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# Isolation, Identification and Antiviral Activity of Bioactive Compounds of *Kampheria Rotunda*

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**Abstract.** The aim of the research are to isolation, identification and antiviral activity of bioactive compounds of Kunci pepet (Kaemferia rotunda).Isolation was done by maceration the dry powder of Kunci pepet (Kaemferia rotunda) by using methanol, and fractination of the methanol extract by using the solvent n-hexane, chloroform, and ethyl acetate respectively. The identification of structures by using NMR Spectroscopy. Antiviral activity test carried out on the AI virus H5N1, performed test according to OIE (2008) with modifications. The isolation of chemical compounds from the hexane fraction showed the presence of one compound that is pinostrombin or 6-hydroxyl, 8-methoxy-flavanones. While isolated from the ethyl acetate fraction of Kunci pepet (Kaemferia rotunda) obtained three compounds, i.e. 4'-hydroxy-8-methoxy-flavanones, 6- hydroxy-8-methoxy-flavanones, and 4 ', 8-dihydroxy-flavanones. The activity against the AI virus H5N1 from extracts Kaemferia rotunda, showed that the methanol extract has antiviral activity but not significant, while hexane extracts of Kaemferia rotunda showed high antiviral activity. The activity against the AI virus H5N1 from extracts Kaemferia rotunda, showed that the methanol extract has antiviral activity but not significant, while hexane extracts of Kaemferia rotunda showed high antiviral activity.

**Keywords:** *Kaemferia rotunda*, Anti viral, H5N1

## 1. Introduction

Disease caused by infection or a virus remains a concern and priority in both Indonesia and the world at large. Because of the tendency of resistance and immunity against the use of bacterial or viral drugs continuously [1]. Thus the discovery of new bioactive compounds that can be used as lead compounds from medicinal plants herbal archipelago is very interesting to do.

Some herbs that have been used as a cure measles, influenza, or herpes, among others, i.e. mengkudu (*Morinda citrifolia* Lin), cangkring (*Erythrina fusca* Lour), brotowali (*Tinospora crispa* Miers), temulawak (*Curcuma xanthorrhiza* Roxb), kunyit (*Curcuma domestica* Val), Kunci pepet (*Kaemferia rotunda* L), tapak liman (*Elephantopus scaber* Linn), temugiring (*Curcuma heyneana* Val), galangal (*Alpinia galanga* Sw), and temuireng (*Curcuma aeruginosa* Roxb). However, in this study will be selected Kof Zingiberaceae family of plants that have not been investigated thoroughly, that are *Kaemferia rotunda* [2-6].

Some studies indicate that further investigations of a plant to produce chemical compounds that are very useful in the field of medicine. For example the discovery of alkaloids such as vinblastine and vincristine from *Catharanthus roseus* plant (vinca) as a cure for cancer, as well as the discovery of taxol from Taxus brevifolia plants as well as uterine cancer drugs. This research spurred pharmaceutical companies to explore the bioactive compounds from plants as lead compounds of new drug discovery [2-6].

## 2. Materials and Methods

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## 2.1. Preparation of Samples of Plant Extracts

Plant tissue used for the treatment such as *kunci pepet* (*Kaemferia rotunda*) was collected, washed, dried, and made powder. Then performed maceration of sample with methanol for 24 hours and repeated 2 times. Filtrate and concentrated using a vacuum evaporator. Concentrated extract of kunci pepet (*Kaemferia rotunda*) then had partition using the solvent n-hexane, chloroform, and ethyl acetate respectively.

## 2.2. Isolation and Structural Identification of Bioactive Compounds

Isolation and purification of chemical compounds conducted by chromatographic techniques, such as vacuum liquid chromatography (VLC), gravity column chromatography (GCC), column chromatography press (CCP), and centrifugal chromatography (Cromatotron), and crystallization for compounds that can form crystals. The identification and structure elucidation performed using analysis of spectral data UV, IR, $^{1}$H NMR and $^{13}$C one and two-dimensional (HMQC, HMBC, Cosy and Noesy), and FAB MS.

## 2.3. Toxicity Test

All test material created concentration of 0.01; 0.1; and 1%, then inoculated into embryonated chicken eggs aged 10 days. Eggs were incubated in the incubator for 24 hours, then observed whether the embryos remain alive or dead. Evaluation of toxicity test: if toxic material is said to cause embryonic death within 24 hours. Safe material used in case of no cause of death of embryos within 24 hours of incubation.

## 2.4. Antiviral Activity Assay

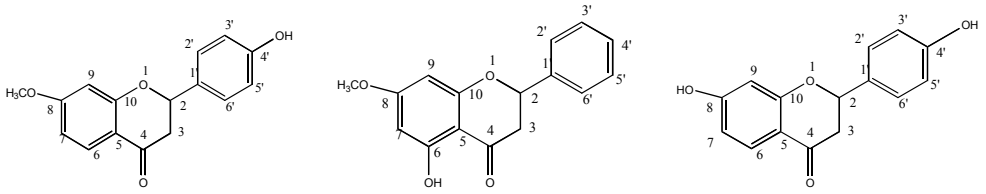
As much as 0.1 mL H5N1 AI virus were added by 0.5 mL extract 1%; 10,000 IU / mL antibiotic penicillin, and 10 mg / mL streptomycin, then incubated $37^{0}$C for 30 minutes. Suspension of the virus as much as 0.1 mL was inoculated on the TAB through korioalantois space. Eggs were incubated in the incubator for 3 days. Embryo mortality was observed whether or not there every day. Eggs stored in refrigerator for 24 hours, then the liquid alantois harvested to tested its hemaglutination titter (HA). Hemagglutination test performed according to OIE (2008) modification of premises. In principle alantois fluid 0.05 mL diluted series 2 times and then reacted with 0.5% chicken erythrocytes. HA positive to say in case of hemagglutination. HA titter is the reciprocal of the highest dilution that still showed a positive reaction. Extract is said to have antiviral activity when the HA titter differs significantly from the control or even a HA titter of approximately $2^{0}$. Eggs inoculated with the H5N1 AI virus suspension without extract was used as control.

# 3. Results and Discussion

The hexane extract was evaporated using the evaporator Buchii, appears yellowish crystals (weight 1.2 g), then separated and recrystallized using methanol. Crystals after tested TLC showed a single spot. UV spectroscopic data in methanol show wavelengths at 220 and 289 nm, while the IR spectrophotometer data (in KBR pellet) showed absorption at 3459, 1646, 1381, and 1158 cm-1. Data 1H NMR (400 MHz) in CDCl3 solvent showed: δ 2.82 (1H, dd, J = 3, 3.6 Hz) ppm; 3.09 (1H, d, J = 3, 13 Hz) ppm; 3 , 8 (3H, s) ppm; 5.44 (1H, dd, J = 3, 13 Hz) ppm, 6.08 (2H, dd, J = 2.5 Hz) ppm; 7.3 (5H, br s) ppm: 12.02 (1H, br s) ppm. Data 13C NMR (400 MHz) equipped with Dept. 135 in CDCl3 solvent showed: δ 43.38 ppm (CH2), 55.68 ppm (CH3), 79.1 ppm (CH), 94.26 ppm (CH); 95 , 14 ppm (CH), 102.3 ppm (C), 103.13 ppm (C), 126.12 ppm (2 CH), 126.86 (2 CH), 138.35 ppm (C); 162, 77 ppm (C), 164.14 ppm (C), 167.98 ppm (C), 195.75 ppm (C). From these data indicate that the hexane fraction of the isolated compounds are flavonoids that have metoksil and hydroxyl groups found previously that 6-hydroxy-8-methoxy-flavanones (2) (Fig.1).

The ethylacetate concentrated extract (30 g) separated by chromatography (VLC) followed repeatedly by gravity chromatography using various solvent mixture produced three pure compounds, namely 4'-hydroxy-8-methoxy-flavanones (1), (1.2 g) white crystals, 6-hydroxy-8-methoxy-flavanones (2) (1.5 g) of white crystals yellowish, and 4',8-dihydroxy-flavanones (3) (1.8 g) in the form of greenish-white powder. UV spectroscopy 4'-hydroxy-8-methoxy-flavanones showing wavelength at 213 and 283 nm, IR showed absorption at 3452, 1614, 1579, and 1108 cm-1. 6-Hydroxy-8-methoxy-flavanones UV spectroscopy showed wavelength at 213 and 287 nm, IR showed absorption at 3444, 1645, 1621, 1381, 1302, 1158, and 799 cm-1. Furthermore, UV spectroscopic data 4 ', 8-dihydroxy-flavanones showed the absorption at a

wavelength of 213 and 248 nm, showed IR absorption at 3450, 3093, 1631, 1487, 1302, 1168, and 1089 cm-[1]. Data analysis of proton and carbon NMR spectroscopy and two-dimensional one contained in Table 1.



1 2 3

Fig. 1:Structure of compound isolated from hexane and ethyl acetate fraction of kunci pepet (4'-hydroxy-8-methoxy-flavanon (1) ; 6-hydroxy-8-methoxy-flavanon (2) ; 4', 8-dihydroxy-flavanon (3))

Table 1: Data spectroscopy proton and carbon NMR one and two dimensional of compounds isolated of *Kaemferia rotunda*

| No | 1 | | | 2 | | | 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | δ C ppm | Δ H (∑ H; m; J Hz) | HMBC (H→C) | δ C ppm | Δ H (∑ H; m; J Hz) | HMBC | δ C ppm | Δ H (∑ H; m; J Hz) | HMB C |
| 1 | - | | | - | | | | | |
| 2 | 79.80 | 5.48 (1H, d, 12,6 | C4; C-1'; C3 | 77.45 | 5.43 (1H,d, 2,8) | C4; C-1'; C3 | 79.47 | 5.56 (1H, dd,2.9; 12.6 | C4; C-1'; C3 |
| 3 | 46.48 | 2.67 (1H,d, br s); 2.97 (1H,d, 12.6) | C4; C2 | 43.5 | 3.08 (1H, t, ); 2.84 (1H,d) | C4; C2 | 43.63 | 2.82 (1H, dd, 2.9; 12.6); 3.18 (1H, dd, 2.9; 12.6) | C4; C2 |
| 4 | 187.84 | - | - | 195.93 | - | | 196.85 | - | |
| 5 | 106.18 | - | - | 103.3 | - | | 103.29 | - | |
| 6 | 129.22 | 7.37 (1H,d, 8) | C5;C-7 | 164.3 | - | C5;C-7 | 129.51 | 7.42 (1H, d, 8) | C5;C-7 |
| 7 | 96.65 | 6.15 (1H,d,8) | C8;C5 | 95.3 | 6.06 (1H, br s) | C8;C5 | 96.96 | 5.98 (1H,d, 8). | |
| 8 | 165.06 | - | | 168.3 | - | | 165.33 | - | |
| 9 | 94.23 | 6.09 (1H,br s) | C10;C5 | 94.43 | 6.06 (1 H, br s) | C10;C5 | 95.91 | 6.01 (1H, br s) | C10;C5 |
| 10 | 163.79 | - | | 163.14 | - | | 164.19 | - | |
| 1' | 140.67 | - | | 138.54 | - | | 140.06 | - | |
| 2' | 129.47 | (1H, d, 8.6) | C1';C2 | 126.3 | 7.43 (1 H, br s) | C1';C2 | 129.45 | 7.45 (1 H, d, 8,0) | C1';C2 |
| 3' | 127.23 | (1H, d,8.6) | | 129.0 | 7.42 (1H, brs) | | 127.32 | 7.56 (1H, d,8.0) | |
| 4' | 165.60 | - | | 126.3 | 7.43 (1 H, br s) | | 167.38 | - | |
| 5' | 127.23 | (1H, d,8.6) | | 129.0 | 7.42 (1H, brs) | | 127.32 | 7.56 (1H, d,8,0) | |
| 6' | 129.47 | (1H, d, 8.6) | C1';C2 | 126.3 | 7,43 (1 H, br s) | | 129.45 | 7.45 (1 H, d, 8.0) | |
| OH | - | 9.42 | | - | 12.03 | | | 9.63 12.16 | |
| OCH3 | 56.14 | 3.79 | | 55.85 | 3.81 | | | | |

Toxicity and antiviral activity test performed in the laboratory of Microbiology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta. Toxicity test should be done on the extract to determine the toxixity character of the material against chicken egg embryos. As much as 0.1 mL extract in a suitable solvent concentration i.e . 1; 0.1; and 0.01%, was inoculated into embryonated chicken eggs aged 10 days. Eggs were incubated in the incubator for 24 hours. Chicken embryos then released and observed presence or absence of abnormalities due to test material. The toxicity test showed that all test materials are not toxic, this is indicated because of all the chicken embryo is still alive and there is no embryo abnormalities or organ damage, thus further test the antiviral activity can be performed on extract concentration up to 1% .

Antiviral activity test carried out on the AI virus H5N1. As much as 0.1 mL H5N1 AI virus was added by 0.5 mL extract 1%, 10,000 IU / mL antibiotic penicillin, and 10 mg / mL streptomycin, then was

incubated 370C for 30 minutes. Suspension of the virus as much as 0.1 mL was inoculated on the TAB through korioalantois space. Eggs were incubated in the incubator for 3 days, replicated in every study is 5 times. Embryo mortality was observed whether or not there every day. Eggs stored in refrigerator for 24 hours, then the liquid alantois harvested to tested of hemaglutination titter (HA). HA positive to say in case of hemagglutination. HA titer is the reciprocal of the highest dilution that still showed a positive reaction. Extract is said to have antiviral activity when the HA titter differs significantly from the control or even a HA titter of approximately $2^0$. The fluid of alantonis chicken embryos inoculated with only the suspension of the virus (HA titer $2^8$) used as control. The test results of antiviral activity against methanol extracts (1%) is $2^5$; hexane extracts (1%) is $2^0$. HA positive to say in case of hemagglutination, under control of the chicken embryo erythrocytes. The virus will bind to the erythrocyte shape hemaglutinat, if there is an active ingredient that test, the test materials will kill the virus by way of entry into the membrane lipid bilayer. HA titter results of methanol and hexane extracts of *Kaemferia rotunda* have smaller than the controls. The activity against the AI virus H5N1 from extracts Kaemferia rotunda, showed that the methanol extract has antiviral activity but not significant, while hexane extracts of Kaemferia rotunda showed high antiviral activity.

## 4. Conclusion

The isolated chemical compounds from the hexane extracts showed the presence of one compound that is 6 - hydroxy-6-methoxy-flavanones (2), while isolated from the ethyl acetate fraction of Kaemferia rotunda obtained three compounds, namely 4'-hydroxy-8-methoxy-flavanones (1), 6 - hydroxy-8-methoxy-flavanones (2), and 4 ', 8-dihydroxy-flavanones (3). The activity against the AI virus H5N1 from extracts Kaemferia rotunda, showed that the methanol extract has antiviral activity but not significant, while hexane extracts of Kaemferia rotunda showed high antiviral activity.

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