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Amomum tsao-ko Crevost & Lemarié: a comprehensive review on traditional uses, botany, phytochemistry, and pharmacology

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Abstract Tsaoko Fructus, the dried ripe fruit of *Amomum tsao-ko* Crevost & Lemarié, is used as both medicinal material and food additive. This review summarized the traditional uses, botany, phytochemistry, and pharmacological progress on Tsaoko Fructus. One classical prescription and the other 11 representative prescriptions containing Tsaoko Fructus were reviewed. The indications of these prescriptions are major in treating spleen and stomach disorders and epidemic febrile diseases including malaria. At least 209 compounds have been isolated and identified from Tsaoko Fructus, most of which belong to terpenoids, phenylpropanoids, and organic acids. Essential oil, crude extract, and some compounds were observed to have pharmacological activities such as anti-biotics, anti-inflammation, antioxidant, mostly via in vitro experiments. However, the mechanism of its medicinal uses remains unclear. This review provides a comprehensive understanding of Tsaoko Fructus, which will be beneficial to exploring the mechanism and potential medicinal applications of Tsaoko Fructus, as well as developing a rational quality control system for Tsaoko Fructus as a medicinal material in the future.

Keywords *Amomum tsao-ko* · Traditional use · Botany · Phytochemistry · Pharmacological and biological activity

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Introduction

Amomum tsao-ko Crevost & Lemarié is a perennial Zingiberaceae herb, mainly growing in the warm and humid southwestern China and northern Vietnam. Its dried ripe fruit, called Tsaoko Fructus (Caoguo in Chinese), smells aromatic and spicy and has been used as both folk medicine and food additive. The earliest record of the medicinal application of Tsaoko Fructus can be dated back to *Official Prescription of the Royal Medical Prescriptions (Taiping Huimin Heji Ju Fang*,

Ju Fang in brief) and *Summary of Medicinal Herbs in Baoqing* (*Baoqing Bencao Zhezhong*) in the Song Dynasty (Chen 2007; Gao and Wang 2007). Since then, the actions, compatibility, and prescriptions of Tsaoko Fructus had been developed and recorded in successive ancient medical books such as *Yanshi Ji Sheng Fang* (*Ji Sheng Fang* in brief) in the South Song dynasty, *Wen Yi Lun* in the Ming dynasty, and *Wen Bing Tiao Bian* in the Qing dynasty (Yao 2002; Shi et al. 2013). Five prescriptions consisting of Tsaoko Fructus were included in *Pharmacopoeia of the People's Republic of China* (*China Pharmacopoeia* in brief) (Chinese Pharmacopoeia Commission 2015).

A number of prescriptions composed of Tsaoko Fructus were recorded in ancient medicinal books and *China Pharmacopoeia.* Those prescriptions were major in treatment of abdominal pain, diarrhea, hemorrhoids, throat infections, and malaria (Gao and Wang 2007). In recent years, Tsaoko Fructus-containing prescriptions have been developed and used to treat Hepatitis B, influenza, the Severe Acute Respiratory Syndromes (SARS), and the Coronavirus Disease 2019 (COVID-19) (Hu 1993; Yao 2002; Zhang and Chen 2008; Ding et al. 2020; Shen et al. 2020; Zong et al. 2020). Phytochemical research revealed at least 209 compounds belonging to terpenoids, flavonoids, diarylheptanoids, and organic acids were present in Tsaoko Fructus (Hong et al. 2015; Lee et al. 2019; He et al. 2020d). Some of them have anti-biotic, anti-inflammatory, anti-tumor, anti-diabetic, and neuroprotective activities (Zhang et al. 2014, 2015; Kim et al. 2016; Lee et al. 2019; He et al. 2020a, d).

However, the mechanism of the medicinal uses of Tsaoko Fructus has not been elucidated. As a result, there are some unreasonable points in the present quality control system of Tsaoko Fructus. In fact, its value as a medicinal material had received less attention than as a condiment (Gao and Wang 2007), although it has a long history of clinical applications.

In this review, the information related different aspects of *A. tsao-ko* was collected from reviewing peer-reviewed journals covering 1981–2021. Pubmed, SciFinder, Web of Science, EBSCO Medline, Baidu Scholar, and CNKI were used for electronic retrieval of the information. Based on this information, we gave a comprehensive review of *A. tsao-ko*, aiming to provide information for better understanding its pharmacological mechanism and the potential medicinal applications, and for developing a rational quality control system of Tsaoko Fructus as medicinal material in the future.

Traditional uses of Tsaoko Fructus

According to the theory of traditional Chinese medicine (TCM), Tsaoko Fructus has a mild property and has effects on removing dampness and warming the spleen and stomach (Yuan et al. 2000). It is indicated to treat interior obstruction of *cold-dampness*, distending pain in the epigastrium and abdomen, vomiting, malaria with cold and fever, and pestilence fever (Chen 2007; Gao and Wang 2007; Chinese Pharmacopoeia Commission 2015).

In traditional uses, Tsaoko Fructus or the seed of Tsaoko Fructus was commonly used in combination with other medicinal materials. One classical prescription and the other 11 representative ones were listed in Table 1. The main function and indication of these prescriptions covers two aspects. One is digestive system disorders resulted from *cold* and *dampness* of spleen and stomach, and the other is epidemic diseases such as malaria caused by epidemic pathogen infection (Table 1). Among the 12 prescriptions, Caoguo decoction (Caoguo Yin) from J*u Fang*, Guofu decoction (Guofu Tang), and Qingpi decoction (Qingpi Yin) from *Ji Sheng Fang*, Dayuan decoction (Dayuan Yin) from *Wen Yi Lun*, Caoguo Zhimu decoction (Caoguo Zhimu Tang) from *Wen Bin Tiao Bian* had effects on both digestive system disorders and epidemic febrile diseases. Changshan decoction (Changshan Yin) from *Ju Fang* was intended for curing malaria. Suopi decoction (Supi Yin) from *Ju Fang* and four prescriptions from *China Pharmacopoeia* (Jiebai Pills, Lige Pills, Piweishu Pills, and Piwei Xiaozhi Pills) were adopted in treating disorders of digestive system. Although it seems some prescriptions had similar functions, their specific applicable indications were not exactly the same. For example, regarding to the treatment of malaria, Dayuan Yin was used to treat early malaria, Guofu Tang could prevent attack of malaria, and Changshan Yin suited for curing all types of malaria including the chronic one. It was worth mentioning that Ershiwuwei Zhenzhu Pills (Ershiwuwei Zhenzhu Wan) documented in *China Pharmacopoei*a had different indications from the other 11 ones. It was effective for the treatment of

Table 1 Twelve representative prescriptions composed of Tsaoko Fructus recorded in literatures

Prescriptions	Ingredients*	Functions and Indications	Sources
Changshan Yin[#]	Anemarrhenae Rhizoma, Dichroae Radix, **Tsaoko Fructus**, Glycyrrhizae Radix Et Rhizoma (stir-baked with liquid), Alpiniae Officinarum Rhizoma, Mume Fructus (without core) (In the mass ratio of 10:10:**10:**10:6:5, 9 g a dose, dipped in 150 ml water and boiled to 100 ml, one dose a day)	Functions and indications: Treating all types of malaria including chronic malaria	Taiping Huimin Heji Ju Fang, Vol. 8, Song dynasty, 1151
Caoguo Yin[#]	Perillae Folium, **Seeds of Tsaoko Fructus**, Chuanxiong Rhizoma, Angelicae Dahuricae Radix, Alpiniae Officinarum Rhizoma (stir-baked), Citri Reticulatae Pericarpium Viride (without flesh, stir-baked), Glycyrrhizae Radix Et Rhizoma (stir-baked) (In the mass ratio of 1:1:1:1:1:1:1, 6 g a dose, dipped in 150 ml water and boiled to 100 ml, three doses on the first day, then one dose a day)	Functions and indications: Warming spleen and stomach, dispersing *cold* and *heat*, regulating *qi*, treating *cold* spleen and preventing attack of malaria	Taiping Huimin Heji Ju Fang, Vol. 3, Song dynasty, 1151
Suopi Yin[#]	Amomi Fructus, Mume Fructus (without core), **seeds of Tsaoko Fructus**, Glycyrrhizae Radix Et Rhizoma (stir-baked with liquid), Puerariae Lobatae Radix, Lablab Semen Album (stir-baked, without cortex) (In the mass ratio of 2:2:**2**:2:1:1, 12 g a dose, dipped in 250 ml water and boiled to 200 ml, taken as frequently as tea)	Functions and indications: Reducing fever and fidgetiness after cholera, treating *heat*- and *dampness*-resulted vomit	Taiping Huimin Heji Ju Fang, Vol. 2, Song dynasty, 1151
Guofu Tang[#]	**Seeds of Tsaoko Fructus**, Aconiti Lateralis Radix Praeparata (Processed, without cortex) (In the mass ratio of 1:1, 25 g a dose, dipped in a bottle of water and boiled with 7 pieces of ginger and one Jujubae Fructus, taken anytime)	Functions and indications: Warming spleen and stomach, preventing attack of malaria	Yanshi Ji Sheng Fang, Vol. 18, South Song dynasty, 1253
Qingpi Tang[#]	Citri Reticulatae Pericarpium Viride (without flesh), Magnoliae Officinalis Cortex (stir-baked with ginger), Atractylodis Macrocephalae Rhizoma, **Seeds of Tsaoko Fructus**, Bupleuri Radix (without stem), Poria (without cortex), Pinelliae Rhizoma (Soaking in the water seven times), Scutellariae Radix, Glycyrrhizae Radix Et Rhizoma (stir-baked with liquid) (Equal proportion, 12–20 g a dose, dipped in 220 ml water and boiled with five pieces of ginger to 150 ml, taken anytime)	Functions and indications: Expelling phlegm, removing *dampness*, harmonizing stomach, and preventing attack of malaria	Yanshi Ji Sheng Fang, Vol. 18, South Song dynasty, 1253
Dayuan Yin[#]**	Arecae Semen, Magnoliae Officinalis Cortex, **Seeds of Tsaoko Fructus**, Anemarrhenae Rhizoma, Paeoniae Radix Alba, Scutellariae Radix, Glycyrrhizae Radix Et Rhizoma (In the mass ratio of 4:2:**1**:2:2:2:1, 21 g a dose, dipped in 200 ml water and boiled to about 160 ml, one dose a day)	Functions: Eliminating pathogens between interior and exterior, eliminating fetid and turbid-transmission. Indications: Pathogens, early malaria, pathogens between interior and exterior, aversion to cold and high fever, fullness in the chest, vomiting, headache, and restlessness	Wen Yi Lun, Ming dynasty, 1642
Caoguo Zhimu Tang[#]	**Tsaoko Fructus**, Anemarrhenae Rhizoma, Pinelliae Rhizoma, Magnoliae Officinalis Cortex, Scutellariae Radix, Mume Fructus, Pollen (In the mass ratio of **3**:4:6:4:3:3:3, 39 g a dose, dipped in 1000 ml of water and boiled with 25 ml of ginger juice to 400 ml, divided into two parts and taken twice a day)	Functions: Relieving *cold* in the back, fullness and discomfort of *qi* in the chest, preventing attack of malaria	Wen Bing Tiao Bian, Vol. 2, Qing dynasty, 1798

Table 1 continued

Prescriptions	Ingredients*	Functions and Indications	Sources
Jiebai Wan[$]	Chebulae Fructus, Calcitum, Pterocephali Herba, Trogopteri Faeces Extract, Inulae Radix, Punicae Granati Fructus, Chaenomelis Fructus, Aquilariae Lignurn Resinatum, Caryophylli Flos, Pulveratum Calx, Carthami Flos, Myristicae Semen, Alpiniae Katsumadai Semen, **Seeds of Tsaoko Fructus** (In the mass ratio of 60:35:14:30:4:4:4:3:3:2:1:2:2:**2**)	Functions: Fortifying the spleen, harmonizing the stomach, relieving epigastric pain and vomiting, separating the clear and excrete the turbid. Indications: Distension and fullness in the chest and the abdomen, indigestion, hiccup, diarrhea, and inhibited urination	Pharmacopoeia of the People's Republic of China, 2015
Lige Wan[$]	Raphani Semen (stir-baked), Arecae Semen, Rhei Radixet Rhizoma (processed with wine), Magnoliae Officinalis Cortex (baked with ginger), Crataegi Fructus, Massa Medicata Fermentata (stir-baked), Amomi Fructus, Platycodonis Radix, Citri Reticulatae Pericarpium Viride (processed with vinegar), Aurantii Fructus (stir-baked with bran), Hordei Fructus Germinatus (stir-baked with bran), Aucklandiae Radix, Citri Reticulatae Pericarpium, Atractylodis Rhizoma (stir-baked with bran), Pogostemonis Herba; **Seeds of Tsaoko Fructus**; Glycyrrhizae Radixet Rhizoma (In the mass ratio of 4:4:4:2:2:2:1:2:2:2:2:2:2:2:2:**2**:2)	Functions: Soothing the chest and diaphragm, eliminating accumulation and relieving pain. Indications: *Qi* stagnation and constraint, distension and fullness in the chest and the diaphragm, pain in the epigastrium and abdomen, and retained fluid	Pharmacopoeia of the People's Republic of China, 2015
Piweishu Wan[$]	Trionycis Carapax (processed), Astragali Radix Praeparata, Citri Pericarpium Reticulatae, Aurantii Immaturus Fructus, Paeoniae Radix Alba, Macrocephalae Rhizoma (stir-fried with bran), Cyperi Rhizoma (processed with vinegar), **Tsaoko Fructus**, Mume Fructus (stir-baked), Chuanxiong Rhizoma, Arecae Semen Tostum, Magnoliae Officinalis Cortex (Equal proportion)	Functions: Soothing the liver, regulate *qi*, fortifying the spleen, harmonizing the stomach, eliminating accumulation, and promoting digestion. Indications: Indigestion, poor appetite, epigastric upset, abdominal distention, borborygmus, nausea, vomiting, sloppy stool, distending pain in the hypochondrium, irritability, insomnia and dream-disturbed sleep; Chronic gastritis, chronic hepatitis and early stage liver cirrhosis with the symptoms described above	Pharmacopoeia of the People's Republic of China, 2015
Tiaowei Xiaozhi Wan[$]	Officinalis Cortex Magnoliae (stir-baked with ginger juice), Notopterygii Rhizoma et Radix, Guangdong Shenqu, Aurantii Fructus, Cyperi Rhizoma (processed), Pinelliae Rhizoma (stir-baking with ginger juice), Saposhniloviae Radix, Peucedani Radix, Chuanxiong Rhizoma (stemming with distillate spirits), Angelicae Dahuricae Radix, Menthae Haplocalycis Herba, AmomiFructus, **Tsaoko Fructus**, Aucklandiae Radix, Amomi Rotundus Fructus, Poria, Atractylodis Rhizorna (macerate), Pogostemonis Herba, Linderae Radix (steaming with vinegar), Glycyrrhizae Radix (et Rhizoma), Perillae Folium, Citri Reticulatae Pericarpium (In the mass ratio of 10:10:10:5:1:10:10:10:1:10:10:10:**5**:1:10:10:10:1:10:5:10:10)	Functions: Dispersing wind, releasing the exterior, dissipate cold, resolving dampness, invigorating the stomach, and promoting digestion. Indications: Common cold due to wind-cold with dampness and internal food stagnation, manifested as chills, fever, headache, body heaviness with difficult movement, reduced food intake, fetid belching, acid reflux, abdominal pain, and diarrhea	Pharmacopoeia of the People's Republic of China, 2015

Table 1 continued

Prescriptions	Ingredients*	Functions and Indications	Sources
Ershiwuwei Zhenzhu Wan[$]	Margarita, Margaritifera Concha, Myristicae Semen, Calx Pulveratum, Carthami Flos, **Tsaoko Fructus**, Caryophylli Flos, Dalbergiae Odoriferae Lignum, Amomi Fructus Rotundus, Chebulae Fructus, Santali Albi Lignum, Phyllanthi Fructus, Aquilariae Lignum Resinatum, Cinnamomi Cortex, Terminaliae Billericae Fructus, Eriocheir seu Potamon, Aucklandiae Radix, Malvae Fructus, Piperis Longi Fructus, Fragariae Herba, Micae Lapis Aureus, Bovis Calculus Sativus, Cumni Cymini Fructus, Croci Stigma, Nigellae Semen, Moschus Artifactus, Bubali Cornu. (Proportion unavailable)	Functions: Tranquilizing the mind and opening the orifices. Indications: Apoplexy manifested as hemiplegia, deviated eyes and mouth, coma, disordered consciousness, delirious speech, and mania etc	Pharmacopoeia of the People's Republic of China, 2015

*Tsaoko Fructus and the related information in these prescriptions is highlighted in bold. The medicinal material names are referred in China Pharmacopoeia (Chinese Pharmacopoeia Commission 2015). The sources of these medicinal material are listed in Table S1. The mass ratio of the ingredients and the usage of the decoction are indicated in brackets

**Dayuan Yin is one of the classical prescriptions to treat malaria.

[#] To prepare the decoction, a certain amount medicinal material was precisely weighted and firstly dipped into appropriate volume of water, then boiled to a certain volume. The filtered solution was taken when it was warm

[$] The preparation and usage of these prescription can be retrieved from *China Pharmacopoeia*

apoplexy manifested as hemiplegia, deviated eyes and mouth, coma, disordered consciousness, delirious speech, and so on. Information of these prescriptions including ingredients, functions, indications and others was listed in Table 1.

In most of the prescriptions, Tsaoko Fructus acts as the main medicine due to its efficacy on invigorating the spleen and stomach, promoting *qi* to disperse stagnation, and eliminating pathogens (Gao and Wang 2007). For instance, Dayuan Yin is the classic prescription formulated by Wu Youke in the Ming dynasty to treat malaria (Fang and Yue 2021). In this prescription, Tsaoko Fructus acts as one of the "minister" medicines to cooperate with Arecae Semen, the "monarch", and Magnoliae Officinalis Cortex, the other "minister", to eliminate the pathogens between interior and exterior, according to the "monarch, minister, assistant, and guide" formula theory of TCM (Fang and Yue 2021; Li 2021). The other four ingredients of the prescription, Anemarrhenae Rhizoma, Paeoniae Radix Alba, Scutellariae Radix, and Glycyrrhizae Radix Et Rhizoma do not directly clear away the pathogens but serve as reconciliation agents, the "assistant" and the "guide", to recover the balance of the body (Fang and Yue 2021; Li 2021). To prepare this decoction, a total of 21 g of the medicinal materials at a ratio of 4:2:1:2:2:2:1 are firstly dipped in 200 ml water and then boiled to about 160 ml. Then all the debris are discarded and the filtered solution is taken as medicine once a day.

Recently, Dayuan Yin has been used to prevent and treat SARS and the COVID-19, two epidemic diseases that cause severe damage to the respiratory system. When it was used to treat 112 confirmed SARS cases during 2003, more than 93.7% of patients had experienced noticeable symptom relief and recovery (Ren et al. 2021). When used for mild and common cases of COVID-19 combined with antiviral drugs, it could relieve symptoms of cough, asthma, and dry throat, improve prognosis of COVID-19 patients, and shorten disease progression (Ren et al. 2021). The volatile oil of Tsaoko Fructus was speculated as one of the effective ingredients to treat these epidemic diseases due to their anti-inflammatory and antibacterial properties (Zhang et al. 2020).

Botany of *A. tsao-ko*

A. tsao-ko is of forest understory habitat in the tropical and subtropical regions (Fig. 1a) and mainly distributes in the southwestern of China including Yunnan, Guangxi, and Guizhou provinces, and the northern Vietnam. *A. tsao-ko* herb typically grows about 2–2.5 m high. Its leaves are green, smooth, slightly sharp, and oval-shaped, approximately 40–70 cm in length and about 10–20 cm in width (Fig. 1a). Its anthotaxy is spica, and the yellow or white flowers are serried inserted on the thick rachis (Fig. 1b). Its fruits are oval-shaped red capsules, densely packed together when fresh (Fig. 1c). The dried ripe capsules are roughly 2.5–4.5 cm in length, the pericarps of which are grayish-brown to brown with longitudinal furrows and ribs without hair or spikes (Fig. 1d). The seeds grow in clusters in the capsule, generally divided into three parts by rows, and are wrapped by pulp. Seeds are conical polyhedral, reddish-brown, covered with grayish-white membranous aril (Wu et al. 2014). The ripe capsules are harvested before crack usually during September to November when becoming grey to brown. The harvested capsules are dried into brown Tsaoko Fructus (Fig. 1d) in the sun or in a thermostat.

A. tsao-ko is a cultivated herb. It has different cultivated populations that have varied phenotypes. According to the morphological characteristics, for example, the shape of the capsules, *A. tsao-ko* has at least five cultivars, the spheroidal-, the near spheroidal-, the spindle-, the ellipsoid-, and the cone-shaped fruit groups (Fig. 1d) (Zhang et al. 2011; Lu et al. 2019; Wei et al. 2019). Tsaoko Fructus of different shape have different chemical profiles. Taken *A. tsao-ko* cultivated in Xishuangbanna as an example, the ellipsoid shape Tsaoko Fructus contained 3.55 mL/100 g of essential oil (EO) with 20.33% of geraniol (**5**), the spindle-shaped ones had 2.75 mL/100 g of EO with 14.40% of geraniol (**5**), and the spheroidal-, the near spheroidal-shaped ones had 4.00 mL/100 g of EO with 17.86% of geraniol (**5**) and 3.33 mL/100 g of EO with 16.87% of geraniol (**5**), respectively (Ma et al. 2008). The varied phenotypes suggest that *A. tsao-ko* has morphologic and genetic diversity among populations.

Genetic diversity assay based on phenotypic traits revealed that *A. tsao-ko* cultivars clustered into a

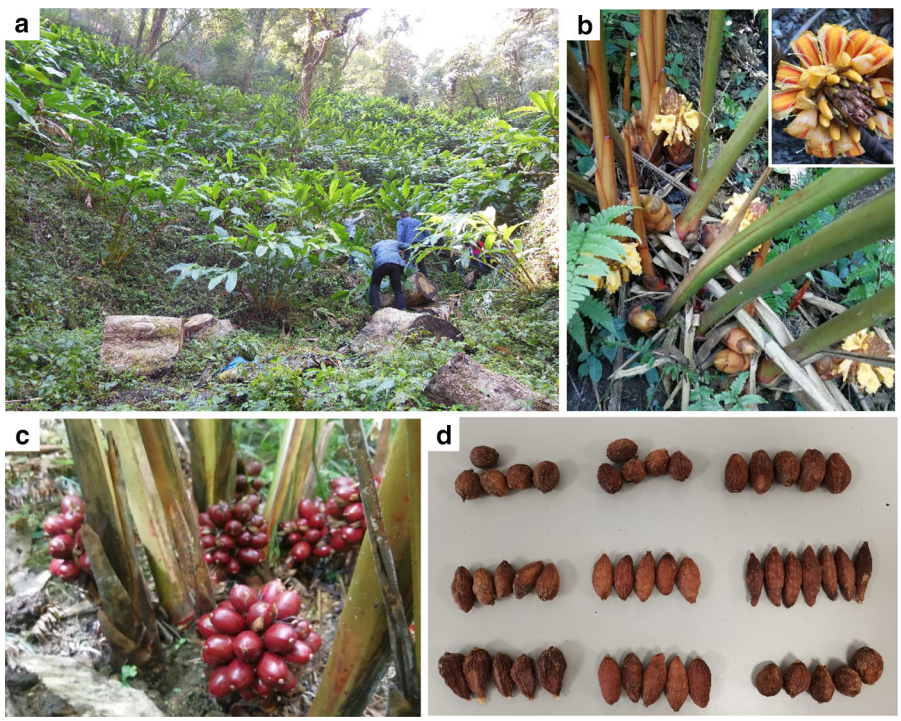

Fig. 1 *Amomum tsao-ko* plant and its growing environment, flowers, and fruits. **a** *A. tsao-ko* plant and its growing environment. **b** flowers. **c** fresh fruits. **d** dried ripe fruits

number of large groups and sub-groups, indicating *A. tsao-ko* germplasm has high genetic diversity (Yan 2012; Yang et al. 2014; Ma et al. 2017b, a, 2020; Hu et al. 2018a, 2019a, b; Xie et al. 2018; Lu et al. 2019; Ma and Lu 2020). As mentioned above, the concentration of EO varied in different shape fruits (Ma et al. 2008), which indicated that the chemical profiles may be related to morphological characteristics such as fruit forms. Sim et al. (2019) also found that *A. tsao-ko* fruits distilled for EO **A** showed a more conical shape, while pods used for EO **B** had an elliptic form. The composition of main ingredients in EO **A/B** had significant variations in concentration, e.g., eucalyptol (**10**), 4-indanecarbaldehyde (**16**), and (2*E*)-decenal (**200**) in EO **A/B** was 28.1%/22.6%, 4.3%/2.3%, and 3.0%/6.1%, respectively (Sim et al. 2019). However, the relationship between the morphological characteristics such as fruit forms and the chemical profiles, especially the characteristics of active ingredients, is still unclear. Such work is important for selecting high quality Tsaoko Fructus germplasm resources, and worth exploring in the future. There are 23 novel microsatellite markers found in *A. tsao-ko* (Lu et al. 2021) and several site variations in *matK*, *psbA-trnH* and *ycf1* sequences of *A. tsao-ko* cultivars (Hu et al. 2019b). These DNA markers may help develop molecular tools for the germplasm characterization, and the selection and breeding of good germplasm *A. tsao-ko*.

Some Zingiberacea plants have similar capsules to that of *A. tsao-ko* and also have overlaps in the distribution. As a result, they are often mixed up with *A. tsao-ko* and incorporated into Tsaoko Fructus containing medicines (Shi et al. 2013), thus adding difficulty to its regular use as medicinal material. The commonly confused species include other *Amomum* genera such as *A. paratsao-ko*, *A. Koenigii, A. kravanh, A. subulatum,* and *A. xanthoides,* the *Alpinia* genera such as *A. galanga, A. katsumadai*, and *A. zerumbet*, and *Elettaria Maton* specie like *E. cardamomum* (Shi et al. 2013; Wu et al. 2014). The species of Zingiberaceae was usually identified by the seed and fruit features, according to the macroscopic morphological characteristics and the microscopic features of their seeds and fruits (Table S2, Table S3) (Shi et al. 2013; Wu et al. 2014). The chemical profiles of EO of *A. tsao-ko* fruits are different from that of other species like *A. paratsaoko*. For example, the dried fruit of *A. tsao-ko* is rich in 1,8-cineole (**10**) and citral, which are 19.50% and 14.95%, respectively, whereas the dried fruit of *A. paratsaoko* only contains 0.25% of 1,8-cineole and undetectable citral (Huang et al. 2014). Thus, chemical profiles can also be used to distinguish Tsaoko Fructus from other easily-confused species.

In these years, DNA molecule labeling technology has been explored to study the genetic property of *A. tsao-ko* and the related plant species. The Internal Transcribed Spacer (ITS), Random Amplified Polymorphic DNA Markers (RAPD), Simple Sequence Repeat (SSR) or Microsatellite sequence (MS), and complete chloroplast genome of *A. Tsaoko* have been explored (Yan 2012; Yang et al. 2014; Ma et al. 2017b, a, 2020; Hu et al. 2018a, 2019a, b; Xie et al. 2018; Lu et al. 2019, 2021; Ma and Lu 2020). DNA barcoding sequence analysis revealed that *ITS*, *matK*, *psbA-trnH* and *ycf1* could accurately distinguish *A. tsao-ko* from 18 other *Amomum* genus (Hu et al. 2019b). Chloroplast genome was also workable, as revealed by phylogenetic analysis using complete chloroplast genome of *A. tsao-ko* and 16 other related species (Ma and Lu 2020).

Phytochemistry

Phytochemicals are the medicinal basis substances of medicinal plants, as well as an important reservoir for candidate drug development. To date, more than 300 compounds have been detected in Tsaoko Fructus (Tables 2, S4), at least 209 of which have been isolated and identified (Table 2, Figs. 2, 3, 4, 5, 6, 7, 8). According to the characteristics of core structure, these compounds can be classified as terpenoids, phenylpropanoids, organic acids, and other compounds (Table 2). Overall, there are 32 terpenoids (**1–31**), 157 phenylpropanoids (**32–188**), 19 organic acids (**189–208**), and one pyrrole (**209**). Since Tsaoko Fructus has an aromatic and spicy odor, its volatile oil, also called essential oil (EO), has attracted much attention (Yang et al. 2008; Feng et al. 2010; Min et al. 2010; He et al. 2013; Cui et al. 2017; Sim et al. 2019). *A. tsao ko* EO contains terpenoids, phenolic acids, and organic acids.

Table 2 Isolated and identified compounds from *A. tsao-ko* fruits

Compd. no.	Chemical name	Molecular formula	References
Terpenoids			
Monoterpene hydrocarbons			
1	**Limonene**	$C_{10}H_{16}$	Wang et al. (2014)
Oxygenated monoterpenes			
2	Myrcenol	$C_{10}H_{18}O$	Wang et al. (2014)
3	**8-hydroxy-2,6-dimethyl-1,6-octadien-3-one**	$C_{10}H_{16}O_2$	Lee et al. (2019)
4	(2*E*,6*E*)-8-(acetyloxy)-2,6-dimethyl-2,6-octadienal	$C_{12}H_{18}O_3$	Lee et al. (2019)
5	**Geraniol**	$C_{10}H_{18}O$	Dai et al. (2016a)
6	Geraniol acetate	$C_{12}H_{20}O_2$	Yang et al. (2009)
7	(2*E*,6*E*)-8-hydroxy-2,6-dimethyl-2,6-octadienal acetate	$C_{12}H_{18}O_3$	Yang et al. (2009)
8	(2*E*,6*E*)-8-hydroxy-2,6-dimethyl-2,6-octadienal	$C_{10}H_{16}O_2$	Yang et al. (2009)
9	**8-oxogeraniol**	$C_{10}H_{16}O_2$	Lee et al. (2008)
10	**1,8-cineole (eucalyptol)**	$C_{10}H_{18}O$	Wang et al. (2014), Dai et al. (2016b)
11	***p*-menth-1-ene-5,6-diol**	$C_{10}H_{18}O_2$	Lee et al. (2008)
12	**3α-hydroxycarvotagenone**	$C_{10}H_{16}O_2$	Lee et al. (2008)
13	**Tsaokoin**	$C_{10}H_{14}O_2$	Moon et al. (2004), Yang et al. (2009), Kim et al. (2019b)
14	**Isotsaokoin**	$C_{10}H_{14}O_2$	Moon et al. (2004)
15	**5-indanecarbaldehyde (5-Indancarboxaldehyde)***	$C_{10}H_{10}O$	Jin et al. (2013), Sim et al. (2019)
16	4-indanecarbaldehyde*	$C_{10}H_{10}O$	Jin et al. (2013), Wang et al. (2014), Dai et al. (2016b), Sim et al. (2019)
17	**6-hydroxyindan-4-carbaldehyde (6-hydroxy-4-aldehydeindene)**	$C_{10}H_{10}O_2$	Lee et al. (2008), Yang et al. (2009), Jin et al. (2013)
18	**6,7-dihydroxy-indan-4-carbaldehyde**	$C_{10}H_{10}O_3$	Lee et al. (2008), Jin et al. (2013)
19	(1*RS*,5*SR*,6*RS*)-5-hydroxybicyclo[4.3.0]non-2-ene-2-carbaldehyde	$C_{10}H_{14}O_2$	Yang et al. (2009)
20	*trans*-2,3,3a,7a-tetrahydro-1*H*-indene-4-carbaldehyde (*trans*-dihydroindane-4-carboxylaldehyde)*	$C_{10}H_{10}O$	Starkenmann et al. (2007), Sim et al. (2019)
21	*trans*-2,3,3a,7a-tetrahydro-1*H*-indene-5-carbaldehyde	$C_{10}H_{10}O$	Sim et al. (2019)
22	*cis*-2,3,3a,7a-tetrahydro-1*H*-indene-4-carbaldehyde (*cis*-dihydroindane-4-carboxylaldehyde)*	$C_{10}H_{10}O$	Starkenmann et al. (2007), Sim et al. (2019)
23	*cis*-2,3,3a,7a-tetrahydro-1*H*-indene-5-carbaldehyde	$C_{10}H_{10}O$	Sim et al. (2019)
Sesquiterpenoids			
24	(3*S*,6*E*)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (*trans*-nerolidol)	$C_{15}H_{26}O$	Hong et al. (2015), Lee et al. (2019)
Diterpenoids			
25	**Coronadiene**	$C_{17}H_{26}O_2$	Liu et al. (2018)
26	(3*E*)-4-[(1*S*,4a*S*,8a*S*)-decahydro-5,5,8a-trimethyl-2-methylene-1-naphthalenyl]-3-buten-2-one	$C_{18}H_{28}O$	Lee et al. (2019)
27	Amotsaokonal A	$C_{20}H_{30}O$	Hong et al. (2015)
28	Amotsaokonal B	$C_{20}H_{32}O$	Hong et al. (2015)
29	Amotsaokonal C	$C_{20}H_{32}O$	Hong et al. (2015)
Steroids			
30	β-sitosterol	$C_{29}H_{50}O$	Martin et al. (2000), Zhang et al. (2014)
31	**β-sitosterol-3-O-glucoside (daucosterol)**	$C_{35}H_{60}O_6$	Martin et al. (2000), He et al. (2020c)
Phenylpropanoids			

Table 2 continued

Compd. no.	Chemical name	Molecular formula	References
Phenolic acids			
32	**Catechol**	$C_6H_6O_2$	Wang et al. (2009), Jin et al. (2013), Zhang et al. (2014)
33	**Hydroquinone**	$C_6H_6O_2$	Jin et al. (2013)
34	Pyrogallic acid	$C_6H_6O_3$	Wang et al. (2009)
35	4-hydroxy-benzaldehyde	$C_7H_6O_2$	Yang et al. (2009)
36	**Protocatechualdehyde**	$C_7H_6O_3$	Martin et al. (2000), Liu et al. (2018), Choi et al. (2018)
37	p-hydroxybenzoic acid	$C_7H_6O_3$	Martin et al. (2000), Wang et al. (2009)
38	Protocatechuic acid	$C_7H_6O_4$	Martin et al. (2000), Wang et al. (2009)
39	3,5-dihydroxybenzoic acid	$C_7H_6O_4$	Jin et al. (2013)
40	**Vanillic acid (4-hydroxy-3-methoxy-benzoic acid)**	$C_8H_8O_4$	Martin et al. (2000), Wang et al. (2009), Liu et al. (2018), Choi et al. (2018)
41	**3-*O*-methylgallic acid**	$C_8H_8O_5$	Liu et al. (2018)
42	**3,4-dihydroxybenzoic acid**	$C_7H_6O_4$	Liu et al. (2018)
43	Anisole	C_7H_8O	Jin et al. (2013)
44	**3-methoxy-catechol**	$C_7H_8O_3$	Jin et al. (2013)
45	**2-methoxy-hydroquinone**	$C_7H_8O_3$	Jin et al. (2013)
46	**2-methoxy-resorcinol**	$C_7H_8O_3$	Jin et al. (2013)
47	**4-methoxy-catechol**	$C_7H_8O_3$	Jin et al. (2013)
48	4-methoxybenzaldehyde	$C_8H_8O_2$	Jin et al. (2013)
49	2-methoxy-benzaldehyde	$C_8H_8O_2$	Jin et al. (2013)
50	3-methoxy-benzaldehyde	$C_8H_8O_2$	Jin et al. (2013)
51	3-methoxy-4-hydroxy-benzaldehyde	$C_8H_8O_3$	Jin et al. (2013)
52	4-hydroxy-2,5- dimethoxy-benzaldehyde	$C_9H_{10}O_4$	Jin et al. (2013)
53	4-methoxy-3-hydroxy-benzaldehyde	$C_8H_8O_3$	Yang et al. (2009), Jin et al. (2013)
54	3-hydroxybenzoic acid	$C_8H_8O_3$	Jin et al. (2013)
55	**4-(2-hydroxypropyl)phenol**	$C_9H_{12}O_2$	Jin et al. (2013)
56	(*E*)-p-coumaric acid	$C_9H_8O_3$	Liu et al. (2018), Choi et al. (2018)
57	**2,6-dimethoxy-4-[(1*R*)-1-methoxyethyl]-phenol**	$C_{11}H_{16}O_4$	Lee et al. (2019)
58	**2,6-dimethoxy-4-[(1*R*)-1-methoxypropyl]-phenol**	$C_{12}H_{18}O_4$	Lee et al. (2019)
59	1,3-dimethoxybenzene	$C_8H_{10}O_2$	Jin et al. (2013)
60	**2,6-dimethoxy-phenol**	$C_8H_{10}O_3$	Lee et al. (2019)
61	**2,6-dimethoxy-4-methyl-phenol**	$C_9H_{12}O_3$	Lee et al. (2019)
62	**2,6-dimethoxy-4-(methoxymethyl)-phenol**	$C_{10}H_{14}O_4$	Lee et al. (2019)
63	**2,6-dimethoxy-4-(2-propen-1-yl)-phenol**	$C_{10}H_{14}O_4$	Lee et al. (2019)
64	1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone	$C_{11}H_{14}O_4$	Lee et al. (2019)
65	**4-hydroxy-3-methoxy-benzaldehyde**	$C_8H_8O_3$	Lee et al. (2019)
66	1-(4-hydroxy-3-methoxyphenyl)-ethanone	$C_9H_{10}O_3$	Lee et al. (2019)
67	1-(4-hydroxy-3-methoxyphenyl)-1-propanone	$C_{10}H_{12}O_3$	Lee et al. (2019)
68	**3,4-dimethoxy-benzoic acid**	$C_9H_{10}O_4$	Lee et al. (2019)
69	3,3',5,5'-tetramethoxy-[1,1'-biphenyl]-4,4'-diol	$C_{16}H_{18}O_6$	Lee et al. (2019)
70	Myrciaphenone A	$C_{14}H_{18}O_9$	(Choi et al. 2018)
Flavonoids			
71	**(+)-afzelechin**	$C_{15}H_{14}O_5$	He et al. (2021)

Table 2 continued

Compd. no.	Chemical name	Molecular formula	References
72	8-aldehyde-catechin	$C_{16}H_{14}O_7$	He et al. (2021)
73	**(-)-catechin**	$C_{15}H_{14}O_6$	Martin et al. (2000), Jin et al. (2013), Choi et al. (2018), He et al. (2021)
74	(-)-epi-afzelechin	$C_{15}H_{14}O_5$	He et al. (2021)
75	**(+)-epicatechin**	$C_{15}H_{14}O_6$	Martin et al. (2000), Zhang et al. (2014), Choi et al. (2018), He et al. (2021)
76	(2*R*,3*R*,4*R*)-3',5'-dimethoxy-3,4,7,4'-tetrahydroxy-flavan	$C_{17}H_{18}O_7$	Jin et al. (2013)
77	**Quercetin**	$C_{15}H_{10}O_7$	Zhang et al. (2014)
78	**quercetin-3-*O*-β-D-glucopyranoside**	$C_{21}H_{20}O_{12}$	Wang et al. (2009), Zhang et al. 2014), Rahman et al. 2017)
79	**quercetin-7-*O*-β-glucoside**	$C_{21}H_{20}O_{12}$	Zhang et al. (2014), Rahman et al. (2017)
80	Rutin	$C_{27}H_{30}O_{16}$	Wang et al. (2009), Dai and Peng 2011)
81	3',7-dihydroxy-4'-methoxy-flavan	$C_{16}H_{16}O_4$	Jin et al. (2013)
82	Abyssinoflavanone VII	$C_{25}H_{28}O_6$	Jin et al. (2013)
83	Alpinetin	$C_{16}H_{14}O_4$	Kim et al. (2019b)
84	Naringenin-5-O-methyl ether	$C_{16}H_{14}O_5$	Kim et al. (2019b)
85	Naringenin	$C_{15}H_{12}O_5$	Kim et al. (2019b)
86	Hesperetin	$C_{16}H_{14}O_6$	Kim et al. (2019b)
87	4',7-dihydroxy-3',6-diprenylflavone	$C_{25}H_{26}O_4$	Jin et al. (2013)
88–91	**Geranylated pyranoflavanones**	-	Kim et al. (2019a)
92–95	**Farnesylated pyranoflavanones**	-	Kim et al. (2019a)
96	**4'-hydroxy-2'-methoxychalcone**	$C_{16}H_{14}O_3$	Jin et al. (2013)
97	4-hydroxy-4'-methoxychalcone	$C_{16}H_{14}O_3$	Jin et al. (2013)
98	**2',4',6'-trihydroxy-4-methoxy chalcone**	$C_{16}H_{14}O_5$	Kim et al. (2019b)
99	**4-hydroxy-2'-methoxychalcone**	$C_{16}H_{14}O_3$	Jin et al. (2013)
100	4'-hydroxy-4-methoxychalcone	$C_{16}H_{14}O_3$	Jin et al. (2013)
101	**2',4'-dihydroxy-4-methoxy-chalcone**	$C_{16}H_{14}O_4$	Jin et al. (2013)
102	4,4'-dimethoxychalcone	$C_{17}H_{16}O_3$	Jin et al. (2013)
103	2',4,4'-trimethoxychalcone	$C_{18}H_{18}O_4$	Jin et al. (2013)
104	**Boesenbergin B**	$C_{26}H_{28}O_4$	Kim et al. (2019b)
105	**4-hydroxyboesenbergin B**	$C_{26}H_{28}O_5$	Kim et al. (2019b)
106,107	**Farnesylated pyranochalcones**	-	Kim et al. (2019a)
108	3',5'-di-C-β-D-glucopyranosylphloretin	$C_{21}H_{26}O_9$	Wang et al. (2009), Hussain et al. (2018)
109	2-(4-hydroxy-3-methoxybenzoyl)-4-methoxy-benzaldehyde	$C_{15}H_{14}O_5$	Jin et al. (2013)
110	**Flavanocoumarin**	$C_{18}H_{14}O_7$	He et al. (2021)
111	**Sappanone B**	$C_{16}H_{14}O_6$	He et al. (2021)
112	**Brazilin**	$C_{16}H_{14}O_5$	He et al. (2021)
113	Epi-catechin-(4β → 8,2β → O → 7)-epi-afzelechin	$C_{30}H_{24}O_{11}$	He et al. (2021)
114	**Proanthocyanidin A-2**	$C_{30}H_{24}O_{12}$	He et al. (2021)
Flavanol-menthane conjugates			
115	**Amomutsaokin A**	$C_{25}H_{28}O_6$	He et al. (2021)
116	**Amomutsaokin B**	$C_{25}H_{28}O_6$	He et al. (2021)
117	**Amomutsaokin C**	$C_{25}H_{28}O_6$	He et al. (2021)

Table 2 continued

Compd. no.	Chemical name	Molecular formula	References
118	Amomutsaokin D	$C_{25}H_{28}O_6$	He et al. (2021)
119	**Amomutsaokin E**	$C_{25}H_{30}O_7$	He et al. (2021)
120	**Amomutsaokin F**	$C_{25}H_{30}O_7$	He et al. (2021)
121	**Amomutsaokin G**	$C_{25}H_{30}O_7$	He et al. (2021)
122	**Amomutsaokin H**	$C_{25}H_{30}O_7$	He et al. (2021)
Flavanol-fatty alcohol hybrids			
123	**Tsaokoflavanol A**	$C_{23}H_{28}O_7$	He et al. (2020a)
124	**Tsaokoflavanol B**	$C_{25}H_{32}O_7$	He et al. (2020a)
125	Tsaokoflavanol C	$C_{25}H_{32}O_7$	He et al. (2020a)
126	Tsaokoflavanol D	$C_{25}H_{30}O_8$	He et al. (2020a)
127	Tsaokoflavanol E	$C_{27}H_{32}O_9$	He et al. (2020a)
128	**Tsaokoflavanol F**	$C_{27}H_{36}O_7$	He et al. (2020a)
129	Tsaokoflavanol G	$C_{23}H_{28}O_7$	He et al. (2020a)
130	Tsaokoflavanol H	$C_{23}H_{28}O_7$	He et al. (2020a)
131	Tsaokoflavanol I	$C_{23}H_{28}O_7$	He et al. (2020a)
132	**Tsaokoflavanol J**	$C_{25}H_{32}O_7$	He et al. (2020a)
133	**Tsaokoflavanol K**	$C_{25}H_{32}O_7$	He et al. (2020a)
134	**Tsaokoflavanol L**	$C_{25}H_{32}O_7$	He et al. (2020a)
135	Tsaokoflavanol M	$C_{25}H_{32}O_7$	He et al. (2020a)
136	Tsaokoflavanol N	$C_{25}H_{30}O_8$	He et al. (2020a)
137	Tsaokoflavanol O	$C_{25}H_{30}O_8$	He et al. (2020a)
138	Tsaokoflavanol P	$C_{27}H_{32}O_9$	He et al. (2020a)
139	Tsaokoflavanol Q	$C_{27}H_{32}O_9$	He et al. (2020a)
140	**Tsaokoflavanol R**	$C_{27}H_{36}O_7$	He et al. (2020a)
141	**Tsaokoflavanol S**	$C_{27}H_{36}O_7$	He et al. (2020a)
Flavanol-monoterpenoid hybrids			
142	**Tsaokol A**	$C_{25}H_{26}O_7$	He et al. (2020b)
143	**Tsaokol B**	$C_{25}H_{26}O_7$	He et al. (2020b)
Diarylheptanoids			
144	**(+)-hannokinol**	$C_{19}H_{24}O_4$	Martin et al. (2000), Lee et al. (2008), Liu et al. 2018), Choi et al. 2018)
145	(3*R*,5*R*)-3-acetoxy-5- hydroxy-1,7-bis(4-hydroxyphenyl) heptane	$C_{21}H_{26}O_5$	He et al. (2020d)
146	(3*R*,5*R*)-3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane	$C_{19}H_{24}O_5$	He et al. (2020d)
147	(3*R*,5*R*)-3,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)heptane	$C_{20}H_{26}O_5$	He et al. (2020d)
148	***meso*-hannokinol**	$C_{19}H_{24}O_4$	Martin et al. (2000), Lee et al. (2008), Zhang et al. (2014), Liu et al. (2018), Choi et al. (2018), He et al. (2020d)
149	*rel*-(3*R*,5*S*)- 3,5-dihydroxy-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptane	$C_{19}H_{24}O5$	He et al. (2020d)
150	(3*R*,5*S*)-3,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxy phenyl)heptane	$C_{20}H_{26}O_5$	He et al. (2020d)
151	*rel*-(3*R*,5*S*)-3,5-dihydroxy-1-(4-hydroxy-3-methoxy phenyl)-7-(3,4-dihy- droxyphenyl)heptane	$C_{21}H_{26}O_5$	He et al. (2020d)

Table 2 continued

Compd. no.	Chemical name	Molecular formula	References
152	(4*E*,6*E*)-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one	$C_{19}H_{18}O_3$	Zong et al. (2020), He et al. (2020d)
153	**Tsaokoarylone**	$C_{20}H_{20}O_4$	Lee et al. (2008), Yang et al. (2009), Jin et al. (2013), Kim et al. (2019b), He et al. (2020d)
154	**(4*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one/1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-hepta-dien-3one**	$C_{21}H_{22}O_5$	Lee et al. (2008), He et al. (2020d)
155	1,7-bis(3,4-dihydroxyphenyl)-hepta-4*E*,6*E*-dien-3-one	$C_{19}H_{18}O_5$	Hussain et al. (2018), He et al. (2020d)
156	4-[(3*S*,5*E*)-3-hydroxy-7-(4-hydroxyphenyl)hept-5-en-1-yl]-2-methoxyphenol	$C_{20}H_{24}O_4$	He et al. (2020d)
157	Amomutsaokol G	$C_{19}H_{22}O_4$	He et al. (2020d)
158	Amomutsaokol H	$C_{20}H_{24}O_5$	He et al. (2020d)
159	Amomutsaokol I	$C_{22}H_{26}O_5$	He et al. (2020d)
160	**1,7-bis(4-hydroxyphenyl)-3-hepten-5-one (1,7-bis(4-hydroxyphenyl)-4*(E)*-hepten-3-one)**	$C_{19}H_{20}O_3$	Jin et al. (2013), He et al. (2020d)
161	**hannokinin**	$C_{19}H_{22}O_4$	Lee et al. (2008)
162	Amomutsaokol J	$C_{20}H_{20}O_4$	He et al. (2020d)
163	Amomutsaokol K	$C_{20}H_{24}O_5$	He et al. (2020d)
164	Amomutsaokol C	$C_{20}H_{22}O_6$	He et al. (2020d)
165	Amomutsaokol D	$C_{20}H_{18}O_6$	He et al. (2020d)
166	Amomutsaokol E	$C_{20}H_{20}O_6$	He et al. (2020d)
167	Amomutsaokol A	$C_{31}H_{34}O_{11}$	He et al. (2020d)
168	Amomutsaokol B	$C_{29}H_{32}O_6$	He et al. (2020d)
169	Amomutsaokol F	$C_{22}H_{26}O_8$	He et al. (2020d)
170	**2,3-dihydro-2-(4′-hydroxy-phenylethyl)-6-[(3″,4″-dihydroxy-5″-methoxy)phenyl]-4-pyrone**	$C_{20}H_{20}O_6$	Zhang et al. (2015, 2016)
171	**4-dihydro-2-(4′-hydroxy-phenylmethyl)-6-[(3″,4″-dihydroxy-5″-methoxyphenyl)methylene]-pyran-3,5-dione**	$C_{20}H_{18}O_7$	Zhang et al. (2015, 2016)
172	**Tsaokopyranol A**	$C_{28}H_{32}O_9$	He et al. (2020c)
173	**Tsaokopyranol B**	$C_{29}H_{34}O_{10}$	He et al. (2020c)
174	Tsaokopyranol C	$C_{21}H_{26}O_8$	He et al. (2020c)
175	**Tsaokopyranol D**	$C_{22}H_{28}O_9$	He et al. (2020c)
176	**Tsaokopyranol E**	$C_{20}H_{24}O_6$	He et al. (2020c)
177	**Tsaokopyranol F**	$C_{20}H_{24}O_6$	He et al. (2020c)
178	Tsaokopyranol G	$C_{21}H_{26}O_8$	He et al. (2020c)
179	**Tsaokopyranol H**	$C_{20}H_{24}O_5$	He et al. (2020c)
180	**Tsaokopyranol I**	$C_{20}H_{24}O_6$	He et al. (2020c)
181	**Tsaokopyranol J**	$C_{21}H_{24}O_7$	He et al. (2020c)
182	**Tsaokopyranol K**	$C_{20}H_{24}O_6$	He et al. (2020c)
183	Tsaokopyranol L	$C_{20}H_{22}O_4$	He et al. (2020c)
184	(2*R*,6*R*)-3,4-dehydro-4'-de-*O*-methyl centrolobin	$C_{19}H_{22}O_3$	He et al. (2020c)
185	Tsaokopyranol M	$C_{20}H_{22}O_4$	He et al. (2020c)
186	(2*R*,6*S*)-3,4-dehydro-1,7-bis(4-hydroxy phenyl)-4'-de-*O*-methyl centrolobine	$C_{19}H_{22}O_3$	He et al. (2020c)
187	**Phaeoheptanoxide**	$C_{19}H_{22}O_5$	He et al. (2020c)
188	**Engelheptanoxides C**	$C_{20}H_{24}O_5$	He et al. (2020c)

Table 2 continued

Compd. no.	Chemical name	Molecular formula	References
Phenylethanoid glycosides			
189	2-methoxy-1,4-biphenol-1-*O*-[6-*O*-(3-methoxy-4-hydroxybenzoyl)]-β-d-glucopyranoside	$C_{21}H_{24}O_{11}$	Wang et al. (2009)
Organic acid			
Fatty acids			
190	**6,7-dihydroxy-3,7-dimethyloct-2-enoic acid**	$C_{10}H_{18}O_4$	Lee et al. (2008)
191	**(2*E*,7*Z*,10*Z*,13*Z*)-hexadeca-2,7,10,13-tetraenoic acid**	$C_{16}H_{24}O_2$	Liu et al. (2018)
192	**(2*E*,7*Z*)-tetradeca-2,7-dienoic acid**	$C_{14}H_{24}O_2$	Liu et al. (2018)
193	***(E)*-dodec-2-enoic acid ((2*E*)-2-dodecenoic acid)**	$C_{12}H_{22}O_2$	Liu et al. (2018), Lee et al. (2019)
194	***(E)*-tetradec-2-enoic acid ((2*E*)-2-tetradecenoic acid)**	$C_{14}H_{26}O_2$	Liu et al. (2018), Lee et al. (2019)
195	(11*R*)-hydroxyhexadeca-(2*E*,7*Z*,9*E*)-trienoic acid	$C_{16}H_{26}O_3$	Lee et al. (2019)
196	(9*S*,10*E*,12*Z*)-9-hydroxy-10,12-octadecadienoic acid	$C_{18}H_{32}O_3$	Lee et al. (2019)
197	**(9*S*,6*Z*,10*E*,12*Z*)-9-hydroxy-6,10,12-octadecatrienoic acid**	$C_{18}H_{30}O_3$	Lee et al. (2019)
198	(2*E*)-dodecenoic acid	$C_{12}H_{22}O_2$	Hong et al. (2015)
Aliphatic aldehydes			
199	2*E*-decenal	$C_{10}H_{18}O$	Yang et al. (2009)
200	Hexadecanal	$C_{16}H_{32}O$	Yang et al. (2009)
201	(2*E*,8*E*)-10-hydroxy-decadienal	$C_{10}H_{16}O_2$	Yang et al. (2009)
Aliphatic alcohols			
202	*(E)*-2-decen-1-ol/(2*E*)-decenol	$C_{10}H_{20}O$	Yang et al. (2009)
203	**2,8-decadiene-1,10-diol (DDO)**	$C_{10}H_{18}O_2$	Kim et al. (2016)
204	**(2*E*,8*E*)-2,8-decadiene-1,10-diol**	$C_{10}H_{18}O_2$	Lee et al. (2019)
205	**1-acetate 2,8-decadiene-10-ol**	$C_{12}H_{20}O_3$	Lee et al. (2019)
206	**(2*E*)-1-acetate 2-dodecen-1-ol**	$C_{14}H_{26}O_3$	Lee et al. (2019)
Aliphatic esters			
207	(2*E*)-dodecenyl acetate	$C_{14}H_{26}O_2$	Hong et al. (2015)
208	1,10-diacetate-2,8-decadiene-1,10-diol (acetoxytsaokol A)	$C_{14}H_{22}O_4$	Lee et al. (2019)
Other Compounds			
Pyrroles			
209	Pyrrole-2-carboxylic acid	$C_5H_5NO_2$	Hong et al. (2015)

The compounds labeled with an asterisk (**15, 16, 20, 22**) are species-specific components in *A. tsao-ko*. Those with certificated biological activities are highlighted in bold

Terpenoids

Terpenoids are abundant in *A. tsao-ko* EO (Tables 2, S4, Fig. 2). There was one monoterpene hydrocarbon (**1**), 22 oxygenated monoterpenes (**2–23**), one sesquiterpenoid (**24**), five diterpenoids (**25**–**29**), and two sterols (**30, 31**) isolated and identified from *A. tsao-ko*. All these compounds are present in Tsaoko Fructus. Limonene (**1**) and 1,8-cineole (**10**) also exist in *A. tsao-ko* stems and leaves (Yang 2019).

Among the 23 monoterpenes, 1,8-cineole (eucalyptol, **10**) accounts for the highest proportion, taking 34.6%-45.24% (Feng et al. 2010; Cui et al. 2017; Rahman et al. 2017; Gu et al. 2018; Liu et al. 2018; Sun et al. 2018; Sim et al. 2019). Specially, there were four indanecarbaldehydes, 5-indanecarbaldehyde (**15**), 4-indanecarbaldehyde (**16**), *trans*-

dihydroindane-4-carboxylaldehydes (**20**), and *cis*-dihydroindane-4-carboxylaldehydes (**22**). These compounds have not been found in any other species, but only in *A. tsao-ko* from both China and Vietnam, thus could be used as chemical marker of *A. tsao-ko* species probably regardless of the growing regions (Sim et al. 2019).

Sesquiterpenoids are the condensation products of three isopentenyl pyrophosphate molecules. one linear sesquiterpenoid, (3*S*,6*E*)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, also named *trans*-nerolidol (**24**), has been isolated from Tsaoko Fructus (Hong et al. 2015; Lee et al. 2019).

The five diterpenoids included two labdane-type trinorditerpenes, namely coronadiene (**25**) and (3*E*)-4-[(1*S*,4a*S*,8a*S*)-decahydro-5,5,8a-trimethyl-2-methylene-1-naphthalenyl]-3-buten-2-one (**26**) (Liu et al. 2018; Lee et al. 2019), two cycloterpenals, namely amotsaokonal B (**28**) and amotsaokonal C (**29**), and one benzaldehyde, amotsaokonal A (**27**) (Hong et al. 2015). It should be noted that amotsaokonal A (**27**) may be formed through the dehydrogenation of **29** from the perspective of biosynthetic pathway, so it is regarded as diterpenoids here.

There were two steroids isolated from Tsaoko Fructus. They were β-sitosterol (**30**) and its glycosylation product, β-sitosterol-3-O-glucoside (daucosterol, **31**).

Besides the 31 terpenoids mentioned above, there were other 85 terpenoids detected by LC–MS or GC–MS in the extracts of *A. tsao-ko* fruits (Table S4) (Feng et al. 2010; Hong et al. 2015; Hu et al. 2018b; Sim et al. 2019).

Phenylpropanoids

Phenylpropanoids are the large group of secondary metabolites in plants. At least 157 phenylpropanoids

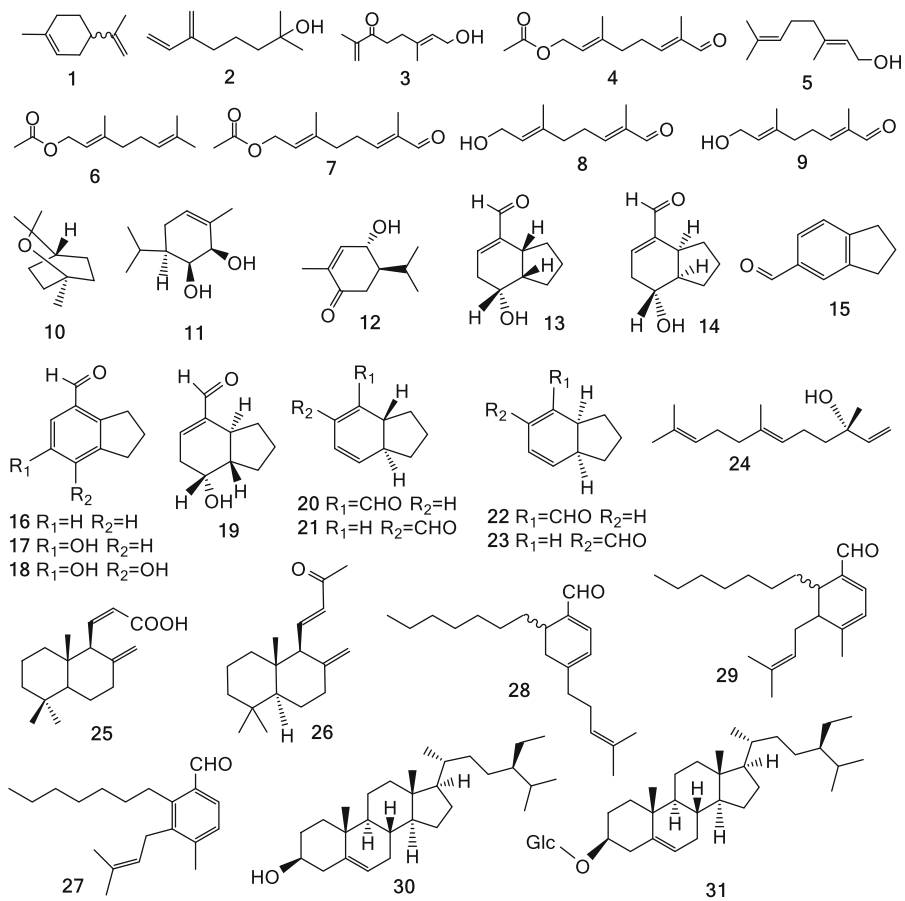

Fig. 2 Terpenoids isolated and identified from *A. tsao-ko* fruits

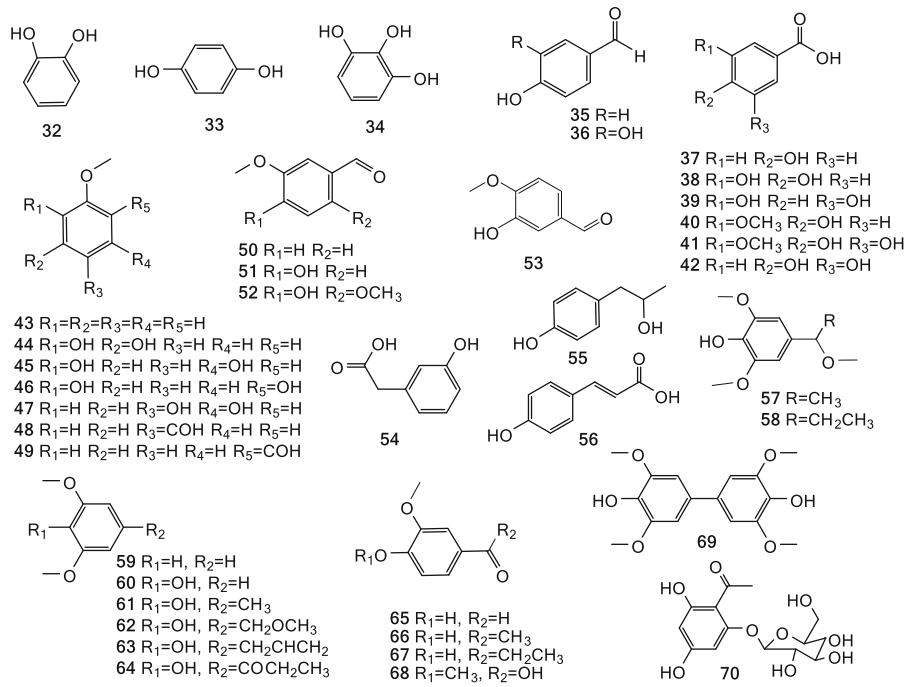

Fig. 3 Phenolic acids isolated and identified from *A. tsao-ko* fruits

have been isolated and identified from Tsaoko Fructus (Table 2). These phenylpropanoids included simple phenolic acids (**32–70**, Fig. 3), typical flavonoids (**71–114**, Fig. 4), and flavonoid derivatives including flavanol-menthane conjugates (**115–122,** Fig. 5) (He et al. 2020c), flavanol-fatty alcohol hybrids (**123–141,** Fig. 5) (He et al. 2020a), flavanol-monoterpenoid hybrids (**142**, **143,** Fig. 5) (He et al. 2020b), diarylheptanoids (**144–188**, Fig. 6, Fig. 7), and phenylethanoid glycoside (**189**, Fig. 7). Six other phenolic acids have also been detected but not separated from Tsaoko Fructus (Table S4).

Out of the phenolic acids from Tsaoko Fructus, 40 are simple phenolic acids that have one aromatic ring with hydroxyl-, aldehyde-, carbonyl-, methoxy-, or carboxyl-groups attached to it (**32–70**, Fig. 3).

The flavonoids (**71–114**) isolated from Tsaoko Fructus included flavan-3-ols (**71–75**), flavan-3,4-diol (**76**), flavonols and their corresponding glycosylated derivatives (**77–80**), flavan (**81**), flavanones (**82–95**), chalcones (**96–107**), dihydrochalcone (**108**), and flavanol conjugates with other groups such as flavanocoumarin (**110**), epi-catechin-(4β → 8,2β → O → 7)-epi-afzelechin (**113**), proanthocyanidin A-2 (**114**) (He et al. 2021). Other flavonoid derivatives like sappanone B (**112**), brazilin (**113**), flavanol-menthane conjugates (**115–122**), flavanol-fatty alcohol hybrids (**123–141**), and flavanol-monoterpenoid hybrids (**142**, **143**) were also reported (He et al. 2020a, b, 2021) (Fig. 4, Fig. 5). In particular, there were nine flavonoids that were geranylated or farnesylated at the A ring of the skeleton, including geranylated pyranoflavanones (**88–91**), farnesylated pyranoflavanones (**92–95**), and farnesylated pyranochalcones (**106**, **107**) (Kim et al. 2019a).

Diarylheptanoids are another type phenylpropanoids, which are characterized with a 1,7-diphenylheptane core Lee et al. (2008; He et al. 2020c, d). These compounds are widespread in Zingiberaceae. Twenty linear diarylheptanoids (**144–163;** Fig. 6) and 25 cyclic diarylheptanoids (**164–188**; Fig. 6, Fig. 7) have been isolated and identified from Tsaoko Fructus Lee et al. (2008; He et al. 2020c, d).

One phenylethanoid glycoside, 2-methoxy-1,4-biphenol-1-O-[6-O-(3-methoxy-4-hydroxybenzoyl)]-β-d-glucopyranoside (**189**), has also been identified from the dried fruits of *A. tsao-ko*(Wang et al. 2009).

Organic acids

Organic acids from Tsaoko Fructus included fatty acids (**190–198**), aliphatic aldehydes (**199–201**), aliphatic alcohols (**202–206**), and aliphatic esters

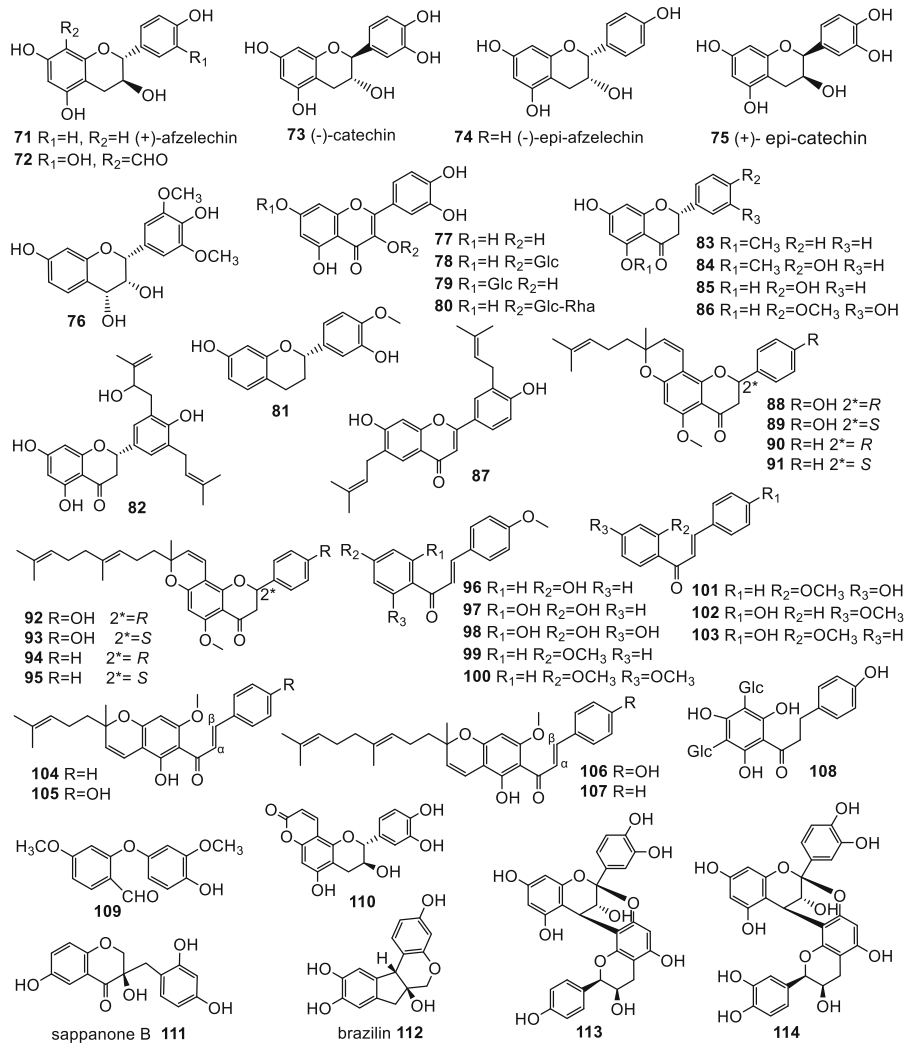

Fig. 4 Flavonoids isolated and identified from *A. tsao-ko* fruits

(**207–208**) (Fig. 8)(Yang et al. 2008; Min et al. 2010; He et al. 2013; Hu et al. 2018b; Liu et al. 2018; Lee et al. 2019; Sim et al. 2019; Xu et al. 2019). These compounds were also rich in *A. tsao-ko* EO (Li et al. 1998; Ma et al. 2008). For example, 2*E*-decenal (**199**) accounts for 3.41%-10.92% of *A. Tsaoko* EO (Ma et al. 2008). In addition, 49 other organic acids were also detected in *A. tsao-ko* EO (Table S4).

Other compounds

Besides the main constituents of Tsaoko Fructus listed above, pyrrole-2-carboxylic acid (**209**, Table 2, Fig. 8) (Hong et al. 2015), alicyclic compounds, furan compounds, and heterocyclic compound were also reported (Table S4).

Pharmacological and biological activities

Biological activities of EO, extracts, and isolated compounds of *A. tsao-ko* fruits have been investigated by several research groups. Most of the bioactivities were evaluated by in vitro experiments, including antibiotic, anti-tumor and anti-cancer, anti-inflammatory, anti-diabetes, neuroprotective, plasma and liver triacylglycerol decreasing activities (Table 3).

Antibiotic activity

Antibiotic activity of Tsaoko Fructus is extensively studied through the inhibition of various microbes such as fungi, protozoa, and both Gram-positive and Gram-negative bacteria, and against insects like

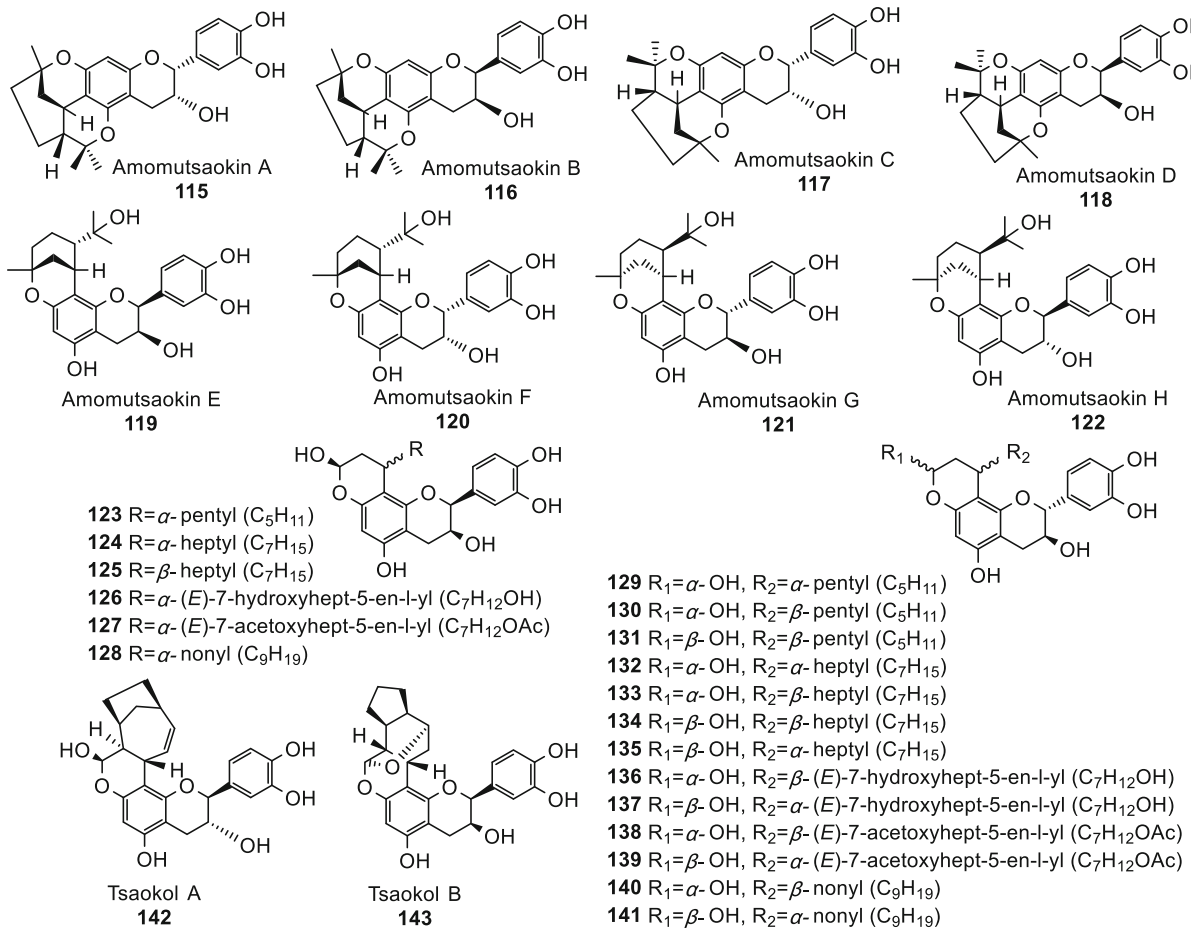

Fig. 5 Flavanol-menthane, -fatty alcohol, and -monoterpenoid hybrids isolated and identified from *A. tsao-ko* fruits

Tribolium castaneum, mainly using agar dilution/diffusion or liquid/broth dilution methods in vitro.

Both the ethanol extracts (EtOH Ex.) and the ethyl acetate extracts (EtOAc Ex.) of *A. tsaoko* fruits showed inhibitory activities against *Staphylococcus aureus, Salmonella Typhimurium, Pseudomonas aeruginosa*, and *Klebsiella pneumonia,* a gram-negative bacterium caused pneumonia with high morbidity and mortality (Rahman et al. 2017; Liu et al. 2018). The Minimum Inhibitory Concentration (MIC) of EtOH Ex. for *S. aureus, S. Typhimurium,* and *P. aeruginosa* was 1, 2, and 2 mg/mL, which was 25%, 25%, and 12.5% of the positive control (vanillin), respectively (Rahman et al. 2017). The MIC for *K. pneumonia* was 5 μg/mL, equal to the chloramphenicol positive control (Liu et al. 2018). Investigation with purified compounds showed that MIC values of fatty acids (**191–194**), phenolic acids (**40**–**42**), and coronadiene (**25**) for *K. pneumonia* ranged from 5 to 50 μg/mL, 100% to 10% of the positive control, proving that the anti-microbial activity of EtOH Ex. and EtOAc Ex. was most likely contributed to the synergistical effects of these components (Liu et al. 2018).

A. tsao-ko EO also showed antibiotic activity in vitro. It had an inhibitory effect on a broad spectrum microbial organisms, including gram-positive and gram-negative bacteria such as *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa,* with MIC ranging from 22.49 to 1438.91 μg/mL (Dai et al. 2016b). It could also suppress the growth of *Trichomonas vaginalis* Tv1 and Tv2, with IC_{50} values of 22.49 μg/mL and 44.97 μg/mL, respectively; the IC_{50} values of metronidazole positive control were 2.44 μg/mL and 4.88 μg/mL, respectively (Dai et al. 2016a). Observation under transmission electron microscopy (TEM)

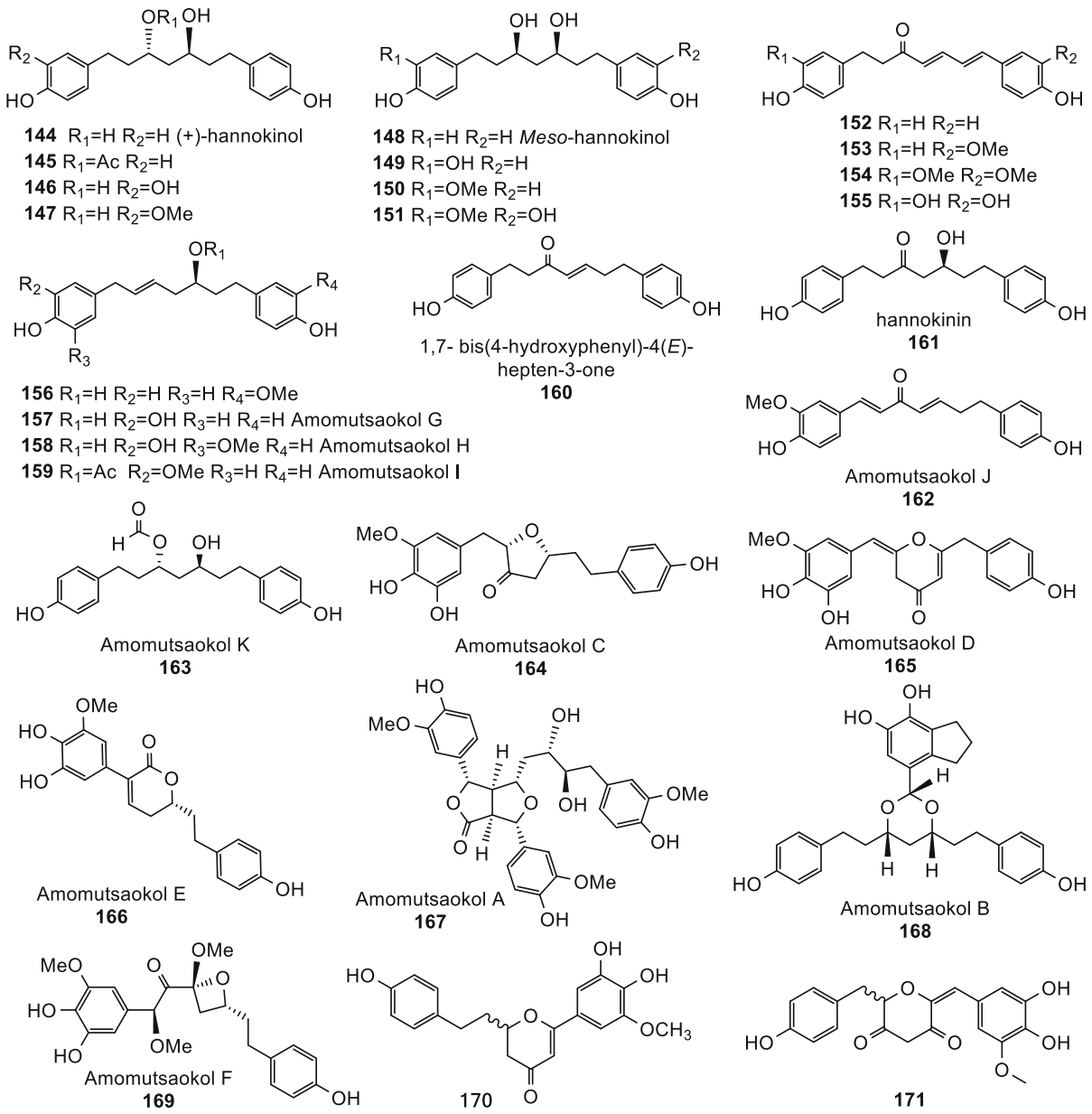

Fig. 6 Diarylheptanoids (**144–171**) isolated and identified from *A. tsao-ko* fruits

showed the anti-*T. vaginalis* activity was possibly due to the damage of membrane structure, reduction or disappearance of ribosomes, organelles disintegration, cell disintegration and necrosis (Dai et al. 2016a). Geraniol (**5**) was thought to be the most effective substance due to its high content in *A. tsao-ko* EO (13.69%) and its inhibitory activity against *T. vaginali* (IC_{50} = 171.48 μg/mL for both Tv1 and Tv2) (Dai et al. 2016a).

A. tsao-ko EO had considerable toxicity on stored-product insects, *T. castaneum* (Herbst) and *Lasioderma serricorne* (Fabricius) (Wang et al. 2014). Further isolation led to two components, limonene (**1**) and eucalyptol (**10**). Both compounds showed pronounced contact toxicity against *T. castaneum* and *L. serricorne*. The LD_{50} values of **1** for *T. castaneum* and *L. serricorne* were 14.97 μg/adult and 13.66 μg/adult, respectively; those of **10** were 18.83 μg/adult and

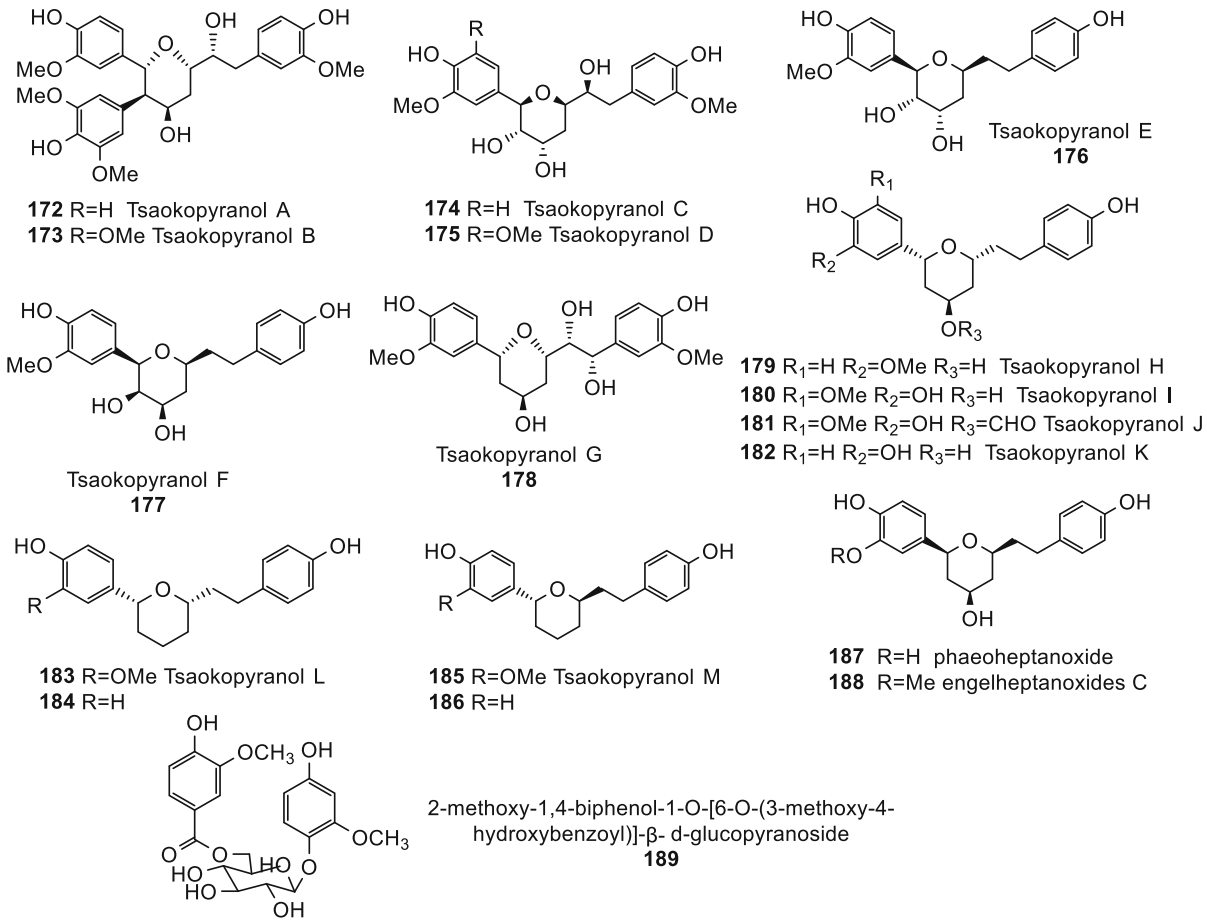

Fig. 7 Diarylheptanoids (**172–188**) and phenylethanoid glycoside (**189**) isolated and identified from *A. tsao-ko* fruits

15.58 μg/adult, respectively (Wang et al. 2014). These two components also possessed strong fumigant toxicity against both insect species. The LC_{50} values of **10** for *T. castaneum* and *L. serricorne* were 5.47 mg/L air and 5.18 mg/L air, and those of **1** were 6.21 mg/L air and 14.07 mg/L, respectively (Wang et al. 2014).

Isotsaokoin (**14**), another *A. tsao-ko* EO component, showed antifungal activity against *Trycophyton mentagrophytes* a little better than the positive control (amphotericin B) (Moon et al. 2004). The inhibition zone of **14** was 1.5 and 2.0 mm at 20 and 40 μg/disk, respectively, whereas that of amphotericin B was 2.5 and 3.0 mm at 20 and 40 μg/disk, respectively (Moon et al. 2004).

In vivo experiment also demonstrated that *A. tsao-ko* EO had antibiotic activity. When intramuscularly supplied 0.92 g/kg/d, *A. tsao-ko* EO could protect the mice from the infection of *S. aureus* or *Escherichia coli*, showing 100.00% survival rates (Dai et al. 2016b).

The broad-spectrum antibiotic activities of Tsaoko Fructus against microorganisms especially pathogenic microbes makes Tsaoko Fructus a promising and potential natural source for developing broad-spectrum antibiotics, which also gives a hint to the probable mechanism of its clinical application such as curing malaria and diarrhea.

Anti-inflammatory activity

It was proved that EtOH Ex. and some purified compounds from Tsaoko Fructus had anti-inflammatory activities. Lipopolysaccharide (LPS)-treated RAW 264.7 macrophage cells and BV2 microglial cells are commonly used to evaluate the effects and to

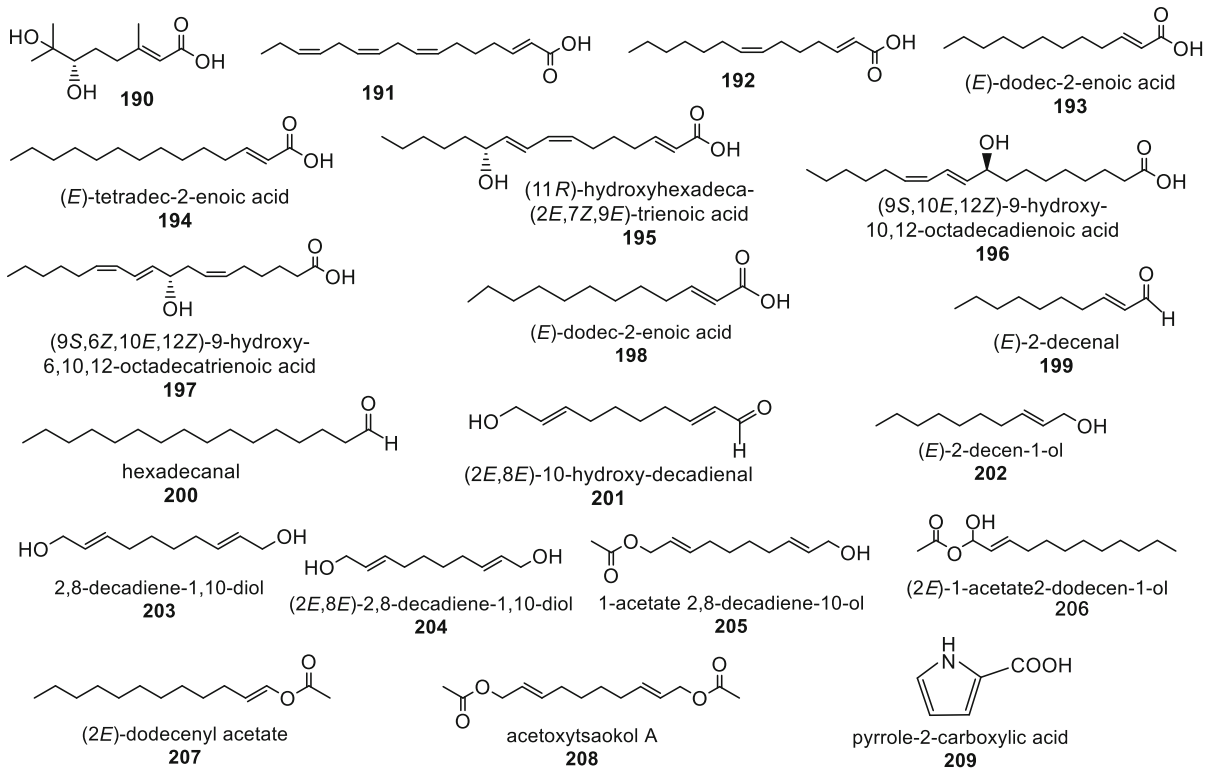

Fig. 8 Fatty acids, aliphatic ketones, and hydrocarbons isolated and identified from *A. tsao-ko* fruits

explore the possible molecular mechanism of anti-inflammatory activities.

NNMBS227, the 70% EtOH Ex. of *A. tsao-ko* seeds (At-EE), was reported to suppress the expression of two pro-inflammatory mediators, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), in the LPS-induced inflammatory responses in RAW264.7 cells and exhibited IC_{50} values of 194.92 ± 1.38 μg/mL and 151.00 ± 1.06 μg/mL, respectively (Li et al. 2014). Another research also revealed that the 80% EtOH Ex. of *A. tsao-ko* decreased LPS-induced NO production in RAW 264.7 cells with an IC_{50} value of 59.5 ± 1.8 μg/mL, and the N-Monomethyl-L-arginine positive control had an IC_{50} value of 27.3 ± 0.3 μM (Choi et al. 2018). Bioassay guided purification and inhibitory effect assay led to the finding of two active compounds, (-)-catechin (**73**) and (+)-epicatechin (**75**) (Choi et al. 2018). Both showed high activity (IC_{50} = 70.6 μM and IC_{50} = 73.3 μM, respectively) against NO production without cytotoxicity (Choi et al. 2018). Pharmacological research with purified compounds revealed that diarylheptanoids (**170**, **171**) and aliphatic alcohol (2,8-decadiene-1,10-diol, DDO, **203**) from the ethanol extracts, and oxygenated monoterpenes (**9**, **11–14**, **17**, **18**), flavonoids (**88–95**, **98**, **104–107**), diarylheptanoids (**144**, **148**, **153**, **154**, **161**), and fatty acid (**190**) from the methanol extracts also had considerable inhibitory effect against LPS-induced inflammatory response, with IC_{50} values or the inhibition effects equivalent to that of the positive control Lee et al. (2008) (Table 3).

Western blot, RT-PCR, and ELISA analysis proved that (-)-catechin (**73**) and (+)-epicatechin (**75**) inhibited the NO production in LPS-stimulated RAW 264.7 cells through suppressing the expression of iNOS and the translocation of nuclear factor kappa-B (NF-κB) and reducing the production of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-10 (Choi et al. 2018). For **203**, besides reducing NO production and iNOS transcription, it could also inhibit the production of prostaglandin E2 (PGE2) and pro-inflammatory cytokines such as IL-6 and TNF-α. Such biological effect was resulted from the inactivation of the MAPKs such as extracellular signal-regulated kinase, c-Jun-N-terminal kinase and

Table 3 The pharmacological activities of extract, essential oil, and isolated compounds of *A. tsao-ko* fruits*

Activity	Extract/ EO/ compound	Measure of activity	Positive control and activity	Cell line/strain/Model	Method	References
Antibiotic activity	EtOH Ex	MIC: 1, 2, and 2 mg/mL for *S. aureus, S. Typhimurium,* and *P. aeruginosa*, respectively	Vanillin: MIC: 250 μg/mL	*Staphylococcus aureus* ATCC 6538 *Salmonella Typhimurium* ATCC 50,013 *Pseudomonas aeruginosa* ATCC 9027	Agar disk diffusion method Flask incubation assay	Rahman et al. (2017)
	EtOH Ex., EtOAc Ex., **25**, **40–42**, **191–194**	MIC: 5, 5, 5, 5, 10, 10, 10, 50, 50, 50 μg/mL, respectively	Chloramphenicol: MIC: 5 μg/mL	*Klebsiella pneumoniae*	Broth-dilution method	Liu et al. (2018)
	EO	In vitro: MIC: 22.49 to 1438.91 μg/mL In vivo: 0.92 g/kg/d (intramuscularly): 100.00% survival rate for *S. aureus* and *E. coli* infected mice 1.84 g/kg/d (intragastrically): 100.00% and 70% survival rates for *S. aureus* and *E. coli* infected mice, respectively	Cefradine for mice infected with *S. aureus* Cefminox for mice infected with *E. coli*	Reference strains: *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Pseudomonas aeruginosa* NCTC 10662, *E. coli* CMCCB 44102, *S. aureus* CMCCB 26003, *S. pneumoniae* ATCC 49619 Clinical isolated strains: 85 *S. aureus, E. coli, P. aeruginosa, Proteus vulgaris, Shigella flexneri, Enterobacter cloacae* Mouse peritonitis model: Infected with *S. aureus* or *E. coli*	Agar dilution method In vivo anti-infectious efficacy	Dai et al. (2016b)
	EO **5**	MLC = 44.97 μg/mL; IC_{50} = 22.49 μg /mL for *T. vaginalis* isolate Tv1; MLC = 89.93 μg/mL; IC_{50} = 44.97 μg/mL for Tv2 MLC = 342.96 μg/mL; IC_{50} = 171.48 μg/mL for both Tv1 and Tv2	Metronidazole: MLC = 4.88 μg/mL; IC_{50} = 2.44 μg /mL for Tv1; MLC = 9.77 μg/mL; IC_{50} = 4.88 μg /mL for Tv2; Ornidazol: MLC = 2.44 μg/mL; IC_{50} = 1.22 μg /mL for Tv1; MLC = 4.88 μg/mL; IC_{50} = 2.44 μg /mL for Tv2;	Clinically isolated strains: *Trichomonas vaginalis* isolates Tv1, Tv2	Liquid dilution method TEM	Dai et al. (2016a)
	14	The inhibition zone was 1.5 and 2.0 mm at 20 and 40 μg/disk, respectively	Amphotericin B: The inhibition zone was 2.5 and 3.0 mm at 20 and 40 μg/disk, respectively	*Trycophyton mentagrophytes* KCTC 6085	Paper-disk agar diffusion method	Moon et al. (2004)

Table 3 continued

Activity	Extract/EO/ compound	Measure of activity	Positive control and activity	Cell line/ strain/Model	Method	References
	EO	LD_{50} = 16.52 µg/adult; LC_{50} = 5.85 mg/L air against *T. castaneum* LD_{50} = 6.14 µg/adult; LC_{50} = 8.70 mg/L air against *L. serricorne*	Pyrethrins: LD_{50} = 0.26 µg/adult against *T. castaneum* Pyrethrins: LD_{50} = 0.24 µg/adult against *L. serricorne* Methyl bromide: LC_{50} = 1.75 mg/L air against *T. castaneum* Phosphine: $LC_{50} = 9.23 \times 10^{-3}$ mg/L air against *L. serricorne*	*Tribolium castaneum* (Herbst) *Lasioderma serricorne* (Fabricius)	Contact toxicity bioassay Fumigant toxicity bioassay	Wang et al. (2014)
	1	LD_{50} = 14.97 µg/adult; LC_{50} = 6.21 mg/L air against *T. castaneum* LD_{50} = 13.66 µg/adult; LC_{50} = 14.07 mg/L air against *L. serricorne*				
	10	LD_{50} = 18.83 µg/adult; LC_{50} = 5.47 mg/L air against *T. castaneum* LD_{50} = 15.58 µg/adult; LC_{50} = 5.18 mg/L air against *L. serricorne*				

Table 3 continued

Activity	Extract/EO/ compound	Measure of activity	Positive control and activity	Cell line/ strain/Model	Method	References
Anti-inflammatory activity	EtOH Ex. (seeds)	No cytotoxic effect below 400 μg/mL IC_{50} = 194.92 μg/mL for iNOS IC_{50} = 151.00 μg/mL for COX-2	-	LPS-induced RAW264.7 macrophages	MTT assay, Nitrite assay using the Griess reaction ELISA, Western Blot, Immunofluorescence Microscopy	(Li et al. 2014)
	203	No cytotoxic effect on up to 300 μM IC_{50} = 136.66 μM for NO production Inhibition on iNOS, COX-2, IL-6, NF-κB, and MAKPs at 200 μM, and TNF-α at 100 μM	NG-methyl-L-arginine (L-NMMA, 100 μM)	LPS-induced RAW264.7 macrophages	MTT assay, Nitrite assay using the Griess reaction ELISA, Western blot, Immunofluorescence Microscopy	Kim et al. (2016)
	EtOH Ex	IC_{50} = 59.5 μg/mL for NO production and MTT > 100%	N-Monomethyl-L-arginine: IC_{50} = 27.3 μM for NO production and MTT > 100%	LPS-induced RAW264.7 macrophages	MTT assay, Nitrite assay using the Griess reaction ELISA, Western blot	(Choi et al. 2018)
	73 **75**	IC_{50} = 73.32 μM for NO production and MTT > 100% IC_{50} = 70.57 μM for NO production and MTT > 100%	N-Monomethyl-L-arginine: IC_{50} = 25.29 μM for NO production and MTT > 100%			
	75	Inhibition on iNOS, TNF-α, IL-1β, IL-10 at 25–100 μM	Dexa: Inhibition on iNOS, TNF-α, IL-1β, IL-10 at 10 μM			
	170, 171	Cell viability: 80.34% and 69.82% at 50 μg/mL, respectively NO inhibition: 60.46% and 48.62% at 100 μg/mL, respectively	Vitamin C: Cell viability: 84.80% at 50 μg/mL	LPS-stimulated macrophage RAW 264.7 cells H_2O_2-treated PC-12 cells	MTT assay, Nitrite assay using the Griess reaction	(Zhang et al. 2016)
	9, 11, 13, 14, 17, 18, 144, 148, 153, 154, 161, 190	68.8% to 1.1% NO inhibition at a concentration ranging from 1 μM to 100 μM	NAME (ω-nitro-L-arginine methyl ester): 58.5% to 11.2% for NO inhibition at a concentration ranging from 1 μM to 100 μM	LPS-induced BV2 microglial cells	MTT assay, Nitrite assay using the Griess reaction	Lee et al. (2008)
	98, 104, 105, 153	IC_{50} = 10.9 to 22.5 μM	Aminoguanidine: IC_{50} = 21.4 μM	LPS-induced RAW 264.7 macrophages	MTT assay, Nitrite assay using the Griess reaction	Kim et al. (2019b)
	88–95, 106, 107	IC_{50} = 10.6 to 41.5 μM	Aminoguanidine: IC_{50} = 21.7 μM	LPS-induced RAW 264.7 macrophages	MTT assay, Nitrite assay using the Griess reaction	Kim et al. (2019a)

Table 3 continued

Activity	Extract/EO/ compound	Measure of activity	Positive control and activity	Cell line/strain/Model	Method	References
Anti-tumor and anti-cancer	Ethyl acetate fraction of EtOH Ex	Inhibition rate at 400 μg/mL: 71.4% against SMMC-7721; About 60% against HepG-2, Hela and A549	5-fluorouracil: Inhibition rate at 400 μg/mL: About 70% against SMMC-7721 and A549; About 60% against HepG-2 and Hela	HepG-2, SMMC-7721, Hela and A549 human cancer cells	MTT assay	Zhang et al. (2015)
	Petroleum ether fraction of EtOH Ex	Inhibition rate at 400 μg/mL: About 70% against SMMC-7721 and A549; About 60% against HepG-2 and Hela				
	170	IC_{50} = 91.23, 89.08, 117.83, 79.77 μg/mL to SMMC-7721, HepG-2, Hela, A549 cells, respectively	5-fluorouracil: IC_{50} = 59.83, 73.89, 65.89, and 72.29 μg/mL to SMMC-7721, HepG-2, Hela, and A549 cells, respectively			
	171	IC_{50} = 44.66, 97.18, 71.71, and 80.95 μg/mL to SMMC-7721, HepG-2, Hela, A549 cells, respectively				
	14	IC_{50} = 72.14 μg/mL to Hela cells				
	Hexane fraction of EtOH Ex. (seeds)	SPHK1 and SPHK2 inhibition: inhibited 39% and 67% of the control, respectively, at 100 μg/mL	-	BV2 microglial cells	MTT assay SPHK1/2 activity inhibition assay	Lee et al. (2019)
	61–63, 68, 194, 197, 204–206	No significant cell death at 10 μM SPHK1 inhibition: 59.75% (**205**) to 77.51% of the control	Resveratrol for SPHK1 inhibition: 85.43% of the control SKI-II for SPHK2 inhibition: 75.35% of the control			
	3, 57, 58, 65, 193, 204, 205	No significant cell death at 10 μM SPHK2 inhibition: 22.75% (**3**), 25.40% (**205**) to 58.20% of the control				
	EO	IC_{50} = 31.80 μg/mL (for HepG2)—600 μg/mL (for A549, no obvious cytotoxicity) IC_{50} = 163.91 and 272.41 μg/mL for HUVEC and HL-7702, respectively	Mitomycin: IC_{50} = 5.93 μg/mL for HepG2 IC_{50} = 2.54 and 16.04 μg/mL for HUVEC and HL-7702, respectively	Carcinoma cell lines: Human HepG2, Hela, Bel-7402, SGC-7901, PC-3 Normal cell lines: Human HUVEC and HL-7702	MTT assay, DNA content and cell cycle analysis	Yang et al. (2010)

Table 3 continued

Activity	Extract/EO/ compound	Measure of activity	Positive control and activity	Cell line/strain/Model	Method	References
Antioxidant activity	Ethyl acetate fraction of 95% EtOH Ex	> 90% DPPH radicals inhibition rate at 200 μg/mL	Vitamin C: > 90% DPPH radicals inhibition rate at 200 μg/mL	HepG-2, SMMC-7721, Hela, and A549 human cancer cells	DPPH radical- scavenging activity assay	Zhang et al. (2015)
	171	About 80% DPPH radical inhibition rate at 100 μg/mL	Vitamin C: > 80% DPPH radicals inhibition rate at 100 μg/mL			
	40–42, 144, 148, 191, 192	MIC: 5, 100, 100, 100, 100, 100 μg/mL; > 80%, > 80%, > 80%, 77.08%, > 70%, > 70%, 60.83%	Vitamin C: MIC: 5 μg/mL, > 90%	-	DPPH radical scavenging activity assay	Liu et al. (2018)
	36, 60, 73, 75	IC_{50} = 12.55, 12.66, 15.89, 14.39 μM, respectively	α-Tocopherol: IC_{50} = 12.57 μM	-	DPPH radical scavenging activity assay	Martin et al. (2000)
	EO (obtained by M-SFME)	IC_{50} = 5.27 mg/mL for DPPH assay IC_{50} = 0.63 mg/mL for β-carotene/linoleic acid bleaching assay	Vitamin C for DPPH assay: IC_{50} = 0.046 mg/mL BHT for β-carotene/ linoleic acid bleaching assay: IC_{50} = 0.02 mg/mL	-	DPPH radical scavenging activity assay, β-carotene/linoleic acid bleaching assay	Cui et al. (2017)
	EO	IC_{50} = 5.12 mg/mL for DPPH assay IC_{50} = 0.04 = mg/mL for TAB test FRAP = 24.27 μM Fe^{2+}/mg	L-ascorbic acid for DPPH: IC_{50} = 2.17 μg/mL BHT for TBA test: IC_{50} = 0.05 μg/mL L-ascorbic acid: FRAP = 10.33 mM Fe^{2+}/mg	Human HepG2, Bel-7402, Hela, A549, SGC-7901, and PC-3 cancer cell lines, and HL-7702, HUVEC normal cell lines	DPPH radical scavenging activity assay, TBA test (Lipid peroxidation inhibition assay), FRAP assay	Yang et al. (2010)

Table 3 continued

Activity	Extract/EO/ compound	Measure of activity	Positive control and activity	Cell line/strain/Model	Method	References
Anti-diabetic activity	Aqueous Ex. of *A. tsao-ko* seeds	IC_{50} = 1.04 mg/mL for α-amylase IC_{50} = 1.4 mg/mL for α-glucosidase	Acarbose: IC_{50} = 2.1 mg/mL for α-amylase IC_{50} = 1.90 mg/mL for α-glucosidase	-	α-amylase inhibition assay α-glucosidase inhibition assay	Hussain et al. (2018)
	Aqueous Ex. of *A. tsao-ko* rinds	IC_{50} = 1.24 mg/mL for α-amylase IC_{50} = 2.4 mg/mL for α-glucosidase				
	MeX Polar fraction of MeX	IC_{50} = 0.02 mg/mL for α-glucosidase No IC_{50} for α-amylase and lipase Plasma glucose: About 100 mg/dL of polar fraction	Diet control: Plasma glucose: About 200 mg/dL	Male mice of the Crlj:CD-1 (ICR) strain	In vitro: α-amylase, α-glucosidase, and lipase activity assay In vivo: Plasma glucose assay	Yu et al. (2010)
	50% EtOH Ex	IC_{50} = 38.6 μg/mL	Acarbose: IC_{50} = 219.0 μM	-	α-glucosidase inhibitory assay	He et al. (2020c)
	176, 179–182, 187	IC_{50} = 59.4 to 97.0 μM				
	172, 173, 175, 177, 188	IC_{50} = 100.1 to 179.5 μM				
	110, 114, 116, 117, 120	IC_{50} = 201.45 to 317.51 μM	Suramin sodium: IC_{50}:199.39 μM	-	PTP1B inhibitory assay TCPTP assay	He et al. (2021)
	71, 112, 114–117, 119–122	IC_{50} = 3.73 to 76.23 μM	Acarbose: IC_{50} = 193.77 μM	-	α-glucosidase inhibitory assay	He et al. (2021)
	123, 124, 128, 133, 140	IC_{50} = 5.2 to 9.0 μM,	Acarbose: IC_{50} = 180.0 μM	-	α-glucosidase inhibitory assay	He et al. (2020a)
	128, 132–134, 141	IC_{50} = 56.4 to 80.4 μM	Suramin sodium: IC_{50} = 200.5 μM	-	PTP1B inhibitory assay TCPTP assay	He et al. (2020a)
	142	IC_{50} = 18.8 μM	Acarbose: IC_{50} = 213 μM	-	α-glucosidase inhibitory assay	He et al. (2020b)
	143	IC_{50} = 38.6 μM				

Table 3 continued

Activity	Extract/ EO/ compound	Measure of activity	Positive control and activity	Cell line/strain/ Model	Method	References
Lipid reducing activity	MeX polar fraction of MeX	Body lipid: About 12.5%	Diet control: Body lipid: about 20%	Male mice of the Crlj:CD-1 (ICR) strain	Plasma and liver lipid analysis Plasma TBARS concentration assay	(Yu et al. 2010)
	191–194	MIC: 50 μg/mL, 50.07%; MIC: 50 μg/mL, 61.56%; MIC: 50 μg/mL, 59.37%; MIC: 50 μg/mL, 49.32%	Orlistat: MIC: 5 μg/mL, 58.78%	-	Lipase inhibition assay	Liu et al. (2018)
Neuroprotective activity	**170** **171**	80.34% cell viability at 50 μg/mL 69.82% cell viability at 50 μg/mL	Vitamin C: 84.80% cell viability at 50 μg/mL	LPS-stimulated macrophage RAW 264.7 cells H_2O_2-treated PC-12 cells	MTT assay, Nitrite assay using the Griess reaction	Zhang et al. (2016)
	77	up to 78.9% cell viability at 50 μg/mL DPPH radical-scavenging activity at 100 μg/mL	Hydrogen peroxide: > 50% cell viability	H_2O_2-treated PC-12 cells	MTT assay, Nitrite assay using Griess reaction, DPPH radical scavenging activity assay	Zhang et al. (2014)
Anti-complementary activity	**33** **160**	CH_{50}: 0.55 mM; AP_{50}: 0.53 mM CH_{50}: 0.42 mM; AP_{50}: 0.66 mM	Heparin: CH_{50}: 40 mM; AP_{50}: 97 mM	Sheep erythrocytes	In vitro test for complement- inhibitory properties against CP and AP, In vitro hemolytic assays	Jin et al. (2013)

*All the extracts, EO, and isolated compounds were from the dried fruits of *A. tsao-ko*, except where specified

ELISA Enzyme-Linked Immunosorbent Assay, *EtOAc Ex.* Ethyl Acetate Extracts, *EtOH Ex.* Ethanol Extracts, IC_{50} 50% inhibitory concentration, LC_{50} 50% Lethal Concentration, LD_{50} 50% Lethal Dose, *LPS* Lipopolysaccharide, *MAPK* Mitogen-Activated Protein Kinase, *MBC* Minimum Bactericidal Concentration, *MeX* Methanol Extracts, *DPPH* 2,2-Diphe- Nyl-1-Picrylhydrazyl, *MIC* Minimum Inhibitory Concentration, *MLC* Minimum Lethal Concentration, *MLD* Minimal Lethal Dose, *MTT* 3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide, *TBARS* Thiobarbituric Acid Reactive Substances, *TCPTP* T-Cell Protein Tyrosine Phosphatase, *TEM* Transmission Electron Microscopy

$p38^{MAPK}$ and the suppression of the NF-κB pathway such as degradation of κB-α and NF-κB inhibitors (Kim et al. 2016). It appeared that the anti-inflammatory activities of Tsaoko Fructus extracts and the isolated compounds were possibly achieved through the downregulation of the mitogen-activated protein kinase (MAPK) pathway and the NF-κB pathway.

The anti-inflammatory activity of Tsaoko Fructus indicates that it could be used as a potent therapeutic agent for the treatment of inflammatory disorders. It also implies that the medicinal value of Tsaoko Fructus such as its treatment on spleen and stomach disorders might be at least partially due to its anti-inflammatory activity.

Anti-tumor/cancer and antioxidant activity

The anti-tumor and anti-cancer activities were usually evaluated by MTT assay against human cancer cells such as hepatoma cells HepG-2 and SMMC-7721, cervical cancer cell Hela, and lung cancer cell A549 in vitro. Besides, since oxidative stress is among the main causes of cancer-related death and the chemoprevention is defined as the use of antioxidants to prevent cancer formation or cancer progress, antioxidative activity assay such as DPPH radical scavenging ability assay was also used to screen potential anti-tumor/cancer chemicals (Zhang et al. 2015).

It was reported that the ethyl acetate fraction and the petroleum ether fraction of 95% ethanol extracts of *A. tsao-ko* fruits showed > 60% inhibition rate at 400 μg/mL against several cancer cell lines including Hela, HepG-2, SMMC-7721, and A549 (Zhang et al. 2015). Bioactivity-guided separation led to the isolation of isotsaokoin (**14**) and two diarylheptanoids (**170** and **171**) (Zhang et al. 2015). **170** and **171** inhibited the proliferation of HepG-2, SMMC-7721, Hela and A549 cells with IC_{50} ranging from 44.66 μg/mL to 117.83 μg/mL, nearly equal to that of the positive control (5-fluorouracil: 59.83–73.89 μg/mL), while **14** only had inhibitory activity against Hela cells (IC_{50} = 72.14 μg/mL) (Zhang et al. 2015). **171** also showed DPPH scavenging ability, equivalent to vitamin C (Vc) (Zhang et al. 2015).

Sphingosine kinases 1 and 2 (SPHK1/2) are considered rate limiting enzymes for the formation of sphingosine 1 phosphate (S1P), which serves an important function in cellular and physiological processes Lee et al. (2019). So, the inhibition of SPHK1/2 may induce cell cycle arrest and apoptosis, exerting anticancer effects Lee et al. (2019). Research revealed the hexane fraction of 50% EtOH Ex. of *A. tsao-ko* seeds showed inhibitory effect on SPHK1 and SPHK2 by 39% and 67% of the control, respectively Lee et al. (2019). Under the guidance of this assay, 25 compounds had been isolated, of which phenolic acids **61, 62, 63** and **68**, fatty acids **194** and **197**, aliphatic alcohols **204**, **205** and **206** showed inhibition against SPHK1 activity up to 20%, and monoterpene **3**, phenolic acids **57**, **58** and **65**, fatty acid **193,** aliphatic alcohols **204** and **205** had inhibition against SPHK2 activity up to 40% compared with the control Lee et al. (2019) (Table 3). Compound **205** had the highest potency to inhibit the activity of SPHK1, by 59.75%, and compound **3** showed the highest potency in suppressing SPHK2 activity, by 22.75%, in comparison with the control, where both exhibited higher inhibitory effect than the corresponding positive control (Resveratrol for SPHK1: 85.43%; SKI-II for SPHK2: 75.35%) Lee et al. (2019). Docking modeling analysis indicated that **205** and **3** bind into the hydrophobic substrate-binding pocket of SPHK1 and SPHK2, respectively, suggesting they might act as substrate-competitive inhibitors of SPHK1/2 enzymes Lee et al. (2019).

Compounds from the 95% EtOH extract or 70% acetone fraction of dichloromethane extracts of Tsaoko Fructus had antioxidative activity. These compounds included phenolic acids **36**, **40**, **41** and **42**, flavonoids **60**, **74** and **76**, diarylheptanoids **145** and **149**, and fatty acids **192** and **193** (Martin et al. 2000; Liu et al. 2018). Among them, **36**, **60**, **73** and **75** had the IC_{50} values of 12.55–15.89 μM, equivalent to that of the positive control (α-tocopherol: IC_{50} = 12.57 μM) (Martin et al. 2000), and **42** exhibited almost excellent DPPH scavenging activity at a concentration of 100 μg/mL (DPPH radical inhibition rate > 90%), which was very close to that of vitamin C at the same concentration (DPPH radical inhibition rate about 95%) (Liu et al. 2018).

A. tsao-ko EO was also proved to have antioxidative and anti-tumor activities. Cytotoxicity analysis by MTT assay showed that *A. tsao-ko* EO was cytotoxic to HepG2, Hela, Bel-7402, SGC-7901 and PC-3 cell lines. And the lowest IC_{50} of 31.80 ± 1.18 μg/mL was obtained for HepG2 carcinoma cell lines, compared to 5.93 ± 0.30 μg/mL of the positive control (mitomycin) (Yang et al. 2010). It

had weak antioxidant activity as measured by DPPH radical assay, thiobarbituric acid (TBA) test, and ferric reducing antioxidant power (FRAP) assay (Yang et al. 2010).

The strong antioxidant and anti-tumor activity against tested tumor cell lines of **171** indicates it is worthy of further study as a potential nutraceutical compound and chemotherapeutic drug. The studies of **205** and **3** on the inhibition of SPHK1 and SPHK2 enzymatic activities also suggest that these compounds could be developed as potential anti-tumor drugs.

Anti-diabetic activity

The anti-diabetic activities of extracts, EO, and isolated compounds of *A. tsao-ko* are usually assessed by assaying the inhibition activity of enzymes such as α-amylase, α-glucosidase, protein tyrosine phosphatase 1B (PTP1B), and T-Cell protein tyrosine phosphatase (TCPTP) through in vitro and in vivo experiments.

The aqueous extracts of *A. tsao-ko* seeds showed obvious inhibitory activities against α-amylase and α-glucosidase in vitro, with IC_{50} of 1.04 mg/mL and 1.4 mg/mL, in contrast to 2.1 mg/mL and 1.90 mg/mL of the positive control (acarbose), respectively (Hussain et al. 2018). The methanol extracts (MeX) and the polar fraction of MeX of *A. tsao-ko* fruits inhibited α-glucosidase activity with an IC_{50} of 0.02 mg/mL in vitro (Yu et al. 2010). Dietary feeding experiments in mice proved that feeding the polar fraction of MeX can reduce plasma glucose to about 50% of the negative control, indicating that the polar fraction of MeX had effective hypoglycemic activity in vivo (Yu et al. 2010).

Recently, it has been demonstrated that 50% ethanol–water extract of *A. tsao-ko* dried fruits had significant α-glucosidase inhibitory activity (IC_{50} = 38.6 μg/mL) (He et al. 2020c). Bioactivity-guided isolation on the active fraction afforded seventeen 2,6-epoxy diarylheptanoids (**172**–**188**)(He et al. 2020c) Among them, tsaokopyranols E, H, I, J, K (**176**, **179**–**182**) and phaeoheptanoxide (**187**) showed obvious α-glucosidase inhibitory activity with IC_{50} below 100 μM, much lower than the positive control (acarbose: IC_{50} = 219.0 μM). Tsaokopyranols A, B, D, F (**172**, **173**, **175**, **177**) and engelheptanoxide C (**188**) exhibited moderate activity with IC_{50} ranging from 100.1 to 179.5 μM, comparable to the positive control (He et al. 2020c). Applying similar approaches, the same research group also isolated a series of flavonoids and flavonoid derivatives from the EtOH extract of *A. tsao-ko* fruits and demonstrated that some of them had anti-diabetic activities (He et al. 2020a, b, 2021). These compounds included flavonoids, namely (+)-afzelechin (**71**), flavanocoumarin (**110**), sappanone B (**111**), brazilin (**112**) and proanthocyanidin A-2 (**114**), rare flavanol-menthane conjugates, namely amomutsaokins A-C and E–H (**115**–**117**, **119**–**122**), new flavanol-fatty alcohol hybrids, namely tsaokoflavanols A, B, F, J-L, R and S (**123**, **124**, **128**, **132**–**134**, **140**, **141**), and two unusual flavanol-monoterpenoid hybrids, tsaokols A (**142**) and B (**143**) (He et al. 2020a, b, 2021) (Table 3). Tsaokols A (**142**) and B (**143**) showed significant α-glucosidase inhibitory effect with IC_{50} values of 18.8 and 38.6 μmol/L (He et al. 2020b). Compounds **110**, **114**, **116**, **117** and **120** exhibited PTP1B selective inhibition with IC_{50} values of 201.45–317.51 μM, and **71**, **111**, **112**, **114**–**117** and **119**–**122** displayed α-glucosidase inhibitory effect with IC_{50} values ranging from 3.73 to 76.23 μM (He et al. 2021). Tsaokoflavanols A, B, F, K and R (**123**, **124**, **128**, **140**) exhibited inhibitory activity against α-glucosidase with IC_{50} values of 5.2–9.0 μM, 20–35 times stronger than the positive control (acarbose: IC_{50} = 180.0 μM). And tsaokoflavanols F, J-L and S (**128**, **132**–**134**, **141**) were PTP1B/TCPTP selective inhibitors with IC_{50} values of 56.4–80.4 μM, 2–4 times stronger than the positive control (suramin sodium: IC_{50} = 200.5 μM)(He et al. 2020a). Enzyme kinetics study indicated that compounds **123**, **124**, **128** and **133** were α-glucosidase and PTP1B mixed-type inhibitors with Ki values ranging from 2.9 to 13.0 μM and 39.2 to 142.3 μM, respectively (He et al. 2020a). Using docking simulation they proved that the hemiacetal hydroxy, the orientation of 3,4-dihydroxyphenyl, and the length of alkyl were essential in binding with α-glucosidase and PTP1B (He et al. 2020a).

Lipid reducing activity

Methanol extracts (MeX) of *A. tsao-ko* fruits could reduce the body lipid in mice at about 50% of the control, and (+)-epicatechin (**75**) was believed to be the main active component (Yu et al. 2008, 2010). Through in vitro assay, Liu et al. (2018) proved that

fatty acids **191**, **192**, **193** and **194** exhibited inhibition effects on lipase activity, and when the concentrations were at 50 μg/mL, their inhibition rates were 50.07%, 61.56%, 59.37% and 49.32%, respectively. The inhibition effects of (2*E*,7*Z*)-tetradeca-2,7-dienoic acid (**192**) and (*E*)-tetradec-2-enoic acid (**193**) on lipase were even better than the positive control (orlistat: 58.78%) at a concentration of 50 μg/mL (Liu et al. 2018).

Neuroprotective activity

The neuroprotective effect is closely in correlation with the antioxidant activity, just as anti-inflammatory and anti-tumor activities (Zhang et al. 2015, 2016). H_2O_2 induced nerve injury of PC-12 cells were commonly used to assay the neuroprotective activity.

Besides the activity against inflammation, diarylheptanoids **170** and **171** also showed significant neuroprotective activity by reversing the loss of cell viability induced by H_2O_2, with nearly equal activity to the Vc control (Zhang et al. 2016). The 95% EtOH and the ethyl acetate fraction of *A. tsao-ko* fruits also showed potent protective effect on the damage to PC-12 cells induced by H_2O_2 (Zhang et al. 2014). Bioactivity-guided separation led to the isolation of six active compounds including quercetin (**77**), daucosterol (**31**), (+)-epicatechin (**75**), quercetin-7-*O*-β-glucoside (**79**), quercetin-3-*O*-β-D-glucopyranoside (**78**), *meso*-hannokinol (**148**). Quercetin (**77**) exhibited the strongest neuroprotective effect, and the cell viability was up to 78.9% at a concentration of 50 μg/mL. The other five compounds **31**, **75**, **78**, **79** and **148** also showed protective effects. The cell viability was 75.6%, 70.4%, 68.1%, 68.1% and 63.8% after treatment with these compounds, respectively (Zhang et al. 2014). Quercetin (**77**) exhibited good DPPH radical-scavenging activity at a concentration of 100 μg/mL (DPPH radical inhibition rate $> 80\%$), very close to Vc at the same concentration (about 83%) (Zhang et al. 2014). But, quercetin (**77**) is a widespread natural product in plants and can interact with many proteins in vitro (Gertsch 2009). Whether it works as the effective ingredient of Tsaoko Fructus or not requires further exploration.

Anti-complementary activity

In the effort to search for anti-complementary agents under the guidance of bioactivity-directed fractionation and isolation, Jin et al. (2013) obtained 14 compounds (**15, 18, 32, 33, 44–47, 55, 96, 99, 101, 153, 160**) from the ethanolic extract of *A. tsao-ko* dried fruits. All the 14 compounds exhibited anti-complementary activities against the classical pathway (CP) and the alternative pathway (AP) through in vitro evaluation (Jin et al. 2013). Among them, hydroquinone (**33**) and 1,7-bis(4-hydroxyphenyl)-4(E)-hepten-3-one (**160**) showed the strongest anti-complementary activity. The CH_{50} and AP_{50} values of **33** and **160** were 0.55 ± 0.11 mM and 0.53 ± 0.15 mM, 0.42 ± 0.15 mM and 0.66 ± 0.11 mM, respectively; compared to the positive control, heparin, the CH_{50} and AP_{50} of which were 40 μg/mL and 97 μg/mL, respectively (Jin et al. 2013). Hemolytic assays indicated that **160** blocked C1q, C2, C3, C4, C5 and C9 in the complement system, and **33** acted on C1q, C2, C3, C5 and C9 (Jin et al. 2013). The anti-complementary activity of *A. tsao-ko* extracts and the purified compounds, in particular **33** and **160**, suggests that they have the potency to be complement inhibitors.

Although most of the pharmacological activities of the extracts and compounds from Tsaoko Fructus were obtained only by in vitro experiments at present, the advanced achievements have provided certain evidences for elucidating the therapeutic mechanism. It also makes Tsaoko Fructus an expected potential health care product and medicinal source such as dietary supplements for reducing blood glucose and lipid levels or as new anti-diabetic drug candidates. Moreover, it has been demonstrated that the ethanol extract of Tsaoko Fructus showed no toxic and no-observed adverse effects in mice when fed with the extract at 2000 mg/kg/day (Park et al. 2015).

Quality control of Tsaoko Fructus as a medicinal material

Quality control of medicinal materials is of great importance to keep the clinical efficacy and safety. There is no international standard of Tsaoko Fructus at present. The current quality control of Tsaoko Fructus is based on the content of eucalyptol (**10**), besides the

Table 4 Compounds with certificated biological activities from *A. tsao-ko* fruits

Pharmacological activity	Compounds isolated and identified from *A. tsao-ko* fruits
Anti-biotic activity	Monoterpenoids (**1, 5, 10, 14**), Diterpenoids (**25**), Phenolic acids (**40–42**), Fatty acids (**191–194**)
Anti-inflammatory activity	Monoterpenoids (**9, 11–14, 17, 18**), Phenolic acids (**73, 75**), Flavonoids (**88–95, 98, 104–107**), Diarylheptanoids (**144, 148, 153, 154, 161, 170, 171**), Fatty Acids (**190**), Aliphatic alcohols (**203**)
Anti-tumor and anti-cancer activity	Monoterpenoids (**3, 14**), Phenolic acids (**57, 58, 61–63, 65, 68**), Diarylheptanoids (**144, 170, 171**), Fatty acids (**193, 194, 197**), Aliphatic alcohols (**204–206**)
Antioxidant activity	Phenolic acids (**36**, **40–42, 60**), Flavonoids (**73**, **75**), Diarylheptanoids (**144, 148, 171**), Fatty acid (**191**)
Anti-diabetic activity	Flavonoids (**71**, **110, 112, 114**), Flavanol-menthane conjugates (**115–117**, **119–122**), Flavanol-fatty alcohol hybrids (**123, 124, 128, 132–134, 140, 141**), Flavanol-monoterpenoid hybrids (**142, 143**), Diarylheptanoids (**172, 173, 175–177, 179–182, 187, 188**)
Lipid-reducing activity	Fatty acids (**191–194**)
Neuroprotective activity	Phenolic acids (**77**), Diarylheptanoids (**170, 171**)
Anti-complementary properties	Phenolic acid (**33**), Diarylheptanoid (**160**)

normal morphological detection and authentication, according to the newly published *Pharmacopoeia of the People's Republic of China (2020)*. Yet, eucalyptol (**10**) universally exists in the volatile oil of many plants, not unique to Tsaoko Fructus. More importantly, besides the anti-biotic activity, eucalyptol (**10**) has little bioactivities (Table 4), which makes it improper as the "quality" standard index. A more proper standard should be developed to focus on either *A. tsao-ko*-specific compounds such as **15, 16, 20,** and **22** (Sim et al. 2019) (Table 2) or components relevant to its clinical efficacy like **14** that has anti-inflammatory and anti-tumor activities.

Additionally, since Tsaoko Fructus has multiple pharmacological activities (Tables 3, 4) and there are a great variety of chemicals in Tsaoko Fructus (Tables 2, S4), it will be better to study the biological activities of a fraction or extract and establish a specific chemical fingerprint correlated with a certain clinical efficacy or bioactivity, instead of just focusing on one or two particular compounds.

Conclusion and perspective

The dried fruits of *A. tsao-ko* (Tsaoko Fructus) are valuable medicinal materials that have been used clinically more than one thousand years ago. Its traditional uses in treating malaria have contributed to the successful application of Tsaoko Fructus-containing prescriptions in the treatment and prevention of the current epidemic diseases, SARS and COVID-19. Nowadays, epidemic has become the greatest threat to people's health and life. Novel viruses and multiple antibiotic-resistant bacteria keep emerging, posing an unprecedented challenge to the health even life security of human. People are in urgent need of safe and effective medicines. The extracts, EO, and isolated compounds of Tsaoko Fructus exhibited a broad-spectrum inhibition against multiple microbes, which makes it a potential source of safe and natural antibiotics, especially in an era that pathogens have become the greatest enemy to us.

Pharmacological studies of the extracts, EO, and isolated compounds of Tsaoko Fructus provided a certain basis for its mechanism of medicinal function and potential application. The inhibition activities on a broad-spectrum of microorganism of Tsaoko Fructus may account for its suppression of many pathogen-related diseases such as malaria, diarrhea, throat infections, pathogen induced fever and other pathogen infections. The relief of abdominal pain and the elimination of phlegm is possible related to its anti-inflammatory activities. The anti-diabetic and lipid-reducing activities of *A. tsao-ko* make it a potential resource to prevent or relieve age or life-style related diseases such as diabetes, obesity and hypertension.

Behind its various biological activities lies an abundant number of phytochemicals. At least 209 components have been separated and identified including terpenoids, flavonoids, aromatic compounds and a diversity of simple organic molecules, some of which have already been tested for their bioactivities.

However, there still exist some research gaps to date. Firstly, current research has revealed many

biological activities of Tsaoko Fructus, but those bioactivities haven't been well linked to its medical usage. More study on the relationship between the bioactivity and medical uses should be done in the future. Investigation at molecular and cellular levels and in vivo experiments are expected to reveal what exactly are functioning as key components and how they work, considering the bioavailability of the compounds. This can subsequently help us better understand the mechanism to treat disease and make better use of this medicinal material. Secondly, in many phytochemical research, there exist some unidentified signals in the results of LC–MS or GC–MS profiles, which might be due to instrumental accuracy and precision, or some compounds that were not listed in the current database. This can be a point to dig into, which may help to discover some new compounds with biological activities. At last, according to *Pharmacopoeia of the People's Republic of China (2020)*, a more appropriate quality control system on the basis of the unique components and more relevant to its pharmacological activity or clinical efficacy should be developed for Tsaoko Fructus. Using a specific chemical feature related with a certain pharmacological activity or clinical efficacy may be more rational, because there are a great number of compounds in Tsaoko Fructus and it has multiple bioactivities and clinical applications.

In conclusion, Tsaoko Fructus has a long historical clinical use to treat a number of disorders. The present studies revealed that it contains hundreds of compounds and has multiple biological activities. These achievements indicate a bright future of Tsaoko Fructus as a natural source of next-generation medications, and also lay foundation for further elucidating the therapeutic mechanism, and revealing the relationship between clinical usage, chemical composition and pharmacological activity of Tsaoko Fructus in the future.

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Declarations

Conflict of interest The authors have no conflicts of interest.

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