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学 位 論 文 題 名

Isolation and Structural Elucidation of Anti-Babesial Compounds from Medicinal Plants

（薬用植物に含まれる抗バベシア化合物に関する有機化学的研究）

学位論文内容の要旨

Introduction

Canine babesiosis is a tick-borne disease caused by the protozoal parasites *Babesia gibsoni* and *B. canis*. They infect the red blood cells of dogs and typically cause hemolytic anemia. *B. gibsoni* is distributed in many regions throughout the world, including Asia, Africa, Europe, America, and Australia. The treatment of acute infection by anti-babesial drugs or the spontaneous recovery from the disease fails to clear the organism from the host, resulting in a carrier stage. The animals that recover are a reservoir for tick-transmitted infections and are at risk for recrudescent infection. No drugs have been proven effective for the elimination of *B. gibsoni* organisms from infected dogs. Some anti-babesial drugs reduce the severity of clinical signs and the mortality associated with the disease. However, these drugs usually cause pronounced and severe side effects. Therefore, an alternative chemotherapeutic agent with fewer side effects is urgently needed for the treatment of *B. gibsoni* infection.

Anti-babesial compounds from *Berberis vulgaris* [3)]

Based on the hypothesis, in which medicinal plants used to treat malaria might have anti-babesial activity, nine North African medicinal plants were examined for their anti-babesial properties. Some of these are used for the treatment of malaria and others as traditional antifebritics. Extracts prepared from *Berberis vulgaris* and *Rosa damascena* showed more than 90% inhibition at a concentration of 100 μg/mL, and extracts prepared from *Marrubium vulgare* and *Taraxacum officinale* showed more than 85% inhibition at 1000 μg/mL. The remaining five plants extracts, *Tamarindus indica*, *Balanites aegyptica*, *Innula viscose*, *Vinca minor*, and *Helianthus annus*, showed more than 50% inhibition at 1000 μg/mL. The active ingredients of *B. vulgaris* were elucidated to be *E*-coniferyl alcohol (**1**), (–)-simulanol (**2**), *p*-hydroxybenzaldehyde (**3**), 3-hydroxy-4,5-dimethoxybenzoic acid (**4**), *trans*-ferulic acid (**5**), syringic acid (**6**), vanillic acid (**7**), *cis*-ferulic acid (**8**), syringaresinol-β-D-glucoside (**9**), berberine (**10**), and jatrorrhizine (**11**). Compounds **1-9** were tested for their *in vitro* anti-babesial activity against *B. gibsoni*. Compound **5** showed the strongest activity, with an IC_{50} value of 7.33 μg/mL, followed by **8** and **9**, with IC_{50} values of 134.84 and 51.60 μg/mL, respectively. Compounds **1–4**, **6**, and **7** showed very weak activities against *B. gibsoni in vitro*, with IC_{50} values > 250 μg/mL, compared with the standard drug diminazene aceturate (Ganaseg, IC_{50} = 0.60 μg/mL). Interestingly, *trans*-ferulic acid (**5**) is 20 times more active than its *cis* isomer (**8**). Although compounds **1-9** are all known, this study describes the first isolation of them from *B. vulgaris*. On the other hand, berberine (**10**) and jatrorrhizine (**11**) have been reported to possess higher activity than the standard anti-babesial drug with IC_{50} values of 0.45 and 0.57 μg/mL, respectively.

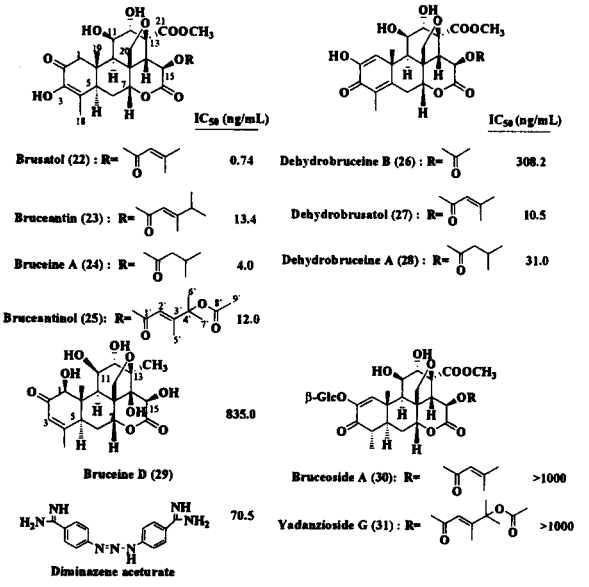
Anti-babesial compounds from *Rosa damascena* [2)]

Bioassay-guided investigation of extracts of the flowers of *Rosa damascena* Mill. led to the

isolation of four anti-babesial compounds, 3,4-dihydroxy benzoic acid (**12**), gallic acid (**13**), 2-phenylethyl 6-*O*-galloyl-β-D-glucopyranoside (**14**), and quercetin 3-*O*-β-D-(6-*O*-acetyl)-glucopyranosyl-(1→4)-α-L-rhamnopyranoside (**20**), with IC_{50} values of 78.00, 15.74, 11.78, and 90.88 μg/mL, respectively. In addition to seven compounds, kaempferol 3-*O*-β-D-glucopyranoside (**15**), kaempferol 3-*O*-β-D-xylopyranoside (**16**), kaempferol 3-*O*-α-L-rhamnopyranoside (**17**), quercetin 3-*O*-β-D-glucopyranoside (**18**), quercetin 3-*O*-α-L-rhamnopyranoside (**19**), 5-hydroxymethyl-2-furfural (**21**), and vanillic acid (**7**), which possessed week anti-babesial activity with IC_{50} values >100 μg/mL.

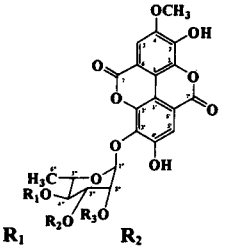
Anti-babesial quassinoids from the fruits of *Brucea javanica* [1]

The medicinal plant *Brucea javanica* (L.) Merr. (Simaroubaceae), grown in China, was examined for anti-babesial properties. The anti-babesial activity of the fruit, collected in Indonesia, was found to be attributed to its quassinoid constituents in a previous study, and ten active compounds were isolated and purified in this study. The identities of these compounds were confirmed from NMR spectroscopic and mass spectral data as brusatol (**22**), bruceantin (**23**), bruceine A (**24**), bruceantinol (**25**), dehydrobruceine B (**26**), dehydrobrusatol (**27**), dehydrobruceine A (**28**), bruceine D (**29**), bruceoside A (**30**), and yadanzioside G (**31**). When tested *in vitro* against *Babesia gibsoni*, compounds **22-31** had IC_{50} values of 0.74, 13.4, 4.0, 12.0, 308.2, 10.5, 835.0, >1000, and >1000 ng/mL, respectively. Compounds **22-25**, **27**, and **28** had far higher activity than the commercial anti-babesial drug diminazene aceturate, which possesses an IC_{50} value of 70.5 ng/mL



Anti-babesial ellagic acid rhamnosides from the bark of *Elaeocarpus parvifolius* [4]

Bioassay-guided investigation of the bark of *Elaeocarpus parvifolius* led to the isolation of four new ellagic acid derivatives, 4-*O*-methylellagic acid 3'-α-rhamnoside (**33**), 4-*O*-methylellagic acid 3'-(3"-*O*-acetyl)-α-rhamnoside (**34**), 4-*O*-methylellagic acid 3'-(2"-*O*-acetyl)-α-rhamnoside (**35**), and 4-*O*-methylellagic acid 3'-(4"-*O*-acetyl)-α-rhamnoside (**36**) in addition to one known ellagic acid derivative, 4-*O*-methylellagic acid 3'-(2",3"-di-*O*-acetyl)-α-rhamnoside (**32**). Their structures were elucidated on the basis of ^1H NMR, ^{13}C NMR, HMQC, HMBC, and MS spectral data. Compounds **32-34** and **36** were evaluated for their growth-inhibitory effect on *Babesia gibsoni in vitro*. Compounds **33** and **36** showed very weak activity, while compounds **32** and **34** showed moderate activity, with IC_{50} values of 28.5 and 52.1 μg/mL, respectively.



	R_1	R_2	R_3
32	H	CH_3CO	CH_3CO
33	H	H	H
34	H	CH_3CO	H
35	H	H	CH_3CO
36	CH_3CO	H	H

Conclusion

In conclusion, four medicinal plants were examined in this study for their anti-babesial properties, from which 36 compounds were isolated as anti-babesial compounds. Some of the isolated compounds are promising as new candidates for the treatment of the symptoms caused by *B. gibsoni* infection due to the closeness of their anti-babesial activity to that of the standard anti-babesial drug (Ganaseg) and also due to their origin from medicinal plants.

1) **Elkhateeb**, et al. "**Anti-babesial quassinoids from the fruits of *Brucea javanica***" *Natural Product Communications* (**2008**), 3 (2), 145-148.

2) **Elkhateeb**, et al. "**Anti-babesial compounds from *Rosa damascena***" *Natural Product Communications* (**2007**), 2 (7), 765-769.

3) **Elkhateeb**, et al. "**Anti-babesial compounds from *Berberis vulgaris***" *Natural Product Communications* (**2007**), 2 (2), 173-176.
4) **Elkhateeb**, et al. "**Anti-babesial ellagic acid rhamnosides from the bark of *Elaeocarpus parvifolius***" *Phytochemistry* (**2005**), 66 (21) 2577-2580.

学位論文審査の要旨

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学位論文題名

Isolation and Structural Elucidation of Anti-Babesial Compounds from Medicinal Plants

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本論文は英文274頁，図22，表15，5章からなり，参考論文4編が付されている。

マラリア原虫がハマダラ蚊を媒介者としてヒト赤血球に侵入しマラリア症を引き起こす事はよく知られている。これと同様に、バベシア原虫はダニを媒介者として犬，家畜類の赤血球に侵入しマラリア症と同様な症状を呈するバベシア症を引き起こす。希ではあるがヒトへの感染例も知られており、本疾病は発展途上国に於いて甚大な被害をおよぼし、大きな問題となっている。大手の製薬メーカーが顧みない「neglected disease」の代表例でもある。本論文はバベシア原虫汚染地域で古くから用いられる薬用植物を実験材料とし、抗バベシア活性を有する植物成分の単離構造決定，生理活性を明らかとたものである。

【1】*Berberis vulgaris*からの抗バベシア化合物の単離とその生理活性

北アフリカで用いられる数十種の薬用植物のスクリーニングから2種の植物(*B. vulgaris, Rosa damascena*)に抗バベシア活性を明らかとした。生物検定として犬赤血球にバベシア原虫を感染させた *in vitro* 系を用いた。*B. vulgaris* より活性化合物として *E*-coniferyl alcohol (**1**), (-)-simulanol (**2**), *p*-hydroxybenzaldehyde (**3**), 3-hydroxy-4,5-dimethoxybenzoic acid (**4**), *trans*-ferulic acid (**5**), syringic acid (**6**), vanillic acid (**7**), *cis*-ferulic acid (**8**), syringaresinol-β-D-glucoside (**9**), berberine (**10**), jatrorrhizine (**11**)の単離構造決定に成功した。化合物 **10**，**11** 以外では生物活性に関しては化合物 **5** がもっと強い生理活性（IC_{50}= 7.33 μg/mL）を示した。

【2】*Rosa damascena*からの抗バベシア化合物の単離とその生理活性

R. damascena Mill. より 3,4-dihydroxy benzoic acid (**12**、IC_{50}= 78 μg/mL), gallic acid (**13**, IC_{50}= 15.7 μg/mL), 2-phenylethyl 6-*O*-galloyl-β-D-glucopyranoside (**14**, IC_{50}= 11.8 μg/mL), quercetin 3-*O*-β-D-(6-*O*-acetyl)-glucopyranosyl-(1→4)-α-L-rhamnopyranoside (**20**, IC_{50}= 91 μg/mL)を単離した。また、IC_{50}> 100 μg/mL であったが、kaempferol 3-*O*-β-D-glucopyranoside (**15**), kaempferol 3-*O*-β-D-xylopyranoside (**16**), kaempferol 3-*O*-α-L-rhamnopyranoside (**17**), quercetin 3-*O*-β-D-glucopyranoside (**18**), quercetin 3-*O*-α-L-rhamnopyranoside (**19**), 5-hydroxymethyl-2-furfural (**21**), and vanillic acid (**7**)を単離した。

【3】*Brucea javanica*からの抗バベシア化合物の単離とその生理活性

*B. javanica*は東南アジアで用いられる薬用植物であるが、本植物より brusatol (**22**), bruceantin (**23**), bruceine A (**24**), bruceantinol (**25**), dehydrobruceine B (**26**), dehydrobrusatol (**27**), dehydrobruceine A (**28**), bruceine D (**29**), bruceoside A (**30**), yadanzioside G (**31**)を精製し、その抗バベシア活性はそれぞれ、**IC_{50}**= 0.74, 13.4, 4.0, 12.0, 308.2, 10.5, 835.0, >1000, >1000 ng/mL であった。Brusatol (**22**)の活性は市販の抗バベシア剤の 100 倍の活性であった。

【4】*Elaeocarpus parvifolius*からの抗バベシア化合物の単離とその生理活性

*E. parvifolius*はインドネシアで用いられる薬用植物であるが、本植物より 4-*O*-methylellagic acid 3'-(2",3"-di-*O*-acetyl)-α-rhamnoside (**32**)、4-*O*-methylellagic acid 3'-α-rhamnoside (**33**), 4-*O*-methylellagic acid 3'-(3"-*O*-acetyl)-α-rhamnoside (**34**), 4-*O*-methylellagic acid 3'-(2"-*O*-acetyl)-α-rhamnoside (**35**), 4-*O*-methylellagic acid 3'-(4"-*O*-acetyl)-α-rhamnoside (**36**) を活性物質として単離した。化合物**33**－**36**に関しては新規化合物であった。化合物**32**、**34**の活性はそれぞれ、**IC_{50}**= 28.5, 52.1 μg/mLを示し、他の化合物は>100 μg/mLであった。

以上，本研究では主として有機化学的手法を用いて、4 種の薬用植物から、抗バベシア活性を有する化合物、36 種の単離構造決定を行なった。このうち 4 種については新規化合物であった。また、*B. javanica* より精製した化合物の中には市販の抗バベシア剤の 100 倍の活性を有する化合物を発見し、今後の有望な抗バベシア剤の候補化合物の発見につながった。

よって審査員一同は，Elkhateeb Ahmed Mohamed が博士（農学）の学位を受けるのに十分な資格を有するものと認めた.