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Potential of n-hexane and chloroform extracts from *Melochia umbellata* (Houtt) Stapf var bark. *Visenia* as dengue antivirus

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Potential of n-hexane and chloroform extracts from *Melochia umbellata* (Houtt) Stapf var bark. Visenia as dengue antiviral

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Abstract. A study was conducted on the identification of the group of compounds from from n-hexane and chloroform extract of bark of *M. umbellata* (Houtt.) Stapf var. Visenia (Houtt.) and its activity test against dengue antiviral causes *dengue hemorrhagic fever* (DHF). The method used through several stages are maceration gradually starting from n-hexane and then followed chloroform, to stem bark powder *M. umbellata* (Houtt.) Stapf var. Visenia (Houtt.). Phytochemical test was done to know the group of compounds in each extract. In both extracts tested its activity against dengue virus. From the phytochemical test it is known that the n-hexane extract contain terpenoid, steroids and alkaloids, while in chloroform extract contain of steroids and alkaloids. From this study it was found that n-hexane and chloroform extracts were very active against dengue virus with IC_{50} values of 2.39 and 2.34 $\mu\text{g/mL}$. Thus, n-hexane and chloroform extracts can be used to help cure of Dengue Fever (DHF) disease

1. Introduction

Aedes aegypti and *Aedes albopictus* mosquitoes are the spreaders of dengue virus [1]. This is a public health problem in the world because more than 100 countries in the tropic and subtropic exposed to the risk of dengue fever (DHF) every year. There has been no effective medicine for diseases, a lot of research have been conducted to find alternative medicine from plants. *Melochia* is one of the plants that have long time been used by the community as traditional medicine. *Melochia* consist of 65 species. In the many countries, *Melochia* is widely known and used in traditional medicines to treat some diseases, such as bronchitis, cough, dysentery, swelling the stomach, abdominal pain, hypertension, lever, wound, stop vomiting, cancer, and inflammation [2–6]. In Brazil this plant is used by the community as a traditional medicine for tumors, wounds, colds and anti hypertension [7]. Based on previous research, methanol extract of *Melochia corchorifolia* potentially as an antioxidant [8]. Leaf of *Melochia corchorifolia* extract has moderate activity against HCT-116 (Human colon cancer) cell line with IC_{50} value is 138.48 $\mu\text{g/ml}$. The extract contains of phenol and flavonoid groups compounds [9]. Another *Melochia* plant is *M. umbellata* (Houtt.) Stapf consist of two varieties of *M. umbellata* (Houtt.) Stapf var. Degrabrata K and *M. umbellata* (Houtt.) Stapf var. Visenia. This paper will report on the activity of n-hexane and chloroform extracts to dengue virus and it's related to the compounds contained in the extract.



2. Methods

2.1. Material

The materials used in this research are stem bark powder of *M. umbellata* (Houtt) Stapf var. *Visenia*, some organic solvents such as technical methanol, technical n-hexane, chloroform, ethyl acetate, technical acetone, Liebermann-Buchard phytochemical reagent, Dragendorff, Wagner, iron (III) chloride medium MEM, Fetal Bovin Serum (FBS), MEM medium, Vero cell ((derived from the kidney of an African green monkey), cell line C6/36, DENV-2, Phosphate-Buffered Saline (PBS), 1% L-glutamine, 1.5% CMC, tetramethylbenzidine (TMB), NaHCO₃, H₂SO₄ 0,1 M.

2.2. Instrument

The tools used in this research are glass, blender, funnel, separating funnel, Buchner funnel, rotary evaporator, digital scales, Vigreux distillation device, concave tube, T25 flask, CO₂ incubator, incubator without CO₂, centrifuge 4°C, freezer, hemocytometer, microwell plate, inverted microscope, ELISA reader (Benchmark).

2.3. Preparation of Plant Extract

50 g dried powder of stem bark *M. umbellata* was macerated with n-hexane for 1 x 24 hours 4 times. The macerate collection obtained was then concentrated to obtain a concentrated n-hexane extract. The residue was macerated with chloroform solvent 4 time.

2.4. Phytochemical test

Phytochemical tests of n-hexane and chloroform extracts were tested flavonoids, alkaloids, steroids, and terpenoids. Flavonoid test uses 1% iron (III) chloride solution in water, alkaloid test uses the Dragendorff, Wegner, and Meyer reagent, while steroid and terpenoid test using Liebermann-Burchard reagents. Dragendorff reagent (0.8 g Bi (NO₃) was added 10 mL CH₃COOH and 40 mL water, then mixed with solution made from 8 g KI in 10 mL water), Wegner's reagent (2.5 g of iodine was added 2 g KI and 10 mL of water then diluted with distilled water to 200 mL), Meyer reagent (1.36 g HgCl₂ added 0.5 g KI dissolved and diluted with distilled water to 100 mL). Terpenoid and steroid tests used 10 drops of acetic anhydride and 3 drops of H₂SO₄ [10].

2.5. Bioassay

The extract obtained was tested toxicity to *Artemia salina* using Meyer method [11] and dengue Antivirus test is done by using Focus Formation Unit Reduction Assay method (FFURA). Dengue activity test procedure refer to references [11,12].

3. Results and Discussion

The results of phytochemical tests were performed on n-hexane and chloroform extracts of stem bark *M.umbelata* (Houtt) Stapf var *Visenia* including steroid and alkaloids. Steroid compounds have been identified from the extract of n-hexane from root of *M.umbellata*, ie. stigmasterol (5,22-stigmastadien-3β-ol) (a) [12]. Furthermore, β-sitosterol (b) was also successfully isolated from the n-hexane extract of *M. umbellata* (Houtt) stapf var. *Degrabrata* K. Research on the bioactivity of compounds as antivirus has been carried out on steroid group compounds belonging to the oleanene group of 2,3-dihydroxy-12-oleanen-28-oat (c). The compound was isolated from the *Kleinhovia hospita* Linn and was cytotoxic against P-388 cancer cells with IC₅₀ value 15.0 µg/mL [13].

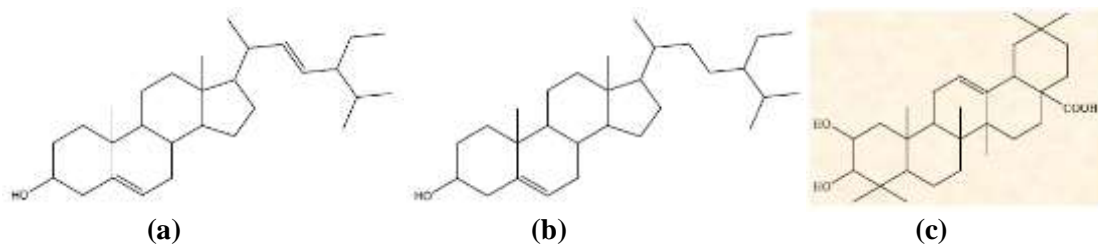


Figure 1. Isolated bioactive component from root of *M.umbellata*, ie. stigmasterol (5,22-stigmastadien-3 β -ol) (a), β -sitosterol (b), and oleanene group of 2,3-dihydroxy-12-oleanen-28-oat (c).

Some research of antivirus bioactivity on alkaloids compound group also have carried out to waltherrone C (a) from chloroform extract heard wood *M. umbellata* (Houtt) Stapf var. *Degrabrata* K. The compound have anti HIV activity [14]. Have isolated of 4-quinolon alkaloid of *n*-heksan extract from stem bark *W. douradinha*, waltherrion B (b) and vanassin which are active against *A. salina* and also antibacterial[14]. Two compounds of 4-quinolon alkaloid have isolated from methanol extract *M. odorata*, waltherrion A (c) and (a). Both of compounds have significant activity as anti-HIV with value 0,84 and 56,2 μ M and also against HIVP24 with value 0,95 and 1,7 μ M, respectively [15]. Based on the activity data of steroid and alkaloid, was this study was anti virus *dengue* test to *n*-hexane and chloroform extract from stem bark *M.umbellata* (Houtt) Stapf var. *Visenia*. The activity data of both extract can be seen on Table 1.

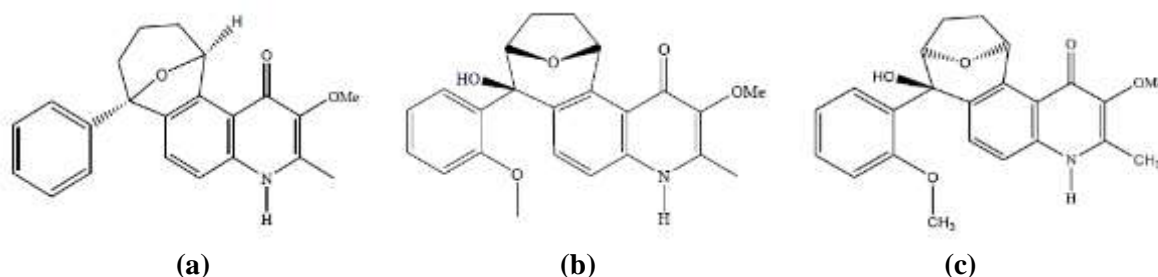


Figure 2. Isolated bioactive component from chloroform extract heard wood *M. umbellata* (Houtt) Stapf var. *Degrabrata* K (a), isolated of 4-quinolon alkaloid of *n*-heksan extract from stem bark *W. douradinha*, waltherrion B (b), two compounds of 4-quinolon alkaloid have isolated from methanol extract *M. odorata*, waltherrion A (c) and (a).

Most dengue virus causing dengue fever in Indonesia is DENV-2, so the anti- virus test is conducted on both *n*-hexane and chloroform extracts. To ensure the bioactivity of dengue virus, the two extracts were also tested against vero cells.

Table 1. Bioassay test of *n*-hexane and chloroform extract.

Extract	<i>A. salina</i> (μ g/mL)	DENV-2 (μ g/mL)	Vero Cell (μ g/mL)
<i>n</i> -hexane	0,02	2.39	12.20
Chloroform	53,28	2.34	33.19

Based on the data in Table 1, it shows that n-hexane extract very active and chloroform extracts moderate active against DENV-2. Bioactivity of both of extracts as strong relation to the content of existing compounds of steroids and alkaloids.

4. Conclusion

The cytotoxic n-hexane extract very active and chloroform extracts moderate active against DENV-2 with IC_{50} value 2.39 and 2.34 $\mu\text{g/mL}$ and against vero cell with IC_{50} value 12.20 and 33.19 $\mu\text{g/mL}$. Both of extracts contain compounds that can be useful for Dengue Fever (DHF) disease.

Acknowledgment

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