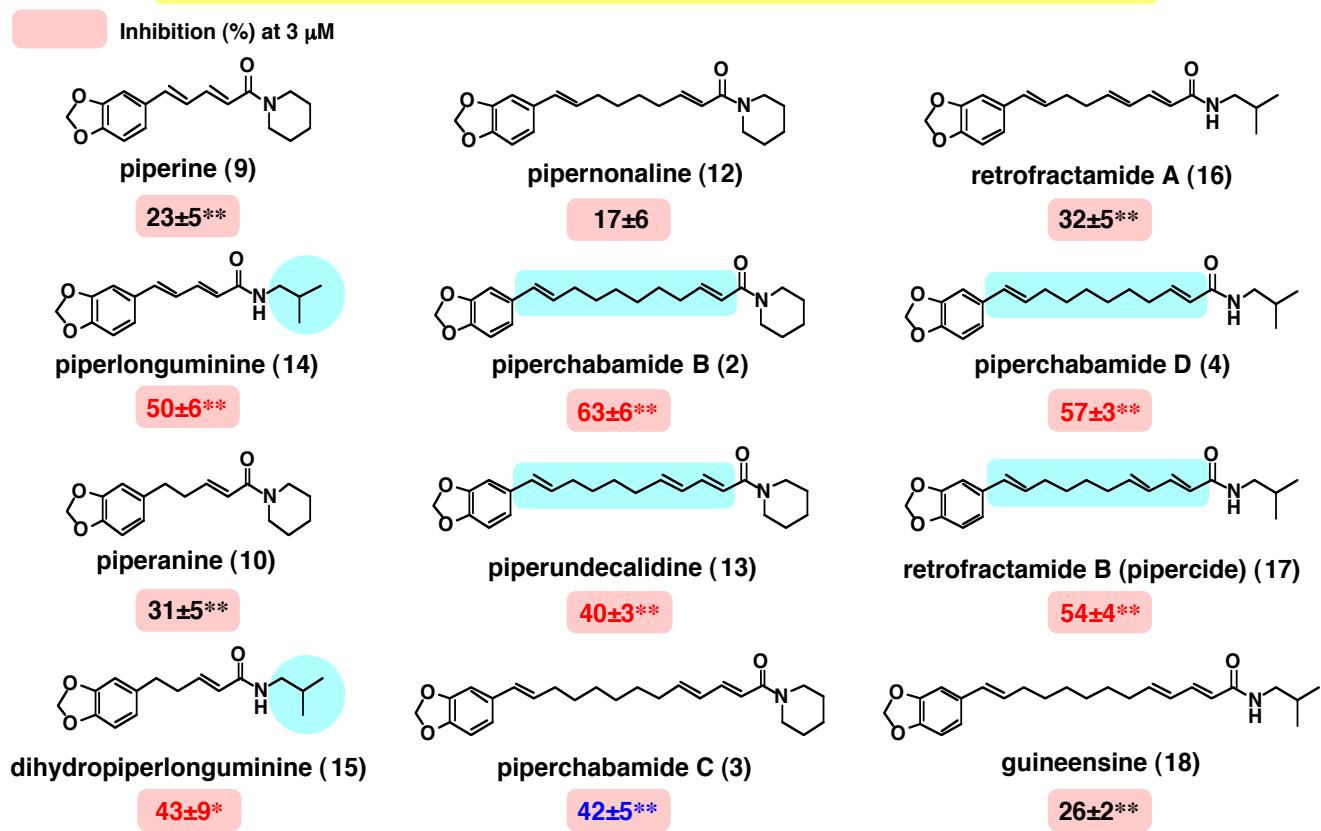


Effects of Constituents of the Fruit of *P. chaba* on D-GalN/TNF- α -Induced Cell Death in Primary Cultured Mouse Hepatocytes – 3

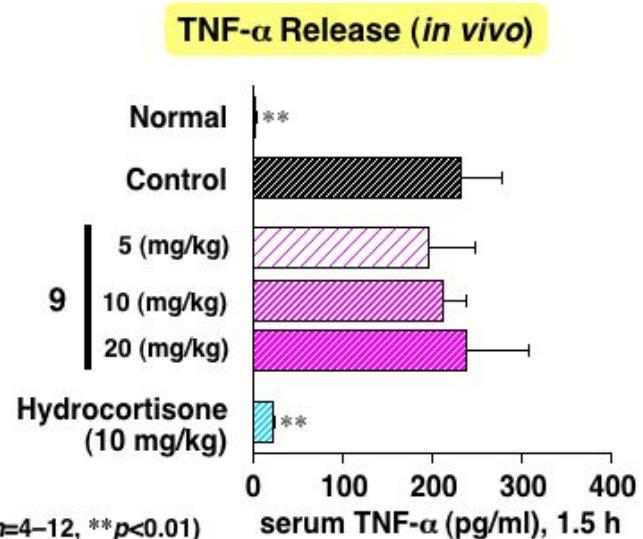
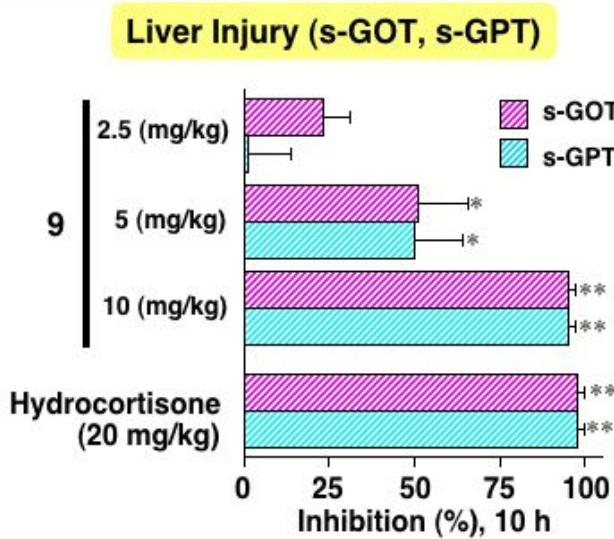
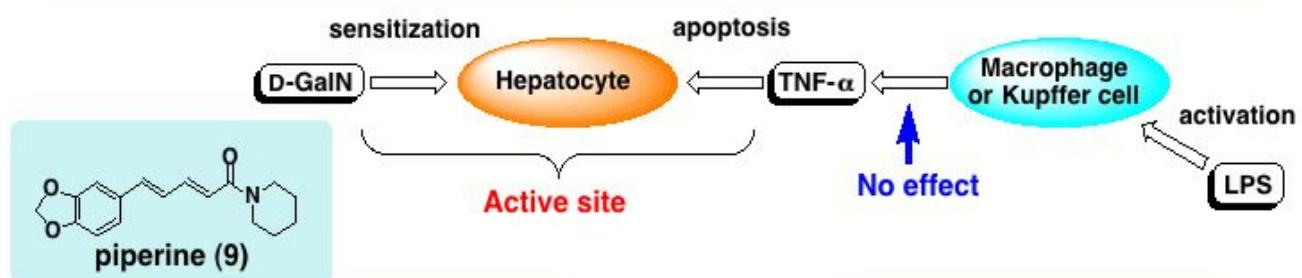
Inhibition (%) at 3 μ M



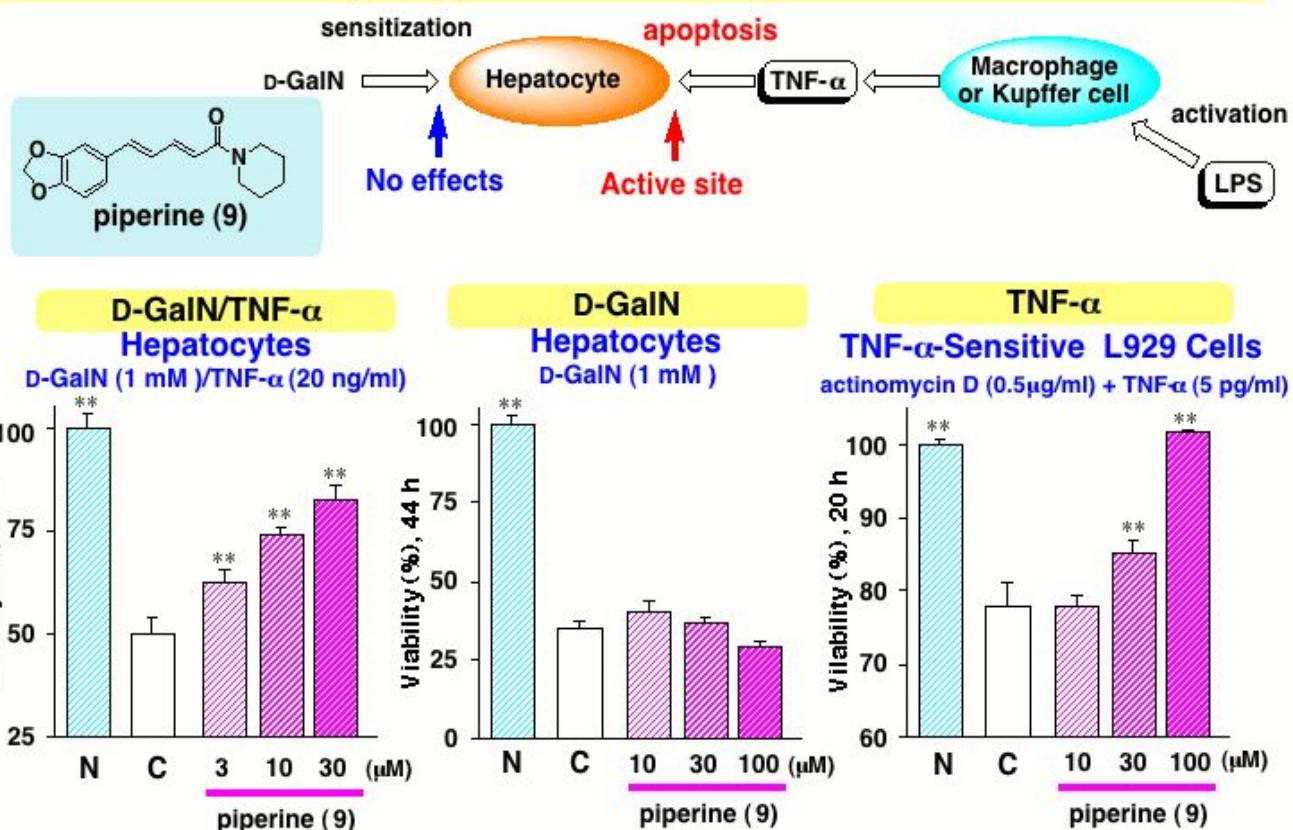
Structure-Activity Relations of Amide Constituents with 3,4-Methylenedioxyphenyl Group for the Activity



Effects of Piperine (9) on Liver Injury and Serum TNF- α Elevation Induced by D-GalN/LPS in Mice



Effects of Piperine (9) on D-GaIN/TNF- α - or D-GaIN-Induced Cell Death in Primary Cultured Mouse Hepatocytes and TNF- α -Induced Cell Death in L929 Cells

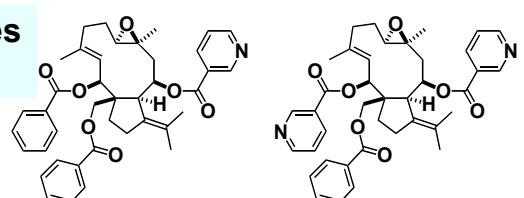


Bioorg. Med. Chem. Lett., **18**, 2038–2042 (2008); *Bioorg. Med. Chem.*, **17**, 7313–7323 (2009).

Biofunctional Molecules of Several Egyptian Herbal Medicines

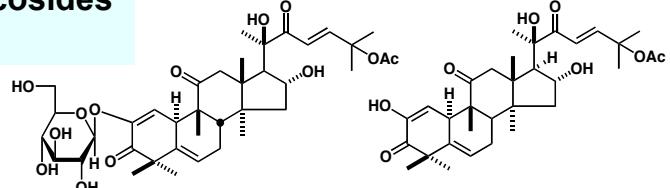
1. Black Cumin (the seed of *Nigella sativa*)

Reduces triglyceride levels in mouse hepatocytes
... Dolabellane-type diterpenes



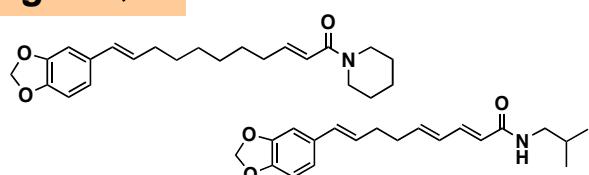
2. Colocynth (the fruit of *Citrullus colocynthis*)

Antiallergic activity ... Triterpene glycosides
Cytotoxic activity ... Triterpenes



3. Black Pepper (the fruit of *Piper nigrum*)

Hepatoprotective activity,
PPAR γ agonist-like activity ... amides



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