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Bioactive compound from *Goniothalamus andersonii*

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Abstract

Studies on the crude bark extract of *Goniothalamus andersonii* have enabled the isolation of stigmasterol, goniothalamin and two mixtures of sesquiterpenes.Their chemical structures were determined by using spectroscopic techniques like ^{1}H NMR, ^{13}C NMR, GC-MS and Infrared spectroscopy.The crude n-hexane and etanol stem bark extracts of *Goniothalamus andersonii* were tested for their larvicidal activity against the larvae of *Aedes aegypti.* The n-hexane and methanol extracts of Goniothalamus *andersonii* were susceptible to the larvae with an LC_{50} value 42.3 µg/ml. This indicated very strong larvicidal activity. The ethanol extract showed larvicidal activity with an LC_{50} values of 58.1 µg/ml. This is also good larvicidal activity.

Keywords: *Goniothalamus andersonii*, *Aedes aegypti*, goniothalamin, sesquiterpenes and larvicidal activity

Introduction

Generally the plant from the genus *Goniothalamus* are widely known as 'Mempisang' or 'Penawar Hitam'. The timbers are often aromatic. The bark is though, and some species are used for rough ropes in the Philippine Islands and Sumatera. The fruits are oblong, with 1.5 to 2.0 cm lengths and usually one seeded. It has germination durian. The cotyledons are non-emergent and its hypocotyls elongated. Its leaves are alternated and distichously. The shoots are plagiotropic and the leader is self-straightening in a zone of curvature behind the apex (Ng, 1991).

Plants from the genus *Goniothalamus* are widely used as traditional medicine by the native of Malaysia. The Malay find uses for them in connection with childbirth, but not in a clearly consistent way, for they are used in attempts to produce abortion, though possibly actually to mitigate the violence of abortient, and they are given after childbirth. An undetermined species mentioned under the name "kayu bukit" is used after childbirth. The sakai of Bentong use 'Selada' for treating cases where blood is passed in the urine (Hassan, 1994).

Materials and Methods

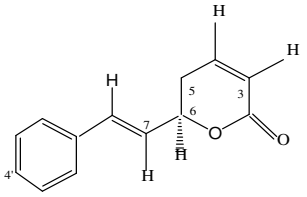
The stem barks of Goniothalmus Andersonii were collected from Sri Aman division, East Malaysia. The plants were identified at the Herbarium, Forest Department Headquarters, and Kuching Sarawak.The finely powdered stem bark (≈ 1.0 kg) was extracted with distilled hexane. The crude extracts were filtered and after removal of the solvents by vacuum evaporation yielded dark residues weighing 12.8g. The crude extract was subjected to a series of column chromatography over Si gel column using hexane, ethyl acetate and methanol of increasing polarity as an eluting solvent and was further purified by preparative TLC, mini column chromatography and recrystalization. Column chromatography has been used to get all the compounds.

Results and Discussion

Goniothalamin, **1** (1.8g) was crystallized from the crude hexane and ethyl acetate extracts as colorless crystals with melting point of 83-85°C (Li.85°C) (Jewers *et al* 1972). Mass spectra showed a molecular in peak at m/z 200 expected for $C_{13}H_{12}O_2$. Strong IR absorption at 1720, 1247.0cm¹ were a carbon double bond.

This structure of **1** was also supported by ^{1}H NMR (400 MHz, $CDCL_3$). A ddd at δ 6.07 (J 9.78 Hz and 1.5 Hz) and a dt at (J 9.78, 1.68 Hz) were assigned to the H-4 and H-3 of an α, β-unsaturated δ-lactone moiety. A proton multiplet at δ 2.49 and at δ6.25 (J16.Hz and 6.3Hz) and doublet at δ6.71 (J15.8Hz) were assigned to H-7 and H-8 of the styryl pyrone. The large coupling constant value of 15.6Hz shows that the two have *trans* configuration. Another multiplet at δ7.27 was assigned to the protons of the phenyl ring. The ^{1}H NMR assignments were done by comparison with ^{1}H NMR published data (Ee.G.C.L. et al., 1998). The ^{13}C (400 MHz, $CDCL_3$) indicated a

total of thirteen carbons.The ^{13}C NMR assignment for the 13 carbons which accur at C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-1`, C-2`, C-6`, C-3`-C-5`, and C-4 of the compound are at δ 163.8, 121.1, 44.6, 29.9, 77.9, 125.6, 133.1, 135.1, 26.7, 128.7, 128.3.



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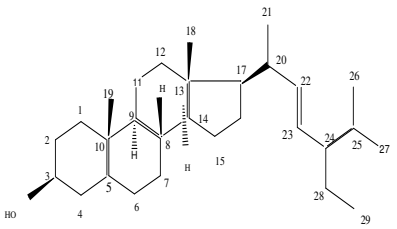
Stigmasterol **2** (96.85mg) was crystallized from hexane as needle shaped crystals with melting point of 164-167.5ºC (Lit 166-168ºC) (Schwartz and Wal, 1955). The R_f value for this compound is 0.6 using a mixture of 20% ethyl acetate and 80% hexane. It is not a UV active compound. Mass spectral data obtained from GCMS showed a molecular ion peak at m/z 394 implying the existence of one hydroxyl group in the molecule. Other significant fragments were at 369, 351, 300, 271, 25, 213, 173, 159,145, 133, 119, 105, 91, 81, 69, and 55 respectively. The existence of a hydroxyl group was further confirmed by the strong IR absorption observed at 3436cm[1]. The absorption observed at 2957cm[1] and 2935cm[1] were assigned to the carbon carbon-hydrogen stretching for CH_3 and CH_2 and 1637cm1 to the carbon-carbon double bond. This deduction was confirmed by ^1H NMR (400 MHz, $CDCL_3$). Two doublet of doublet at δ 5.02 (J 15.1Hz) and δ 5.15 (*J* 15.1 Hz, 8.5Hz) were assigned to H-23 and H-22 of the chain respectively. The large coupling constant value of 15.1Hz shows that the two protons have a trans configuration. The ^{13}C NMR assignments were done by comparison with ^{13}C NMR published data (Holland et al., 1978). Nine CH2 carbons were shown in the DEPT spectrum was consistent with the number of CH2`s present in the structure of stigmasterol and are matched with the $^{13}C$ chemical shift values that were reported earlier $^{13}C$ NMR assignment for nine CH2`s which accurred at C-1, C-2, C-7, C-11, C-12, C-15, C-15, C-16 and C-28 of the compound are at δ 37.3, 31.7, 42.3, 31.9, 24.4, 28.9 and 25.4 respectively.



2

3 was isolated from the hexane extract and ethyl acetate extracts respectively. This compound gave a purple coloured spot on the TLC plate under UV light with wavelength of 366nm. The R_f value for this compound is 0.55 using 40% Hexane and 60%ethyl acetate solvent system. Analysis work was carried out by using GC-MS which indicated that **2** was an inseparable sesquiterpenes mixtures**.** The GCMS spectrum gave peaks with rentation time 5.4, 10.567, 6.942, 7.317, 11.308, 5.417 minutes respectively. Three sesquiterpenes were identified by comparison with the MS library search; they were naphthalene, germacrene D, and alpha-cubebene.

Table 1 ^1H NMR (δ)assignments and J values of goniothalamin

Proton	δ ppm	δ ppm *(Lit.)	J(Hz)	J (Hz) *(Lit.)
H-3	6.07 ,dt	6.07, dt	9.8,1.7	9.8, 1.9
H-4	6.90, ddd	6.90, ddd	9,6, 3.8, 1.7	9.8, 4.2, 1.5
H-5a	2.49, m	2.49 , m		
H-5b	2.49, m	2.49 , m		
H-6	5.08, ddd	5.07, ddd	6.3, 8.0 , 2.9	6.3, 8.0, 2.9
H-7	6.25, dd	6.25 , dd	16.2, 6.3	15.6, 6.3
H-8	6.71, d	6.71, d	15.8	15.6

*Lit: Alkofahi, 1989

The larvae of Aedes aegypti were susceptible to the crude hexane extract with an LC_{50}=42.3 µg/ml and and LC_{50}=87.9 µg/ml.

The table below shows the comparison of the larvicidal activity with preview results obtained from *Goniothalamus species*

Plant	LC_{50}	LC_{90}
G.andersonii (bark, hexane extract)	42.3	87.9
G.andersonii (bark, ethanol extract)*	58.1	171.8
G.malayanus (bark, rthanol extract)*	33.9	110.9
G.velutines (bark, ethanol extract)*	12.2	-

*Ee G.C.L.,1996 Ph D Thesis

The larvae activity of Aedes aegypti was susceptible to hexane and ethanol extract of the plants with an LC_{50} values of less than 200 µg/ml.

Conclusions

The stem bark of *G.andersonii* provided four compounds. They are goniothalamin, stigmasterol, and two mixtures of terpenes.
Goniothalamin **1** and Stigmasterol **2** and three terpenes **3** were isolated from hexane and ethanol extract.Copaene, alpha cubebene, germacrene-D, alphacrayophylene naphthalene and caryophylene.

The larvae of *Aedes aegypti* were susceptible to the crude ethanol extract with LC_{50}=58.1C µg/ml. A dosage of 171.8 µg/ml was required for 90% mortality. The LC_{50}=42.3 µg/ml and LC_{50}=87.9 µg/ml for the crude hexane crude extract.

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References

Alkofahi A., W.W.Ma, A.T. McKenzie, S.R. Byrn, and J.L.McLaughlin. 1989. Goniothalamin *Goniothalamus andersonii*. J.Nat.Prod., 52(6):1371-13373.

Axcell, B., L. Kruger, & G. Allan. 1988. Some investigative studies with yeast foods, p. 201–209. *In* Proceedings of the 20th Convention of the Institute of Brewing (Australia and New Zealand Section). Institute of Brewing, Sydney, Australia.

Ee. G.C.L. 1996, Ph.D Thesis, Uninersity of Malaya

Holland H.L.,Diakow P.R. P.and Taylor G.J. 1978.13C Nuclear Magnetic Resoanance Spectra of Some C-19 hydroxy, C-5,6, spoxy and C-24 ethyl steroids. Can J. Chem.,56:3121-3127.

Imai, T., & T. Ohno. 1995. Measurement of yeast intracellular pH by image processing and the change it undergoes during growth phase. J. Biotechnol. 38:165–172.

Jewers J.R., Davis J.B., Dougan J., Machanda A.H., Blunden G., Kyi A and Wetchapinan S.1972. Goniothalamin and its distribution in four *Goniothalamus* Species. *Phytochem.,* 11:2025

Sinclair J.1985. A New Species of Goniothalamus from Peat Swamp Forest in Borneo. *The Gardens ' Bulletin Singapore.* XIV 423, 149.

van Uden, N. 1989. Effect of alcohols on the temperature relations of growth and death in yeasts, p. 77-88. In N. van Uden (ed.), Alcohol toxicity in yeasts and bacteria. CRC Press, Inc., Boca Raton, Florida.

Warth, A. D. 1988. Effect of benzoic acid on growth yields of yeasts differing in their resistance to preservatives. Appl. Environ. Microbiol. 54:2091–2095.