STI 2018 Indah (Tahongai)

by Herlina Herlina

Submission date: 07-Jun-2023 01:44PM (UTC+0700)
Submission ID: 2110852889
File name: STI_2018_Indah_Tahongai.pdf (328.07K)
Word count: 3726
Character count: 19670

Science & Technology Indonesia
p-ISSN: 2580-4405 e-ISSN: 2580-4391
Sci. Technol. Indonesia 3 (2018) 14-18

Science & Technology Indonesia

Research Article http:sciencetechindonesia.com

Standardization of Ethanolic Extract of Tahongai Leaves (*Kleinhovia hospita* L.)

Indah Solihah[1*], Mardiyanto[1], Soilia Fertilita[2], Herlina[1], Oktia Charmila[1]

[1] *Departement of Pharmacy, Faculty of Mathematic and Natural Sciences, Sriwijaya University, Indonesia*
[2] *Departement of Medical, Faculty of Medical, Sriwijaya University, Indonesia*

**Corresponding author e-mail : indahsolihah26614@gmail.com*

ABSTRACT

Extract is basic material for herbal drug. Herbal drugs formulation requires consistent of biological activity, a consistent chemical profile, or simply a quality assurance programs that can be achieved by standardizing the extracts. The leaves of tahongai (*Kleinhovia hospita* L.) have been traditionally used in Komering ethnic groups as phytotherapy to cure the inflammation related diseases including cancer, furuncle, polyps, and tonsillitis. The aim of this study was to standardize the quality of ethanolic extract of tahongai leaves by determining the specific and non specific parameters of the extract. The preliminary phytochemical analysis revealed presence of alkaloids, flavonoids, saponins, tanins, and steroids in the extract. The result of specific parameters analysis of the extracts showed that the organoleptic properties of ethanolic extract of tahongai leaves were thick, brownish black, has distinctive odor, astringent with slightly bitter taste, the water and ethanol soluble extractive content were 19.263% ± 0.95 and 18.30% ± 0.51 respectively. The nonspecific parameters analysis of the extract showed that the extract's density was 1.413 g/mL ± 0.04, the water content value was 21.16% ± 0.55, total ash content was 15.64% ± 0.75, acid insoluble ash content was 8.282% ± 0.28, Pb contamination content was 3.67 ppm, Cd contamination content was <0,0043 ppm, total bacteria contamination was 90.5 x 10^1 colony/g, and the total mold and yeast contamination was 1 x 10^1 colony/g.

Keyword: Standardization, Kleinhovia hospita L, extract.

1. INTRODUCTION

Tahongai (*Kleinhovia hospita* L.) has been widely used as traditional medicine by Komering ethnic groups, South Sumatera, Indonesia to treat inflammatory diseases such as tumors, ulcers, polyps, tonsils, and dysmenorrhea. Tahongai leaves have been proven to have strong antioxidant activity against DPPH free radical agents (Arung et al., 2009). Decocta of tahongai leaves also have the activity as treatment for acute liver disease (Raflizar and Sihombing, 2009). Raflizar (2009) proved that tahongai leaves was safe, did not cause toxicity on the liver or kidneys based on the animal experiments. Because of the potency of tahongai as herbal medicine, it is necessary to standardize the tahongai extract.

Standardization is a system to ensure that every packet of medicine that is being marketed has the correct substances in the correct amount and will induce its therapeutic effect (Ekka et al., 2008). It is an important step to maintain the consistency of biological activity, chemical profile, or simply a quality assurance programs for production and manufacturing of herbal drugs preparation (Bajpai et al, 2012). Furthermore, extract standardization can also increase the economic value of herbal medicine producers (Saifudin et al., 2011). This standardization is carried out by spesific and non spesific parameters based on generalized standardization parameters of medicinal plant extract issued by Indonesian Ministry of Health.

2. EXPERIMENTAL SECTION

2.1. Chemicals

The chemicals used of this study were ethanol, aquadest, Mayer reagent, Wagner reagent, Dragendorff reagent, concentrated sulfuric acid, ammonia, chloroform, concentrated hydrochloric acid, magnesium powder, sodium hydroxide, iron (III) chloride, anhydrous acetic acid, formic acid, acetic acid, peptone, plate count agar (PCA), distilled water agar (DWA), potato dextrose agar (PDA).

2.2. Plant materials

The tahongai leaves were collected from Belitang, Ogan Komering Ulu District of South Sumatera, Indonesia. The sample was determined at Herbarium Department of Biology, Faculty

Article History:
Received 16 October 2017; revised 11 January 2018; accepted 11 January 2018
http://doi.org/10.26554/sti.2018.3.1.14-18

of Mathematics and Natural Science, Andalas University with letter number of 332/K-ID/ANDA/VIII/2017. Sampel was thoroughly washed with tap water, sorted while wet, dried in the shade, and grinded into powder.

2.3. Preparation of extract

Five hundred grams of powdered simplicia was weighed, then added ethanol 70% as much as 3.5 L and macerated for 48 hours at room temperature while stirred occasionally. Next, the obtained macerate was filtered using a paper-coated funnel to obtain the filtrate and then the residue was remacerated again twice for 24 hours to maximize the withdrawal of the unextracted chemical compounds in the previous maceration process. The obtained filtrate was concentrated using rotary evaporator at 70°C until thick extract from tahongai leaves obtained (Dewi et al, 2014). The thick extract was weighed and yield percentage of extract calculated by using equation 1.

$$\% \text{ Yield} = \frac{\text{Obtained thick extract}}{\text{Simplisia used in extraction}} \text{x} 100 \quad \text{.....(1)}$$

2.4. Alkaloid Test

One g of sample was crushed in the mortar, a small amount of chloroform and sand were added, then 5 mL of 0.05 N ammonia solutions in chloroform was added. The mixture was shaken for several minutes, then filtered into the test tube. H_2SO_4 2N was added into the filtrate and shaken regularly, two layers are formed. The top solution (water phase) is separated and tested with Mayer, Wagner, and Dragendorff reagents. Sediment formed indicates the presence of alkaloid group compounds (Al-Daihan and Bhat, 2012).

2.5. Flavonoid Test

A total of 0.5 g of sample was put into the test tube, added 5 mL of ethanol, and heated for 5 mins. The extract then filtered and the filtrate was added a few drops of concentrated HCl. Next, added 0.2 mg of magnesium powder approximately. If it appears red, it shows the presence of flavonoid compounds (Al Daihan and Bhat, 2012).

2.6. Saponin Test

A total of 500 mg sample was added into 10 mL of hot water. Then cooled and shake firmly. If there was a stable foam as high as 1 cm or more it showed the presence of saponin group compounds. Furthermore, addition of 1 drop of HCl 2 N will not make the foam disappear (Indonesian Ministry of Health, 1977).

2.7. Tannin Test

A total of 500 mg of sample was added into 50 mL of distilled water, then boiled for 15 minutes and chilled. 5 mL of filtrate were taken and dripped with $FeCl_3$ 1%. If the color turned into greenish black, it shows the presence of tannin class compounds (Al-Daihan and Bhat, 2012).

2.8. Steroid and Triterpenoid Test

A total of 2 g samples were crushed in mortar, a small amount of chloroform and sand was added, then added 5 mL of 0.05 N ammonia solutions into chloroform. The mixture was shaken for several minutes, then filtered into the test tube. H_2SO_4 2N was added into the filtrate and shaken regularly, leaving it to form two layers. The bottom solution was separated and dropped onto the drop plate, allowed to dry. After drying, anhydrous acetic acid was added and stirred evenly. Subsequently inserted 3 drops of concentrated sulfuric acid and observed the color that occurred. If the color was blue or green, then this indicates the presence of steroid compounds (Al-Daihan and Bhat, 2012). If the color was orange or purple, it shows the existence of triterpenoid group compounds (Malla et al., 2013).

2.9. Specific Parameter Determination of Extract

Organoleptic Analysis of the Extract

The organoleptic parameters of tahongai leaves extract were described about the shape, color, odor, and taste. Shape parameters include solid, dry powder, thick, and liquid. Color parameters such as yellow and brown. Parameters of aromatic odor or non odor and taste parameters include sweet, bitter, and others (Indonesian Ministry of Health, 2000).

2.10. Water Soluble Extractive Content

Samples were weighed 5 g then filled into closed flask. A total of 100 mL of chloroform saturated water was added into the flask. Stirring was done repeatedly for the first 6 hours and for the next 18 hours extract was ignored. The filtrate of 20 mL from soaking result was evaporated. The filtrate was then heated at temperature of 105°C to a constant weight. The percentage of water soluble extract was calculated by using Equation 2 (Indonesian Ministry of Health, 2000).

$$\text{Water Soluble} = \frac{\text{Dried filtrate (g)}}{\text{Extract (g)}} \text{ x } 100\% \quad \text{_____(2)}$$

Ethanol Soluble Extractive Content

The sample was carefully weighed 5 g, put into a flask and 100 mL of 95% ethanol was added. Stirring was done repeatedly for the first 6 hours and left it for the next 18 hours. Twenty mL of filtrate from the soaking result was evaporated in a preheated cup. The filtrate was then heated at a 105°C to a constant weight. Percentage of ethanol soluble extractive content was calculated using Equation 3 (Indonesian onMinistry of Health, 2000).

$$\text{Ethanol Soluble} = \frac{\text{Dried filtrate (g)}}{\text{Extract (g)}} \text{ x } 100\% \quad \text{.....(3)}$$

2.11. Non Specific Parameter Determination of Extract

Density of extract

Clean and dry pycnometer was weighed (W0). Then calibrate by determining pycnometer weight and water at 25°C then weighed (W1). The ethanolic extract of tahongai leaves was set to 20°C and put into empty pycnometer, remove the excess extract, set pycnometer containing extract temperature at 25°C then weighed (W2). The density of extract was calculated based on Equation 4 (Indonesian Ministry of Health, 2000).

$$\text{Density} = \frac{W2 - W0}{W1 - W0} \quad \text{.....(4)}$$

Tabel. 1 Phytochemical screening result of Tahongai leaves

Chemical Substance	Screening Result	
	Simplicia	Ethanolic Extract
Alkaloid	+	+
Flavonoid	+	+
Saponin	+	+
Tanin	+	+
Triterpenoid	-	-
Steroid	+	+

2.12. Water Content

Water content was determined using gravimetric method. Ten grams of extract was carefully weighed. The extract was dried at 105°C for 5 hours and weighed. The process was continued and weighed after 1 hour until the difference between 3 consecutive weighings was no more than 0.25% (Ministry of Health, 2000).

$$\text{Water Content} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100\% \quad \text{.....(5)}$$

2.13. Total Ash Content

A total of 2 - 3 g of extract was put into the furnace. The temperature was gradually increased up to 600°C and left for 4 hours, then cooled in desiccator and weighed. The total ash content was calculated based on the weight of the residue and sample, expressed using Equation 6 (Indonesian Ministry of Health, 2000).

$$\text{Total Ash Content} = \frac{\text{Residue (g)}}{\text{Sample (g)}} \times 100\% \quad \text{.....(6)}$$

2.14. Acid Insoluble Ash Content

The ash sample from total ash content analysis was boiled in 25 mL of dilute chloride acid for 5 minutes. The insoluble part of the acid-ash mixture was filtered through ash-free filter paper, washed with hot water, and chilled until the weight was fixed. The acid insoluble ash content was calculated on the weight of the test material, expressed using Equation 6 (Indonesian Ministry of Health, 2000).

2.15. Total Pb and Cd content

Total Pb and Cd content on ethanolic extract were determined with wet destructive methode using AAS (Atomic Absorption Spectroscopy) (Indonesian Pharmacopeia, 1979). Sample were tested in the Integrated Testing Laboratory, Faculty of Mathematics and Natural Science Sriwijaya University.

2.16. Microbial Contamination

Total Plate Count

A total of 5 tubes were filled with 9 mL peptone dilution fluids (PDF). The homogenization results from the preparation of the sample were plated by 1 mL dilution into the tube containing the first PDF diluent until 10^{-2} dilution was obtained and shaken to homogeneous, further diluting until reached the 10^{-6} dilution. One mL of each dilution was poured into a petri dish and duplicated, then poured 15 - 20 mL of medium plate count agar (PCA). Petri dish was shaken and treated in such way until the suspension spread evenly. To determine the sterility of the media and diluent, a control test (blank) was made. After the media was solidified, the petri dish was incubated at 35 - 37 °C for 24 - 48 hours with the upside down position. The number of growing colonies was observed and calculated (Indonesian Ministry of Health, 2000).

2.17. Mold and yeast count

Filled a total of 3 pieces of tube with 9 mL distilled water agars (DWA) 0.05%. The homogenization of the sample preparation was 1 mL dilution of 10^{-1} dilution into the first DWA tube until 10^{-2} dilution was obtained and shaken until homogeneous, then further dilution was made up to 10^{-4}. A total of 0.5 mL of each dilution was poured on the surface of the PDA, immediately shaken while rotated around the suspension to spread evenly and made duplo. To determine the sterility of the media and diluent, blank test was made by pouring the media on one petri dish and another petri dish filled with medium and diluent, then left to solidify. All petri dishes were incubated at 20 – 25°C for 5 - 7 days. After 5 days of incubation, a growing number of fungal colonies were observed and also at 7 days incubation. The plate with 40 - 60 colonies of mold/yeast was observed (Indonesian Ministry of Health, 2000).

3. RESULT AND DISCUSSION

3.1. Extraction and phytochemical screening results

The thick extract obtained from maceration of 500 g tahongai leaves powders using 70% ethanol was 86.9 g with the yield percentage of 17.38%. The value of this yield percentage was influenced by the duration of extraction and the amount of solvent

Table. 2 Standardization result of ethanolic extract of tahongai (*Kleinhovia hospita* L.) leaves

Parameter	Result	Requirement
Specific parameter		
Organoleptic	thick, brownish black in color, has distinctive odor, astringent with slightly bitter taste	-
Water soluble extractive content	19.263% ± 0.95	-
Ethanol soluble extractive content	18.30% ± 0.51	-
Non specific parameter		
Density	1.413 ± 0.04	-
Water content	21.16% ± 0.55	5 – 30% [a]
Total ash content	15.64% ± 0.75	-
Acid insoluble ash content	8.282% ± 0.28	-
Pb content	3.67 ppm	< 10 ppm [b]
Cd content	<0.0043 ppm	< 0.3 ppm [b]
Total plate count	90.5 x 10^3 colony/g	<1 x 10^4 colony/g [b]
Mold and yeast count	1 x 10^3 colony/g	1 x 10^3 colony/g [b]

*a (Voight, 1994)
b (Indonesian FDA, 2014)

used. Yield percentage is the percentage of raw materials that can be utilized from total raw materials, the higher the value of yield percentage indicates that the raw material has a greater chance of utilization (Kusumawati et al., 2008)

Phytochemical testing for the presence of various chemical constituents of simplicia and ethanolic leaves extract was performed using standard tests and procedures. The data reveals the presence of alkaloids, flavonoids, saponins, tannins, and steroids (Table 1).

3.2 Standardization of ethanolic extract of Tahongai (*Kleinhovia hospita* L.) leaves

Standardization of ethanolic extract of tahongai (*Kleinhovia hospita* L.) leaves was done to guarantee the quality of the final product (medicine, extract, extract product) and has certain determined constant parameter values (Indonesian Ministry of Health, 2000). Standardization of ethanolic extract of tahongai leaves was done by determining specific and nonspecific parameters of extract. The result of specific and nonspecific parameter of ethanolic extract of tahongai (*Kleinhovia hospita* L.) leaves can be seen in Table 2.

Determination results of this standardization require a reference to indicate that the extract meets the requirements that have been set. Ethanolic extract of tahongai leaves has no official standardization reference published yet by the Indonesian Ministry of Health and other sources. General values of extract requirement by Indonesian Food and Drug Administration was used as reference for the nonspecific parameters.

Tests of specific parameters are extract's organoleptic, water soluble extractive content, and ethanol soluble extractive content. The organoleptic test of extract aims as initial introduction to describe the shape, smell, color, and taste of the extract (Indonesian Ministry of Health, 2000). The results of the organoleptic test of the extract can be seen in Table 2. Other specific parameters tests were the determination of soluble compounds in certain solvents using ethanol and water. The results showed that the extract had 18.30% soluble compound in ethanol and 19.263% in water. Determination of soluble compounds in water and ethanol aims as a rough estimate of the polar (water soluble) active compounds and semi-polar to non-polar active compounds (soluble ethanol) (Saifudin et al., 2011).

Determination of nonspecific parameters of extract are extract's density, moisture content, total ash content, acid insoluble ash content, Pb and Cd content, and microbial contamination. Determination of extract density aims to provide limits of the mass size per unit volume as well as the type weights also related to the purity of extract and contamination (Indonesian Ministry of Health, 2000). On the measurement of this extract density was using pycnometer with value of 1.413 g/mL.

Measurements of water content of the extracts were performed to provide minimum limit or ranges of the amount of water content remaining in the extract after drying process (Indonesian Ministry of Health, 2000). Result of determination showed that water content of ethanolic extract of tahongai leaves equal to 21.16%, its set as viscous extract (Voight, 1994). The result was exceeding the maximum limit allowed for water content in extract by Indonesian Ministry of Health (limit value <10%). The extract was hygroscopic, so it must be dried again before usage and must be stored in low humidity to prevent microbial contamination (Isnawati and Arifin, 2006). The determination of ash content aims to provide an overview of internal and external mineral content derived from the initial process until the formation of the extract. The total ash content obtained from the extract was 15.236% and acid insoluble ash content was 8.282%. The result of high ash content is suspected because the level of inorganic elements such as minerals contained in the extract are quite high as well and the level of inorganic elements that are insoluble in acid such as silica obtained from the soil or sand where the plants grow. Determination of Pb and Cd content aims to guarantee that the heavy metal content of the extract does not cross the limit from generalized standardization parameters of medicinal plant extract by Indonesian Ministry of Health, because of its toxicity. Pb content of extract was 3.67 ppm and Cd content was <0.0043 ppm.

Microbial contamination testing aims to provide assurance that the extract should not contain pathogenic and non-pathogenic microbes beyond the specified limits because it affects the stability of the extract and is dangerous for health (toxic) (Indonesian Ministry of Health, 2000). Microbial contamination tests are based on the total plate numbers and yeast number. The total plate number obtained from ethanolic extract of tahongai leaves was 90.5 x 10^1 colonies/g. The result was still in the permitted range as it is below the maximum limit of 1 x 10^4 colonies/g that set in the book of Monographic Extract of Medicinal Plants by Indonesian FDA. The determination of yeast number obtained was 1 x 10^1colony/g also does not exceeded the requirements set by Indonesian FDA of 1 x 10^3 colonies/g. The low growth of bacteria and mold/yeast can also caused by the active compound flavonoid contained in tahongai leaves extract inhibit the growth of bacteria or microbes contained in the extract.

CONCLUSION

Phytochemical screening of ethanolic extract of tahongai leaves (*Kleinhovia hospita* L.) in Belitang, South Sumatera Indonesia shows presence of alkaloids, flavonoids, tannins, saponins, and steroids. Specific and nonspecific parameters of extract standardization mostly qualified based on the parameter that set in generalized standardization parameters of medicinal plant extract by Indonesian Ministry of Health. Except water content of extract that exceed the limit, it makes the extract must be stored in low humidity or dried again before further processing.

ACKNOWLEDGMENT

Authors would like to express their gratitude toward Sriwijaya University PNBP Sateks Research Grant that made this research possible.

REFERENCES

Al-Daihan, S., & Bhat, R.S. (2012). Antibacterial activities of extracts of leaf, fruit, seed, and bark of *Phoenix dactylifera*, *Afr J Biotechnol*, *11*(42), 10021-10025

Arung, E. T., Kusuma, I. W., Purwatiningsih, S., Roh, S. S., Yang, C. H., Jeon, S., Kondo, R. (2009). Antioxidant Activity and Cytotoxicity of the Traditional Indonesian Medicine Tahongai (*Kleinhovia hospita* L.) Extract. *Journal of Acupuncture and Meridian Studies*, *2*(4), 306–308.

Bajpai R., Jain N., & Pathak A.K.. (2012). Standardization of ethanolic extract of *Cucurbita maxima* seed, *Journal of applied pharmaceutical science*, *2*(8), 92-95

Dewi, N.W., Puspawati, N.M., Swantara, I.M., Asih, I.A. & Rita,

W.S. (2014). The antioxidant activity of flavonoid compound in ethanolic extract of *Solanum betaceum*, syn inhibiting the fat peroxidation. *Chemical chakra*, *2*(1), 7-16.

Ekka N.R., Namdeo K.M., Samal P.K..(2008). Standardization strategies for herbal drugs an overview. *Res J Pharm Tech*, *1*(4), 310-312

Indonesian FDA. (2014). *Quality parameters of traditional medicine.* Jakarta, Indonesia : Indonesian FDA

Indonesian Ministry of Health. (1977). *Materia Medika Indonesia.* Jakarta, Indonesia : Indonesian FDA

Indonesian Ministry of Health. (2000). *Standard parameters of medicinal plant extracts.* Jakarta, Indonesia : Indonesian FDA

Indonesian Pharmacopeia. (1979). *Indonesian Pharmacopeia* (3rd ed.). Jakarta, Indonesia : Indonesian Ministry of Health

Isnawati, A., & Arifin, K.M. (2006). Study of characteristic of *Gloria superba* L. leaves from phytochemical aspects, *Media research and medical development*, *16*(4), 8-14

Kusumawati R., Tazwir, & Wawanto A. (2008). The effect of rendement in hydrochloric acid on gelatin quality of red snapper (*Lutjanus sp.*), *Marine and fisheries postharvest and biotechnology Journal*, *3*(1), 63-38

Malla M.Y., Sharma M., Saxena R.C., Mr, M.I., Mir, A.H., & Baht S.H. (2013). Phytochemical screening and spectroscopic determination of total phenolic and flavonoid content of *Edipta alba* Linn, *J Nat Prod Plant Resour*, *3*(2), 86-91

Raflizar, D., & Sihombing, M. (2009). Paliasa Leaves (*Kleinhovia hospita* Linn) Extract For Treatment of Acute Hepatitis. *Health of ecology J*, *8*(2), 984–993.

Raflizar. (2009). Sub Chronic Toxicity test from alkohol extract paliasa leaves (*Kleinhovia Hospita* Linn) to hepar/Liver and Kidney of Experimental mice. *Media research and development, 19*(4), 204–212.

Saifudin, A., Rahayu, & Teruna. (2011). *Standardization of natural medicine ingredients*. Yogyakarta, Indonesia : Graha Ilmu

Voigt, R. (1994). *Pharmaceutical technology textbook.* (5th ed.). Yogyakarta, Indonesia : Gadjah Mada University Press

STI 2018 Indah (Tahongai)

ORIGINALITY REPORT

12%	8%	7%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	litbang.kemenperin.go.id Internet Source	1%
2	d.researchbib.com Internet Source	1%
3	Rennie Puspa Novita, Herlina, Arya Prasetya Beumaputra, Arini Alfa Hidayah. "The Rationality of Antihypertensive and Oral Antidiabetic Patients with Chronic Kidney Disease with Hypertension and or Type 2 Diabetes Mellitus in RSI Siti Khadijah Palembang", Journal of Physics: Conference Series, 2019 Publication	1%
4	erepo.unud.ac.id Internet Source	1%
5	Neza Rahayu Palapa, Muhammad Said. "Keggin type polyoxometalate H4[αSiW12O40].nH2O as intercalant for hydrotalcite", 'ARTS Publishing', 2017 Internet Source	1%

6	Anup Kalra, K. Ravikanth, M. J. Saxena. "Chapter 19 Herbals for Animal Health and Better Production", Springer Science and Business Media LLC, 2018 Publication	1 %
7	Submitted to Florida Institute of Technology Student Paper	1 %
8	Submitted to King Saud University Student Paper	1 %
9	jurnal.uii.ac.id Internet Source	1 %
10	Submitted to iGroup Student Paper	1 %
11	krishikosh.egranth.ac.in Internet Source	1 %
12	mail.phcogj.com Internet Source	1 %
13	www.academicjournals.org Internet Source	1 %
14	Sutomo Sutomo, Arnida Arnida, Fachrida Yulistati, Normaidah Normaidah, Mohammad Rizki Fadhil Pratama. "PHARMACOGNOSTIC STUDY AND ANTIOXIDANT ACTIVITY OF SUNGKAI (PERONEMA CANESCENS JACK.) METHANOL EXTRACT FROM INDONESIA",	1 %

Bulletin of Pharmaceutical Sciences. Assiut, 2022

Publication

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		