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理 事 長　　中 島 章 殿

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Ⅱ．過 去 の 研 究 歴

平成6年6月～9年3月 サボテン科植物のトリテルペンの研究

平成9年4月～現在　新しい鎮吐活性スクリーニングの開発と天然由来抗嘔吐物質の検索

Ⅲ．過 去 の 研 究 実 績

サボテン科植物から14種のトリテルペンを単離した。そのうち、4種は新規化合物、2種ははじめてサボテン科植物から単離されたトリテルペン、8種は既知トリテペンである。

(論文) ① New Triterpenes from Machaerocereus eruca, a Cactaceous plant. J. Nat. Prod. (印刷中)

② Machaerocerlic acid, A Novel Germanicane, from Machaerocereus eruca. Phytochemistry. (印刷中)

Ⅳ．本 年 度 の 研 究 業 績

(1) 学会、研究会等においての口頭発表（学会名・内容）

(学会) International Symposium on Natural Medicines (KYOTO)

(内容) Anti-emetic principles of Alpinia Katsumadai (2P-035) P177

(学会) 日本薬学会第118年会(京都)

(内容) ソウズクの鎮吐活性成分の研究 (01[XH]14-5, プログラム P55)

(2) 学会誌等に発表した論文　　無 ・ (有)　（雑誌名・論文名）

(雑誌名) Phytomedicine

(論文名) Anti-emetic principles of Pogostemon cabin (Blanco) Benth (投稿中)

(雑誌名) Natural product Sciences

(論文名) New Assay Method for Anti-Emetic Compounds from Natural Sources. (印刷予定)

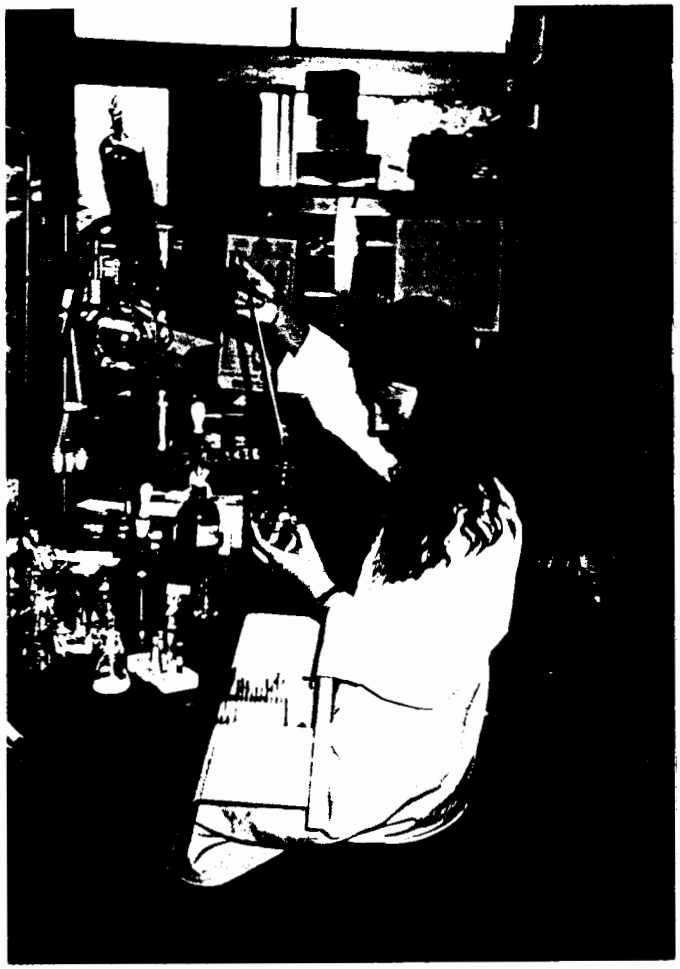
Ⅴ．今後の研究計画及び希望

1) 鎮吐作用を示した化合物の構造－活性相関について検討する。そして、その結果に基づいて、多くの誘導体を合成し、医薬品の開発への基礎研究とする。

2) 抗癌剤シスプラチンによる嘔吐モデルの確立して、今までに単離された生薬由来の鎮吐物質の有効性について検討する。

Ⅵ. 研　究　報　告（日本語、又は英語で書いて下さい。 4,000字以上で記載して下さい。別紙可）

別紙添付



Ⅶ. 指導教官の意見

楊燁氏は平成6年4月より明治薬科大学生薬学研究室の研究生となり、サボテン科植物のトリテルペンの研究でスタートしました。そして、天然物化学の手法を身につけ、本人の強い希望により、薬学博士の学位を取得するため、平成9年度より研究テーマを鎮吐作用天然物質の研究を始めました。本人は、大変真面目で、研究態度も良く、別紙研究報告に見られるように、次々と成果をあげてきました。この研究は、1つは化合物の単離精製、もう1つはヒヨコを用いた動物試験があり、とても大変なところがありますが1つ1つ確実に結果を出しております。来年度もひき続き本学の研究生となり、目標に向って進んで行くことになります。以上のことから貴財団補助金を受けるのに十分な資格があったことを認めます。

研究報告

1）生薬「霍香」（カッコウ）（*Pogostemon cabin* Benth）の抗嘔吐活性成分の研究

「霍香」はシソ科植物：広霍香の全草である。鎮吐、飲食を促進する作用があるとされ、鎮吐の目的とした処方もいくつか知られている。和剤局方の「カッコウ正気散」はその一つである。薬理学の研究として抗真菌作用と抗スピロヘータ作用の報告がある。また、霍香は胃腸神経に対して鎮静作用があり、胃液の分泌を促進し、消化力を増強する。

「霍香」をヘキサン、クロロホルム、メタノールで順次抽出し、濃縮した各エキスのヒヨコ硫酸銅誘発嘔吐抑制活性を検討したところ、ヘキサンエキスは有意なレッチング回数減少を認められた。さらに、このヘキサンエキスの活性成分を詳細に検討し、5種の活性化合物を単離した。それぞれpatchouli alcohol, pogostol, sigmast-4-en-3-one, retusin, pachypodolである。その内、sigmast-4-en-3-oneが「霍香」から単離されたのは初めてである。この５種の化合物の構造と鎮吐活性結果はそれぞれ図1と表１に示された。

2）生薬「ソウズク」（*Alpinia katsumadai*）の鎮吐活性成分の研究

「ソウズク」はショウガ科植物であり、消化器の疾患に常用される薬物である。辛、温、芳香の薬性を持つことによって、胃腸の蠕動を強め、胃液の分泌を促進して、止痛、消化通気、止嘔の効果をあげる。薬理学の研究として、ソウズクの水煎剤（0.25-0.75％）はモルモットの摘出腸管にたいして興奮作用があり、剤量をふやす（1-1.25％）と抑制作用を示す。

「ソウズク」をヘキサン、クロロホルム、メタノール、水で順次抽出し、得られた各エキスについて鎮吐活性試験を行ったとこる、ソウズクのヘキサン、クロロホルム、メタノールエキスがヒヨコの硫酸銅誘発嘔吐に有意なレッチング回数の減少を認められた。さらに、そのクロロホルムエキスの活性成分を詳細に検討し、8種の活性化合物を単離した。その内、４種の構造が明らかにした（図２）。それぞれcadamomin, *trans, trans*-1, 7-diphenyl-4, 6-heptadie-3-one, *trans, trans* - fanesol, (5R)-*trans*-1, 7-diphenyl-6-hydroxy-6-hepten-3-oneである。この8種化合物の鎮吐活性結果は表２に示された。

3）生薬「ビワヨウ」（*Eriobotrya japanica*）と「ピャクズク」（*Amomum kravanh*）の鎮吐活性について研究した。今まで２種の活性化合物を単離した。それぞれsqualeneと5-hydroxy-3,7,4'-trimethoxyflavonolである（図３）。

4）フラボノイドの鎮吐活性と構造相関について検討してきた。漢方薬に鎮吐作用を目的の一つとしてよく配合される生薬「枳実」と「陳皮」の成分であるフラバノンの鎮吐活性と構造相関を検討した。その結果、naringin　prunin（図４）は有意な鎮吐活性を示し、活性発見には糖部の構造が関係していることを明らかにした。

5）鎮吐活性スクリーニングの開発

抗ガン剤シスプラチンのよる嘔吐は体内でラジカル生成するのが原因と見なされている。このことを指標にヒヨコによる新しいスクリーニング法を検討してきた。種種のラジカル発生剤（過酸化水素ー塩化第二鉄、ピロガロール、水溶性ラジカ

ル誘発剤アAAPHと脂溶性ラジカル誘発剤AMVN）を試験した。この試験法はさらに検討中である。

Table 1. Anti-emetic effect of compound**1**-**5** on copper sulfate induced emesis in young chicks

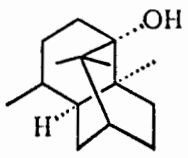
Drugs	Dose (mg/kg b.w.)	No. of young chicks	No. of retches (means±S.E)	Inhibition (%)
control		6	60.0±4.73	
patchouli alcohol (**1**)	10	6	54.5±7.58	9.3
	20	6	50.3±6.90	16.2
	50	6	36.0±3.69**	40.0
	70	6	25.4±2.80***	57.7
control		5	67.2±2.81	
pogostol (**2**)	10	5	40.8±4.79**	21.8
	20	5	38.8±8.73 *	42.2
	50	5	38.2±6.13**	43.2
control		6	55.7±4.6	
stigmast-4-en-	10	6	54.9±8.80	1.4
3-onc (**3**)	20	6	39.6±7.48	28.9
	50	6	24.7±1.21***	55.7
control		6	67.5±6.63	
retusin (**4**)	10	6	49.0±6.97	27.4
	20	6	48.0±4.97*	28.9
	50	6	18.5±2.25***	72.6
control		5	56.6±3.93	
pachypodol (**5**)	10	5	53.4±3.93	5.6
	20	5	44.6±7.27	21.2
	50	5	28.0±3.09***	50.6

Significantly different from the control value, *p<0.05, **p<0.01, ***p<0.001

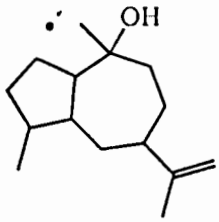
Table 2 Anti-emetic effect of compound 1-8 isolated from *Alpinin katsumadai* on copper sulfate induced-emesis in young chicks

Drugs	Dose (mg/kg)	No.of young chicks	No. of retches (means±S.E)	Inhibition (%)
control		6	84.8±1.31	
compound 1	10	6	67.0±3.29**	31.0
	20	6	53.8±4.01**	36.6
	50	6	39.4±2.84***	53.6
control		6	81.7±1.52	
compound 2	10	6	74.2±4.96	9.2
	20	6	40.0±6.85**	51.0
	50	6	48.3±6.75**	40.8
control		6	75.8±2.33	
compound 3	10	6	60.3±3.00**	20.4
	20	6	54.7±4.74*	27.9
	50	6	33.5±3.71***	55.8
control		6	72.8±3.27	
compound 4	50	6	42.7±3.60**	41.4
control		6	75.2±4.80	
compound 5	10	6	68.6±4.0	8.8
	20	6	64.3±5.91	14.5
	50	6	37.4±3.35**	50.2
control		6	87.5±1.15	
compound 6	10	6	57.5±10.5*	34.3
	20	6	34.7±4.69***	60.4
	50	6	40.7±6.68***	53.5
control		6	82.2±2.27	
compound 7	10	6	52.5±4.25**	36.1
	20	6	49.2±3.14**	40.2
	50	6	36.5±2.20***	55.6
control		6	74.2±4.76	
compound 8	10	6	50.1±3.64*	32.4
	20	6	32.8±4.15**	55.7
	50	6	21.7±1.19***	70.8

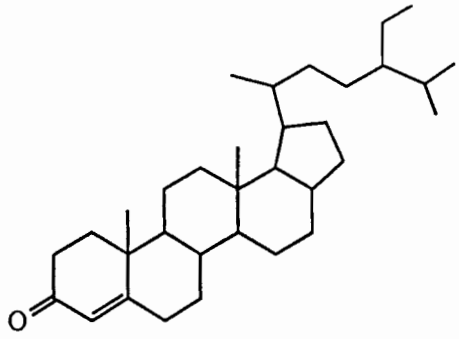
Significantly different from the control value, *p<0.05, *p<0.01, *p<0.001



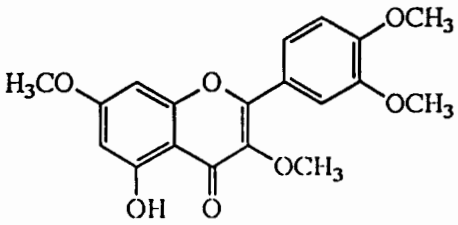
patchouli alcohol (1)



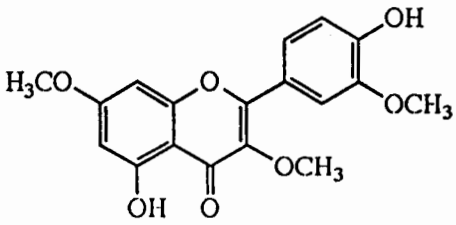
pogostol (2)



sigmast-4-en-3-one (3)

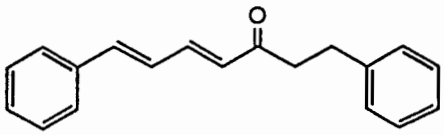


retusin (4)

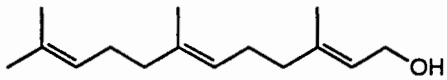


pachypodol (5)

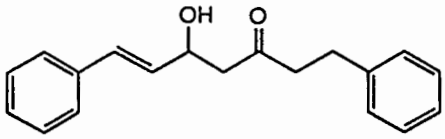
図 1



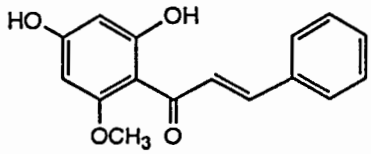
(trans, trans -1,7-Diphenyl-4,6-heptadin-3-one)



(trans,trans - Farnesol)

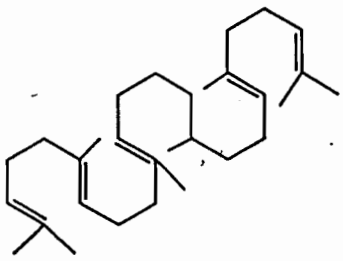


[(5R)- trans -1,7-Diphenyl-5-hydroxy-6-hepten-3-one]

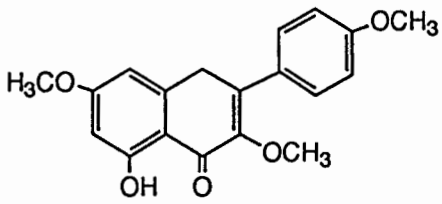


(Cardamomin)

図 2

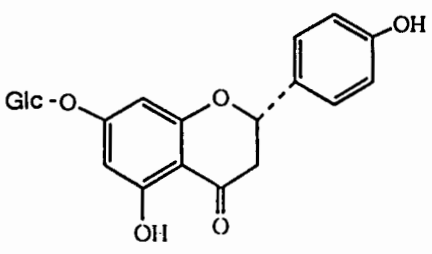


squalene

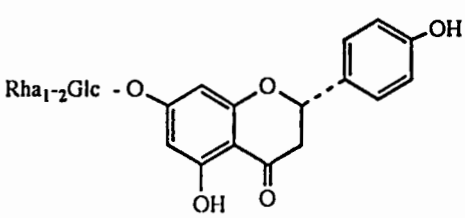


5-hydroxy-3,7,4'-trimethoxyflavonol

図 3



Prunin



naringin

図 4

Anti-emetic principles of *Pogostemon cabin* (Blanco) Benth

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Summary

Bioassay-guided fractionation of anti-emetic constituents of 8 traditional Chinese herbal drugs was performed, and the 20 extracts showed anti-emetic activity on copper sulfate induced-emesis in young chicks. From n-hexane extract of Pogostemon cabin, pachouli alcohol (**1**), pogostol (**2**), stigmast-4-en-3-one (**3**), retusin (**4**), and pachypodol (**5**) were isolated, and they exhibited anti-emetic effects.

Key words: anti-emetic, Pogostemon cabin, pachouli alcohol, pogostol, stigmast-4-en-3-one, retusin, pachypodol

Introduction

Nausea and vomiting are noted as concomitant side effects of many cancer chemotherapeutic agents. They cause an unavoidable problem in cancer chemotherapy. To prevent theos side effects traditional Chinese herbal drugs which may exhibit anti-emetic effects are examined. However, anti-emetic active principles of these crude drugs are not so much known supported by the modern pharmacological evidence. One of the difficulties for testing anti-emetic activity of natural products is that only a few mammals vomit in response to emetic drugs and require a large dose even if they are responsive.

In the previous papers, we reported anti-emetic principles from *Magnolia obovata* bark, *Zingiber officinale* rhizome (Kinoshita et al., 1996) and *Poria cocos* (Tai et al., 1995) using frogs in screening their anti-emetic activities. However, a long emetic latency of frogs induced by the emetic agent is not suitable for testing many samples within a short period of time. Therefore, we studied a new screening method using chicks for the anti-emetic activity (Akita et al., 1998) to overcome the above-mentioned difficulties.

The present paper describes the screening of 31 extracts from 8 traditional Chinese herbal drugs for anti-emetic activities using young chicks, and the identification of active principles contained in *Pogostemon cabin* (Blano) Benth.

Materials and Methods

Materials

The crude drug materials were commercial products purchased from Kinokuniya Kanyakkyoku (Tokyo, Japan) and Kotaro Pharmaceutical Co., Ltd. (Osaka, Japan).

reagents

Copper sulfate anhydride (Yokuhata Pure Chemicals Co., Ltd., Tokyo, Japan) was used as the emetic agent. Dimethyl sulfoxide (DMSO), saline and Tween 80 (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) are commercial products.

Spectrometry and chromatography

^{1}H- and ^{13}C-NMR spectra were recorded using a JEOL GSX-400 spectrometer in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as an internal standard. Kieselgel 60 F_{254} (MERCK)

precoated plates were employed for thin-layer chromatography (TLC). Column chromatography was carried out on 70-230 mesh silica gel (MERCK). HPLC was performed using an SSC-3100-J pump with an Oyo-Bunko Uvilog 7 UV detector. HR-MS and EI-MS were obtained using a JEOL JMX-DX 302.

Animals

Male young chicks (4 days of age) weighing 25-35 g were purchased from Goto Furanjo Co., Ltd.(Saitama, Japan).

Bioassay of anti-emetic activity

The young chicks were divided into 1-3 groups consisting of six each, and each young chick were set aside for 10 min to stabilize in large beakers at 38°C. The sample was dissolved in saline contented 5% DMSO and 1% Tween 80 and administered abdominally at volume of 0.1 ml/10g b.w.. After 10 min, copper sulfate anhydride was administered orally at 50mg/kg b.w., then the number of retches (an emetic action without vomiting gastric materials) was recorded during the next 10 min. The results were judged by the decrease in number of retches in contrast with those of control. The inhibition (%) was calculated as follows:

$$\text{Inhibition (\%)} = [(A-B)/A] \times 100$$

A: control frequency of retching

B: frequency retching after sample treatment

Statistical analysis

All numerical data were expressed as the mean ± S.E.. The statistical significance of the difference was determined by an unpaired student's *t*-test .

Isolation and purification of the anti-emetic principles from the Pogostemon cabin

The crude drug (1.0 kg) was extracted successively with *n*-hexane, $CHCl_3$, MeOH and each extract was examined for anti-emetic activity using young chicks. As shown in Table 1, three extracts significantly inhibited the emetic-action, respectively. The *n*-hexane extract which showed the highest anti-emetic activity (58.6%) was chramatographed on a silica gel column (*n*-hexane: EtOAc), and 8 fractions (fr. 1-8) were obtained.

Each fraction was tested for anti-emetic activity, and fr.2, fr.3 and fr.7 showed positive activity (Table 2). Fr.2 was chromatographed on HPLC using a silica gel column

[Silica-4251-N 10 ϕ × 250mm, *n*-Hexane-EtOAc (11:1)], and compound **1** (1.72g) was obtained and identified as pathouli alcohol by comparison with published spectral data (Barton et al., 1987).

An active fraction, fr.3, was chromatographed on a silica gel column (*n*-hexane : acetone) and HPLC using a silica gel column [Silica-4251-N 10 ϕ × 250mm, *n*-Hexane-EtOAc (5:1)]. Compounds **2** (79.7 mg) and **3** (41.3 mg) were obtained as active principles and identified as pogostol and sigmast-4-en-3-one by comparison with published spectral data (Hikino et al., 1986; Greca et al., 1990). Compound **3** was first isolated from *Pogostemon cabin.*

Fr.7 was chromatographed on HPLC using a silica gel column [(Silica-4251-N 10 ϕ × 250mm, *n*-Hexane-acetone (75 : 25)], and compounds **4** (62.6 mg) and **5** (48.0 mg) were obtained and examined by MS, ^{1}H- and ^{13}C-NMR, DEPT experiment, ^{1}H-^{13}C cosy, ^{1}H-^{13}C long range cosy (COLOC) and the difference of Overhauser enhancement (nOe) spectra. These observations suggested that compound **4** and **5** are retusin and pachypodol, respectively.

Results and Discussion

As shown in Table 1, 20 extracts significantly inhibited emetic action induced by $CuSO_4$ in young chicks. The *n*-hexane, $CHCl_3$, MeOH extracts of *Pogostemon cabin* showed significant inhibition at a dose of 300mg/kg b.w.. Patchouli alcohol (**1**), pogostol (**2**), stigmast-4-en-3-one (**3**), retusin (**4**) and pachypodol (**5**) were isolated from *n*-hexane extract of *Pogostemon cabin* as anti-emetic principles.

Patchouli alcohol (**1**) showed anti-emetic activities at two doses of 50mg/kg b.w. and 70mg/kg b.w.. Pogostol (**2**) significantly inhibited emetic action at three doses of 10mg/kg-50mg/kg b.w.. Stigmast-4-en-3-one (**3**) and pachypodol (**5**) showed anti-emetic activity at a dose of 50mg/kg b.w.. Retusin (**4**) inhibited emetic action at two doses of 20mg/kg and 50mg/kg b.w..

Pogostemon cabin is one of the traditional Chinese medicines used mainly for the treatment of dyspepsia, vomiting, diarrhea and poor appetite. On the other hand, it was known that excessive contraction of digestive organ muscles may cause of vomiting and diarrhea, and patchouli alcohol showed Ca^{2+}antagonist activity (Ichikawa et al.,1989). Therefore, the anti-emetic activity of patchouli alcohol is caused by depressing excessive excitation of smooth

muscles as a result of inhibition of inward Ca^{2+} influx through the cell membranes. Patchouli alcohol contained abundantly in *Pogostemon cabin* (>0.1%) may play a main role in anti-emetic effect clinically in view of its traditional use in Chinese medicine.

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Table 1. Screening test of medicinal plants for anti-emetic activities on copper sulfate induced emesis in young chicks

Crude drugs	Extract	No. of young chicks	No. of ratches (mean±S.E.)	Inhibition (%)
control		6	69.3±3.19	
Pogostemou cabin	*n*-hexane	6	28.9±4.31***	58.6
	$CHCl_3$	6	45.8±6.75*	34.4
	MeOH	6	47.8±6.39*	31.5
control		6	70.6±1.25	
Alpinia katsumadai	*n*-hexane	6	52.4±3.39**	25.8
	$CHCl_3$	6	28.0±5.09***	60.1
	MeOH	6	18.4±0.93***	73.9
	H_2O	6	46.2±8.12	34.6
control		6	48.8±4.34	
Amonum tsaoko	*n*-hexane	6	46.6±3.29	4.5
	$CHCl_3$	6	36.6±5.39	25.6
	MeOH	6	22.2±3.28***	54.5
	H_2O	6	39.3±7.23	19.4
control		6	75.8±1.38	
Amomum cardamonum	*n*-hexane	6	78.6±3.14	-3.6
	$CHCl_3$	6	36.0±5.96**	52.6
	MeOH	6	70.0±11.0	7.7
	H_2O	6	52.0±2.98*	31.4
control		6	64.5±4.46	
Amonum xanthioides	*n*-hexane	6	41.8±9.05*	35.2
	$CHCl_3$	6	29.8±3.94***	53.8
	MeOH	6	34.8±8.47*	46.0
	H_2O	6	43.5±7.70*	32.5
control		6	68.2±2.31	
Eupatorium fortunei	*n*-hexane	6	61.3±6.68	10.1
	$CHCl_3$	6	46.3±7.32	32.1
	MeOH	6	50.8±2.42***	25.5
	H_2O	6	54.2±3.16*	20.5
control		6	86.8±3.69	
Nelumbo nueifera	*n*-hexane	6	75.3±9.81	13.2
	$CHCl_3$	6	63.2±3.06**	27.2
	MeOH	6	77.6±2.98	10.6
	H_2O	6	71.6±5.83	17.6
control		6	44.5±2.33	
Alpinia officinarum	*n*-hexan	6	37.5±4.19	15.7
	$CHCl_3$	6	24.2±3.23***	45.6
	MeOH	6	32.7±3.74*	26.5
	H_2O	6	31.2±4.26*	29.9

Significantly different from the control value, *$p<0.05$, **$p<0.01$, ***$p<0.001$

Table 2. Anti-emetic effect of the fractions from the *n*-hexane extract on copper sulfate induced emesis in young chicks

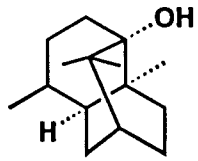
Drugs	Dose (mg/kg b.w.)	No. of young chicks	No. of retches (means±S.E)	Inhibition (%)
control		6	47.8±6.14	
Fr. 1	150	6	37.8±5.17	21.8
Fr. 2	150	6	11.6±7.08**	75.7
Fr. 3	150	6	7.6±1.25***	84.1
control		6	45.0±8.45	
Fr. 4	150	6	31.2±3.36	30.7
Fr. 5	150	6	27.7±6.92	42.9
Fr. 6	150	6	28.4±6.95	36.9
control		6	47.8±6.14	
Fr. 7	150	6	21.6±4.25**	54.8
Fr. 8	150	6	38.2±7.87	20.0

Significantly different from the control value, **$p<0.01$, ***$p<0.001$

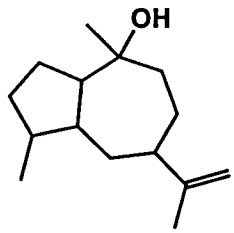
Table 3. Anti-emetic effect of compound **1-5** on copper sulfate induced emesis in young chicks

Drugs	Dose (mg/kg b.w.)	No. of young chicks	No. of retches (means±S.E)	Inhibition (%)
control		6	60.0±4.73	
patchouli alcohol (**1**)	10	6	54.5±7.58	9.3
	20	6	50.3±6.90	16.2
	50	6	36.0±3.69**	40.0
	70	6	25.4±2.80***	57.7
control		5	67.2±2.81	
pogostol (**2**)	10	5	40.8±4.79**	21.8
	20	5	38.8±8.73 *	42.2
	50	5	38.2±6.13**	43.2
control		6	55.7±4.6	
stigmast-4-en-	10	6	54.9±8.80	1.4
3-one (**3**)	20	6	39.6±7.48	28.9
	50	6	24.7±1.21***	55.7
control		6	67.5±6.63	
retusin (**4**)	10	6	49.0±6.97	27.4
	20	6	48.0±4.97*	28.9
	50	6	18.5±2.25***	72.6
control		5	56.6±3.93	
pachypodol (**5**)	10	5	53.4±3.93	5.6
	20	5	44.6±7.27	21.2
	50	5	28.0±3.09***	50.6

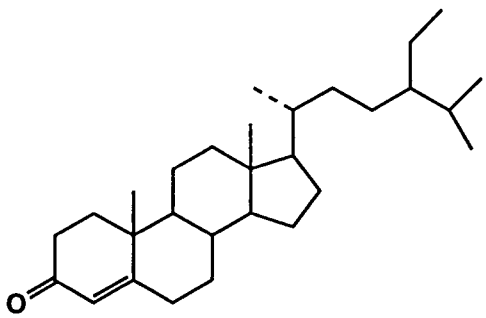
Significantly different from the control value, *$p<0.05$, **$p<0.01$, ***$p<0.001$



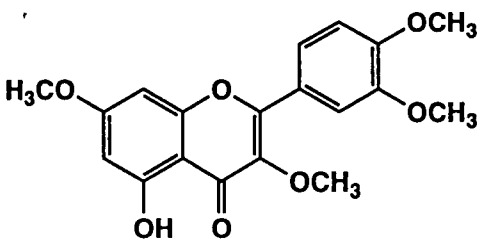
patchouli alcohol (**1**)



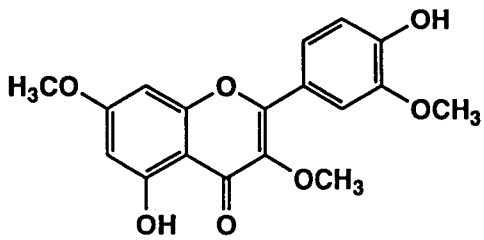
pogostol (**2**)



sigmast-4-en-3-one (**3**)



retusin (**4**)



pachypodol (**5**)

Fig. 1. The structures of active compounds isolated from *Pogostemon cabin* Benth