Total phenolic content, total flavonoid content, and antioxidant activity of water and ethanol extract from Surian ([*Toona sinensis*]) leaves

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**Abstract.** Surian (*Toona sinensis*) is a forest plant that is widespread in Asia. In Indonesia, this plant only used and known as a commodity for carpentry but in other nation, saurian is known as spices and traditional medicine because of its natural antioxidant potential. The objective of this study was assayed total phenolic content, total flavonoids content, and antioxidant activity from Surian leaves. Surian leaves were extracted by water and ethanol solvent. Water extraction was conducted by reflux in 90 °C then ethanol extraction was conducted by maceration, which is through various concentration 70%, 80%, 90%, and 96% of ethanol. Total phenolic content varied from 276.62 to 444.68 (mg GAE/g) and total flavonoid content ranged from 209.23 to 324.61 (mg QE/g). Antioxidant activity of the water and ethanol extracts was evaluated using DPPH (1,1-diphenyl-2-picryl-hydrazil) radical scavenging method. Total phenolic and flavonoid content of ethanol 90% extract and ethanol 96% extract had a higher amount than the other. The ethanol 96% extract had the strongest DPPH inhibition concentration 50% (IC50), which is 3.38 μg /mL while ascorbic acid is 3.90 μg /mL as a comparison. Antioxidant activity had a strong correlation with total flavonoid content.

1. Introduction

Reactive oxygen species (ROS) are reactive chemical species containing oxygen for examples peroxides, superoxide, hydroxyl radical. Free radicals are an atom or molecule that has one or more unpaired electrons in its outer orbitals. Free radicals are unstable and have high reactivity, so they can take electrons from other molecules in an effort to get their electron pairs. They are unstable molecule and are extremely reactive with ability to oxidize and change other molecules nearby to gain an electron to return to a ground state. This reactive property can be resulting from the destruction of cell and tissue damage that contribute to many health problem and diseases [1].

In physiological levels ROS naturally formed as the result of respiration and other metabolic processes. These molecules in normal condition contribute to assist normal cell maintenance. Endogenous antioxidant (e.g. glutathione peroxidase, superoxide dismutase, catalase, thioredoxin) have a role in maintenance redox balance in a physiological cellular level. Imbalance occurs when ROS levels are in excess and antioxidants are under strain to control them. Accumulation ROS excess in the body known as oxidative stress condition [2]. These conditions can affect physiological processes like oxygen sensing, genomic stability, enzyme work, cellular sturdiness, and cell proliferation. When the body’s endogenous antioxidant can’t suppress radical excess, it requires exogenous antioxidant (dietary intake) to counterbalance. Mainly small molecules have a role to bring back the balance of redox body’s state [3].
Most of antioxidant exogenous usually obtained from fruits, vegetables, and others part of plant product. The animal product contains antioxidant but in the small quantity [4]. Plant antioxidant molecules mostly are secondary metabolites. The plant produces these compound as adaptive responses [5]. The quality and quantity of secondary metabolites compound depend on species, genetics, and the environment in which they are grown. In Asia, many plants can be founded to have beneficial properties for health. One of them is Toona sinensis or in Indonesia known as surian. T. Sinensis (Meliaceae) widely distributed in Asia. From southern North Korea through most eastern, central, and south-western parts China to Thailand, Myanmar, India, Malaysia, and Indonesia [6].

In China, this plant especially has been used as traditional medicine. The parts of a plant which have beneficial properties are bark, oil, seed, flower, and leaves. The young fresh leaves were used as vegetable and spices. The leaves of T. sinensis were consumed to treat oriental diseases such as halitosis, vomiting, dysentery, lack of appetite, enteritis, and itchiness. Beneficial properties of T. Sinensis leaves potentially to be explored. In Indonesia, T. sinensis only known as carpentry commodity surian trees. This plant generally lives with an altitude between 350 m to 2500 m above sea level, widely planted in a community forest. They were planted as border trees at sengon (Paraserianthes falcata) plantation and at tea and coffee crops to protect stem borer attack [9]. In Indonesia, there are a similar species with surian, namely suren (Toona sureni), but surian is mostly planted by people in their community forest. The purpose in this study was to evaluate total phenolic content, total flavonoids content, and antioxidant activity from surian leaves as basic information for developing the study of this plant in food, health, pharmacological, and medicine area.

2. Materials

The following reagents were obtained from Sigma Chemical Co. (USA): Gallic acid, Quercetin, DPPH, and MES buffer. The ascorbic acid standard was obtained from HiMedia Ltd. Toona sinensis leaves were sourced from Rancaekek Sumedang, West Java, Indonesia.

3. Methods

3.1 Preparation and Extraction Toona Sinensis Leaves

Toona sinensis leaves were dried at 50 °C (EYELA NDO-700, Japan) and powdered. Water extraction with reflux method with 50g in 3× 500 mL or 3× 1:10 (w/v) and for ethanol extraction, were used four degree ethanol concentration (70%, 80%, 90%, and 96%) with 50g in 3× 500 mL or 3× 1:10 (w/v) maceration method. All extraction filtrate evaporated by vacuum evaporator (OSK 6513 universal reduced pressure-concentration still apparatus Ogawa Seiki Co. Japan) under constant pressure at 60 °C until powdered form. Dried extracts stored in a refrigerator at 5 °C before use.

3.2 Total Phenolic Content Measurement

Total phenolic content was estimated using the Folin–Ciocalteau method. The extract was prepared by extracting samples for concentration 200 ppm in dimethyl sulfoxide (DMSO). About 0.1 mL sample was transferred to 10 mL test tube, Na₂CO₃ 75% in aquadest 1 mL was added and the solution was re-mixed. Folin–Ciocalteau’s phenol reagent 1.25 mL was added to the mixture and the solution was re-mixed. After mixing, mixture incubated at 45 °C for 15 minutes. The absorbance of the reaction mixtures was recorded at 765 nm. Gallic acid in DMSO was used as standard with the following concentration 20, 40, 60, 80, 100, 120, 140, and 160 ppm.

3.3 Total Flavonoids Content Measurement

Total flavonoid content was estimated using aluminum chloride method. The extract was prepared by extracting samples for concentration 200 ppm in dimethyl sulfoxide (DMSO). About 0.5 mL sample was transferred to 10 mL test tube, aluminum chloride (AlCl₃ 2%) was added 0.5 mL and the mixture was incubated for 15 minutes. The absorbance of the reaction mixtures was recorded at 415 nm.
Quercetin in DMSO was used as standard with following concentration 15, 30, 45, 60, 75, 90, 105, and 120 ppm.

3.4. DPPH Radical Scavenging Activity Measurement
A solution of DPPH in methanol 0.4 mM was prepared. Methanol 20% and MES buffer (2-(morpholino)ethanesulfonic acid) pH 6.0 were prepared for reagent mixture[11]. Ascorbic acid and samples were prepared in methanol with the following concentration 1.25, 2.5, 3.75, 5, and 6.25 ppm. About 1 mL sample for each concentration was transferred to the test tube and start from the lowest concentration. Mixture reagent (DPPH: Methanol 20%: buffer MES) 1:1:1 was added 1 mL start to reacting from the highest concentration. Samples were incubated in dark condition about 20 minutes and absorbance of the reaction mixtures was recorded at 520 nm start from the highest concentration sample. The percentage of remaining DPPH was calculated using the following equation : (% DPPH remaining = 100 × ([DPPH]sample / [DPPH]blank ) and inhibition percentage (% Inhibition = 100 − % DPPH remaining).

3.5. Statistical Analysis
Linear equations are calculated by Microsoft Excel 2010. Mean data values are presented with standard error. Statistical analysis was using Statistical Analysis Software (SAS). Analysis of variance (ANOVA) followed by Duncan test for data comparison was used for statistical validation of the data, with significance defined as $p < 0.05$.

4. Results and Discussion

4.1. Extraction Toona Sinensis Leaves
The yield of extraction shows that through the extraction process, it can produce the yield range of compounds taken from 10.743% to 14.516%. Sequentially the highest amount of yield was obtained from extraction with 70% ethanol > water extract > 90% ethanol > ethanol 96% > 80% ethanol ‘Figure 1’. These results show that the number of compounds that can be separated from the material through the extraction process is related to the solvent polarity and concentration. The polarity of water can attract the compounds contained in surian leaves as much as 13.429% while ethanol solvents with various concentrations can attract secondary metabolites start from 10.743% to 14.516% which is also the highest yield. The extraction is the process of separating the compounds contained in the material with the help of solvents [12]. In this case, the material used was the simplicity of T. sinensis leaves. Based on the polarity of solvents were used, water solvents are the most polar solvents in this study.

The yield in ‘Figure 1’ looks diverse result. The diversity that occurs related to metabolite compounds that can be taken through the extraction process. Water solvent extraction did not get the most yield, meaning that the amount of yield that can be obtained is not related to the level of polarity [13]. These result showed that the highest yield of T. Sinensis extract was in ethanol 70% solvent and lowest in ethanol 80%. Water polarity is seen from the polarity index, molecular dipole moment, and dielectric constant [14]. Water solvents are used as a model for the application of surian leaf utilization as a tea while ethanol solvents with various concentrations are used as an optimization approach to the acquisition of bioactive compounds which will be tested for their content and activity [14]. Based on previous phytochemical investigation T.sinensis leaves potentially to have a chemical compound that can act as antioxidants such as Polyphenol [15] methyl gallate [16] and gallic acid [17]. In this study, all of the extracts was used as a sample with the aim that all activities can be known and compared.
4.2. Total Phenolic Content

Phenolics are the most investigated group of secondary metabolites so far. They are a large class of chemical compounds that show a diversity of structures, from rather simple ones like phenolic acids to polyphenols such as flavonoids. Plant phenolic compounds are natural secondary metabolites in plant [18]. Polyphenol is one of three major groups in secondary metabolites and the others are terpenoid and alkaloid [19]. Polyphenols are produced in plant cells through secondary metabolism and function in plant reproduction and growth [20]. Plant secondary metabolites derived exclusively from the shikimate derived phenylpropanoid and/or the polyketide pathways, featuring more than one phenolic ring and being devoid of any nitrogen-based functional group in their most basic structural expression [21]. Polyphenol also had substantial health potential in multiple research and medical use [22].

Total phenolic compounds were measured by the Folin-Ciocalteau method with gallic acid as a comparison. These methods are based on formation molybdenum-blue from oxidation with a phenolic group [23]. The data showed that the highest phenolic total was obtained from the extract of *T. Sinensis* leaves with 90% ethanol solvent. Sequentially obtained the number of ethanolic phenolic compounds 90% extract > ethanolic 80% extract > ethanolic 70% extract > ethanolic 96% extract > water extract. The result was significantly different in statistic Duncan test with \( p < 0.05 \). These results indicate that the total yield obtained is not directly correlated to the total phenolic content. However, the highest total phenolic content was obtained from extracts with 90% ethanol solvent and it is the second best in yield. Total phenolic content assayed as an approach to get the quantitative data antioxidant potential compound. Polyphenol presents in plant secondary metabolites and is able to react with radicals [24]. They contain benzene rings with one or more hydroxyl substituents which potentially have antioxidant activity with hydrogen electron donor [25]. Total phenolic content varied from 276.62 to 444.68 (mg GAE/g) ‘Figure 2’. These result showed a large range of total phenolic content while other studies in China have found *T.sinenses* leaves reach 262.09 mg GAE/g [26]. In this study, it’s expected to have better antioxidant activity as well based on the data. Their antioxidant activity seems to be related to their molecular structure, more precisely to the presence and number of hydroxyl groups, and to double bond conjugation [27]. The major antioxidant components of these common foods are the phenolic compounds. Diet rich in fruits, vegetables, cereals, and olive oil can prevent cardiovascular diseases and certain forms of cancer [27]. Well-known that phenolic compounds not only contribute in providing health beneficial effects and serve defense mechanisms to counteract reactive oxygen species (ROS) but also contribute to the quality and nutritional value in terms of modifying color, taste, aroma, and flavor [28]. Total phenolic content results are still not able to show the content of compounds that are more specific in the extract obtained. Total flavonoid testing still needs to be done as a presumption for more specific chemical group compound.
4.3. **Total Flavonoid Content**

In Nature, flavonoids are the largest group of phenolic compound. Flavonoids are based on a C15 skeleton including chroman ring bearing an aromatic ring in position 2, 3, or 4. Most of the flavonoid compound known as glycosides and relatively in small number aglycones. Flavonoids are the most typical polyphenols which have C₆-C₃-C₆ carbon core, the aromatic ring in the benzopyran moiety is called the A ring, the aromatic ring attached to the benzopyran moiety is called the B ring, and the O-heterocyclic ring is called the C ring ‘Figure 3’ [29]. The presence of numerous phenolic OH groups makes these compounds more potential to have antioxidant activity. Total flavonoid compounds showed different data trends compared to total phenolic data. Flavonoid compounds are one of the compounds that belong to the class of phenolic or polyphenol compounds [30]. Flavonoids are the most studied group of polyphenols. This group has a basic structure consists with commonly have two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle [31].

The test results showed that the highest total phenolic was found in extracts with 96% Ethanol solvent ‘Figure 4’. Sequentially obtained that the total flavonoid content showed that ethanol 96% extract > ethanol 90% extract > ethanol 80% extract > water extract > ethanol 70% extract. These showed a correlation that in the ethanolic extract, the additional water to increase the polarity solvent can affect to decrease total flavonoids content. The result statistically had the same result with total phenolic content. test The data significantly different for each sample with the Duncan test. Total flavonoid content in this study ranged from 209.23 to 324.61 (mg QE/g) while another study T.sinensis in China was 108.57 mg RE/g (rutin equivalents). These results indicate that the number of compounds obtained from the sample in this study is more than that of the study. The standard difference used can even indicate that the content of the active compound obtained is higher antioxidant potential because quercetin has more active hydroxyl groups than rutin whose glycosylated form of quercetin [31]. These result showed that the increase of ethanol concentration have a correlation with total flavonoids contain extracted. Based on properties of solvent polarity in this study ethanol 96% have got more flavonoids compound than others solvent.

![Figure 2. Total phenolic content T. Sinensis leaves extract.](image-url)
4.4. DPPH Radical Scavenging Activity

The best inhibitory strength is shown by Ethanol 96% extract sample which is characterized by the lowest concentration needed to inhibit 50% radical DPPH [32]. Therefore, sequentially the best inhibitory activity is shown by the ethanol 96% extract > ethanol 90% extract > ascorbic acid > water extract > ethanol 80% extract > ethanol 70% extract ‘Figure 5’. These results indicate that the total yield of extract and the total phenolic extract is not directly proportional correlation to the ability of antioxidant activity obtained. The ability of antioxidant activity is more in accordance with the total flavonoid data contained in the extract because it can be observed by increasing the flavonoid content in the extract proportional to the observed antioxidant ability [14]. Ethanol 96% extract had the highest total value of flavonoid content with a value of 324.61 QE/g of antioxidant activity and with an IC50 value of 3.38 ppm. This value was better than ascorbic acid control. IC50 concentration showed ability compound to inhibit radical. Ascorbic acid as a positive control was used as an antioxidant in dietary supplement reach 3.90 ppm in this study. This is weaker than ethanol 90% and 96% extract which can reach lower value with concentration 3.70 ppm and 3.38 ppm respectively and it is not significantly different. Statistically, the data can be grouped into three groups: strong, moderate, and weak. The strongest one is ethanol 96% extract followed by ethanol 90% extract and ascorbic acid control. Moderate activity was water extract and ethanol 80% extract which is 4.98 ppm and 5.49 ppm respectively. The weak one is ethanol 70% extract which is 7.4966 ppm. This grouping of antioxidant capability is relative only in these study based on Duncan statistic test but all of the IC50 concentration in this study was good because the ascorbic acid standard in most recently DPPH reach the range (5.85–11.85 ppm) [33].
Scavenging radical DPPH activity showed by the decrease of absorbance that shows the decrease of DPPH percentage [34]. Various concentrations were used to make a linear equation for determining Inhibition Concentration 50% (IC50). The evaluation of DPPH scavenging activity based on a reaction between the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical with an active compound in samples [35]. DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule [36]. The odd electron of a nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine [34]. Antioxidant activity was measured by the decrease of absorbance in 520nm. The decrease of free radical showed an increase in antioxidant activity and hydrazine non-radical formation [37]. Antioxidant activity correlated with their chemical structure and the content of phenolic and flavonoids compounds [38]. Phenolic and flavonoids compound obtained are determined by the extraction solvent [39]. Many compounds can influence antioxidant activity, several compounds can be found in T. sinensis are quercetin-3-O-α-Lrhamnopyranoside, kaempferol-3-O-α-L-rhamnopyranoside, 1,2,3,4, 6-Penta-O-galloyl-β-D-glucopyranose, and ethyl gallate) [40].
Figure 6. Chemical structures isolated from T. sinensis leaves. A: QLR (quercetin-3-O-α-L-rhamnopyranoside); B: KLR (kaempferol-3-O-α-L-rhamnopyranoside); C: PGG (1,2,3,4, 6-Penta-O-galloyl-β-D-glucopyranose); D: EG (ethyl gallate) [40].

5. Conclusion
Water and ethanol extraction in this study obtain yield crude extract that has high total phenolic and total flavonoids content which is significantly different \( p < 0.05 \) statistically. 90% ethanol is the best solvent that can extract the highest total phenolic and flavonoid content with the strongest scavenging DPPH activity.

References
[1] Alfadda A A and Sallam RM 2012 Reactive Oxygen Species in Health and Disease.
[2] Lee MT, Lin W C, Yu B and Lee T T 2017 Asian-Australasian J. Anim. Sci. 30 299.
[3] Procházková D, Boušová I and Wilhelmová N 2011 Fitoterapia 82 513.
[4] Beta T 2014 J. Agric. Food Chem. 2014.
[5] Wang DS 2012 Secondary Metabolites from Plants 1–52
[6] Yang CJ, Chen YC, Tsai YJ, Huang MS and Wang CC 2014 Kaohsiung J. Med. Sci. 30 279.
[7] Yang W, Cadwallader K R, Liu Y, Huang M and Sun B 2018 Food Chem.
[8] Peng W, Liu Y, Hu M, Zhang M, Yang J, Liang F, Huang Q and Wu C 2018 Brazilian J. Pharmacogn. 29 111.
[9] Falah S and Andrianto D 2012 Characterization and Antioxidant Activity of Mahogany Bark Extract-loaded Chitosan Nanoparticles 3.
[10] Wahyuni TS, Tumewu L, Permanasari AA, Apriani E, Adianti M, Rahman A, Widyawaruyanti A, Lusida MI, Fuad A, Soetjipto, Nasromudin, Fuchino H, Kawahara N, Shoji I, Deng L, Aoki C and Hotta H 2013 Virol. J. 10 259.
[11] Andrianto D 2015 J. For. Biomass Util. Soc. 1 19.
[12] Harborne JB 2011 Phytochem. Methods 1.
[13] Sultana B, Anwar F and Ashraf M 2009 Molecules 14 2167.
[14] Chen YC, Lin KY, Kumar KJS, Cho HJ, Huang HC, Wang MT, Yang HL, Chen SC, Wang L and Hseu YC 2011 J. Ethnopharmacol. 137 669.
[15] Feng W, Wang M, Cao J, Sun J and Jiang W 2007 Process Biochem. 42 1155.
[16] Hsieh TJ, Liu TZ, Chia YC, Chern CL, Lu FJ, Chuang M, Mau SY, Chen SH, Syu YH and Chen CH 2004 Food Chem. Toxicol. 42 843.
[17] Chen CC, Hsieh YC, Yuan SS, Hsu HK, Chang FR, Chia YC, Wu YC and Chen HM 2009 Cancer Lett. 286 161.
[18] Zhong J J 2011 Plant Secondary Metabolites 3 (Elsevier B.V.)
[19] Pandey KB and Rizvi SI 2009 Oxid. Med. Cell. Longev. 2 270.
[20] Vogt T 2000 Recent Adv. Phytochem. 34 317.
[21] Quideau S, Deffieux D, Douat-Casassus C, and Pouységú L 2011 Angew. Chemie Int. Ed. 50 586.
[22] Gorelick J and Bernstein N 2014 Elicitation: An underutilized tool in the development of medicinal plants as a source of therapeutic secondary metabolites vol 124 (Elsevier Inc.).
[23] Agbor G A, Vinson J A and Donnelly P E 2014 Int. J. Food Sci. Nutr. Diet. 3 147.
[24] Pourreza N 2013 Jundishapur J. Nat. Pharm. Prod. 8 149.
[25] Huyut Z, Beydemir Ş and Gülçin İ 2017 Biochem. Res. Int. 2017 7616791.
[26] Jiang SH, Wang CL, Chen ZQ, Chen MH, Wang YR, Liu CJ, Zhou QL and Li ZJ 2009 J. Food Biochem. 33 425.
[27] Bendary E, Francis R R, Ali H M G, Sarwat M I and El Hady S 2013 Ann. Agric. Sci. 58 173.
[28] Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z and Ercisli S 2009 Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants 22 102.
[29] Castellano G, González-Santander J L, Lara A and Torrens F 2013 Phytochemistry 93 182.
[30] Perez-Vizcaino F and Fraga C G 2018 Arch. Biochem. Biophys. 646 107.
[31] Pietta P 2000 Flavonoids as Antioxidants 1035.
[32] Kedare S B and Singh R P 2011 J. Food Sci. Technol. 48 412.
[33] Mishra K, Ojha H and Chaudhury N K 2012 Food Chem. 130 1036.
[34] Xie J and Schaich KM 2014 J. Agric. Food Chem. 62 4251.
[35] Nariya P B, Bhalodia N R, Shukla V J, Acharya R and Nariya M B 2013 Ayu 34 124.
[36] Mahmoud M A A, Chedea V S, Detsi A and Kefalas P 2013 Food Res. Int. 51 907.
[37] Apak R, Özyürek M, Güçlü K and Çapanoğlu E 2016 J. Agric. Food Chem. 64 997.
[38] Andzi Barhé T and Feuya Tchouya G R 2016 Arab. J. Chem. 9 1.
[39] Hseu YC, Chang WH, Chen CS, Liao JW, Huang CJ, Lu FJ, Chia YC, Hsu HK, Wu JJ and Yang HL 2008 Food Chem. Toxicol. 46 105.
[40] Luo YQ, Fu X, You LJ, Li C, Zhang W and Chen YS 2014 J. Funct. Foods 10 427.