Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(4): 179-184
n harves: 12-09-2015
Accepted: 13-10-2015

Lianah Kuswanto
IAIN Walisongo, Jl Walisongo 4-5, Semarang, Indonesia 50185.

Krisantini
Bogor Agricultural University, Kampus IPB Darmaga, Indonesia 16680.

Peter Sopade
Centre for Nutrition and Food Science, the University of Queensland, St Lucia QLD 4072, Australia.

Correspondence:
Lianah Kuswanto
IAIN Walisongo, Jl Walisongo 4-5, Semarang, Indonesia 50185.

Status of traditional herb *Tetrastigma glabratum* (Blume). Planch in Mt Prau, Central Java, Indonesia

Lianah Kuswanto, Krisantini, Peter Sopade

Abstract
Medicinal plants growing in Mt Prau, Central Java, Indonesia were surveyed, and their traditional uses and preparation methods were documented. The entire study area was stratified into three zones based on altitudes, and a total of 23 medicinal species were identified and recorded. *Tetrastigma glabratum* (Blume) Planch was the main herbal used in Mt Prau for at least three generations. The exudate from large stem is believed to cure several ailments and diseases. *T. glabratum* was found in small numbers at >1300m above sea level and was not found in the lower altitudes. Preliminary phytochemical analysis in *T. glabratum* plant extracts confirmed the presence of diverse group of phytochemicals (steroids, terpenoids, flavonoids, saponins, and tannins). There is a need to conserve *T. glabratum*, and recommendations are made.

Keywords: medicinal plants, traditional uses, conservation, habitat degradation

1. Introduction
People in Indonesia have been using plants as a source of traditional herbal remedies for generations. In Surakarta, Central Java alone, 1,734 traditional plant-based formulas for healing have been documented [17]. Harvesting medicinal plants from the rainforest have been a common practice in countries, such as India [8, 17], Nigeria (Osemeobo, 2010) and Indonesia [17]. Harvesting and trades in medicinal plants taken from natural habitats generated huge incomes in Nigeria [18] but often resulted in destruction of natural forests.
Mt Prau, Central Java, Indonesia is home to diverse plant species including medicinal plants, unique ornamental carnivorous plants *Nepenthes* and black lip orchid *Coelogyne pandurata* Lindl. [13]. It is also home to endemic animals such as leopard (*Panthera pardus*), Javan hawk-eagle (*Nisaetus bartelsi*), Javan rusa (*Cervus timorensis)*, and black monkey (*Presbytis comate*) [13]. Amongst many plant species with ethno-pharmacological uses in Mt Prau, is *Tetrastigma glabratum*, and its local names include *walikadep*, *gangputih*, *akar darik-darik*, *bantengan*, *oyod epek*, and *oyonwaliran* [19, 13]. The exudate from *T. glabratum* stem is believed to cure a number of ailments and diseases, has refreshing and stimulating effects, and increases appetites.
Tetrastigma is a widespread genus of approximately 100 species occurring from Asia to Oceania [9, 18]. The species are found in subtropical and tropical regions of Asia and Australia, and suited to grow in hillsides and valleys of shady and moist primary rainforests [20]. *Tetrastigma* is the sole host species of the *Rafflesia* (Rafflesiaceae), a parasitic genus that has the largest flower of all flowering plants [15]. Despite the long history of traditional uses of plants in Central Java, Indonesia, limited pharmacological studies of these plants exist. Abdiyani (2008) reported the diversity of medicinal plants in Dieng Plateau, Central Java. Medicinal plants produce secondary metabolites that have a broad range of therapeutic properties. The present study describes the identification and uses of traditional herbs growing in protected rainforests at Mt Prau, Candiroto, North Kedu, Central Java, Indonesia, with particular interests in the distribution, population and ecology of *T. glabratum*. Preliminary phytochemical profiling is presented.

2 Materials and Methods
2.1 Study area
The study was conducted in the protected rainforests at Mount Prau, Candiroto, North Kedu (7°42”S, 3°45”E), Central Java, Indonesia. Mt Prauis located about 95km North East of Semarang city [2]. Mt Prau area has a relative humidity of 80%, soil pH of 6.9, slopes of up to 60°, day and night temperatures of 20-25 °C and 16-20 °C respectively, and a precipitation of

3,394 mm per year (BMKG, 2014). The study area was stratified into three altitudinal zones (1,000, 1,300, and 1,300-1,600 m above sea level) with a study area of 2,000m^2 per zone.

2.2 Ethnobotanical survey

The Ethnobotanical survey was conducted throughout Mt Prau area in 2013 and 2014. Two villages, Jiwan and Blumah, located in the study area were selected. The respondents were selected randomly from 468 families from the two villages with a total respondent of 48 people that included two elderlies, who have been harvesting medicinal plants from the protected forest for more than 30 years. Unstructured and semi-structured questionnaires were administered, and information was collected on the herbal plants in use, plant parts used, local names of the plant species, and preparation methods of the herbs. Field visits were made with the two elderlies for identification and verification of the plant species in use.

2.3 Characteristics of population ecology of T. glabratum

Sampling was done in three plots of 20x100 m placed randomly in each zone, and the number of individual *T. glabratum* plants was calculated in each plot within each zone. The following characteristics were recorded per plant: (i) plant size, (ii) diameter at breast height (mm), (iii) stage of growth (vegetative or generative), (iv) the main accompanying higher plants, (v) estimated density (plant per ha), calculated from the number of plants found in the study area.

2.4 Phytochemical analysis

Steroid, terpenoid, flavonoid, caffeine and nicotine contents were analysed using Thin Layer Chromatography (TLC). Fresh leaves and stems of *T. glabratum*, at the vegetative (non-flowering) stage, were collected. The plant materials were washed and rinsed with distilled water, and dried (60 ºC) in an oven before grinding into a fine powder. From each plant sample, 100g powder was macerated with ethanol (1:5) in a sealed container for three days at room temperature with occasional shaking. Extracts of each sample were filtered separately through What man No.1 filter paper, evaporated to dryness and stored at 5 ºC until analysis. The prepared plant extracts were applied on pre-coated reactivated (105°C for 30 min.) TLC plates (Silica gel 60 F254 pre-coated TLC aluminium sheets, Merck Art. 5554) by using capillary tubes, and developed in a TLC chamber (Chromato-Vue cabinet CC-20 of Ultra-violet Products, Inc.) using a suitable mobile phase. The developed TLC plates were air-dried and observed under UV light at 254 nm and 366 nm. They were later sprayed with different reagents for the development of colour in separated bands. The movement of the analyte was expressed by its retention factor (R_f):

R_f (Retention factor)=Distance travel by solute/Distance travel by solvent

A mobile phase of toluene and ethyl acetate (93:7) was used to identify terpenoids with terpineol as a comparator, and a red violet colour indicating the presence of terpenoids. Steroids identification used benzene and ethyl acetate (70:30) mobile phase, detection with Liebermann-Burchard added to beta-sitosterol as a comparator. A red violet colour indicated the presence of steroids. Flavonoids identification used ethyl acetate: methanol: formic acid (95:5:0.5) as a mobile phase, detection with aluminium chloride and quercetin as comparators, and a yellow colour indicated the presence of quercetins. Detection of nicotines used mobile phase of toluene, ethyl acetate and diethyl amine (70:20:10) and Dragendorff's reagent as detector, with nicotine as a comparator; a chocolate orange colour indicated the presence of nicotines. Caffeins were detected using mobile phase of ethyl acetate, methanol and H2O (100:13.5:10) with caffeine as a comparator [5].

Alkaloids, phenolics, saponins were identified using the method of Harborne (1984). Dragendorff 'sreagent was used to test for alkaloids. The reagents consisted of (a) 0.85g of potassium bismuth substrate dissolved in a solution of 10 mL of acetic acid and 40 mL of water, and (b) 8g of potassium iodide (KI) dissolved in 20 mL of water. A stock solution was prepared by mixing equal parts of solutions (a) and (b). The spray reagent was prepared by mixing 1 mL of the stock solution with 2 mL of glacial acetic acid and 10 mL of water. To 1 mL of each extracts, 1 mL of Dragendorff's reagent was added. The presence of alkaloids was detected by orange-brown spots on yellow background [6].

Powdered plant leaves and stem of the test plant were weighed (1g) into a beaker and 10 mL of distilled water was added. The mixture was boiled for 5 min. Two drops of 5% ferric chloride ($FeCl_3$) were then added. Production of a greenish precipitate indicated the presence of tannins. For identification of phenols the TLC plates were sprayed with 1 N Folin-Ciocalteu reagent and the plates were heated at 80 °C for 10 min. The colour and Rf values of the spots were recorded under visible light. Blue coloured spots indicated the presence of phenols [6]. The presence of saponins was tested by shaking 500 mg of the plant extracts in a test tube for 5 min., with foams indicating saponins [6].

3. Results and Discussion

3.1. Ethnobotanical survey

The survey revealed that 26 plant species grown in the protected forests of Mt Prau have been used as traditional herbs. The species, local names, uses and traditional methods of processing the plant parts, are listed in Table 1. *T. glabratum* is one of the medicinal species that has been consistently searched for use, particularly the plants with stem diameter of >50mm. *T. glabratum* is a climbing perennial vine with tendrils, whose leaves consist of five leaflets, serrated and arranged palmately compound, fig. 1A. The young stems are reddish in colour and have tendrils, fig. 1B.

The liquid extracted from large stems of *T. glabratum* is used for medicinal purposes, and it is usually collected and stored in bottles, or is drunk directly from the freshly-cut stems. Only large stems of around 30-50 mm in diameter are believed to contain enough liquid. However, this practice of harvesting large stems has killed most large size *T. glabratum* plants in the area.

Table 1: Medicinal plant species, habitat and their traditional uses in Mt Prau, Central Java, Indonesia

Species	Local name	Uses	Part of plants used	Altitude(m, above sea level)	Traditional method of processing
Curcuma sp.	Jahe-jahean	Rheumatics	Rhizomes of medium to mature plants	1000-1300	Grind to fine powder, disperse in boiled water
Bergenia	Sukma	Coughing	Mature leaves	1300-1600	Grind to fine powder, disperse in boiled water
Cinchona	Kina	Anti-malaria	Barks and stems	1000-1600	Grind, disperse in water
T. glabratum	Walikadep	Coughing, refreshment	Stem exudates from >50mm stem diameter	1300-1600	Collect liquid in bottle, or drink from freshly-cut stems
Centella asiata	Pegagan	Nutrition	Young leaves	1000-1300	Boil young leaves in water, or eat fresh
Nasturtium microphyllum	Selada air	Anti-cancer	Leaves and stem of medium to mature plants		Boil leaves in water prior to drinking
Impatiens balsamina	Pacar air	Lower blood cholesterol	Leaves of medium to mature plants	1000-1300	Boil leaves prior to drinking
Piper nigrum	Lada	Cure impotency, rheumatics, anti-malaria	Fruits		Grind, disperse in water
Piper beetle	Sirih	Coughing, bronchitis, cure eye itchiness, nose bleed	Fully developed Leaves	1000-1300	Boil leaves in water
Solanum torvum	Ter	Back pain, swelling from a fall or bumping, irregular periods, skin infection	Fruit and leaves	1000-1300	Boil fruits in water or eat fresh Grind leaves, disperse in water prior to drinking
Zingiber officinale	Jahe	Hypertension, skin itchiness due to insect bites, reduce blood vessel clogging	Rhizomes from mature plants	1000-1300	Grind prior to drinking
Curcuma domestica	Kunyit	Anti-typhoid, diabetes and anaemic	Rhizomes from medium to mature plants	1000-1300 1300-1600	Grind prior to drinking
Hedyotis corymbosa	Rumput mutiara	Colds and body warmer	Roots from mature plants	1000-1300	Grind roots, and use externally
Sida rhombifolia	Sidaguri	Anti-rheumatics, various skin infection and rash, oedema	Leaves from mature plants		
Medinilla speciosa	Parijoto	Mouth ulcer, diarrhoea	Fruits	1300-1600	
Anredera cordifolia	Binahong	Promote fast recovery after major surgery	Leaves from mature plants		
Amomum compactum	Kapulogo	Pain killer, avoid osteoporosis	Fruits		Grind prior to drinking
Blumea balsamifera	Sembung	Rheumatics, menstrual pains, regulate irregular menstruation, anti-bloating, increase appetite, anti-worm	Leaves and Roots	700-1000	Grind and boil in water prior to drinking
Boesenbergia rotunda	Temu kunci	*Panas dalam*	Rhizomes from medium age to mature plants	700-1000	Grind
Orthosiphon aristatus	Kumis kucing	Diuretic	Leaves from medium age to mature plants	700-1000	Boil leaves prior to drinking
Isotoma longiflora	Kitolod	Eye drops	Flowers		Drops from liquid of the basal parts of the flower
Cinnamomun burmannii	Kayu manis	Diabetes, relax muscles	Barks from medium age to mature trees	1000-1300 1300-1600	Boil barks prior to drinking
Phyllanthus niruri		Increase appetite, diarrhoea, hepatitis, mouth ulcer, gouts, prevent infection from dog bites	All parts of plants (leaves, stem, roots, fruits)		Boilprior to drinking

Fig 1: Three-month-old *Tetrastigma glabratum* propagated by cutting (A); leaves, young red stems and tendrils (B)

3.2. Status of *T. glabratum* in Mt Prau

T. glabratum was found only in small numbers in the surveyed area sat 1300 and 1600m altitudes (Table 2). All large *T. glabratum* plants in Mt Prau were found climbing on *Mranak* trees (*Castanopsis acuminatissima* (Bl.) A. DC), which was the predominant tree species growing at higher elevations on Mt Prau. These trees grow 20-40m high with a large canopy, and distinctly characterised by the presence of a specific mushroom growth on the old trunks, with a local name of 'jamurpete'. Two companion species that were found to grow in the proximity of *T. glabratum* were *Imperata cylindrica* and *Lantana camara*. During the survey, none of the mature plants had flowers, and no seedlings or small *T. glabratum* plants were found in all the study areas. *T. glabratum* was found to grow in localised patches and was not distributed uniformly. We suspect that the large plants had been harvested before they reached reproductive stage.

Large *T. glabratum* canopy had large coverage and shaded the area underneath it, which limited the number of plants and plant species that grewaround them. There was no *T. glabratum* found in the lower altitudes, and this was presumably due to heavy extractions of the plants. It needs about five years to grow from cuttings until the diameter of the plant stem reaches 20-30mm before it can be harvested for its liquid content [13]. Unfortunately, local agencies have not taken measures for systematic protection, propagation and planting back into the natural habitats. We, therefore, propose that a protected area be established to grow this species for long-term benefits to the local community. The remaining areas in the wild should be protected for *in situ* conservation by banning or reducing exploitation of this species from its natural habitat.

Table 2*: T. glabratum* population in Mt Prau, Indonesia[1]

	Altitude (m, above the sea level)		
	1000	1300	1600
Slope	30-45%	45-60%	45-60%
Number of individual *T. glabratum* plants	none	3	7
Size	Large	Large	Large
Estimated plant density (plant per ha)	0	5	11

[1]Large: stem diameter >50mm, coverage >9m^2.

3.3. Phytochemical studies

The TLC profiling of *T. glabratum* plant extracts confirmed the presence of a diverse group of phytochemicals: terpenoids, steroids and flavonoids (Table 3), but nicotines and caffeines were absent. Colour visualisation test indicated the presence of saponins and tannins in the leaf and stem extracts, which did not reveal caffeines, nicotines and alkaloids. The TLC of the stem extract of *T. glabratum* revealed two terpenoid compounds having R_f values of 0.31 and 0.63, and three compounds having R_f values of 0.31, 0.91, and 0.94 in the leaf extracts. Both TLC tests used a solvent phase of toluene and ethyl acetate (93:7). The TLC of the stem extracts of *T. glabratum* also showed that three steroid compounds having R_f values of 0.67, 0.74, and 0.96 were present, while the leaf extracts indicated the presence of five compounds with R_f values of 0.67, 0.74, 0.84, 0.89, and 0.96 (Table 3). Both TLC tests used a solvent phase of benzene and ethyl acetate (70:30). One flavonoid with $R_f$valueof0.91 was identified from both stem and leaf extracts using a solvent phase of ethyl acetate-methanol-formic acid (95:5:0.5) (Table 3).

Many studies have reported a variety of biochemical components of *Tetrastigma* worldwide, for example in China [10, 11, 12]. and Malaysia [4]. *T. hemsleyanum* possesses the functions of antipyretic, detoxification, anti-inflammatory, improving blood circulation, and relieving pain [12]. Its antiviral and antitumor properties have been described [22, 24].Ten chemical compounds had been isolated from *T. hypoglaucum*, which has been traditionally used to treat fracture, traumatic injury and swelling pain [12]. Din *et al*. (2002) reported that *T. dubium* Planch, *T. hookeri* Planch and *T. pedunculare* Planch of East Malaysia contained saponins. Despite these studies and knowledge on *Tetrastigma*, a few studies have been conducted on *Tetrastigma* in Indonesia [25] which is the centre of biodiversity of *Rafflesiaceae* [16]. From their studies in Riau, East Sumatra, Indonesia, Sofiyanti *et al*. (2008) reported that *Rafflesia hasseltii* and its host, *T. leucostaphylum*, had the similar alkaloid and phenolic compounds (i.e. nicotines, caffeines, catechins, leucoanthocyanins, and phenolic acids) as *Tetrastigma* elsewhere. The TLC profiling of the *T. glabratum* extracts in the present study, confirmed the presence of a number of phytochemicals with different retention factors in different solvents. These preliminary results provide important direction in understanding their polarity for further identification, isolation and characterisation of these bioactive compounds.

Table 3: Phytochemical analysis of stem and leaf extracts of *T. glabratum*bythinlayerchromatography[1]

Chemical Name	Solvent	Plant part	R_f values
Steroids	B: EA (70:30)	Stem	0.67; 0.74; 0.96
		Leaf	0.67; 0.74; 0.84; 0.8; 0.96
Terpenoids	T: EA (93:7)	Stem	0.31; 0.63
		Leaf	0.31; 0.91; 0.94
Flavonoid	EA: M: FA (95:5:0.5)	Stem	0.91
		Leaf	0.91

[1]T: toluene, M: methanol, B: Benzene, EA: Ethyl acetate, FA: Formic acid, adsorbent: Silicagel 60F254 (Al-sheet)

4. Conclusions

The remaining *T. glabratum* plants in Mt Prau grow on altitudes 1,300 and 1,600 m above sea level, and no *T. glabratum* plants, seedlings and mature, were found in the lower altitudes. *Ex-situ* conservation is important to protect this species from extinction, and to maintain a sustainable supply for its use by the inhabitants. The present study shows the presence of a number of phytochemicals in *T. glabratum*, thereby providing a valuable phytomarker for further studies of its bioactive compounds.

Acknowledgements

The author thanks ISDB and University of Walisongo, Indonesia for their funding of the research. The information on local knowledge of *Tetrastigma* growth, ecology and traditional uses from Mr Supangat, the late Mr Solikhin of Blumah village and Mr Parso of Tawang village, is highly appreciated.

References

1. Abdiyani S. Keanekaragaman Jenis Tumbuhan Bawah Berkhasiat Obat di datarantinggi Dieng. Jurnal Penelitian Hutandan Konservasi Alam 2008; 5:79-92.
2. Anon Profil KPH Kedu Utara. BKPH Candiroto, Perum Perhutani KPH Kedu Utara, Kedu, 2009.
3. Bhawani SA, Sulaiman O, Hashim R, Mohamad Ibrahim MN. Thin-Layer Chromatographic Analysis of Steroids: A Review, Tropical Journal of Pharmaceutical Research. 2010; 9:301-313.
4. Din LB, Yusoff NI, Samsudin MW, Suki U, Salleh KM, Ibrahim ZA. A Preliminary Phytochemical Survey of Plants in Crocker Range, Sabah, Malaysia. ASEAN Review of Biodiversity and Environmental Conservation, Malaysia, 2002.
5. Drozd J. TLC of Psychostimulants In: Komsta L, Waksmundzka-Hajnos M, Sherma J. (Eds), Thin Layer Chromatography in Drug Analysis. CRC Press, 2014, 406-407.
6. Harborne JB. Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Edn2. Chapman and Hall, 1984.
7. Hyne K. Tumbuhan Berguna Indonesia Vol III. Balitbang Kehutanan, Jakarta, 1987.
8. Kala CK. Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya. Biological Conservation 2000; 93:371-379.
9. Li CL, Wu ZY. A taxonomical study on Tetrastigma (Miq.) Planch. In China. Chinese Journal of Applied Environmental Biology 1995; 1:307-333.
10. Liu D, Yang JS. A study on chemical components of *Tetrastigma hemsleyanum* Diels et Gilg. native to China. Zhongguo-Zhong-Yao-Za-Zhi 1999; 24:611-612.
11. Liu D, Ju JH, Lin G, Xu XD, Yang JS, Tu GZ. New C-glycosyl flavones from *Tetrastigma hemsleyanum* (Vitaceae). Acta Botanica Sinica 2002; 44:227-229.
12. Liu D, Ju JH, Yang JS. Studies on chemical constituent on *Tetrastigma hypoglaucum*. Chinese Traditional Herbal and Drugs 2003; 34:4-6.
13. Lianah. Implikasi Lingkungan Pemanfaatan Tumbuhan Walikadep (*Tetrastigma glabratum*) untuk Bahan Obat Traditional. Unpublished PhD Thesis, University of Diponegoro, 2013.
14. Liu D, Ju JH, Lin G, Xu XD, Yang JS, Tu GZ. New C-glycosyl flavones from *Tetrastigma hemsleyanum* (Vitaceae). Acta Botanica Sinica, 2002; 44:227-229.
15. Meiyer W. Rafflesiaceae. Flora Malesiana Series 1997; 13:1-42.
16. Nair J. Rafflesia of the World. Natural History Publication, Malaysia, 2010.
17. Osemeobo GJ. Can the rainforests of Nigeria sustain trade in medicinal plants? International Journal of Social Forestry. 2010; 3:66-80.
18. Prapti IY. Implementation of Herbal Medicine (Jamu) Networking. Country Report Indonesia, 2011.
19. Rai LK, Prasad P, Sharma E. Conservation threats to some important medicinal plants of the Sikkim Himalaya. Biological Conservation 2000; 93:27-33.

20. Ren H, Wen J. TetrastigmaIn, Wu ZY, Raven PH. (Eds), Flora of China. Beijing. St. Louis Missouri Botanical Garden Press, 2007, 195-208.
21. Sofianti N, Wahibah NN, Purwanto D, Syahputra E, Mat-Salleh K. Alkaloid andphenolic compounds of *Rafflesia hasseltii*Suringar and its host *Tetrastigma leucostaphylum* (Dennst.) Alston ex Mabb. in Bukit Tigapuluh National Park, Riau: a preliminary study. Biodiversitas 2008; 9:17-20.
22. Xu C, Ding GQ, Fu JY, Meng J, Zhang RH, Lou, XM. Immunoregulatory effects of ethyl-acetate fraction of extracts from *Tetrastigma hemsleyanum* Diels et. Gilg on immune functions of ICR mice. Biomedical and Environmental Science 2008; 21:325-331.
23. Yamuna DM, Wesely EG, Johnson M. Chromatographic studies on the tannins of *Aerva lanata* (L.) Juss. Ex Schultes. IOSR Journal of Pharmacy. 2012; 2:2250-3013.
24. Yang XL, Luo J, Sun SB. Research on anti-virus function of *Tetrastigma hemsleyanum*. Hubei Journal of Traditional Chinese Medicine. 1989; 4:40-41.
25. Zuhud EAM, Hikmat A, Jamil N. Rafflesia Indonesia: Keanekaragaman, Ekologi dan Pelestariannya. Yayasan Pembinaan Suaka Alamdan Suaka Margasatwa Indonesia dan Laboratorium Konservasi Tumbuhan, Departemen Konservasi Sumberdaya Hutan, Fakultas Kehutanan, Institut Pertanian Bogor, 1998.