PHARMACOGNOSTICAL STUDIES AND DETERMINATION OF TOTAL FLAVONOIDS OF PAITAN (Tithonia diversifolia (Hemsl.) A. Gray

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PHARMACOGNOSTICAL STUDIES AND DETERMINATION OF TOTAL FLAVONOIDS OF PAITAN (*Tithonia diversifolia (*Hemsl.) A. Gray

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Abstract

Tithonia diversifolia (Hemsl.) A. Gray is one of the plants used in traditional medicine and belongs to Compositae family. In different parts of Indonesia, it is commonly known as " *paitan* and *kembang bulan*" .The study provides an early description of *Tithonia diversifolia* (Hemsl.) A. Gray) and complete the monographs data extract. This plant prospects to the main source of the raw material for the herb-drug product and some parameters identified were needed to ensure the safety, quality and efficacy of the product. The present study is to evaluate macros-and microscopic characteristic of the Indonesian plant and its quality parameter including fluorescence, physicochemical characteristics and phytochemical screening. Moisture content, ethanol and water soluble extract was determined, and were discovered to be 11,27%, 4,73% and 18,01%. Total ash value and acid insoluble ash value were determined which was 10.29 and 0.72 % respectively. Phytochemical screening of aqueous ethanol extract of *Tithonia diversifolia* showed the presence of alkaloids, flavonoids, tannins, saponins and triterpenoids. The result showed that average content of flavonoid total is 69.1653 mg QE/g extract.

Keywords: Pharmacognostical, Physicochemical, Phytochemical, *Tithonia diversifolia* (Hemsl.) A. Gray).

INTRODUCTION

Tithonia diversifolia is potensial prospect as traditional medicine, a study for quality and quantity control is needed to provide a scientific base about pharmacognostic specification. *Tithonia diversifolia* is a plant species belonging to the Compositae family. In different parts of Indonesia, people commonly known as " *paitan and kembang bulan*" and also used in Indonesia traditional medicine This plant is native to Mexico and Central America . *Tithonia diversifolia* can grow naturally in Indonesia and other Southeast Asia countries . *Tithonia diversifolia* leaves have antioxidant compounds because of its flavonoids (Hanifa et al 2015). The flavonoid compounds present in this "*paitan* "leaf are pentahidroksiflavonol (Zirconia *et al*., 2015). In addition to flavonoids, these plants also contain alkaloids, tannins, saponins and triterpenoids (Ezeonwumelu *et al*., 2012).

Based on several studies it has been shown that *paitan* leaves have cytotoxic effects on colon cancer cells (Mardihusodo *et al*., 2011), antihyperglycemic (Darmawi *et al*., 2015) and antidiarrheal activity that may inhibit intestinal motility (Ezeonwumelu *et al*., 2012) because of its flavonoid content. Flavonoids are one of the most common groups of secondary metabolite compounds found in plant tissues and have many important functions for health, such as

antioxidants (Redha 2010), antibacterial, antiinflammatory, allergic and antithrombotic (Rais 2015).

Considering the potency of *Tithonia diversifolia* as medicinal plants, it is necessary to make the pharmacognostical studies and phytochemical screening. In this study, the specific and non-specific parameters were tested to determine the quality of the extract using methods described in the Farmakope Herbal Indonesia (FHI is the Indonesian Herb Pharmacopoeia, 2008) and to determine the level of flavonoids in *paitan* leaf extract. This preliminary study helped for standardization of the crude drug as well as further processing of the sample with some indication regarding the nature of chemical coumpounds present in it .

MATERIALS AND METHODS

Collection and Authentication

The plant of *Tithonia diversifolia* (Hemsl.) A. Gray) were collected from Botanical Garden, Bogor, West –Java, Indonesia .The taxonomical identification and authentication of the plant was done by Herbarium Bogoriense, Bogor Indonesia.

PHARMACOGNOSTICAL EVALUATION

Macroscopic study

Determining the characteristics of *paitan* (*Tithonia diversifolia* (Hemsl.) A. Gray) plants by direct observation of the physical form of fresh plants includes roots, stems, leaves, flowers, fruits and seeds .

Microscopic study

Microscopic study of the transverse sections of fresh leaves *paitan* and material powder. Using chloralhydrate and fluoroglusin reagens and then it was heated on spirit lamp. Specific fragment were observed under the microscope and were photographed (Wallis TE, 1984).

Extraction Method

Extraction is done by maceration of one part of paitan leaf with 10 parts of ethanol 70%. Soak for the first 6 hours while stirring 3 times every 2 hours, then let it rest for 18 hours. The extraction was filtered and concentrated using a vacuum rotary evaporator and the obtained extracts were concentrate.

Physicochemical Parameter Study

a) *Moisture content*

Determination of water content is done by distillation toluene (Depkes RI, 2008).

b) *Total Ash Value*

Weigh accurately 2 g of extract and put it into crushed silicate crucible which was previously ignited and weighted. Spread the extract material in a fine even layer at the bottom of the crucible and ignite it by gradually increasing the heat to 400 ^{0} C until it changes color to white. The crucible was cooled in a desiccator and weighed. Procedure was repeated until constant weights were obtained (Depkes RI, 2008).

c) Acid Insoluble Ash Content

The total ash obtained above was boiled with 25 ml dilute hydrochloric acid for 5 minutes. The insoluble ash was collected on ash free filter and washed with hot water, then transferred into pre weighed silica crucible, ignited, cold and weighed. The same procedure was repeated until the constant weight was obtained (Depkes RI, 2008).

d) Water soluble extract value

Firstly, 5 grams of extract were put into a glass-stopper conical flask, then adding 100 ml of chloroform saturated water, was shaked repeatedly for the first 6 hours, and then leave it for 18 hours. Afterwards 20 ml of filtrate dried at 105ºC and weighed. The percentage value of water soluble extract was calculated with reference to the air dried drug (Depkes RI, 2008).

e) Ethanol soluble extract value

Firstly, 5 grams of extract were put into a glass-stopper conical flask. Macerated with 100 ml of 95% ethanol , was shake repeatedly for the first 6 hours, and then leave it for 18 hours. Afterwards, 20 ml of filtrate dried at 105ºC and weighed. The percentage value of soluble extract was calculated with reference to the air dried drug (Depkes RI, 2008).

FLUORESCENCE CHARACTERISTICS

Fluorescence characteristic of powdered and extract material was observe with different chemical reagents. The samples were inserted on the plate and dripped different chemical reagents. The reagents were 2 N hydrochloric acid, 50% sulfuric acid, 50% nitrite and 5% sodium hydroxide and then observed under visible and ultraviolet (254 and 366 nm) light (Kokashi *et al*., 1958).

PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was carried out using 20 g powdered material and subjecting it to successive extraction in a reflux apparatus with 200 ml ethanol 70% for 30 minutes. The extraction was filtered and concentrated using a rotary evaporator. This extract were being tested for its alkaloids content using Dragendorff, Mayer and Bouchardat reagents, flavonoid test (Shinoda and ammonia test), tannin test (test with gelatin and FeCl3), saponin test (foam test) and steroid and terpenoid test (Liebermann Burchard test).

CHROMATOGRAPHIC PROFILE

Chromatographic profile were performed on hexane extract, dichloromethane extract (DCM), 70% ethanol extract by maceration. The extraction was continued until the solvent became colourless. All of the 3 extracts were concentrated using a rotary evaporator then analyzed by TLC. Silica gel 60 GF 254 TLC plates were used for the chromatographic profile. Each extract was lightly dissolved in ethanol and capillary tube were used to uniformly apply the dissolved samples on the plates and allowed to dry. The plates were developed in a chromatographic tank using the different solvent system as Hexane-DCM (4:6), Kloroform-Metanol (9:1) and Etil asetat-etanol (5:5). The plates were dried and observed under visible light and ultraviolet light 366 nm and by spraying with 10 % sulfuric acid followed by heating at 105° C for 5 minutes in an oven (Wagner S,1984). The retention factor (Rf) value was calculated using following formula.

$$Rf = \frac{\text{Distance moved by the solute/compound}}{\text{Distance moved by the solvent front}}$$

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DETERMINATION OF TOTAL FLAVONOID CONTENT

Qualitative analysis

The standard solution of quercetin and ethanol extract of *paitan* leaves was slightly dissolved in ethanol and capillary tubes were used to apply the dissolved`sample on the plates in uniformity and allowed` to dry. The plates`were developed in a chromatograhic tank using solvent system as ethyl acetate - acetic acid- water (7:2:1).

Quantitative analysis

1. **Preparation of standard solutions**

Determination of total flavonoid levels contained in the extract was done using quercetin as standard to make the calibration curve. Quercetin was weighed to 10 mg dissolved in 96% ethanol in order to achieve 10 ml (1000 µg / ml) solution, then diluting 40, 35, 30, 25, and 20 µg / ml. Out of these concentrations were taken sequentially 0.4; 0.35; 0.3; 0.25; and 0.2 ml of solution, added 3 ml of 96% ethanol, 0.2 ml AlCl3 10%, 0.2 ml of 1 M natrium acetate, and add the aquades until it's up to 10 ml . The solution mixture was incubated for 30 minutes at room temperature, measured its absorbance at a 440 nm wavelength using a UV-Vis spectrophotometer.

2. Preparation of sample solution

Preparation of sample using 1 gram of *paitan* leaf extract, add 96% ethanol until it's up to 100 ml volume (100.000 µg / ml). Then taken 1 ml and diluted 5,000 µg / ml, then taken 1.0 ml to add 10 ml of 96% ethanol, 0.2 ml AlCl3 10%, 0.2 ml of 1 M natrium acetate and add the aquades it's up to 10 ml of value. The solution mixture was incubated for 30 minutes at room temperature, then measured its absorbance at 440 nm wavelength using UV-Vis Spectrophotometer. The result obtained is calculated by linear regression equation between quercetin concentration relationship with absorbance. (Chang et al., 2002).

$$y = bx \pm a$$

Notes:
y = Absorbance of sample
x = Total flavonoid concentration (µg / ml)
a, b = Constanta

RESULTS AND DISCUSSION

Macroscopic images of *Tithonia diversifolia*

Macroscopic characters is done by observing the physical form of *paitan* plants that aims to determine the characteristics of *paitan* plants. The results showed that the plant has a characterisc a single leaf shape, alternatus leaf and inflorecentia cymosa has two types of flowers are sterile flowers and fertilized tube flowers. Microscopic observation results show on longitudinal sectional paitan powder have specific fragments of glandula hair and anomocytic type stomata.

Macroscopic images of *Tithonia diversifolia*

a. Leaf

b. Plants

c. Root

d. Fruits

e. Flower

f. Seeds

Microscopic images of *Tithonia diversifolia*

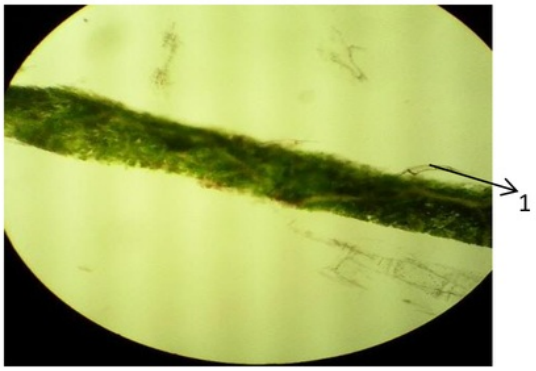

Fig 1. multicellular trichomes on longitudinal sectional paitan leaf

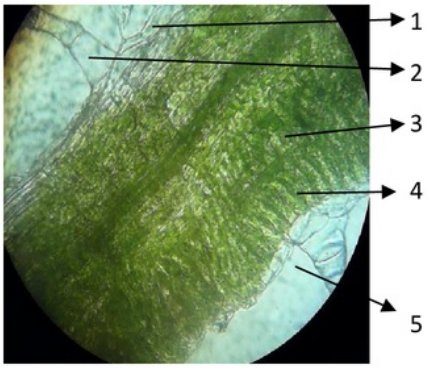

Fig 2. :
(1) upper epidermis
(2) multicellular trichomes
(3) coral flower tissue
(4) palisade
(5) lower epidermis

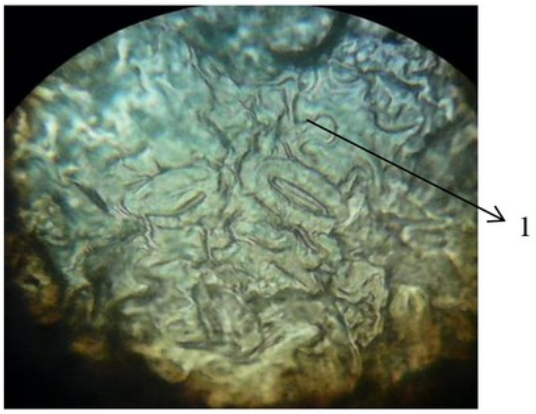

Fig 3. Anomocytic type stomata on transversal sectional (1)

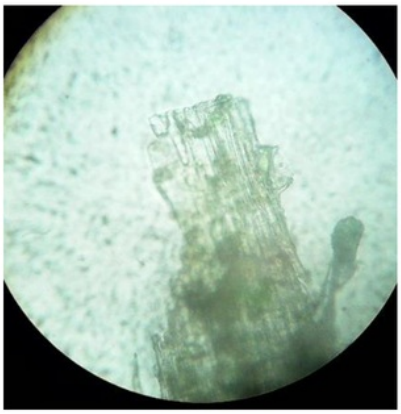

Fig 4. Mesophyl fragmen on transversal sectional

Fig 5. Multicellular trichomes

Fig 6. Calcium oxalate crystals

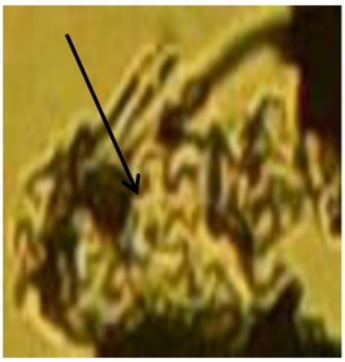

Fig 7. Epidermis

Phytochemical screening was performed to observe the secondary metabolite in *Tithonia diversifolia* leaf extract. Results showed the extract contains alkaloids, flavonoids, saponins, tannins and triterpenoids.

Tabel 1. Phytochemical screening of material plant extracts of *Tithonia diversifolia*

Phytoconstituens	Test Performed/ Reagents	Result
Alkaloids	Bouchardat	+
	Dragendorff	+
	Mayer	+
Flavonoids	Shinoda	+
	Ammonia	+
Tannins	Gelatin	+
	Lead acetate	+
Saponins	Foam	+
Steroids and terpenoids	Liebermann Burchard	+

Physicochemical parameters of ethanol extract of 70% *paitan* leaf include total ash value, acid insoluble ash value, water soluble extract value, ethanol soluble extract value and moisture content can be seen in table 2. Determination of total ash value was done in order to give description of content internal and external minerals derived from the initial process until the formation of the extract. Determination of water soluble concentration and ethanol was conducted to give an early study of the number of compounds that can be dissolved with water solvents and ethanol from a simplicia (Depkes RI, 2000).

Tabel 2. Physicochemical parameter of powder of *Tithonia diversifolia*

No	Parameter	Result
1	Moisture content loss on drying	11.27%
2	Ash Value ➢ Total Ash ➢ Acid insoluble ash	 10.29% 0.72%
3	Extractive values ➢ Ethanol soluble extractive ➢ Water soluble extractive	 4.73% 18.01%

Chromatographic profile of n-hexane extract, dichloromethane (DCM), and 70% ethanol extract was done. Determination of chromatographic profile aims to know the characteristics of chromatography based on the level of polarity. The result above shows that chromatographic profile of n-hexane extract obtained 6 spots and the most dominant spot had a Rf value of 0.675. On the other hand the dichloromethane extract obtained 7 spots and the most dominant spot had Rf value of 0.8625.Whereas ethanol extract 70% got 3 spots and the most dominant Rf value of 0.625.

Tabel 3.Chromatographic evaluation of different extract *Tithonia diversifolia*

Extract	Solvent system (Ratio)	No.of spots	Visual	Spraying reagent (10% H2SO4)	Rf (UV 366 nm)
Hexane	Hexane-DCM (4:6)	6	1.Green 2.Yellow 3.Yellow 4.Green 5.Green 6.Yellow	Brown Yellowish Pink Yellowish Light brown Pink	0.05 0.25 0.40 0.45 0.68 0.95
DCM	Chloroform-Methanol (9:1)	7	1.Green 2.Green 3.Yellow 4.Yellow 5.Green 6.Green 7.Blue	Light blue Light blue Yellowish Yellowish Yellowish Pink Light blue	0.06 0.23 0.26 0.35 0.38 0.55 0.86
Etanol	Etil asetat-Etanol 96% (5:5)	3	1.Green 2.Green 3.Green	Light blue Light blue Light blue	0.63 0.78 0.93

Flourescence character of powder and ethanol extract 70% used reagent NaOH 5%, H_2SO_4 50%, HCl 2N and HNO_3 50%. The results observation of powder visually with green color and ethanol extract 70% with brown color. Fluorescence provided by a drug is one of the several methods used for analyzing crude drugs. The different compounds produce specific fluorescence characteristics which are help full for preliminary chemical study as well as for standardization of specific plant materials. The colour was seen at day light and ultraviolet rays at 254 nm and 366 nm for observing any specific fluorescence.The results of the fluorescence of the powder and extract of *Tithonia diversifolia* are summarized in Table 4.

Table 4. Fluorescent analysis of various powder and extract *Tithonia diversifolia*

Material Powder	Day Light	Short UV (254 nm)	Long UV (366 nm)
+Aquadest	Dark green	Green	-
+2N HCL	Dark brown	Light green	Green
+50% H2SO4	Brown	Green	Brown
+50% HNO3	Yellowish brawn	Yellowish green	Brown
+ 5% NaOH	Greenish brawn	Greenish brawn	Brown
Ethanol Extract			
+ Aquadest	Brown	Light yellow	Yellow
+2N HCl	Brown	Yellow	Yellow
+ 50% H2SO4	Brown	Yellow	Yellow
+50% HNO3	Yellowish brown	Yellowish brown	Yellowish brown
+5% NaOH	Yellowish brown	Yellowish brown	Yellowish brown

Flavonoid determination of ethanol extract 70% leaf *paitan* done qualitatively and quantitatively. From qualitative results, quercetin compounds have a value of Rf = 0.9 equivalent to flavonoid compounds from *paitan* leaf ethanol extract. In the quantitative analysis, the determination of total flavonoid levels uses UV-VIS Spectrophotometry. The result also obtained the spesific maximum wavelength of quercetin compound which is 440 nm wavelength. The data analysis show the total flavonoid content that were contained in *paitan* leaf ethanol extract (*Tithonia diversifolia* (Hemsl.) Gray) was 69.1653 mg calculated as quercetin / g extract.

CONCLUSION

Pharmacognosy study is an important starting point for the standardization of traditional medicines because only good quality of simplicia produces a good quality of traditonal medicines. Authenticity is a general introduction of simplicia which are sensoric characteristics, macroscopic and microscopic evaluation. The result of physicochemical parameter test showed that the total was 10.29 % ash value, 0.72% acid insoluble ash value, 4.73 % ethanol soluble extract value, 18.01 % water soluble extract value and 11.27 % moisture

content. Phytochemical screening tests found alkaloid, flavonoids, tannins, saponins and terpenoids. The chromatography profile of n-hexane extract was obtained 6 spots, dichloromethane extract obtained 7 spots and ethanol extract 70% got 3 spots. Levels of flavonoids contained in *paitan* leaf ethanol extract was 69.1653 mg calculated as quercetin / g extract.

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