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Alkaloids of *Fagraea fragrans* (Tembesu) Fruits

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Abstract

The aim of this paper is to visualize the alkaloids of *Fagraea fragrans* fruits therefore the fruits can be scientifically used as herb traditional medicines and phytocosmetics. Three alkaloids have successfully been identified from those *Fagraea fragrans* fruits, Loganiaceae. They are gentialutine, gentianine, and isaindigotone. The alkaloids were alternately explored by means of ether and 2% H_2SO_4 extractions. The sulphuric acid phase was naturalized with ammonium chloride and then extracted with ethyl acetate. The residue were then subjected to silica gel G60 (70-230 mesh) column chromatography and eluted with 40% ethyl acetate in *n*-hexane. The LC-MS spectral of alkaloids gave the protonated molecular ion peaks at m/z (*r.t. minute*) = 150.08 (*1.39*), 176.06 (*4.80*), and 351.37 (*5.24*) respectively.

Keywords: Alkaloids, Fagraea fragrans, tembesu, fruits.

Abstrak (Indonesian)

Tujuan dari paper ini adalah untuk memvisualisasi kandungan alkaloid buah tembesu (*Fagraea fragrans*) sehingga buah tembesu ini dapat diterima secara ilmiah sebagai obat tradisional herbal dan fitokosmetika. Tiga alkaloid telah berhasil diidentifikasi dari buah tembesu, *Fagraea fragrans*, Loganiaceae. Alkaloid tersebut adalah gentialutin, gentianin, and isaindigoton. Alkaloid tersebut secara ber-urutan dieksplorasi dengan cara ekstraksi menggunakan pelarut eter dan H_2SO_4 2 %. Fase asam sulfat ini dinetralisir dengan ammonium khlorida dan kemudian diekstrak lagi dengan etil asetat. Residunya dipisahkan dengan menggunakan kolom kromatografi berisi silica gel G60 (70-230 mesh) dengan menggunakan eluen 40% etil asetat di dalam *n*-heksana. Spektral LC-MS dari alkaloid tersebut memberikan puncak puncak ion molekul terprotonasi pada m/z (*r.t. minit*) = 150.08 (*1.39*), 176.06 (*4.80*), and 351.37 (*5.24*)

Kata Kunci: Alkaloid, Fagraea fragrans, tembesu, buah.

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INTRODUCTION

Phytochemical works were conducted on many parts of *Fagraea Fragrans* Roxb, Loganiaceae, such as isolated gentianine for antimalarial and antiamoebic from leaves, fruits, and twigs [1, 2], and isolated fagraldehyde for antiplasmodial from bark and leaves [3]. The secondary metabolites of *F. fragrans* fruits have also mapped recently [4] and now we report three simple alkaloids from these fruits in order to boost our people and industries to use these for both medicinal and phytocosmetics. In addition the fruits are available in big scale in Sumatran and Borneo Islands meanwhile they also contain phyto-chemical ingredients, i.e. 3.1% ursolic and oleanolic acids [5]. Alkaloids are basic nitrogenous compounds of plants or animal origin and generally possessing a marked physiological action on man and animals. The nitrogen is usually contained in heterocyclic ring system and it mainly derived from amino acid. As a result the human beings are understanding the vitality of using natural products such as these fruits to lead a healthy life for both cosmetics and traditional medicines including Sumatran ladies [4, 5] and Tahitian women [6].

Alkaloids play an important role in the ecology of organisms which synthesize them. They are also playing an important role in the defense systems against pathogens and animals as well. The applications of alkaloids are not limited to biological control of herbivores but also have pharmacological, veterinary and medical importance.

Alkaloids showed strong biological effects on animal and human organisms in small doses. Alkaloids are present not only in daily life in food and drinks but also as stimulant drugs. They showed anti-inflammatory, anticancer, analgesics, local anaesthetic and pain relief, neuropharmacologic, antimicrobial, antifungal, and many other activities. Alkaloids are useful as diet ingredients, supplements, and pharmaceuticals, in medicine and other applications in human life. Alkaloids are also important compounds in organic synthesis for searching new semisynthetic and synthetic compounds with possibly better biological activity then parent compounds [7, 8]. In the last ten years we have used the methanol extracts of *Fagraea Fragrans* Roxb fruits as phytocosmetics [4, 5] and now we are reporting their alkaloids. In addition the *Fagrae fragrans* is a potential and multipurpose indigenous plant species in Indonesia, especially in South Sumatra, West Borneo and Central Java and its fruits are also abundant to produse herb medicines and cosmetics in the future [9, 10].

MATERIALS AND METHODS

Materials

Tembesu fruits (Figure 1), diethyl ether, n-hexane, ethyl acetate, silica gel plate, silica gel G60 (70-230 mesh) and dragendrof reagent.

Instrumentals

LC-MS Spectrometer, Xevo G2-XS QTOF [Channel: TOF MS^E (50-1200) 6eV ESI+ - Low CE (BPI)], and Gamax UV Lamp (254 nm and 366 nm).

Methods

LCMS operation

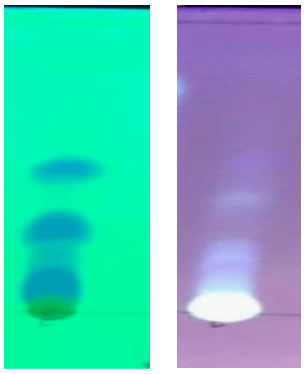
Mass spectrometry was performed on a LCMSMS Xevo, G2-XS Qtof (waters MS Technologies). Ionisation type is ESI. The scan range was from 100 to 1200 m/z. The capillary and cone voltage was at 0.8 kV and 30 kV, respectively and was used positive electron spray mode. The desolvation gas was set to 1000 L/h at a temperature of 500 ^oC and the cone gas was set to 50 L/h and the source temperature was set to 120 ^oC.

The UPLC analysis was perfomed using a Water Acquity Ultra Performance LC system. Chromatographic separation was carried out on an ACQUITY UPLC HSS T3 column (100 mm x 2.1 mm, 1.7 µm) at a column temperature of 40 ^oC. The mobile phase consisted of solvent A (0.1% formic acid in water, v/v and solvent B (0.1% formic acid in acetonitrile), with gradient polarity from 95:0.5(A:B) to 0.5:95(A:B). The flow rate was set at 0.3 mL/min. The column and auto sampler were maintained at 40 ^oC and 20 ^oC, respectively. The injection volume was 1 µL. The data acquisition and processing were performed using UNIFI. The parameter was retention time (RT) in the range of 1- 16 min.

Figure 1. Tembesu (*Fagraea fragrans*) fruits

Extraction of alkaloids

The dried powder of the *F. fragrans* fruits (1.2 kg) is basified with ammonium hydroxide and then macerated with ether (3 x 2.5 L) for 3 x 24 hours. The total ethers were concentrated to be 400 mL under reduced pressure and dropped to 1 L separating funnel. The 2.5 % sulphuric acid (500 ml) was added to the separating funnel and shake for 2 hours. The acid phase is moved to 1 L beaker glass, basified with ammonium hydroxide (pH 9-10), and then extracted with ethyl acetate (3 x 500 mL) by shacking in the separating funnel. The ethyl acetate (1.5 L) was dried with Na_2SO_4 anhydrous and evaporated by rotary evaporator under reduced pressure. The alkaloid residue is subjected to 70-230 mesh silica gel G60 column (20 gr) and eluted with 40% ethyl acetate in n-hexane (2 L), see Figure 1. Seventy vials, each vial containing 10 mL eluent, were collected during this process. The vial number of 11 to 19 were combined and dried. It was checked by means of TLC, see figure 2 and then analyzed by LC-MS.

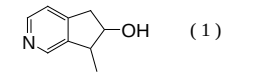


(a) (b)

Figure 2. TLC of alkaloids in 40% ethyl acetate in n-hexane under UV (a) 254 nm and (b) 366 nm.

Data Analysis

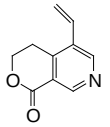
The results of LC-MS spectral analysis of alkaloids come from Fagraea fragrans fruits is given in Figure 3. The figure comprises structures, names, molecular weight (Mol.Wt.), percentage elementary (C, H, N, O in %), and molecular formula of alkaloids.



(1)

$C_9H_{11}NO$
Exact Mass: 149,08
Mol. Wt.: 149,19
C, 72,46; H, 7,43; N, 9,39; O, 10,72

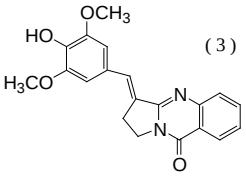
7-Methyl-6,7-dihydro-5*H*-[2]pyridin-6-ol



(2)

$C_{10}H_9NO_2$
Exact Mass: 175,06
Mol. Wt.: 175,18
C, 68,56; H, 5,18; N, 8,00; O, 18,27

5-Vinyl-3,4-dihydro-pyrano[3,4-*c*]pyridin-1-one



(3)

$C_{20}H_{18}N_2O_4$
Exact Mass: 350,13
Mol. Wt.: 350,37
C, 68,56; H, 5,18; N, 8,00; O, 18,27

3-(4-Hydroxy-3,5-dimethoxy-benzylidene)-2,3-dihydro-1*H*-pyrrolo[2,1-*b*]quinazolin-9-one

Figure 3. The correlation between chemical structures, molecular weight, and empirical formula of isolated alkaloids of *Fagraea fragrans* fruits.

RESULTS AND DISCUSSION

It was obvious that *Fagraea fragrans* (tembesu) fruits contained some advantageous alkaloids for herbal medicine candidates, they were gentialutine, 7-Methyl-6,7-dihydro-5H-[2]pyridin-6-ol *(structure-1)* ; gentianine, 5-Vinyl-3,4-dihydro-pyrano[3,4-c]pyridin-1-one *(structure-2)* ; and isaindigotone, 3-(4-Hydroxy-3,5-dimethoxy-benzylidene)-2,3-dihydro-1H pyrrolo-[2,1-b]-quinazolin-9-one *(structure-3)*, see Figure 3.

Compound-1; 7-Methyl-6,7-dihydro-5H-[2]pyridin-6-ol called as gentialutine or venoterpine : The molecular ion M^+ of 1 in the positive ion mode was observed at m/z 151.09 (10%) [M+2H], 150.09 (75%) $[M+H]^+$, 120 (100%) [M-(HCHO)]. Compound 1 had empirical formula $C_9H_{11}NO$ in accordance with the molecular weight (Mol.Wt.) = 149, see Fig. 3 and its mass spectral is given in Figure 5 while its fragmentation reactions in Figure 4. It is still need to discuss if this compound is artefact of gentianine that is formed during exploration process.

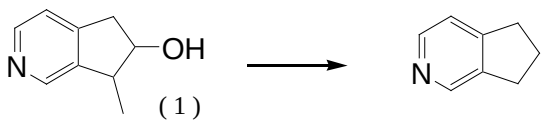


Figure 4. Fragmentation of 1, Mol.Wt. = 149

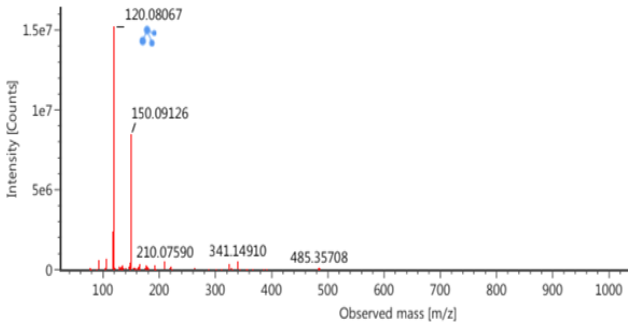


Figure 5. Mass spectral of 1 with Mol.Wt. =149.

Compound-2; 5-Vinyl-3,4-dihydro-pyrano - [3, 4-c]-pyridin-1-one called as gentianine: The molecular ion M^+ of 2 in the positive ion mode was observed at m/z 363.09(15%) $[2M+Li+5H]^+$, 351 $[2M+H]^+$ please see our previous work for this m/z = 351 [4], 177.07 (8%) [M+2H], 176.07 (100%) $[M+H]^+$, and fragment peaks at m/z 146.05 (50%) (experimental) see figure 7., 147.05 (calculated) see figure 6 for $[M-(C=O + 2H)]^+$ or $[M-HCHO]^+$ and the two of other unnumbered fragment peaks 133 (10%) and 120 (25%). Compound 2 had empirical formula $C_{10}H_9NO_2$ in accordance with the the molecular weight (Mol.Wt.) = 175, see Figure 3 and its mass spectral is given in Figure 7 while its fragmentation reactions in Figure 6.

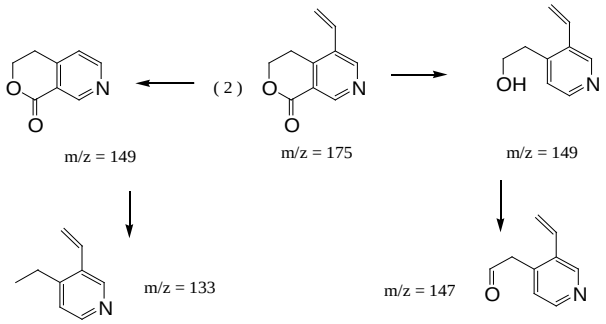


Figure 6. Fragmentation of 2, Mol.Wt. = 175.

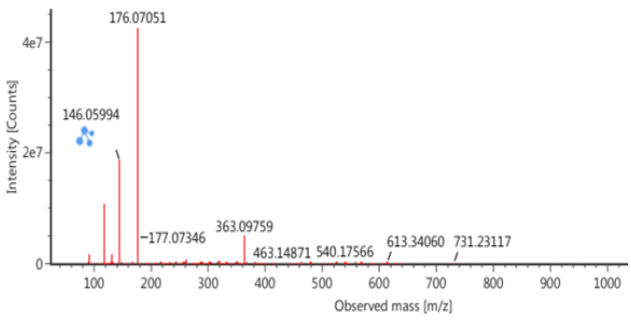


Figure 7. Mass spectral of 2 with Mol.Wt. = 175.

This compound was reported to have anti-diabetic, antipsychotic, hypotensive, diuretic and anti-inflammatory actions. It could be used as a safe

antihypertensive drugs [11], antimalarial agents [2, 12], antibacterial and antifungal [13].

Compound-3;.3-(4-Hydroxy-3,5-dimethoxy-benzyl idene)-2,3-dihydro-1H-pyrrolo[2,1-b]quinazolin-9-one called as isaindigotone with new class of cytotoxic agents [14]: The molecular ion M^+ of 3 in the positive ion mode was observed at m/z = 723.24 (3%) [2M+Na], 524.18 (12%), 352.13 (25%) [M+2H], 351.13 (100%) $[M+H]^+$, 224.11 $[223.11 + H]^+$, (72%), 222.11 (78%) $[M-C_6O_3H_7 - 2H]^+$, 176.07 (95%) $[M- (phenyl\text{-}CH{=}C + 2OCH_3 + OH)]^+$, 146.05 (70%) $[M-(phenyl\text{-}CH{=}C\text{-}CH_2\text{-}CH_3{+}2OCH_3{+}OH)]^+$.
Compound 3 had empirical formula $C_{20}H_{18}N_2O_4$ in accordance with the molecular weight (Mol.Wt.) = 350, see Figure 3 and its mass spectral is given in Figure 9 while its fragmentation reactions in Figure 8.

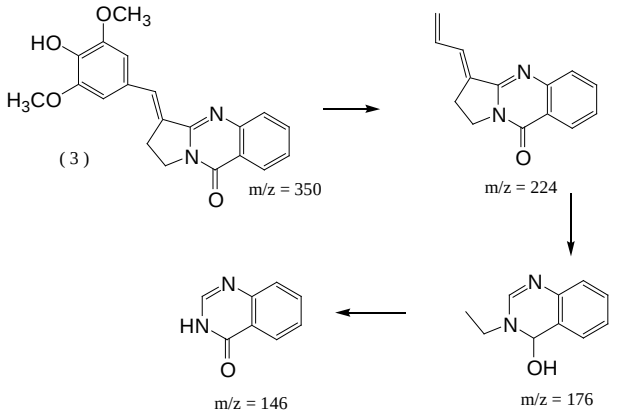


Figure 8. Fragmentation of 3, Mol.Wt. = 350

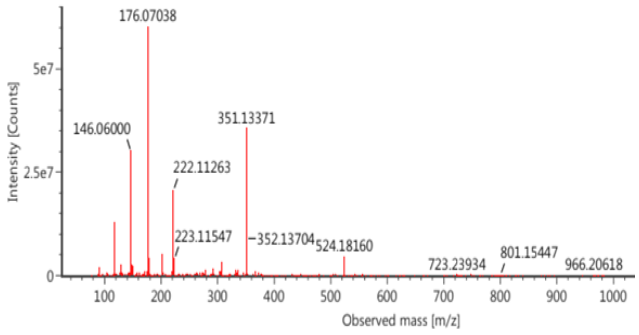


Figure 9. Mass spectral of 3 with Mol.Wt. = 350

Isaindigotone which a compound comprises a pyrrolo [2,1-*b*] quinazoline moiety conjugated with a benzylidene group [15] was reported highly selective ligands for telomeric G-quadruplex DNA [16]. Isaindigotone is an alkaloid isolated from the root of the traditional Chinese herb *Isatis indigotica* Fort. This compound is reported to exhibits excellent effects against influenza, epidemic hepatitis, and epidemic encephalitis [17]. Isaindigotone also inhibited 5-lipoxy-genase activity and leukotriene B(4) production [18, 19]. Now we first report the compound 3 come from *Fagraea fragrans* Roxb fruits.

CONCLUSION

The alkaloids of *Fagraea fragrans* (Tembesu) fruits, i.e. gentianine and isaindigotone, were boosting the application these fruits to be our traditional medicines for herb candidate due to those compounds were well known anti-malarial, anti-diabetic, anti-influenza agents or as phytocosmetics with their efficacy as anti-cancer, anti-inflammatory,and anti-microorganism.

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