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REVIEW ARTICLE

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Resources and bioactive substances from Taiwania (*Taiwania cryptomerioides*)

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Abstract Taiwania (*Taiwania cryptomerioides* Hayata), native to Taiwan, is one of the most economically important tree species grown there. In this article we summarize the current results of phytochemistry and bioactivity of Taiwania extracts, including antifungal, antitermite, antibacterial, and antimite activities as well as cytotoxicity against three tumor cells. The resources of Taiwania are also addressed.

Key words *Taiwania cryptomerioides* · Bioactivity · Cytotoxicity · Resources

Introduction

The family Taxodiaceae, as traditionally defined, includes 10 genera and about 16 species that are widely distributed in northern temperate to subtropical regions of both the Old and New Worlds, with one genus present in the Southern Hemisphere. Widely ranging genera are *Taxodium* (from the northeastern United States to Florida and Mexico), *Cunninghamia* (northern China to Taiwan), *Taiwania* (southern China to Taiwan and northern Myanmar), and *Cryptomeria* (China and Japan). The rest are local, with *Sequoia* and *Sequoiadendron* in western North America, *Glyptostrobus* and *Metasequoia* in southern and central China, and *Athrotaxis* in Tasmania. All the genera of this family are either monotypic or consist of small, closely related species groups (e.g., *Athrotaxis*).[1] *Taiwania* is treated by most taxonomists as a monotypic genus comprising the only Taiwania species (*Taiwania cryptomerioides* Hayata). In 1904 Konishi first collected Taiwania in the Wushonken area, a side branch of the Alishan range, on the western slope of Mt. Yushan. The collected specimen then was sent to Hayata, who found that it differed from other genera known at that time. After careful study, Hayata proposed a new genus and assigned the name *Taiwania cryptomerioides* to this species.[2] Taiwania, named after its native island of Taiwan, is a close relative to *Cryptomeria*, with similar foliage and general appearance except that Taiwania has stiffer, sharper, more widely angled needles. The heartwood of Taiwania is yellowish-red with distinctive purplish-pink streaks. In the climate of Taiwan, Taiwania has an excellent decay resistance; and it is one of the most economically important tree species grown in Taiwan.

With the beginnings of phytochemistry in 1931, Kafuka and Kato began to characterize the constituents of essential oils from Taiwania heartwood. By now, according to Kuo et al.[3] and Wang et al.,[4] more than 100 compounds, including terpenoids, lignans, isoflavones, and other compounds, have been isolated from this species during the past 70 years. Much of this compound identification should be credited to the research groups of Lin, Chen, and Kuo. Since 1993 we have characterized the putative bioactivities of specific constituents of Taiwania and evaluated the potential usages of the phytochemicals isolated from Taiwania for pharmacological applications. Although the identification of compounds and bioactive constituents is still ongoing, in this article we summarize the current results regarding the phytochemistry and bioactivity of Taiwania extracts. The resources of Taiwania are also reported herein.

Distribution and resources of Taiwania in Taiwan

Taiwan Island is covered by a central mountain system including the Central range, Shueshan range, and Yushan range. Taiwania is distributed broadly over the mountain areas of Taiwan, although it is sparse in most localities.

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According to the investigation by Liu and Su, Taiwania is found throughout the middle elevations of Taiwan in more than 30 localities.[2] Two-thirds of these habitats range from 1500 to 2400 m in elevation. Generally, Taiwania grows well on all exposures except the sunny south-facing slopes. It seems that there is no geographical restriction as to the distribution of Taiwania in Taiwan, and its occurrence is conditioned by the microclimate, soil factors, and forestry distribution. Unfortunately, because of extensive logging, it is now difficult to find a large area of natural Taiwania forestry.

Chen et al. studied the growing stock and growth estimation of Taiwania plantations in the Liukuie area,[5] predicting that the average cumulative annual increment (CAI) of Taiwania is 12.5 m^3/ha, and the annual growth rate is 5.8%. Taiwania is a fast-growing tree species relative to other conifers in Taiwan, and it is native to Taiwan. Thus, Taiwania is one of the most important plantation tree species selected by the government since 1967. According to the most current forestry resource and land use inventory in Taiwan by the Council of Agriculture (December 1995), the total forestry standing volume is 358 744 km^3. Of this, the standing volume of Taiwania is 724 km^3, including natural stands (461 km^3) and plantation stands (263 km^3).

Bioactive constituents of Taiwania

Although many compounds have been isolated from Taiwania, the bioactivities of these constituents have been little investigated until recently. To develop a comprehensive understanding of bioactive constituents of Taiwania, an integral research team, including forestry scientists, phytochemists, organic chemists, entomologists, and pharmacologists, have been working together over the past years. We have characterized the putative bioactivity of some specific chemical constituents (Fig. 1) isolated from the heartwood of Taiwania, including antifungal and antitermitic activity. In addition, we have studied the antibacterial and antimite activities of the heartwood extractives, essential oils, and their derived compounds from the plant. Our research group has also evaluated the potential usages of the phytochemicals isolated from Taiwania in pharmacological applications. Our results are summarized as follows.

Bioactive compounds contributing to the wood decay resistance of Taiwania

To develop methods to prolong the service life of wood is one of the challenges of wood utilization researchers. With regard to environmental protection, finding the bioactive constituents in this highly durable species and understanding their mechanisms are appropriate approaches to achieve protection of the wood without polluting the environment. Fungi and termites are two of the most harmful organisms for wooden structures worldwide. Taiwania is a species with excellent antifungal and antitermitic properties.[6–9] Figure 2 shows the antifungal activity of the dominant compounds isolated from Taiwania, including taiwanin A (**1**), savinin (**2**), taiwanin C (**3**), helioxanthin (**5**), ferruginol (**9**), hinokiol (**10**), sugiol (**11**), α-cadinol (**12**), and cedrol (**15**). Among them, α-cadinol has been demonstrated to have the most antifungal effectiveness.[8] It completely inhibited the growth of *Coriolus versicolor* (white rot) and *Laetiporus sulphureus* (brown rot) at levels as low as 100 ppm. Because cadinol exhibits the strongest antifungal activity, the amounts of this cadinane-type compound and its structure–activity relations were further studied. The results showed that the total amount of cadinols extracted from heartwood with n-C_6H_{14} (6.49 mg/kg, based on wood weight) was much larger than that of the essential oils that were collected by water distillation from leaves (0.04 mg/kg), sapwood (0.36 mg/kg), or heartwood (1.77 mg/kg).[8] Compared with the antifungal activities of cadinanes, α-cadinol still demonstrates the best antifungal effectiveness. Due to the low antifungal properties of T-muurolol, it

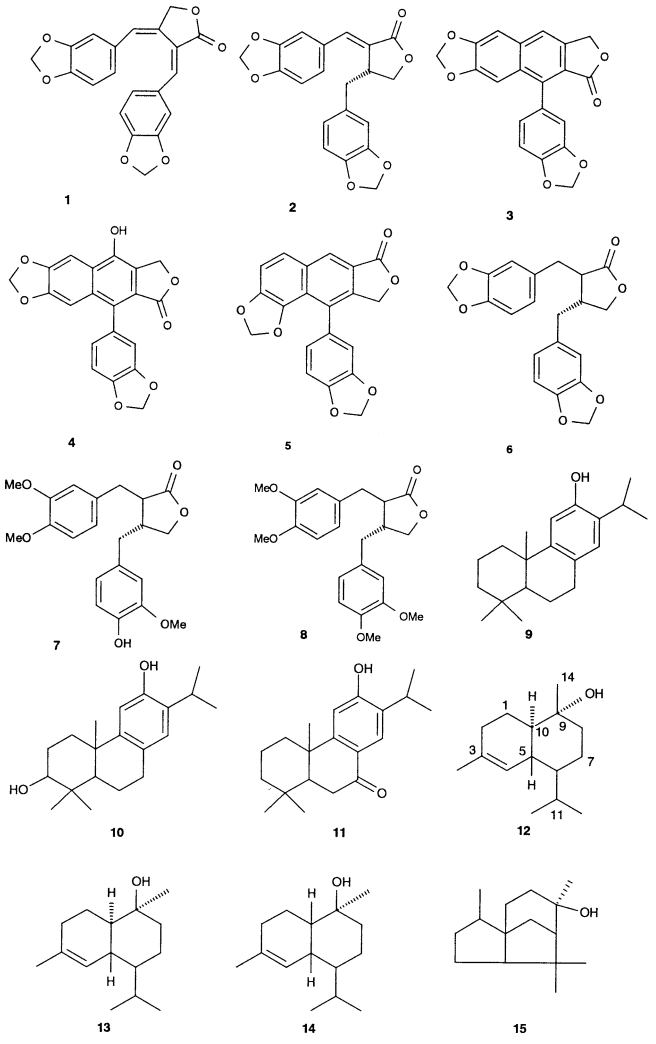

Fig. 1. Bioactive compounds isolated from *Taiwania cryptomerioides*. **1** Taiwanin A. **2** Savinin. **3** Taiwanin C. **4** Taiwanin E. **5** Helioxanthin. **6** Hinokinin. **7** Arctigenin. **8** Dimethylmatairesinol. **9** Ferruginol. **10** Hinokiol. **11** Sugiol. **12** α-Cadinol. **13** T-Cadinol. **14** T-Muurolol. **15** Cedrol

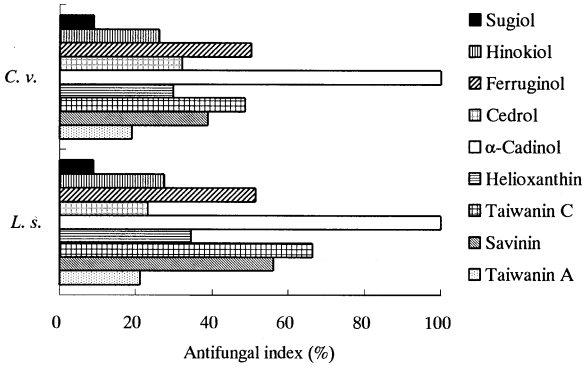

Fig. 2. Antifungal indices of extractives isolated from Taiwania heartwood. *C. v.*, *Coriolus versicolor* (white rot fungus); *L.s.*, *Laetiporus sulphureus* (brown rot fungus)

Table 1. Cytotoxicity of lignans and sesquiterpenes isolated from Taiwania heartwood

Compounds	ED_{50} (μM), by cell line		
	MCF-7	A-549	HT-29
Lignans			
Taiwanin A	0.16	0.15	0.11
Savinin	0.52	6.70	1.52
Taiwanin C	4.06	5.82	14.31
Taiwanin E	0.50	1.23	0.55
Hinokinin	4.87	9.24	4.02
Dimethylmatairesinol	1.82	1.91	1.35
Sesquiterpenes			
α-Cadinol	2.47	3.13	0.67
T-Cadinol	2.49	5.43	7.93
T-Muurolol	0.60	3.21	1.78

seems that the cadinane skeletal sesquiterpenoid with a *cis* configuration and an axial hydroxyl group at C-9 has the lowest antifungal activity. In contrast, α-cadinol has an equatorial hydroxyl group at C-9 and a *trans* configuration at the ring junction (C-5 connects to C-10) and exhibited the strongest antifungal activities.

In addition to illustrating the antifungal activities of compounds from Taiwania, we examined the antitermitic activities of the three dominant compounds, namely α-cadinol, cedrol, and ferruginol.[9] The order of antitermitic activity of these compounds against *C. formosanus* was cedrol followed by α-cadinol and then ferruginol. Termite mortality rates (at 5 mg/g after 14 days) were 50%, 30%, and 12%, respectively, for the three compounds. Moreover, the antitermitic activities of α-cadinol and cedrol (10 mg/g) increased after 14 days, whereas no dosage-dependent effects occurred with ferruginol. The decreasing order of termite mortality at 10 mg/g dosage for 14 days of testing was cedrol (100%) > α-cadinol (70%) > ferruginol (12%). Overall, cedrol had the strongest antitermitic activity.

Antibacterial and antimitic active compounds from Taiwania

Plant-derived essential oils have long been used as flavoring agents in food and beverages; and owing to the presence of antimicrobial compounds, they have potential as natural agents for food preservation. The antibacterial activities of the essential oils and the extractives obtained from Taiwania heartwood, sapwood, and leaves were previously investigated.[10] In addition, four compounds (α-cadinol, T-cadinol, T-muurolol, ferruginol) were isolated from the hexane-soluble fraction of methanol extractives of Taiwania heartwood, and their minimum inhibitory concentrations (MICs) against bacteria were determined. According to the results of the antibacterial test, the essential oil of Taiwania heartwood and its components inhibited the growth of gram-positive bacteria.[10] The MICs of the essential oil of heartwood for *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and methicillin-resistant *S. aureus* (MRSA) were 250, 250, 1000, and 500 μg/ml, respectively. The antibacterial activities of three cadinane-type sesquiterpenoids (α-cadinol, T-cadinol, T-muurolol) were the same against four tested bacteria, although they have different configurations. The MICs of those three compounds for the four bacteria were 250, 250, 500, and 250 μg/ml, respectively. The ferruginol was highly effective in inhibiting the growth of the four bacteria at doses as low as 50, 100, 50, and 100 μg/ml, respectively.

In addition, it has been confirmed that house dust mites are causative agents of many allergic diseases, such as bronchial asthma, allergic rhinitis, and atopic dermatitis.[11] Particularly, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* have been recognized as two major allergenic species of house dust mites. The antimite activity of the essential oils and their components obtained from Taiwania against *D. pteronyssinus* and *D. farinae* were investigated.[11] Results from the antimite tests demonstrated that the essential oil extracted from Taiwania heartwood had miticidal activity against *D. pteronyssinus* and *D. farinae* with mortality rates of 67.0% and 36.7%, respectively, at a dosage of 12.6 μg/cm^2 after 48 h. α-Cadinol had the strongest antimite activity compared with other components of the Taiwania heartwood essential oil. The mortality rates for *D. pteronyssinus* and *D. farinae* were 100% for α-cadinol at a dosage of 6.3 μg/cm^2. The order of antimite activity of the three cadinane skeletal sesquiterpenoids was α-cadinol > T-muurolol > T-cadinol.

Potential antitumor compounds from Taiwania

To study the cytotoxicity of the major chemical ingredients of Taiwania, we first assessed the effects of eight lignans [taiwanin A (**1**), savinin (**2**), taiwanin C (**3**), taiwanin E (**4**), helioxanthin (**5**), hinokinin (**6**), arctigenin (**7**), dimethylmatairesinol (**8**)] and three sesquiterpenoids [α-cadinol (**12**), T-cadinol (**13**), T-muurolol (**14**)] on the viability of three human tumor cells including A-549 lung carcinoma, MCF-7 breast adenocarcinoma, and HT-29 colon adenocarcinoma.[12,13] The results are summarized in Table 1. Although the cytotoxicity of lignans and sesquiterpenoids of Taiwania showed little differential cytotoxicity, some of them (taiwanin A, taiwanin E, dimethylmatairesinol) exhibited significant cytotoxicity

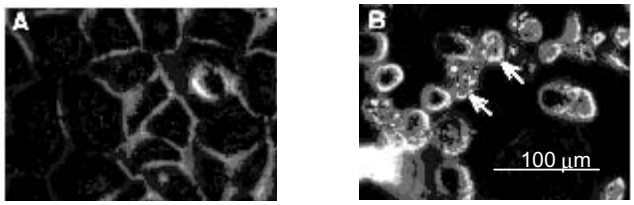

Fig. 3. Morphological changes in MCF-7 cells examined 24 h after addition of 0 μM (**A**) or 4 μM (**B**) of taiwanin A to the medium. MCF-7 cells were plated in a 96-well plate and photographed under a phase contrast microscope. *White arrows* indicate apoptotic bodies

against all of the human tumor cells tested. Overall, taiwanin A is the most cytotoxic compound. The median effective doses (ED_{50}) of taiwanin A against A-549, MCF-7, and HT-29 cells were 0.2, 0.2, and 0.1 μM, respectively.[13]

Based on the distinct morphological and biochemical changes of the dying cells, two modes of cell death have been described: apoptosis and necrosis. A number of anticancer drugs have been shown to induce apoptosis in cancer cells. Apoptosis, a genetically controlled response by which cells destroy themselves, has become a major focus in the study of cancer biology. It was thus important to establish the mode of cell death induced by taiwanin A. Figure 3 shows the morphological changes in MCF-7 cells examined 24 h after addition of 0 μM (control, Fig. 3A) or 4 μM (Fig. 3B) of taiwanin A.[13] At a dosage at 4 μM, the tumor cells show that chromatin condensation and apoptotic bodies (such as the one indicated by a white arrow in Fig. 3B) were widespread throughout the entire population. These morphological changes suggested that taiwanin A induced apoptotic cell death in MCF-7 cells. In addition to the morphological identification, flow cytometric analyses indicated that 44.3% of cells (sub-G_1 peak) experienced apoptosis when MCF-7 cells were treated for 24 h with 2 μM of taiwanin A.[13] In the control cells, only a minor fraction of the cell population (4.6%) experienced apoptosis. Finally, the DNA fragmentation assay was used to confirm that the mode of cell death induced by taiwanin A on MCF-7 was apoptosis.[13] On the basis of results noted above, it was determined that the tumor cell death induced by taiwanin A was due to apoptosis. The mechanisms for inducing apoptosis in tumor cells require further elucidation in future studies.

Conclusions

The plant kingdom represents an extraordinary reservoir of a wide spectrum of diverse and unique bioorganic molecules. During the past few years worldwide, the market availability and public use of a broad range of phytochemicals speculated or reputed to be useful as pharmaceuticals or nutraceuticals have drastically increased. As a positive but cautious note on this fact, herbal medicines and nutraceuticals/food, as an alternative approach to synthetic drugs, apparently have played an important role at the basic, public health care level in various countries, especially in Asia. As shown by the results summarized in this article, a well-coordinated research team has been investigating bioactive compounds from Taiwania, an indigenous tree in Taiwan. Although much work remains to be done, this good collaboration and integrated research group will continue to work closely to find more significant uses for Taiwania.

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