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**Research Article**

# Distribution and Phytocomponent in the Ethanol Extract of *Globba candida* Gagnep. (Zingiberaceae) by GC-MS Analysis

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**ABSTRACT**

*Globba* is the third largest genera in the Zingiberaceae family, distributed in tropical and sub-tropical regions with the center of biodiversity in Southeast Asia. The study about phytochemical contents *Globba* is an interesting especially *Globba candida* Gapnep which have been never reported. The aims of this study were to observe distribution and phytochemical compounds of ethanol extract of *G. candida* Gapnep. found on Bali Island. Hydrodistillation was used to determine the presence of its essential oil and a Gas Chromatography-Mass Spectrometry (GC-MS) was used to carry out the phytochemical analysis of the essential oil and ethanol extracts of *G. candida* Gapne. The phytochemical analysis of the rhizomes and leaves ethanol extracts of *G. candida* Gapnep revealed 36 and 8 identified chemical compounds representing 85,82% and 85,47% of the total analyzed extracts. However, there was no essential oil constituent found in *G. candida* Gapnep. The main compounds of the rhizome extract of *G. candida* Gapnep were levoglucosan 19.07%, allylhydrazone acetaldehyde 5.52%, trans-2,3-epoxybutane 6.30%, butan-3-enoic acid methyl ester 4.36%, 2-methylcyclopentanone 4.02%, and 2-n-propyl-oxetan 4.00%. While the dominant contents of the leaves extract of *G. candida* Gapnep were *pinostrobin chalcone* 75.63%.

*Keywords: Globba candida Gapnep, GC-MS, ethanol extract, chemical compounds, essential oil*

## Introduction

Zingiberaceae commonly known as ginger is an aromatic plant, perennial herbs consisting of more than 1,300 species and 52 genera with the tuberous rhizomes and creeping horizontal, distributed from tropical Asia, Africa and America [1]. Among them, the distribution of ginger predominantly was found in tropical Asia with approximately about 1000 species occur in this area. Traditionally, Ginger has been used by people around the world for various purpose as food, traditional medicine, spice, condiment, dye and flavor [2]. Ethnobotany study about medicinal plants in the Buyan-Tamblingan Lake area, Bedugul, Bali revealed that Zingiberaceae was the largest family used by the local people as a traditional medicine, among other as antitoxin of scorpion, treatment for body warmer, anti-inflammatory, rheumatism, skin diseases, fever, weak heart, and painkiller [3]. In Ayurveda (a system of traditional medicine with historical roots in India), ginger was used for many kinds of treatment, as anti-inflammatory decoctions, a cardiac stimulant carminative, rheumatoid arthritis, inflammations, stomatopathy, pharyngopathy, cough, asthma, hiccough, dyspepsia, stomachalgia, obesity, diabetes, cephalalgia, tubercular glands and intermittent fevers [4].

Phytochemical and phytopharmacological studies had also proven that many species in Zingiberaceae family contained various secondary metabolites that had great potential in the pharmaceutical field, such as an anti-inflammatory, antioxidant, antimutagenic, antidiabetic, antibacterial, hepatoprotective, expectorant and anticancer properties [5], antiplatelet, anti-ulcer, anti-convulsive, provides analgesic effects, diarrhea medication, dermatosis disorder, rheumatism, and expec-

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torant [2]. The previous study showed that *Alpinia hookeriana* Val., *Alpinia mutica* Roxb., *Alpinia nutans* Rosc., *Alpinia rafflesiana* Wall. ex. Bak., *Alpinia vitellina* Ridl. (Lindl.), *Costus discolor* Rosc., *Costus megalobractea* K. Schum., *Costus spiralis* Rosc., *Costus villosissimus* Jacq contained some phenolic compounds which exhibited positive antioxidant activity [6]. In the other hand, Verma *et al.* [7] reported that the essential oil of *Zingiber montanum* contained DMPBD, terpinen-4-ol and sabinene as dominant compound and these constituents could be used as a potential source for several pharmaceutical products. The biological activity assay also performed that this essential oil had strong antibacterial and antifungal activity against several human pathogen bacterial and fungal.

*Globba* is the third largest genera in the Zingiberaceae family, consisting of more than 100 species. The *Globba* genus is widely distributed in tropical and sub-tropical regions, including Asia (India, China) with the highest diversity center in the Southeast Asia region (Thailand, Myanmar, Malaysia, and Indonesia) [8]. It has been a long time since phytochemical studies of *Globba* have been an interesting object to be explored by scientists. The presence of major chemical contents of *Globba schomburgkii* Hook. F. and *Globba ophioglossa* Wight had been detected by Raj *et al*. [9] among others cyperene 3.1 % (Gs), 0.9% (Go), b-caryophyllene 31.7% (Gs), 1.0% (Go), aromadendrene 5.5% (Gs), a-humulene 5.3% (Gs), 0.7% (Go), caryophyllene oxide 10.3% (Gs), 21.8% (Go), (E)-nerolidol 5.7% (Go), humulene epoxide* 5.9 % (Go), zerumbone 22.0% (Go), 13,14,15,16-tetranor labd-8(17)-en-12-al 6.6% (Gs), 2.2% (Go), hexadecanoic acid 7.6% (Gs) 19.6% (Go). But in reality, there is still a lot of information about chemical compounds records in *Globba* genera that have not been revealed. One of them was *G. candida* Gapnep, a native species in Indo-china region [10]. Three accessions of *G. candida* Gapnep were found by researchers from Eka Karya Botanical Garden during explorations in the natural habitat (not planted) in Bali and Sumbawa, and these new distributions are still unclear records. The aims of this present study were to investigate the real distribution of *G. candida* Gapnep by comparing literature reviewed and its exploration histories. This study also to observe phytochemical composition in the ethanol extract of *G. candida* Gagnep from Bali Island.

## Material and Methods

### *Plant material*

*G. candida* Gapnep was collected from the border area between natural forests and community gardens at Pangkung Jangu Village, a traditional village in Mendoyo Sub-district, Jembrana Regency, Bali Province, Indonesia on March 2007 and planted at Eka Karya Botanical Garden on February 2009. In the natural habitat, this plant grows in the open area with altitude 150 masl. In Eka Karya Botanical garden, this plant was planted at USADA block area, with temperature ranging between 18 – 20°C, 70 – 90% humidity and altitude of 1,250 – 1,450 masl [11]. A voucher of herbarium specimen with number Put 54 was identified by a taxonomist of Eka Karya Bali Botanical Garden, I B K Arinasa, M.Si. and deposited in the Tabanan Hortus Botanicus Baliense (THBB).

### *Isolation of essential oil*

The essential oil content was observed and isolated from the fresh material of the aerial part of *G. candida* Gapnep. Respectively, 100 g fresh sample was harvested on March 2016 and extracted by hydrodistillation for five hours using a Pudak Scientific apparatus. The essential oil was separated from hydrosol by using a Duran Schott separator [12].

### *Extraction process*

Fresh material of the rhizome and leaves of *G. candida* Gapnep were harvested on March 2016 and chopped in small size and dried several days at room temperature and avoided the sun irradiance until the materials were completely dry. One hundred grams of each dried materials of *G. candida* Gapnep were extracted with ethanol by the maceration method and the extract suspension was filtered by filter paper [13]. These extract suspensions were analyzed with GC-MS to determine the chemical contents.

### *GC-MS analysis*

The phytochemical composition of the aerial part essential oil and the ethanol extracts of rizomes and leaves of *G. candida* Gapnep were analyzed by GC-MS equipment model Shimadzu GC-MS – QP2010 with Rtx 5 ms and capillary

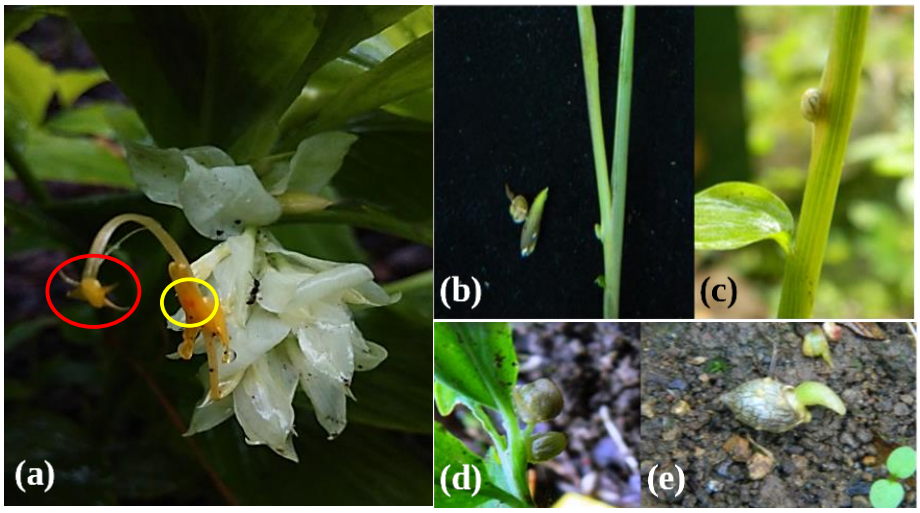


Figure 1. The inflorescence and the bulbil of *G. candida* Gapnep: terminal inflorescence with open flower and large white bractea; anther with 4 triangular appendages (red line) and red spot in the center of labellum (yellow line) (a), bulbil between leaf midribs (b and c), bulbils at the position of the last flower in cincinni (d), and the buds emerge from the bulbil (e).

column 60.0 m × 25 mm with 0.25 µm thickness. The carrier gas was helium UHP with the condition of GC setting and it based on similar method from another research [12].

***Data analysis***

The chemical compounds were identified by mass spectra fragmentation patterns and their identity was approved by comparing their retention time indices and their spectral data with those from computer library WILEY7.LIB and open published literature.

## Results and Discussion

***Distribution of G. candida Gapnep***

*G. candida* Gapnep was reported as an accepted name among of *Globba* Species with original publication detail was published in Bull. Soc. Bot. France 54: 112. 1907 and this name were approved by World Checklist of Monocotyledons Database in ACCESS: 1-54382 [10]. The first reported about the distribution of this species was native in Indo-china, especially in Cambodia [10, 14]. But the database the Global Biodiversity Information Facility (GBIF) published that the *G. candida* Gapnep occurred in Cambodia, Thailand, Indonesia and Lao People's Democratic Republic (Laos) [15]. Base on exploration records which had been conducted by researchers from Eka Karya Bali Botanical Garden, the occurrence of *G. candida* Gapnep in Indonesia had been reported three times, specifically in Bali and Sumbawa. On March 2007, an accession of *G. candida* Gapnep was found in an open area, altitude 150 masl, in Pangkung Jangu Village, Mendoyo Sub-district, Jembrana Regency, Bali Province. On July 2014, this species was found flowering in the slope of Batukaru Mountain, altitude about 700 masl, near Batu Asri Temple, Pujungan Village, Pupuan Sub-District, Tabanan Regency, Bali. On Mei 2015, an accession of *G. candida* Gapnep was also found in Punik Natural Forest, Batu Dulang Village, Batulanteh Regency, Sumbawa Island, West Nusa Tenggara (S 08°35'.866"E117°14'501"; altitude 965 masl) [16]. There is no publication in scientific journal reporting on *G. candida* Gapnep new distribution in Indonesia.

To observe the possibility of *G. candida* Gapnep spreading through cultural and religious factors, deeply literature searching was conducted. Based on reports of Siregar *et al*. [17] and Adiputra [18] about the ceremonial plant diversity in Bali Hindu Community, there was no information that mentions the use of *G. candida* Gapnep in Balinese ceremonial requirements. While the use of *G. candida* Gapnep in traditional medicinal plants Balinese community was also not found in the book of Lontar Husada [19].

An In-depth interview with respondents of local community was used to collect data of local-nknowledge about the local name and ethnobotanical function of plants collected around the Punik

Table 1. The Phytochemical compounds of ethanol extract of the rhizomes *G. candida* Gapnep

| No. | Chemical Compound | Mol. Formula | R. Time | Rel. Conc. % |
|---|---|---|---|---|
| 1. | Butan-3-enoic acid Methyl Ester | - | 3.42 | 4.36 |
| 2. | Diacetyl | $C_4H_6O_2$ | 4.06 | 1.03 |
| 3. | Acetic Acid | $C_2H_4O_2$ | 4.83 | 3.93 |
| 4. | Trans-2,3-Epoxybutane | $C_4H_8O$ | 5.39 | 6.30 |
| 5. | Methyl Pyruvate | $C_4H_6O_3$ | 8.18 | 3.26 |
| 6. | Dioxadiene | $C_4H_8O_2$ | 8.46 | 0.95 |
| 7. | Allyl butyrate | $C_7H_{12}O_2$ | 9.02 | 0.59 |
| 8. | Methallylacetone | $C_7H_{12}O$ | 9.89 | 3.10 |
| 9. | 3,4-Dimethyl-4-penten-1yn-3-ol | $C_7H_{12}O$ | 10.83 | 0.60 |
| 10. | 2-Methylcyclopentanone | $C_6H_{10}O$ | 11.36 | 4.02 |
| 11. | Pinacolone | $C_6H_{12}O$ | 11.59 | 0.99 |
| 12. | 3-Methyl-2-cyclopentenone | $C_6H_8O$ | 11.90 | 1.14 |
| 13. | Methylcyclopentenolone | $C_6H_8O2$ | 12.99 | 1.97 |
| 14. | Ethyloctynol | $C_{10}H_{18}O$ | 13.21 | 0.70 |
| 15. | 1-Acetylcyclohexene | $C_8H_{12}O$ | 13.61 | 1.77 |
| 16. | m- cresol | $C_7H_8O$ | 13.74 | 1.21 |
| 17. | 2-Propyloxetane | $C_6H_{12}O$ | 14.06 | 4.00 |
| 18. | 2H-Pyran-3(4H)-one, dihydro-6-methyl- | $C_6H_{10}O_2$ | 14.42 | 1.01 |
| 19. | 2H-Pyran-3(4H)-one, dihydro- | $C_5H_8O_2$ | 14.64 | 1.26 |
| 20. | Caprylene | $C_8H_{16}$ | 15.01 | 0.70 |
| 21. | Allylhydrazone acetalde-hyde | $C_5H_{10}N_2$ | 15.35 | 5.52 |
| 22. | 4-Ethylguaiacol | $C_9H_{12}O_2$ | 16.09 | 1.37 |
| 23. | Beta.Damascone | $C_{13}H_{20}O$ | 16.38 | 0.84 |
| 24. | Longipinane, Trans | $C_{15}H_{26}$ | 16.51 | 0.57 |
| 25. | 1,2,4-Cyclopentanetriol | $C_5H_{10}O_3$ | 16.63 | 0.60 |
| 26. | D-Glucose, 4-O-(3-acetyl-1-(trimethylsilyl)-1H-indolyl)-2,3,5,6-tetrakis-O-(trim) | - | 16.99 | 0.81 |
| 27. | Farnesol | $C_{15}H_{26}O$ | 17.36 | 2.55 |
| 28. | Cycloisosativene | $C_{15}H_{24}$ | 17.87 | 0.65 |
| 29. | Levoglucosan | $C_6H_{10}O_5$ | 18.80 | 19.07 |
| 30. | (5-alpha)-Cholest-7-EN-3-one | $C_{27}H_{44}O$ | 20.39 | 1.86 |
| 31. | Cyclohexene, 4-(4 Ethylcy-clohrxyl)-1-Pentyl | $C_{19}H_{34}$ | 22.18 | 0.75 |
| 32. | Lanost-7-en-3-one | $C_{30}H_{50}O$ | 23.13 | 3.27 |
| 33. | 4-2',2'-Dimethyl 6'methyli-den-1'cyclohexyliden)-3-methyl-2-butanone | $C_{14}H_{22}O$ | 23.75 | 2.72 |
| 34. | 3beta-Bromocholest-5-ene | $C_{27}H_{45}Br$ | 24.04 | 0.56 |
| 35. | Olean-12-en-3-one | $C_{30}H_{48}O$ | 24.67 | 1.21 |
| 36. | Naphthalene, ar, ar' ar"me-thylidynetris (Decahydro- | - | 26.74 | 0.58 |
| | Total | | | 85.82 |

Note: Mol Formula: Molecular Formula; R. Time: Retention Time; Rel. Conc.: Relative concentration

Table 2. The Phytochemical compounds of ethanol extract of the rhizomes *G. candida* Gapnep

| No. | Chemical Compound | Mol. Formula | R. Time | Rel. Conc. % |
|---|---|---|---|---|
| 1. | 2-Methylfluorene | $C_{14}H_{12}$ | 19.59 | 0.69 |
| 2. | Palmatic Acid | $C_{16}H_{32}O_2$ | 20.89 | 1.70 |
| 3. | Alpha-ionone | $C_{13}H_{20}O$ | 21.84 | 0.70 |
| 4. | Ambrettolide | $C_{16}H_{28}O_2$ | 22.68 | 0.62 |
| 5. | 1-(1-(Hydroxy-Phenyl-Methyl)-Cyclopropyl)-2-Phenyl-Ethano | - | 22.92 | 2.40 |
| 6. | Benzenepentanal | $C_{11}H_{14}O$ | 23.57 | 2.08 |
| 7. | Dibenzylacetone | $C_{17}H_{18}O$ | 24.29 | 1.65 |
| 8. | Pinostrobin chalcone | $C_{16}H_{14}O_4$ | 25.44 | 75.63 |
| | Total | | | 85.47 |

Note: Mol Formula : Molecular Formula; R. Time: Retention Time; Rel. Conc.: Relative Concentration

Natural Forest, Batu Dulang Village, Batulanteh Regency, Sumbawa Island, West Nusa Tenggara, during exploration by the researchers of Bali Botanic Garden on Mei 2015, and the results showed that there were no records of the local name and ethnobotanical function of *G. candida* Gapnep [16]. So, it is still a big question whether the distribution of *G. candida* Gapnep found in Bali and Sumbawa is natural distribution or dispersed due to other human activities.

### *Description of G. candida Gapnep*

*G. candida* Gapnep was a small perennial herb from 50 – 70 cm tall (Figure 1). Rhizomes were tuberculous, 20 – 25 mm length, 3 – 4 mm in diameter, white with the white color internally. Leafy shoots were densely clumped, 5 – 7 leaves in a stem, stems bright green, sometimes red green in the lower stem. Leaves were glabrous and soft, only midvein of the ventral surface pubescent, lamina 8 – 17 × 3.5 – 5.5 cm elliptic bright green adaxially, pale green and hairy abaxially, margin entire, base cuneate, apex acuminate with long acumen. Size of petiole was 3 – 5 mm, and fluffy smooth, green. Ligule was small, 2 – 3 mm in length, and hirsutulous. Inflorescence was terminal on leafy shoots with striking feature a large and white bractea. The color of flowers was orange and had a red spot in the center labellum. Anther consisted of 4 appendages; appendages triangular. Bulbils was at the position of the last flower in cincinni and between leaf midribs, many roots and shoots but there was only one who was developed into bamboo-like shoot. Fruit and seeds were unknown.

### *The Essential oil contents of G. candida Gapnep*

This present study was the first report about the essential oil observation in *G. candida* Gapnep. After five hours extraction by hydrodistillation, the result showed that there was no essential oil content found in the aerial part of *G. candida* Gapnep. The presence of essential oils in *Globba* spp. had been reported in several studies. Meno and and Dan [20] found the essential oil contents

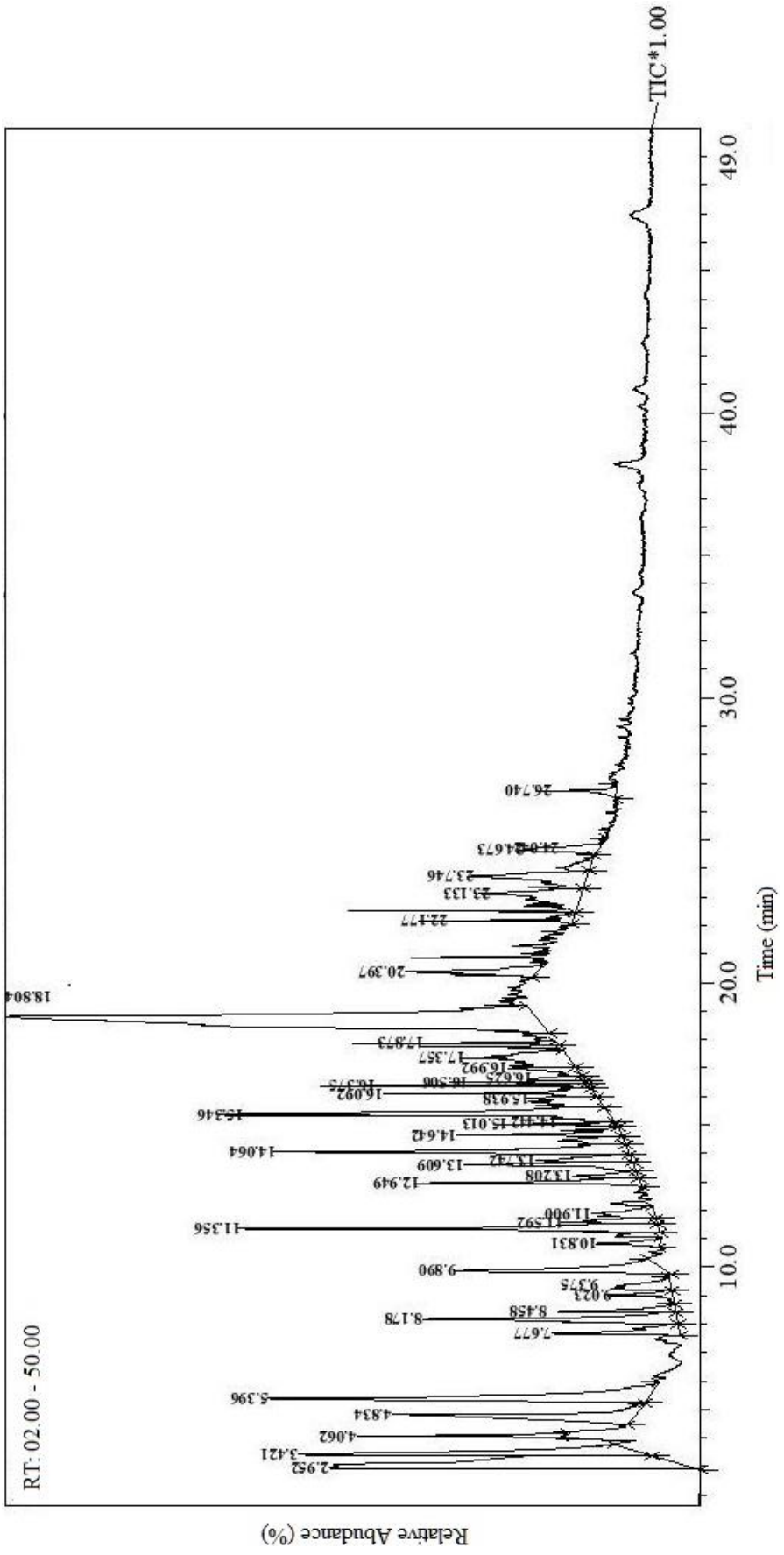


Figure 2. GC-MS chromatogram of the rhizomes extracts of *G.candida* Gapnep (Note: RT: Retention Time; TIC: Total Ion Chromatogram)

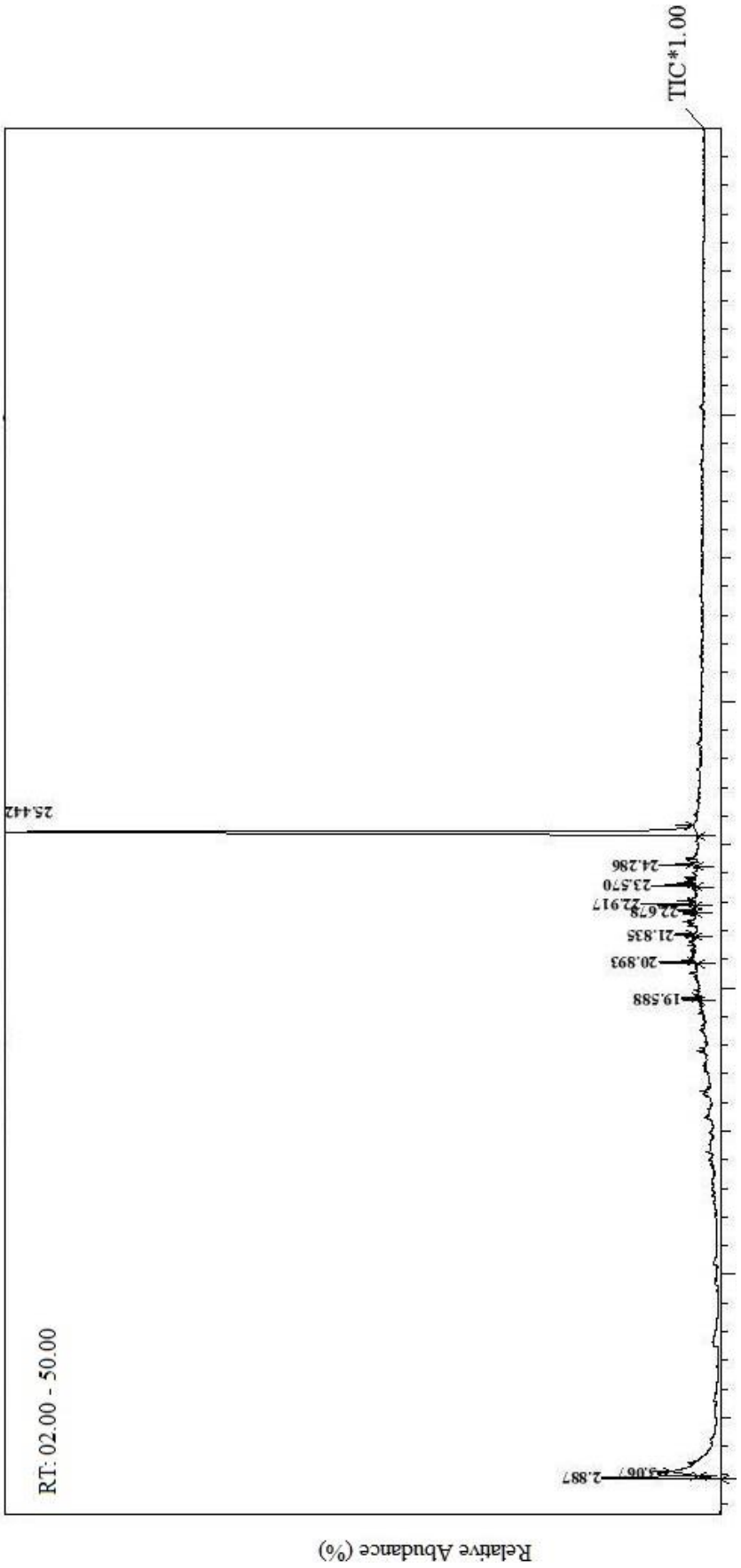


Figure 3. GC-MS chromatogram of the leaves extracts of *G. candida* Gapnep (Note: RT: Retention Time; TIC: Total Ion Chromatogram)

in three species of *Globba* with various concentration, namely *G. marantina* (0.007% (V/W)), *Globba ophioglossa* (0.009% (V/W)), and *Globba cernua* (0.006% (V/W)). Consecutively, the essential oil of *Globba marantina*, *G. ophioglossa*, and *G. cernua* comprised of 13, 16, and 10 identified chemical constituents accounting 74.8%; 16%; and 10% of the total oils. The main compounds of *G. marantina* were b-caryophyllene 19.3%, a-humu-lene 14.2%, (Z) neroli-dol 7.5%, isoborneol 7.3%, and b-bisabolene 7.2%. While the major constituents of *G. ophioglossa* were b-caryophyllene 20.1%, (Z,Z)-farnesol 9.8%, cedrol 7.3%, (e)-anethole 6.3%, neryl acetate 6.8% and the dominant compounds of *G. cernua* were b-caryophyllene 24.2%, Z)-nerolidol 7.8%, caryophyllene oxide 7.7%, (Z,Z)-farnesol 9.8%, (E)-anethole 6.3% [20].

The essential oils content of *G. schomburgkii* and *G. ophioglossa* were also detected by Raj et al [9] in the low concentration 0.01% (v/w). This report showed that *G. schomburgkii* oil contained 22 identified constituents, comprising of 95.2 % of the total oil composition with the major compounds of this species were b-caryophyllene (31.7%), caryophyllene oxide (10.3%), hexadecanoic acid (7.6%) and a labdane-type diterpene 13,14,15,16-tetranor labd-8(17)- en-12-al (6.6%). In the other hand, the essential oil of *G. ophioglossa* consisted of 16 identified constituents representing 88.3% of the analyzed oil with zerumbone 22.0%, caryophyllene oxide 21.8%, and hexadecanoic acid 19,6% as major constituents. Kumar *et al*. [21] also found the essential oil contents in rhizome of *Globba sessiliflora* Sims with comprising of 35 determined contents (representing 98.1% of the oil) and exhibiting β-eudesmol (27.6%), (E)-β-caryophyllene (24.3%), α-humulene (3.0%), (6E)-nerolidol (4.1%), caryophyllene oxide (9.7%), γ-eudesmol (6.4%) and τ-muurolol (8.3%) as major compound.

### *The Phytochemical compounds of ethanol extract of the rhizome and leaves of G. candida Gapnep*

The rhizome and leaves extract of *G. candida* Gapnep consisted of 36 and 8 identified chemical contents, representing 85.82% and 85.47% of the total analyzed extracts. The complete results of the phytochemical compounds can be seen in Table 1 and Table 2 and their GC-MS chromatograms in Figure 1 and Figure 2. Among of them, the major compounds of the rhizome extract of *G. candida* Gapnep were levoglucosan 19.07%, allylhydrazone acetaldehyde 5.52%, trans-2,3-epoxybutane 6.30%, butan-3-enoic acid methyl ester 4.36%, 2-Methylcyclopentanone 4.02%, and 2-N-Propyl-Oxetan 4.00%. While the dominant contents of the leaves extract of *G. candida* Gapnep were pinostrobin chalcone 75.63%.

As far as the author's investigation, there is no previous study pertaining to the chemical contents in *G. candida* Gapnep has been carried out. However, prior phytochemical studies on the genus *Globba* have revealed several contents as main compounds, among other the presence sesquiterpene and diarylheptanoid in *G. malaccensis* Ridl (A synonym for *G. variabilis* subsp. Variabilis) [22]; two new chemical compounds of lipids, three new labdane-type diterpenes and two new steroids from the rhizomes of *G. reflexa* [23]; triterpene and steroids from *G. racemosa* Sm; phenolic compound, steroid, labdane, diterpene and benzofuran from *G. pendula* Roxb. [24]; heptadecane, pinocarvone, l-linalool, alloaromadendrene, β –caryophyllene, α- humulene, terpineol, lavandulol, 2,6-dimethyl-1,5,7-octatrien-3-ol in methanol crude extract from *G. marantina* [25] and steroids, alkaloids, flavonoids, cardiac glycosides, saponins, tannins, terpenoids, phlobatinins, fatty acids, coumarins and phenols compounds from ethanolic tuber extract of *G. bulbifera* [26].

## Conclusion

*G. candida* Gapnep is one species in the genus *Globba* that has not been widely studied, especially in the analysis of chemical content and its potential. The phytochemical constituents of rhizomes and leaves extract of this species was done for the first time. This present investigation will be helpful for further research of *G. candida* Gapnep both related to chemotaxonomic or for phytochemical analysis

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