

Total Phenolic, Total Flavonoid and Phytochemical Screening by FTIR Spectroscopic of Standardized Extract of *Mikania micrantha* Leaf

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History

- Submission Date: 01-07-2020;
- Review completed: 30-07-2020;
- Accepted Date: 03-08-2020.

DOI : 10.5530/pj.2020.12.193

Article Available online

<http://www.phcogj.com/v12/i6>

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ABSTRACT

Background: *Mikania micrantha* is a great plant that has been used as raw material for traditional medicines. **Objective:** This paper aims to evaluate total phenols, total flavonoids, and phytochemical screening by FTIR spectroscopy of standardized extract of *Mikania micrantha* leaf to confirm its medicinal values. **Materials and Methods:** The leaves were extracted by maceration method using ethanol 96% and evaporated by rotary evaporator. The determination of total phenolic and total flavonoid were performed by spectroscopic method. The phytochemical constituent was screened through the bioactive group of the chemical by FTIR analysis. **Results:** The total phenolic of extract of *Mikania micrantha* leaf ranged from 13.19±0.74 to 34.24±1.24 mg gallic acid equivalent/g and total flavonoid ranged from 1.11±0.11 to 20.63±0.16 mg quercetin/g. FTIR analysis confirmed the presence of O-H, aliphatic CH, and C=O functional group. **Conclusion:** the result of this study confirm that *Mikania micrantha* possesses the potential of bioactive compounds which are responsible for the biological activities that are useful for raw material of traditional medicines.

Key words: *Mikania micrantha*, Total Flavonoid, Total Phenolic, FT-IR.

INTRODUCTION

Medication using medicinal plants is believed safer because it has lower side effects than synthetic drugs¹. Medicinal plants are known to have medicinal properties and have been used in traditional medicine for generations. Herbal medicines are used by 80% of the people worldwide due to its high efficiency, cheap cost, nonnarcotic nature and fewer side effects². So, the development of medicinal plants as raw materials for traditional medicines needs to be conducted. The use of raw materials or simplicia must be standardized for the sake of guaranteed sustainability. This is approved by identity substances and biochemical compositions with specifications related to monographs as quality requirements related to the Materia Medika³. It is necessary to extract standardized to ensure the quality and safety of the extract⁴.

Mikania micrantha is Asteraceae family and can be found in plenty from its natural habits, which makes the plant readily available for traditional treatment. In Indonesia, this plant known as 'Sembung Rambat'⁵ and widely distributed in North Sumatera, Indonesia. *Mikania micrantha* leaves are commonly used in the traditional medicine of insect bites or scorpion stings, skin diseases such as rashes and skin itches⁶, diabetes, stroke, hypercholesterolemia, hypertension⁷, analgetic, skin bleeding wound, healing sores, antimicrobial, skin infection and ulcers⁸.

Considering the broad medicinal effect consuming *Mikania micrantha* and the widespread this plants on different regions in North Sumatera, it is important

to ensure the quality of standardized extracts based on physicochemical and phytochemical screening responsible for biological action. This study is an essential starting point for the standardization of traditional medicines because only good quality of simplicia produce a good quality of traditional medicines⁹. The specific and non-specific parameters were tested to determine the quality of the extract using methods described in *Farmakope Herbal Indonesia*. This study aims to evaluate total phenols, total flavonoids, and phytochemical screening by FTIR spectroscopy of standardized extract of *Mikania micrantha* leaf to confirm its medicinal values. This preliminary study helped for standardization of the crude extract as well as further processing of the sample with some indication regarding the nature of chemical compounds present in it.

MATERIAL AND METHODS

Plant materials

The Leaves of *Mikania micrantha* were obtained from three different regions in North Sumatera Province, that is Deli Serdang, Tapanuli Selatan and Langkat. The plant was authenticated by *Herbarium Medanese*, FMIPA, Universitas Sumatera Utara.

Methods

Extractions

Mikania micrantha leaves were dried at room temperature and smashed manually. Each sample was extracted by maceration method with ethanol 96% at room temperature for 5 x 24 h. The liquid extract obtained was then evaporated with a rotary

Cite this article: Sumantri IB, Wahyuni HS, Mustanti LF. Total Phenolic, Total Flavonoid and Phytochemical Screening by FTIR Spectroscopic of Standardized Extract of *Mikania micrantha* Leaf. Pharmacogn J. 2020;12(6):1395-401.

evaporator. Viscous extracts are packaged in dark bottles then stored in a refrigerator at 4°C and can be used for further analysis.

Phytochemical screening and physicochemical analysis

All extracts were subjected to phytochemical screening by identification using different spraying reagent for particular compounds, such as dragendorff for alkaloids, AlCl_3 for flavonoid, FeCl_3 for tannin, Lieberman Burchard for steroid and sulfuric acid for saponin/triterpenoid. The extracts were also analyzed for physicochemical properties, such as water and ethanol-soluble extract, water content, total ash content and acid-insoluble ash content according to Farmakope Herbal Indonesia.

Determination of total phenol

Total phenolic content was determined with the Folin-Ciocalteu reagent. A calibration curve was obtained by using gallic acid as standard. 5 mg gallic acid dissolved in 100 ml methanol as a standard solution. Then diluted to 62.5; 125; 500 µg/ml. 10 mg sample was diluted in 10 ml methanol on the test tube. Both of 0.1 ml of standards and samples were taken and mixed with 0.5 ml of Folin-Ciocalteu and 7.9 ml of distilled water, vortexed for ± 1 minute, and added 1.5 mL of Na_2CO_3 20%, then incubated for 90 minutes. The absorbance of all standards and samples were measured at 400 nm to 800 nm using Shimadzu 1800 UV-Vis spectrophotometer and the results expressed as milligrams of gallic acid equivalents (GAE) per gram of extract.

Determination of total flavonoid

Total flavonoid was analyzed using aluminium chloride colourimetric method. Quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in 100 ml ethanol 96% and diluted to 6, 10, 14.5, 19, and 23.5 µg/mL. 25 mg sample was diluted in 25 ml ethanol 96%. 2 ml of each concentration of standard solutions, as well as 1 ml of each sample solution, were mixed with 3 mL ethanol 96%, 0.2 mL of aluminium chloride, 0.2 mL potassium acetate 1 M and 5.6 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured at 440 nm using Shimadzu 1800 UV-Vis spectrophotometer and the results expressed as weight of quercetin equivalent (QE) per gram of extract.

FT-IR extract analysis

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tools for identifying the types of chemical bonds (functional groups) present in compounds. All of the different extracts of *Mikania micrantha* was used for FTIR analysis. The extract is mixed

with powder which has been mashed, homogenized and put into the sample container, the powdered sample of each extract was loaded in FTIR Spectrophotometer (Shimadzu prestige 21).

RESULT AND DISCUSSION

Extraction

Mikania micrantha leaves processing is done as shown in Figure 1A-B. the leaves are processed into simplicia then extracted with ethanol.

In this present study, the extraction of *Mikania micrantha* leaf from Langkat has higher percentage yield compared to Deli Serdang and Tapanuli Selatan. The result of percentage yield extraction of *Mikania micrantha* leaf shown in Table 1.

Physicochemical properties and phytochemical screening

The physicochemical and phytochemical screening performed on *Mikania micrantha* leaf. The result of physicochemical analysis such as water and ethanol-soluble extract, water content, total ash content and acid-insoluble ash content shown in Table 2. Further phytochemical screening showed in Table 3.

Total phenolic and total flavonoid

The result of total phenolic showed that the extract *Mikania micrantha* from Langkat is higher than in other regions. Whereas the result of total flavonoid showed that the extract of *Mikania micrantha* from Deli Serdang is higher than other regions (Table 4). Determination of total phenolic and total flavonoid use gallic acid and quercetin as standards where the calibration curve equation obtained were $y = 0.001233x + 0.032114$ ($R^2 = 0.996382$) and $y = 0.031619x - 0.00836$ ($R^2 = 0.999029$).

FTIR extract analysis

The FTIR spectrum of ethanol extract of *Mikania micrantha* leaf is presented in Table 5: Figures 2-4. The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the extract are represented in Table 5. The region of IR radiation helps to identify the functional groups of the active component present in extract based on the peaks values of the FTIR spectrum. When the extract was passed into the FTIR, the functional groups of the components separated based on the ratio of its peak. The results of FTIR



Figure 1: *Mikania micrantha* leaves. A: Fresh leaves. B: Simplicia leaves.

Table 1: The percentage yield of extraction in each region.

No	Region	The percentage yield of extraction
1	Deli Serdang	11.67%
2	Tapanuli Selatan	12.76%
3	Langkat	13.86%

Table 2: Physiochemical analysis of *Mikania micrantha* leaf.

Parameters	Mikania micrantha leaf		
	Deli Serdang	Tapanuli Selatan	Langkat
Water content	8.33%	7.61%	6.67%
Total ash content	5.93%	7.88%	8.34%
Acid Insoluble ash content	4.44%	5.42%	5.89%
Water-soluble extract	27.82%	23.93%	23.66%
Ethanol soluble extract	18.57%	19.17%	16.39%

Table 3: Phytochemical screening of *Mikania micrantha* leaf.

Phytochemical constituent	Mikania micrantha leaf		
	Deli Serdang	Tapanuli Selatan	Langkat
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Glikosides	+	+	+
Terpenoids/Steroids	+	+	+

Table 4: Total Phenolic and Total Flavonoid.

Region	Total Phenolics (mg/g) in GAE	Total Flavonoid (mg/g) in QE
Deli Serdang	13.19 ± 0.74	20.63 ± 0.16
Tapanuli Selatan	32.77 ± 1.21	4.26 ± 0.15
Langkat	34.24 ± 1.24	1.11 ± 0.11

Table 5: FTIR spectral wavenumber's values and functional groups obtained from an extract of *Mikania micrantha* leaf.

	Peak Values	Functional Groups
Deli Serdang	3348.42 cm ⁻¹	-OH group
	2924.09 cm ⁻¹	C-H stretching aliphatic
	2858.51 cm ⁻¹	C-H stretching aliphatic
	1724.36 cm ⁻¹	C=O carbonyl group
	1056.99 cm ⁻¹	C-O group
Tapanuli Selatan	3344.57 cm ⁻¹	-OH group
	2927.94 cm ⁻¹	C-H stretching aliphatic
	2858.51 cm ⁻¹	C-H stretching aliphatic
	1450.47 cm ⁻¹	C-H stretching aliphatic
	1728.22 cm ⁻¹	C=O carbonyl group
Langkat	3340.71 cm ⁻¹	-OH group
	2927.94 cm ⁻¹	C-H stretching aliphatic
	2858.51 cm ⁻¹	C-H stretching aliphatic
	1624.06 cm ⁻¹	C=O carbonyl group

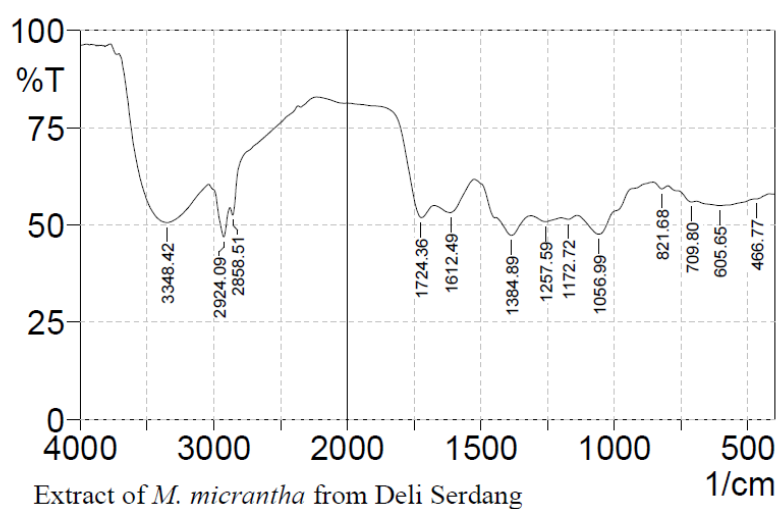


Figure 2: FTIR spectrum of Extract of *Mikania micrantha* leaf from Deli Serdang.

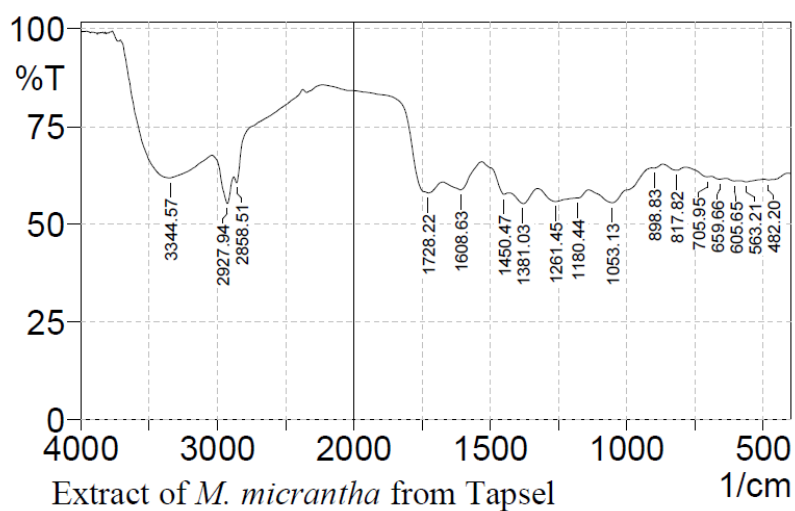


Figure 3: FTIR spectrum of Extract of *Mikania micrantha* leaf from Tapanuli Selatan.

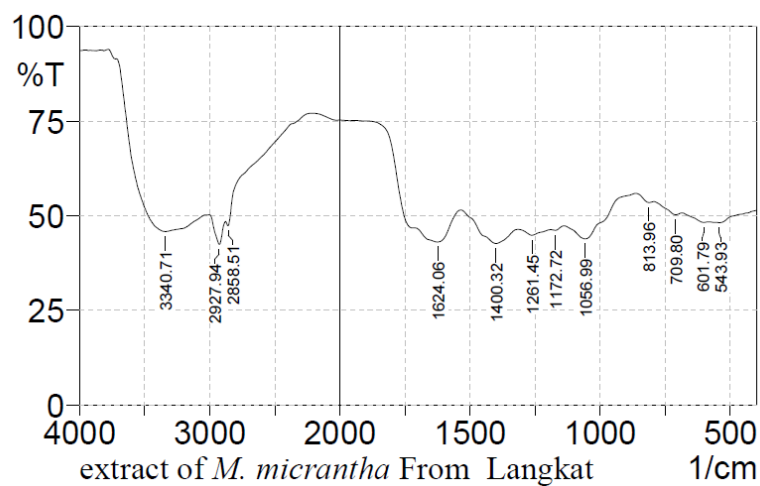


Figure 4: FTIR spectrum of Extract of *Mikania micrantha* leaf from Langkat.

analysis confirmed the presence of hydroxyl, aliphatic hydrocarbon and carbonyl group.

DISCUSSION

The plant is a very source of potentially useful as raw material for traditional medicines. The biological and pharmacological properties of many plants have active compounds that the potential pharmacologically. *Mikania micrantha* is a great plant that has been used as herbal medicines for diverse pharmacology activity.

The extraction of *Mikania micrantha* leaf from three different regions by maceration using ethanol 96% solvent did not show a significant difference in the yield. The result of extraction (Table 1) is not less than 11.67%.

The physicochemical analysis is responsible for ensuring the quality and purity of *Mikania micrantha*¹⁰. The value of water content showed values less than 10 % that means sample will not allow for microbial growth. The water-soluble extractive matters are not less than 23.66%, and the ethanol extractive matters are not 16.39%. The total ash content is not more than 8.34%, and acid insoluble ash content is not more than 5.89% (Table 2). 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. Total ash was determined to describe the content of internal and external minerals derived from the initial process until the formation of the extract¹¹. It is an essential point for the standardization of traditional medicine because only good quality of simplicia produces a good quality of traditional medicine⁹.

The phytochemicals are responsible for the therapeutic activities of the plants. The results showed that the extract contains alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides. Pharmacological properties of *Mikania micrantha* are used as antioxidant, anthelmintic, antidiabetic, antispasmodic, antimicrobial, antiprotozoal, antitumour, anti-inflammatory, analgesic, anticancer and antiviral activities¹². In addition, Alkaloids can also be used as an anti-inflammatory, analgesic and anticancer^{13,14}. Alkaloids also can heal wounds by stimulating the formation of fibroblast phase, and steroids can help the formation of new skin in the open wound, terpenoids can help the wound healing process¹⁵. Flavonoids are known as a wide range of therapeutic activities such as antihypertensive, antioxidant, antimicrobial and anticancer properties^{16,17}. Flavonoids, tannins and phenols are acting as antimicrobial agents¹⁸. Steroids/terpenoids can help the formation of new skin cells and wound healing process¹⁹. Therefore, this plant can be used as a raw material for traditional medicine.

The extract of *Mikania micrantha* leaf was standardized for total phenolics and flavonoids content. The total phenolics content was in descending order from Langkat > Tapanuli Selatan > Deli Serdang (Table 4). The phenolic content of extract of *Mikania micrantha* leaf varied between 13.19 ± 0.74 to 34.24 ± 1.24 mg/g GAE. The phenolic compound is a class of antioxidant agents which as free radical terminators and their abilities to chelate metals, inhibit lipoxigenase and scavenge free radicals²⁰.

The total flavonoids content was in descending order from Deli Serdang > Tapanuli Selatan > Langkat (Table 4). The total flavonoid content varied between 1.11 ± 0.11 to 20.63 ± 0.16 . The extract of *Mikania micrantha* leaf showed the total phenolics and total flavonoids content present in these extracts. Flavonoid and phenolic compounds exhibited the most significant antioxidant activity thus can be explored to be a new drug. The antioxidant activity of a plant has an important role in indicating the possibility of other biological activities. Antioxidants have properties that can overcome metabolic disorders and pathological

conditions such as cardiovascular, respiratory, infection, inflammation, carcinogenesis and the ageing process²¹.

The FT-IR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. The IR absorption bands spectrum of *Mikania micrantha* leaf extracts from Deli Serdang, Tapanuli Selatan and Langkat confirmed the presence of -OH, C-H stretching aliphatic, C = O carbonyl group (Table 5, Figures 1-3). Overall, the peaks contained in the three ethanolic extracts of *Mikania micrantha* leaf have almost the same functional groups but different absorbances. FTIR spectroscopy provides a spectral fingerprint that uniquely identifies chemical compounds or extracts and their functional group. Fingerprinting is superior to other analytical methods because no two compounds or extract have the same infrared spectrum²².

CONCLUSION

The result of this study shows the total phenol, total flavonoid determination, phytochemical screening and analysis by FTIR confirmed that *Mikania micrantha* extract leaf possesses the potential of bioactive compounds which are responsible for the biological activities as a raw material of traditional medicines. Standardized extract of *Mikania micrantha* leaf from Deli Serdang, Tapanuli Selatan and Langkat contain alkaloids, flavonoids, tannins, saponins, glycosides, steroids/triterpenoids. The total phenolic of extract of *Mikania micrantha* leaf ranged from 13.19 ± 0.74 to 34.24 ± 1.24 mg gallic acid equivalent/g and total flavonoid ranged from 1.11 ± 0.11 to 20.63 ± 0.16 mg quercetin/g. FTIR analysis confirmed the presence of O-H, aliphatic CH, and C=O functional group.

ACKNOWLEDGEMENT

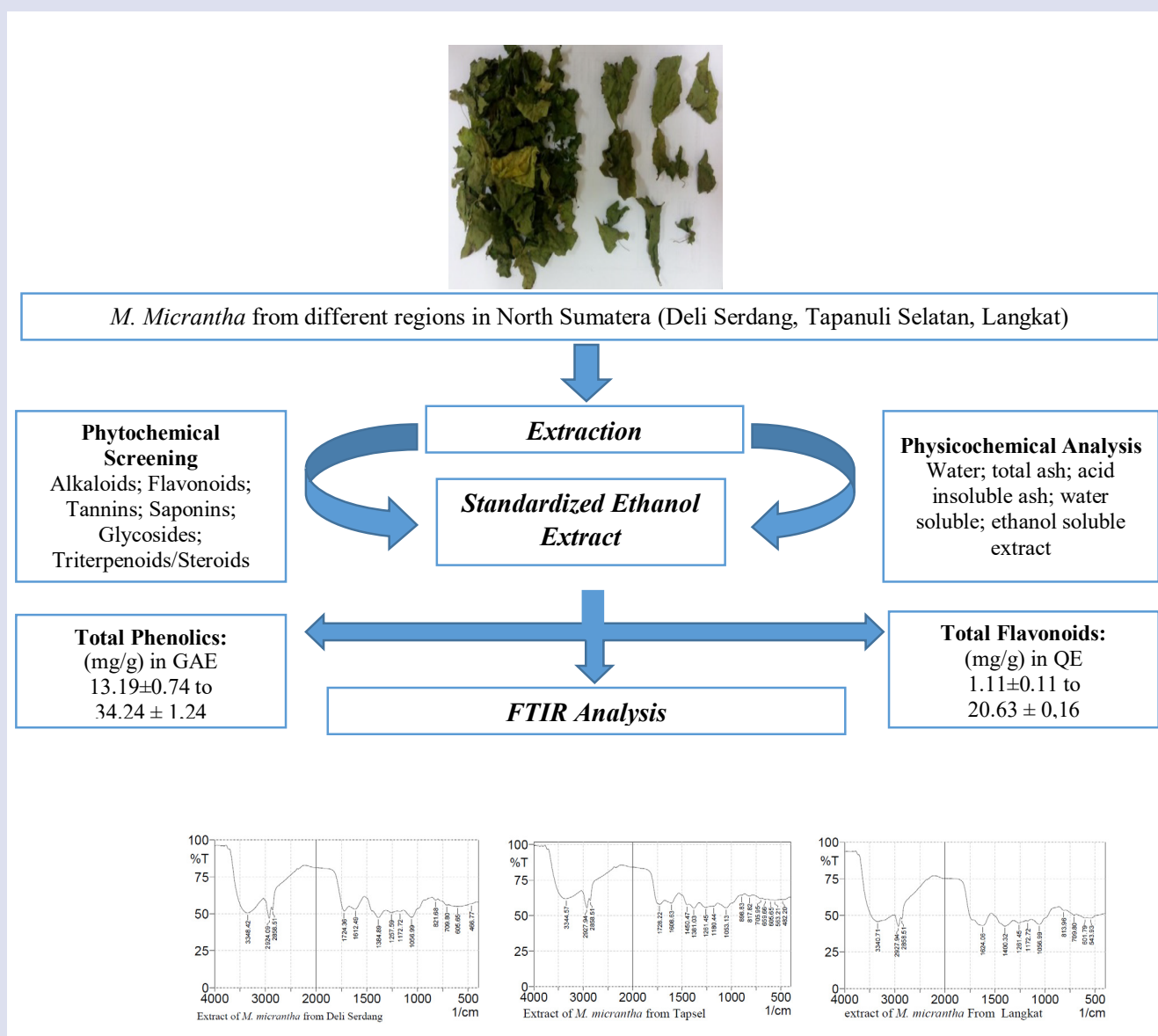
This study is supported by USU Grant (Kontrak Penelitian Talenta USU Tahun Anggaran 2018 No. 2590/UN5.1.R.PP/2018 tanggal 16 Maret 2018).

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GRAPHICAL ABSTRACT



SUMMARY

- Standardized ethanol extract of *M. Micrantha* leaf from three different regions in North Sumatera i.e Deli Serdang, Tapanuli Selatan and Langkat with percentage yield is not less than 11.67%.
- Phytochemical screening showed that all of extract contain alkaloids, flavonoids, tannins, saponins, glycosides, triterpenoids/steroids. Physicochemical analysis showed that water content is no more than 8.33%; total ash content is not more than 8.34%; acid insoluble ash content is not more than 5.89%; water-soluble extractive are not less than 23.66%, and ethanol extractive matters are not less than 16.39%.
- Total phenolic of ethanol extract of *M. Micrantha* leaf from Deli Serdang, Tapanuli Selatan and Langkat are respectively 13.19 ± 0.74 ; 32.77 ± 1.21 ; 34.24 ± 1.24 mg/g in GAE.
- Total flavonoid of ethanol extract of *M. Micrantha* leaf from Deli Serdang, Tapanuli Selatan and Langkat are respectively 20.63 ± 0.16 ; 4.26 ± 0.15 ; 1.11 ± 0.11 mg/g in QE.
- FTIR Analysis showed the spectrum of extract ethanol of *M. Micrantha* extracts from Deli Serdang, Tapanuli Selatan and Langkat confirmed the presence of –OH, C-H stretching aliphatic, C = O carbonyl group.

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Cite this article: Sumantri IB, Wahyuni HS, Mustanti LF. Total Phenolic, Total Flavonoid and Phytochemical Screening by FTIR Spectroscopic of Standardized Extract of *Mikania micrantha* Leaf. Pharmacogn J. 2020;12(6):1395-401.