PHYTOCHEMICAL ANALYSES AND FREE RADICAL SCAVENGING ACTIVITY FROM TUIS (*Nicolaia speciosa,* HORAN)

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ABSTRACT

Kojong *et al.,* 2010. Phytochemical Analyses and Free Radical Scavenging Activity From Tuis (Nicolaia speciosa, Horan)

Tuis (Nicolaia speciosa, Horan) is a plant which used by people to give taste and odor on food, and it also used as herbal.The objective of this research was to determine phytochemical content and free radical scavenging activity from Tuis. Phytochemical content in Tuis was determined by its alkaloid, steroid, flavonoid, phenolic, tannins and saponim content. Free radical scavenging activity from Tuis was determined using 1,1-difenil-2-pikrilhidrazil (DPPH) method.Tuis contain phytochemical such as alkaloids, terpenoid, saponim and tannins, although flavonoid appear only in leaves part. The highest free radical scavenging activity from Tuis was leaves extract, with IC_{50} 1,77. Phenolic content in Tuis was believed act as antioxidant.

Keywords : tuis (*Nicolaia speciosa,* Horan), phytochemical, free radical scavenging, antioxidant

INTRODUCTION

Our environment tread on have provided resource which so abundance so that we earn to fulfill requirement of life. Requirement of life which progressively complex stimulate human being to be more develop science and technology, but progress of science and technology which fast progressively in the reality unable to shift traditional drug's role which have there is but both equiping each other. This matter is proved with life style which is flange return to meaningless nature of old fashion.

Exploiting natural resources in the form of traditional drug represent assessed by alternative more economic, though don't know active chemical compound consisting are in it. This matter is caused the lack of adequate information hit compound in used as by plant drug ingredient. On that account require to be conducted by research to know chemical compound from plant medicine specially plant which can be used as traditional drug.

Phytochemical screening represent matter which need to be conducted to know chemical compound in plant, specially plant medicine later on can be used to determine active compound which there are in it. Such active compound is metabolit secundary compound. Metabolit secundary compound is compound which don't synthesis in human being body but very required by human being body and this compound is usually found at animal or plant. The metabolit secundary compound for example alkaloid, steroid, terpenoid, flavonoid, tanin and saponin (Harbone, 1987).

Nicolaia speciosa, HORAN or which is in Minahasa language more knowledgeable by the name of *Tuis* represent wild plant which is life at height 600 - 1200 m from sea level. This plant besides used by society to give to feel and aroma at food, is also used as drug plant. Perry (1978), please report that this plant have medication like curing earache, cleaning hurt and deodorize body.

Till now there is no newest information about phytochemical content and free radical scavenging activity found on *Tuis* plant. Considering free radical very reactive and can oxidize protein, fat, DNA (Deoksiribonucleic Acid) and vitamin and also can generate threat the happening of degenerative disease. This research aim to to determine phytochemical found on *Tuis* (*Nicolaia speciosa*, HORAN) and test free radical scavenging activity from *Tuis* parts which is extraction by maseration using ethanol.

MATERIALS AND METHODS

Material

Tuis (part of root, leaf and steam) obtained from Malalayang. Used chemicals in this research is professional analyses : chloride aluminium, ammonia, aquades, acetate acid, chloride acid, sulphuric acid, iron (III) chloride, subnitrat bismuth, ethanol 95%, iodium, potassium iodida, paper filter Whatman No. 42, chloroform, vanilin, magnesium, merkuri (II) chloride, sodium carbonate, sodium chloride, Folin-

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Ciocalteu reagen and tocopherol obtained from MERCK (Darmstadt, Germany). Galac acid, quersetin, chatekin, 1.1-diphenyl-2-picrylhydrazil (DPPH) obtained from SIGMA Chemical Co. (St. Lois, MO).

Used appliance at this research is: PYREX glass appliance, EYELA N-1000 rotary evaporator and UV-VIS LEYBOLD Milton Roy spectrophotometer 501.

Preparation of Sampel

Tuis (*Nicolaia speciosa*, HORAN) was taken away from district of Malalayang, Manado – North Sulawesi. *Tuis* different pursuant to part of root, leaf and bar, is later cleared of by other dirt (such as like land, insect and dirt) by using water. After got wanted shares, the sampel of dry during ± 1 week.

Extraction Plant

Sampel which have run dry counted 20 g is later attenuated by blender become powder and kept in place of closed. Sampel deliberated by each (part of root, leaf and bar) counted 5 gram later extraction by maseration, using ethanol during 24 hour. Sample is later filtered so that obtained by filtrate 1 and debris 1. Debris 1 soaked with same solvent during 24 hour is later filtered so that obtained by filtrate 2 and debris 2. Filtrate 1 and 2 joined and filtered using paper filter Whatman No. 42. The filtrate is later evaporated using rotary evaporator with ambient temperature 40 ^{o}C. Harsh extract which obtained was kept in freezer (cooler cupboard) at temperature -20 ^{o}C before analyse and activity test.

Phytochemical Screening (Houghton and Raman, 1998)

Phytochemistry screening was done to know the existence of alkaloid compound, steroid, terpenoid, flavonoid, tannin and saponin in Tuis (*Nicolaia Speciosa*, HORAN).

Determination of total phenolic

The content of total phenolic was measured using the method of Jeong *et al*., 2002. Briefly, the tuis extract (1 mL) was mixed with 1 mL of the 50% Folin-Ciocalteu reagen and 1 mL of 2% Na_2CO_3 and centrifuged at 13400 g for 5 min. The absorbance of extracts was read at 750 nm with Spectrophotometer Milton Roy 501 after 30 min of incubation at room temperature. The results were expressed as gallic acid equivalents.

Determination of total flavonoid

The total flavonoid content of tuis extracts was determined according to Zhishen *et al*., 1999. Briefly, Distilled water was added to make 5 mL and 0,3 mL $NaNO_2$ (1:20) were added to make 5 mL $AlCl_3$ (1:10) were added and the total was made up to 10 mL with distilled water. The solution was mix well again and the absorbance was measured against a blank at 510 nm with a Milton Roy 501 Spectrophotometer.

Determination of condensed tannin

Condensed tanin was determined according to Julkunen-Tiitto method (1985). Counted 0,1 sampel condensation mL packed into wrapped reaction tube, then enhanced by 3 mL vanilin 4% (b/v) than mixed using vortex mixer. So soon as enhanced by 1,5 condensed HCL mL and mixed. Absorbance of sample was read at λ 500 nm after incubation during 20 minute at room temperature. Obstetrical of condensed tannin was expressed as cathekin equivalent in mg/extract. Curve calibrate to be drawn up by cathekin as standard.

Determination of Free Radical Scavenging Activity

Determination of free radical scavenging activity of DPPH according to Burda and Oleszek (2001). Drawn up by counted 2 mL DPPH 93 µM in ethanol and enhanced by 1 mL tuis extract for every part of root, steam, leaf and as comparator used tocopherol. The changing of condensation color it from purple to turning yellow to show radical scavenging efficiency. Last five minute from 30 minute, absorbance measured at λ 517 nm using UV-VIS Milton Roy spectrophotometer 501. Ranked among free radical scavenging activity of percentage decrease DPPH color it by using equation:

$$\text{Activity (\%)} = 100 \times \frac{1 - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

From percentage of free radical scavenging which obtained, we made by curve between free radical scavenging activity to test condensation concentration. From equation of the linear regression can be determined by IC_{50} value, that is test condensation inhibition concentration capable to scavenging 50% free radical of DPPH.

RESULTS AND DISCUSSION

Screening of Alkaloid

Censorship of alkaloid, addition of Mayer reagent cause to be formed its white chromatic sediment which show result which are positive contain alkaloid compound. The same process also done by addition of Dragendorf reagent and Wagner. Dragendorf reagent will be formed by rose colored sediment of orange while for the Wagner reagent will be formed by tan sediment.

Principle from method analyses of this reaction of precipitation that happened caused by ligand replacement. Nitrogen atom having free electron pairs at alkaloid can change iodo ion in the reagent. Mayer reagent contain chloride mercury and iodida potassium. Dragendorf reagent contain iodida potassium and subnitrat bismuth in glacial acetate acid. Wagner reagent contain iodida potassium and iod. In the field of pharmacy according to Solomon in Makang (2005), alkaloid have effect in the form of nerve system, lessening to feel pain and as antimicroba.

Screening of Saturated Terpenoid/Steroida

Terpenoid/Steroid content in plant tested by using Liebermann-Buchard method that will showed red or purple for the terpenoid and blue for the steroid. Lieberman-Buchard reagent contain molecule of acetate and sulphate acid that bonding with terpenoid/steroid compound so that produce color change.

Result of screening of steroid and terpenoid had been done, all part of plant contain terpenoid compound. In the field of terpenoid pharmacy represent active component in plant medicinize to cure diabetes disease, trouble menstruate, snake pecking, husk trouble, damage liver, malaria, antivirus and antibacteria, while steroid relate to cholesterol, D vitamin, hormone and antibiotic (Robinson, 1995).

Screening of Flavonoid

According to result that part of leaf contain flavonoid compound. Robinson (1995), please express that addition of magnesium powder and chloride acid examination of flavonoid will cause existing flavonoid compound were reducted causing reaction ruddle representing characteristic of existence of flavonoid at sample. In the field of flavonoid pharmacy can be used as antioksidan, curing liver function trouble and as antihypertense (Robinson, 1995).

Screening of Saponin

Arcuri in Makang (2005), expressing saponin have functioning glicosyl as polar bunch and terpenoid bunch/steroid as non-polar bunch. Compound owning polar bunch and non-polar have the character of is active of surface so that moment shaked with saponin water can form misell. At misell structure, polar bunch face out while its bunch face. This situation see like spume, in consequence in this analysis can be seen by ability of sample form spume.

In screening of saponin through test foam at sample from parts of Tuis plant formed by spume in number a few at surface of dilution, so that can be concluded that each part of Tuis plant contain saponin. In the field of saponin pharmacy can be used as antimicroba (Robinson, 1995).

Screening of Tannin

At addition of iron condensation (Chloride III) 10% estimated by this condensation react wrongly one hydroxyl bunch exist in tannin compound. The result of reaction finally generated color. Iron (III) Chloride utilized widely to identify phenol compound is including tannin. Result of conducted by examination at reaction tube using iron condensation (Chloride III) show green to black color. In the field of tannin compound pharmacy can be used as antioxidant and pursue growth of tumor (Robinson, 1995).

Total Content of Phenolic, Flavonoid and Condensed Tannin

Quantitative analysis of total content of phenolic, flavonoid, and condensed tannin showed on Table 1 below.

Table 1. Obstetrical total phenolic, flavonoid and condensed tannin from Tuis

No	Sample*	Phenolic (mg/kg)	Flavonoid (mg/kg)	Condensed Tannin (mg/kg)
1	Steam	51,22	ND**	126,8
2	Root	77,52	ND**	121,3
3	Leaf	132,7	2,51	140,8

* : Concentration 100 ppm
**ND : No detected

Obstetrical Determination Total Phenolic

Obstetrical determination total phenolic conducted to know free radical scavenging potency in extract. In this research, total phenolic in Tuis extract measured with gallic acid standard (mg/kg). Total of

phenolic in extract determined pursuant to ability of phenolic compound in Tuis extract reacting with fosfomolibdat-fosfotungstat acid in Folin-Ciocalteu reagen (natural yellow) change of colour become blue colour. Total content of root extract phenolic, leaf and steam from Tuis successively is 51.22; 77.52 and 132.70 mg/kg (Figure 1).

Obtained extract from part was characterized that leaf have total content of highest phenolic (132.70 mg/kg) compared to part of root (51,22 mg/kg) and steam (77.52 mg/kg). Level of total content of leaf phenolic anticipated because phenolic compound in leaf extract have the character of polar so that phenolic component in dissolve extract in ethanol become more.

Its obstetrical total phenolic in Tuis extract in direct corollation to free radical scavenging activity from the each part of Tuis extract. Ability of free radical scavenging activity of Tuis extract because of existence of chemical compound which can personate free radical scavenger.

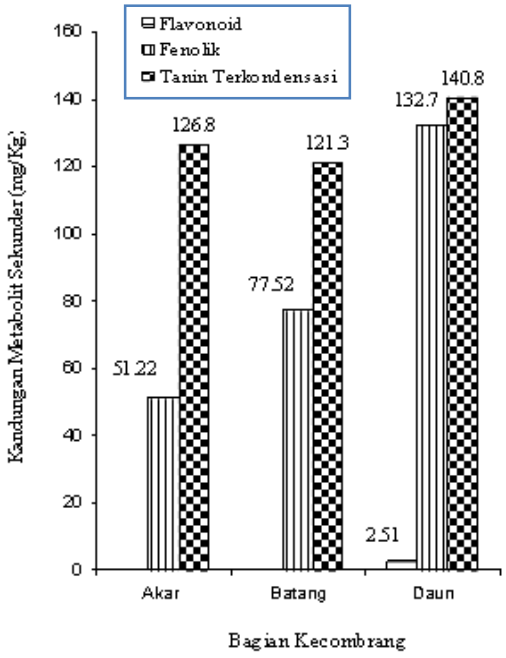

Figure 1. Obstetrical Bar diagram of secondary metabolite from parts of Tuis

Obstetrical Determination Total Flavonoid

Total content of flavonoid in Tuis extract indicate that leaf extract have total content of flavonoid, while at part of steam and root do not contain flavonoid compound. Total of leaf extract flavonoid is 2.51 mg/kg. This matter because of leaf represent place the happening of photosynthesis process at plant.

Determination of Condensed Tannin

Tannin content condensed to be to be expressed as cathekin ekuivalen in mg/kg extract. Total of condensed tannin highest found at part of leaf, steam and root successively is 140.8; 126.8 and 121.3 mg/kg. Leaf extract contain the amount of condensed tannin by larger ones than steam and root.

Tannin represent to secondary metabolite owning characteristic feel tan and bitter and also dissolve naturally in water form polyphenolic complex which attend in many plant is including husk and seed. A research about tannin had been reported that tannin 15-30 times more effective as radical arrester of peroksil than simple phenolic compound and trolox. Therefore, tannin has potency as important free radical scavenger (Shahidi and Naczk, 1995).

Determination of Free Radical Scavenging Activity of DPPH

Free radical scavenging activity from Tuis can be known through change of colour that happened, that is from purple become to turn yellow (Table 2).

Table 2. Radical scavenging activity from part of Tuis and also tocopherol as comparator by some concentration

Sampel	Concentration (ppm)					
	25	50	75	100	150	200
Steam	1.7	2.9	6.2	9,9	25.6	30.7
Root	2.3	7.0	10.6	12,8	37.1	40.9
Leaf	28.5	36.3	53.9	65	83.9	89.8
Toco	42.1	75.7	94.0	93,9	93.4	93.3

From free radical scavenging price which obtained, curve calibration between free radical scavenging percentage was made to test condensation concentration. From equation of the linear regression can be determined by $IC_{50.}$

Equation of regression ($y = ax \pm b$), can be calculated by IC_{50} value. To get IC_{50} value, hence y value at each equation filled with value 50so that got by x value (IC_{50}) (Table 3).

Table 3 Regression equation of and IC_{50} value from Tuis extract with tocopherol as comparator

No	Sample	Equation	R^2	IC_{50}	Concentration (ppm)
1	Steam	y = 33.34x – 50.616	0.804	3.01	1023.29
2	Root	y = 44.42x – 66.113	0.808	2.61	407.38
3	Leaf	y = 73.226x – 79.833	0.954	1.77	58.88
4	Tocopherol	y = 54.853x – 22.331	0.759	1.31	20.41

Inhibition concentration 50% from Tuis extract can be seen at table above. Value IC_{50} indicated that free radical scavenging activity of DPPH was decrease in sequence following: steam - root - leaf. With inhibisi concentration (IC_{50}) 58.88 ppm of Tuis leaf extract have effective earn scavenging 50% free radical. Later then followed by extract grow on and Tuis steam successively is 407.38 and 1023,29 ppm.

Determination of Free Radical Scavenging Activity of DPPH

Free radical scavenging activity from Tuis extract evaluated with radical examination 1.1-diphenyl-2-picrylhydrazil (DPPH). Radical Compound of DPPH is usually used as subtrate to evaluate free radical scavenging activity. Radical DPPH is unstable free radical and accept one hydrogen or electron to stable molecule (Matthaus, 2002). Examination of free radical scavenging activity of DPPH use spectrophotometer conducted with reacting extract with DPPH condensation. Absorbance at λ 517 nm used to measure scavenging effect from extract to radical DPPH. Absorbance at λ 517 nm, decrease as reaction between radical and free radical scavenging molecule.

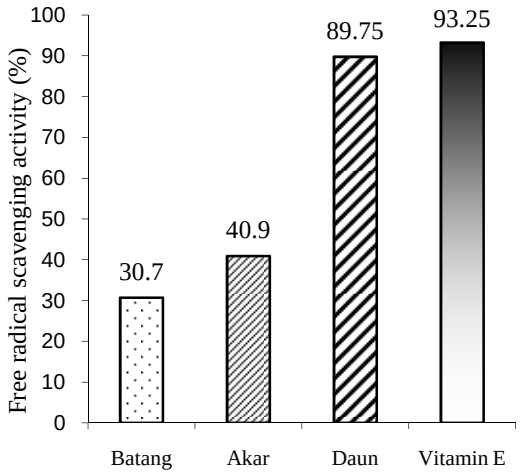

Figure 2. Comparison diagram between free radical scavenging activity of DPPH from part of Tuis to tocopherol at concentration 200 ppm

Therefore is, quicker of degradation of absorbance more have extract potency as free radical scavenger. This matter is shown also with change of color from purple become to turn yellow.

Such as those which seen at figure above, third part of Tuis extract have ability as free radical scavenging of DPPH. This data indication that any part of Tuis extract have potency as free radical scavenging of DPPH and also have ability to discharge hydrogen atom to diphenylpicrylhydrazil radical (violet) become difenilpikrilhidrazin non-radical compound (yellow) (Molyneux, 2004).

Leaf extract (89,75%) in this research show compared to higher level activity of root extract (40,9%) and Tuis steam (30,7%). In comparison with other antioxidant compound (tocopherol), at concentration which is same to be obtained by free radical scavenging activity equal to 93,25 % (Figure 2).

For the value of IC_{50}, smaller its value hence progressively lower also required concentration from part of Tuis plant as free radical scavenger. Table 4, can be seen that part of leaf have the lower IC_{50} value that is 1,77 so that at concentration 58,88 ppm have earned free radical scavenging. Part of root, owning IC_{50} value equal to 2,61 is so that required by concentration equal to 407,38 ppm. Part of steam, owning highest IC_{50} value that is 3,01 is so that required by concentration equal to 1023,29 ppm to be able to free radical scavenger. Equally, leaf at concentration 58,88 ppm more have potency as free radical scavenger compared to part of root (407,38 ppm) and steam (1023,29 ppm). As comparator used tocopherol.

CONCLUSION

From this research can be known that Tuis plant can be made drug pursuant to secondary metabolite content and potency as free radical scavenger especially part of Tuis leaf. Considering free radical can generate threat the happening of disease in the form of cancer, ageing, neurodegenerative disease (alzheimer), lung trouble, kidney and liver, diabetes etcetera.

REFERENCES

Burda, S. and W. Oleszek. 2001. Antioxidant and Antiradical Activities of Flavonoids. *J. Agric. Food Chem*. 49: 2774-2779.

Harbone, J.B. 1987. *Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan*. ITB. Bandung.

Houghton, P.J. and A. Raman. 1998. *Laboratory Handbook for the Fractionation of Natural Extracts*. Chapman & Hall. London.

Jeong, S.M., S.Y. Kim, D.R. Kim, S.C. Jo, K.C. Nam, D.U Ahn and S.C. Lee. 2004. "Effect of Heat Treatment on the Antioxidant Activity of Extracts from Citrus Peels". *J. Agric. Food Chem.* 52: 3389-3393.

Julkunen–Tiitto, R. 1985. Phenolics Constituens in the Leaves of Northern Willows : Methods for the analysis of Certain Phenolics. *J. Agric. Food Chem*. 33: 213-217.

Makang, V.M.A. 2005. Analisis Fitokimia Tumbuhan Obat di Kabupaten Minahasa Utara. *Skripsi* FMIPA UNSRAT. Manado.

Matthaus, B. 2002. Antioxidant Activity of Extracts Obtained from Residues of Different Oilseeds. *J. Agric. Food Chem*. 50: 3444-3452.

Meda, A., C.E. Lamien, M. Romito, J. Miliogo and O.G. Nacoulina. 2005. Determination of the Total Phenolic., Flavonoid, and Proline Contents in Burkina Fasan Money, as well as their Radical Scavenging Activity. *J. Agric. Food Chemistry*. 91: 571-577.

Molyneux, P. 2004. The use of The Stable Free Radical Diphenylpicrylhidrazyl (DPPH) for Estimating Antioxidant Activity. Songklanakarin. *J. Sci Technol*. 26: 211-219.

Perry, L.M. 1978. *Medicinal Plants of East and Southeast Asia : Attributed Properties and Uses*. The MIT Press. London.

Rababah, T.M., K.I. Ereipej and L. Howard. 2005. Effect of Ascorbic Acid and Dehydration on Concentrations of Total Phenolics, Antioxidants Capacity, Anthocyanins and Color in Fruits. *J. Agric. Food Chem*. 53: 4444-4447.

Robinson, T. 1995. *Kandungan Organik Tumbuhan Tinggi*. ITB. Bandung.

Shahidi, F. and M. Naczk. 1995. *Food Phenolics*. Technomic Pub. Co. Inc. Lancester, Basel.

Sumampouw, A. GO. 2003. Radikal Bebas dan Antioksidan. Diperoleh dari : http://www.medikaholistik.com. Diakses tanggal : 6 September 2008.

Yen, G. C. and P. Duh. 1994. Antioxidative Properties of Methanolic Extracts from Peanut Hulls. *J. Agric. Oil Chem. Soc*. 70: 383-386.

Zhishen, J., T. Mengcheng dan W. Jianming. 1999. The determination of Flavonoid Contents in Mulberry and their Scavenging Effects on Superoxide Radikal. *Food Chemistry*. 64: 555-559.