

**CHALCONE CONTENT IN THE ETHANOL EXTRACT OF *Angelica keiskei*
LEAVES
BY SPECTROPHOTOMETRIC METHOD**

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ABSTRACT

Angelica keiskei Koidzumi, known as ashitaba or Japanese celery, has been the focus of interest in Asia. This plant has been reported to possess antituberculosis activity against Mtb H37Rv and antioxidant activity equals to vitamin E. It was predicted that those activities were caused by the plant's secondary metabolites, e.g. flavonoids and polyphenols. Xanthoangelol (XAG) and 4-hydroxyderricin (4-HD), two chalcones or chalconoids (flavonoids with an open C-ring), are contained in the stems of *A. keiskei*. This work was aimed to determine the chalcone level, calculated as XAG, in the leaves of *A. keiskei* planted in Mount Rinjani, Lombok island, Indonesia. The plant was taxonomically identified at Herbarium Bandungense, Bandung Institute of Technology, Indonesia. The leaves were extracted using ethanol 70% and were screened for its phytochemical contents. The determination of the chalcones was carried out by employing standard addition UV spectrophotometric method. Results indicated that phenols, flavonoids, alkaloids, tannins and saponins were contained in both the dried leaves and the leaves extract. The chalcones, calculated as XAG, were 1.959% w/w.

Keywords: antioxidant, ashitaba, chalcone, xanthoangelol

INTRODUCTION

Angelica keskei Koidzumi, which belongs to Asian celery family (Endang and Lingganingrum, 2018), contains chalcones, flavanones, and coumarins (Caesar and Cech, 2016). Xanthoangelol (XAG) and 4-hydroxyderricin (4-HD) are two chalcones contained in this plant (Adinata et al., 2012; Bektur et al., 2013). Chalcones are able to increase the production of red blood cells, growth hormones, and furthermore, these compounds could increase the body's defense against infectious diseases (Made, Wayan and Budiasa, 2003). Chalcones have been proven in exhibiting various pharmacological activities, such as antiinflammatory, antibacterial, analgesic, antiplatelet, anticancer, and antioxidant properties (Shahid and Subhan, 2014). 4-HD isolated from the sap of this plant, could inhibit dipeptidyl peptidase-IV by building hydrogen bonds with Glu206 and Phe357 in the binding pocket of the enzyme (Aulifa et al., 2019). Prenylated chalcones, 22 of them, were reported present abundantly either in the root bark or the stem of *A. keskei* (Caesar and Cech, 2016), however, their presence in the leaves had not been explored. This work was aimed to determine the chalcone level in the ethanol extract of *Angelica keskei* Koidzumi leaves planted in Mount Rinjani, Lombok island, Indonesia.

METHODS

Instrument. Instruments used in this work were micropipets (Accumax, Lab Technology), cuvettes (Quartz SUPRASIL®), Genesys 10S UV-visible spectrophotometer (Thermo).

Plant materials. *A. keskei*. was obtained from Mount Rinjani, Lombok, Indonesia. Taxonomic determination was conducted by a certified botanist at the Herbarium Bandungense, Bandung Institute of Technology (ITB).

Chemicals. Ethanol 70% and other chemicals used in this work were purchased from Brataco, Bandung. Xanthoangelol was isolated from the sap of *A. keskei* and purified by Dr. Diah Lia Aulifa at Bandung Institute of Technology.

Extract Preparation

A. keskei leaves (Figure 1) were washed under tap water, sundried, ground, and sieved to mesh-60 powder. 50 g of *A. keskei* leaves powder was put into the macerator, soaked using 70% ethanol, allowed to stand at room temperature (± 26 °C) for 3 x 24 hours, and filtered. The filtrates were collected and the solvent was rotary-vaporated at 60 °C 85 rpm until a viscous extract was obtained. The viscous extract was evaporated in a water-bath at 60 °C.

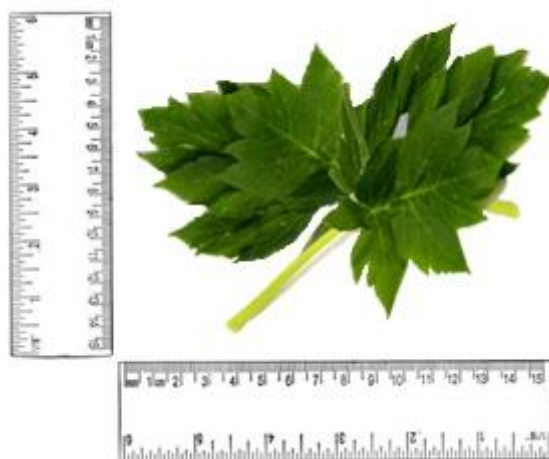


Figure 1. The leaves of *Angelica keskei*

Phytochemical Screening

Phytochemical screening of the dried leaves and the extract was carried out according to Tiwari et al. (2011) method (Tiwari et al., 2011).

Determination of chalcones by using standard addition UV spectrophotometric method

The standard addition method is a method in which the sample to be analyzed is added to a standard solution whose concentration is known to minimize errors caused by various matrices. The determination of chalcone in the leaves extract of *A. keiskei* was initiated by weighing 30 mg of the extract and dissolving it in 25 ml of ethanol (1200 µg/ml). XAG was accurately weighed at 2 mg and dissolved in 10 ml of ethanol (200 µg/ml). The XAG solution in ethanol was scanned at 200 to 800 nm against ethanol in a UV-visible spectrophotometry (Figure 2).

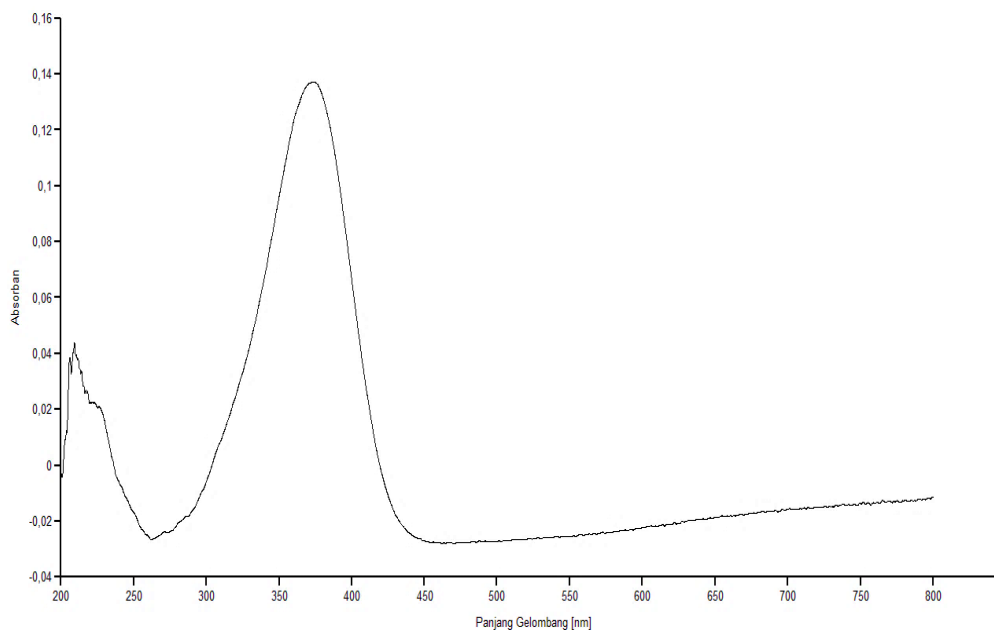


Figure 2. The absorption spectrum of xanthoangelol in ethanol (isolated from *A. keiskei* sap)

Meanwhile, various solutions were prepared as provided in Table 1 and were measured their absorbance at 369.7 nm (the λ_{max} of XAG). A standard addition curve was obtained by plotting the absorbance of the solutions against the concentration (Figure 3).

Table 1. XAG-standard addition series of the extract

No.	Extract (mL)	XAG (mL)	Ethanol added (mL)	Final concentration of XAG standard (µg/mL)
1	1.0	0.0	4.0	0.0
2	1.0	0.1	3.9	4.0
3	1.0	0.2	3.8	8.0
4	1.0	0.3	3.7	12.0
5	1.0	0.6	3.4	24.0

RESULT AND DISCUSSION

The yield of the viscous extract was 22.764%, compared to that previously obtained by Sembiring and Manoi (5.75%) and Wirasisya et al. (17.52%) (Sembiring and Manoi, 2011; Wirasisya et al., 2018). Furthermore, the phytochemical screening indicated the presence of phenols, flavonoids, alkaloids, tannins and saponins in both the dried leaves and the leaves extract.

Another work reported that the stem extract of *A. keiskei* contained secondary metabolites e.g. flavonoid, polyphenol, tannin, monoterpenoid and sesquiterpen, quinon, and saponin (Kusuma et al.,

2018). Our result confirmed that ethanol is a universal solvent that attracts polar plant metabolites such as tannins, phenols, alkaloids, flavonoids, and saponins.

The standard addition curve ($y = 0.0296x + 0.4144$; $R^2 = 0.9975$) is provided in Figure 3. The concentration of total chalcones calculated as XAG was 1.959%.

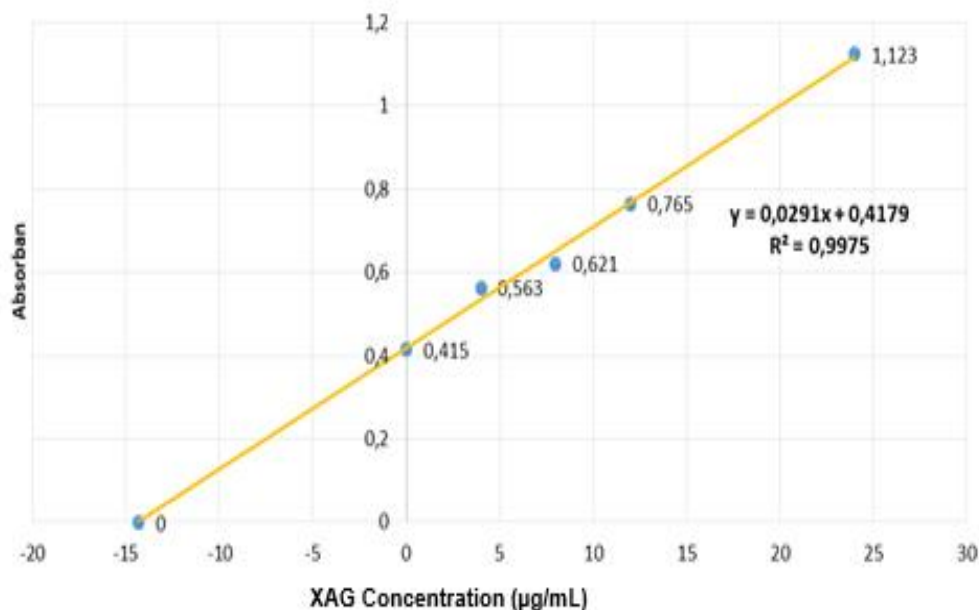


Figure 3. Standard addition curve of the ethanol extract of *A. keiskei* leaves

CONCLUSION

The ethanol extract of *Angelica keiskei* leaves planted at Mount Rinjani, Lombok island, Indonesia, positively contains chalcones, which concentration, calculated as xantoangelol, is 1.959%. This work could be beneficial to add scientific knowledge about the leaves of *A. keiskei* which, at present, is still limited. The leaves of this plant are interesting to be further explored for its pharmacological activity.

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