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CONSTITUENTS AND BIOACTIVITIES OF *CLAUSENA EXCAVATA*

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Abstract - *Clausena excavata* Burm. f. (Rutaceae) is a medicinal plant which is used in folklore medicine for treatment of cold, malaria, AIDS, dermatopathy, abdominal pain, and snake-bite. This plant is a rich source of coumarins and carbazole alkaloids. So far, fifty-three coumarins and fifty-eight carbazole alokaloids were isolated from *C. excavata*. Furthermore, a small group of tetranortriterpenoids, steroids, flavonoids, and essential oils were also obtained from this plant. *C. excavata* showed diverse therapeutic activities which are antibacterial, antifungal, antiplatelet, antiplasmodial, antitumor, antinociceptive, immunomodulatory, antimycobacterial, and anti-HIV-1 activities. The incidence of HIV-1 infection leading to AIDS has increased every year, and fungal and bacterial infections, particularly TB-causing mycobacteria are prevalent in HIV-infected patients. So *Clausena excavata* which showed inhibition of these diseases, is very promising to be developed for treatment of AIDS.

INTRODUCTION

Clausena excavata Burm. f. (Rutaceae) (Figure 1) is a wild shrub which is widely distributed in southern and southeastern Asia. Local Thai people usually call it by the name “Sun Soak” which is used in folklore medicine for treatment of cold, malaria, AIDS, dermatopathy, abdominal pain, snake-bite and as a detoxification agent.[1] *C. excavata* is a rich source of coumarins and carbazole alkaloids, however a small group of tetranortriterpenoids, steroids and flavonoids has been reported. In addition, its leaves contain essential oils such as β-elemene (95.8%), β-caryophyllene (25.3%), safrole (82%), nerolidol (4.7%) and germacrene (11.8%).[2] Some isolated substances from *Clausena excavata* as well as other *Clausena* genus have been reported to exhibit diverse biological activities, antibacterial,[3] antiplatelet,[4] antiplasmodial,[5] antitumor,[6] antimycobacterial and antifungal activity.[7] *C.excavata* extracts from the leaves showed

antinociceptive activity[8] and that from the woods had immunomodulatory activity.[9] Furthermore, the rhizomes and roots of *C. excavata* showed anti-HIV-1 activity.[10,11]

Figure 1. *Clausena excavata* Burm.f (Rutaceae)

In Thailand, the incidence of HIV-1 infection leading to acquired immunodeficiency syndrome (AIDS) has dangerously increased every year, and fungal and bacterial infections, particularly TB-causing mycobacteria, often are associated with HIV-infected patients. Currently, there is no effective remedy for curing AIDS. In traditional Thai medicine, some patients with AIDS drink the extract of *C.excavata* obtained by soaking the roots and rhizomes in Thai whiskey that contains approximately 35% ethanol.

The crude 35% ethanol extract of the roots and rhizomes showed anti-HIV-1 activity with EC_{50} values of 8.67 μg/mL, IC_{50} values of 32.83 μg/mL and potential therapeutic index (PTI) values of 3.79. Whereas the water extract showed less activity with EC_{50} = 73.9 μg/mL and IC_{50} > 250 μg/mL and PTI = >3.38.[11b]

CONSTITUENTS ISOLATED FROM *CLAUSENA EXCAVATA*

Compounds isolated from *Clausena excavata* belong to different classes such as coumarins, carbazole alkaloids, and limonoids (tetranortriterpenoids) as shown in Tables 1 – 3.

2.1 Coumarins

So far, fifty-three coumarins were isolated from various parts of *Clausena excavata* as shown in Table 1, Figures 2 and 3. Root barks, stem barks, leaves, twigs, branch, rhizomes and roots contain quite a large amount of coumarins. Hexane, acetone, chloroform, ethyl acetate, methanol, and ethanol were used for extraction. Clausenidin (**4**) showed significant anti-HIV-1 activity.[11]

2.2 Carbazole alkaloids

Fifty-eight alkaloids were isolated from *C. excavata* as shown in Table 2 and Figure 4. Three carbazoles, clauzoline J (**95**), 3-formyl-2,7-dimethoxycarbazole (**101**), and *O*-methylmukanol (**109**) possessed anti-HIV-1 activity.

2.3 Limonoids (Tetranortriterpenoids)

Six limonoids were isolated from the ethanol extracts of aerial parts and rhizomes as shown in Table 3 and Figure 5. Clausenolide-1-ethyl ether (**117**) isolated from the rhizome of *C.excavata* showed anti HIV-1 activity.[10]

Table 1. Coumarins isolated from *Clausena excavata*

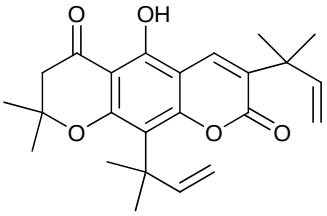
Substance	Part of plant	Solvent for extraction	Reference
claucavatin-A (**1**)	root barks	acetone	12
claucavatin-B (**2**)	root barks	acetone	12
clausarin (**3**)	root barks	EtOH	3
	root barks	acetone	12
	stem barks	MeOH	14
	roots	acetone	15
clausenidin (**4**)	root barks	EtOH	3
	rhizome	hexane	7 and 11
	roots	acetone	15
	root barks	acetone	12 and 13
clausenidinaric acid (**5**)	root barks	EtOH	3
clauslactone-A (**6**)	leaves	acetone	6
clauslactone-B (**7**)	leaves	acetone	6
clauslactone-C (**8**)	leaves	acetone	6
clauslactone-D (**9**)	leaves	acetone	6
clauslactone-F (**10**)	leaves	acetone	6

Table 1. (continued)

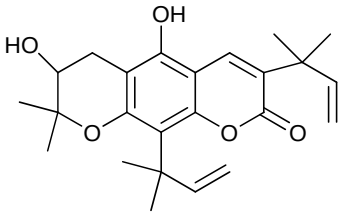
Substance	Part of plant	Solvent for extraction	Reference
clauslactone-G (**11**)	leaves	acetone	6
clauslactone-H (**12**)	leaves	acetone	6
clauslactone-I (**13**)	leaves	acetone	6
clauslactone-J (**14**)	leaves	acetone	6
clauslactone-K (**15**)	leaves and twigs	acetone and MeOH	16
clauslactone-L (**16**)	leaves and twigs	acetone and MeOH	16
clauslactone-M (**17**)	leaves and twigs	acetone and MeOH	16
clauslactone-N (**18**)	leaves and twigs	acetone and MeOH	17
clauslactone-O (**19**)	leaves and twigs	acetone and MeOH	17
clauslactone-P (**20**)	leaves and twigs	acetone and MeOH	17
clauslactone-Q (**21**)	leaves and twigs	acetone and MeOH	17
clauslactone-R (**22**)	leaves abd Stems	EtOH	18
clauslactone-S (**23**)	leaves abd Stems	EtOH	18
clauslactone-T (**24**)	leaves abd Stems	EtOH	18
dentatin (**25**)	roots	acetone	15
	rhizomes	$CHCl_3$	7
nordentatin (**26**)	root barks	EtOH	3
	rhizomes	$CHCl_3$	7
	roots	acetone	15
	root barks	acetone	12 and 13
	stem barks	MeOH	14
excavacoumarin-A (**27**)	leaves	EtOH	19
excavacoumarin-B (**28**)	aerial parts	EtOH	20
excavacoumarin-C (**29**)	aerial parts	EtOH	20
excavacoumarin-D (**30**)	aerial parts	EtOH	20

Table 1. (continued)

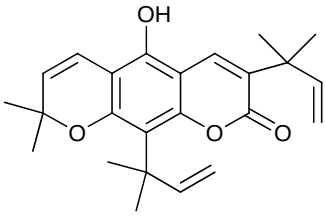
Substance	Part of plant	Solvent for extraction	Reference
excavacoumarin-E (**31**)	aerial parts	EtOH	20
excavacoumarin-F (**32**)	aerial parts	EtOH	20
excavacoumarin-G (**33**)	aerial parts	EtOH	20
excavacoumarin-H (**34**)	aerial parts	EtOH	21
excavacoumarin-I (**35**)	aerial parts	EtOH	21
excavatin-A (**36**)	leaves	MeOH	19 and 22
excavatin-B (**37**)	leaves	MeOH	22
excavatin-C (**38**)	leaves	MeOH	22
excavatin-D (**39**)	leaves	MeOH	22
excavatin-E (**40**)	leaves	MeOH	22
excavatin-F (**41**)	leaves	MeOH	22
excavatin-G (**42**)	leaves	MeOH	22
excavatin-H (**43**)	leaves	MeOH	22
excavatin-I (**44**)	leaves	MeOH	22
excavatin-J (**45**)	leaves	MeOH	22
excavatin-K (**46**)	leaves	MeOH	22
excavatin-L (**47**)	leaves	MeOH	22
excavatin-M (**48**)	leaves	MeOH	22
scopoletin (**49**)	leaves	$CHCl_3$	4
xanthoxyletin (**50**)	root barks	EtOH	3
	rhizomes	$CHCl_3$	7
	root barks	acetone	12 and 13
	stem barks	MeOH	14
	roots	acetone	15
xanthyletin (**51**)	roots	acetone	15
	root barks	acetone	12
dicoumarin Type			
cladimarin A (**52**)	branch	acetone and MeOH	23
cladimarin B (**53**)	branch	acetone and MeOH	23



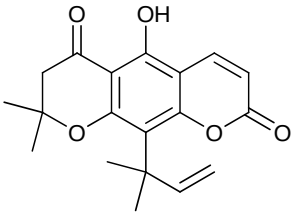
claucavatin-A
(**1**)



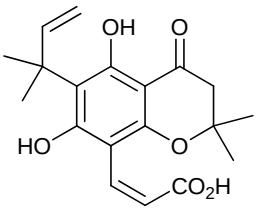
claucavatin-B
(**2**)



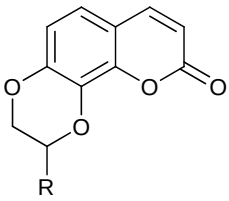
clausarin
(**3**)



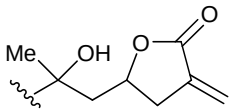
clausenidin
(**4**)



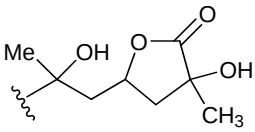
clausenidinaric acid
(**5**)



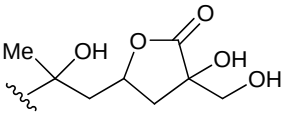
clauslactone-A (**6**) R =



clauslactone-B (**7**) R =



clauslactone-C (**8**) R =



clauslactone-D (**9**) R =

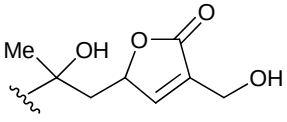


Figure 2. Coumarins isolated from *Clausena excavata*

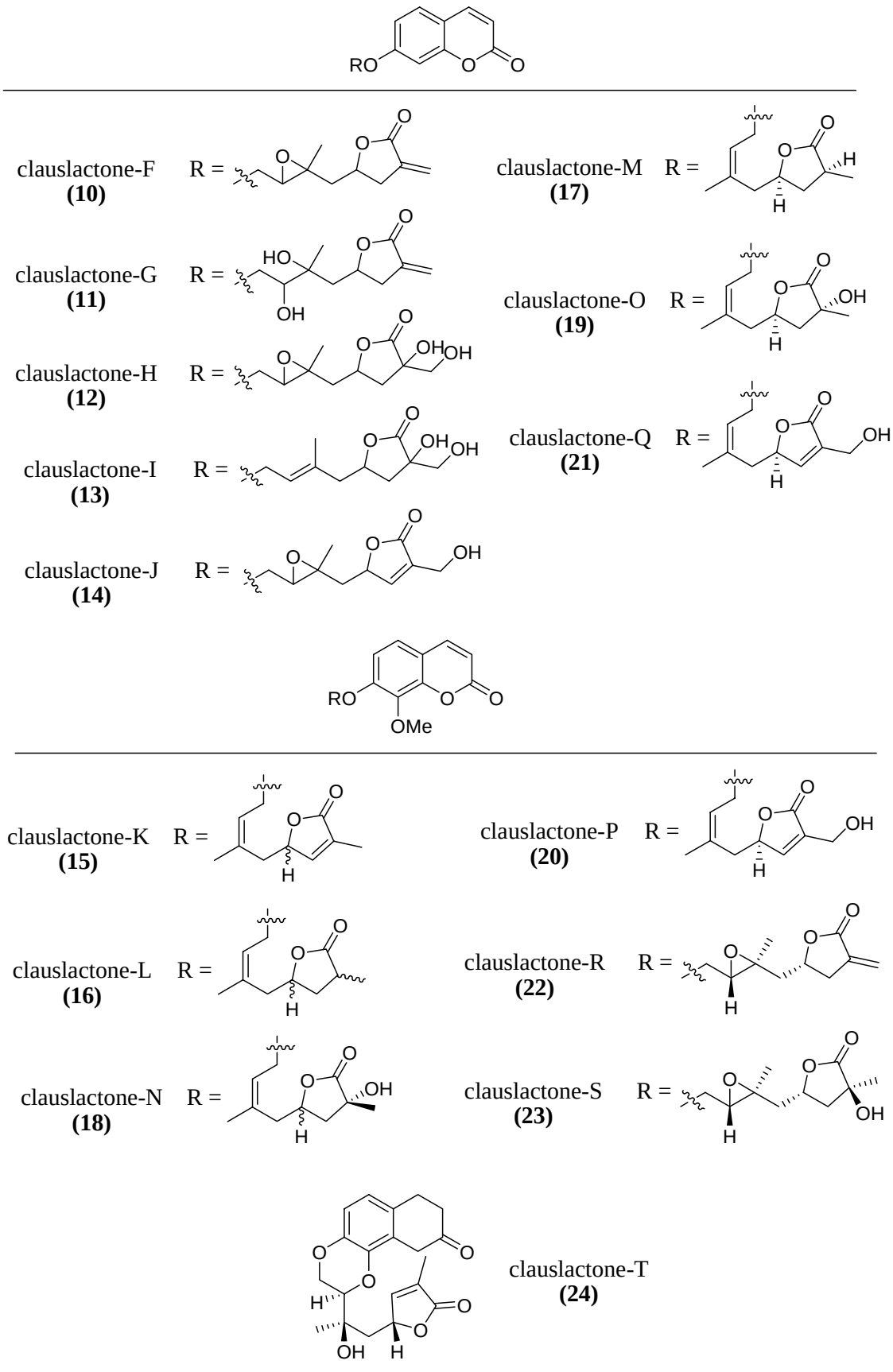
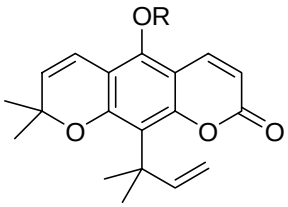
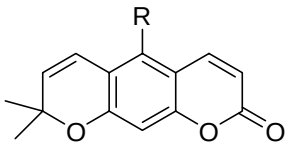


Figure 2. (continued)



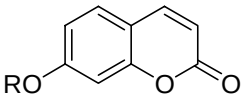
dentatin **(25)** R = OMe

nordentatin **(26)** R = H



xanthoxyletin **(50)** R = OMe

xanthyletin **(51)** R = H



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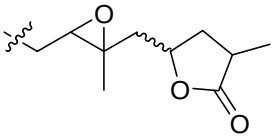
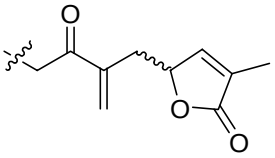
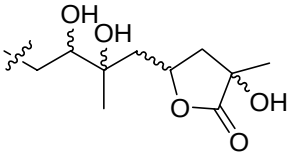
excavacoumarin-A R = **(27)**



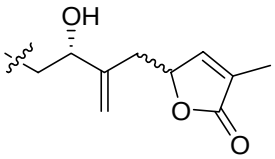
excavacoumarin-B R = **(28)**



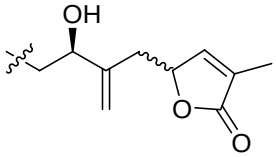
excavacoumarin-C R = **(29)**



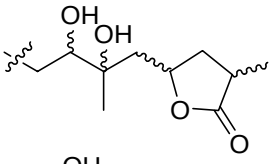
excavacoumarin-D R = **(30)**



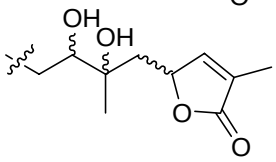
excavacoumarin-E R = **(31)**



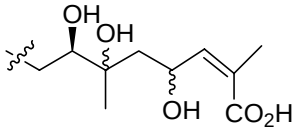
excavacoumarin-F R = **(32)**



excavacoumarin-G R = **(33)**



excavacoumarin-H R = **(34)**



excavacoumarin-I R = **(35)**

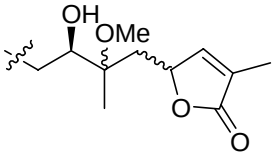
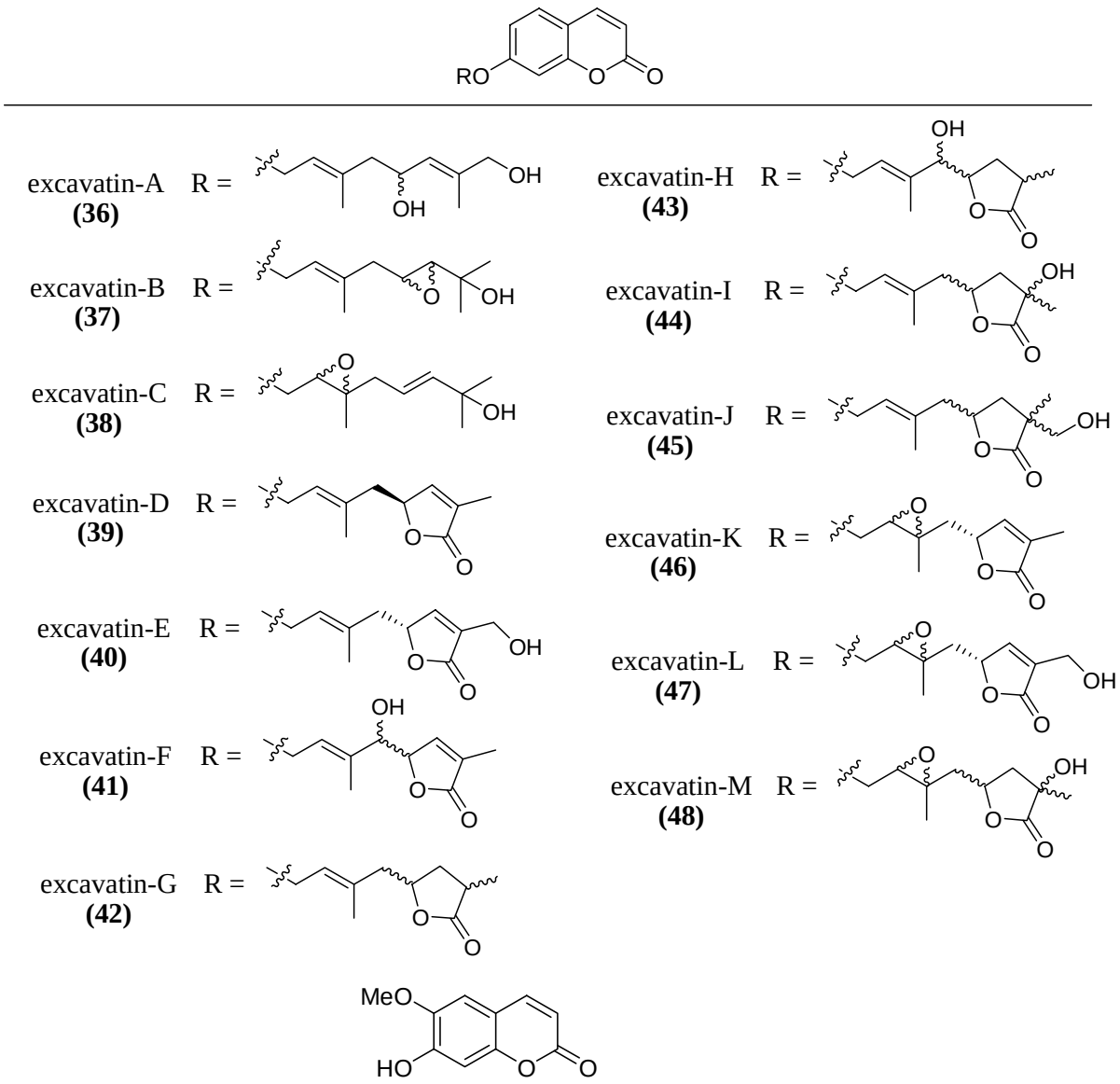


Figure 2. (continued)



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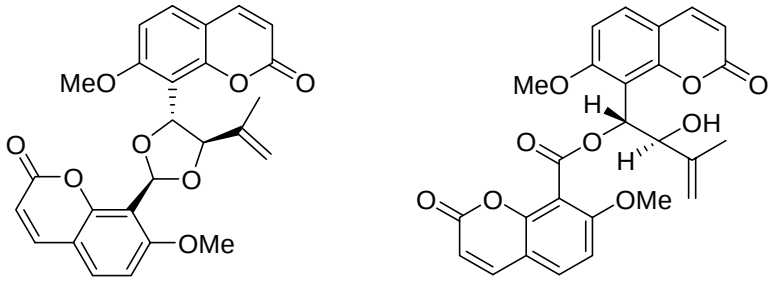


cladimarin A (**52**) cladimarin B (**53**)

Figure 3. Dicoumarin isolated from *Clausena excavata*

Table 2. Carbazole Alkaloids isolated from *Clausena excavata*

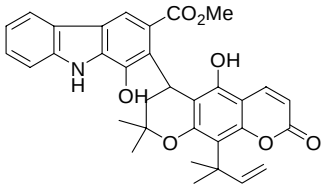
Substance	Part of plant	Solvent for extraction	Reference
carbazomarin-A (**54**)	root barks	acetone	24
clausamine-A (**55**)	root barks	acetone	25 and 13
clausenamine-A (**56**)	stem barks	MeOH	24
clausenatine-A (**57**)	root barks	acetone	13
clausevatine-D (**58**)	root barks	acetone	25 and 13
clausevatine-E (**59**)	root barks	acetone	25
clausevatine-F (**60**)	root barks	acetone	25 and 13
clausevatine-G (**61**)	root barks	acetone	25 and 13
clausine-A (**62**)	stem barks	MeOH	26
clausine-B (**63**)	stem barks	MeOH	14
clausine-C (**64**)	stem barks	MeOH	26
	root barks	acetone	13
clausine-D (**65**)	stem barks	acetone and MeOH	27 and 14
	roots	acetone	15
clausine-E (**66**)	root barks	acetone and MeOH	14 and 13
clausine-F (**67**)	stem barks	acetone	27
		MeOH	14
clausine-G (**68**)	stem barks	MeOH	26
clauusine-H (**69**)	stem barks	MeOH	14
clauusine-I (**70**)	stem barks	MeOH	14
clausine-J (**71**)	stem barks	MeOH	26
clausine-K (**72**)	stem barks	MeOH	14
	root barks	acetone	13
clausine-L (**73**)	leaves	MeOH	4
	rhizomes	$CHCl_3$	7b
	stem barks	acetone	28
clausine-M (**74**)	root barks	acetone	13
clausine-N (**75**)	root barks	acetone	13

Table 2. (continued)

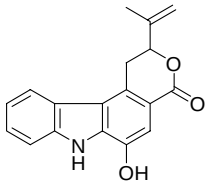
Substance	Part of plant	Solvent for extraction	Reference
clausine-O (**76**)	root barks	acetone	13
clausine-P (**77**)	root barks	acetone	13
clausine-Q (**78**)	root barks	acetone	13
clausine-R (**79**)	root barks	acetone	13
clausine-S (**80**)	root barks	acetone	13
clausine-T (**81**)	root barks	acetone	29 and 13
clausine-U (**82**)	root barks	acetone	13
clausine-V (**83**)	root barks	acetone	13
clausine-W (**84**)	root barks	acetone	13
clausine-TY (**85**)	stem barks	EtOAc	30
clauszoline-A (**86**)	stem barks	acetone	28
clauszoline-B (**87**)	stem barks	acetone	28
clauszoline-C (**88**)	stem barks	acetone	28
clauszoline-D (**89**)	stem barks	acetone	28
clauszoline-E (**90**)	stem barks	acetone	28
clauszoline-F (**91**)	stem barks	acetone	28
clauszoline-G (**92**)	stem barks	acetone	28
clauszoline-H (**93**)	roots	acetone	15
clauszoline-I (**94**)	roots	acetone	15
clauszoline-J (**95**)	rhizomes	EtOH	11
	roots	acetone	15
		$CHCl_3$	7
clauszoline-K (**96**)	stem barks	acetone	15
clauszoline-L (**97**)	stem barks	acetone	15
clauszoline-M (**98**)	leaves	acetone	15 and 6
O-demethyl-	roots	acetone	15
murrayanine (**99**)	rhizomes	EtOH	7b
3-formylcarbazole (**100**)	root barks	acetone	3
	rhizomes and	$CHCl_3$	7
	roots		

Table 2. (continued)

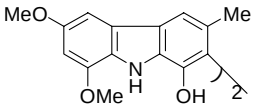
Substance	Part of plant	Solvent for extraction	Reference
3-formyl-2,7-	root barks	acetone	13
dimethoxycarbazole (**101**)	roots	acetone	15
	rhizomes	$CHCl_3$	11
furoclausine-A (**102**)	root barks	acetone	29 and 13
furoclausine-B (**103**)	root barks	acetone	29 and 13
heptaphylline (**104**)	root barks	EtOH	3
	rhizomes	$CHCl_3$	7b
	stem barks	acetone	13
	roots	acetone	28
		MeOH	14
		acetone	15
2-hydroxy-3-formyl-7-	stem barks	acetone	15
methoxycarbazole (**105**)	roots	$CHCl_3$	7
3-methylcarbazole (**106**)	stem barks	MeOH	14
	root barks	acetone	13
methyl carbazole-3-	stem barks	MeOH	14
carboxylate (**107**)	rhizomes	$CHCl_3$	7
	root barks	acetone	13
mukonal (**108**)	stem barks	MeOH	14
	root barks	acetone	13
	rhizomes	$CHCl_3$	7
O-methylmukonal (**109**)	rhizomes	$CHCl_3$	11
mukonine (**110**)	root barks	acetone	13
	rhizomes	$CHCl_3$	7b
murrayanine (**111**)	stem barks	MeOH	14
	rhizomes	$CHCl_3$	7b
	root barks roots	acetone	13
		acetone	15



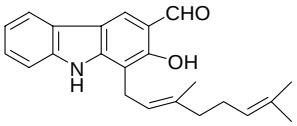
carbazomarin-A
(**54**)



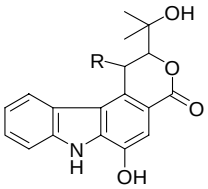
clausamine-A
(**55**)



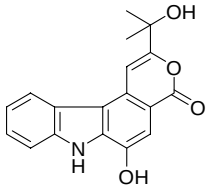
clausenamine-A
(**56**)



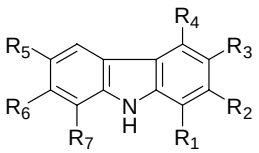
clausenatine-A
(**57**)



clausenatine-D (**58**) R = H
clausenatine-E (**59**) R = *cis*-OH
clausenatine-F(**60**) R = *trans*-OH

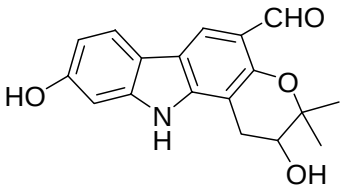


clausenatine-G
(**61**)

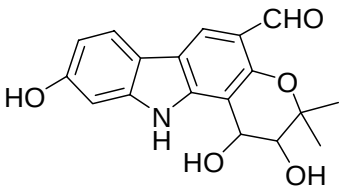


	R_1	R_2	R_3	R_4	R_5	R_6	R_7
clausine-A (**62**)	H	OH	CHO	H	H	H	H
clausine-B (**63**)	H	OH	CHO	H	OMe	H	OMe
clausine-C (**64**)	H	H	CO_2Me	H	H	OMe	H
clausine-D (**65**)	OH	H	CHO		H	H	H
clausine-E (**66**)	OH	H	CO_2Me	H	H	H	H
clausine-F (**67**)	OH	H	CO_2Me		H	H	H
clausine-G (**68**)	OH	H	CO_2Me	H	OMe	H	H
clausine-H (**69**)	H	OMe	CO_2Me	H	H	OMe	H
clausine-I (**70**)	OH	H	CHO	OMe	H	H	H
clausine-J (**71**)	OH	H	CHO	H	OMe	OH	H
clausine-K (**72**)	H	OMe	CO_2H	H	H	OMe	H
clausine-L (**73**)	H	OMe	CO_2Me	H	H	H	H
clausine-M (**74**)	H	H	CO_2Me	H	H	OH	H
clausine-N (**75**)	H	H	CO_2H	H	H	OMe	H
clausine-O (**76**)	H	OH	CHO	H	H	OMe	H
clausine-P (**77**)	H	OMe	Me	H	H	H	OMe
clausine-Q (**78**)	OMe	H	CHO	H	H	OH	H
clausine-R (**79**)	OH	H	CO_2Me	H	H	OH	H
clausine-S (**80**)		OH	CHO	H	H	H	H
clausine-U (**82**)		OH	CHO	H	H	OH	H
clausine-V (**83**)	H	OMe	H	H	H	OMe	H
clausine-TY (**85**)	H	OH	CO_2Me	H	H	OMe	H

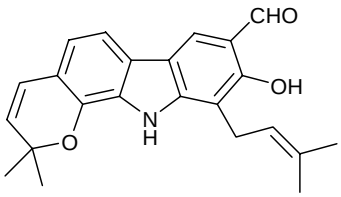
Figure 4. Carbazoles isolated from *Clausena excavata*



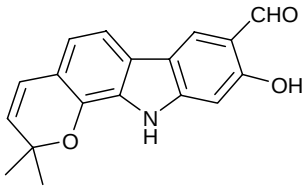
clausine-T (**81**)



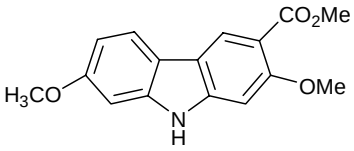
clausine-W (**84**)



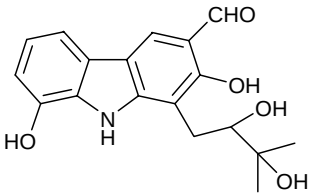
clauszoline-A (**86**)



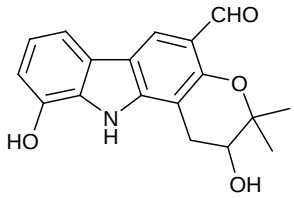
clauszoline-B (**87**)



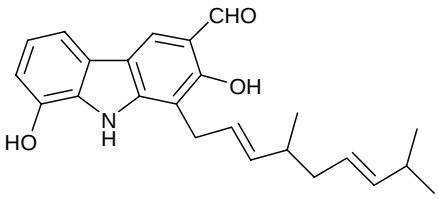
clauszoline-C (**88**)



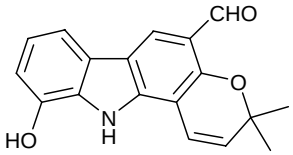
clauszoline-D (**89**)



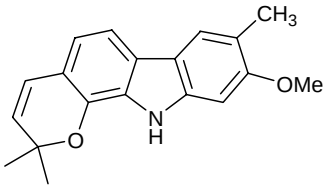
clauszolineE (**90**)



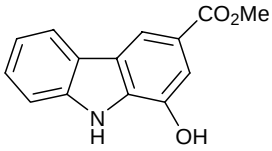
clauszoline-F (**91**)



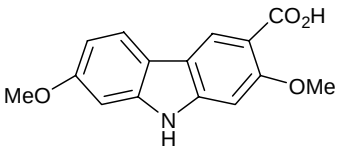
clauszoline-G (**92**)



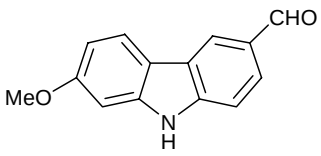
clauszoline-H (**93**)



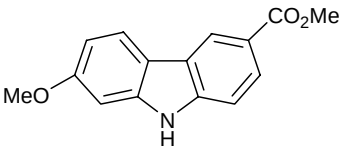
clauszoline-I (**94**)



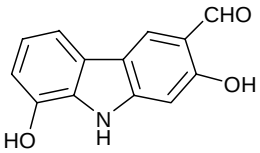
clauszoline-J (**95**)



clauszoline-K (**96**)

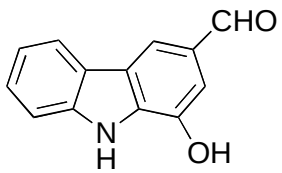


clauszoline-L (**97**)

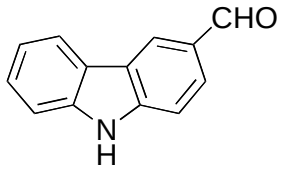


clauszoline-M (**98**)

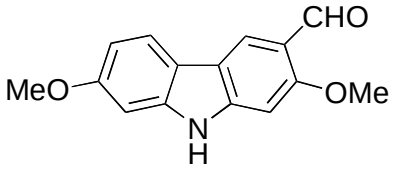
Figure 4. (continued)



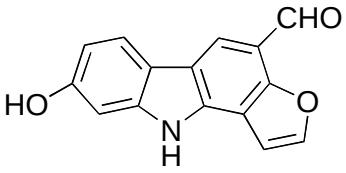
O-demethylmurrayanine
(**99**)



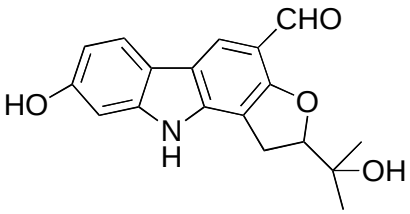
3-formylcarbazole
(**100**)



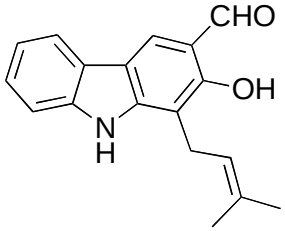
3-formy-2,7-dimethoxycarbazole
(**101**)



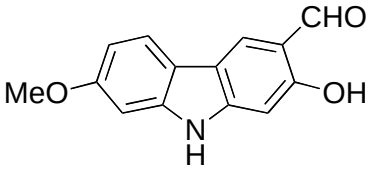
furoclausine-A
(**102**)



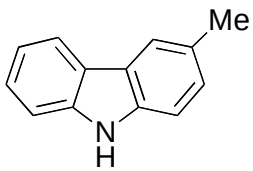
furoclausine-B
(**103**)



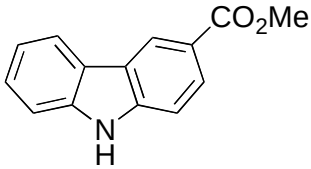
heptaphylline
(**104**)



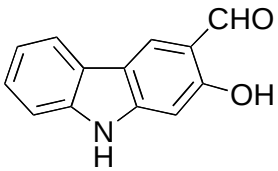
2-hydroxy-3-formyl-7-
methoxycarbazole (**105**)



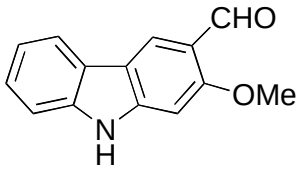
3-methylcarbazole
(**106**)



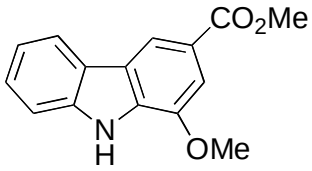
methyl carbazole-3-carboxylate
(**107**)



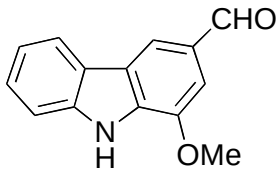
mukonal
(**108**)



O-methylmukonal
(**109**)



mukonine
(**110**)

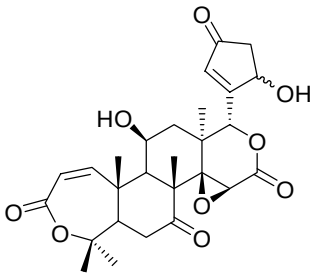


murrayanine
(**111**)

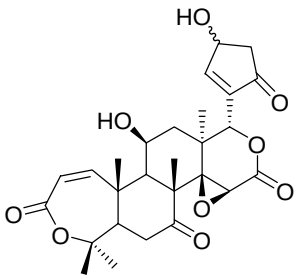
Figure 4. (continued)

Table 3. Limonoids (tetranortriterpenoids) isolated from *Clausena excavata*

Substance	Part of plant	Solvent for extraction	Reference
(11β)-21,23-dihydro-11,21-dihydroxy-23-oxoobacunone (**112**)	aerial parts	EtOH	31
(11β)-21,23-dihydro-11,23-dihydroxy-21-oxoobacunone (**113**)	aerial parts	EtOH	31
(1α, 11β)-1,2,21,23-tetrahydro-1,11,23-trihydroxy-21-oxoobacunone (**114**)	aerial parts	EtOH	31
(1α, 11β)-23-ethoxy-1,2,21,23-tetrahydro-1,11-dihydroxy-21-oxoobacunone (**115**)	aerial parts	EtOH	31
(11β)-1,2,21,23-tetrahydro-11,23-dihydroxy-21-oxoobacunoic acid (**116**)	aerial parts	EtOH	31
clausenolide-1-ethyl ether (**117**)	rhizomes	EtOH	10

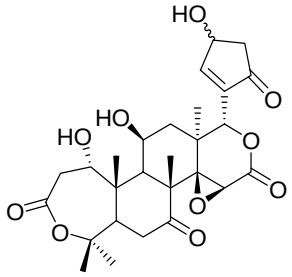


(11β)-21,23-dihydro-11,21-dihydroxy-23-oxoobacunone (**112**)

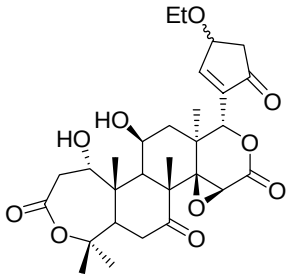


(11β)-21,23-dihydro-11,23-dihydroxy-21-oxoobacunone (**113**)

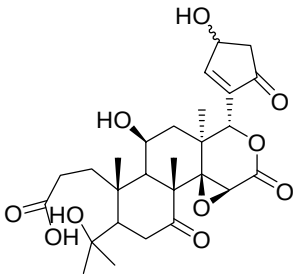
Figure 5. Limonoids isolated from *Clausena excavata*



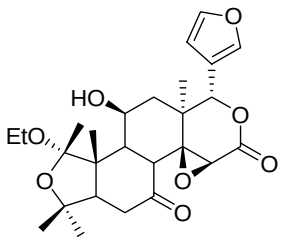
(1α, 11β)-1,2,21,23-tetrahydro-1,11,23-oxoobacunone (**114**)



(1α, 11β)-23-ethoxy-1,2,21,23-tetrahydro-1,11-dihydroxy-21-oxoobacunone (**115**)



(11β)-1,2,21,23-tetrahydro-11,23-dihydroxy-21-oxoobacunoic acid (**116**)



clausenolide-1-ethyl ether (**117**)

Figure 5. (continued)

BIOACTIVITIES

Clausena excavata is used in diverse aspects of traditional medicine in Asian countries. Several bioassays were done on this plant and many properties were found which support the folk medicine. The therapeutic activities of this plant are:

3.1 Antinociceptive activity

3.2 Antiplatelet activity

3.3 Antimicrobial activity

3.3.1 Antifungal activity

3.3.2 Antibacterial activity

3.4 Immunomodulatory activity

3.5 Antimycobacterial activity

3.6 Anti HIV-1 activity

3.1 Antinociceptive activity

In 2002, Rahman and coworkers[8] have found the ethanol extract of *C.excavata* leaves showed significant antinociceptive activity on acetic acid-induced writhing in mice by oral at doses of 125.25 and 500 mg/kg body weight. The ethanol extract was obtained by maceration of dried leaves in ethanol (4.08% yield). The report has not mentioned about the active constituents responsible for this activity. It was just stated that phytochemical screening indicated the presence of coumarins, flavonoides and glycosides. This plant is used in Bangladesh traditional medicine; for example, the sap of the leaves reduced the muscular pain; the root is used to reduce malarial fever; stems and roots are used for treatment of stomach problem; leaves and stem barks are used as tonic, diuretic and astringent.

3.2 Antiplatelet activity

Safrole, a volatile oil, has been isolated from the leaves of *C.excavata*[4] and inhibited rabbit platelet aggregation in 70% at the dose of 20 μg/mL, which were induced by arachidonic acid (100 μM). When collagen (10 μg/mL) was used instead, safrole only inhibited in 48% at a dose of 50 μg/mL. A carbazole, clausine-D (**65**) isolated from the leaves of *C. excavata* also exhibited antiplatelet activity *in vitro*. It inhibited platelet aggregation and released reaction of washed rabbit platelets. The inhibitory effect varied depending on the types of aggregation inducers. Clausine-D (**65**) inhibited most strongly when induced by arachidonic acid (AA) and inhibited less when induced by collagen whereas there was no effect when induced by U46619, PAF, and thrombin.[4]

The IC_{50} values of clausine-D (**65**) on arachidonic acid (AA) and collagen-induced platelet aggregation were 9.0 ± 1.1 and 58.9 ± 0.9 μM, respectively. Clausine D (**65**) also inhibited increased intracellular concentration of calcium in platelet aggregation caused by AA and collagen. In human citrated platelets-rich plasma, cluasine-D (**65**) inhibited the secondary phase, but not primary phase of aggregation induced by epinephrine and ADP. The results showed that the antiplatelet effect of clausine D (**65**) was due to inhibition of thromboxane A_2 formation.

3.3 Antimicrobial activity

3.3.1 Antibacterial activity

In 1982, Wu and Furukawa[3] have investigated that a coumarin, nordentatin possessed inhibitory affect against the bacteria *Bordetella brochiseptica* 4614, *Bacillus subtilis* 6633, *Pneumococcus*, *Staphylococcus aureus* 6538-P and *Pseudomonas aeruginoso* NCTC 10490 at more than 10 ppm concentration.

3.3.2 Antifungal activity

In 2002, we reported[7] that four carbazoles isolated from the rhizome and roots of *C.excavata*; 3-formylcarbazole (**100**), 2-hydroxy-3-formyl-7-methoxylcarbazole (**105**), 3-methoxycarbonylcarbazole (**107**) and mukonal (**108**) showed antifungal activity against *Candida albicans* with IC_{50} values of 13.6, 2.8, 9.5 and 29.3 μg/mL, respectively. In addition, these four carbazoles did not exhibit cytotoxicity against KB and BC-1 cell lines. This indicates a good sign for modification of these compounds by derivatization and evaluation for their antifungal activity.

3.4 Immunomodulatory activity

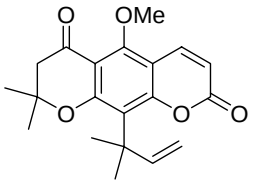
In 1998, Manosroi's group[9] found that the Thai folklore extract from wood of *C.excavata* increased CD_4 and CD_8 level as well as the CD_4/CD_8 ratio in cancer patients. They have proved the immunomodulatory activity of this plant using aqueous extract, acetone extract and Thai folklore extract (35% aqueous ethanol solution) to test *in vitro* mouse macrophage phagocytosis and splenocyte proliferation assay. The results showed as follow; all extracts showed phagocytic modulation, but no dose-response relationships. The aqueous extract (62.5 μg/ml) gave the most effective nitroblue tetrazolium (NBT) dye reduction[9a] which was approximately 30% and 20% more than that from the acetone and the folklore extracts, respectively. The aqueous extract (162.5 μg/ml) also exhibited potential lysosomal enzyme activity approximately 80% and 40% higher than those from the acetone and the folklore extracts, respectively. All extracts showed cell proliferation simulation with no dose-response relationship. The 35% aqueous ethanol extract gave the maximum proliferation enhancement both with and without mitogens by the MTT assay. All extracts did not cause cell death, with the percentage of viability of macrophages and splenocytes of more than 90% and 80%, respectively. All extracts stimulated phagocytic activity on lysosomal enzyme activity higher than NBT dye reduction. Although this work did not mention about the active constituents which had immunomodulatory activity, it is still useful to support the folklore medicine.

In addition, the same group[9b] has reported in 2004 that the hot aqueous extract and the acetone extract were more splenocyte-proliferation active than the folklore extract (35% aqueous ethanol extract). The following year, this group[9c] showed the effect *in vivo* of the crude extracts of *C.excavata* on the production of haemagglutinating antibodies (HA) in mice by intraperitoneal administration and oral route. They found that both aqueous extract and 35% aqueous ethanol extract gave the maximum antibody titer of 1/800 which was two times of the control. Oral administration appeared to reach the maximum heamagglutinating antibody (HA) titer[9c] faster than the intraperitoneal administration. Antibodies produced by orally administration of both extracts decreased with times without maintaining similar to

cimetidine. Phenolic compounds in the aqueous extract and 35% aqueous ethanol extract were preliminary observed by TLC spraying with $FeCl_3$ solution. Therefore the immunomodulating activity in both extracts might be due to the presence of phenolic groups. The aqueous extract and 35% aqueous ethanol extract (folklore) from wood of *C. excavata* showed potent in vitro and in vivo immunomodulating activity in mice. The folklore extract exhibited effective antibody production stimulation whereas the aqueous extract demonstrated superior cell-mediated immune (CMI) response.[9c]

3.5 Antimycobacterial activity

We reported[7] the antimycobacterial activity in 2003. The crude chloroform extract from the rhizomes of *C.excavata* exhibited antimycobacterial activity with a minimum inhibitory concentration (MIC) of 25 μg/ml. Further isolation by vacuum liquid chromatography (VLC) and then column chromatography (CC) gave dentatin (**25**), nordentatin (**26**), 3-formylcarbazole (**100**), methylcarbazole-3-carboxylate (**107**), and mukonal (**108**). The hexane extract of *C.excavata* rhizomes gave clausenidin. The crude chloroform extract of *C.excavata* roots were further isolated by VLC and CC to give clauszoline-J (**95**) and 2-hydroxy-3-formyl-7-methoxycarbazole (**105**). *O*-Methylated clausenidin (**118**) was obtained from methylation reaction of clausenidin (**4**) using methyl iodide in the presence of potassium carbonate. The antimycobacterial activity of courmarins and carbazoles were shown in Table 4.



O-methylated clausenidin (**118**)

Table 4. Antimycobacterial activity of coumarins and carbazoles from the rhizomes and roots of *Clausena excavata*

Compound	Antimycobacterium (MIC) (μg/mL)[a]
dentatin (**25**)	50
nordentatin (**26**)	100
clausenidin (**4**)	200
O-methylated clausenidin (**118**)	50
3-formylcarbazole (**100**)	100
mukonal (**108**)	200

methylcarbazole-3-carboxylate (**107**)	50
2-hydroxy-3-formyl-7-methoxycarbazole **(105**)	100
clauszoline J (**95**)	100
kanamycin sulfate	2.0-5.0[b]

[a] Data are typical values from replicate experiments

[b] Ranges of MIC (n=3)

Among these, dentatin (**25**), methylcarbazole-3-carboxylate (**107**) and *O*-methylated clausenidin (**118**) showed significant antimycobacterial activity (MIC = 50 μg/mL).

3.6 Anti-HIV-1 Activity

Some thai AIDS patients consume the extract of *C.excavata* obtained by soaking the rhizomes and roots in thai whisky (35% ethanol). Therefore we[10,11] have searched for anti HIV-1 coustituents from the extract of *C.excavata* rhizomes and roots since 2000. It was found that one limonoid,[10] clausenolide-1-ethyl ether (**117**); one pyranocoumarin,[11] clausenidin (**4**) and three carbazole alkaloids,[11] clauszoline-J (**95**), 3-formyl-2,7-dimethoxycarbazole (**101**), *O*-methylmukonal (**109**) inhibited HIV-1 viruses. The IC_{50} values of the inhibition and PTI are shown in Table 5.

Table 5. Anti-HIV-1 activity and cytotoxicity of limonoid, coumarins and carbazoles isolated from *C.excavata* and AZT as determined by syncytium and toxicity assays in 1A2 cell lines.

Compound	Syncytium Assay		
	EC_{50} (μM)	IC_{50} (μM)	PTI
clausenidin (**4**)	5.3	37.2	7.0
O-methylmukonal (**109**)	12.0	680.0	56.7
3-formyl-2,7-dimethoxycarbazole (**101**)	29.1	232.0	8.0
clauszoline J (**95**)	34.2	54.1	1.6
clausenolide-1-ethyl ether (**117**)	34.4	~548.0	~16.0
AZT control[b]	$2.3x10^{-3}$	>1	>430

[a] Potential therapeutic index (PTI) = IC_{50}/EC_{50}

[b] Results are averaged from two experiments

The above promising results spurred us to investigate the synthesis and modification of the structure of these compounds in order to increase the anti-HIV-1 activity.

In addition, determination of these compounds in the extracts from different regional sources in Thailand was investigated. We have found that the extracts from various sources of Thailand showed different amounts of the anti-HIV-1 compounds and anti-HIV-1 activity.[32]

We have found optimum conditions for quantitative determination of the anti-HIV-1 compounds in the extract (without purification) by HPLC which would be very useful for AIDS patients.

CONCLUSION

In conclusion, *Clausena excavata* is a magic plant which showed several therapeutic activities especially anti-HIV-1, antimycobacterial, immunomodulatory and antimicrobial activities. The activities of this plant is so promising that it could be developed for anti-HIV drug. Moreover, this plant showed no toxicity to normal Vero cells. Finally, this plant is very worthy to develop for the treatment of AIDS.

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