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Investigation into the Phytochemical Profiles, Antibacterial and Antioxidant Potentials of *Toona sinensis* Stem Bark Part

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ABSTRACT: *Toona sinensis* has been used as a traditional medicine to treat diarrhea, dysentery, and fever. In the present study, the stem bark extracts of *T. sinensis* were investigated to determine the antibacterial activity, total phenolic content, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, and chemical constituent using gradual extraction by maceration method with hexane, ethyl acetate, and methanol respectively. In vitro, antibacterial activity was evaluated against *Staphylococcus aureus* and *Escherichia coli* by agar well diffusion method. The hexane fraction was more sensitive to *S. aureus* and the ethyl acetate and methanol fractions were more sensitive to *E. coli*. The methanol extract showed the highest total phenolic content and antioxidant activity. Phytochemically, *Toona sinensis* stem bark extract contained phenolic, flavonoid, steroid, triterpenoid, and coumarin. Investigation of ethyl acetate extract produced 5,7,3',4'-tetrahydroxyflavan-3-ol (catechin).

Keywords: Antibacterial, antioxidant, catechin, *Toona sinensis*, total phenolic content

INTRODUCTION

Meliaceae family has a huge diversity of plants containing 51 genera and 550 species (Pennington et al., 1975). This family is widespread in China, Indonesia, Vietnam, Malaysia, and the Philippines and Indonesia is one of the most distributed areas of this plant family. Some plants of this family have been reported to provide interesting biological activity as antimalarials (MacKinnon et al., 1997), antifeedant (Abdelgaleil et al., 2003), antidiarrheals (Maiti et al., 2007), antiplasmodial (Júnior et al, 2012), antihyperglycaemic (Hashim et al., 2013), and anticancer (Zhang et al., 2007). *Toona sinensis* plant is one of the plants of the Meliaceae that is widely distributed in Indonesia (Sumatra, Java, and Sulawesi) and known as *Surian* or *Suren*. The *T. sinensis* plant has long been used to treat diarrhea, dysentery, and fever. The previous study reported that *T. sinensis* had unique secondary metabolites, such as limonoid, phytol derivatives, triterpenoid, quercetin-3-*O*-rutinoside, quercetin-3-*O*-*β*-D-glucopyranoside, 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucopyranoside, quercetin-3-*O*-*α*-L-rhamnopyranoside, kaempferol-3-*O*-*α*-L-rhamnopyranoside and some essential oils which the main compounds were germacrene-D, germacrene-B, α-terpinene, α-humulene, β-caryophyllene, bicyclo germacrene, and α-copaene (Hu et al 2016); (Luo et al., 2000); (Santoni et al 2008); (Harneti et al., 2013).

However, the stem bark of this plant has not been widely studied for its potential activity, especially as an antibacterial and antioxidant. The aim of this study is to investigate the antioxidant and antibacterial properties of several extracts of *T. sinensis* stem bark. In addition, this study also isolates the secondary metabolite compound from the extract.

EXPERIMENTAL SECTION

Materials

The materials used in this research were stem bark of *T. sinensis* was collected in Padang West Sumatra, Indonesia. The plant was identified at Herbarium of Andalas University, hexane (Merck), ethyl acetate (Merck), methanol (Merck), Folin-Ciocalteau reagent (FC), sodium carbonate (20%, w/v), gallic acid (Merck), DPPH (2,2-diphenyl-1-picrylhydrazyl) and aquadest, DMSO (Dimethylsulfoxide) (Merck), MHA (*Muller Hinton Agar*) (Merck). *Escherichia coli* bacteria and *Staphylococcus aureus* bacteria were purchased from the Biotechnology Laboratory, Faculty of Agricultural Technology, Universitas Andalas.

Extraction and Purification

To get extracts with different polarities, a total of 2.8 kg of dried *T. sinensis* stem bark was first extracted (maceration method) several times with hexane. The mixture was filtered and the solvent was evaporated using a rotary evaporator yielding crude hexane extract. The residue was then extracted with ethyl

acetate and methanol, respectively using the same procedures. During the extraction process, ethyl acetate produced the largest quantity of extract and in general, can extract the most phenolic and flavonoid compounds. To get a pure compound, ethyl acetate extract was separated by liquid vacuum chromatography, column chromatography, chromatotron, and re-crystallization techniques. The pure compound was obtained from about 200 mg with yellowish-white color and needle shapes. The isolated compound was characterized by NMR spectroscopy.

Phytochemical Screening

The chemical constituents of hexane, ethyl acetate, and methanol extracts of the stem bark of *T. sinensis* were determined by the standard method of Harborne (J.B. Harborne et al., 1973).

Determination of Total Phenolic Content

The phenolic content of all fractions of *T. sinensis* stem bark was investigated by Folin-Ciocalteu (FC) method with slight modification. The sample was dissolved in methanol (100 μg/mL). The sample solution of 0.5 mL was mixed with 0.5 mL of FC reagent. After 5 minutes of the mixing time, 1 mL of sodium carbonate (20%, w/v) was added to the mixture. The mixed solution was diluted in a volumetric flask (10 mL) by adding distilled water and then incubated for 2 hours at room temperature. The absorbance was measured at 765 nm and gallic acid (20-120 mg/L) was used as the calibration curve. The total polyphenol content was presented as gallic acid equivalent (GAE) of the dry weight of the extracts (Okselni et al 2019).

Radical Scavenging Activity by DPPH Assay

The antioxidant activity was tested by DPPH (2,2-diphenyl-1-picrylhydrazyl) method with slight modification. The sample of 2 mL in various concentrations of methanol was mixed with 3 mL of DPPH solution (0.1 mmol/L) and incubated for 30 minutes in a dark place at room temperature. The absorbance was measured at 517 nm. The inhibition concentration was obtained from the regression equation (Okselni et al 2019).

Antibacterial Activity Test

A disc diffusion method by Balouiri et al, 2016 was applied with slight modification to investigate the antibacterial activity of *T. sinensis* stem bark against *Staphylococcus aureus (S. aureus)* for Gram-positive bacteria and *Escherichia coli (E. coli)* for Gram-negative bacteria. The bacterial suspension of 200 μL was added to a petri dish containing MHA (Mueller-Hinton Agar) medium. A cotton swab was used to distribute the tested bacteria. A paper disc (diameter of 5 mm) containing the extract solution (1000 μg/mL) was placed on the surface of the agar plate. The plate was allowed to stand for 24 h at room temperature. The antibacterial activity was determined by measuring the clear inhibition zone (including the diameter of the disc). Amoxicillin (62.5 μg/mL) was applied as a positive control.

RESULTS AND DISCUSSION

Phytochemical Profiles

The phytochemical screening had been conducted by the standard method to know the chemical constituent of the *T. sinensis* stem bark and the result was shown in **Table 1**. The determination of the chemical constituent of *T. sinensis* stem bark is based on the specific reaction between the chemical constituent and the specific reagent[13]. As shown in **Table 1**, the hexane extract was dominated by steroid compounds, whereas the ethyl acetate and methanol fractions had similar chemical content, such as phenol, flavonoid, triterpenoid, and coumarin. The chemical constituents present in each extract need to be identified because they play an important role in the biological activities of the extracts.

Total Phenolic Contents

The phenolic content of *T. sinensis* stem bark was obtained from a calibration curve of gallic acid ($y = 0.0072x + 0.0552$, $R^2 = 0.9907$) and the results were shown in **Table 2**. The phenolic content of *T. sinensis* was determined by Folin-Ciocalteau (FC) method. This method is based on the forming of blue complex compounds as the result of the Folin-Ciocalteau reagent and phenolic compounds[16]. This study showed that the ethyl acetate and methanol extracts of *T. sinensis* stem bark had a high amount of phenolic content. The previous study reported the total phenolic content of the aqueous leaf extract of *T. sinensis* was 130 ± 26 mg GAE/g dry weight (DW) of the sample (Yang et al., 2006). This study exhibited that the stem bark of *T. sinensis* has a higher amount of phenolic content than the *T. sinensis* leaves.

Antioxidant Activity by DPPH Assay

The antioxidant activity of *T. sinensis* stem bark (**Table 3**) was evaluated by the colorimetric method using the free radical of DPPH (2,2-diphenyl-1-picrylhydrazyl). The DPPH method is based on the reaction of the compound that gives an electron to radical DPPH so the (Miguel, 2010). This study showed that the ethyl acetate and methanol extracts give strong antioxidant activity which is close to the antioxidant value of ascorbic acid as a positive control. The previous study reported the IC_{50} of the aqueous leaf extract of *T. sinensis* was 50 μg/mL (Hseu, et al., 2008). It indicated that the antioxidant activity of the stem bark of *T. sinensis* was better than that of *T. sinensis* leaves. Many studies evaluate that TPC directly contributes to antioxidant activity. These compounds are related to strong chain-breaking antioxidants (Dobrinas et al., 2021).

Table 1. A Chemical constituent of *T. sinensis* stem bark

Chemical Constituent	Extracts		
	Hexane	Ethyl acetate	Methanol
Phenol	-	+	+
flavonoid	-	+	+
Saponin	-	-	-
Triterpenoid	-	+	+
Steroid	+	-	-
Alkaloid	-	-	-
Coumarin	-	+	+

(+) indicates the presence of a constituent, and
(–) indicates the absence of constituent

Table 2. Total phenolic content of *T. sinensis* stem bark

Extracts	TPC (mg GAE/g DW)
Hexane	54.81 ± 0.80
Ethyl acetate	262.22 ± 0.00
Methanol	301.11 ± 1.39

The values were presented as average ± SD (Standard deviation), n = 3

Table 3. Antioxidant activity of the *T. sinensis* stem bark

Extracts	IC_{50} (μg/mL) ± SD
Hexane	>1000
Ethyl acetate	22.32 ± 5.35
Methanol	15.28 ± 1.48
Ascorbic acid	10.59 ± 0.08

The values were presented as average ± SD (Standard deviation), n = 3.

Antibacterial activity

The antibacterial activity of *T. sinensis* stem bark was investigated against *E. coli* and *S. aureus* bacteria and the result was shown in **Table 4**. The disc diffusion method was used to evaluate the antibacterial activity of *T. sinensis* stem bark against *E. coli* and *S. aureus* bacteria. The result (**Table 4**) indicates that the hexane extract has good antibacterial activity compared to all fractions for Gram-positive bacteria. It could be due to the chemical constituent of hexane extract which was dominated by steroid (**Table 1**). The steroid compounds are known to have a great effect on Gram-positive bacteria (Smania et al., 1999). On the other hand, the ethyl acetate and methanol extracts were more sensitive for Gram-negative bacteria. It might be related to the chemical constituent of ethyl acetate and methanol extracts containing phenol, flavonoid, triterpenoid, and coumarin compounds (Table-1). These compounds provide a positive correlation to the antibacterial activity of Gram-negative bacteria (Saleem et al., 2010). Different studies investigate the inhibitory effects of plant flavonoid-rich extracts against some pathogenic bacteria. Various mechanisms have been proposed for the antibacterial activities of flavones. As a mechanism, flavones form a complex with the cell wall components and consequently inhibit further adhesions and microbial growth as well (Farhadi et al., 2018).

Isolation of 5,7,3',4'-tetrahydroxyflavan-3-ol

This compound was obtained as yellowish-white needle shapes with its molecular formula of $C_{15}H_{14}O_6$ (m/z 290 Hz) and a melting point of 170-172°C. The UV spectrum showed that this compound gives the maximum absorption at λ_{max} = 281, 232, 205nm which indicated the presence of chromophore groups of flavonoids. The IR spectrum of this compound gave absorption at V_{max}: 3396 cm^{-1} (O-H), 2923 cm^{-1} and 2852 cm^{-1} (aliphatic C-H), 1627 cm^{-1}, 1524 cm^{-1} and 1467 cm^{-1} (aromatic C=C). The ^{1}H-NMR data (Table-5) showed the presence of protons at δ 4.57 (1H, d, J = 8.2 Hz, H-2), 3.95 (1H, t, H-3), 2.54 and 2.92 (2H, tb, dd, H-4) which are characteristic of a flavan-3-ol, at δ 6.03 (1H, d, J = 2.0 Hz, H-6) and 5.88 (1H, d, J = 2.0 Hz, H-8) which indicated the substitution of O-H groups in meta-position at C-5 and C-7. The ^{13}C-NMR (**Table 5**) and DEPT spectrum showed the presence of 3 carbon of sp^3 hybridization at δ = 82.7, 68.3, and 29.7 ppm and 12 carbon of sp^2 at δ = 95 - 157 ppm. This data had been recognized by the previous study (Watanabe et al., 1988).

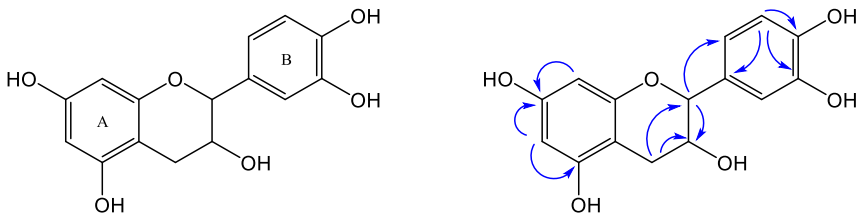
Table 4. Antibacterial activity of the stem bark of *T. sinensis*

Extracts	Inhibition Zone (mm)	
	Staphylococcus aureus	*Escherichia coli*
Hexane*	8.1	6.6
Ethyl acetate*	6.5	7.1
Methanol*	6.1	7.1
Amoxicillin**	9.0	10.5

*Concentration (1000 μg/mL)
**Concentration (62.5 μg/mL)

Table 5. NMR chemical shift of isolated compound

	δc (ppm)	δ_H (ppm)
2	82.7	4.57 d (J = 8.2 Hz)
3	68.3	3.95 t
4	29.7	2.54 tb
		2.92 dd
5	156.42	-
6	95.32	6.03 d (J = 2.0 Hz)
7	156.93	-
8	94.39	5.88 d (J = 2.0 Hz)
9	156.08	-
10	99.81	-
1′	131.34	-
2′	114.42	6.90 d (J = 1.4 Hz)
3′	144.83	-
4′	144.91	-
5′	114.86	6.81 d (J = 8.3 Hz)
6′	119.23	6.77 dd (J = 1.4, 8.3 Hz)



(a) (b)

Figure 1. Catechin (a) and catechin with key HMBC (b)

The HMBC spectrum showed the correlation of H-2 to C-3 and C-6′, H-4 to C-2 and C-3, H-6 to C-5 and C-7, H-8 to C-7, and H-5′ to C-1′, C-3′, and C-4′. Those data indicated that the isolated compound was a flavonoid compound of 5,7,3',4'-tetrahydroxyflavan-3-ol with the trivial name as catechin (**Figure 1**).

CONCLUSIONS

The *T. sinensis* stem bark contains phenol, flavonoid, steroid, triterpenoid, and coumarin compounds. The quantitative determination of phenolic content presented that the ethyl acetate and methanol fractions of *T. sinensis* stem bark had great potential as the source of phenolic compound. Biological activity investigation led to the discovery that the potential of *T. sinensis* stem bark as an antioxidant and antibacterial agent. These activities are affected by the chemical constituents contained in the stem bark of *T. sinensis.* The flavonoid compound of 5,7,3',4'-tetrahydroxyflavan-3-ol or catechin had been successfully isolated by column chromatography technique from the stem bark of *T. sinensis*.

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