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Ethnomedicinal study, phytochemical characterization, and pharmacological confirmation of selected medicinal plant on the northern slope of Mount Wilis, East Java, Indonesia

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Abstract. Bhagawan WS, Suproborini A, Putri DLP, Nurfatma A, Putra 2022. Ethnomedicinal study, phytochemical characterization, and pharmacological confirmation of selected medicinal plant on the northern slope of Mount Wilis, East Java, Indonesia. Biodiversitas 23: 4303-4313. The traditional usage of plants in ethnomedicine is a valuable resource for human primary healthcare. The effectiveness of herbal medicines and the creation of new drugs are proven by pharmacological and phytochemical verification. This study documented the traditional ethnomedicine knowledge (TEK) on the northern slope of Mount Wilis community utilizing medicinal plants and revealed the ethnopharmacology relevance of selected medicinal plants. Data on TEK and its practices were collected through in-depth and semi-structured interviews with informants who were selected using purposive sampling and snowball sampling techniques. The phytochemical compounds of the selected medicinal plant were identified using the UPLC-Qtof-MS/MS instrument, while their pharmacological activity was measured in antibacterial tests. The results showed that the community continued to apply TEK that had been passed down by the previous generation through a method called *Gethok Tular*. The medicinal plant species in the study area were diverse, 82 species from 39 families with various modes of preparation were used to treat seven disease categories. *Tithonia diversivolia* had the highest use value of 0.65 and it contained 23 phytochemical compounds. In addition, this plant has high pharmacological potential for antibacterial agents.

Keywords: Ethnomedicinal study, Mount Wilis, pharmacological activity, phytochemical compounds, *Tithonia diversivolia*

INTRODUCTION

Plants have been used in primary health care in the world since ancient times (James et al. 2018). The use of plants for treatment has been implemented across generation, until traditional ethnomedicine knowledge (TEK) was formed (Reyes-García 2010). UNESCO acknowledges TEK as an intangible cultural heritage that should be preserved (Vecco 2010), especially in tropical countries where shifts in traditional culture occur rapidly (Vandebroek and Balick 2012). In the last decade, efforts to preserve TEK have been carried out by many researchers in various tropical countries (de Santana et al. 2016; Gumisiriza et al. 2021; Leonti et al. 2010; Purushothaman et al. 2020), including Indonesia (Silalahi et al. 2019; Sujarwo et al. 2014).

TEK is a popular basis for the development of drugs or traditional drugs (Newman and Cragg 2016). The development of new traditional drugs has employed TEK, for example, field study in Papua followed by laboratory testing to develop new antifertility drugs for males (Bhagawan et al. 2017; Prajogo 2014). The benefits of ethnomedicinal research also must be given to the local community itself (Heinrich et al. 2017). Ethnomedicine study is also useful for preserving the cultural heritage of medicinal plants (Indrawati et al. 2022). In an interesting

example, several researches were conducted in West Timor to find a conventional method of malaria treatment using plants in Tetun community (Taek 2020; Taek et al. 2021). In Indonesia, TEK varies in each community, depending on the type of specific disease, plant biodiversity, human-environment interaction, and geographical conditions (Taek et al. 2019, 2018). Indonesia has high biodiversity, diverse indigenous cultures, and a large variety of landscapes, where its mountainous areas have abundant plant species and TEK (Brambach et al. 2017; Batoro et al. 2019; Iswandono et al. 2016).

The community living around Mount Wilis has been practicing TEK for generations. Kare sub-district, which consists of eight villages located on the northern slope of Mount Wilis, with an altitude between 700 and 2,100 meters above sea level has diverse botanical potentials. The community has been using plants grown around them or from the forest for health care (Indonesian Central Bureau of Statistics 2020). Over the past decade, research on ethnomedicine has shown various uses of medicinal plants in mountainous areas on Java Island, for example: Bromo Tengger Semeru National Park (Batoro and Siswanto 2017; Bhagawan et al. 2021, 2020; Bhagawan and Kusumawati 2021; Jadid et al. 2020; Shalas et al. 2021), around the slope of Mount Merapi (Nahdi et al. 2016; Nahdi and Kurniawan 2019), and Mount Gede Pangrango (Rosita et

al. 2015; Sihotang 2011; Susiarti et al. 2018). However, the TEK and practices by the community in the slope area of Wilis Mountain have never been documented. Beginning with a field study to determine the plant with the most citations, followed by laboratory studies is the most effective approach to finding new drug compound candidates (Andrade-Cetto and Heinrich 2011). In addition, scientific evidence on the efficacy of using herbal medicines by local communities is important (Heinrich et al. 2017; Muchtaromah et al. 2021; Widyowati and Agil 2018). Regarding the aforementioned facts, this research was conducted to: (1) document traditional ethnomedicine knowledge of medicinal plants on the northern slope of Mount Wilis, (2) reveal selected medicinal plants based on ethnomedical field studies, (3) profile phytochemical compounds in selected medicinal plant, and (4) confirm the pharmacological potential of selected medicinal plants.

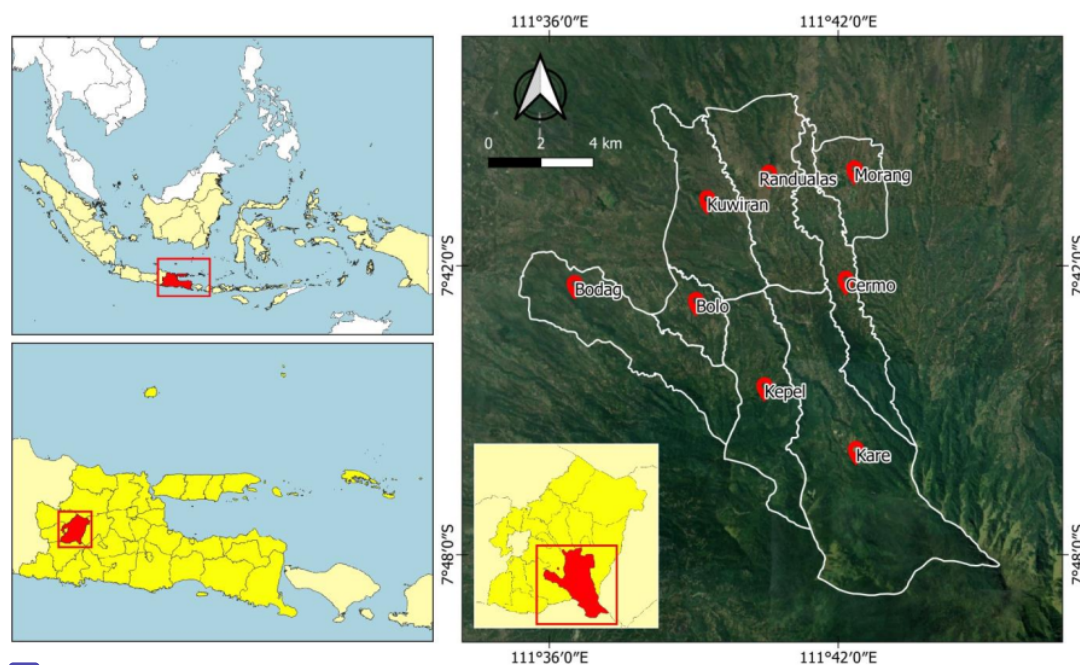
MATERIALS AND METHODS

Ethnomedicinal field study

This research took place at the northern slope of Mount Wilis, Kare Sub-District, Madiun District which has high biodiversity. Eight villages (namely: Bodag, Bolo, Cermo, Kare, Kepel, Kuwiran, Morang, and Randualas villages) were designated as research sites (Figure 1). Ethnomedicinal data were collected for eleven months

from March 2020 to January 2021 based on a preliminary study conducted by the first author in September 2019. Written ethical consent was also obtained from the government of Madiun District (No. 072/232/402301) and verbal consent to conduct the study was requested from village heads. People who know and use at least one species of medicinal plant are referred to as traditional healers in this research. There were 189 traditional healers identified and registered. From this list, one elderly member in each village was selected as a key informant. After that, key informants were asked to recommend other informants in the snowball sampling method. Semi-structured interviews and direct observations were administered to collect ethnomedicinal data.

Informants' demographic data (among others: age, gender, education level, main occupation), TEK sources and transfers, names of plants, types of human diseases treated, plant parts used, preparation method, and route of drug administration were collected during the interview and observation. Plant taxonomic identification was carried out by the second author based on the Flora of Java book (Backer and Steenis 1973). Plants' scientific names were also determined in reference to a website <http://www.theplantlist.org/>. Meanwhile, plants that could not be identified by the second author were photographed and herbariumed for scientific name identification by taxonomists in Materia Medika, Batu.



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Figure 1. Location of the study area, eight villages on the northern slope of Mount Wilis, East Java, Indonesia

Quantitative ethnomedicinal data analysis

Use Value (UV) index was used to quantify and determine plants with the strongest pharmacological and phytochemical relevance. Plants with high UV levels indicate the potential of these plants to be developed as new drugs. This index was first proposed by Phillips and Ge (1993a, 1993b), which was adopted in the research of de Albuquerque et al. (2007). The following formula was used to calculate plant citations in the field study.

$$UV = \sum U_i / ns$$

Where; U_i is the total citation for certain species divided by the number of informants (ns).

Preparation of the plant extract

The leaves of *Tithonia diversifolia* were collected, dried and made into powder. Extraction was performed in remaceration method using 100 g of simplicia dry powder of *T. diversifolia* leaves. The powder was soaked in 5 L of 96% ethanol solvent for 24 hours before being filtered using filter paper to take only the filtrate. The second immersion was carried out using 2.5 L of 96% ethanol solvent in the same way, which was then repeated for the third immersion until reaching 10 L of solvent. The filtrate was evaporated in a rotary evaporator at a temperature of 55°C and 120 rpm until the extract thickened. The thickened extract was then put into an oven at 50°C to remove the solvent. The 96% ethanol extract was ready for phytochemical analysis and in vitro pharmacology test.

Phytochemical characterization using UPLC-QToF-MS/MS

Phytochemical characterization was performed at the Central Police Forensic Laboratory, Jakarta. A 96% ethanol extract of *T. diversifolia* of 100 ppm was prepared in methanol and 5 µL of the extract was then injected into the ACQUITY UPLC® H-Class System (Waters, USA) tandem detector MS Xevo G2-S QToF (Waters, USA). Samples were separated in ACQUITY BEH C18 (1.7 µm 2.1x50 mm) using 0.05% acetonitrile : 0.05% and formic acid as mobile phase with a flow rate of 0.2 mL/min. The results of the UPLC-MS analysis were processed using the Masslynx version 6.1 application, resulting in the chromatogram and spectra of each detected peak. The prediction of the compound was made on the ChemSpider website.

In vitro pharmacological confirmation

A pharmacological activity test was performed using the agar disk-diffusion method against gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*) bacteria according to the CLSI (2012) procedure. The in vitro antibacterial testing was carried out at the Microbiology Laboratory of the State University of Malang. One mL of *B. subtilis* and *E. coli* cultures were put on a sterile petri dish. The cooled Mueller-Hinton Agar (MHA) was melted and poured into a petri dish containing the inoculum and stirred well. After compaction, wells were drilled using a sterile cork borer (6 mm in diameter) into the agar plate containing inoculum. Then, 30 µL of each extract concentration (2.5 mg/mL; 5 mg/mL; 10

mg/mL; 20 mg/mL; and 40 mg/mL) was added to each well. The plates were placed in the refrigerator for 30 minutes to allow the extract to diffuse well to the agar. The plate was then incubated at 37°C for 24 hours. The diameter of the inhibition growth zone was measured before using chloramphenicol at a concentration of 0.5 mg/mL as positive controls and DMSO at a concentration of 10% as negative controls.

RESULTS AND DISCUSSION

Demographic characteristics and information sources of TEK

There were 189 traditional healers consisting of 155 men (82.02%) and 34 women (17.98%) who participated as informants. Most of them were aged > 40 years (59.26%), followed by adults (39.68%), and adolescents (1.06%). The data showed that the younger generation is less interested in using traditional medicine. Most of the traditional healers were elementary school graduates (42.86%) and most of them worked as farmers (85.71%) (Table 1). These demographic characteristics are similar to the ethnomedicinal studies in Bromo Tengger Semeru National Park (Bhagawan and Kusumawati 2021; Jadid et al. 2020). However, it is different from the results obtained in Mount Merapi, which reported that women were more active in utilizing medicinal plants for family health care (Nahdi et al. 2016; Nahdi and Kurniawan 2019).

Mount Wilis community receives their TEK from their ancestors. This knowledge is inherited and subsequently preserved across generations. TEK was mostly passed down from older family members (85.18%) (see Table 1) through a knowledge transfer system called *Gethok Tular*, which is defined as a knowledge transfer system orally from generation to generation. There are no ancient manuscript records discussing TEK in this community. Our results are also in accordance with other studies in the Tenggerese (Bhagawan and Kusumawati 2021) and in the economic capital of Casablanca, Morocco (Zougagh et al. 2019).

Medicinal plant biodiversity and diseases treated

There were 83 plant species from 39 families documented in this research (see Table 2). Zingiberaceae family dominated with 13.25%, followed by four families with the same proportion of 4.82%, namely: Apiaceae, Asteraceae, Piperaceae, and Rutaceae. The dominance of Zingiberaceae has been found in previous ethnomedicinal studies in the mountains of Java, such as in Tengger tribe (Jadid et al. 2020) and Mount Merapi (Nahdi and Kurniawan 2019).

Various medical conditions can be cured using medicinal plants (Table 3). Most medicinal plants have more than one therapeutic property, such as *Acorus calamus* L. can be used to treat fever and headache. On the other hand, several diseases require a combination of a variety of plants to cure. For instance, erectile dysfunction is treated with *Gynura procumbens*, *Piper betle*, *Piper retrofractum*, *Alpinia galanga*, and *Kaempferia rotunda*. Diseases can be categorized into seven; dermatological

diseases, gastrointestinal disorders, internal medical diseases, reproductive health problems, respiratory problems, and skeletal-muscular disorders. Internal medical diseases have the highest number of diseases (eight diseases) including diabetes, fever, high cholesterol, hypertension, insomnia, kidney stones, obesity, low immune system, and nose bleeding.

Preparation mode, administration route, and part used of medicinal plant

Single therapy using a single plant is predominant, although the use of combined plants is also common. In diabetes treatment, two plant species are used; the decoction of *Catharanthus roseus* leaf and *Cinnamomum verum* bark. Three plant species are used in treating dysmenorrhea; the decoction of *Foeniculum vulgare* leaf, *Curcuma longa* rhizome, and *Zingiber officinale* rhizome.

Decoction appeared to be the most common method used in preparing medicinal plants (62.65%) as it is simpler, easier and cost-efficient (Amri et al. 2014). In addition, this preparation method allows more phytochemical compounds to be extracted, thereby increasing the pharmacological effect of the plant (Barkaoui et al. 2017). The administration of medicinal plants after preparation is

mostly done orally, but a small proportion of topical and nasal applications were also identified.

Table 1. Demographic characteristics and information sources of traditional ethnomedicine knowledge in the northern slope of Mount Wilis community

Variable	Category	No. of informants	Percentage
Gender	Female	34	17.98%
	Male	155	82.02%
Age	Adolescent (13-19 years)	2	1.06%
	Adult (20-40 years)	75	39.68%
	Elderly (>40 years)	112	59.26%
Education	Illiterate	2	1.06%
	Primary education	81	42.86%
	Tertiary education	72	38.10%
	University education	34	17.98%
Main profession	Civil servant	14	7.41%
	Village head	8	4.23%
	Village staff	5	2.65%
	Farmer	162	85.71%
Information source	Hereditary	161	85.18%
	Empirical	15	7.94%
	Formation	13	6.88%

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Table 2. Medicinal plants used in the northern slope of Mount Wilis community including: family, species name, local name, part used, diseases treated, preparation, administration and use value

Family	Species name	Local name	Part used	Diseases treated	Preparation, administration	UV
Acanthaceae	<i>Andrographis paniculata</i> (Burm.f.) Nees	Sambiloto	Leaf	Cough, fever	Decoction, oral	0.32
	<i>Strobilanthes crispus</i> Blume	Keji beling	Leaf	Kidney stones	Decoction, oral	0.10
Acoraceae	<i>Acorus calamus</i> L.	Dringu	Leaf	Fever, headache	Decoction, oral	0.38
Amaranthaceae	<i>Amaranthus hybridus</i> L.	Bayam	Leaf	High cholesterol, diabetes	Decoction, oral	0.02
Amarillydaceae	<i>Allium ampeloprasum</i> L.	Bawang pre	Leaf	High cholesterol	Decoction, oral	0.10
	<i>Allium cepa</i> L.	Brambang	Tuber	Fever, flatulence	Decoction, oral	0.22
10	<i>Allium sativum</i> L.	Bawang putih	Tuber	Cough, high cholesterol	Decoction, oral	0.32
	<i>Annona muricata</i> L.	Sirsak	Fruit	Diabetes	Raw, oral	0.12
Annonaceae	<i>Annona squamosa</i> L.	Srikoyo	Fruit	Diarrhea	Raw, oral	0.04
	<i>Cananga odorata</i> (Lam.) Hook f. & Thomson	Kenanga	Flower	Dry skin	Powdered, topical	0.25
Apiaceae	<i>Apium graveolens</i> L.	Seledri	Leaf	Hypertension	Raw, oral	0.10
	<i>Centella asiatica</i> (L.) Urban	Pegagan	Leaf	Insomnia	Decoction, oral	0.06
33	<i>Coriandrum sativum</i> L.	Ketumbar	Fruit	Diarrhea, flatulence	Decoction, oral	0.22
	<i>Foeniculum vulgare</i> Mill	Adas	Leaf	Cough, fever, menstrual pain	Decoction, oral	0.42
Apocynaceae	<i>Alstonia scholaris</i> (L.) R. Br.	Pule	Bark	Fever	Decoction, oral	0.02
	<i>Calotropis gigantea</i> (L.) Dryand.	Babakoan	Root	Fever	Decoction, oral	0.02
Araceae	<i>Catharanthus roseus</i> L.	Tapak doro	Leaf	Diabetes, hypertension	Decoction, oral	0.10
	<i>Colocasia esculenta</i> (L.) Schott	Talas	Leaf	Obesity	Decoction, oral	0.02
Araliaceae	<i>Polyscias scutellaria</i> (Burm.f.) Fosberg	Mangkakan	Leaf	Wound infection	Decoction, oral	0.02
Arecaceae	<i>Areca catechu</i> L.	Jambe	Seed	Intestinal worms	Decoction, oral	0.22
Asteraceae	<i>Elephantopus scaber</i> L.	Tapak liman	Leaf	Kidney stones	Decoction, oral	0.08
	<i>Gynura procumbens</i> (Lour.) Merr	Sambung nyowo	Leaf	Erectile dysfunction	Decoction, oral	0.02
	<i>Sonchus arvensis</i> L.	Tempuyung	Leaf	Kidney stones	Decoction, oral	0.02
	<i>Tithonia diversifolia</i> (Hemsl.) A. Gray	Insulin	Leaf	Diabetes, wound infection, osteoporosis	Decoction, oral	0.65
Basellaceae	<i>Anredera cordifolia</i> (Ten.) Steenis	Binahong	Leaf	Mouth ulcer	Raw, oral	0.12
Brassicaceae	<i>Raphanus raphanistrum</i> L.	Lobak	Tuber	Obesity	Decoction, oral	0.04
Bromeliaceae	<i>Ananas comosus</i> (L.) Merr.	Nanas	Fruit	Diarrhea	Squeezed, oral	0.04

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Compositae	<i>Cosmos caudatus</i> Kunth	Kenikir	Leaf	Hypertension	Decoction, oral	0.02
	<i>24</i> <i>Canthus annuus</i> L.	Bunga matahari	Seed	Obesity	Burn, oral	0.04
Cucurbitaceae	<i>Citrullus lanatus</i> (Thunb.) Matsum	Semangka	Fruit	Hypertension	Raw, oral	0.26
	<i>Cucumis melo</i> L.	Melon	Fruit	Hypertension	Raw, oral	0.25
	<i>Cucumis sativus</i> L.	Timun	Fruit	Hypertension	Raw, oral	0.22
Cluseaceae	<i>Garcinia × mangostana</i> L.	Manggis	Fruit	Mouth ulcer	Raw, oral	0.12
Euphorbiaceae	<i>Manihot utilisima</i> Pohl	Singkong	Leaf	Low immune system	Boiled, oral	0.04
Fabaceae	<i>Caesalpinia sappan</i> L.	Secang	Stem	Diabetes, diarrhea	Decoction, oral	0.38
	<i>Parkia speciosa</i> Hassk.	Petai	Fruit	Menstrual pain	Raw, oral	0.02
Lamiaceae	<i>Mentha × piperita</i> L.	Min	Leaf	Asthma	Burn, nasal	0.24
	<i>Ocimum citriodorum</i> Vis.	Kemangi	Leaf	Diarrhea	Decoction, oral	0.04
	<i>Orthosiphon aristatus</i> (Blume) Miq.	Kumis kucing	Leaf	Kidney stones	Decoction, oral	0.38
Lauraceae	<i>Cinnamomum verum</i> J.Presl	Kayu manis	Bark	Diabetes	Decoction, oral	0.10
	<i>Persea americana</i> Mill.	Alpukat	Fruit	High cholesterol	Raw, oral	0.08
Leguminosae	<i>Erythrina variegata</i> L.	Dadap srep	Leaf	Fever	Raw, topical	0.25
	<i>Tamarindus indica</i> L.	Asem	Fruit	Diabetes	Decoction, oral	0.16
Liliaceae	<i>Aloe vera</i> Mill.	Lidah buaya	Leaf	Hair problems, skin burn	Squeezed, topical	0.32
	<i>Sansevieria trifasciata</i> Prain.	Lidah mertua	Leaf	Diarrhea	Decoction, oral	0.02
Loranthaceae	<i>Dendrophthoe pentandra</i> (L.) Miq.	Kemladean	Leaf	Fever	Decoction, oral	0.03
Marantaceae	<i>10</i> <i>Arundinaceae</i> L.	Garut	Tuber	Diarrhea	Decoction, oral	0.02
Meliaceae	<i>Swietenia mahagoni</i> (L.) Jacq.	Mahoni	Seed	Diabetes	Decoction, oral	0.06
Menispermaceae	<i>Tinospora crispa</i> (L.) Hook. f. & Thomson	Brotowali	Leaf	Osteoporosis	Decoction, oral	0.04
Moraceae	<i>Morus alba</i> L.	Krokot	Leaf	Obesity	Decoction, oral	0.02
Moringaceae	<i>Moringa oleifera</i> Lam.	Kelor	Leaf	Low breast milk production, low immune system	Boiled, oral	0.44
Muntingiaceae	<i>Muntingia calabura</i> L.	Kersen	Fruit	Hypertension	Raw, oral	0.02
Myrtaceae	<i>Psidium guajava</i> L.	Jambu klutuk	Leaf	Diarrhea, hemorrhoid	Raw, oral	0.32
	<i>Syzygium polyanthum</i> (Wight) Walp.	Salam	Leaf	Diabetes	Decoction, oral	0.34
Oxalidaceae	<i>Averrhoa bilimbi</i> L.	Blimbing wuluh	Fruit	Mouth ulcer	Raw, oral	0.14
Phyllanthaceae	<i>Phyllanthus niruri</i> L.	Meniran	Leaf	Hepatitis	Decoction, oral	0.12
	<i>Sauropus androgynus</i> (L.) Merr	Katuk	Leaf	Low breast milk production	Boiled, oral	0.26
Piperaceae	<i>Piper betle</i> L.	Sirih	Leaf	Erectile dysfunction, flatulence, wound infection	Decoction, oral	0.40
	<i>Piper cubeba</i> L.f.	Kemukus	Fruit	Asthma, leucorrhea	Decoction, oral	0.14
	<i>Piper ornatum</i> N.E.Br.	Sirih merah	Leaf	Low immune system	Decoction, oral	0.47
	<i>23</i> <i>P. retrofractum</i> Vahl	Cabe jawa	Fruit	Erectile dysfunction	Decoction, oral	0.43
Poaceae	<i>Cymbopogon citratus</i> (DC.) Stapf	Sereh	Leaf	Diarrhea	Decoction, oral	0.11
	<i>Imperata cylindrica</i> (L.) Raeusch.	Alang-alang	Root	Nose bleeding	Squeezed, topical	0.06
Rubiaceae	<i>Morinda citrifolia</i> L.	Bentis/Pace	Fruit	Diabetes, high cholesterol	Decoction, oral	0.41
	<i>21</i> <i>deria foetida</i> L.	Sembukan	Leaf	Leucorrhea	Decoction, oral	0.05
Rutaceae	<i>Citrus aurantiifolia</i> (Christm.) Swingle	Jeruk nipis	Fruit	Cough	Raw, oral	0.37
	<i>Citrus hystrix</i> DC.	Jeruk purut	Fruit	Cough	Raw, oral	0.25
	<i>Citrus limon</i> (L.) Osbeck	Jeruk lemon	Fruit	Cough	Raw, oral	0.14
	<i>Citrus maxima</i> (Burm.) Merr.	Jeruk bali	Fruit	Cough	Raw, oral	0.02
Solanaceae	<i>Physalis angulata</i> L.	Ceplukan	Fruit	Diarrhea	Raw, oral	0.04
	<i>Solanum melongena</i> L.	Terong	Fruit	High cholesterol	Raw, oral	0.15
	<i>Solanum tuberosum</i> L.	Kentang	Tuber	Dry skin	Powdered, topical	0.13
Zingiberaceae	<i>Alpinia galanga</i> (L.) SW.	Laos	Rhizome	Erectile dysfunction, low immune system	Decoction, oral	0.20
	<i>Curcuma aeruginosa</i> Roxb.	Temu ireng	Rhizome	Rheumatoid arthritis	Decoction, oral	0.04
	<i>Curcuma alba</i> L.	Kunir putih	Rhizome	Dry skin	Powdered, topical	0.15
	<i>Curcuma heyneana</i> Valetton & <i>23</i>	Temu giring	Rhizome	Intestinal worms, rheumatoid arthritis	Decoction, oral	0.18
	<i>Curcuma longa</i> L.	Kunir	Rhizome	Menstrual pain	Decoction, oral	0.45
	<i>Curcuma xanthorrhiza</i> Roxb.	Temulawak	Rhizome	Low immune system, hepatitis	Decoction, oral	0.46
	<i>Kaempferia galanga</i> L.	Kencur	Rhizome	Cough	Decoction, oral	0.32
	<i>Kaempferia rotunda</i> L.	Kunci pepet	Rhizome	Erectile dysfunction	Decoction, oral	0.24
	<i>21</i> <i>giber cassumunar</i> Roxb.	Bangle	Rhizome	Fever, headache	Decoction, oral	0.04
	<i>Zingiber officinale</i> Roscoe	Jahe	Rhizome	Cough, menstrual pain	Decoction, oral	0.36
	<i>Zingiber zerumbet</i> (L.) Roscoe ex Sm.	Lempuyang	Rhizome	Leucorrhea, hypertension	Decoction, oral	0.18

Table 3. Disease categories and health-related problems treated by medicinal plants on the northern slope of Mount Wilis community

Desease categories	Specified disease name
Dermatological diseases	Dry skin, Hair problems, Skin burn, Wound infection
Gastro-intestinal disorders	Diarrhea, Flatulence, Intestinal worms
Internal medical diseases	Diabetes, Fever, High cholesterol, Hypertension, Insomnia, Kidney stones, Obesity, Low immune system, Nose bleeding
Reproductive health problems	Erectile dysfunction, Leucorrhea, Low breast milk production, Menstrual pain
Respiratory problems	Asthma, Cough, Mouth ulcer
Skeleto-muscular disorders	Headache, Osteoporosis, Rheumatoid arthritis

Table 4. Phytochemical profile of *T. diversifolia* leaf extract

No.	RT (min)	% area	Measured m/z	Compound formula	Compound name	Pharmacological activity
1.	0.909	0.28%	151.0359	C ₃ H ₇ ClN ₄ O	5-(aminomethyl)-2,4-dihydro-3H-1,2,4-triazol-3-one hydrochloride	-
2.	1.303	2.62%	156.0434	C ₂ H ₉ N ₃	Guanidine methanesulfonate	Cystic fibrosis therapy (Hirsh et al. 2008)
3.	1.914	0.06%	132.1023	C ₆ H ₁₃	2-Isobutoxyacetamide	-
4.	2.55	0.02 %	174.0555	C ₁₀ H ₇	5-Quinolincarboxylic acid	-
5.	3.412	1.42%	100.0766	C ₅ H ₉ NO	1-Cyclopropyl-N-hydroxyethanimine	-
6.	4.115	2.05%	301.1661	C ₁₅ H ₂₄ O ₆	1,13-propylene glycol diacrylate	-
7.	4.797	0.87%	427.2547	C ₂₃ H ₃₇ NO ₉	(4S,5R,8S,9R,10S,12R)-1,5,9-trimethyl-11,14,15,16-tetraoxatetracyclo[10.3.1.04.13.08.13] hexadec-10-yl 4-[(2,2-dimethoxyethyl)amino]-4-oxobutanoate	-
8.	5.956	1.60%	484.2552	C ₂₄ H ₃₇ NO ₉	(2S,3S,4S,5S,6R)-5-(2-Cyclopentyl-4-hydroxyphenox)-6-ethoxy-3,4,5-trihydroxy-6-[(2-methyl-2-propanyl)amino]methyl tetrahydro-2H-pyran-2-carboxylic acid	-
9.	6.68	1.31%	500.2867	C ₂₅ H ₄₁ NO ₉	Aconine	Anti-osteoporosis (Zeng et al. 2016)
10.	7.187	2.89%	484.2192	C ₂₇ H ₃₃ NO ₅ S	Octyl (βS)-β-hydroxy-N-(2-naphthylsulfonyl)-D-phenylalaninate	-
11.	8.115	31.96%	369.1920	C ₁₉ H ₂₈ O	Tagitinin A	Anti-inflammatory and antitumor (Chagas-Paula et al. 2012)
12.	9.170	9.46%	243.1028	C ₁₉ H ₂₄ O ₆	Tagitinin C	Anti-inflammatory, cytotoxic, antiviral, antibacterial, antifungal, allelopathic activities (Au et al. 2021)
13.	10.33	14.63%	353.1964	C ₁₅ H ₃₂ N ₂ O ₃ S ₂	1,3-Bis[6(methylsulfinyl)hexyl]urea	Inhibit the enzyme HIV-I protease (Gupta et al. 1998)
14.	11.40	1.85%	445.2122	C ₂₇ H ₂₈ N ₂ O ₄	Ethyl 4-[[[(3,4-dimethoxyphenyl)(2-methyl-1H-imidazol-3-yl)methyl]amino]benzoate	-
15.	11.89	3.25%	247.1705	C ₁₆ H ₂₂ O ₂	(2-Ethyl-2-methyl-tetrahydro-pyran-4-yl)-methyl-methanone	-
16.	12.57	1.82%	227.2175	C ₁₈ H ₂₈ O ₂	25-Phenyldecanoic acid	Antiviral (Parang et al. 1997)
17.	13.76	4.24%	653.4047	C ₃₉ H ₅₆ O ₈	2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl tetracosanoate	-
18.	14.267	2.70%	635.3948	C ₃₉ H ₅₄ O ₇	(3β)-27-[[[(2E)-3-(3,4-Dihydroxyphenyl)-2-propenoyl]oxy]-3-hydroxyolean-12-en-17-yl]ic acid	-
19.	15.427	1.70%	611.3547	C ₃₂ H ₄₆ N ₆ O ₆	N-[(3S,4S)-3-Hydroxy-6-phenyl-4-[[N-(2-pyridinylcarbonyl)-L-valyl]amino]hexanoyl]-L-alanyl-L-leucinamide	-
20.	15.849	1.03%	256.2646	C ₁₆ H ₃₃ NO	Palmitamide	Anti-osteoporosis (Liu et al. 2015)
21.	16.875	0.01%	515.3935	-	Unknown	-
22.	17.958	2.02%	872.7714	-	Unknown	-
23.	18.879	0.50%	139.9888	-	Unknown	-

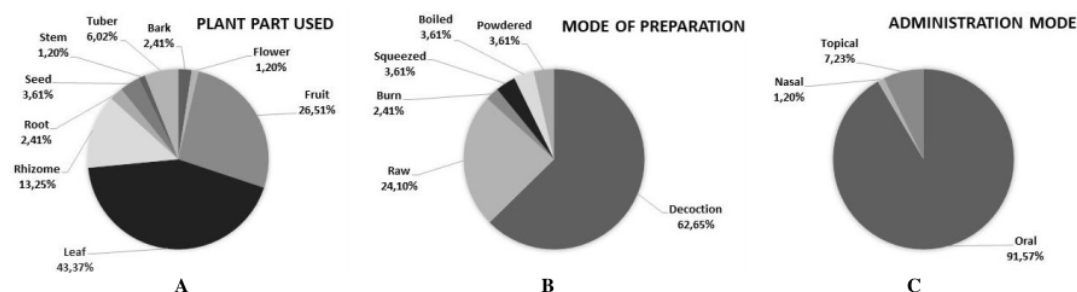


Figure 2. Plant part used (A), preparation (B), and administration (C) of medicinal plants in the northern slope of Mount Wilis community

Parts of plants that are commonly used in producing traditional medicine are roots, leaves, bark, stems, flowers, tubers, fruits, and rhizomes. In this research, leaves are dominated with 36 species, followed by fruit (22 species) and rhizomes (11 species). The dominant use of the leaf relates to the survival rate of the plant. Removing leaf biomass at a reasonable amount does less damage to the plants, compared to the collection of stems, roots, or whole plants. In addition, leaves produce various secondary plant metabolites (Zahoor et al. 2017). Our results of the preparation, administration route, and part used of medicinal plants found in this research are similar to the findings of previous research done in other mountainous areas of Java (Bhagawan and Kusumawati 2021; Jadid et al. 2020; Nahdi et al. 2016; Nahdi and Kurniawan 2019).

As explained by Sujarwo and Caneva (2016), use value (UV) determines which species are considered most culturally important in a particular community. There were ten species of medicinal plants with the highest UV found in Mount Wilis which included: *Tithonia diversifolia* (0.65), *Piper ornatum* (0.47), *Curcuma xanthorrhiza* (0.46), *Curcuma longa* (0.45), *Moringa oleifera* (0.44), *Piper retrofractum* (0.43), *Morinda citrifolia* (0.41), *Piper betle* (0.40), *Acorus calamus* (0.38), and *Caesalpinia sappan* (0.38). In addition, this quantitative index is able to select plant species for ethnopharmacological relevance tests in the laboratory (Andrade-Cetto and Heinrich 2011). The highest UV level was found in *T. diversifolia*, and the sample of the plant was sent to the laboratory to be tested for ethnopharmacological relevance. We tested the profile of the phytochemical compounds using the UPLC-QtoF-MS/MS instrument, while the pharmacological activity was measured in an antibacterial test.

Phytochemical compounds of selected medicinal plant

Based on the analysis of the metabolite profile of *T. diversifolia* extract, we identified 23 phytochemical compounds. Twenty compounds could be identified by name, while three were unknown. The failure in identifying these unknown compounds might be due to impurity that was detected by the instrument, or because these unknown compounds are new compounds that have not been listed in the ChemSpider database. The three major phytochemical compounds include Tagitinin A (31.96%); 1,3-

Bis[6(methylsulfinyl)hexyl]urea (14.63%); and Tagitinin C (9.46%), each of which showed pharmacological potential. Tagitinin A has anti-inflammatory and antitumor properties (Chagas-Paula et al. 2012). Bis[6(methylsulfinyl)hexyl]urea compounds contain strong anti-HIV properties which inhibit the enzyme HIV-1 protease (Gupta et al. 1998). Tagitinin C has a wide range of pharmacological functions including anti-inflammatory, cytotoxic, antiviral, antibacterial, antifungal, and allelopathic properties (Au et al. 2021).

Some other minor phytochemical compounds are also known to have pharmacological properties. Guanidine methanesulfonate is a potent treatment for cystic fibrosis (Hirsh et al. 2008). Aconin has an anti-osteoporosis compound that works by inhibiting osteoclast differentiation (Zeng et al. 2016) and 12-Phenyldodecanoic acid also possesses antiviral properties (Parang et al. 1997), while Palmitamide functions as an anti-osteoporosis (Liu et al. 2015).

Pharmacological potential of *T. diversifolia*

The results showed that the 96% ethanol extract of *T. diversifolia* efficiently suppressed the growth of microorganisms *B. subtilis* and *E. coli* at different potential levels.

Table 5 shows that *T. diversifolia* extract at all concentrations including: 2.5 mg/mL; 5 mg/mL; 10 mg/mL; 20 mg/mL; and 40 mg/mL showed inhibition zones against *B. subtilis* of 11.68 ± 0.46 mm, 12.01 ± 0.32 mm; 13.02 ± 0.74 mm; 13.86 ± 1.10 mm; and 16.85 ± 0.83 mm respectively. In addition, all concentrations of *T. diversifolia* extract also had a higher inhibition zone than the positive control (8.91 ± 0.35 mm). Similarly, *T. diversifolia* extract at all concentrations showed inhibition zones for *E. coli* (14.86 ± 0.68 mm; 16.08 ± 0.46 mm; 17.05 ± 0.95 mm; 17.13 ± 0.74 mm); and 21.53 ± 1.30 mm) that are higher than the positive control (7.48 ± 0.46 mm). It implies that *T. diversifolia* extract is highly effective antibacterial against gram-positive and gram-negative bacteria. As explained by Balouiri et al. (2016), in vitro antibacterial method offers simpler and cost-efficient pharmacological plant extract screening. As found in this research, the in vitro antibacterial test was able to represent the high pharmacological potential of *T. diversifolia*.

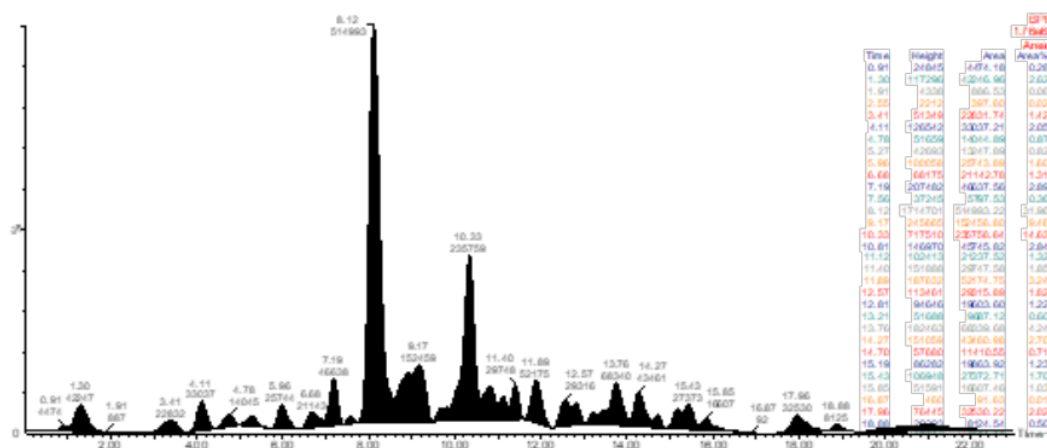


Figure 3. Chromatogram profile of *T. diversifolia* extract using the UPLC-QToF-MS/MS

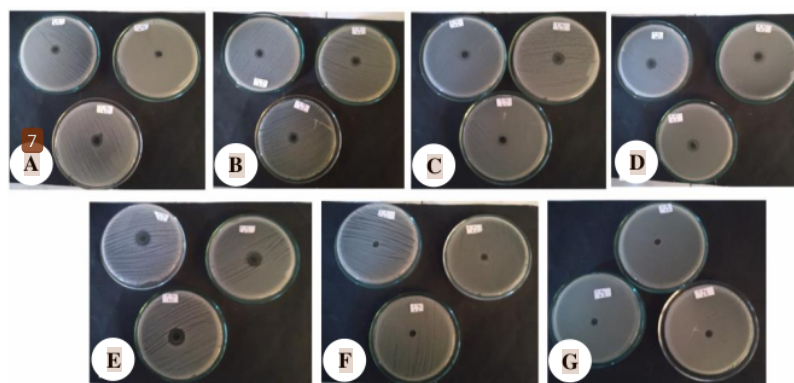


Figure 4. The results of the antibacterial activity test of *T. diversifolia* extract against *B. subtilis*. A. Concentration 2.5 mg/mL, B. Concentration 5 mg/mL, C. Concentration 10 mg/mL, D. Concentration 20 mg/mL, E. Concentration 40 mg/mL, F. Positive control (chloramphenicol), and G. Negative control (DMSO)

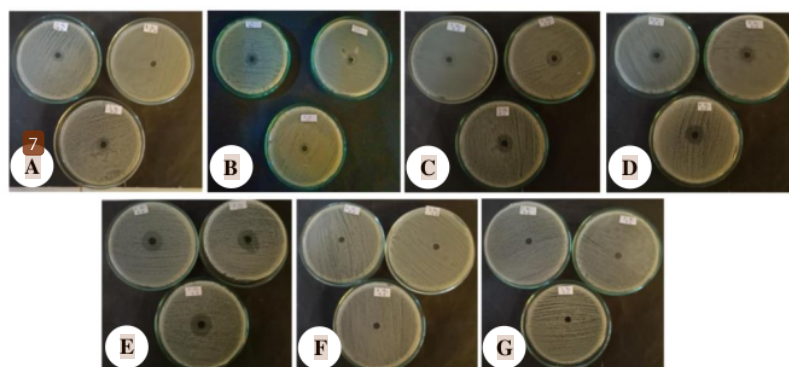


Figure 5. The results of the antibacterial activity test of *T. diversifolia* extract against *E. coli*. A. Concentration 2.5 mg/mL, B. Concentration 5 mg/mL, C. Concentration 10 mg/mL, D. Concentration 20 mg/mL, E. Concentration 40 mg/mL, F. Positive control (chloramphenicol), and G. Negative control (DMSO)

Table 5. Antimicrobial screening test of *T. diversifolia* ethanolic plants extract against positive and negative bacterial

Samples	Concentration (mg/mL)	Inhibition zones (mm)	
		Gram positive bacteria <i>B. subtilis</i>	Gram negative bacteria <i>E. coli</i>
<i>T. diversifolia</i> extract	2.5	11.68 ± 0.46	14.86 ± 0.68
	5	12.01 ± 0.32	16.08 ± 0.46
	10	13.02 ± 0.74	17.05 ± 0.95
	20	13.86 ± 1.10	17.13 ± 0.74
	40	16.85 ± 0.83	21.53 ± 1.30
Positive control	0.5	8.91 ± 0.35	7.48 ± 0.46
Negative control	10	0 ± 0.00	0 ± 0.00

Note: Data are means of three replicates (n = 3) ± standard error

In addition, *T. diversifolia* leaves extract has been reported to decrease leptin and BG concentrations by increasing IR expression and reducing pancreatic tissue necrosis by suppressing macrophage CD14 concentrations in rats with STZ-induced diabetes (Muniroh 2022). This result is correlated with its traditional use as antidiabetic. A similar study also conducted by Ramadhan (2022) confirmed the ethnopharmacological use of selected plants from East Kalimantan might have potential as an antidiabetic and natural antioxidant. This study has limitations on the interpretation of the pharmacological activity and phytochemical compounds of all cited plants.

In conclusion, our results highlighted the use of medicinal plants as traditional medicine among traditional healers on the northern slope of Mount Wilis. A total of 83 medicinal plant species have been extensively used for treatment in this community. Insulin (*Tithonia diversifolia*) has been confirmed to contain phytochemical compounds that have pharmacological properties and this plant extract is proven to have pharmacological potential. On this basis, *T. diversifolia* is a potential source to be developed for new drugs. Information about the safety and efficacy of this plant should be disseminated to support traditional medicine in the local community. We suggest that future research analyze the pharmacological activity and phytochemical compounds in plants with high UV values.

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