Tropical Journal of Pharmaceutical Research January 2017; 16 (1): 161-164
**ISSN:** 1596-5996 (print); 1596-9827 (electronic)


Available online at http://www.tjpr.org
**http://dx.doi.org/10.4314/tjpr.v16i1.21**

**Original Research Article**

# Identification of active anti-inflammatory principles of beta-beta wood (Lunasia amara Blanco) from Siawung Barru-South Sulawesi, Indonesia

**Hasnaeni[1]*, Sudarsono[2], Arief Nurrochmad[3] and Sitarina Widyarini[4]**
*[1]Faculty of Pharmacy, Universitas Muslim Indonesia, Urip Sumoharjo Makassar, 90231, [2]Department of Pharmaceutical Biology, [3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, UGM, Sekip Utara, [4]Department of Pathology Anatomy, Faculty of Veterinary Medicine, UGM, Karangmalang Yogyakarta, Indonesia-55281*

**For correspondence: **Email:** hasnaeniyahya@yahoo.co.id; **Tel:** +6285255714072*

*Received: 4 May 2016* *Revised accepted: 10 December 2016*

## Abstract

***Purpose:** To identify the anti-inflammatory components of beta-beta (Lunasia amara Blanco.) wood.*
***Methods:** The wood material was extracted with 96 % ethanol and fractionated with dichloromethane using a liquid-liquid continuous extraction (LLCE). The fractions were subjected to silica gel column chromatography. Components of the extracts were identified by thin layer chromatography (TLC) scanner and UV-visible spectroscopy, using scopoletin as standard.*
***Results:** TLC results for Lunasia amara extract showed the same spot as standard scopoletin. UV-visible spectrum for scopoletin displayed maximum absorption at 213, 228, 255 and 344 nm, while beta-beta wood extract showed characteristic bands at 344, 336, 299 and 255 nm. The results indicate that the main components of the extracts are scopoletin and its derivatives.*
***Conclusion:** The active anti-inflammatory compound in beta-beta (Lunasia amara) wood is scopoletin.*

***Keywords:** Beta-beta wood (Lunasia amara Blanco.), Scopoletin, Thin layer chromatography*

**Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts**

## INTRODUCTION

Beta-beta wood is the general name for plants of the genus *Lunasia,* and family Rutaceae. The family Rutaceae contains essential oils, alkaloids, amides, coumarin, flavonoids, benzoic acid, tannins, lignin, phenolics, terpenes, pentacyclic saponins, carbohydrates, scopilatin and mucin [1].

Scopoletin (Figure 1) is known to have anti-inflammatory properties. It is derived from coumarin, a phenylpropane compound [2].

Research on the anti-inflammatory activity of scopoletin has revealed that it inhibits cyclooxygenase and suppresses pro-inflammatory Tumor Necrosis Factor alpha (TNF-α) and Interleukin (IL) - 1β [3,4]. Compounds with basic coumarin skeleton ring structure reduce trauma or disease-induced tissue inflammation due to their antioxidant, anti-inflammatory and immunosuppressive activities [5].

The present study focuses on the anti-inflammatory potential of beta-beta timber extracts, and identification of the bioactive compounds via TLC Scanner and UV-visible spectroscopy.

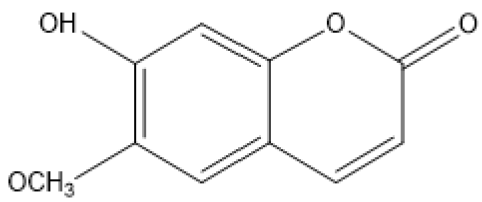

**Figure 1:** Chemical structure of scopoletin (7 – hydroxyl – 6 - methoxy coumarin)

## EXPERIMENTAL

### Materials

Beta-beta wood (*Lunasia amara* Blanco.) was collected at Siawung, South Sulawesi province of Indonesia in March 2014. Taxonomic identification of the plant was performed by Drs. Joko Santoso. College of Biology Pharmacy Faculty of Pharmacy, Univesitas Gadjah Mada, Indonesia. A voucher specimen (no. BF/204/Ident/Det/V/2014) was deposited at the herbarium of Department of Pharmaceutical Biology Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia for future reference.

### Extraction and isolation of compounds

Beta-beta (*Lunasia amara* Blanco.) wood powder (1 kg) was extracted by maceration at room temperature with 96 % ethanol for 72 h. The maceration process was repeated three times with the same solvent. The extract was collected and evaporated in a vacuum evaporator to obtain a dense residue which was dried and weighed. The dried ethanol extract was dissolved in distilled water and fractionated with dichloromethane using a continuous liquid-liquid extraction (LLCE). Fractionation was done repeatedly until the last fraction was clear. Each fraction obtained was concentrated and weighed, and then subjected to gravitational column chromatography (GCC) using silica gel G 60 (0.04 to 0.83 mm) with dichloromethane: ethyl acetate (97:3, v/v) as eluent. KOH (10 %) reagent was used to observe the spots, as well as UV absorption at 366 nm and 254 nm. Fractions with similar $R_f$ values were combined. The fractions were identified by UV-visible spectroscopy and assayed using a TLC scanner. The levels of scopoletin in the ethanol extract and fractions were determined from a scopoletin standard curve. The fractions from column chromatography were identified by UV-Visible spectroscopy in the wavelength range of 200 - 400 nm. Identification of fractions was also done by "spiking". Equal volumes and equal concentrations (0.25 mg/mL) of fractions from column chromatography, and scopoletin were spotted on TLC plates, and after development, absorption spectra were obtained. The area under curve (AUC) was measured for scopoletin standard, and for fractions spiked with scopoletin, by densitometry. The AUCs were then compared.

## RESULTS

### Yield and identity of chemical components

The yield of the crude extract was 1.54 %. Figure 2 shows the TLC chromatogram of the crude extract and its solvent fractions. Figure 3 and Table 1 show the UV-Visible spectra of the crude extract and scopoletin. From these results, the main chemical component of *Lunasia amara* was identified as scopoletin.

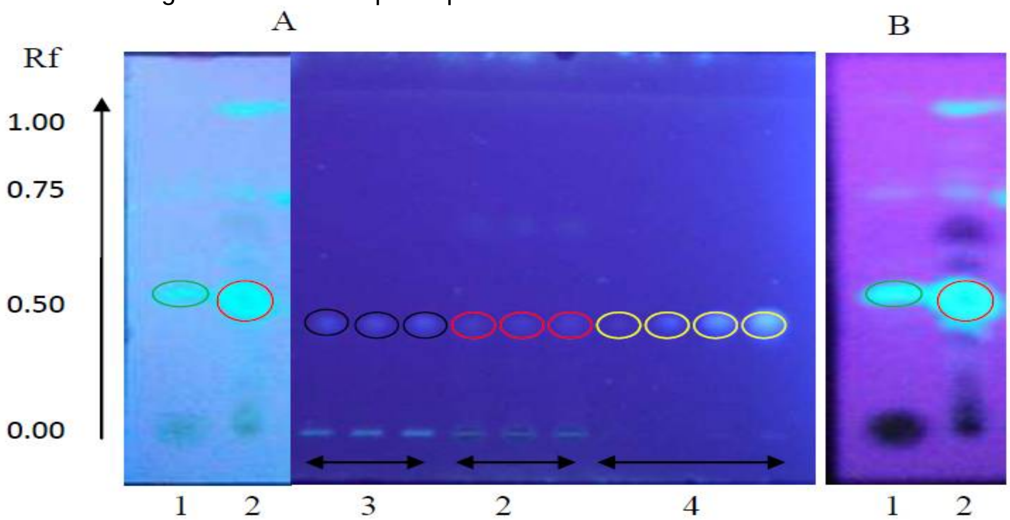


**Figure 2:** Thin-layer chromatogram: (1) Ethanol extract; (2) Dichloromethane fraction; (3) Beta-beta wood isolate; (4) Scopoletin standard; A: Observation at UV 366 nm; B: Spots seen when sprayed with KOH 10 %

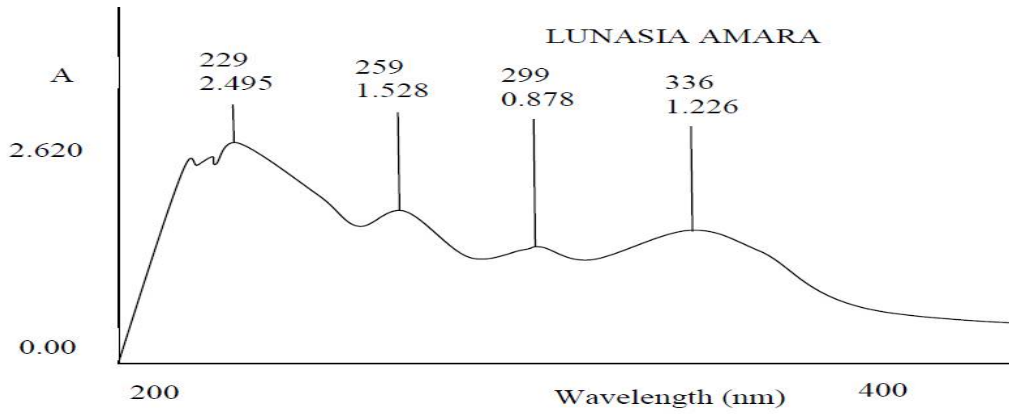


**Figure 3:** UV-Vis spectra of Beta-beta wood extract fractions

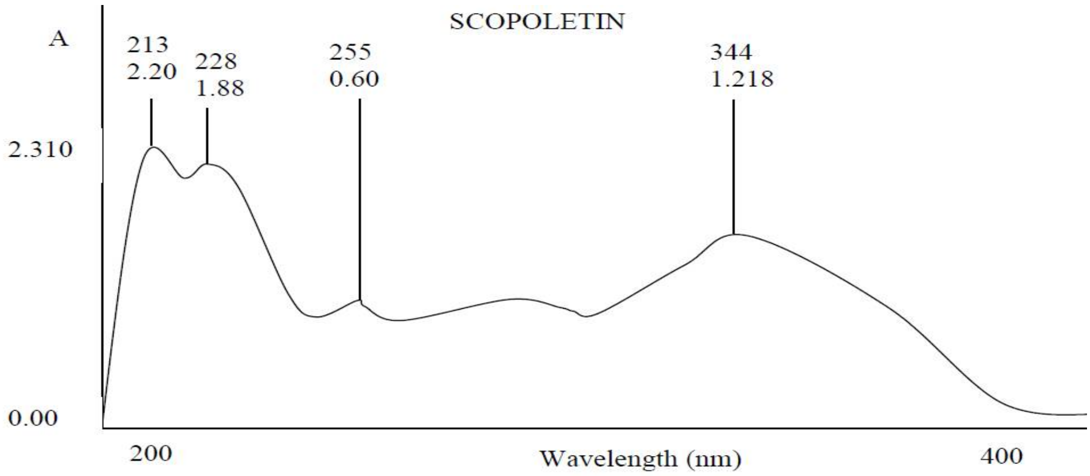


**Figure 4:** UV-Vis spectra of scopoletin standard

**Table 1:** UV-Vis spectra of beta-beta wood extract fractions and standard scopoletin

| Sample | Wavelength (nm) | Absorption |
|---|---|---|
| Scopoletin standard | 344 | 1.218 |
| | 255 | 0.609 |
| | 228 | 1.887 |
| | 213 | 2.204 |
| Extract fraction | 336 | 1.226 |
| | 299 | 0.879 |
| | 259 | 1.528 |
| | 229 | 2.459 |

Scopoletin compounds absorb maximally at wavelengths of 231 nm, 255 nm, 299 nm, 340 nm and 347 nm [6]. Results from UV-visible spectroscopy for scopoletin indicate maximum absorption at 213, 228, 255 and 344 nm, while beta-beta wood extract and scopoletin standard exhibited characteristic bands at 344 nm and 336 nm, 299 nm, 255 and 259 nm, 228 nm, 229 nm and 213 nm, respectively. This strongly suggests that the beta-beta wood extract and its fractions consist of scopoletin compounds.

## DISCUSSION

Chromatograms of 96 % ethanol extract and dichloromethane fractions produced prominent and intense blue spots after spraying with 10 % KOH. The appearance of dominant blue spots in chromatograms is used to identify scopoletin compounds. Scopoletin is a coumarin derivative present in plants with anti-inflammatory potential. Values of AUC increased when scopletin was added to the fractions. This suggests that the fractions are similar in identity to standard scopoletin. The fractions and crude extract also had similar spectra and $R_f$ values with scopoletin. Thus the anti-inflammatory property of beta-beta wood (*Lunasia amara*) is due its content of scopoletin. Numerous studies have demonstrated the anti-inflammatory potential of scopoletin and scopoletin compounds [7,9].

## CONCLUSION

Based on the results of TLC and UV-visible spectroscopy, it seems that the active anti-inflammatory principles in beta-beta wood

(*Lunasia amara* Blanco) are scopoletin compounds.

## DECLARATIONS

### *Acknowledgement*

The authors are thankful to Directorate General of Higher Education, Ministry of Research Technology and Higher Education for provision of BPPDN scholarship. They are also grateful to Messrs. Yahya and Baharuddin who assisted with the collection of samples of beta-beta wood (*Lunasia amara*).

### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

### *Open Access*



## REFERENCES

1. *Hegnauer R. Chemotaxonomi der Planzen, edisi V. Birkkhauser Verlag. Basel and Stuttgart. 1969.*
2. *Ding Z, Dai Y, Hao H, Pan R, Yao X, Wang Z. Anti-Inflammatory Effects of Scopoletin and Underlying Mechanisms. Pharmaceutical Biology. 2008; 46(12): 854–860.*
3. *Sommerfelt RM, Feuerherm AJ, Jones K, Johansen B. Cytosolic Phospholipase A2 Regulates TNF-Induced Production of Joint Destructive Effectors in Synoviocytes. PLoS ONE. 2013: 8-12.*
4. *Kim HJ, Jang SI, Kim YJ, Chung HT, Yun Y.G, Kang TH, Jeong OK, Kim YC. Scopoletin suppresses pro-inflammatory cytokines and PGE2 from LPS-stimulated cell line, RAW 264.7 cells. Fitoterapia. 2004; 75(3-4): 261–266.*
5. *Iaroshenko V.O, Erben F, Mkrtchyan S, Hakobya A, Vilches-Herrera M, Dudkin S, Bunescu A, Villinger A, Sosnovskikh V, Langer P. 4–Chloro–3-(trifluoroacetyl) and 4– chloro–3-(methoxalyl) coumarins as novel and efficient building blocks for the regioselective synthesis of 3,4-fused coumarins. Tetrahedron. 2011; 67(41): 7946–7955.*
6. *Budavari S. The Marck Index, twelfth edition. Merck Research Laboratories Division of Merck & Co.Inc. New Jersey 07065; p 1445*
7. *Mogana R, Teng-Jin K, Wiart C. Anti-inflammatory, anticholinesterase, and antioxidant potential of scopoletin isolated from Canarium patentinervium Miq. (Burseraceae Kunth). Evid Based Complement Alternat Med. 2013; 2013: 1-7.*
8. *Moon PD, Lee BH, Jeong, HJ, An HJ, Park SJ, Kim HR, Ko SG, Um JY, Hong SH, Kim HM. Use of scopoletin to inhibit the production of inflammatory cytokines through inhibition of the IκB/NF-κB signal cascade in the human mast cell line HMC-1. Eur J Pharmacol. 2007; 555: 218–225.*
9. *Dou Y, Tong B, Wei Z, Li Y, Xia Y, Dai Y. Scopoletin suppresses IL-6 production from fibroblast-like synoviocytes of adjuvant arthritis rats induced by IL-1β stimulation. Int Immunopharmacol. 2013; 17: 1037–1043.*