A Cytotoxic Flavanone from The Pod Peels of Tephrosia vogelii Hook.f.

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Abstract

Tephrosia vogelii Hook.f. is a species of the family Fabaceae (Leguminosae). These plants are termed "Polong-polongan" in Indonesia, and are known to contain active flavonoid groups. Previous studies have shown the isolation of one known flavanone: isolonchocarpin from methanol extract, and the structure obtained was established based on chemical evidence as well as spectroscopic methods, including NMR, and also by a comparison with published data. This research is aimed at evaluating the cytotoxic property of methanol extract against larvae of Arthemia salina Leach, using the Brine Shrimp Lethality Test (BSLT) method. The results show potent cytotoxicity at LC₅₀ of 41.40 ppm.

Keyword: Arthemia salina Leach., cytotoxic activity, Fabaceae, flavanone, Tephrosia vogelii Hook.f.

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1. INTRODUCTION

Tephrosia is one of the 400 species in family Fabaceae, which belongs to the sub-family Papilionoideae, comprising shrubs, herbs and trees (Polhill, et al., 1981), distributed across the tropics and sub-tropics (Tarus, et al., 2002). Previous studies on the intrinsic chemical constituents showed the occurrence of chalcones (Andrei, et al., 2000; Chang, et al., 2000; Gomez-Garibay, et al., 2002; Tarus, et al., 2002), flavanones (Chang, et al., 2000; Jang, et al., 2003; Kishore, et al., 2003), flavones (Prabhakar, et al., 1996; Achmad, et al., 2007), isoflavones (Yenesew, et al., 1989; Dagne, et al., 1992; Chang, et al., 2000), rotenoid (Prashant, et al., 1993; Andrei, et al., 1997; Jang, et al., 2003). Table 1 and Figure 1 demonstrate several highly diverse phytochemical compounds present, as generally observed in most other similar genera, including Cassia from Fabaceae (Kusumaningtyas, et al., 2020).

The phytochemical and pharmacological evaluations showed cytotoxic potentials in several compounds (Roy, et al., 1986; Ganapaty, et al., 2009). Touqeer, et al. (2013) performed similar research and reported various activities from several isolates, including cytotoxicity, as observed in Table 2 (Padmapriya, 2017). This current investigation evaluates the phytochemicals and bioactivities of compounds obtained from Indonesian Tephrosia. The results showed the cytotoxic effects of pod peel methanol extract from T. vogelii against the Arthemia salina Leach larvae, with a Brine Shrimp Lethality Test (BSLT) method with LC₅₀ of 41.40 ppm.
Table 1. Tephrosia chemical constituents

| Chemical constituents          | Plant                        | References          |
|-------------------------------|------------------------------|---------------------|
| Chalcones: tefron (1)         | *T. candida* (seed)          | Tanaka, *et al.*, 1992 |
| Flavanones: maksimalflavanon A (2) | *T. maxima* (root)      | Rao, *et al.*, 1994  |
| Flavones: fulvinervin B (3)   | *T. fulvinervis* (seed)     | Rao, *et al.*, 1985  |
| Isoflavones: kalopogoniumisoflavon B (4) | *T. maxima* (root) | Murthy, *et al.*, 1985 |
| Rotenoid: 9-demetildihidrostemonal (5) | *T. pentaphylla* (root) | Dagne, *et al.*, 1989 |

Figure 1. Structures of chemical constituents in Tephrosia

Table 2. Cytotoxic activity of *Tephrosia purpurea* extracts

| Extracts   | Cytotoxicity (IC₅₀ value µg/mL) |
|------------|---------------------------------|
| Leaves     | 95.73 ± 9.60 *#§                |
| Root       | 382.33 ± 18.78 §                |
| Stem       | 324.80 ± 21.20                  |
| Seed       | 303.97 ± 24.31 §                |

Mean ± SD, n = 3. *P < 0.05 significantly different when compared to root*, stem* and seed*. (Padmapriya, *et al.*, 2017)

2. MATERIALS AND METHODS

Experimental Procedure

The melting points were obtained using a micro Fisher-John, while the NMR data were recorded with JEOL ECA 500 (¹H 500 MHz; ¹³C 125 MHz), where tetramethylsilane served as an internal standard. In addition, mass spectra were obtained on LCT XE ESI-TOF waters, and Centrifugal Thin-Layer Chromatography (Chromatotron) was performed on the silica gel 200 mesh using a precoated silica gel 60 GF₂₅₄ (0.25 mm thickness) with various mobile phases. Subsequently, the spots were visualised by 1.5% cerium sulfate in 2 N sulfuric acid spraying agent, followed by heating. The NMR spectrum was then measured using residual peaks and deuterated solvents CDCl₃.

Plant Material

The *T. vogelli* pod peels were obtained at the Plant Taxonomy Laboratory, Biology Department, FMIPA, Unpad, West Java Province, Indonesia in March 2016. These samples were identified by Drs. Joko Kusmoro, M.P. at the Jatinangor Herbarium, Indonesia, and were deposited at the herbarium with number 183/HB/03/2016.

Brine Shrimp Lethality Assay

The bioassay indicator used to monitor plant extract toxicity during fractionation involved larva *A. salina*. Moreover,
propyleneglycol/Tween 80/water (4:1:4) in 5 mL of saltwater was applied as a negative control, while ten milligrams of potassium dichromate dissolved in propyleneglycol/Tween 80/water (4:1:4) served as the positive control. The assessment procedure was as follows: ten shrimp were transferred to each sample vial containing extracts at varied concentrations of 500, 250, 125, 62.5, 31.2, 15.6, 7.8, and 0 ppm (control). Therefore artificial seawater for hatched Brine shrimp (A. salina) 50 - 2020) were expressed as the st was performed triplicate. A. salina and ≥ 1000 ppm was not toxic. C. Table 3 shows the NMR - isolatedonchocarpin (80 - 2010) was added to each sample vial to make 5 mL. This was followed by test tubes examination, and the number of dead larvae in each bottle was counted after 24 hours. The respective death percentage were determined and the test was performed triplicate. Consequently, statistical analysis used for determining the death percentage (Equation 1) and lethal concentration (LC50). (Sahgal, et al., 2010).

\[ \%PD = \frac{(Tn - An)}{(Tn)} \times 100\% \]

Where PD is percentage of death, Tn is total nauplii and An is alive nauplii. In addition, probit analysis was performed after obtaining mortality rate. This evaluation was conducted to calculate the LC50, defined as the concentration required for a compound to produced 50% death. Subsequently, the results were counted by using linear regression equation \( y = a + bx \), and the statistical analysis (Melina, et al., 2020) were expressed as the mean value ± standard error of mean (SEM), while the variance was evaluated through ANOVA test. The extract toxicity level was organized according to Meyer (1982) classification. In addition, LC50 scores in the range ≤ 30 ppm were defined as highly toxic, while ≤ 1000 ppm were attributed as toxic, and ≥ 1000 ppm was not toxic.

**Extraction and Isolation**

The powdered and dried pod peels of T. vogelii (35 g) were extracted to yield a crude methanol extract (18.2 g). These products were then subjected to vacuum liquid chromatography over silica gel 60 GF254, and was eluted with n-hexane-EtOAc (10:0-0:10) in a polarity gradient manner (Syah, et al., 2006), and a total of fourteen (I-XIV) fractions were produced. Subsequently, fraction I was purified through centrifugal chromatography and eluted with chloroform-EtOAc (8:2) to generate isolatedonchocarpin (80 mg), and the structures were elucidated based on NMR data, and also through comparison with reported spectra values (Athipornchai, et al., 2008).

**3. RESULTS AND DISCUSSION**

The isolates were obtained as a pure colourless and transparent compounds in the form of needle crystals, with a melting point of 114-115 °C. Table 3 shows the NMR spectrum, featuring the signals of two proton double doublets at 2.84 δH and 3.00 ppm, respectively assigned to the H3α and H3β. Also, one proton signal double doublets was observed at δH 5.47 ppm for H-2, and two emerged from the two shielding carbons at δC 79.8 and 44.4 ppm for (C-2 and C-3). These were further determined as characteristic for flavanone group compounds. The nature of this structure was also confirmed by the presence of a carbon signal C = O ketone conjugated at δC 190.6 ppm (Athipornchai, 2008). In addition, the 13C NMR spectrum also demonstrated eighteen signals for twenty carbon atoms, including for one ring unit dimethyl tetrahydropryan (δC 116.0; 129.0; 77.6; 28.5; 28.2 ppm), and two oxyaryl (δC 159.7; 157.7 ppm). Table 3 shows the 1H NMR spectrum, indicating the presence of an aromatic signal for five protons at δH 7.48; 7.43 and 7.39 ppm, corresponding to the unsubstituted B ring. The two oxygen functional groups are further confirmed to occur in ring A, located at C-8a and C-7, according to the prevalence of the oxygen pattern. This phenomenon facilitates the formation of dimethyl tetrahydropryan ring at C-8 or C-6. In addition, the presence of an ortho-coupled aromatic as a doublet proton signal (J = 8.6 Hz) at δH 7.75 and 6.51 ppm respectively denote chemical shifts initiated at C-8. The HMBC spectrum shows the multiplicities and the weak coupling constants between the signal from the aromatic doublet proton at δH 7.75 ppm (H-5) and a C = O ketone conjugated carbon at δC 190.6 ppm (C-4). Also, a correlated was established between the aromatic double protons at δH 6.51 ppm (H-6) with oxyaryl carbon signals at δC 159.7 ppm (C-7), alongside two quaternary carbons at δC 109.5 and 114.8 ppm, respectively situated by C-8 and C-4a. According to the
coupling constant in H-2 / H-3, the configurations at C-2 and C-3 were determined to be trans (\(J = 13.2\) Hz), with absolute stereochemistry assumed to follow the prevalence of 2S, 3R-flavanone. Table 3 shows the evidence of this outcome, indicated by the coupling constant in H-2 / H-3, as (\(J_{H-2 / H-3\beta} = 13.2\) Hz), (\(J_{H-2 / H-3\alpha} = 3.0\) Hz) and (\(J_{gem\, H-3\alpha / H-3\beta} = 16.8\) Hz). Based on the spectroscopic data comparison with previous research conducted Athipornchai, et al. (2008), the compound is identified as isolonchocarpin.

![Figure 2. Chemical structure of isolonchocarpin](image)

Table 3. NMR data for isolonchocarpin

| Position | \(\delta_H\) (mult., \(J\) Hz ppm) isolonchocarpin | \(\delta_C\) (ppm) isolonchocarpin | \(\delta_C\) (ppm) isolonchocarpin* | HMBC \(^1\text{H} \leftrightarrow ^{13}\text{C}\) | \(^2J\) | \(^3J\) |
|----------|--------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|--------|--------|
| 2        | \(5.47\) (dd, 3.0, 13.2)                         | 79.8                            | 79.8                            | 2'                              | 4      |
| 3\(\beta\)| \(3.00\) (dd, 13.2, 16.9)                       | 44.4                            | 44.4                            | 2, 4                            | 1'     |
| 3\(\alpha\)| \(2.84\) (dd, 3.0, 16.8)                        | 44.4                            | 44.4                            | 4                               | 4a     |
| 4        |                                                 | 190.6                           | 190.6                           |                                  | -      | -      |
| 4\(a\)|                                                 | 114.8                           | 114.8                           |                                  | -      | -      |
| 5        | \(7.75\) (d, 8.6)                               | 127.9                           | 127.9                           |                                  | 4      |
| 6        | \(6.51\) (d, 8.6)                               | 111.3                           | 111.3                           |                                  | 8, 4a  |
| 7        |                                                 | 159.7                           | 159.7                           |                                  | -      |
| 8        |                                                 | 109.5                           | 109.5                           |                                  | -      |
| 8\(a\)|                                                 | 157.7                           | 157.7                           |                                  | -      |
| 1'       |                                                 | 139.1                           | 139.0                           |                                  | -      |
| 2'       | \(7.48\) (dd, 2.0, 7.9)                         | 126.1                           | 126.0                           | 3'                              | 2, 6'  |
| 3'       | \(7.43\) (dd, 7.9, 8.2)                         | 128.9                           | 128.8                           |                                  | 5'     |
| 4'       | \(7.39\) (dt, 2.0, 8.2)                         | 128.7                           | 128.6                           | 3', 5'                          | -      |
| 5'       | \(7.43\) (dd, 7.9, 8.2)                         | 128.9                           | 128.8                           |                                  | -      |
| 6'       | \(7.48\) (dd, 2.0, 7.9)                         | 126.1                           | 126.0                           | 5'                              | 2'     |
| 4''      | \(6.66\) (d, 10.1)                              | 116.0                           | 115.9                           | 8                               | 7, 8a  |
| 5''      | \(5.57\) (d, 10.1)                              | 129.0                           | 128.9                           | 6''                             | 8      |
| 6''      |                                                 | 77.6                            | 77.6                            |                                  | -      |
| 7''\(\text{CH}_3\)| \(1.47\) (s)         | 28.2                            | 28.1                            |                                  | 5'', 8''|
| 8''\(\text{CH}_3\)| \(1.45\) (s)         | 28.5                            | 28.4                            |                                  | -      |

*Athipornchai et al. (2008); \(^1\text{H}\) (300 MHz); \(^{13}\text{C}\) (75 MHz), CDCl\(_3\)

Table 4. LC\(_{50}\) value of methanol extract and n-hexane fraction with maceration method

| Extract / Fraction | Replication | LC\(_{50}\) (ppm) | Mean ± SD (ppm) |
|--------------------|-------------|-------------------|-----------------|
| Methanol           | 1           | 42.05             | 41.40 ± 0.63    |
|                    | 2           | 41.38             |                 |
|                    | 3           | 40.78             |                 |
| n-Hexane:EtOAc (10:0) | 1         | 37.05             |                 |
|                    | 2           | 38.41             | 38.16 ± 1.41    |
|                    | 3           | 36.53             |                 |
The average LC$_{50}$ value obtained through maceration method was 41.40 ppm, with a deviation standard of 0.63. This result indicates the potential toxic effect of $T$. vogelii pod peels methanol extract at below 1,000 ppm (Meyer et al., 1982). Based on statistical analysis, the $n$-hexane fraction (fraction I) yielded an LC$_{50}$ of 38.16 at 1.41 ppm deviation standard. Therefore, the results indicate the relatively higher toxicity in fraction I compared to the methanol extract, due to the smaller value. Furthermore, the pure isolonchocarpin subsequently isolated from Fraction I is assumed to also demonstrate toxicity.

4. CONCLUSION

This study highlights valuable data on the cytotoxic effect of $T$. vogelii pod peels. The flavanone investigated and identified from the $n$-hexane fraction was isolonchocarpin, which demonstrated an LC$_{50}$ with greater toxicity than the methanol extract. Hence, this yield is assumed to also possess strong cytotoxic property. However, further evaluation is needed to determine further effects, in order to provide the complete safety profile as a phytotherapeutic agent for generally recommended use.

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REFERENCES

Achmad SA, Hakim EH, Makmur L, Syah YM, Juliawaty LD, Mujahidin D. 2007. Tumbuh-tumbuhan Obat Indonesia. Bandung(ID): Penerbit ITB, Kebudayaan, Universitas Terbuka: 107-119.

Andrei CC, Ferreira DT, Faccione M, de Moraes LAB, de Carvalho MG, Braz-Filho R. 2000. C-prenylflavonoids from roots of $Tephrosia$ tunica. Phytochemistry. 55: 799-804.

Andrei CC, Vieira P, Fernandes JB, Da Silva MFGDF, Fo ER. 1997. Dimethylchromene Rotenoids From $Tephr$ osia candida. Phytochemistry. 6: 1081-1085.

Athipornchai A, Pancharoen O. 2008. Chemical Constituents From Leaves and Pods of Milletia brandisiana. Thesis Master of Science. Department of Chemistry. Silpakorn University, 60.

Chang LC, Chávez D, Song LL, Farnsworth NR, Pezzuto JM, Kinghorn AD. 2000. Absolute configuration of novel bioactive flavonoids from $Tephrosia$ purpurea. Organic letters. 2: 515-518.

Dagne E, Mammo W, Sterner O. 1992. Flavonoids of $Tephrosia$ polyphylla. Phytochemistry. 31: 3662-3663.

Dagne E, Bekele A, Waterman PG. 1989. The flavonoids of Milletia ferruginea subsp. Darassana in Ethiopia. Phytochemistry. 28: 3207-9.

Ganapaty S, Lakshminarayana K, Lakshmi P. Thomas PS. 2009. Antiprotozoal and cytotoxicity assays of the isolates of $Tephrosia$ tinctoria. Asian Journal of Chemistry. 21: 1007-1010.

Gomez-Garibay F, Tellez-Valdez O, Moreno-Torres, Calderon JS. 2002. Flavonoids from $Tephrosia$ major. A new Prenyl-β-hydroxychalcone. Z. Naturforsch. 57c: 579-583.

Jang DS, Park JU, Kang YH, Hawthorne ME, Vigo JS, Graham JS, Pezzuto JM, Kinghorn AD. 2003. Potential cancer chemopreventive flavonoids from the stem of $Tephrosia$ toxicaria. J. Nat. Prod., 66: 1166-1170.

Kishore PH, Mopuru VBR, Duvvuru G, Madugula MM, Cristelle C, Bernard B. 2003. A new
Coomestan from Tephrosia calophylla. Chemical Pharmacognosy Bull., 51: 194-196.

Kusumaningtyas VA, Syah YM, Juliawaty LD. 2020. Two stilbenes from Indonesian Cassia grandis and their antibacterial activities. Research Journal of Chemistry and Environment. 24(1): 61-63.

Melina, Putra EK, Witanti W, Sukrido, Kusumaningtyas VA. 2020. Design and Implementation of Multi Knowledge Base Expert System Using the SQL Inference Mechanism for Herbal Medicine. Journal of Physics: Conference Series. 1477(2): 1-9. https://doi.org/10.1088/1742-6596/1477/2/022007

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. 1982. Brine shrimp. A convenient general bioassay for active plant constituents. Planta Med. 45: 31-4.

Murthy MSR, Rao VE. 1985. Maxima isoflavone J: a new 0-prenylated isoflavone from Tephrosia maxima. Journal of Natural Products. 48: 967-968.

Padmapriya R, Ashwini S, Raveendran R. 2017. In vitro antioxidant and cytotoxic potential of different parts of Tephrosia purpurea. Research in Pharmaceutical Sciences. 12(1): 31-37.

Prabhakar P, Vananganudi A, Gandhidasan R, Venkateswara PR. 1996. Hookerianin: a flavone from Tephrosia hookeriana. Phytochemistry. 43: 315-316.

Prashant A, David KGL. 1993. Dehydro-6-hydroxyrotenoid and lupenone from Tephrosia villosa. Phytochemistry. 32: 484-486.

Polhill RM, Ravan PH, Stirton CH. 1981. Evolution and Systematics of Evolution of Leguminosae, in Advances in Legume Systematics. Part I. Royal Botanic Gardens, Kew, 1.

Rao EV, Rajendra PM, Sree RM. 1994. A prenylated flavanone from Tephrosia maxima. Phytochemistry. 37: 111-112.

Rao EV, Venkataratnam G, Vilain C. 1985. Flavonoids from Tephrosia fulvinervis. Phytochemistry. 24: 2427-2430.

Roy M, Mitra SR, Bhattacharyya A, Adityachaudhury N. 1986. Candidone, a flavanone from Tephrosia candida. Phytochemistry. 25: 961-962.

Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM. 2010. Brineshrimp lethality and acute oral toxicity studies on Swietenia mahagoni (Linn.) Jacq. Seedmethanolic extract. Pharmacognosy Research. 2: 25-220.

Sasidharan S, Darah I, Jain K. 2008. In vivo and In vitro toxicity study of Gracilaria changii. Pharm Biol. 46: 413-7.

Syah YM, Hakim EH, Makmur L, Kurdi VA, Ghisalberti EL, Aimi N, Achmad SA. 2006. Prenylated 2-arylbenezofurans from two species of Artocarpus. Natural Product Communications. 1(7): 549-552. https://doi.org/10.1177/1934578x0600100706

Tanaka T, Iinuma M, Yuki K, Fujii Y, Mizuno M. 1992. Flavonoids in root bark of Pongamia pinnata. Phytochemistry. 31: 993-998.

Tarus PK, Machocho AK, Lang'at-Thoruwa CC, Chhabra SC. 2002. Flavonoids from Tephrosia aequilata. Phytochemistry. 60: 375-379.

Touqeer S, Saeed MA, Ajaib M. 2013. A review on the phytochemistry and pharmacology of Genus Tephrosia. Phytopharmacology. 4(3): 598-637.

W Kalala, A Mwakigonja, S Maregesi, Z. Msengwa, R Mahunnah. 2015. Brine shrimp lethality and acute oral toxicity of Commiphora swynertonii (Burtt) exudate. Pyrex Journal of Medicinal Plant Research. 1(3): 010-018.

Yenesew A, Dagne E, Waterman PG. 1989. Flavonoids from the seed pods of Tephrosia pumila. Phytochemistry. 28: 1291–1292.