doi: 10.11594/jtls.08.01.12
THE JOURNAL OF TROPICAL LIFE SCIENCE
OPEN ACCESS Freely available online
VOL. 8, NO. 1, pp. 68 – 74, January 2018
Submitted November 2016; Revised January 2017; Accepted October 2017

Tetracera scandens as a Medicinal Plant: Secretory Structures, Histochemistry, and Antibacterial Activity

Evi Muliyah [1*], Sulistijorini [1], Yohana Cecilia Sulistyaningsih [1], Mohamad Rafi [2]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor, Indonesia
[2] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor, Indonesia

ABSTRACT

Tetrascera scandens, a member of Dilleniaceae, is used for traditional medicine; the stem is utilized by the *Anak Dalam* tribe of Jambi Province, Sumatera island, Indonesia, to treat diarrhea symptoms. The aims of this study were to identify the secretory structures, histochemical aspects, and the antibacterial potency of *T. scandens* stem. Histological study of the secretory structures of *T. scandens* stem was carried out. The species has idioblast cells and trichomes as its secretory structures. Histochemical analysis indicated the substance secreted by *T. scandens* idioblast cells mainly contains alkaloids, terpenoids, and phenols. Trichomes of *T. scandens* only contain flavonoids. The antibacterial activity of methanol extracts was tested against *Staphyllococcus aureus* and *Escherichia coli*. Different concentration of extracts was tested using the well diffusion method. According to the results, 100 mg/mL *T. scandens* extract showed the best inhibitory activity with a maximum inhibition zone of 17.7 mm against S. aureus and of 12.5 mm against *E. coli*. This study provides scientific evidence that the stem of *T. scandens* has antibacterial activity and justifies its use by the local community.

Keywords: *Tetrascera scandens, diarrhea medicine, secretory structures, histochemistry, antibacterial activity*

INTRODUCTION

Tetracera scandens is a shrub species of which various parts have been used for traditional medicine. It belongs to the family *Dilleniaceae* and spreads all over China, India, Malaysia, Vietnam, Philippines, Myanmar, Thailand, and Indonesia. In Vietnam, the root and stem are used to treat hepatitis, swelling, and gout [1]. The *Anak Dalam* tribe, who inhabit Bukit Duabelas National Park, utilize the stem of the species to treat diarrhea. The tribe knows *T. scandens* by its local name *akosempalay*. To treat the diarrhea, the stem is boiled with water and the decoction is drunk. The liquid and methanol extracts of *T. scandens* leaves indicate anti-diabetic potential by reducing glucose in diabetic rats [2]. The methanol extract of *T. scandens* exhibits xanthine oxidase inhibitory activity [3] and its components show highly desirable activities against T2 diabetes with significantly stimulated uptake of glucose in L6 myotubules [4]. The ethanol extract of *T. scandens* has anti-HIV activity and high inhibitory activity against HIV-1 reverse transcriptase activity [5]. The methanol extract of *T. Scandens* stem yields new nor-lupane triterpene capable of exhibiting significant concentration-dependent xanthine oxidase inhibitory activity [1]. However, the potential of *T. scandens* as diarrhea medicine was unknown, but in another extract *Dilleniace* member, i.e aqueous and methanolic extracts of *Dillenia indica* showed anti-diarrheal activity [6].

Dickison (2000) [7] reported that most medicinal plants have secretory structures that contribute in metabolites production. Certain various substances and chemical compounds such as essential oils, resins, latex, mineral salts, alkaloids, and glycosides are produced by such structures. Secretory structures are classified into external and internal ones. Studies on secretory structures, e.g. the structure, ultrastructure, size, density, his-

*Corresponding author:
Yohana Cecilia Sulistyaningsih
Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University
Kampus Dramaga, Jalan Raya Dramaga, Bogor, Indonesia 16680
E mail: yo_sulistya@yahoo.com

How to cite:
Muliyah E, Sulistijorini, Sulistyaningsih YC, Rafi M (2018) Tetracera scandens as a Medicinal Plant: Secretory Structures, Histochemistry, and Antibacterial Activity. J. Trop. Life. Science 8 (1): 68 – 74.

tochemistry, and chemical points of view of such structures, have been carried out plenty, particularly on that of *Lamiaceae* and *Asteraceae* [8, 9]. Kjaer *et al.* (2012) [10] studied the size and density of secretory structure (glandular trichomes) in *Artemisia annua*. Using histochemical assay, the secretory structure of *Salvia officinalis* is known containing terpenoids, alkaloids, tannin, flavonoids, and essential oils that are the characteristics of *Salvia* as aromatic plant [11].

Some species of *Dilleniaceae* previously reported are found having potency as antibacterial agent plant such as *Dillenia elliptica* and *Dillenia nitida*, however, the potential of *T. scandens* was unknown. Therefore, this study aimed to identify the secretory structures, histochemical aspects, and the antibacterial potency of the stem of *T. scandens*, which is used by *Anak Dalam* tribe to treat diarrhea.

MATERIALS AND METHODS

Collection of plant materials

The stems of *T. scandens* were obtained from Bukit Duabelas National Park of Jambi Province, Sumatera island, Indonesia. The plant materials were collected at September 2015. *T. scandens* located at altitudes of 73.8 m to 125.4 m above mean sea level. The fresh samples were collected and used for histochemical test. For antibacterial activity test, fresh samples were dried under sun light for 3 days, and then dried using oven at 50°C for 5 days.

Light microscope observation

To observe the presence of secretory structure in stem, transversal section of stem using freeze microtome was prepared. The size and density of secretory structures were then calculated. The metabolite contents were identified based on histochemical test. The density of secretory structure was calculated using the following formula (Modified from Willmer 1983) [12]:

$$D = \frac{Sc}{f}$$

Note:

D : The density of secretory structures

Sc : The number of secretory structures

F : The area of field of view (mm^2)

Histochemical analysis

For histochemical analysis, fresh stems were tranversely sectioned, at 20 – 25 µm by using a dual-purpose microtome (Yamato RV-240). Stem sections were then treated with various reagents and observed by a light microscope to identify the presence of terpenoids, alkaloids, phenols, and flavonoids. Terpenoids in the stem tissues were identified by soaking the section in 5% cupric acetate solution [13]. The positive result is indicated by the appearance of yellow or brownish yellow colour. Alkaloids presence was tested by soaking the section in Wagner reagent. The positive result is indicated by the presence of reddish brown or yellow deposits [14]. For phenol test, sample sections were soaked in 10% ferric trichloride and added with several flakes of sodium carbonate, then were incubated for 15 minutes in room temperature. The positive result is indicated by the appearance of dark green or black colour [15].

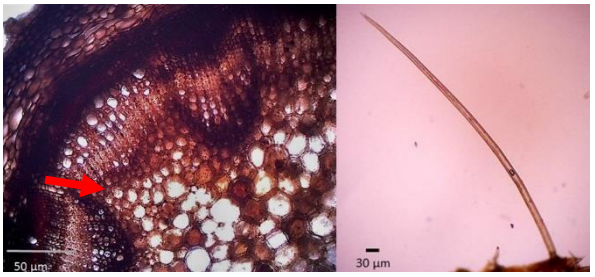
For flavonoids content, sample section was treated with 5% of aluminum trichloride ($AlCl_3$) in 85% ethanol and observed by fluorescence microscope. The positive result is indicated by the appearance of yellow, greenish yellow, or blue colour [16].

Plant extraction

Dry samples were cut into small pieces and grinded into powder. The powder was extracted using maceration method with methanol as its solvent. The extraction result was then evaporated using rotary evaporator. The extract was then diluted using 10% dimethyl sulfoxide (DMSO) into 25, 50, 75, and 100 mg/mL concentrations.

Antibacterial test

Antibacterial activity was tested using well diffusion methods [17]. The pure cultures of *Escherichia coli* and *Staphyllococcus aureus* were grown in sterile nutrient agar media and suspended in sterile nutrient broth media. The culture was incubated at 37°C for 24 hours and then resuspended in 1% liquid nutrient agar. Agar



(a) (b)

Figure 1. Secretory structures (a) and idioblast cells (red arrow); trichomes (b)

wells were prepared by using sterilized cork-borer with 7 mm diameter. As much as 50 µL of different concentrations of *T. scandens* stem extracts (100, 75, 50, and 25 mg/mL) were added to the wells in the plate. In addition, 50 µg/mL tetracycline antibiotic was used as positive and 10% DMSO as the negative control. The culture was incubated for 24 hours at 37°C. The antimicrobial activity was measured as the diameter (mm) of clear zone of growth inhibition. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

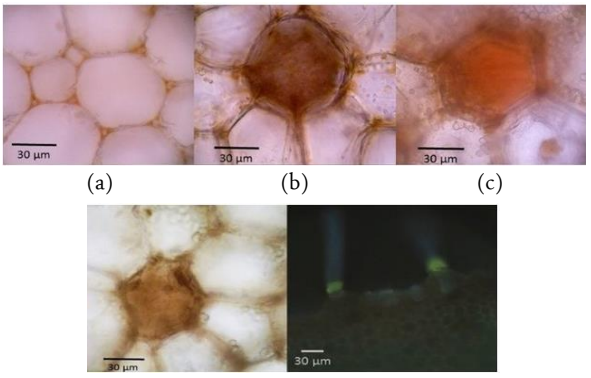
Secretory structures

In the stems of *T. scandens*, we observed two types of secretory structures i.e. idioblast cells and glandular trichomes (Figure 1). The idioblast cells are classified as internal secretory structures while trichomes are external structures [7]. Idioblast cells may be morphologically indistinguishable from their neighbors except that they contain secreted material, or they may differ to some extent. It is spherical, ellipsoidal, or branched and may contain carbohydrates, lipids, and phenolic derivatives. Idioblast cells are varied in shape and size. For example, in suspension culture of *Peganum harmala* L. cells, it is reported that idioblasts containing alkaloids are spherical, oval, and elongated cell shapes [18]. Idioblast cells in *T. scandens* stem are hexagonal and spread in pith area. Iranbakhsh (2006) [19] found that the idioblast cells in Datura stramonium semi-hyaline callus are of spherical or oval form. They have thick cell wall and large central vacuole.

Trichomes are outgrowths of the epidermis cells and vary in size and complexity, including scale and other structures and maybe glandular or stinging types [7]. The glandular trichomes of *T. scandens* are unicellular and located at the epidermal surface. Secretory structures in the form of trichomes have been widely studied, particularly in the Lamiaceae and Asteraceae. *Salvia aurea* that belongs to family Lamiaceae has two types of glandular trichomes, i.e. peltate and capitate trichomes [20]. Monteiro (2001) [21] reported the presence of ten-celled biseriate glandular trichome on both leaf surfaces of *Stevia rebaudiana* that belongs to family Asteraceae.

Histochemical analysis

The histochemical test of the idioblast cells showed a positive result for terpenoids confirmed by the brownish yellow color with cupric acetate reagent. The presence of alkaloids was shown by the formation of a positively containing phenols shown by the formation of



(a) (b) (c)

(d) (e)

Figure 2. Light (a – d) and fluorescence micrographs under UV light (e) showing the response of idioblast cells and trichomes of *T. scandens* stem to histochemical tests. Control (a); Wagner's reagent for alkaloids (b); cupric acetate for terpenoid (c); ferric trichloride for phenolic compounds (d); and aluminium trichloride for flavonoid (e)

reddish-brown deposit with Wagner reagent. Idioblast cells cells positively containing phenols shown by the formation dark colour when the samples were added with Ferric trichloride reagent (Figure 2). Such results are in accordance with Kulip *et al.* (2010) [22] that has reported *T. scandens* contains alkaloids, terpenoids, and phenols. Histochemical analysis has been widely studied on idioblast cells. Idioblast cells in *Catharanthus roseus* were reported synthesize alkaloids in the form of vindoline [23] and the idioblast cells of *Sambucus racemosa* accumulate phenols in the form of tannins [24]. In this study, histochemical analysis on glandular trichome demonstrate the presence of flavonoids, confirmed with the appearance of yellow colour after the sample was added with aluminum trichloride (Table 1). Histochemical analysis of trichome has been widely studied in family Lamiaceae. Gersbach (2001) [25] reported that *Thymus vulgaris* and *Oreganum vulgare* have peltate trichome containing phenols. Peltate trichome in Salvia officinalis contains alkaloids, terpenoids, and flavonoids [11].

Idioblast containing alkaloids has the same form with other that containing terpenoids, all of them are hexagonal. However, they are different in their size and density. Idioblast cells containing terpenoids were slightly larger than that of containing alkaloids, and they were two times larger than that of containing phenols (Table 2). The length and width idioblast cells containing terpenoids were slight larger than the others. The results are in accordance with Lima *et al.* (2014) [26] that described Dilleniaceae family has terpenoids and flavono-

Table 1. Histochemistry result of secretory structures of *T. scandens* stem

Reagent	*Target compounds*	*Observed colour*	*The presence of metabolites*
Wagner reagent	Alkaloids	Reddish brown or yellow	+
Ferric trichloride	Phenols	Dark-green	+
Cupric acetate	Terpenoids	Yellow-Brownish yellow	+
Aluminum trichloride	Flavonoids	Yellow-green yellow or blue	+

Note: (+) = positive result; and (-) = negative result

Table 2. Size and density of idioblast cells containing secondary metabolites

Secretory structures (Idioblast cells containing)	*Density (mm^{-2})*	*Size (µm)*	
		Length	*Width*
Alkaloids	61.5 ± 2.8	75.9 ± 3.6	66.6 ± 2.4
Phenols	31.5 ± 2.8	88.0 ± 4.1	69.5 ± 1.9
Terpenoids	66.8 ± 3.1	108.0 ± 6.27	82.1 ± 5.2

ids as its main secondary metabolite compounds. Other species from the same family, *Dillenia pentagyna*, produced two types of flavonoids glycosides, naringenin 7-galactosyl (1+4) glucoside and dihydroquercetin 5-galactoside [27].

Antibacterial activity of T. scandens stem extract

The extract of *T. scandens* stem has inhibition activity against *S. aureus* and *E. coli*, showed with the appearance of inhibition zone (Figure 3). According to Aneja *et al.* (2012) [28], a metabolite compound is considered has inhibition activity against bacterial growth once the size of its inhibition zone is bigger than the well diameter. Test result showed the maximum zone of inhibition against both bacteria at 100 mg/mL extract concentration of 17.7 mm and 12.5 mm, respectively (the well diameter was 7 mm) (Table 3). At the lowest concentration (25 mg/mL), the stem extract still showed inhibition activity against both bacteria with maximum zone of inhibition, 12.3 mm and 9.0 mm, respectively. *Piper betle* known have a strong antimicrobial activity. Methanol extract of *P. betle* at 500 mg/mL concentration showed the maximum zone of inhibition against *S. aureus* and *E. coli*, 25 mm and 15 mm, respectively [29].

The stem extract of *T. scandens* shows higher inhibition activity against *S. aureus* growth than against *E. coli*. Antibacterial inhibition activity against gram-positive bacteria is more apparent than against gram-negative one. There is, in part, a morphological basis for the differential susceptibilities. *E. coli*, as gram-negative bacteria, have outer membrane composed mainly of lipopolysaccharide, which is rather impermeable to lipophilic molecules, as suggested by the strong resistance of wild type strains to hydrophobic antibiotics, detergents, and to hydrophobic dyes [30]. The outer membrane also acts as a selective barrier to hydrophilic molecules with an exclusion limit of around 600 Da for sugars and peptides [31, 32]. Gram-positive bacteria lack this outer membrane but have a much thicker peptidoglycan layer, which is not an effective permeability barrier to hydrophilic solutes as its exclusion limit approximates 105 Da [33].

Antibacterial compounds are capable of being bacteriostatic, bactericidal, and bacteriolytic. Most antibacterial agents used for the treatment of bacterial infections may be categorized according to their principle mode of action. The most common modes of action are damaging cell wall, inhibiting protein and nucleic acid syntheses, hindering cell permeability, and inhibiting enzyme activity [34]. Such mechanisms inhibit bacterial growth, indicated by clear zone on media containing plant extract suspected containing anti-bacterial compound. Antibiotic used as positive control in this study was tetracycline, a wide-spectrum compound that able to inhibit both gram-positive and gram-negative bacteria by inhibiting protein synthesis. Negative control used was 10% DMSO that is solvent for stem extract. This solvent was used as comparison to observe the effect of the solvent on inhibition zone produced by the extract.

Studies on antibacterial activities of family Dilleniaceae are plenty. Wiart *et al.* (2004) [35] reported

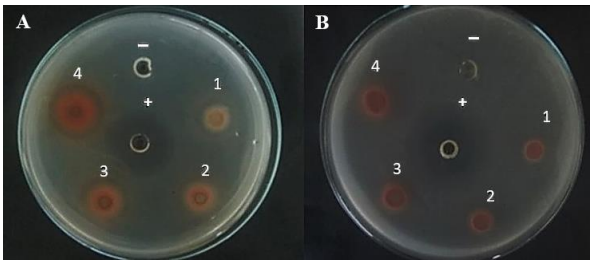


Figure 3. Antibacterial activity of *T. scandens* stem extraction to *S. aureus* (A) and *E. coli* (B). The extract concentrations are 25 mg/mL (1); 50 mg/mL (2); 75 mg/mL (3); 100 mg/mL (4); tetracyclin 30 µg/mL (+); DMSO 10% (-)

Tabel 3. Antibacterial activity of *T. scandens* stem extract against *S. aureus* and *E. coli*

Concentration (mg/mL)	*Diameter of the inhibition zone (mm)*	
	S. aureus	*E.coli*
25	12.3 ± 0.1	9.0 ± 0.0
50	13.5 ± 0.1	11.0 ± 0.1
75	16.0 ± 0.1	12.0 ± 0.1
100	17.7 ± 0.1	12.5 ± 0.1
50 µg/mL Tetracyclin *	20.7 ± 0.0	17.7 ± 0.0
10% DMSO **	-	-

Note: (*) as a positive control, (**) as a negative control

that *Dillenia suffruticosa* is capable of inhibiting *Bacillus cereus*, *B. subtilis*, *Candida albicans*, and *Pseudomonas aeruginosa*. Methanol extracts of *Davilla elliptica* and *Davilla nitida* leaves are potential to inhibit *Helicobacter pylori* [36]. The capability of *T. scandens* stem extract in inhibiting bacterial activity is because of its metabolite content. Histochemical test revealed that *T. scandens* stem contains phenols, alkaloids, terpenoids, and flavonoids. According to Ahmad *et al.* (2014) [37], flavonoids in the form of 3',4',5,7 tetramethoxyflavone are able to inhibit the growth of S. aureus. Djoukeng *et al.* (2005) [38] reported that the leaf extract of *Syzygium guineense* (Myrtaceae) contains terpenoids in the form of asiatic acid and a mixture of terminolic acid, made it able to inhibit the growth of *E. coli*, *B. subtilis*, and *Shigella sonnei*. Phenols in the form of hydroquinone are capable of inhibiting the growth of *S. aureus* and *E. coli* [39]. These properties may explain the mechanisms of action of the plant extracts. Further purification of the antibacterial principles in the extracts are needed. Such substances in the purified state may be useful for the treatment of this conditions.

CONCLUSION

Stem of *T. scandens* has idioblast cells and unicellular glandular trichomes as its secretory structures. Histochemical reactions indicate the substance secreted by *T. scandens* idioblast cells mainly contain alkaloids, terpenoids, and phenols. Glandular trichomes of *T. scandens* only contain flavonoids. *T. scandens* stem extract have a potency as antibacterial agent against *S. aureus* and *E. coli*. Stem extract at concentration of 100 mg/mL showed the best inhibitory activity.

ACKNOWLEDGMENT

The authors express the deepest appreciation to Directorate General of Higher Education through BOPTN funding of 2015 for providing financial support for this research.

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