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# Phytochemical and chemotaxonomic studies on the twigs of *Cinnamomum cassia* (Lauraceae)

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ABSTRACT

A phytochemical investigation on the twigs of *Cinnamomum cassia* led to the isolation of 39 compounds, including 12 flavonoid glycosides (**1**−**12**), three cinnamic acid amides (**13**–**15**), 12 lignans (**16**–**27**), five sesquiterpenoids (**28**–**32**), three cinnamaldehyde derivatives (**33**–**35**), two phenols (**36** and **37**), and two indole derivatives (**38** and **39**). Their structures were elucidated on the basis of spectroscopic data as well as by comparison with the reported spectroscopic data. Among them, 32 compounds (**1**–**17**, **19**, **21**, **24**–**29**, **31**, **32**, and **35**–**39**) are reported from this plant for the first time, while 23 compounds (**1**–**8**, **12**, **13**, **17**, **19**, **25**–**27**, **29**, **31**, **32**, and **35**–**39**) and ten compounds (**2**–**6**, **8**, **26**, **27**, **32**, and **38**) were isolated from the genus *Cinnamomum* and the family Lauraceae for the first time, respectively. The chemotaxonomic significance of these compounds was summarized.

## 1. Subject and resource

The genus *Cinnamomum* belongs to the family Lauraceae and comprises about 250 species. It is mainly distributed in tropical and subtropical Asia, Australia, and the Pacifica islands (Wei et al., 2017). There are 46 *Cinnamomum* species grown in China, and several of these species have been commonly used in folk medicine to treat amenorrhea, cardiac palpitations, edema, rheumatoid arthritis and tussis (Zeng et al., 2014).

Ramulus Cinnamomi, the dried twigs of *Cinnamomum cassia*, is widely cultivated in Southern China and used as traditional Chinese medicines for treating dyspepsia, gastritis, blood circulation disturbances, and inflammatory diseases (Chaudhry and Tariq, 2006). The dried twigs of *C. cassia* were collected from Yulin, Guangxi Province of China, in January 2016, and authenticated by one of the authors (G.-Y. Zhu). The voucher specimen (CC-201601) was deposited in the State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology.

## 2. Previous work

Previous phytochemical investigations on *C. cassia* led to the isolation of various type of natural products such as sesquiterpenoids (Guoruoluo et al., 2017), diterpenoids (Zhou et al., 2017), proanthocyanidins (Killday et al., 2011), geranylphenylacetate glycosides (Liao et al., 2009), cinnamic aldehyde derivatives (Liu et al., 2009), phenylpropanoids (Li et al., 2013; Liu et al., 2018), phenols (Shin et al., 2017), phenolic glycosides (Tanaka et al., 1989), lignans (Liu et al., 2018), butyrolactones (He et al., 2016; Kim et al., 2017; Liu et al., 2018), phenylmethanol glycosides (Guoruoluo et al., 2018), flavonoids (Liu et al., 2018), and polysaccharides (Kanari et al., 1989; Zhao et al., 2013). However, chemotaxonomic studies on *C. cassia* are rarely reported.

## 3. Present study

The dried twigs of *C. cassia* (5.0 kg) were powdered and extracted with 80% EtOH four times (4 × 10 L) under reflux. After filtration, combination and evaporation, the residues were suspended in water, and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract (102.6 g) was subjected to a silica gel column chromatography (CC) eluted with a gradient of increasing acetone (0–100%) in petroleum ether to afford six fractions (A–F).

Fraction B (3.5 g) was subjected to a reverse-phase (RP) $C_{18}$ column eluted with MeOH–$H_2O$ (30:70 → 100:0, v/v) and further purified by semipreparative HPLC eluted with MeCN–$H_2O$ to yield compounds **24**

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(2.5 mg), **32** (2.5 mg), **33** (5.0 mg), **34** (5.0 mg), **37** (2.5 mg), **38** (2.0 mg), and **39** (2.5 mg). Compounds **21** (2.5 mg), **28** (1.5 mg), **29** (1.0 mg), **30** (1.5 mg), **31** (1.5 mg), **35** (2.0 mg), and **36** (2.5 mg) were obtained from fraction C (2.9 g) using the similar separation processes. Fraction E (13.6 g) was subjected to CC over RP $C_{18}$ column eluted with MeOH–$H_2O$ (20:80 → 100:0, v/v), and further purified by semi-preparative HPLC eluted with MeCN–$H_2O$ to yield compounds **1** (4.0 mg), **2** (2.5 mg), **3** (3.5 mg), **6** (150.0 mg), **7** (2.7 mg), **8** (2.0 mg), **14** (2.0 mg), **15** (2.0 mg), **17** (1.5 mg), **18** (2.5 mg), **19** (2.0 mg), **20** (1.0 mg), **23** (3.5 mg), and **27** (1.5 mg). Fraction F (7.3 g) was separated in a similar manner to fraction E to afford compounds **9** (3.5 mg), **10** (4.5 mg), **11** (3.5 mg), and **12** (1.5 mg). Fraction D (7.6 g) was subjected on a RP $C_{18}$ column eluted with MeOH–$H_2O$ as the gradient solvent, and then was purified by semipreparative HPLC to afford compounds **4** (1.0 mg), **5** (2.5 mg), **13** (3.5 mg), **16** (4.0 mg), **22** (250.0 mg), **25** (1.5 mg), and **26** (2.0 mg).

The structures of the isolated compounds were identified by spectroscopic data and by comparison with those published data. Their chemical structures were elucidated as quercetin 3-*O*-(3″,4″-di-*trans*-*p*-coumaroyl)-α-L-rhamnopyranoside (**1**) (Jagan Mohan Rao et al., 2002), quercetin 3-*O*-(2″,4″-di- *trans*-*p*-coumaroyl)-α-L-rhamnopyranoside (**2**) (Jagan Mohan Rao et al., 2002), 3″-*trans*-*p*-coumaroylquercitrin (**3**) (Yahagi et al., 2012), 4″-*trans*-*p*-coumaroyl-kaempferol-3-*O*-α-L-rhamnoside (**4**) (Feng et al., 2013), 4″-*cis*-*p*-coumaroyl-kaempferol-3-*O*-α-L-rhamnoside (**5**) (Feng et al., 2013), kaempferol 3-*O*-(3″,6″-di-*trans*-*p*-coumaroyl)-ß-D-glucopyranoside (**6**) (Zhong et al., 2009), tiliroside (**7**) (Lee et al., 2005), kaempferol 3-*O*-(3″,6″-di-*trans*-*p*-coumaroyl)-ß-D-galactopyranoside (**8**) (Vega et al., 2007), kaempferol 3-*O*-ß-D-glucopyranoside (**9**) (Wang et al., 2014b), quercetin 3-*O*-ß-D-glucopyranoside (**10**) (Wang et al., 2014b), quercetin 3-*O*-α-L-rhamnopyranoside (**11**) (Liao et al., 2012), quercetin 3-*O*-α-D-arabinopyranoside (**12**) (De Almeida et al., 1998), (*E*)-3-(4-hydroxy-3-methoxyphenyl)-*N*-phenthylacrylamide (**13**) (Shi et al., 2013), *N*-*trans*-feruloylmethoxytyramine (**14**) (Hong et al., 2011), *N*-*cis*-feruloylmethoxytyramine (**15**) (Hong et al., 2011), secoisolariciresinol (**16**) (Chen et al., 2010), 5,5′-dimethoxysecoisolariciresinol (**17**) (Yi et al., 2015), isolariciresinol (**18**) (Liu et al., 2015), 5-methoxy-isolariciresinol (**19**) (Zhu et al., 2013), lyoniresinol (**20**) (Wang et al., 2014a), pinoresinol (**21**) (Subehan et al., 2008), 5-medioresniol (**22**) (Zhao et al., 2013), syringaresinol (**23**) (Zhao et al., 2013), yangambin (**24**) (Li et al., 2015), 4-ketopinoresinol (**25**) (Mi et al., 2013), ficusesquilignan A (**26**) (Li and Kuo, 2000), buddlenol C (**27**) (Liu et al., 2011), blumenol A (**28**) (Jing et al., 2014), dehydrovomifoliol (**29**) (Zan et al., 2016), grasshopper ketone (**30**) (Kuang et al., 2008), boscialin (**31**) (Lu et al., 2014), 1-(3-indolyl)-2,3-dihydroxypropan-1-one (**32**) (Cao et al., 2011), cinnamaldehyde (**33**) (Ragasa et al., 2013), sinapaldehyde (**34**) (Subehan et al., 2008), *trans*-3,4,5-trimethoxycinnamic alcohol (**35**) (Yang et al., 2010), 3,4-dihydroxybenzoate (**36**) (Shi et al., 2014), ethyl 3,4-dihydroxybenzoate (**37**) (Gao et al., 2009), 3-glyceroylindole (**38**) (Dramae et al., 2013), and indole-3-carboxaldehyde (**39**) (Chang et al., 2014) (Figs. 1 and 2).

## 4. Chemotaxonomic significance

This phytochemical study on the twigs of *C. cassia* resulted in the isolation and identification of 39 compounds including 12 flavonoid glycosides (**1**−**12**), three phenolic amides (**13**–**15**), 12 lignans (**16**–**27**), five sesquiterpenoids (**28**–**32**), three cinnamaldehyde derivatives (**33**–**35**), two phenols (**36** and **37**), and two indole derivatives (**38** and **39**). To the best of our knowledge, 32 compounds (**1**–**17**, **19**, **21**, **24**–**29**, **31**, **32**, and **35**–**39**) are reported from this plant for the first time, while 23 compounds (**1**–**8**, **12**, **13**, **17**, **19**, **25**–**27**, **29**, **31**, **32**, and **35**–**39**) were isolated firstly from the genus *Cinnamomum*. Compounds **2**–**6**, **8**, **26**, **27**, **32**, and **38** were isolated from the family Lauraceae for the first time.

Among eight flavonol *p*-coumaric acylglycosides (**1**–**8**), compounds **2**–**6** and **8** were isolated from the Lauraceae family for the first time. Compounds **1** and **7** were previously reported from *Litsea glutinosa* and *L. japonica* (Lauraceae) (Xu et al., 2016; Lee et al., 2005), respectively, indicating that similar metabolic pathways for flavonol acylglycosides exist in these species of the Lauraceae family. It is worth mentioning that compounds **1**, **6**, and **8** were previously obtained from plants of the Pinaceae family, such as *Abies fabri*, *A. delavayl*, and *Pinus sylvesris* (Li et al., 2015; Yang et al., 2014; Ivanova et al., 1978). The presence of these compounds suggested that a close chemotaxonomic relationship existed in these two families. These specific flavonol *p*-coumaric acyglycosides isolated from the *C. cassia* may serve as new potential chemotaxonomic markers for *C. cassia*.

Flavonoid glycosides are common secondary metabolites in many plant species. Four flavonoid glycosides (**9**−**12**) were isolated from *C. cassia* for the first time. Compounds **9**, **10**, and **11** have been reported from *C. camphora* and *C. parthenoxylon*, which confirms the chemotaxonomic relationship between *C. camphora*, *C. parthenoxylon*, and *C. cassia* (Wang et al., 2014b; Liao et al., 2012; Wei et al., 2017). Compound **12** has been previously found in *Cryptocarya mandioccana* (de Moraes et al., 2007), a species belonging to the Lauraceae family, therefore there may be phylogenetic associations between these Lauraceae species. Although three phenolic amides (**13**–**15**) were isolated from *C. cassia* for the first time, this type of cinnamic acid amides have also been found in the genera *Lindera* (Wei et al., 2016), *Litsea* (Tanaka et al., 2009), *Cinnamomum* (Hong et al., 2011), and *Neolitsea* (Kim et al., 2013) of the Lauraceae family. These compounds expanded the chemical diversity of *C. cassia*.

Compared to flavonoid glycosides, lignans show a more taxonomically interesting pattern. Twelve lignans including two simple lignans (**16** and **17**), three cyclolignans (**18**–**20**), and seven bisepoxylignans (**21**–**27**) were obtained from *C. cassia*. Among them, compounds **17**, **19**, **25**, **26**, and **27** have not been found in any species belonging to the genus *Cinnamomum*. Compound **16** was previously isolated from *C. osmophloeum* (Chen et al., 2010), and compounds **21** and **24** were recorded from *C. burmanni* and *C. macrostemon*, respectively (Subehan et al., 2008; Li et al., 2015). The presence of these types of lignans supports the taxonomic position of the genus *Cinnamomum* in the Lauraceae family. Interestingly, 4-ketopinoresinol (**25**) was isolated from *L. chinpingensis* (Yang et al., 2009). The presence of compounds **1**, **7**, **25** and the cinnamic acid amides in both *Cinnamomum* and *Litsea* genus suggested that there may be a close relationship between the two genera. Two bisepoxylignans combined with a phenylpropanoid unit (**26** and **27**) were also firstly isolated from the *Cinnamomum* genus, which can be used to differentiate *C. cassia* from the other species in this genus.

Sesquiterpenoids are the characteristic metabolites of the genus *Cinnamomum*. Three megastigmane sesquiterpenoids (**28**, **29**, and **31**) have never reported from any species of the genus *Cinnamomum*. Several megastigmane sesquiterpenoids have been described in other species of the genus *Cinnamomum* including *C. subavenium*, *C. wilsonii* and *C. burmannii*, and this may indicate a close relationship between these species (Hao et al., 2015; Shu et al., 2013; Subehan et al., 2008). It is worth noting that compounds **28** and **29** have been isolated from *L. glutinosa* and *L. sessilis*, respectively (Xu et al., 2016; Chung et al., 2009), and these similarities in chemical constituents may further support the close relationship between the *Cinnamomum* and *Litsea* genera. Compound **32** is the first example of bicyclic sesquiterpenoids from the Lauraceae species and may be used as a new reference marker for the chemical taxonomy of *C. cassia*. Cinnamaldehyde derivatives are primary components of *C. cassia*, and are common in other species of the Lauraceae family. Compounds **34** and **35** were isolated previously from *C. burmannii* and *L. cubeba*, respectively (Subehan et al., 2008; Yang et al., 2010). Moreover, the occurrence of compound **35** in the genus *Litsea* and the genus *Cinnamomum* is in agreement with the speculation that taxonomically *Litsea* and *Cinnamomum* are closely related.

Two phenols (**36** and **37**) and two indole derivatives (**38** and **39**) were also obtained from the genus *Cinnamomum* for the first time.