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

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Abstract. A study was conducted on the identification of the group of compounds from n-hexane and chloroform extract of bark of M. umbelatta (Houtt.) Stapf var. Vicenia (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. umbelatta (Houtt.) Stapf var. Vicenia (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 IC50 values of 2.39 and 2.34 μg/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 IC50 value is 138.48 μg/ml. The extract contains of phenol and flavonoid groups compounds [9]. Another Melochia plant is 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 pa, ethyl acetate, technical acetone, Liebermann-B 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, hemositometer, 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 (II) chloride solution in water, alkaloid test uses the Dragendorf, Wegner, and Meyer reagent, while steroid and terpenoid test using Lieberman-Buchard reagents. Dragendorf 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) stap var. Degabrata 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-3β-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 waltherione C (a) from chloroform extract heard wood M. umbellata (Houtt) Stapf var. Degabrata K. The compound have anti HIV activity [14]. Have isolated of 4-quinoln alkaloid of n-heksan extract from stem bark W. douradinha, waltherion B (b) and vanassin which are active against A. salina and also antibacterial[14]. Two compounds of 4-quinoln alkaloid have isolated from methanol extract M. odorata, waltherion A (c) and (a). Both of compounds have significant activity as anti-HIV with value 0.84 and 56.2 µM and also against HIVP24 with value 0.95 and 1.7 µM, 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.umbelata (Houtt) Stapf var. Visenia. The activity data of both extract can be seen on Table 1.

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      | A. salina (µg/mL) | DENV-2 (µg/mL) | Vero Cell (µg/mL) |
|--------------|-------------------|----------------|-------------------|
| n-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 $\mu$g/mL and against vero cell with $IC_{50}$ value 12.20 and 33.19 $\mu$g/mL. Both of extracts contain compounds that can be useful for Dengue Fever (DHF) disease.

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References
[1] Mulyatno K C, Kotaki T, Yotopranoto S, Rohmah E, Churotin S, Sucipto T, Amarullah I, Wardhani P, Soegijanto S and Kameoka M 2017 Detection and serotyping of dengue viruses from Aedes aegypti and Aedes albopictus (Diptera: Culicidae) collected in Surabaya, Indonesia 2008 - 2015 Jpn. J. Infect. Dis. 71
[2] Agra M de F, Baracho G S, Nurit K, Basfio I and Coelho V P M 2007 Medicinal and poisonous diversity of the flora of “Cariri Paraibano”, Brazil J. Ethnopharmacol. 111 383–95
[3] Shanmugam S, Rajendran K and Suresh K 2012 Traditional uses of medicinal plants among the rural people in Sivagangai district of Tamil Nadu, Southern India Asian Pac. J. Trop. Biomed. 2 S429–34
[4] Pullaiah T 2014 Ethnobotany, Phytochemistry and Pharmacology of Melochia corchorifolia L. Int. Res. J. Pharm 5 543–5
[5] Liogier H A 1988 Descriptive flora of Puerto Rico and adjacent islands, Spermatophyta. Vol. 2. Descr. flora Puerto Rico Adjac. islands, Spermatophyta. Vol. 2.
[6] Dias G O C, Porto C, Stüker C Z, Graessler V, Burrow R A, Dalcol I I, Silva U F and Morel A F 2007 Alkaloids from Melochia chamaedrys Planta Med. 73 289–92
[7] Rüegg T, Calderón A I, Queiroz E F, Solis P N, Marston A, Rivas F, Ortega-Barría E, Hostettmann K and Gupta M P 2006 3-Farnesyl-2-hydroxybenzoic acid is a new anti-Helicobacter pylori compound from Piper multiplinervium J. Ethnopharmacol. 103 461–7
[8] Mamatha B S, Palaksha M N, Gnanasekaran D, Senthilkumar G P and Tamizmani T 2018 MELOCHIA CORCHORIFOLIA L: A REVIEW
[9] Palaksha M N, Ravishankar K and Sastry V G 2015 Evaluation of in-vitro Anticancer Activity and Quantitative Estimation of Phenolics and Flavonoids of Melochia corchorifolia and Saccharum officinarum Leaf Extracts Eur. J. Biomed. Pharm. Sci. 2 1410–20
[10] Harborne J B 1973 Phytochemical methods mechanism and Hall Ltd. London 4 49–188
[11] Soekamto N H, Liong S, Fauziah S, Wahid I, Tabo P and Ahmad F 2018 Dengue antiviral activity of polar extract from Melochia umbellata (Houtt) Stapf var. Visetenia Journal of Physics: Conference Series vol 797 (IOP Publishing) p 12017
[12] Fasola T R, Adeyemo F A, Adeniji J A and Okonko I O 2011 Antiviral potentials of Enantia chlorantha extracts on yellow fever virus J Nat Sci 9 99–105
[13] Soekamto N H, Alfian N, Iwan D, Hasriani A, Ruhma R and Agustono A 2012 Dua senyawa triterpenoid dari tumbuhan paliasa (Kleinhovia hospita L.) famili sterculiaceae J. Sains MIPA Univ. Lampung 8
[14] Noor A, Soekamto N H, van Altena I and Syah Y M 2014 Waltherione C and cleomiscosin from Melochia umbellata var. Degrabrata K.(Malvaceae), biosynthetic and chemotaxonomic significance Biochem. Syst. Ecol. 358–61
[15] Jadulco R C, Pond C D, Van Wagoner R M, Koch M, Gideon O G, Maitainaho T K, Piskaut P and Barrows L R 2014 4-Quinolone alkaloids from Melochia odorata J. Nat. Prod. 77 183–7