

Chemical Study of the Ghanaian Parasitic Plant *Thonningia sanguinea* Vahl

(ガーナ産寄生植物 *Thonningia sanguinea* Vahl の成分に関する化学的研究)

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[目的]

Thonningia sanguinea Vahl (Balanophoraceae) is a parasitic plant that has found itself as an important part of traditional medicine in many African cultures. In Ghana, the plant has been used extensively for the management of pain, infections and inflammatory conditions like bronchial asthma, arthritis, dysmenorrhea, gastroenteritis, and both male and female infertility. Despite the interesting clinical use in Ghana, as well as the reported pharmacological activities of the extracts from the plant, there is no detailed study on the chemical composition of the plant. The study therefore aimed to use a combination of biological and chemical approaches as valuable tools to investigate the chemical profile of the methanolic extract and fractions from *T. sanguinea* Vahl, to better understand these reported pharmacological activities.

[実験方法と結果]

The whole plant of *T. sanguinea* was collected from the Eastern region of Ghana. The crude methanolic extract (423 g) obtained from the plant was fractioned into the *n*-hexane (20 g), ethyl acetate (260 g), *n*-butanol (88 g) fractions. The resultant aqueous layer (30 g) was applied on a Diaion HP-20 column and eluted serially with appropriate solvents to yield 20% MeOH, 50% MeOH, 80% MeOH, 100% MeOH, 80% acetone and 100% acetone fractions. The crude extract and its fractions were tested against the standard gram-negative bacterial strain *Vibrio parahaemolyticus* in the paper disc method. HPLC fingerprints of the crude methanolic extract, its fractions and isolated compounds [(1 – 13), TSC-1 and TSC-2] were applied as quality control to standardize the herbal medicinal product from *T. sanguinea*.

**Antimicrobial activity of the crude methanolic extract of *T. sanguinea* and its fractions
(Chapter 2-1)**

The antimicrobial activity of the crude methanolic extract of *T. sanguinea* and its fractions were tested against the standard bacterial strain *Vibrio parahaemolyticus*. All fractions except for the 50% MeOH showed moderate to significant antibacterial action against the test microbe.

Compounds isolated from the *n*-hexane fraction of *T. sanguinea* (Chapter 2-2)

The *n*-hexane fraction (20 g) was subjected to repeated column chromatographic procedures to isolate and characterise one sphingosine-type cerebroside (**TSC-1**) and one phytosphingosine-type cerebroside (**TSC-2**), with both containing mainly a 2-hydroxy fatty acid and β -D-glucopyranose moieties, and one β -Sitosteryl-3 β -D-glucopyranoside-6'-*O*-fatty acid methyl esters molecular species (**TSS-1**). β -Sitosterol-3 β -D-glucopyranoside-6'-*O*-palmitate (**1**), β -sitosterol (**2**), β -sitosterol-3 β -D-glucopyranoside (**3**), β -stigmasterol (**4**), β -stigmasterol-3 β -D-glucopyranoside (**5**), cholesterol (**6**), betulinic acid (**7**), one saturated fatty acid methyl ester (**14**) and a saturated fatty acid (**15**) were also isolated. Their structures were clarified on the basis of chemical methods, spectroscopic techniques (IR, ^1H NMR, ^{13}C NMR experiments and FAB-MS) and comparison with appropriate literature data.

Compounds isolated from the ethyl acetate fraction of *T. sanguinea* (Chapter 2-3)

A series of chromatographic separation of the ethyl acetate fraction (70 g) of *T. sanguinea* led to the isolation and characterization of five known lignans: (+)-epipinoresinol (**8**), (+)-pinoresinol (**9**), (+)-cycloolivil (**10**), (+)-secoisolariciresinol (**11**) and (+)-isolariciresinol (**12**), one known flavanone (+)-eriodictyol (**13**), one saturated fatty acid (**16**) and one unsaturated fatty acid (**17**). Their structures were also clarified on the basis of spectroscopic techniques (^1H NMR, ^{13}C NMR experiments and FAB-MS) and comparison with appropriate literature data.

Development of HPLC fingerprints of the crude methanolic extract, its fractions, isolated compounds and Herbal Medicinal Product from *T. sanguinea* (Chapter 3-1)

The HPLC fingerprints of the crude methanolic extract, its fractions, the isolated compounds [(**1** – **13**), **TSC-1** and **TSC-2**] and the herbal medicinal product from *T. sanguinea* were studied. A comparative analysis of the HPLC fingerprints of the isolated compounds and the crude methanolic extract, with the aqueous Herbal Medicinal Product was then conducted for quality control purposes. All these compounds were also present in the herbal medicinal product since their retention times corresponded exactly with that seen in the crude methanolic extract. Hence, they can be used as analytical markers for quality assurance of batch-to-batch preparations of the product. Chromatographic fingerprints have

also been provided for *T. sanguinea* and its herbal product for standardization and quality assurance.

[結論]

In summary, the moderate to significant activity of the *n*-hexane, *n*-butanol, and aqueous fractions from *Thonningia sanguinea* against *Vibrio parahaemolyticus* in the antibacterial assay gives scientific basis for its use as an anti-infective agent. The study also led to the isolation of one sphingosine-type cerebroside (**TSC-1**), one phytosphingosine-type cerebroside (**TSC-2**), one β -sitosteryl-3 β -D-glucopyranoside-6'-*O*-fatty acid methyl esters molecular species (**TSS-1**), seven triterpenes (**1** – **7**), five lignans (**8** – **12**), one flavanone (**13**), one saturated fatty acid methyl ester (**14**) and three fatty acids (**15** – **17**) from the *n*-hexane and ethyl acetate fractions of the plant. To the best of our knowledge, all the isolated compounds in this study are being reported for the first time in this genus. They can be developed as analytical markers for *T. sanguinea*-based herbal medicinal products. These compounds have a wide range of biological activities and may act individually or in synergy to produce these biological actions. They may therefore be responsible for these biological actions, giving credence to the use of the *T. sanguinea* in Ghanaian traditional medicine.

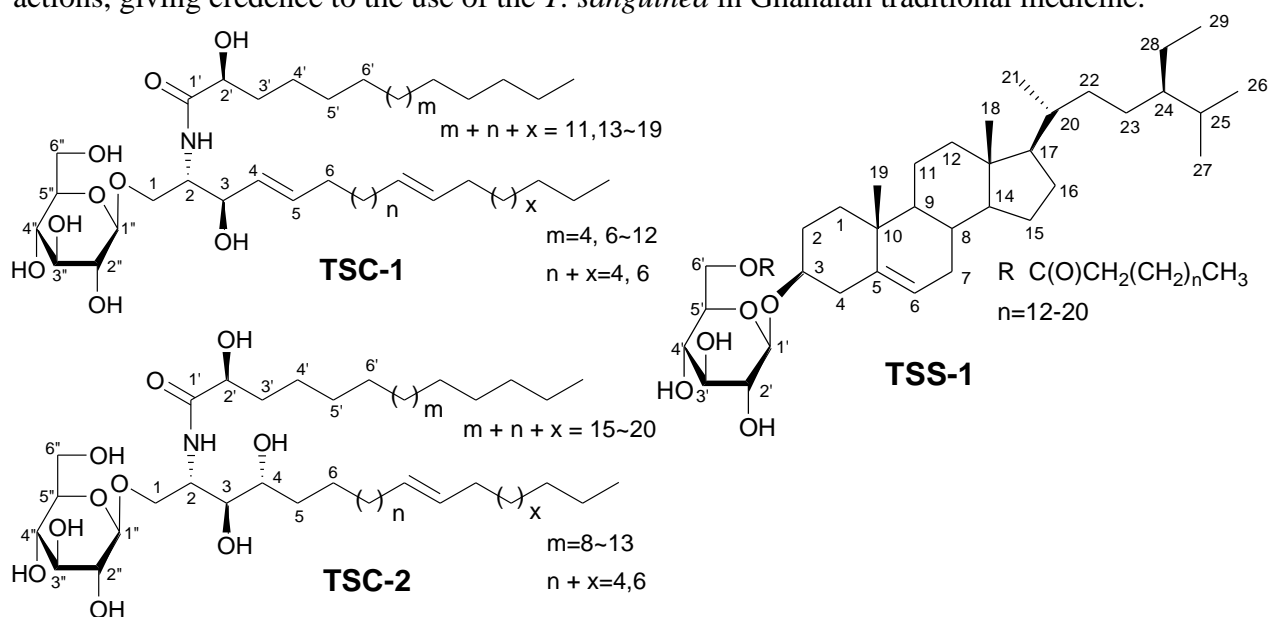


Figure 1. Structures of selected isolated compounds

[基礎 となった学術論文]

1. Thomford A. K., Abdelhameed R. F. A., Yamada K. Chemical studies on the parasitic plant *Thonningia sanguinea* Vahl. *RSC Adv.* **8**, 21002-21011 (2018).