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論文摘要內容:

Paederia foetida,俗稱雞屎藤,為茜草科植物。傳統上,它 Unive 用於治療腸腫大、腸炎、胃腸脹氣、胃萎縮、風濕病、鼻炎、糞 java 血症、疼痛、胃痛和牙痛。在現代藥理學研究中,據文獻指出 P. ^{ve}foetida 的萃取物具有抗菌、止瀉、抗發炎、抗痙攣、驅蟲、鎮咳 w和保肝活性。本研究目的為分離與鑑定 P. foetida 萃取物之化學成 分,及探討其主要的藥理活性,並將具生物活性之化合物進行抗 菌活性分析。P. foetida 之甲醇粗萃物依序經由乙酸乙酯 (EtOAc) 及正丁醇 (n-BuOH) 進行分配萃取。將 EtOAc 層萃取物進一步分 離純化,得到十個化合物,包含兩種固醇類、四種蔥醌類、兩種 unive 香豆素及雨種芳香類。化合物的的結構則是由核磁共振光譜 ava (Nuclear Magnetic Resonance, NMR) 及質譜 (Mass Spectrometry, MS) 來鑑定, 分別為 β-sitosterol (S1)、stigmastan-3-one (S2)、 Unive morindaparyin [Av(An1)] and 1,3-dihydroxy-2-methoxyanthraquinone java Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Ilniversitas Rrawijava Ilniversitas Rrawijava Ilniversitas Rrawijava Ilniversitas Rrawijava

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya (An2) alizarin (An3) digiferrol (An4) scopoletin (C1) awijaya fraxidin (C2)、 ferulic acid (A1) 與 vanillic acid (A2)。 awijaya awijaya Universitas P. foetida 之分配萃取物及 EtOAc 層分液之抗菌活性將使用瓊山和 awijaya awijaya 脂擴散法分析,而刀天青還原試驗則用來做最小抑菌濃度 awijaya (minimum inhibitory concentration, MIC)的確效。結果顯示 EtOAc awijaya Unive 層比 n-BuOH 層及水層具有較顯著的抑制大腸桿菌 (11 mm) 及葡 java awijaya awijaya 萄球菌 (11.5 mm), 並發現 1,3-dihydroxy-2-methoxyanthraquinone awijaya (An2)、digiferrol (An4) 和 vanillic acid (A2) 具葡萄球菌之抑制活。 awijaya awijaya 性,其 MIC 為 4.68-100 µg/mL。P. foetida 甲醇粗萃物的 EtOAc 層 awijaya 富含蒽醌類化合物,為抗菌的活性成分,因此其萃取物及分液具 awijaya awijaya Univer有抗菌感染的潛力。 awijaya awijaya

> 光譜分析、 關鍵字:雞屎藤 (Paederia foetida Linn)、分離 純化、

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijaya Abstract awijaya Universitas Brawijaya Universitas Brawijaya Unive Student ID: M10618032s Brawijava Universitas Brawijava Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Title of Thesis: Studies of Biological Properties of Major Bioactive Compounds from the Stems of Paederia foetida. Linn UniverTotal Page: 81 Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awiiava awijaya Name of Institute: Department of Biological Science and Technology, National Pingtung University of Science and Universitas Brawilava awijaya Technology awijaya Graduate Date: January 11, 2019 Degree Conferred: Master awijaya

Name of Student: Aryandi Ramadhan

The Contents of Abstract in This Thesis:

Paederia foetida Linn locally known as skunk vine or Chinese fever vine from Rubiaceae Family. Traditionally, it is used to treat enteromegaly, enteritis, flatulence, gastromegaly, rheumatism, rhinitis, and toothache. copraemia, stomachache sore, In modern University pharmacological studies, the extract of *P. foetida* has been reported to possess antimicrobial, antidiarrheal, anti-inflammatory, antispasmodic, anthelminthic, antitussive, and hepatoprotective activities. In this study, Unive P. foetida extract was investigated for bioactive constituents and their Universitas Brawijaya main pharmacological properties. The research purpose was to isolate and identify the chemical constituents of the stem of *P. foetida* and Unive evaluate their antibacterial activities. The crude methanol extract was lave sequentially partitioned with ethyl acetate (EtOAc) and n-butanol (n-BuOH). The EtOAc layer was further isolated and purified to obtain ten Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Compounds including two steroids, four anthraquinones, two coumarins, and two aromatics. The compounds were identified using Universitas Brawijava Universitas Brawijava Universitas Brawijava

Advisor: Chi-I Chang (Ph.D.)

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) analysis. The ten compounds were identified as β -sitosterol (S1), stigmastan-3-one (S2), morindaparvin A (An1), 1,3-dihydroxy-2methoxyanthraquinone (An2), alizarin (An3), digiferrol (An4), awijaya scopoletin (C1), fraxidin (C2), ferulic acid (A1), and vanillic acid (A2). Unive The antibacterial activity of the partitions and fractions were evaluated laya awijaya awijaya using agar well diffusion assay. The resazurin assay was used to awijaya Unive determine the minimum inhibitory concentration (MIC). The result of java awijaya awijaya agar diffusion assay showed that the EtOAc layer has significant inhibition against Escherichia coli (11 mm) and Staphylococcus aureus (11.5 mm) than the *n*-BuOH and water layer. S. aureus was very awijaya awijaya to, 1,3-dihydroxy-2-methoxyanthraquinone (An2), unive susceptible awijaya awijaya digiferrol (An4), and vanillic acid (A2) with MIC values ranging from awijaya 4.68-100 µg/mL. The EtOAc layer of methanol crude extract contains awijaya awijaya mainly anthraquinones as a dominant bioactive compound. The extract awijaya awijaya and fractions of *P. foetida* could be considered as prospective awijaya ersitas Brawijaya candidates for the prevention of bacterial infections. awijaya

Unive Keywords:

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Paederia foetida Linn, isolation, spectroscopic analysis, bioactive properties

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Acknowledgment Universitas Brawijaya Universitas Brawijaya Universitas I would like to express my gratitude to all those who contributed University and support me during my study for Master program. First and always, java all praise to Allah SWT, our Lord and the Creator of the universe, for His Blessing, Mercy, guidance, and a chance for me to complete my study. Second, Muhammad SAW, the Messenger of God, who leads us to the light of this era, may the peace and blessings of Allah upon him. Universitas B I would like to express my gratitude to my adviser Dr. Chi-I Chang for the opportunity to study and research in his laboratory. I am really grateful for his patience, encouragement, and support helped me complete my thesis. I could not have imagined having a pleasant adviser and I amassed a wealth of knowledge from him. My sincere and thanks also go to my adviser Dr. Agustin Krisna Wardani for her support ersitas Brawijaya during my study.

For my beloved mother, father, brothers, thank you for the neverending love and support. Without them, I could not have finished my study.

For my lab mates of BT201 Laboratory, Bongani Sicelo Dlamini, Ya-Lin Chang, Luo Ci, and Desy Ambar Sari, thank you for all the moments you shared with me. They made it easy for me to adapt and performing my experiment in BT201. My gratitude also goes to Dr. Revathi for her help and Dr. Douglas J. Shyu from BT 306 for allowing me to conduct part of my experiment in his laboratory. To all Indonesian students in NPUST, thank you very much for their effort and support. Lastly, special thanks to the National Council Taiwan, Double Degree Program of NPUST-UB for providing me the scholarship in my graduate study.

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya 1. INTRODUCTION awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Univer1.1 Backgroundversitas Brawijaya Universitas Brawijaya Universitas Brawijaya Over the past decade, natural products have gained importance awijaya University in the health system. Although modern medicine is advanced in most java awijaya parts of the world. Many developing countries still use traditional awijaya awijaya Unive medicine to meet their primary health needs, which relies mostly on lave awijaya medicinal plants. Even though modern medicine may exist side-by-side awijaya with such traditional practice, natural products have often retained its Universtatus for historical and cultural reasons. Herbal medicines have come jaya awijaya awijaya from numerous source materials including microorganisms, marine awijaya organisms, and plants. awijaya Paederia foetida L. locally known as skunk vine or Chinese awijaya awijaya

fever vine from Rubiaceae Family. The species name foetida is Latin for "stinky" or "foul smelling". The name was derived from the odor of carbon disulfide from the leaves. Various traditional treatments have been linked to this plant. It is used to treat enteromegaly, enteritis, flatulence, gastromegaly, rheumatism, rhinitis, copraemia, pain, abdominal pain and toothache (Afroz et al., 2006).

Previous phytochemical studies of the aerial parts of *P. foetida* from Borgohain et al. (2017) showed it contains iridoid glycosides, paederolone, paederone, paederoside, and asperuloside. In addition, Soni et al. (2013) reported that the plant also contains carotene, vitamin C, keto-alcohol, alkaloid, sitosterol, friedelin, campesterol, ursolic acid, hentriacontane, hentriacontanol, vitamin C, ceryl alcohol, palmitic acid, terpenoids, and methyl mercaptan. Soni et al. (2013) reported that fresh and dried plant extracts of *P. foetida* showed their ability to the reactive oxygen species as free radicals using β -carotene bleaching and the ABTS (2,2'-azino-bis (3ethylbenzothiazoline-6-sulfonic acid) method. The percentage of

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya antioxidant activity for fresh and dried plant extract samples was between 58 and 80%. According to Upadhyaya (2013), antioxidant phytochemicals have a strong protective effect against cancer and cardiovascular disease. Furthermore, the leaf extract of this plant possesses antihyperglycemic. Other reported biological activities of the extract of *P. foetida* are an antimicrobial, antidiarrheal, antiinflammatory, antispasmodic, anthelminthic, antitussive, and hepatoprotective activity (Macwan, 2010). as Brawlaya Interestingly, even though P. foetida is known as herbal medicine, it is still rare, threatened in the wild, and needs further investigation. University Although there is some information on the biological activity of *P*. foetida in literature, information is still lacking on the biological activity of the chemical constituents of the plant. Thus, the lack of studies on phytochemical compounds of this plant became the background of this study.

1.2 Research Objective

This research purpose was to isolate and identify the chemical constituent of the stem of *P. foetida*. Then, evaluate and compare the antibacterial activities of crude extract, fractions and chemical constituent of P. foetida with known antibacterial agents.

1.3 Future Impact awijaya awijaya awijaya awijaya industrial level.

Hopefully, P. foetida as a new source of herbal medicine could trigger and improve the medicinal system for human and could be ijava Universitas Brawijaya Universitas Brawijaya developed not only in laboratory scale but also in pharmaceutical Universitas Brawijava Universitas Brawijava ² Iniversitas Brawijava

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya 2. LITERATURE REVIEW 2.1 **Herbal Medicine** Plants and their secondary metabolite constituents have a long ersitas Brawijaya Universitas Brawijaya Universitas Brawijaya history of utilization in cutting-edge medication and in certain frameworks of conventional medication and are the sources of awijaya e imperative drugs. Utilization of natural products in developed countries awijaya has extended strongly within the last half of the twentieth century. They utilize the plants which contain chemical constituents in any of its portion like root, stem, leaf, fruit, and seed. They are classified as traditional herbal medication in the event that they create a definite curing physiological reaction within the treatment of different disease awijaya awijaya in humans and animals (Hamayun et al., 2006). awijaya awijaya

In some cases, the active principles of plant-derived products have been isolated and characterized, and their working mechanisms are understood. Many people have the perception that most herbal products found on the market provide inaccurate information. This is due to the complexity of the preparation and processing of herbal plants. Even though they are not pure compounds. They believe that the combination of several active components in herbal plants has a very beneficial effect (Ajazuddin and Saraf, 2012).

2.2 Paederia foetida. Linn Paederia foetida or Skunk vines are locally prominent plant species in the east and south Asia. The plant was first presented to the USDA field station near Brooksville in 1897. In 1916, it was reported as an invasive weed in the region. The plant was known as a weed that spread on the peninsula of Florida in 1933. In 1977, it was considered a weed that had economic value. In 1993, it was included in the Florida Exotic Pest Plants Invasive Species as Category I, which was defined

Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya as "a species that are invasive and disrupts the native plant communities in Florida". It was also included in the Florida Noxious Weed List (5B-57,007 F.A.C.) in 1999, this plant is illegal to own, move or release in Universitas Brawijaya Unive Florida. (Langeland et al., 2000). a Universitas Brawijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Unive P. foetida was classified following from Macwan (2010) Iniversitas Brawijava Plantae Universitas Brawijaya awijaya Kingdom awijaya Unive Subkingdom Univ: Tracheobionta Universitas Brawijaya awijaya awijaya Superdivison : Spermatophyte inversitas Brawijava awijaya Division : Magnoliophyte awijaya : Magnoliopsida Unive Class awijaya awijaya Subclass : Asteridae awijaya awijaya Order **Rubiales** awijaya Family : Rubiaceae awijaya awijaya : Paederia L. Genus awijaya awijaya Species Paederia foetida L. awijaya awijaya awijaya awijaya awijaya awijaya awijaya 5-7mm (1/4") awijaya awijaya awijaya awijaya Paederia foetida Universitas Brawijava UniverFigure 1. P. foetida L. drawn by (Langeland et al., 2000) Universitas Brawijaya Universitas Brawijaya Universitas Brawijava Universitas Brawijava 4 Iniversitas Brawijava

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya Universite According to Morshed et al. (2012a) describes plants. P. foetida is the awijaya a climber plant, growing between shrubs, hairy or smooth slender awijaya awijaya branches. The height of the lower stem to the tip of the stem can reach awijaya 7 m (23ft) or more, propagating on the tree or fence. The leaves and awijaya stems have an unpleasant odor, especially when it crashed. The flowers Universe small, have grayish or light purple-pink color, in wide or long awijaya awijaya curved clusters, "leafy", and terminals or on axis. The corolla is very awijaya Unive hairy, tubular with 5 lobes usually spreading. Fruits are brown shiny, Java awijaya awijaya round capsules, up to 0.7 cm (0.3 inches) wide, with round black seeds. awijaya awijaya 2.3 Chemical Constituents GUV Universitas I awijaya awijaya

> A variety of chemical constituents are found in *P. foetida* such as iridoids (Shukla et al., 1976), saponins, tannins, phenols, flavonoids, cardiac glycoside, and alkaloids (Upadhyaya, 2013). The chemical ersitas Brawijaya constituents of *P. foetida* are shown in Table 1.

> > BURATION STRUCT

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya Table 1. Chemical constituents of *P. foetida* Linn Universitas Brawija References Universitas Brawijaya Brawijaya Compounds Universitas Brawija Univers7-hydroxy-6-methoxy-2H-1-benzopyran- 2- Br Zhuang et al. (2013) wijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universone (1) wijaya Ethyl (E)-3-(4-methoxyphenyl) prop-2sitas BraUddin et al. (2013) awijaya Brawijaya enoate (2) β -Sitosterol (3) β -Sitosterol (3) Universitas Brawijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya (1974)sitas Brawijaya awijaya Soni et al. (2013) awijaya Stigmasterol (4) awijaya Lupeol (5) awijaya Ellagic Acid (6) awijaya Epifriedelinol (7) awijaya Friedelin (8) awijaya Scandosides (9) awijaya awijaya Asperuloside (10) awijaya Paederoside (11) awijaya Catechin (12) Dasgupta and De Univ awijaya (2007)awijaya Ursolic acid (13) Borgohain et al. awijaya awijaya (2017)awijaya Lupenol (14) ersītas Brawijaya awijaya Z WAXMILES awijaya awijaya awijaya awijaya awijaya awijaya awijaya Jrsitas Brawijaya awijaya awijaya awijaya awijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Rrawijava 6 Iniversitas Rrawijava Universitas Brawijava

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2.4 Pharmacological uses In folklore, *P. foetida* is used to treat enteromegaly, enteritis, flatulence, gastromegaly, rheumatism, rhinitis, sapramia, sore, stomachache, and toothache. Previous studies back up the pharmacological basis for ethnomedical use of the plant. The antidiarrheal activity (Afroz et al., 2006), the anti-inflammatory activity from butanol fraction of a methanol extract (BMEL) (De et al., 1994), antidiabetic and antithrombolytic from methanolic extract (Morshed et al., 2012a), antioxidant and antimicrobial activity from ethanolic extract (Upadhyaya, 2013), antihyperlipidemic and antihyperglycemic from leaf extract (Kumar et al., 2014) have been reported. Ethanolic extract *P. foetida* is also reported to be an aphrodisiac and stimulate sexual behavior (Soni et al., 2012).

2.4.1. Antidiarrheal activity

Afroz et al. (2006) investigated the effect of *P. foetida* ethanol extract as a remedy for diarrhea. The activity was indicated using castor oil and magnesium sulfate-induced diarrhea models in mice. The extract significantly increased the latent period of diarrhea in the models. The effect of extract particularly reduced the purging index (PI) value in a dose-dependent manner in magnesium sulfate-induced diarrhea. Generally, the ethanol extract of *P. foetida* reduced the gastrointestinal motility with barium sulfate milk both in 15 and 30 min time interval. Another result, the extract significantly decreased the cisplatin-induced gastrointestinal motility at both time intervals. The extract also enhanced the morphine-induced reduction of motility. These results indicate that ethanolic extract of *P. foetida* possess antidiarrheal activity by inhibiting intestinal motility and confirm it can be used in traditional medicine as a remedy for diarrhea.

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya Universitas Brawijava The butanol extract of methanol extract (BMEL) of P. foetida leaves was investigated for anti-inflammatory activity by De et al. (1994). The fraction showed significant inhibition of granulation tissue awijaya formation on cotton implanted in mice. The fraction significantly Unive decreased aspartate liver transaminase activity and affected the adrenal java awijaya awijaya body weight and ascorbic acid content, thus ruling out stimulation of awiiava the adrenal-pituitary axis. BMEL also inhibits the increase of awijaya awijaya orosomucoid serum levels in mice, which suggests the possibility of awijaya disease-modifying anti-rheumatic activity. The results show that the awijaya University plant is capable to treat inflammatory disorders. awijaya awijaya

2.4.3. Antidiabetic and antithrombolytic activities

Morshed et al. (2012a) investigated the antidiabetic and antithrombolytic properties of methanolic extracts of the whole plant of P. foetida. Methanolic extracts of the whole plant of P. foetida has moderate antidiabetic activity. It decreased the blood glucose levels by 30% respectively, while the positive control (glibenclamide, 2 mg/kg) decreased blood glucose level by 70%. 100 µl extract of P. foetida exhibited the highest thrombolytic activity with clot lysis value of laya 23.82% whereas standard streptokinase (positive control) and water (negative control) demonstrated clot lysis value of 45.85% and 2.81%, Unive respectively.a Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya 2.4.4. Antimicrobial and cytotoxic activities Universitas Morshed et al. (2012b) reported the antibacterial activities from several fractions of methanolic extracts of the whole plants of P. foetida. n-hexane, chloroform, and ethyl acetate were tested against several pathogenic bacteria using 'disc diffusion method'. The methanolic extract indicated no antimicrobial activity but the other Universitas Brawijava Universitas Brawijava 9 Iniversitas Brawijava Universitas Brawijava awijaya awijaya

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Universitas Brawijaya In the fractions showed moderate to less activity against some organisms as the compared to the standard antibiotic Kanamycin. Then cytotoxicity was unive tested using brine shrimp lethality bioassay. The LC₅₀ result indicated that methanolic extract has significant cytotoxicity. 2.4.5. Antihyperlipidemic and antihyperglycemic activities Universitas BKumar et al. (2014) explained that the leaf extract of P. foetida java shows remarkable antihyperglycemic activity due to the possibility of a sustainability effect involving the mechanism of the pancreas. As long as the antihyperlipidemic activity is given it may reduce the level of lipid profile in the blood and reduce blockages in the heart. The antioxidant activity of the extract was in the form of inhibition of lipid peroxidation and increased superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). It has been confirmed that P. foetida leaf extract has the potential to act as an antidiabetic, antihyperlipidemic and antioxidant.

2.5 Purification and Identification Methods

When isolating active compounds from dried stem *P. foetida*, extraction is the most important stage in the process. The extraction process is divided into several sub-steps. It includes maceration, partition, column chromatography, TLC, separation using HPLC and identification by spectral method (NMR). **2.5.1 Maceration** Maceration is the popular extraction method used with unstable, stable or thermal products. For small-scale extraction, maceration generally consists of several steps. The first step is to reduce plant material into small particles. Secondly, suitable solvents are added to the plant material and leave it to stand for at least 24 hours. Third, the awijaya

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya versitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awiiava liquid is compressed as well as the solids remaining from the extraction process are pressed to recover a large amount of clogged solution. The Univertension and release of the released liquid are separated from the dirt through filtration. Occasional stirring is carried out in maceration to optimize the extraction process in two ways; (a) increase diffusion, (b) Universe release concentrated solution from the surface of the sample and add awijaya new solvents for more extraction yield (Azmir et al., 2013). awiiava awijaya Universitas Brawijava Universitas Dowijava Universitas Brawijava 2.5.2 Partition eitas Rrawilava

Partitions are a technique for separating solutions based on their polarity properties in different liquids or solvents. This is based on the process of transferring solutes from one liquid phase to another liquid phase in accordance with the solubility properties. When the solution is shaken in a separating funnel with two immiscible solvents, the solution will separate itself between two the solvents.

In alcoholic extract (MeOH or EtOH) from plant materials contain various types of polar compounds to moderately polar. Many compounds that cannot dissolve individually but can be extracted with MeOH or EtOH. Usually, the concentrated extract of MeOH and the volume is reduced to an appropriate level so that can be easily partitioned with a separating funnel. Then the concentrated extract is extracted continuously at the same volume using non-polar solvents, nhexane, for example, three times or more, to obtain fractions containing non-polar compounds. This process also called "defatting". MeOH and *n*-hexane are not completely mixed, they can be mixed only to a certain extent. Sometimes, a small amount of water is added to MeOH to get a 95% methanol solution and get the same two layers of partition volume (Otsuka, 2005).

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijava Column chromatography is a purification technique that use glass columns filled with adsorbents (i.e., silica gel, alumina, or C18 phase reversal) in large samples or mixed products to produce large quantities (milligrams to grams) as well. As with thin layer chromatography Unive (TLC), the adsorbent used varies based on the type of separation carried java awijaya out. It is very useful to analyze the mixture by thin layer chromatography before using large-scale column chromatography. For example, to determine the mobile phase suitable for column chromatography, TLC plates can be used to confirm. After compliance, silica gel must be used as an adsorbent for column chromatography and the TLC plate mobile phase can be used for the purification process awijaya awijaya (Meyers, 2001). awijava

Before performing column chromatography, the glass column must be packed with an appropriate adsorbent and solvent. There are two methods for packing columns: wet packing and dry packing. Wet packing is a commonly used technique and is preferred for silica gel columns. While dry packing is less desirable because sometimes the formation of air bubbles is difficult to remove. When this happens, the column must be repackaged. However, when the absorbent is alumina, the method that suitable for use is dry packing. Because if it is packed by wet packing, alumina will be slurry-shaped which is difficult to pour. (Meyers, 2001). ersitas Usually, to separate polar substances, the stationary phase suitable for use is silica gel, because the surface is covered by a hydrated hydroxyl group, which is very polar. It will also interact with polar dipoles. The charge field associated with hydroxyl groups hydrated on silica gel can also produce induced dipoles in solutes by polarization, and thus silica will retain polarized compounds. The difference in university interaction between dissolved compounds and hydrated hydroxyl Ilniversitas Rrawijava Ilniversitas Rrawijava¹²iniversitas Rrawijava

Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya groups in silica gel can occur if more nonpolar solvents are used as the mobile phase. (Scott, 1983). 2.5.4 Thin Layer Chromatography (TLC) Thin layer chromatography is a technique used to separate nonv volatile mixtures. TLC is carried out using sheets of glass, plastic or awijava aluminum foil, which are covered with a thin layer of adsorbent, usually awiiava awijaya in the form of silica gel, cellulose or aluminum oxide (alumina). The adsorbent layer is called the stationary phase. TLC can be used to guarantee the existence of a compound. Then, examine it using UV light. Identification of the presence of mixed compounds must also be awijaya awijaya adjusted to the R_f value (Preethi et al., 2017). According to Poole awijaya awijava (2003), thin layer chromatography is a very simple method of awijava compound characteristic detection. Because the sample is in the awijava chromatogram, thin layer chromatography is the most suitable awijaya awijaya technique for surveying sample properties. awijaya

Different samples are usually applied to the stationary phase before being contacted by the mobile phase that later migrates past it in a particular direction. The motion of the mobile phase in the stationary phase is called the development step. After development, the mobile phase will evaporate and detection is carried out in a stationary phase. detectors used to record plot responses to separation distances are called densitogram (Spangenberg et al., 2011). **2.5.5 High-Performance Liquid Chromatography (HPLC)** is a type of liquid chromatography in which the sample must be changed in liquid conditions. HPLC is used to detect a specific compound on the solution. In HPLC and liquid chromatography, where the liquid sample will interact with the solid or stationary phase and the second liquid (mobile)

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya phase. The difference in polarity interactions can help the separation awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas High-performance liquid chromatography is basically another lava awijaya form of column chromatography that has modern performance. awijaya Solvents entering through a column other than gravity are forced into Unive high pressure up to 400 atmospheres. This process is a lot faster than java awijaya awijaya ordinary column chromatography. awijaya Universitas HPLC also includes a method of separating mixed samples in laya awijaya awijaya shorter periods of time, using a very small particle of samples, having awijaya awijaya a small column diameter, having very high liquid pressures and awijaya universe producing several peaks based on the retention times of each awijaya awijaya compound. Some factors that influence HPLC performance are: awijaya awijaya internal column diameter, column size, high sensitivity, higher pump awijaya pressure, higher separation, sample particle size, the polarity of the awijaya awijaya sample, solvent, and the last factor is temperature. awijaya

The advantages of using HPLC are that it only requires a small sample that has been able to identify with high accuracy and precision and is not destroying the sample during operation compared to GC CEX LEO Unive (Kupiec, 2004).

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya Unive 2.5.6 Nuclear Magnetic Resonance (NMR) Brawijava Nuclear magnetic resonance spectroscopy is a spectroscopic ve technique that utilizes the energy transition from processing atomic lava nuclei in static magnetic fields and alternating (rotating) magnetic field awijaya resonances. This required instrument consists of a direct current or Unive permanent magnete as a supplier of static fields, radio frequency ava awijaya generators for alternating field suppliers, probes that can hold the awiiava sample in a relatively precise position in these two fields, the radio awijaya receiver to detect the signal produced by the hydrogen nucleus in the awijaya sample and recorders to make permanent recordings in the form of awijaya Universpectrum. The static homogeneity of the field must be given a fine band awijaya awijaya and structure that shows the chemical environment in which each awijaya awijaya hydrogen nucleus is located. The spectrum that shows this fine structure awijaya is called a high-resolution spectrum (as opposed to a wide line spectrum awijaya where chemical environmental features are obscured by broad line awijaya awijaya widths). When a sample containing hydrogen is placed in between a awijaya static magnetic field, each hydrogen nucleus will resonate at a frequency determined by the magnetic field. The regulatory process in this field is determined by an electronic system. Thus, various chemical awijaya awijaya environments that exist in the molecule will produce a precession ^e frequency spectrum that will show the chemical properties of various ve parts of the molecule. Then the last is to interpret this frequency lava spectrum into a chemical structure. NMR spectrum appears in the form of a series of vertical peaks or awijaya signals distributed along the x-axis of the spectrum. Each of these awijaya awiiava signals corresponds to the atoms in the observed molecule. then the position of each signal in the spectrum provides information about the laya number of atomic molecules in the surrounding environment that produce signals (Chamberlain, 1974). Jniversitas Brawijaya Ilniversitas Rrawijava Ilniversitas Rrawijava¹⁵iniversitas Rrawijava

Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya 3. MATERIALS AND METHODS Universitas Brawijaya Universitas Brawijaya 3.1. Time and Place Inversitian This research was conducted for fifteen months from September 2017 until December 2018 in the Laboratory of Natural Products at National Pingtung University of Science and Technology in Pingtung awijaya UniverSitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijaya Universitas Dewijaya Universitas Brawijaya awijaya Unive 3.2. Plant material The initial dry weight of the air-dried stem of P. foetida was 22.28 awijaya kg. The plant was collected from Pingtung County, Taiwan. awijaya awijaya awijaya 3.3. Solvents and reagents awijaya awijaya 1. Methanol, n-BuOH, ethyl acetate (EtOAc), dichloromethane, acetone, and isopropanol were purchased from American Tedia awijaya Company awijaya 2. *d*-Chloroform, d_6 -acetone, d_6 -dimethylsulfoxide (DMSO): Bd_{4} -ijaya methanol were purchased from Acros Organics Company. sitas Brawijaya awijaya awijaya 3. Column Chromatography used silica gel (63-200 mesh, Merck) awijaya Inter 4. Thin Layer Chromatography (TLC) used Kieselgel 60 F254 (0.2 mm, Merck) 5. H_2SO_4 (10% v/v in H_2O) awijaya 6. Luria Bertani Broth used acumedia Lab, NEOGEN. 7. Agar used Cyrusbioscience. awijaya awijaya awijaya Unive 8. Mueller Hinton Broth used Mast, Mast Group, Java awijaya 9. Resazurin sodium salt used Acros Organics Company Inversitas Brawijaya 10. Escherichia coli (MTCC 1302) was from IMTECH, India

Univer11. Staphylococcus aureus (MTCC 737) was from IMTECH, India awijaya

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13. Salmonella enterica (ATCC 14028) was from ATCC, America

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12. Bacillus subtilis (NK-1) was isolated from Natto

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awijaya	1. Nuclear Magnetic Resonance Spectrometer (NMF	R) used Varian
awijaya	Universit Mercury Plus 400 MHz NMR Tetramethylsilane (TMS) was used
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awijaya	Universit as a standard for chemical shifts (δppm); singlet (s), doublet (d), _{jaya}
awijaya	Universite triplet (t) the quartet (a) broad (br) and multiplet (n	Universitas Brawijaya
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awijaya	Unive 2. The mass spectrometer (MS) used Bruker Dalton	ics APEX 30e jaya
awijaya	Spectrometer, Finnigan MAT 95S Mass Spectron	neter. Finnigan
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awijaya	MAT 95 XL High-Resolution Mass Spectrometer21	Universitas Brawijaya
awijaya	3. Ultraviolet Spectrometer (UV) used Shimadzu UV	/-1700 Pharma
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awijaya	Unive 4. Infrared Spectrometer (IR) used Bruker Vec	tor 22 to Ft-IR jaya
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awijaya	5. Polarimetry used Optical Activity AA-10R Automat	ic Spectrometer
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awijaya	7. Ultrasonic Cleaner used Ultrasonic Cleaner DC-400	ersitas Brawijaya
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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijava The Figure below, show the first research stage of extraction and Universisolation of bioactive compounds from the air-dried stem of P. foetida. awijaya awiiava Universitas Brawijava Universitas Brawijava EtOAc (Ethyl Acetate) layer Unive Silica gel column $(7 \times 90 \text{ cm})$ awijaya Unive Mobile phase awijaya (n-hexane/EtOAc/ awijaya Unive MeOH) Fraction awijava awijava awijava Figure 2. The First Research Stage

3.5.1. Sample Preparation

awijaya After collected, samples were sliced into a small size and then awijaya awijaya dried. The dry samples were packed into barrels then weighed before it awijaya University matching is macerated by methanol. $M \propto M$ awijaya awijava 3.5.2. Maceration Universitas The plant material was macerated with methanol for 7 days. After Java maceration, the resultant solution was evaporated using rotary evaporation at 50 °C and 55 rpm. After evaporation, the crude methanol awijaya Unive extract was prepared for partition step.versitas Brawijaya Universitas Brawijaya awijaya awijava Univer3.5.3.9 Partition Iniversitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas B The crude methanol extract was partitioned sequentially to obtain have various layers by polarity differences. The crude methanol extract was Unive suspended in water and partitioned sequentially using ethyl acetate and lava Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Ilniversitas Rrawijava Ilniversitas Rrawijava¹⁸iniversitas Rrawijava Ilniversitas Rrawijava

Air dried stem of P. foetida

Methanol crude extract

n-BuOH layer

Extracted with methanol (60 L \times 3)

Partitioned with EtOAc: water 1:1

1:1

Partitioned with

n-BuOH : water

Water layer

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Water layer

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya *n*-butanol. Partition was conducted using a separatory funnel. A part of awijava ethyl acetate or *n*-butanol was added with a part of the water as a solvent awijaya Unive comparison. The water residue of ethyl acetate partition process was awijaya used for the further step to obtain n-butanol layer. This process took 30 awijaya minutes to obtain *n*-butanol layer after the water residue was partitioned Investigation with *n*-butanol. Each step was repeated 6 times. Each layer was available awijaya awijaya concentrated using rotary evaporator at 50°C to obtain concentrate awiiava awijaya Unive residue (brown color) and used for further bioactivity analysis. The Brawlaya Universitas Brawijaya Universitas P iiaya Universitas Brawijaya versitas Brawilava awijaya 3.5.4. Isolation using Column Chromatography awijaya awijaya Compounds of ethyl acetate layer were isolated using column awijaya chromatography. The ethyl acetate layer was subjected on silica gel awijaya awijaya (230-400 mesh, E. Merck) with column size 7 x 90 cm. Hexane and awijaya awijaya ethyl acetate solvent mixtures were used as an eluent by increasing the awijaya polarity and yielded fractions. The mobile phase used for column awijaya awijava chromatography was *n*-hexane \rightarrow hexane/ethyl acetate \rightarrow hexane/ethyl awijaya acetate/methanol \rightarrow ethyl acetate \rightarrow ethyl acetate/methanol \rightarrow awijaya awijaya methanol. Thereafter, fractions were collected in Erlenmeyer flask awijaya awijaya (1000 mL) then concentrated using rotary evaporator and transferred to awijaya Universisting the column was washed with methanol and 10 % Java awijaya water were used to eluate the high polarity sample. Finally, each fraction from column chromatography was confirmed by Thin Layer Unive Chromatography (TLC). awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya 3.5.5. Thin Layer Chromatography (TLC) awijaya awijaya Universitas BThin layer chromatography is a separation method to identify awijaya Universitas Brawijaya Universitas Brawijaya awijaya compounds present in a given mixture. Dried sample on vials was dissolved in dichloromethane (DCM) then pointed into TLC paper. Unive Furthermore, samples were developed with a suitable mobile phase. Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Rrawijava Universitas Rrawijava¹⁹iniversitas Rrawijava Universitas Rrawijava

Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awiiava Compound separation was detected using UV light at 254 nm for short wavelength and 365 for long wavelength. 3.5.6. Separation using High-Performance Liquid Universitas Brawijava Chromatography (HPLC) Universitian B The preparative HPLC equipment was used Hitachis L-7110 ava awijaya awijaya LaChro Elite (Hitachi Inc., Japan) chromatography system, equipped awiiava awijaya with waters R401 Differential Refractometer Detector or Bischoff awijaya Differential Refractive Index Detector RI 8120. The HPLC column was semi-preparative thermo hypersilkeyston BETASIL silica 100 (5µm, awijaya Unive 250 x 10 mm) column. The injection volume was 2 ml at a flow rate of awijaya awijaya 1-2 ml/min. The HPLC results were collected in a vial. Spots and bands awijaya awijaya appearances were confirmed by TLC. Pure compounds show in a single awijaya spot of TLC and the structure was confirmed by NMR analysis. awijava awijaya

3.5.7. Identification by Nuclear Magnetic Resonance (NMR) ¹H NMR and ¹³C NMR spectra were measured using Varian Mercury plus 400 MHz, NMR. TMS (tetramethylsilane) was used as internal standard, δ chemical shifts 's' as single peak (singlet), 'd' as double peaks (doublet), 't' for three peaks (triplet), 'q' for quadrupole peaks (quartet), 'br' stands for broad peak (broad), and 'm' represents the multiple peaks (multiplet).

Hydrogen or carbon nuclear test was conducted by dissolving samples in a deuterated solvent of chloroform-d, kept the sample in 5 mm NMR tube, determined by NMR which yield the peaks as electromagnetic wave absorption signal. Chemical shift is expressed in parts per million (ppm) unit relative to TMS as an internal standard.

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya versitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya 3.5.8. Purification chemical constituent of *Paederia foetida* stem ver3.5.8.1. Universitas Brawijaya ver3.5.8.1. Wijayβ-sitosterol (Compound S1)itas Brawijaya Universitas Brawijaya Universitas Compound S1 was obtained from column chromatography of lava ethyl acetate layer which is fraction 10. After that, it was purified with n-hexane then recrystallized. Compound **S1** also identified the purity university using TLC preparative before examined into NMR. It showed the awijaya awijaya purple spot on TLC paper by mobile phase dichloromethane/ethyl awiiava Univeracetate, 1000:1 (v/v).sitas Brawijaya Universitas Brawijaya awijaya awijaya Universitas Brawilava 3.5.8.2. Morindaparvin A (Compound An1) awijaya Universitas Compound An1 was purified from fraction 15 using column awijaya awijaya chromatography with hexane \rightarrow MeOH as eluent. It was purified with awijaya awijaya hexane then recrystallized. Compound An1 showed as a yellow spot on awijaya TLC with dichloromethane/ethyl acetate eluent (1000:1, v/v). sites Brawijaya awijaya awijaya awijaya 3.5.8.3. **Digiferrol (Compound An4)** awijaya Compound An4 was purified from fraction 17 using column awijaya awijaya chromatography with hexane \rightarrow MeOH as eluent. The compound was awijaya Univerpurified by dichloromethane/acetone subsequently recrystallized. Pure awijaya awijaya compound An4 showed orange spot on preparative TLC with hexane/acetone eluent (5:1, v/v). 3.5.8.4. Stigmastan-3-one (Compound S2) awijaya Compound S2 was obtained from fraction 13. Fraction 13 was

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universitas Brawilaya Universitas Brawilaya Universitas Brawilaya Universitas Brawilaya Universitas Brawilaya Universitas Brawilaya dichloromethane/ethyl acetate (5000:1-1000:1, v/v), subsequently recrystallized. Pure compound S2 was showed pink spot by hexane Jacetone, 5:1 (v/v) on TLC. Ilniversitas Rrawijava Ilniversitas Rrawijava²¹iniversitas Rrawijava

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Universitas Brawijaya **University 3.5.8.5. With a 1,3-dihydroxy-2-methoxyanthraquinone (Compound With a sector)** Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Compound An2 was purified from fraction 15 by normal-phase lave HPLC with gradient elution of dichloromethane/ethyl acetate (500:1-125:1, v/v) subsequently recrystallized. The pure compound An2 was Unive shown as a yellow spot on TLC using hexane/acetone (6:1, v/v). \Rightarrow Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Unive 3.5.8.6. Wijay Alizarin (Compound An3) sitas Brawijaya Compound An3 was purified from fraction 22. Fraction 22 was subjected to normal-phase column chromatography silica gel with dichloromethane/MeOH eluent, (10.000:1, v/v) to give 6 subfractions. awijaya Thereafter, compound An3 was purified by dichloromethane/acetone awijaya from subfraction 3. Pure compound An3 showed yellow spot on TLC using hexane/acetone (6:1, v/v).

3.5.8.7. **Scopoletin (Compound C1)**

Compound C1 was obtained from subfraction 4 of fraction 22. Subfraction 4 was purified by dichloromethane/acetone eluent Universubsequently recrystallized to give compound C1. The pure compound C1 showed light-yellow spot-on TLC using eluent Hexane/Acetone (3:1, v/v).

3.5.8.8. Fraxidin (Compound C2) awijaya versitians Compound C2 was purified from subfraction 5 of fraction 22, awijaya subfraction 5 was subjected over normal-phase column awijaya awijaya chromatography silica gel hexane/acetone eluent (25:1, v/v) to give 2 subfractions. Thereafter, w subfraction tas 1 ray was purified by Java dichloromethane/acetone subsequently recrystallized to give pure compound C2. Compound C2 was shown light green spot on TLC Unive using eluent hexane/ethyl acetate eluent (1:1, v/v). Universitas Rrawijava Universitas Rrawijava²²iniversitas Rrawijava

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya Unive 3.5.8.9. Vanillic acid (Compound A2) Brawijava Compound A2 was obtained from subfraction 2 by purification Unive dichloromethane/acetone/ethyl acetate subsequently recrystallized. Pure compound A2 showed red spot on TLC using hexane/ethyl acetate Jniversitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Jniversitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya Ferulic acid (Compound A1) 3.5.8.10. awiiava awijaya Compound A1 was obtained from subfraction 6 of fraction 22. It awijaya was subsequently fractionated over normal-phase column awijaya chromatography silica gel with hexane/acetone eluent (25:1, v/v) to awijaya University give 3 subfractions. Thereafter, subfraction 3 was purified by awijaya awijaya Dichloromethane/Ethyl acetate to give compound A1. Pure compound awijaya awijaya A1 showed a light pink spot on TLC using dichloromethane/ethyl awijaya acetate (8:1, v/v). awijaya

3.6. Antibacterial assay for the second research stage

3.6.1. Well diffusion assay

The antibacterial of crude extract and fractions was tested using agar well diffusion method based on Revathi et al. (2017). The human pathogens bacteria, such as *Staphylococcus aureus* (MTCC 737) *and Escherichia coli* (MTCC 1302) were selected. *S. aureus* and *E. coli* were obtained from IMTECH, India.

The bacteria strains were inoculated from the stock into Mueller Hinton Agar for 12 hours. After 12 hours, the single colony was inoculated into Luria broth. Then, the 12 hours bacterial culture (10^8 CFU/mL) was continuously inoculated into the agar medium and spread over on the plates. The well was made using 8 mm sterile pipette tip. Then, the well was filled 100 µL (2 mg/mL) of crude extract and fractions. Streptomycin was used as a positive control and DMSO as a negative control. The culture plates were incubated at 37 °C for 24 23

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Unive MIC value.

Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya hours. The clear zone of inhibition around the well was measured in Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya 3.6.2. Minimum Inhibitory Concentration The minimum inhibitory concentration was determined using a Unive microdilution technique with a resazurin as an indicator based on Sarker Java et al. (2007). Four pathogens bacterial were tested. Bacillus subtilis (NK-1, isolated from Natto), and Staphylococcus aureus (MTCC 737) were used for gram-positive while Escherichia coli (MTCC 1302) and Salmonella enterica (ATCC 14028) for gram negative. Universitas BThe bacterial suspension was prepared after 12 hours incubation in Luria broth. Turbidity was adjusted approximately 1 x 10⁶ colonyforming units (CFU/mL). The 96 well plates were prepared under aseptic conditions. The samples for pure compound were dissolved with 100 µL DMSO to give the concentration 10 mg/mL. The working solutions of each compound were prepared by means of serial dilutions from 600 μ g/mL to 4.68 μ g/mL. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use 50 µL the test Universaterial. The resazurin indicator solution was mixed with the broth lava ratio 1:7.5. Finally, 10 µL of bacterial suspension was added to each well (1 x 10^6 CFU/mL). The plates were placed in an incubator set at Unive 37 °C for 6-12 h. The color change was observed visually. Any color change from purple to pink was recorded as positive. The lowest concentration at which color change occurred was considered as the Universitas Brawijaya Universitas Brawijaya Ilniversitas Rrawijava²⁴iniversitas Rrawijava





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awijaya	Table 2. The weights and extraction percentages of P. fostida Linn
awijaya	radie 2. The weights and extraction percentages of <i>F. Joenuu</i> Linn
awijaya	Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya
awijaya	Universitas Brawijaya Universitas Weight (g) versitas Brawijaya
awijaya	Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya
awijaya	Universitas EtOAc Layerversitas Braw174.05 iversitas Brawijaya 0.76 ersitas Brawijaya
awijaya	Universitas n-BuOH Layer ersitas Braw 321.27 liversitas Brawijaya 1.41
awijaya	Water Layer 1085.18 4.76
awijaya	MeOH Crude 1627 7.13
awijaya	Universitas Brawijaya / Universitas Brawijaya / Universitas Brawijaya
awijaya	Universitas Brauguaci Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya
awijaya	Universita Air-dried sample sitas Braw 22800 iversitas Brawijaya Universitas Brawijaya
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awijaya	Universitas Brawijaya Universitas Proviaya Universitas Brawijaya Universitas Brawijaya
awijaya	4.2. Chemical constituents of <i>Paederia foetida</i> stem
awijaya awijaya	Ten compounds were obtained from the EtOAc layer. There are
awijaya	two steroids four anthraquinones two coumarins and two aromatics
awijaya	Universitas
awijaya	University shown in Table 3 and Figure 5. The compounds were identified by
awijaya	Univer risitas Brawijaya
awijaya	Univ comparing the Nuclear Wagnetic Resonance and ta mass jaya
awijaya	spectrophotometric data with previous studies.
awijaya	Un S C S S S S S S S S S S S S S S S S S
awijaya	Unit of the second seco
awijaya	Table 3. Chemical constituents of the stem of <i>P. foetida</i> Linn.
awijaya	Oniv Q Print A Q Print A Print
awijaya	Compounds Reference Isitas Brawijaya
awijaya	Compound S1 Chang et al. (2000)
awijaya	Compound S2 Luo et al. (2009)
awijaya	Compound An1 de Oliveira Figueiredo et al (2014)
awiiava	Compound An? Cimenson and Davil (1096)
awijaya	Simoneau and Paul (1986)
awiiava	Compound An3 Dhananjeyan et al. (2005), Berger et al. (1980)
awijaya	Univer Compound An4 Imre and Ersoy (1978), Permana et al. (1999) wijaya
awijaya	Compound C1 Siddigui et al. (2007). Terra et al. (2013) S Brawijaya
awijaya	Compound C2 Vasuda et al. (2006)
awijaya	Universitas Brawijaya Universitas Brawijaya
awijaya	Universita Universita Wang et al. (2015) tas Brawijaya Universitas Brawijaya
awijaya	Universit Compound A2 Iniversit Phan Duc et al (2016) travilava Universitae Brawilava
awijaya	compound in Than Due et al. (2010)
	Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya
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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Steroids vijaya Universitas Brawijaya Universitas Brawijaya Iniversitas Brawijaya Universitas Brawijaya Brawijaya UniS1: R = OHijaya Universitas Br awiiav niversitas Brawijaya Universitas Brawijaya Iniversitas Brawijaya UniS2: R = O; * = Single bond Brawijaya Universit Universitas Brawijaya UniverAnthraquinones iversitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Rijaya Universitas \mathbf{R}_1 wijay \mathbf{R}_2 Unive \mathbf{R}_3 as \mathbf{R}_4 wijaya Universitas Brawijava Universitas Elawijava 0 Univa OH Hawijaya R_2 OH OCH₃ An₂ Univer Univer neitas Brawijaya An3 OH OH R_3 ersitas Brawijaya Universitas B k⊿ Ô An4 OH CH₂OH HtasOHwijaya An1 Coumarins R_1 **C1**: $\mathbf{R}_1 = \mathbf{OCH}_3$; $\mathbf{R}_2 = \mathbf{OH}$; $\mathbf{R}_3 = \mathbf{H}_{\mathsf{Brawijaya}}^{\mathsf{Brawijaya}}$ R_2 CE'S LECEN **C2**: $R_1 = OCH_3$; $R_2 = OCH_3$; $R_3 = OH^{11}$ Ŕ3 as Brawijaya **Aromatics**ersitas Brawijaya Univer H₃CO_{Bra} HO ersitas Brawijava Universit o rsites Brawijaya Iniversitas Brawij H₃CO n ---/wii `OHversitas Brawijaya UniversitOH Brawijaya Universitas Brawijaya UniA2sitas Brawijaya Universitas BrAdiava Universitas Brawijaya Universitas Brawijaya Ilniversitas Rrawijava²⁷Iniversitas Rrawijava Ilniversitas Brawijava



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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya 4.2.1.1. Compound S1^S Brawijaya Universitas Brawijaya s Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Jan 2/era tas Brawijaya Universitas Brawiłara Brawijaya Universitas Brawija Sniversitas Brawijaya Universitas Brawijaya Universi ijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Davijaya Universitas Brawijaya Universitas Brawijaya

UniverFigure 5. Structure of compound S1 with carbon number.

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S1 $(C_{29}H_{50}O)$ was isolated Universitas BCompound from column java chromatographic over silica gel as white needles with a melting point 138-140 °C. The structure of compound S1 is shown in Figure 5.

The ¹H NMR spectrum of compound **S1** in Table 4 and Figure 6 exhibited for six methyl signals (3H for each) at $\delta_{\rm H}$ 0.65 (s, H-18), 0.78 (d, J = 6.8 Hz, H-26), 0.80 (d, J = 7.2 Hz, H-27), 0.83 (t, J = 7.2 Hz, H-)29), 0.89 (d, J = 6.4 Hz, H-21) and 0.98 (s, H-19). The multiplet signal at $\delta_{\rm H}$ 3.51 assigned to H-3 is a characteristic of the hydroxymethine Univergroup. The broad signal $\delta_{\rm H}$ 5.33 represent an endocyclic double bond. The EI-MS spectrum in Figure 7 showed the molecular ion m/z⁴¹⁴ [M]⁺. The molecular ion and ¹H NMR spectral data of the University compound S1 are corresponding with Chang et al. (2000) as β -

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Universitas Brawijaya Universitas Brawijaya²¹ Universitas Brawijaya Universitas Brawijaya a²⁷ijaya ¹⁷versitas ²⁵awijaya Universitas Bra 16 ersitas ²⁶rawijaya 1Br Universitas Universitas Britovi 8 va¹⁴ Utsiversitas Brawijava Hava Universitas Brawijaya Unive O Universitas Brawijaya Universitas Brawijaya Univer**Figure 8**. Structure of compound **S2** with a carbon number Universitas Brawijava Universitas Brawijaya Universities Compound S2 ($C_{29}H_{50}O$) with melting point 157-160 °C was available isolated as a white needle. The structure is similar to compound **S1**, the

Unive hydroxyl group in carbon three is substituted by ketone. In addition, java compound S2 does not have an endocyclic double bond on carbon position number six. The structure of compound S2 is shown in Figure 8. rsitas

The ¹H NMR spectral data of compound S2 as shown in Table 5 and Figure 8 showed six methyl signals at $\delta_{\rm H}$ 0.65 (s, H-18), 0.79 (d, J = 6.8 Hz, H-26), 0.81 (d, J = 7.6 Hz, H-27), 0.83 (t, J = 7.6, Hz, H-29), 0.89 (d, J = 6.8 Hz, H-21), 0.92 (s, H-19).

The EI-MS spectrum in Figure 10 showed the molecular ion m/z414.4 [M]^{+.} Its molecular ion and the ¹H NMR spectral data for compound S2 is corresponding with Luo et al. (2009) as stigmastan-3-

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Universitas Brawijaya awijaya orv.ub.ac. awijaya 55.0 E+06 2.76 awijaya 100 414.4 awijaya 81.1 255.2 awijaya 145.1 95.1 69.1 80 159.1 awijaya 213.2 awijaya 133.1 400.4 60 awijaya 396<u>.4</u> 273.2 163.1 303 awijaya 329.3 231.2 382.4 173.1 40 awijaya 199.2 awijaya 351.3 awijaya 20 awijaya awijaya 416.4 431.3 awijaya awijaya 300 400 University awijaya vijaya awijaya UniverFigure 10. EI-MS Spectrum of compound S2 awijaya awijaya awijaya awijaya awijaya awijaya awijaya 8 LECENS awijaya Universitas Brawijaya Universitas Brawijaya awijaya Ilniversitas Rrawijava³³Iniversitas Rrawijava **Hniversitas Brawijava**

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Bravilava Universitas Bravilava 4.2.2.1. Compound An1 Universitas Brawijaya O Unive<mark>Osita</mark>s Brawijaya UniversitaOBrawijava as Brawijaya as Brawijaya Universitas ersitas Brawijaya Universitas B Universitas Brawijaya Universitas Brawijaya

UniverFigure 11. Structure of compound An1

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Compound An1 was isolated from column chromatographic over Universilica gel as a yellow powder with a melting point 255-257 °C. The Unive structure of compound An1 is shown in Figure 11.

The ¹H NMR spectrum compound **An1** in Figure 12 and Table 6 showed the presence of two aromatic ortho coupled protons at $\delta_{\rm H}$ 7.12 and 7.96 (1H, J = 8.0 Hz). Four aromatic protons signals appeared as multiplets at δ_H 7.76 (2H, m, H-6, and H-7) and 8.28 (2H, m, H-5, and H-8). A singlet signal at $\delta_{\rm H}$ 6.30 (2H, s) revealed the presence of a methylenedioxy group.

The EI-MS spectrum of compound An1 in Figure 13 showed the molecular ion m/z 252.1 [M]⁺ which is corresponding to the molecular formula $C_{15}H_8O_4$. On the basis of the spectral data of this compound, Unive compound An1 was identified as 1,2-methylenedioxyanthraquinone or jaya Morindaparvin A and its spectral data proved similar to that reported by de Oliveira Figueiredo et al. (2014). Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijava³⁴Iniversitas Brawijava **Hniversitas Brawijava**







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Compound An3 ($C_{14}H_8O_4$) was obtained as an orange powder with a melting point range 186 – 189 °C. Compound An3 was isolated by column chromatography from fraction 22. The structure of compound An3 is shown in Figure 17.

The ¹H NMR spectral data of compound **An3** in Figure 19 and Table 8 revealed the presence of two aromatic ortho coupled protons at $\delta_{\rm H}$ 7.22 (1H, d, J = 8.4 Hz, H-3) and 7.65 (1H, d, J = 8.4 Hz, H-4). Four proton signals (5,6,7,8) correlated at $\delta_{\rm H}$ 7.91 (H-6 and H-7) and 8.21 (H-5 and H-8) in the A-ring which revealed an A₂B₂ system. Two hydroxyl proton signals appeared at $\delta_{\rm H}$ 10.95 (1H, s, 2-OH) and 12.60 (1H, s, 1-OH) in the C-ring.

The ¹³C NMR and DEPT spectral data (Figure 18 and Table 8) showed the presence of six methines at $\delta_{\rm C}$ 135.2, 134.2, 126.8, 126.6, 121.2, 120.9, eight quaternary carbons at $\delta_{\rm C}$ 133.6, 132.9, 123.9 116.3, with ketone groups at $\delta_{\rm C}$ 188.9 and 180.7, and carbon hydroxyl bonds at $\delta_{\rm C}$ 152.8 and 150.8. The EI-MS spectrum showed a molecular ion signal at *m/z* 240 [M]⁺ (Figure 20) corresponds to the molecular formula C₁₄H₈O₄. The compound **An3** was identified as alizarin by comparison of spectroscopic data with reported data from Dhananjeyan et al. (2005) and Berger et al. (1980).







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> The ¹H NMR spectral data of compound **An4** in Figure 23 and Table 9 exhibited three hydroxyl proton signals at $\delta_{\rm H}$ 12.99 (1H, s, 1-OH), 12.73 (1H, s, 4-OH), and 5.60 (1H, t, J = 5.2 Hz, 2'-OH). The spectrum further displayed a two-proton signal at $\delta_{\rm H}$ 4.59 (2H, d, J =2.2 Hz, 2-CH₂). An aromatic proton signal appeared at $\delta_{\rm H}$ 7.33 (1H, d, J = 2.2 Hz, H-3). The remaining aromatic signals are typical for a 6 or 7 substituted A-ring, a pair of multiplets were displayed at $\delta_{\rm H}$ 7.93 (2H, H-6 and H-7) and 8.19 (2H, H-5 and H-8).

The ¹³C NMR and DEPT spectral data (Figure 22 and Table 9) displayed five methines at δ_{C} 135.22, 135.05, 126.76, 126.69, 124.67, nine quaternary carbons, at δ_{C} 157.09, 154.33, 144.74, 133.06, 132.90, 111.77 and 111.00 with two ketone groups at δ_{C} 187.04 and 186.19. The methylene appeared at δ_{C} 57.65. The molecular ion was showed at *m*/*z* 270.0 [M]⁺ (Figure 24) which correspond to the proposed structure. The compound **An4** was identified as digiferrol by comparison of its spectroscopic data with reported data from Imre and Ersoy (1978), and Permana et al. (1999).

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya Univer4.2.3. Coumarin iversitas Brawijaya Universitas Brawijaya Univer**4.2.3.1.** Universitas Brawijaya Universitas Brawijaya awijaya Universitas Forwija rsitas Brawijaya awijaya Universitas Bras Jaya sitas Brawijaya as Brawijaya 9 0 2 0 0 rawijaya UniversitasHO_{iw}ija awijaya 8 Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijava Universitas Brawijava Universitas Brawijava Universitas Brawijaya awijaya UniverFigure 25. Structure of compound C1 with a carbon number awijaya awijaya Universitas Brawijava Universitas awijaya

Compound C1 ($C_{14}H_8O_4$) was obtained as a colorless needle with a melting point 204 °C. The structure of compound C1 is shown in Unive Figure 25.

The ¹H-NMR spectrum in Figure 26 and Table 10 for compound C1 showed the presence of a methoxy group as a singlet at $\delta_{\rm H}$ 3.93. The spectrum further displayed two doublets at $\delta_{\rm H}$ 6.24 (1H, d, J = 9.2 Hz H-3) and 7.57 (1H, d, J = 9.2 Hz, H-4). The remaining signals appeared at $\delta_{\rm H}$ 6.82 (1H, s, H-8) and 6.89 (1H, s, H-5).

The ¹³C NMR and DEPT spectral data (Figure 27 and Table 10) revealed the existence of four methines at $\delta_{\rm C}$ 143.2, 113.3, 107.4, and Unive 103.1. Five quaternary carbons showed at $\delta_{\rm C}$ 161.4, 150.2, 149.6, 143.9, and 111.4. The methoxy group presenced at $\delta_{\rm C}$ 56.3. Universitas The EI-MS spectrum of compound C1 revealed a molecular ion Java Unive at m/z 192.1 [M]⁺ shown in Figure 28. The compound C1 was identified available

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as scopoletin by comparison of its spectroscopic data with reported data
from Siddigui et al. (2007) and Terra et al. (2013).
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showed a pair of methoxy groups at $\delta_{\rm C}$ 55.9 and 60.5. The ketone group appearance at δ 160.1, then the three methines showed at $\delta_{\rm C}$ 114.3 (CH, C-3), 144.6 (CH, C4), and 100 (CH, C-5). The six-carbon quarternaries showed at $\delta_{\rm C}$ 160.1 (C-2), 149.7 (C-6), 140.1 (C-7), 138.3 (C-8), 138.5 (C-9), and 114.5 (C-10).

Universitas BAccording to Yasuda et al. (2006), compound C2 has a molecular lava ion m/z 222.05 [M]⁺ that corresponds to the molecular formula $C_{11}H_{10}O_5$. The ¹H and ¹³C spectrum of compound C2 were identified as fraxidin by spectroscopic data from Table 11 and compared the data

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from Yasuda et al. (2006). Brawijaya Universitas Brawijaya

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Univer 4.2.4. Aromatic niversitas Brawijaya Universitas Brawijaya Compound A1 wijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas 3 avv a Universitas Brawijaya niverOtas Brawilava liaya sitas Brawijava Universitas Brawijaya Universot Brawijaya Univer**Figure 32**. Structure of compound A1 with a carbon number

Compound A1 was purified as a white powder from fraction 22 University a melting point 174 °C. The structure of compound A1 is shown in laya Unive Figure 32.

The ¹H NMR spectrum (Figure 33, Table 12), showed three proton signals at $\delta_{\rm H}$ 6.77 (1H, d, J = 8.0 Hz, H-5), 7.08 (1H, dd, J = 1.6and 8.0 Hz, H-6) and δ 7.25 (1H, d, J = 1.6 Hz, H-2) which display a characteristic of a trisubstituted aromatic ring. Two trans ethenyl protons signals appeared at $\delta_{\rm H}$ 6.34 (H-8) and δ 7.47 (H-7) with a coupling constant of 16 Hz. The spectrum also revealed a methoxy Universignal at $\delta_{\rm H}$ 3.79. The hydroxyl group signal appeared at $\delta_{\rm H}$ 9.56. Brawleya The EI-MS spectrum exhibited molecular ion peak at m/z 194.19 $[M]^+$ (Figure 34) which correspond to the molecular formula $C_{10}H_{10}O_4$. Unive The spectral data of compound A1 is corresponding with that from Java Universitas Brawijaya Wang et al. (2015) for ferulic acid. Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijava55Iniversitas Brawijava Universitas Brawijava




Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya Unive 4.2.4.2. wijay Compound A2 wijaya Universitas Brawijaya awijaya awijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas EHOijaya awijaya awijaya Universitas Brawija Universit H₃CO 3 awijaya Universitas Brawijaya Universed Brawijaya awijaya awijaya awijaya Univer**Figure 35**. Structure of compound A2 with a carbon number awijaya awijaya

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Compound A2 ($C_8H_8O_4$) was isolated as a brown needle with melting point 210-213 °C. The structure of compound A2 is shown in Unive Figure 35.

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The ¹H NMR spectrum of compound A2 (Figure 36, Table 13) ersitas Brawijaya showed a singlet at δ_H 3.79 which indicated the presence of an oxygenated methyl group. Furthermore, the spectrum displayed a multiplet and doublet signals at δ 7.44-7.41 (2H, H-2 and H-6) and 6.83 Unive (1H, H-5) respectively, which indicated a characteristic of an ABX ava system. In addition, a hydroxyl group signal appeared at δ 9.84 and was assigned to 4-OH.

awijaya The molecular formula of compound A2 was $C_8H_8O_4$ that awijaya Univercorresponds to the molecular ion signal at m/z 168 [M]⁺ from the EIawijaya awijaya Universitas Brawijay Unive MS in Figure 37. The compound was identified as vanillic acid by awijaya awijaya comparing its spectral data with the data from Phan Duc et al. (2016). awijaya awijaya awijaya awijaya awijaya Universitas Rrawijava⁵⁸Iniversitas Rrawijava



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Unive 4.3. BThe yiel	d of the chemical c	onstituent Brawijava	Universitas Brawijava
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Universitas The yield	of the chemical co	onstituents obtained	from the crude
Unive methanol extra	ct is displayed in Ta	able 14. The methar	nol crude extract
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unive contained stero	oids, anthraquinones	, coumarins, and arc	matics.sitas Brawijaya
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Table 14. Weig	ghts of isolated comp	pounds tas Brawijaya	Universitas Brawijaya
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Universitas Brawijava	mpound Brawijava	Weight	(mg) versitas Brawijava
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Universitas	An4	97	ersitas Brawijava
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	Universitas Brawijaya Universitas Brawijaya	4.3. The yield of the chemical of methanol extract is displayed in T contained steroids, anthraquinones Table 14. Weights of isolated com Compound S1 S2 An1 An2 An3 An4 C1 C2 A1 A2	4.3. The yield of the chemical constituent The yield of the chemical constituents obtained methanol extract is displayed in Table 14. The methan contained steroids, anthraquinones, coumarins, and arc Table 14. Weights of isolated compounds

The results indicated that the ethyl acetate layer from the crude methanol extract contains the dominant anthraquinone compound as Unive evidenced by the compound An3, it has the highest yield, obtained from Java CE & LE the fraction 22.

According to Duval et al. (2016) and Diaz-Muñoz et al. (2018), Java many anthraquinones can be found in wide range of species, especially Universitas the families' Rubiaceae, Polygonaceae, and Rhamnaceae. Unive Anthraquinones are a group of compounds abundant in the universe of natural substances. They are classified as quinones, and their derivatives are the largest group of natural quinones. Benzoquinones and naphthoquinones are also part of this group. Anthraquinones constitute the largest group of natural pigments, with approximately 700 compounds described. About 200 of these compounds were isolated from plants, while the rest were isolated from lichens and fungi. They are found in all plant parts: roots, rhizomes, fruits, and flowers. Universitas Brawijava Universitas Brawijava⁶¹niversitas Brawijava Universitas Brawijava awijaya

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya versitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Most of these compounds are derived from the basic structure 9,10anthracenedione, a tricyclic aromatic organic compound. Universitas BAnthraquinones also has a broad range of bioactivities such as lave anticancer, anti-inflammatory, immunosuppressive, antimicrobial, diuretic, cathartic, laxative, vasorelaxant, antioxidant and University phytoestrogen activities. Furthermore, the presence of steroids, available awijaya aromatics, and coumarins support the extent of biological activity by awiiava awijaya the extract of the plant in various studies. Jalhan (2017) and Sova (2012) discovered that coumarins and cinnamic acids exhibit a wide range of pharmacological activities, which includes anti-diabetic, anti-viral, University anti-microbial, anticancer, antioxidant, antiparasitic, anti-helminthic, awijaya antiproliferative, anti-inflammatory and antihypertensive activities. In awijaya awijaya addition, steroids were reported to possess antioxidant and antiviral awijava awijaya

(Parvez et al., 2018).

Antibacterial activities 4.4.

4.4.1. Agar well diffusion assay

In this study, the crude extract, partition samples, fractions, and compounds isolated were tested the antibacterial activity against human pathogens following bio-guided fractionation. The results are displayed in Figure 38 and Figure 39.

Iniversitas BThe crude extract was very effective in the inhibition of bacteria java growth against E. coli (9.5 mm) and S. aureus (10.5 mm). In particular, partition samples showed selective activity. The ethyl acetate layer showed the highest activity against bacteria strains with an inhibition zone of 11 mm and 11.5 mm against E. coli and S. aureus respectively. Whereas, BuOH and water layer did not show activity against E. coli and S. aureus. The negative control, DMSO did not show any inhibition zone against all the test strains. The positive control, streptomycin, Ilniversitas Rrawijava Ilniversitas Rrawijava62iniversitas Rrawijava

Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya showed high inhibition zone 20.5 mm against E. coli and S. aureus respectively. Universitas Brawijaya ersitas Brawijaya Universita 20.0 wija Universita Brawija iversitas Brawijaya Universitas Brawijaya niversitas Brawijaya Universitas Brawijaya niversitas Brawijaya Universitas Brawijaya awijaya Brawija ersita awijaya rsitas sitas Brawijaya Universitas Brawijaya ition 10.0 wita iĝ a Brawi 0.0 Water layer DMSO Crude extract EtOAc layer Streptomycin BuOH layer awijaya awijaya E. coli S. aureus awijaya awijaya

Figure 38. The antibacterial activity of crude extract and partitions (2 mg/mL) against *E. coli* and *S. aureus* with streptomycin as control

Meanwhile, most of the fractions showed the antibacterial activity against test strains as shown in Figure 39 and Appendix 2. The high polar fractions (such as Fraction 15 - 26) where most of the compounds isolated from, showed the activity against test strains. The Fraction 21 displayed the highest inhibition zone (15.7 mm for *E. coli*; 16.7 mm for *S. aureus*), then followed by fraction 18 (14.5 mm) against *E. coli*, and fraction 14 (15.3 mm) against *S. aureus*. However, several low polar fractions showed selective activity. Fraction 1 and 5 were active on one of two test strains (*S. aureus* and *E. coli* respectively). Rath and Padhy (2015) reported that the ethyl acetate, ethanol, and methanol extract of *P. foetida* leaf were highly effective at inhibiting eight enteropathogens (*Enterobacter aerogenes, Escherichia coli, K. pneumoniae, S. paratyphi, S. typhi, Shigella dysenteriae, S. sonnei*, and *V. cholerae*).

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coli and S. aureus.

against pathogenic bacteria and the results are shown in Table 15. Table 15. MIC determination using the resazurin assay MIC µg/ mL Universitas BCompound **B**isitas BrawSava **Salmonella** E. coli Univenterica awijaya ubtilis 🛛 🖻 aureus ersitas Brawijaya ^{as} Streptomycin Bransjaya **e**sitas Bra1.875 awijaya 300 600 300 Compound An1 >600 awiiava 300 300 Compound An2 150 18.75 Compound An3 Br150ava Brav150a er300 Brawijava 1150itas Compound An4 Br150aya Uni150 tas Bra 9.37a er300 Brawijaya Compound C1 300 Bra>600 ergi 50 Brawijaya Br150aya ^{er}150 Compound A1 300 300 150 75 300 150150Compound A2 Universitas Brawijava⁶⁴iniversitas Brawijava

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4.4.2. Minimum Inhibitory Concentration (MIC) using resazurin assay

E. coli S. aureus

Figure 39. Antibacterial activity of the fractions (2 mg/mL) against E.

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The MIC was determined using a microdilution technique with resazurin as an indicator of cell growth. Seven compounds isolated from the EtOAc layer which include four anthraquinones, one coumarin, and two aromatics were observed the minimum inhibition concentration

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awiiava The results exhibit that Gram-positive bacteria are selectively inhibited by compounds isolated from the stem of P. foetida. Seven compounds isolated from fractions possessed antibacterial activity against four human pathogens and three of compounds had MIC values less than 100 μ L/mL. Regarding the MIC value, most of the test strains v showed similar susceptibility to the compounds. Except for the S. awijaya aureus strains, was more susceptible to streptomycin as control and awiiava compound An2, An4, and A2. According to Comini et al. (2011), plant compounds are classified as "antimicrobial", if they have MIC values in the range of 100-1000 μ g/mL and others of a higher magnitude than University those of typical antibiotics (0.01-10 μ g/mL). Plants compounds having awijaya MICs equal or lower to 100 µg/mL are considered good antimicrobial awijaya awijaya agents. awijava

The previous study from Lu et al. (2011), the antibacterial activity of the anthraquinone derivatives might be related to the type of substituent groups on the molecular structure. The presence of the polar functional group (carboxyl, hydroxyl, and hydroxymethyl) can increase antibacterial activity. Although compound An2 and An4 have the same amount of polar functional groups, the methoxy in compound An2 might weaken their antibacterial activity. This suggestion also might affect in compound A2 which has methoxy group in its aromatic ring. ve Lu et al. (2011) also reported that anthraquinone derivative such as java emodin can increase membrane permeabilization and caused leakage of intracellular contents. Their study also has demonstrated that unive anthraquinone derivatives could inhibit macromolecular synthesis in Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya as Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya versitas B In addition from Pretto Juliana et al. (2004), the pattern of chemical selectivity to against Gram-positive bacteria is not restricted to compounds from plants but is a general phenomenon observed Univeramong most antibiotics as Brawijaya Universitas Brawijaya Ilniversitas Rrawijava Ilniversitas Rrawijava65 iniversitas Rrawijava

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya However, the low antibacterial activity of isolated compounds awijaya against Gram-negative bacteria also can be explained by Pretto Juliana awijaya awijaya et al. (2004). The outer membrane of Gram-negative bacteria is known awijaya to present a barrier to the penetration of antibiotic agents. The awijaya periplasmic space contains several enzymes which are able to break Unive downs foreign molecules introduced from outside. Several Gram-Java awijaya negative organisms also display high-level resistance to a range of awijaya awijaya awijaya antimicrobial agents and support a role for the outer membrane and awijaya niversitas Brawijaya Universities a barrier to antibiotics. Area Brawline Universities Brawijaya awijaya

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UNIVERSITAS BRAWIJAYA UNIVERSITAS BRAWIJAYA UNIVERSITAS BRAWIJAYA awijaya Universitas Bray awijaya Universitas 5. CONCLUSION awijaya awijaya The crude methanol extract of *P. foetida* has been investigated for antibacterial properties. In this study, ten bioactive compounds Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Unive including four anthraquinones, two coumarins, two aromatics, and two java steroids were isolated from the ethyl acetate layer of the crude extract. awijaya awijaya awijaya The isolated compounds were identified as morindaparvin A, 1,3awijaya dihydroxy-2-methoxyanthraquinone, alizarin, digiferrol, scopoletin, awijaya awijaya rsitas Brawijaya Univerferulic acid, vanillic acid, fraxidin, β -sitosterol, and stigmastan-3-one.wijaya awijaya awijaya The result of the research indicates that the extract of *P. foetida* awijaya awijaya has antibacterial activity against the tested E. coli and S. aureus. This is awijaya awijaya supported by the significant antibacterial effect of the fractions. In awijaya addition, these results indicate that S. aureus is more susceptible to 1,3-/ijaya awijaya awijaya dihydroxy-2-methoxyanthraquinone, digiferrol, and vanillic acid.s Brawijaya awijaya reitae Brawijava awijaya

Nevertheless, the compounds still need to continue investigation such as cytotoxicity before they can be developed into new medical PACE & LEON

Univelagents.

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> BERGER, Y., CASTONGUAY, A. & BRASSARD, P. 1980. Carbon-13 nuclear magnetic resonance studies of anthraquinones Part II—hydroxymethoxyanthraquinones, acetoxymethoxyanthraquinones and naturally occuring anthraquinone analogues. Organic Magnetic Resonance, 14, 103-108.

AHMED, S., M. P., CHOWDHURY, L., BORGOHAIN, BOLSHETTE, N., DEVASANI, K., DAS, T. J., MOHAPATRA, A. & LAHKAR, M. 2017. Renoprotective and antioxidative lava effects of methanolic Paederia foetida leaf extract B on Java experimental diabetic nephropathy Journal of ava in rats. Ethnopharmacology, 198, 451-459. CHAMBERLAIN, N. F. 1974. Introduction. In: The Practice of NMR Universitas BSpectroscopy. Boston: Springer. ersitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya CHANG, F. R., CHEN, C. Y., HSIEH, T. J., CHO, C. P. & WU, Y. C. 2000. Chemical constituents from Annona glabra III. Journal of Universitas Bthe Chinese Chemical Society, 47, 913-920. Java Universitas Brawijava Universitas Brawijaya Universitas Brawijaya Universitas Brawijava Universitas Brawijava⁶⁸ niversitas Brawijava

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DE, S., RAVISHANKAR, B. & BHAVSAR, G. C. 1994. Investigation of the anti-inflammatory effects of *Paederia foetida*. Journal of *Ethnopharmacology*, 43, 31-38.

DHANANJEYAN, M. R., MILEV, Y. P., KRON, M. A. & NAIR, M. G. 2005. Synthesis and Activity of Substituted Anthraquinones against a Human Filarial Parasite, *Brugia malayi. Journal of Medicinal Chemistry*, 48, 2822-2830.

DIAZ-MUÑOZ, G., MIRANDA, I. L., SARTORI, S. K., DE REZENDE, D. C. & DIAZ, M. A. N. 2018. Chapter 11 -Anthraquinones: An Overview. *In:* ATTA UR, R. (ed.) *Studies in Natural Products Chemistry*. Elsevier.
DUVAL, J., PECHER, V., POUJOL, M. & LESELLIER, E. 2016.

Research advances for the extraction, analysis and uses of anthraquinones: A review. *Industrial Crops and Products*, 94, 812-833.
HAMAYUN, M., KHAN, S. A., SOHN, E. Y. & LEE, I.-J. 2006. Folk medicinal knowledge and conservation status of some economically valued medicinal plants of District Swat, Pakistan. *Lyonia*, 11, 101-113.

awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijaya Universitas Brawijaya

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya IMRE, S. & ERSOY, L. 1978. Neue Alkylierungsprodukte von awijaya Chinizarin und seinem Monomethylether. Justus Liebigs Universitas BAnnalen der Chemie, 1978, 2018-2023. Brawijava Universitas Brawijava JALHAN, S. 2017. Review Article: Various Biological Activities of Universitas ^BCoumarin and Oxadiazole Derivatives. Brawlava Universitas Brawlava KUMAR, V., ANWAR, F., AHMED, D., VERMA, A., AHMED, A., Universitas DAMANHOURI, Z. A., MISHRA, V., RAMTEKE, P. W., Universitas BHATT, P. C. & MUJEEB, M. 2014. Paederia foetida Linn. leaf Java awijaya awijaya Universitas Bextract: an antihyperlipidemic, antihyperglycaemic and ava awijaya antioxidant activity. BMC Complement Altern Med, 14, 76. 2004. Quality-control analytical methods: High-

KUPIEC, T. Universitas sperformance liquid chromatography. International journal of lava Universitas Ppharmaceutical compounding, 8, 223-227.

LANGELAND, K. A., STOCKER, R. & BRAZIS, D. 2000. Natural area weeds: skunkvine (Paederia foetida), University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

LU, C., WANG, H., LV, W., XU, P., ZHU, J., XIE, J., LIU, B. & LOU, Z. 2011. Antibacterial properties of anthraquinones extracted from rhubarb against Aeromonas hydrophila. Fisheries Science, 77, 375. $O_{SI} \propto 150$

LUO, J.-R., MA, Q.-Y., ZHAO, Y.-X., YI, T.-M., LI, C.-S. & ZHOU, J. 2009. Palaeophytochemical Components from the Miocene-Fossil Wood of Pinus Griffithii. Journal of the Chinese Chemical Universitas BSociety, 56, 600-605.

MACWAN, C. 2010. Paederia foetida Linn. As a potential medicinal plant : A Review. s Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Unive MEYERS, C. L. F. 2001. Column Chromatography. Current Protocols java Universitas Bin Nucleic Acid Chemistry. John Wiley & Sons, Inc. Versitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya MORSHED, H., BIN SAYEED, M. S., MOSTOFA, A. G. M., ISLAM, S. & PARVIN, S. 2012a. Antithrombolytic and Antidiabetic Universitas Activity of Methanolic Extract of Paederia foetida. Universitas BPharmacognosy Journal, 4, 30-33 sitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Ilniversitas Rrawijava Universitas Rrawijava 70 niversitas Rrawijava Universitas Rrawijava

Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya MORSHED, H., ISLAM, M. S., PARVIN, S., AHMED, M., ISLAM, awijaya M., MOSTOFA, A. & SHAHDAAT BIN SAYEED, M. 2012b. awijaya Universitas PAntimicrobial and Cytotoxic Activity of the Methanol Extract of Java awijaya Universitas BPaederia foetida Linn. (Rubiaceae). tas Brawijava Universitas Brawijava awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya OTSUKA, H. 2005. Purification by Solvent Extraction Using Partition Coefficient. In: SARKER, S. D., LATIF, Z. & GRAY, A. I. University (eds.) Natural Products Isolation. Totowa, NJ: Humana Press. awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya PARVEZ, M. K., ALAM, P., ARBAB, A. H., AL-DOSARI, M. S., awijaya ALHOWIRINY, T. A. & ALQASOUMI, S. I. 2018. Analysis of awijaya antioxidative and antiviral biomarkers *β*-amyrin, *β*-sitosterol, awijaya lupeol, ursolic acid in Guiera senegalensis leaves extract by Universitas Bvalidated HPTLC methods. Saudi Pharmaceutical Journal, 26, ava awijaya awijaya 685-693. awijaya PERMANA, D., LAJIS, N. H., OTHMAN, A. G., ALI, A. M., AIMI, awijaya awijaya N., KITAJIMA, M. & TAKAYAMA, H. 1999. Anthraquinones awijaya from Hedyotis herbacea. Journal of Natural Products, 62, 1430awijaya awijaya 1431. awijaya awijaya PHAN DUC, T., NGUYEN THIEN, T. V., JOSSANG, A., NGUYEN awijaya

KIM, P. P., GRELLIER, P., JAUREGUIBERRY, G. & TON THAT, Q. 2016. New wedelolides, (9R)-eudesman-9,12-olide δlactones, from *Wedelia trilobata*. *Phytochemistry Letters*, 17, 304-309.

POOLE, C. F. 2003. Thin-layer chromatography: challenges and opportunities. *Journal of Chromatography A*, 1000, 963-984.

Unive PRETTO JULIANA, B., CECHINEL-FILHO, V., NOLDIN VÂNIA, awijaya F., SARTORI MARA, R. K., ISAIAS DANIELA, E. B. & awijaya BELLA CRUZ, A. 2004. Antimicrobial Activity of Fractions and awijaya Compounds from *Calophyllum brasiliense* awijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya RATH, S. & PADHY, R. N. 2015. Antibacterial efficacy of five medicinal plants against multidrug-resistant enteropathogenic bacteria infecting under-5 hospitalized children. Journal of Universitas BIntegrative Medicine, 13, 45-57. ersitas Brawijava Universitas Brawijava Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Ilniversitas Rrawijava Ilniversitas Rrawijava⁷¹iniversitas Rrawijava

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awijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya REVATHI, S., GOVINDARAJAN, R. K., RAMESHKUMAR, N., Universitas HAKKIM, F. L., MOHAMMED, A.-B., KRISHNAN, M. & KAYALVIZHI, N. 2017. Anti-cancer, anti-microbial and antioxidant properties of Acacia nilotica and their chemical profiling. Biocatalysis and Agricultural Biotechnology, 11, 322-329._{ava} Universitas Brawijaya awijaya SARKER, S. D., NAHAR, L. & KUMARASAMY, Y. 2007. Microtitre awijaya plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods, 42, 321-324. awijaya Unive SCOTT, R. P. W. 1983. Chapter 4 Column chromatography. In: awijaya awijaya HEFTMANN, E. (ed.) Journal of Chromatography Library. awijaya Elsevier. awijaya awijaya SHUKLA, Y. N., LLOYD, H. A., MORTON, J. F. & KAPADIA, G. J. awijaya awijaya 1976. Iridoid glycosides and other constituents of *Paederia* awijaya foetida. Phytochemistry, 15, 1989-1990. awijaya awijaya SIDDIQUI, B. S., SATTAR, F. A., AHMAD, F. & BEGUM, S. 2007. Isolation and structural elucidation of chemical constituents from awijaya the fruits of Morinda citrifolia Linn. Archives of pharmacal awijaya awijaya research, 30, 919-923. awijaya SIMONEAU, B. & PAUL, B. 1986. Reactions of ketene acetals awijaya 1611Part 15. V. Guay and P. Brassard, Tetrahedron, 1984, 40, Universitas B5039.. The regiospecific synthesis of partially methylated java awijaya Universitas Bpurpurins. Tetrahedron, 42, 3767-3774. Srawijaya Universitas Brawijaya awijaya Universitas Brawijaya SONI, D. K., SHARMA, V., CHAUHAN, N. S. & DIXIT, V. K. 2012. awijaya awijaya Effect of ethanolic extract of *Paederia foetida* Linn. leaves on awijaya Universities sexual behavior and spermatogenesis in male rats. Journal of awijaya Universitas BMen's Health, 9, 268-276.va Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya SONI, R. K., IRCHHAIYA, R., DIXIT, V. & ALOK, S. 2013. Paederia foetida Linn: phytochemistry, pharmacological and traditional University Buses. International Journal of Pharmaceutical Sciences and available Universitas BResearch, 4, 4525; Brawijaya Universitas Brawijaya Universitas Brawijaya

Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya

awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya awijava SOVA, M. 2012. Antioxidant and Antimicrobial Activities of Cinnamic awijaya Universitas BAcid Derivatives. Mini-Reviews in Medicinal Chemistry, B12, Java awijaya Universitas B7491767 Universitas Brawijaya awijaya SPANGENBERG, B., POOLE, C. F. & WEINS, C. 2011. Quantitative awijaya thin-layer chromatography: a practical survey, Springer Science Universitas B& Business Media. Brawijava Universitas Brawijava Universitas Brawijava awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya TERRA, W. D. S., VIEIRA, I. J., BRAZ-FILHO, R., FREITAS, W. R. awijaya D., KANASHIRO, M. M. & TORRES, M. C. M. 2013. awijaya Lepidotrichilins A and B, new protolimonoids with cytotoxic awijaya activity from Trichilia lepidota (Meliaceae). Molecules, 18, awijaya Universitas B12180-12191. ONGTUN awijaya awijaya awijaya TRIPATHI, V. & DASGUPTA, B. 1974. Neutral constituents of awijaya Paederia foetida Linn. Journal. awijaya awijaya UDDIN, N., HOSSAIN, M., HAQUE, M. & HASAN, C. 2013. awijava awijaya Chemical Investigation of *Paederia foetidae* (Rubiaceae). Asian awijaya J. Chem, 25, 1163-1164. awijaya awijaya UPADHYAYA, S. 2013. Screening of phytochemicals, nutritional awijaya status, antioxidant and antimicrobial activity of *Paederia foetida* awijaya Linn. from different localities of Assam, India. Journal of Java awijaya awijaya Pharmacy Research, 7, 139-141. awijaya WANG, W., GUO, J., ZHANG, J., PENG, J., LIU, T. & XIN, Z. 2015. awijaya Isolation, identification and antioxidant activity of bound awijaya Universitas Bphenolic compounds present in rice bran. Food Chemistry, 171, Java awijaya Universitas B40-49. awijaya awijaya YASUDA, T., FUKUI, M., NAKAZAWA, T., HOSHIKAWA, A. & awijaya awijaya OHSAWA, K. 2006. Metabolic Fate of Fraxin Administered awijaya Universities Orally to Rats. Journal of Natural Products, 69, 755-757. S Brawleve awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya ZHUANG, C., WANG, X., MIAO, L., ZHOU, H. & WU, T. 2013. Chemical constituents of Paederia scandens. Chemistry Natural Compounds, 49, 379-380. Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Ilniversitas Rrawijava Ilniversitas Rrawijava⁷³iniversitas Rrawijava Universitas Brawijava

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Appendix Appendix Appendix Appendix Appendix Appendix 1. Summary of the chemical constituents' spectral data β -Sitosterol (S1) White needles; mp 136 °C; ¹H NMR (400 MHz, CDCl₃) δ : 0.65 (3H, s, CH₃-18), 0.77 (3H, d, J = 6.8 Hz, CH₃-26), 0.80 (3H, d, J = 7.2 Hz, CH₃-27), 0.83 (3H, t, J = 7.2 Hz, CH₃-29), 0.89 (3H, d, J = 6.4 Hz, CH₃-21), 0.98 (3H, s, CH₃-19), 3.50 (1H, m, CH-3), 5.33 (1H, br s, H-6). EI-MS (70 eV) m/z (%): 412 [M]⁺ (100), 396 (81), 381 (70), 303 (72), 213 (72), 145 (83), 69 (100).

Stigmastan-3-one (S2)

White needles; mp 157-159 °C; ¹H NMR (400 MHz, CDCl₃) δ: 0.65 (3H, s, CH₃-18), 0.92 (3H, s, CH₃-19), 0.89 (3H, d, *J* = 6.8 Hz, CH₃-21), 0.79 (3H, d, *J* = 6.8 Hz, CH₃-26), 0.81 (3H, d, *J* = 7.6 Hz, CH₃-27), 0.83 (1H, t, *J* = 7.6 Hz, CH₃-29). EI-MS (70 eV) *m*/*z* (%): 414 (94), 400 (62), 396 (46), 382 (36), 351 (26), 329 (36), 303 (40), 273 (46), 255 (88), 231 (38), 213 (70), 199 (30), 173 (36), 163 (42), 159 (74), 145 (82), 133 (68), 95 (80), 81 (88), 55 (100).

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awijaya Unive Morindaparvin A (An1)

awijaya Yellow powder; mp 255-257 °C; ¹H NMR (400 MHz, CDCl₃) δ: 6.30 awijaya awijaya $(2H, s, CH_2), 7.12 (1H, d, J = 8.0 Hz, H-3), 7.76 (2H, m, H-6,7), 7.96$ awijaya (1H, d, J = 8.0 Hz, H-4), 8.28 (2H, m, H-5, 8). EI-MS (70 eV) m/z (%):awijaya awijaya 252 [M]⁺(100), 223 (8), 196 (4), 168 (6), 138 (18), 112 (4), 83 (2), 76 awijaya Univer(5) as Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya 1,3-dihydroxy-2-methoxyanthraquinones (An2) Unive Yellow crystal; mp 218-220 °C; ¹H NMR (400 MHz, CDCl₃) δ: 4.12 awijaya (3H, s, 2-OCH₃), 6.51 (1H, s, 3-OH), 7.76 (2H, m, H-6,7), 7.44 (1H, Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya Ilniversitas Rrawijava Ilniversitas Rrawijava74 niversitas Rrawijava Ilniversitas Rrawijava

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s, H-4), 8.25 (2H, m, H-5,8), 13.11 (1H, s, 1-OH). EI-MS (70 eV) *m/z*. (%): 270 [M]⁺ (100), 252 (58), 227 (52), 199 (22), 196 (24), 115 (20), 77 (6). Alizarin (**An3**) Orange powder; mp 279-283 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.22 (1H, d, *J* = 8.4 Hz, H-3), 7.65 (1H, d, *J* = 8.4 Hz, H-4), 7.91 (2H, m, H-6,7), 8.21 (2H, m, H-5,8), 10.95 (1H, s, 2-OH), 12.60 (1H, s, 1-OH). ¹³C-NMR (100 MHz, DMSO-*d*₆) δ: 150.8 (C-1), 152.8 (C-2), 123.0 (C-3), 120.9 (CH-4), 126.8 (CH-5), 135.2 (CH-6), 134.2 (CH-7), 126.6 (CH-8), 188.9 (C-9), 180.7 (C-10), 121.2 (CH-4a), 133.6 (C-8a), 116.3 (C-9a), 132.9 (C-10a). EI-MS (70 eV) *m/z* (%): 240 (100), 239 (16), 121 (18), 184 (12), 138 (12), 128 (12), 127 (10), 77 (8), 51 (4).

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Digiferrol (An4)

Red powder; mp 200-202 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ: 7.33 (1H, d, J = 2.2 Hz, H-3), 8.19 (2H, m, H-5,8), 7.92 (2H, m, H-6,7), 12.99 (1H, s, 1-OH), 12.73 (1H, s, 2-OH), 4.59 (2H, d, J = 2.2 Hz, 2-CH₂), 5.60 (1H, t, J = 5.2 Hz, 2-OH,). ¹³C-NMR (100 MHz, DMSO- d_6) δ: 154.3 (C-1), 144.7 (C-2), 124.6 (C-3), 126.7 (C-5), 135.0 (C-6), 135.2 (C-7), 126.7 (C-8), 186.1 (C-9), 187.0 (C-10), 111.0 (C-4a), Univer133.0 (C-8a), 111.7 (C-9a), 132.9 (111,7), 157.0 (C-4), 57.6 (2'-CH₂). Java EI-MS (70 eV) m/z (%): 270 (100), 252 (84), 241 (26), 224 (40), 196 ⁽⁶⁰⁾, 185 (18), 168 (30), 139 (42), 77 (18), 66 (16), 51 (8). ⁽¹⁶⁾ Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya versitas Brawijava Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Colorless needle; mp 206-207 °C; ¹H NMR (400 MHz, CDCl₃) δ : 6.24 (1H, d, J = 9.2 Hz, H-3), 7.57 (1H, d, J = 9.2 Hz, H-4), 6.89 (1H, s, H-5), 6.82 (1H, s, H-8), 3.93 (1H, s, 6-OCH₃). ¹³C-NMR (100 MHz, Univ CDCl₃) δ: 161.4 (C-2), 113.3 (CH-3), 143.2 (CH-4), 107.4 (CH-5), ava Ilniversitas Rrawijava Ilniversitas Rrawijava75 niversitas Rrawijava Universitas Brawijava

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Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya Universitas Brawijaya awijaya awijaya 143.9 (C-6), 149.6 (C-7), 103.1 (CH-8), 150.2 (C-9), 111.4 (C-10), 56.3 awijaya (OCH₃). EI-MS (70 eV) *m*/*z* (%): 192 (100), 177 (56), 164 (24), 149 awijaya awijaya Unive (44), 121 (20), 79 (14), 69 (26), 51 (12), rsitas Brawijaya awijaya Universitas Brawijaya awijaya Fraxidin (C2) Universitas Brawijaya Universitas Brawijaya awijaya Unive Brown needle/crystal; mp 196-197 °C; ¹H NMR (400 MHz, DMSO-d₆) ava awijaya awijaya 6.34 (1H, d, J = 9.6 Hz, H-3), 7.90 (1H, d, J = 9.6 Hz, H-4), 6.81 (1H, awijaya unives, H-5), 3.80 (1H, s, 6-OCH₃), 3.75 (1H, s, 7-OCH₃). ¹³C-NMR (100 awijaya awijaya MHz, DMSO-d₆) δ: 160.1 (C-2), 114.3 (CH-3), 144.6 (CH-4), 100.2 awijaya awijaya (CH-5), 149.7 (C-6), 140.1 (C-7), 138.3 (C-8), 138.5 (C-9), 114.5 (Cawijaya 10), 55.9 (6-OCH₃), 60.5 (7-OCH₃). awijaya awijaya

^{nive} Ferulic acid (A1)

White powder; mp 168-172 °C; ¹H NMR (400 MHz, DMSO-*d*₆) & 7.25 (1H, d, *J* = 1.6 Hz, H-2), 6.77 (1H, d, *J* = 8.0 Hz, H-5), 7.08 (1H, dd, *J* = 1.6 and 8.0 Hz, H-6,), 7.47 (1H, d, *J* = 16 Hz, H-7), 6.34 (1H, d, *J* = 16 Hz, H-8), 3.79 (1H, s, 3-OCH₃), 9.56 (1H, s, 4-OH). EI-MS (70 eV) *m*/*z* (%): 194 (100), 179 (20), 177 (8), 133 (18), 77 (8), 51 (6).

Vanilic acid (A2)

Brown needle/crystal; mp 210-213 °C; ¹H NMR (400 MHz, DMSO- d_6) δ : 7.44 (2H, m, H-2 and 6), 6.83 (1H, d, J = 8.4 Hz, H-5), 3.79 (1H, s, 3-OCH₃), 9.84 (1H, s, 4-OH). EI-MS (70 eV) m/z (%): 168 (100), 153 (56), 151 (14), 125 (12), 97 (18), 79 (4), 51 (6). Universitas Brawijaya Univ

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Concentration: Control + : $3.75 - 0.0146 \,\mu g/mL$ Compounds: $37.5 - 0.146 \,\mu g/mL$

> KQ4: Compound An1 KQ6: Compound An2 KQ13: Compound An3 KQ15: Compound C1 KQ20: Compound An4 KQ26: Compound A2 KQ28: Compound A1

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2016-2018:

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