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# Isolation and Characterisation of Alkaloid from Leaves of *Brugmansia Candida*

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**ABSTRACT**

*One of alkaloid compound was isolated from leaves Brugmansia candida. The compound was purified by chromatography column with silica gel as adsorbent. The purity of the isolated compound was tested by TLC, melting point. The structure of the compound was identified by means of spectroscopic include Ultraviolet Spectroscopy, Infrared Spectroscopy, and NMR. All data obtained indicates that the resulting compound is an isoquinolin alkaloid.*

**Keywords:** Isoquinolin, *Brugmansia candida*

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## INTRODUCTION

The use of plant as a medicine related with chemistry compound had by the plant mainly the bioactive one. Bioactive compound can be found at fruit, flower, leaf, root, and trunk. Bioactive compound in the plant is a secondary metabolic compound such as alkaloid, flavonoid, steroid, and saponin [1-3]. One of the bioactive plants is solanaceae *Brugmansia Candida*. This poisonous species is originated from South America [4]. Brugmansia is a genus from flowers belonging to solanaceae known as angel's ''trumpets". Some species well know are: *Brugmansia arborea*, *Brugmansia aurea*, *Brugmansia sanguinea*, *Brugmansia suaveolens*, *Brugmansia versicolor* and *Brugmansia candida* [4]. *Brugmansia candida*, also named *Kecubung Gunung*, is one of member of *Solanaceae* containing slcoloid schopolamin compound, saponin, glikosida flavonoid and polyphenol. It can relieve asthma and can be an analgesic [5]. Some types of compound resulting from isolation of the root of *Kecubung Gunung* are tropane alkaloid such as hyoscyamine, 6β – hydroxyhyoscyamine and skopolamin. It is traditionally used in curing of anticholinergic activity. Indonesians, especially Javanese, use *Kecubung Gunung* for cigarette assortment. The plant is believed to have relaxed effect but there has not been a research about it yet. This condition leads the researcher to know about active compound, alkaloid, containing in the plant.

## EXPERIMENTAL SECTION

### Plant Material

*Brugmansia candida* Pers leaves were compiled on May 20th, 2016 in the nature reserve of Anai valley, West Sumatra, Indonesia. It was identified in Herbarium of Biology Department, Andalas University, Padang, Indonesia.

**Chemical Material**

The chemical used in the research were Acetone, N-hexane, filter paper, ethyl acetate, methanol, dichloromethane, silica gel 60 (230-400 mesh) from Merck company. All chemicals used were in high grade, NaOH, and blue silica gel, distilled water, and sephadex LH-20.

**Instruments**

The instruments used in the research were Rotary evaporator Heidolp WB 2000, the general glassware in organic laboratory, melting point apparatus (John Fisher), oven, and vacuum desiccator. IR spectra were recorded on JASCOFT/IR-460. Plus spectrometer column chromatography (CC) was performed on silica gel 60 N, spherical, neutral, Kanto Chemical Co. Inc. and Sephadex LH- 20LH-20 (GE Health care, Japan), UV spectra on a Spectrometer UV Secoman S 1000. Thin Layer chromatography (TLC) was performed on silica gel 60 F254 for analytical chromatography (200 micrometre layer thickness (Merck). Preparative thin layer (PTLC) was performed on silica gel 60F254 (1 mm layer Thickness, Merck). The entire chemical used in study was purchased from Merck.

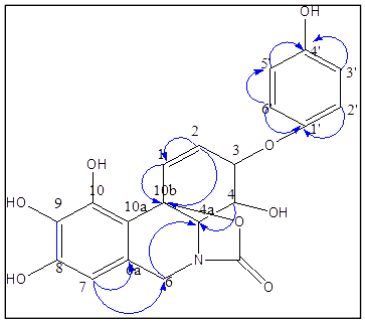
**Extraction, Isolation, and Purification**

Leaf powder of *Brugmansia candida* Pers (3000 grams) was extracted by using maceration method with methanol. The extract was basified to pH 10 with $NH_4OH$ and then fractionated with ethyl acetate extract. The ethyl acetate extract was evaporated in vacuum to give crude (20 g) of ethyl acetate. The crude (20 g) was purified with column chromatography (350 g) on silica gel as the adsorbent (230-400 mesh). It was eluted with the increasing of polarity by using 100% n-hexane to 100% ethyl acetate. Each fraction was monitored with TLC; the same Rf was combined with yield 22 fraction (F1-F22). F22 Fraction, positively containing alkaloid but not pure, was re-purified with re-column chromatography to obtain a pure compound. The isolated compound as yellow crystal (13 mg) was positive as alkaloid compound, gave one spot on TLC and had narrow range of melting point. Furthermore structure was determined by spectroscopy UV, spectroscopy IR, Spectroscopy NMR and mass spectroscopy.

## RESULTS AND DISCUSSION

Based on the result of plant identification at Herbarium, it is known that the plant that was about to be analysed belongs to Solanaceae family and *Brugmansia candida* species (*Kecubung Gunung*). Photochemistry test on *Brugmansia candida* sample shows that the leaf contains alkaloid compound, flavonoid, phenolic, terpenoid, saponin and steroid. Result of purification with chromatographic column gives 13 mg yellow crystal, in which KLT shows single spot around Rf 0, 78 with eluent acetone and ethyl acetate (2:3) and positive result of Dragendorf reagent. This is indicated that it positively contains alkaloid. Melting point test shows that compound resulted from isolation has melting point around 213-214°C. The melting point range of this narrow compound shows pure isolated compound. UV- Vis spectrum gives maximum absorption with wavelength around 204, 267, 303, 314, and 351 nm. The data of UV–Vis spectrum of isolated compound in methanol shows that there is absorption on wavelength more than 200 nm. UV Spectrum of organic compound gotten indicates that isolated compound has double bond conjugation. Infra-red spectrum gives absorption at wavelengths 624, 670, 880, 1046, 1087, 1274, 1328, 1379, 1417, 2883, 2927, 2973 and 3317 $cm^{-1}$. Their spectrum of isolated compound on the wavelength 3317 $cm^{-1}$ indicates the group O-H. On the wavelength of 2973 $cm^{-1}$, there is a group -CH, at 1046 $cm^{-1}$, a group - C - O at wavelength 670 $cm^{-1}$, and a group of the substituted benzene. From the IR spectrum data, it can be seen that the isolated compound has a group C-N, C-O, and the substituted benzene. The spectrum of 13C-NMR (125MHz, CD3OD): 179.731; 166.177; 163.231; 161.791; 158.998; 158.604; 135.702; 132.428; 132.428; 122.753; 116.399; 116.399; 105.802, 104.487, 100.033, 94.888, 74.145, 72.907, 69.096, 66.888 ppm. 13C NMR measurement result shows the number of 20 C atoms contained in the isolated compound. This result shows the primary, secondary, tertiary or quaternary C atom. In the chemical shift 66.888, there is signal of secondary carbon; this is supported by the data of 13C NMR DEPT 1350, which shows such shifts downwards. Then at the chemical shift at 166.177 to 104.487, there are 12 signals of carbon which is typical for aromatic groups. While the chemical shifts of 179.731 is a signal for the ketone group. The result of DEPT 13C NMR spectrum can also show the primary, secondary, tertiary and quaternary C atoms. In 1350 DEPT 13C NMR shows that the secondary C atom forms the spectrum downward, primary and tertiary C atom pointing up; while for C quaternary no longer appear. From the results of

13C NMR and 13C NMR DEPT 1350, it can be concluded that the isolated compound has twenty atom C with one secondary C atom, eleven tertiary C atom and nine quaternary C atom. 1H NMR data indicates the number of protons bounded to a carbon atom. Isolated compound of 1H NMR spectrum shows the number of 12 protons obtained from the integration of the proton spectrum. The spectrum shows two groups of protons, CH2 protons and CH group. 1H-NMR spectrum (500MHz, CD3OD): gives a chemical shift in the chemical shift δ 8063 (s, 1H and 1H); 6.889 (s, 1H and 1H); 6404 (s, 1H); 6203 (s, 1H); 5.148 (d, 1H); 3887 (s, 1H); 3,796 ppm (s, 1H); 3.766 (s, 1H); 3.635 (s, 1H) and 3.419 (s, 1H). This means that the isolated compound has 12 H atoms. The result of 1H NMR, it can be seen at shift, from 416 to 3.423 (1H) and from 3.770 to 3.790 (1H) is a bounded proton to CH2. While the shift from 6.875 to 6.904 and 8.049 to 8.077 is bounded proton to carbon having the same circumstances on the aromatic ring. 1H-1H COSY (Correlated Spectroscopy) shows a correlation between proton and bounded proton to carbon besides. The result shows a correlation between spectrum H1 to H2, H4 and H4a, and H2' and H3'. Data of 1H-13C Heteronuclear Multiple Quantum Coherence (HMQC) isolated compound shows correlation between carbon atom and related proton. NMR spectrum Heteronuclear Multiple Bond Connectivity (HMBC) shows the layout confirmation of proton to carbon by studying the correlation between carbon and proton besides. The result HMBC spectrum and correlation on the structure can be seen on the following structure (Scheme 1).



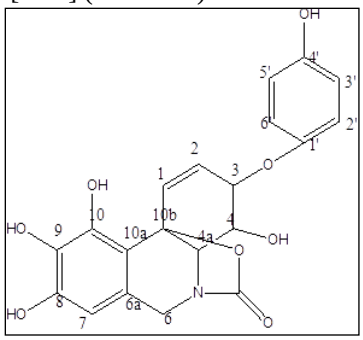

**Scheme 1: HMBC spectrum and correlation**

The results of HMBC spectrum shows correlations between H-2 to C-1 and C-10b, H-1 to C-10b, H-4 to C-4a, H-6, C-4a, H-7 to C-6a and C-6, H-2 'with the C-1', H-3 'with C-4'. This proves that there is a correlation between carbon and proton besides. Furthermore, to support the spectroscopic analysis of the isolated compound above is done by comparison to the results of 1H NMR and 13C NMR of the known compound. Comparative Data can be shown in Table 1.

**Table 1: Chemical shifts data of isolated compound and comparative compound**

| No | Atom C Number | CNMR | | Comparison | |
|---|---|---|---|---|---|
| 1 | 1 | 1,00,033 | CH | 137,4 | CH |
| 2 | 2 | 94,888 | CH | 125,2 | CH |
| 3 | 3 | 69,096 | CH | 68,6 | CH |
| 4 | 4 | 72,907 | CH | 34,7 | CH2 |
| 5 | 4a | 74,145 | CH | 67,7 | CH |
| 6 | 6 | 66,888 | CH2 | 61,8 | CH2 |
| 7 | 6a | 1,35,702 | C | 127,1 | C |
| 8 | 7 | 1,04,487 | CH | 104,5 | CH |
| 9 | 8 | 1,58,998 | C | 147,9 | C |
| 10 | 9 | 1,63,231 | C | 148,4 | C |
| 11 | 10 | 1,58,604 | C | 108,1 | CH |
| 12 | 10a | 1,66,177 | C | 1137,7 | C |
| 13 | 10b | 1,05,802 | C | 51,7 | C |
| 14 | 1’ | 1,61,791 | C | 157,4 | C |
| 15 | 2’ | 1,32,428 | CH | 131,0 | CH |
| 16 | 3’ | 1,16,399 | CH | 116,7 | CH |
| 17 | 4’ | 1,22,753 | C | 130,9 | C |
| 18 | 5’ | 1,16,399 | CH | 116,7 | CH |
| 19 | 6’ | 1,32,428 | CH | 131,0 | CH |
| 20 | | 1,79,731 | C = O | | |

Based on the analysis of spectroscopy UV - Vis, IR, 13C NMR, DEPT 1350, 1H NMR, HMQC, HMBC and COSY as well as the comparison with the results of 13C NMR spectrum of the known compound, it is concluded that the isolated compound is isoquinolin alkaloid [6-11] (Scheme 2).




**Scheme 2: Isoquinolin alkaloid**

## CONCLUSION

Based on data analysis of UV, IR, and NMR, the isolated compound from ethyl acetate fraction of *Brugmansia candida* is isoquinolin alkaloid.

## ACKNOWLEDGEMENTS

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