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Anticancer Effects of Some Medicinal Thai Plants

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http://dx.doi.org/10.5772/67648

Abstract

Ethanolic extracts from thirty Thai edible plants collected from Sa Keao province, Thailand, were screened for in vitro antiproliferative effect on HCT-116 human colon cancer cell line using cell titer 96 aqueous one solution cell proliferation assay. It was found that leaf extract of *Crateva adansnii*, fruit and leaf extracts of *Ardisia elliptica*, shoot extract of *Colocasia esculenta*, leaf extract of *Cratoxylum fomosum*, and leaf extract of *Millettia leucantha* exhibited antiproliferative activities. The fruit extract of *Ardisia elliptica* showed the highest antiproliferative activity. Ethanolic extract of the stems from *C. fenestratum* and its dichloromethane and aqueous fractions showed antiproliferative activity to human colorectal cancer cells (HCT-116) determined by cell growth assay. Berberine, one of the major alkaloid in the stems of *C. fenestratum*, also promoted antiproliferative effect. Extracts from the leaves of three *Azadirachta* species in Thailand, *A. indica*, *A. indica* var. *siamensis*, and *A. excelsa*, were reported to promote in vitro antioxidant effects determined by various methods. Ten *Russula* mushroom collected from northeastern part of Thailand were tested for in vitro antioxidant activities using photochemiluminescence assay for both lipid-soluble and water-soluble antioxidant capacities. *R. medullata* extract exhibited the highest antioxidant effects in both lipid-soluble and water-soluble models.

Keywords: anticancer, *Coscinium fenestratum*, berberine, *Azadirachta*, *Russula*

1. Introduction

Cancer cells uncontrollably divide to form masses of tissue, which are called tumors. Tumors can grow and interfere with the functions of many bodily systems including the digestive, nervous, and cardiovascular systems. Cancer has been reported to be the first in the rank of causes of the death in the Thai population. Liver, colon, and lung cancers are the most prevalent cancers in Thai males, while breast, cervical, and colon cancers are the most prevalent cancers in Thai females [1].
The development of cancer or carcinogenesis occurs through a multistep process involving the mutation, selection of cells with a progressive increasing capacity for proliferation, survival, invasion, and metastasis [2]. The first step in the process, tumor initiation, relates to the genetic alteration leading to the changes in normal cells. Then, in the promotion or development stage, the cells abnormally proliferate leading to the outgrowth of a population of clonally derived tumor cells [2]. This stage can be stimulated by carcinogens, which are a group of substances such as tobacco, asbestos, arsenic, radiation such as gamma and X-rays, sun light, polycyclic hydrocarbons, nitrosamines, and aflatoxins: these substances do not directly cause cancers but promote or aid the development of cancers [2, 3]. After that, tumor progression continues as additional mutations occur within the cells of the tumor population to further advantage the cancer cells, such as more rapid growth, which will allow them to become dominant within the late tumor population. The process is called clonal selection, since a new clone of tumor cells evolves on the basis of its increased growth rate or other properties such as survival, invasion, or metastasis. Clonal selection continues throughout tumor development, so tumors continuously become more rapid-growing and increasingly malignant [2].

2. Cancer therapy

The modern treatments for cancers mainly are surgery, radiation, and chemotherapy. However, most of chemotherapeutic drugs are not specific to only cancer cells, but also cause damage to normal cells, especially bone marrow, mucous glands, mucous membranes, hair, and nails and can lead to the suppression of the immune system [3]. The success of chemotherapy depends on the number of cancer cells, the proliferation rate, the duration of the drug administration, and the therapeutic interval. To avoid drug resistance, polychemotherapy is always used instead of monochemotherapy [3]. The anticancer drugs can also cause some other side effects including nausea, vomiting, agranulocytosis, inhibition of spermatogenesis and ovulation, alopecia, inflammation of mucous membranes, and teratogenesis [3].

Some compounds separated from natural products are now being developed as modern medicines for the treatments of cancers including paclitaxel, catharanthus alkaloids, and derivatives of podophyllumtoxin.

Paclitaxel was separated from the bark of *Taxus brevifolia* Nutt. (Pacific Yew), which is a tree in Taxaceae. Paclitaxel will bind with β-tubulin and stimulate the aggregation of a tubulin subunit to become a nonphysiological microtubule composed of 12 proto-filaments, which cause the inhibition of cell cycles in mitosis and interphase (G2-phase) and lead to cell apoptosis. This compound is normally used in an injection formulation as the adjuvant chemotherapy for the treatments of ovarian, breast, and bronchial cancers [3].

Some alkaloids are separated from the leaves of *Catharanthus roseus* (L.) G. Don., such as vincristine and vinblastine. Vincristine is used for the treatment of lymphatic leukemia, neuroblastoma, and Wilms tumor, while vinblastine is used to treat lymphogranuloma (Morbus Hodgkin), lymphosarcoma, testicular carcinoma, and chorionic carcinoma [3].
Podophyllotoxin was separated from the rhizome of *Podophyllum peltatum* L. or American mandrake. Two derivatives of podophyllotoxin, etoposide and teniposide, are now being developed and used as anticancer drugs. Etoposide is used for the treatment of bronchial cancer, testicular carcinoma, and chorionic carcinoma, while teniposide is used to treat brain or bladder cancers [3]. The chemical structures of some anticancer compounds from natural products are shown in Figure 1.

**Figure 1.** Chemical structures of some anticancer compounds from natural products. A = paclitaxel, B = vinblastine, C = vincristine, D = etoposide, E = teniposide.

### 3. Anticancer effects of medicinal plants and natural products

Natural products from plants, animals, marine sources, and minerals have been used for the treatments of ailments and diseases for a long time. In Thai traditional medicine, the word “cancer” could refer to the symptom of chronic wound, abscess, emaciation, and weak [4]. Active phytochemicals in plants can be classified into two main groups of primary metabolites, which are the compounds necessary for plant growth and development such as carbohydrates, proteins, and fats. Another group is secondary metabolites, which promote the defense mechanisms or support the lives of the plants; they include polyphenolic compounds, flavonoids, terpenoids, and alkaloids [5]. Ethanolic extracts from thirty Thai local edible plants collected from Wang Nam Yen district, Sa Keao province, Thailand were screened for the *in vitro* anti-proliferative effect on HCT-116 human colon cancer cell lines using a cell titer 96 aqueous one solution cell proliferation assay. It was found that six ethanolic plant extracts, including a leaf extract of *Crateva adansnii*, fruit and leaf extracts of *Ardisia elliptica*, a shoot extract of *Colocasia esculenta*, a leaf extract of *Cratoxylum fomosum*, and a leaf extract of *Millettia leucantha* exhibited antiproliferative activities on the HCT-116 cell line. The fruit extract of *Ardisia elliptica* showed the highest antiproliferative activities with an IC$_{50}$ value of 5.12 ± 0.54 μg/ml [6]. The mechanisms of the action of medicinal plants for anticancer effects have been reported as following [4]:

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http://dx.doi.org/10.5772/67648

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3.1. Inhibition of cell division in the cancer cell cycle

Alpha-mangostin from mangosteen (*Garcinia mangostana*) fruit rind promoted inhibitory effects to breast cancer cell line (MDA-MB-231) by inhibition of cell division in G1 and S phases [7]. Methanol extract of *Morus alba* L. leaves inhibited liver cancer cell line Hep G2 by inhibition of cell division in G2/M phase [8]. Cucurbitacin B, a triterpenoid from *Trichosanthes cucumerina* L., also inhibited breast cancer cell division in G2/M phase [9].

3.2. Induction of cancer cell apoptosis

This mechanism includes some minor mechanisms which stimulate anticancer genes, induction of caspase enzymes, induction of free radical formation, inhibition or induction of enzymes relating to histone protein, and the formation of spingosine or ceramide [4]. Dehydrocostus lactone from the root of *Saussurea lappa* induced the apoptosis of liver cancer cells Hep G2 and PLC/PRF/5 via p53 protein [10]. Water extract of the seed from *Sapindus rarak* Candolle. induced lung cancer cells A549 apoptosis through the induction of the caspase enzyme [11], while methanol extract of *Derris scandens* Benth. induced apoptosis of colon cancer cells SE480 by increased caspase-3 activity and down-regulated Bcl-2 and up-regulated Bax protein of SW480 cells; it also significantly induced cell necrosis determined by the release of LDH [12]. Alpha-mangostin separated from the fruit rind of mangosteen also upregulated Bax and down-regulated Bcl-2 proteins in rat liver tissue [13]. Methanol extract from stem bark of *Murrannia loriformis* (Hassk.) R.S.Rao & Kammathy, inhibited breast, lung, colon, and liver cell lines [15].

3.3. Immune stimulation

Methanol extract from the leaves of *Moringa oleifera* Lam. exhibited immune stimulation effect both cell-mediated immunity and humoral immunity by induction of neutrophile production and stimulation of macrophages in animals damaged by the toxicity of anticancer drugs [16].

In Thai traditional medicine, there are some medicinal formulas compose of several plants in different ratios. These formulas are traditionally used for a long time usually for the treatments of cancers in patient with the late stage cancers, patients who cannot improve after treatment with chemotherapy, radiation or surgery, patients with cancers in several organs or patients with recurrent diseases [4]. The sources of anticancer herbal formulas usually come from local traditional doctors or priests in the temples (in Thai, temple is called as “Wat”), with the normal method of preparation being the decoction of plant materials with water [4]. A herbal remedy from Wat Tha-it (Tha-it temple), Ang Thong province, Thailand, composed of several plant materials including *Gelonium multiflorum* A. Juss., *Erycibe elliptilimba* Merrill & Chun, *Balanophora abbreviate* Blume, *Smilax china* L., *Smilax glabra* Wall. ex Roxb., and *Millingtonia hortensis* Linn. was reported to significantly promote synergistic effects on doxorubicin in the treatment of A549 cancer cells by the inhibition of cell divisions in the G2/M phase [4, 17]. Another herbal remedy is from a Thai herbal nursing home, Wat Khampramong, Sakon Nakhon province comprises of several plant materials such as *Rhinacanthus nasutus* (L.) Kurz, *Acanthus ebrateatus* Wall., *Smilax glabra* Wall. ex Roxb.,
Artemisia annua L., Angelica sinensis (Oliv.) Diels, Salacia chinensis L., and Orthosiphon aristatus Miq [18]. This herbal remedy can inhibit the growth of some cancer cell lines such as breast adenocarcinoma MDA-MB 231, synovial sarcoma SW982, hepatocellular carcinoma HepG2, cervical adenocarcinoma HeLa, and lung carcinoma A549 [18].

4. Some potential Thai medicinal plants with anticancer effects

4.1. Coscinium fenestratum (Gaertn.) Colebr

NAG-1 or nonsteroidal anti-inflammatory drug (NSAID)-activated gene was identified in COX-negative cells by PCR-based subtractive hybridization from an NSAID-induced library as a divergent member of the TGF-β superfamily [19]. The overexpression of NAG-1 in cancer cells results in growth arrest and an increase in apoptosis, suggesting that NAG-1 has antitumorigenic activity [20]. NAG-1 expression is also upregulated by a number of dietary compounds, medicinal plants, and anticancer drugs [21–25]. Coscinium fenestratum is one of the medicinal plants that promoted antiproliferative effects on colon cancer cell lines with mechanisms related to NAG-1 [20].

Coscinium fenestratum (Gaertn.) Colebr. is a large climber with yellow wood and sap, known in the Thai language as Hamm or Kamin khruea. The genus Coscinium belongs to the tribe Coscinieae of the family Menispermaceae. This genus comprises two species, which are Coscinium blumeanum Miers. and C. fenestratum (Gaertn.) Colebr. Both of them are stout woody climbers growing in the tropical rain forest regions of Asia [26]. Coscinium species are characterized by the axillary flowers, extra-axillary or cauliflorous in racemiform, or peduncled subumbellate aggregate, of 20–50 cm in length. The inflorescences are axillary or cauliflorous with 6–12 florets. Male flowers are sessile or with pedicels, up to 1 mm. Sepals are broadly elliptic to obovate with the inner 3–6 spreading, yellow, and 1.5–2 mm long. Stamens are 6 with 1 mm long. The Sepals of female flower are as in male flowers. Staminodes are 6 and claviform with 1 mm long. Drupes are subglobose, tomentellous, brown to orange or yellowish, 2.8–3 cm diameter. Pericarp is drying woody. Seeds are whitish and subglobose with the enveloping condyle. The leaves are subpeltate or ovate, large, hard-coriaceous, palmately nerved, reticulate, and densely hairy beneath [26]. Physical characteristic of the Coscinium fenestratum stem (cross section) is shown in Figure 2.

The stem decoction and maceration extracts of Coscinium fenestratum have been traditionally used in the Northeastern part of Thailand for the treatment of various diseases such as cancer, diabetes mellitus, and arthritis [27]. The ethanolic extract of the stems from C. fenestratum and its dichloromethane and aqueous fractions showed antiproliferative activity on human colorectal cancer cells (HCT-116) determined by a cell growth assay. Berberine, one of the major alkaloids in the stems of C. fenestratum, also promoted an antiproliferative effect [20]. The mechanisms of action of the extracts from C. fenestratum were reported as the activation of proapoptotic proteins and pparγ [20]. It was also reported that berberine facilitated the apoptosis of cancer cells, and the molecular targets for its activity are NAG-1 and AFT3 [24]. The chemical structure of Berberine is shown in Figure 3.
Oxidative stress is considered to be of some importance for many ailments and pathologies; including cardiovascular diseases, cancers, rheumatoid arthritis, and Alzheimer’s disease [28]. Polyphenolic compounds have been reported to have important anticancer and chemopreventive effects [29]. Phenolic acids such as gallic acid, ellagic acid, and ferulic acid induce apoptosis in cancer cells, activated caspase, prevented cancer formation, and suppress the angiogenesis of cancer [29–32]. Flavonoids such as quercertin and kaempferol also promote apoptosis, inhibit oncogenes, and generated cell cycle arrest [29, 33–35].

4.2. *Azadirachta* plants

Figure 2. Physical characteristic of *Coscinium fenestratum* stem purchased from Nongkhai province, Thailand (cross section ×1).

Figure 3. Chemical structure of Berberine.

Natural Products and Cancer Drug Discovery
Suttajit et al. [36] studied the antioxidant activities of extracts from many Thai medicinal plants using a ABTS-metmyoglobin assay and reported some plants with high antioxidant activities; including *Uncaria gambier* Roxb., *Piper betle* Linn., *Camellia sinensis* (L.) Kuntze., *Azadirachta indica* A. Juss. var. *siamensis* Valeton., *Curcuma zedoaria* Roxb., *Syzygium aromaticum* (L.) Merr. & Perry and *Tamarindus indica* Linn. When focusing on Thai medicinal plants, the Siamese neem tree (*Azadirachta indica* A. Juss. var. *siamensis* Valeton.) is an interesting plant that showed high antioxidant activity in the screening test [36, 37]. Moreover, there are reports about its antioxidant potential based on the antioxidant content as the butylated hydroxyanisole (BHA) equivalent of Thai indigenous vegetable extracts. From this report, the Siamese neem tree leaf extract appeared to be a high potency antioxidant, containing more than 100 mg BHA equivalent in 100 g fresh weight.

*Azadirachta* plants comprise of three different plant species; *Azadirachta indica* A. Juss or *A. indica* A. Juss var. *indica* (neem), *Azadirachta indica* A. Juss. var. *siamensis* Valeton (Siamese neem tree), and *Azadirachta excela* (Jack) Jacobs. (marrango tree). The Siamese neem tree leaves are wider, longer, and thicker than the leaves of neem, while the marrango tree has the widest, longest, and thickest leaves. The margin of the leaflet of Siamese neem tree is crenate to entire, while the margin of neem is serrate and that of marrango tree is entire to undulate. The colors of the leaflet blade of the Siamese neem tree, neem, and marrango tree are green, light green, and dark shiny green, respectively [38, 39]. The physical characteristics of Siamese neem tree, neem, and marrango tree leaves are shown in Figure 4.

The leaves and flowers of Siamese neem tree and neem have been traditionally used as element tonics and antipyretic and gastric secretion stimulating agents, while the stem bark of all *Azadirachta* plants is used to treat amoebic dysentery and diarrhea [40, 41]. There also reports suggesting that polysaccharides and limonoids found in neem bark, leaves, and seed oil reduce tumors and cancers and showed effectiveness against lymphocytic leukemia [42–44]. Moreover, the young leaves and flowers of the Siamese neem tree are popularly consumed as vegetables [39].

Figure 4. Physical characteristics of *Azadirachta* plants; A = Siamese neem tree (*Azadirachta indica* var. *siamensis*), B = neem (*Azadirachta indica*), C = marrango tree (*Azadirachta excela*).
For the antioxidant effect, *Azadirachta* plants were reported to promote *in vitro* activities tested by various methods. Extracts from the leaves of *A. indica*, *A. indica* var. *siamensis*, and *A. excelsa* were reported to promote *in vitro* antioxidant effects determined by a DPPH scavenging assay, Fremy’s salt assay, ESR detection of POBN spin adducts, and an oxygen consumption assay [45, 46]. The leaf’s aqueous and flower ethanol extracts from the Siamese neem tree provide antioxidant activity on lipid peroxidation formation induced by UV-irradiation of a Chago K-1 bronchogenic cell culture at a concentration of 100 μg/ml determined by the thiobarbituric acid reactive substances (TBARS) method [47].

Cloning and expression analysis of genes involving flavonoid biosynthesis showed that Siamese neem tree leaves total RNA contained nucleotide sequences related to enzymes F3′H, FLS, DFR, and F3′5′H, which could be responsible for the biosynthesis of the antioxidant flavonoids [48]. Some flavonoids that were separated from Siamese neem tree and neem leaves and flowers are kaempferol, myricetin, quercetin, and rutin [39, 49–51]. The chemical structures of some flavonoids found in *Azadirachta* plants are shown in Figure 5.

![Figure 5. Chemical structures of some flavonoids found in *Azadirachta* plants. A = kaempferol, B = myricetin, C = quercetin, D = rutin.](image)

4.3. *Russula* mushrooms

It is well established that many compounds separated from mushrooms can be used as immuno-modulators or as biological response modifiers [52]. Several mushroom species in Basidiomycetes have been reported to possess anti-tumor activity [53, 54].

Many phytochemical compounds have been reported in various mushrooms, and they can be classified into two main groups: high molecular weight compounds such as beta-glucan and other polysaccharides [55] and low molecular weight compounds including polyphenolics, flavonoids, and terpenoids [52]. Polyphenolics such as caffeic acid, chlorogenic acid, ferulic acid, and gallic acid and flavonoids such as myricetin and catechin were found in *Agaricus bisporus*, *Boletus edulis*, *Calocybe gambosa*, and *Cantharellus cibarius* [56]. Triterpenoids
were found in Agaricus bisporus, Ganoderma lucidum, and Russula lepida. Moreover, aristolane sesquiterpenoids were also found in Russula lepida [57]. Polysaccharides were found in Agaricus bisporus, Agaricus brasiliensis, Ganoderma lucidum, and Phellinus linteus [58]. Some polysaccharides such as beta-glucan are reported to promote immunomodulatory effects via CR3, the leukocyte membrane receptor for beta-glucans [59]. The mechanisms of the action of the mushrooms to promote anticancer effects have been reported as NF-kappaB inhibitors, protein kinase inhibitors, protein and DNA alkylating agents, modulators of G1/S and G2/M phases, inhibitors of MAPK protein kinase signaling pathways, aromatase and sulfatase inhibitors, matrix metalloproteinases inhibitors, cyclooxygenase inhibitors, DNA topoisomerases, and DNA polymerase inhibitors and anti-angiogenic substances [52].

A previous study reported the presence of 1147 mushroom species in the Northeast part of Thailand. They are composed of 647 consumed mushroom species, 222 trade mushroom species, and 400 poisonous mushroom species [60]. Thirty-seven species of these mushrooms are used in traditional medicine [60]. However, there are still some mushrooms in Thailand, especially in the Northeastern part of the country, that have never been studied for their biological properties and phytochemical compounds.

The Russula mushroom’s shape resembles an umbrella. There have a clear cap and stem, with the gills underneath the cap. The cap is thin and has an underlying radius arranged around the center. The mushroom has no ring and no latex in the cap. The mushroom is fresh, soft, fragile, and perishable [61]. There are around 750 worldwide species of Russula [62, 63]. The distribution of the Russula species shows that they are present in several countries, including the United States of America, Sweden, France, Norway, Madagascar, Italy, Belgium, Taiwan, China, Japan, and Thailand [64]. In Thailand, Russula mushrooms have been found in 17 provinces in the Northeastern region of Thailand [65]. Numerous Russula mushrooms have been consumed as food such as R. monspeliensis, R. virescens, R. alboareolata, R. medullata, and R. helios [65, 66]. Various Russula mushrooms have been traditionally used for the treatments of various diseases such as R. cyanoantha and R. nobilis, which are used for the treatment of fever; R. luteotacta, which is used for wound healing; and R. delica and R. parazurea, which are used for the treatment of gastritis and high blood pressure, while R. acrifolia is used for treatments of skin cancer [36]. Moreover, some Russula mushrooms have also been traditionally used for tonic purposes such as R. cyanoantha, R. nobilis, R. delica, R. parazurea, R. acrifolia, and R. luteotacta [67]. In addition, Russula luteotacta has been used as a sleep promoting agent [67]. Physical characteristics of some Russula mushrooms found in Thailand are shown in Figure 6.

Ten Russula mushroom collected from northeastern part of Thailand: R. crustosa, R. delica, R. monspeliensis, R. velenovskyi, R. virescens, R. lepida, R. alboareolata, R. paludosa, R. medullata, and R. helios were tested for their in vitro antioxidant activities using a photochemiluminescence assay for both lipid-soluble and water-soluble antioxidant capacities. R. medullata extract exhibited the highest antioxidant effects in both lipid-soluble and water-soluble models with antioxidant capacities of 1.1658 nmol of trolox equivalence and 1.323 nmol of ascorbic acid equivalence, respectively [68].
Some chemical constituents have been reported from *Russula* mushrooms including phenolic acids such as ρ-hydroxy-benzoic acid, chlorogenic acid, ferulic acid, caffeic acid, protocatechuic acid, and coumaric acid and flavonoids such as quercetin, chrysin, and catechin [69–71]. Some terpenoids were also found in *Russula* mushrooms including aristolane and marasmane [57, 72]. The chemical structures of the constituents found in *Russula* mushrooms are shown in Figure 7.

**Figure 6.** Physical characteristics of some Russula mushrooms found in Thailand; A = *Russula crustosa* Peck, B = *Russula delica* Fries, C = *Russula monspeliensis* Sarnari, D = *Russula velenovskyi* Melzer & Zvára, E = *Russula virens* (Sachet) Fries, F = *Russula albaoreolata* Hongo.

**Figure 7.** Chemical structures of some flavonoids found in *Russula* mushrooms. A = ferulic acid, B = chrysin, C = aristolane.

### 5. Conclusion

Natural products have been main sources of drug discoveries including the development of active compounds or formulas for the treatment of cancers. Even though it has become
difficult to discover or synthesize new active components, with the knowledge and intelligence regarding traditional medicine, there are still several ethnomedical herbal formulas and regional plants that could be studied and developed for further medicinal utilizations. Herbal remedies from Wat Tha-it and Wat Khampramong, Thailand, are examples of the efforts to develop anticancer therapies from traditional knowledge. Both remedies can inhibit the growth of various cancer cell lines. The stem extract and active compound, Berberine from the Thai medicinal plant *Coscinium fenestratum*, significantly promoted anti-proliferative activity on human colorectal cancer cells with the mechanism of action via NAG-1 and AFT3. Plants in the genus *Azadirachta* have been traditionally used as a tonic. They promote significant antioxidant activities, which could support the body’s systems and prevent oxidative stress, which is one of the causes of carcinogenesis. *Russula* is the local mushroom species in the Northeastern part of Thailand. They promote significant antioxidant effects in both lipid-soluble and water-soluble models. These plants and natural products have the potential to be sources of anticancer compounds or active extracts for the treatments of cancer. However, standardization and quality control of the extract or active compounds should be performed before studying the toxicity, *in vivo* biological activity tests, and further clinical studies in the future.

Acknowledgements

The authors acknowledge the Thailand Institute of Scientific and Technological Research for the support in supplying the photos of *Russula* mushrooms. The authors would like to thank Dr. Prapaipat Klungsupya for her valuable guidance and support about photochemiluminescence assays. The authors also thank Ms. Charinan Jaengklang for her assistance in the *Russula* mushrooms work.

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