Anti-Syncytium (MC99+1A2) and Anti-Bacterial Activities from Twigs and Stems of *Ficus dubia*

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors WP and PU designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AC, SW, AA and KK managed the analyses of the study. Author NN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the anti-HIVs and anti-bacterial activity and investigate the chemical compositions of the crude extracts and isolated compounds from stems and twig of *Ficus dubia*.

Methodology: Twigs and stems of *Ficus dubia* were collected from Si Sawat District, Kanchanaburi Province, Thailand. Bioactivity assay and phytochemical analysis of *ficus dubia* were processed under standard method including, anti-HIV1-RT, antibacterial activities, and CC chromatography procedures. Structures of the compound were elucidated by spectroscopic techniques.

Results: Bioactivities assay exposed that, the hexane, ethyl acetate, and methanol extracts of *Ficus dubia* were verified for anti-HIV and anti-bacterial activities. The ethyl acetate and
methanol extract showed evidence of anti-HIVs-1RT inhibition at 69.02 % and 69.24 %, respectively. Anti-syncytium (MC99+1A2) among evaluated all three extracts: it afforded the lowest EC$_{50}$; hexane 9.65 (TI 4.39), ethyl acetate 11.15 (TI 3.84), and methanol 43.61 (TI 3.04). Moreover, an antibacterial study on extract was also performed. The antibacterial study was evaluated using nine strains (Staphylococcus aureus, Enterobacter aerogenes, Escherichia coli 0157: H7, Escherichia coli (ETEC), Escherichia coli (EPEC), Proteus mirabilis, Salmonella typhimurium, Shigella flexneri, and Vibrio cholera) by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) method. The MeOH extract was the most effective antibacterial with a MIC in the range >0.1 mg/mL and MBC in the range >0.2 mg/mL. However, the isolated compounds from this plant had not been studied in all bioassay. Phytochemical examination of stems and twigs from Ficus dubia had directed to the isolation of four known compounds, epifriedelanol, β-sitosterol, stigmasterol, and bergapten.

**Conclusion:** The crude extract of twigs and stems of Ficus dubia were specifically active toward MC99+1A2. With these results, it could be concluded that Ficus dubia may be an accomplished candidate in pill and future medicine.

**Keywords:** Ficus dubia; moraceae; anti-HIVs; anti-syncytium (MC99+1A2); anti-bacterial activity.

### 1. INTRODUCTION

**Ficus dubia** Wall. Ex king associated with Moraceae family founded about 110 species, which are woody plants, and widely located among the Borneo island and Llambir hill national park in Malaysia. In Thailand, was founded in Ban Phu Khao Thong, Sukhirin district, narathiwat Province [1] and in some western part of Thailand. General characteristics of Ficus dubia, will have large fruit, large oval to round shape, bright red when ripe, the leaves are medium in size, oblong in shape, about 5-15 cm long, and 4-7 cm wide (Fig. 1). Phytochemical studies on Moraceae family revealed the presence of various bioactive compounds like phenolic compounds, phytosterol, triterpenoids, and volatile compounds. The biological activity of Ficus species is very diverse, such as anti-oxidant, anti-cancer cells, anti-viral, antibacterial, and anti-microbial activity. One of these plants, *Ficus carica*, also known as “fig”, is a tree native to Southwest Asia. The dried fruit of this plant is rich in vitamins, minerals, sugar, organic acids, and phenolic compounds. Fruit, root, and leaf of “fig” are used as a traditional medicine to treat various diseases such as gastrointestinal, respiratory, cardiovascular disorder, and anti-inflammatory remedy [2,3]. Good properties of this plant, therefore, researchers are interested in studying the active substances and bioactivities of the *Ficus dubia* plant, which may discover new substances with good biological activity and lead to future medical uses.

**Fig. 1.** Twigs, leaves and fruits of *Ficus dubia*

### 2. MATERIALS AND METHODS

The melting point was evaluated by Büchi B-540 micro melting point apparatus and uncorrected. Infrared spectra (IR) were recorded on KBr pellets with a Shimadzu 8900 FT-IR spectrophotometer. UV spectra were determined on a Shimadzu UV-2550 spectrometer (Shimadzu, Kyoto, Japan). Column chromatography (CC) was carried using silica gel 60 H (E. Merck. 70-230 mesh ASTM, cat. No. 7734). Pre-coated thin layer chromatography (TLC) aluminum sheets of silica gel 60 PF$_{254}$ were used for analytical purposes and the compounds were visualized under ultraviolet light. Preparative thin-layer chromatography (PLC) was performed on Merck cat. No. 7736,
Kieselgel 60 F<sub>254</sub>/0.50 mm thickness plates. TLC experiments were used by short and long-wavelength ultraviolet light and visualized by spraying the plates with a 4-methoxybenzaldehyde solution followed by heating. <sup>1</sup>H and <sup>13</sup>C-NMR spectra were recorded on NMR Bruker AV-500 (500 MHz for <sup>1</sup>H-NMR, 125 MHz for <sup>13</sup>C-NMR) spectrometer.

### 2.1 Plant Material

*Ficus dubia* Wall. Ex king (BKF192204) was collected from Si Sawat District, Kanchanaburi Province, Thailand, and identified by Dr. Narong Nuntasaen.

### 2.2 Extraction and Isolation

The dry powder of an aerial part of *Ficus dubia* (5.7 kg) was extracted with hexane (4 ×23 L, 3 days) to give the crude hexane extract (33.31 g). Similarly, the crude ethyl acetate (24.53 g) and crude methanol extract (94.00 g) was obtained. The hexane extract was treated with silica gel CC (272 g, Merck 7734, 70-230 mesh) using a stepped gradient elution of hexane-ethyl acetate-methanol (v/v/v, 100:0:0-0:0:100) to produce night fractions (A1-A9). Fraction A3 (5.83 g, eluted with hexane-ethyl acetate, v/v 95:5) was purified by silica gel CC (250 g, Merck 7734, 70-230 mesh) using a stepped gradient elution of hexane-ethyl acetate-methanol (v/v/v, 100:0:0-0:0:100) to give four fractions (E1-E4). Fraction E2 (3.42 g, eluted with hexane-ethyl acetate, v/v 70:30-30:70) was purified by silica gel CC (120 g, Merck, 7631, 86-9 mesh) to afford three fractions (F1-F3). Subfraction F3 (3.57 g, eluted with hexane: ethyl acetate; v/v, 84:16-60:40) was recrystallized with ethanol to give compound 3 (0.68 g) as a white solid.

### 2.3 Anti-HIVs Assay and Cytotoxic Study

An anti-HIV1-RT activity assay, anti-syncytium test and cytotoxic study were done as reported by Pompimon, W., et al. [4,5] and Limjiasahapong, S., et al. [6].

### 2.4 Antimicrobial Susceptibility Test

Antimicrobial activity test was done as reported by Pompimon, W., et al. [7].

### 3. RESULTS AND DISCUSSION

#### 3.1 Bioactivity

##### 3.1.1 Anti-HIVs assay

The result of Anti-HIVs testing of three crude hexane, ethyl acetate, methanol extracts, and pure compounds of stems and twigs from *Ficus dubia* was shown in Table 1.

From Table 1, the ethyl acetate, and methanol extract were moderately inhibited HIV1-RT with % inhibition at 69.02 and 69.24. Then, all of the extracts were active toward anti-syncytium (MC99+1A2) with IC<sub>50</sub> at 42.32-132.45, EC<sub>50</sub> at 9.65-43.61, and TI at 3.04-4.39 respectively. For compounds (1-3) were inactive with the HIV-1RT activity. The results showed that the three crude extracts of *Ficus dubia* were more effective against the HIV1-RT virus and anti-syncytium than those compounds (1-3). These results can be explained that some active anti-HIV1-RT virus and anti-syncytium compounds in the crude extracts were very small and were lost during the purification process. In addition, the synergistic effect of various substances in the crude extract has been shown to be very effective against the HIV virus, that a very common phenomenon in which crude extracts act better than pure compounds [8,9,10].

##### 3.1.2 Antimicrobial Susceptibility Test

The result of the antimicrobial susceptibility test of three crude hexane, ethyl acetate, methanol extracts, and pure compounds of stems and twigs from *Ficus dubia* was shown in Table 2.
and compounds from the twigs and stems of human cancer cell line. Although crude extracts of *Ficus dubia* hexane, ethyl acetate, methanol extracts, and the result of the cytotoxicity study were shown in Table 3.

3.1.3 Cytotoxicity study of crude extracts of the extracts and pure compounds of *Ficus dubia*

The result of the cytotoxicity study of three crude hexane, ethyl acetate, methanol extracts, and pure compounds of stems and twigs from *Ficus dubia* were shown in Table 3.

From Table 3, the extracts and pure compounds of *Ficus dubia* were inactive with all strains of the human cancer cell line. Although crude extracts and compounds from the twigs and stems of *Ficus dubia* do not have cytotoxicity, but the root, leaf and fruits of another *Ficus* species have good cytotoxicity, such as *Ficus pseudopopulina* Blanco [14], *Ficus beecheyana* [15], *Ficus carica* [15], *Ficus racemosa* [15] and *Ficus hispida* Linn [16]. These results revealed that the extract and compounds of twigs and stems of *Ficus dubia* were selective in anti-HIV virus activity.

3.2 Structure Elucidation

Chemical structure analysis of compound 1-3 using spectroscopy techniques was founded that compound 1 was epifriedelanol, compound 2 was a mixture of stigmasterol and β-sitosterol, and compound 3 was bergapten, respectively.

Epifriedelanol (1): white solid, m.p. = 284–285 °C (lit. 281–283 °C) [17,18,19]; IR (KBr), ν_{max} 3474 (OH stretching), 2932, 2853, 1385 cm⁻¹; ¹H NMR data, see Table 4.

![Fig. 2. Chemical structure of epifriedelanol (1), compound (2), and bergapten (3)](image_url)

**Table 1. Anti-HIV activities of the extracts and pure compounds of *Ficus dubia***

| The extracts and pure compounds | HIV-type |
|---------------------------------|----------|
|                                 | Anti-HIV1-RT | Anti-syncytium (MC99+1A2) |
|                                 | % inhibition | Activity | IC₅₀ | EC₅₀ | SI | Activity |
| Hexane extract                  | 22.30       | I        | 42.32 | 9.65 | 4.39 A |
| Ethyl acetate extract           | 69.02       | MA       | 42.80 | 11.15 | 3.84 A |
| Methanol extract                | 69.24       | MA       | 132.45 | 43.61 | 3.04 A |
| Compound 1                      | -12.29      | I        | -    | -    | -   | I        |
| Compound 2                      | -14.34      | I        | -    | -    | -   | I        |
| Compound 3                      | 25.48       | I        | -    | -    | -   | I        |
| AZT                             | -           | -        | >10⁸ | 2.65x10⁹ | >3.78 A |

Anti-HIV1-RT express as % inhibition at 200 µg/mL (radioactive) or 667 µg/mL (non-radioactive): VA = very active, >70% inhibition; MA = moderately active, >50 – 70%; W = weakly active, 30 – 50% inhibition; I = inactive, <30% inhibition (based on ref. [22, 23]). AZT is reference standard.

Syncytium reduction assay: EC₅₀ = dose of compound that reduced 50% syncytium formation by ΔTat/Rev MC99 virus in 1A2 cells. AZT (positive control), averaged from three independent experiments, EC₅₀ 3.18 x 10⁻⁶ µM, IC₅₀ > 10⁻² µM; A = active (SI >1), I = inactive, SI = Selectivity Index (SI = IC₅₀/EC₅₀)
### Table 2. Determination of MIC and MBC of crude extracts and pure compounds of *Ficus dubia*

| The extracts and pure compounds | MIC/MBC (mg/mL) | 
|:--------------------------------|:----------------|
|                                | *S. aureus* | *E. aerogenes* | *E. coli* (0157: H7) | *E. coli* (ETEC) | *E. coli* (EPEC) | *P. mirabilis* | *S. typhimurium* | *S. flexneri* | *V. cholera* |
| hexane extract                 | -           | -              | -                    | -                | -                | -              | -              | -              | -           |
| ethyl acetate extract          | -           | -              | -                    | -                | -                | -              | -              | -              | -           |
| methanol extract               | -           | -              | -                    | -                | -                | -              | -              | -              | -           |
| Compound 1                     | >1.0/ >1.0  | >1.0/ >1.0     | >1.0/ >1.0           | >1.0/ >1.0       | >1.0/ >1.0       | >1.0/ >1.0     | >1.0/ >1.0     | >1.0/ >1.0     | >1.0/ >1.0   |
| Compound 2                     | >1.0/ >1.0  | >1.0/ >1.0     | >1.0/ >1.0           | >1.0/ >1.0       | >1.0/ >1.0       | >1.0/ >1.0     | >1.0/ >1.0     | >1.0/ >1.0     | >1.0/ >1.0   |
| Compound 3                     | >1.0/ >1.0  | >1.0/ >1.0     | >1.0/ >1.0           | >1.0/ >1.0       | >1.0/ >1.0       | >1.0/ >1.0     | >1.0/ >1.0     | >1.0/ >1.0     | >1.0/ >1.0   |
| Chloramphenicol                | <0.03/ <0.03| <0.03/ <0.03   | <0.03/ <0.03         | <0.03/ <0.03     | <0.03/ <0.03     | <0.03/ <0.03   | <0.03/ <0.03   | <0.03/ <0.03   | <0.03/ <0.03 |
|                              | 0.125       | 0.125          | 0.125                | 0.125            | 0.125            | 0.125          | 0.125          | 0.125          | 0.125       |

* Chloramphenicol is reference standard

### Table 3. Cytotoxicity study of the extracts and pure compounds of *Ficus dubia*

| The extracts and pure compounds | Result of ED<sub>50</sub> test in Cell Line (µg/mL) | 
|:--------------------------------|:----------------------------------|
|                                | Cell lines                        |
|                                | KKU-M213 | FADu | HT 29 | MDA-MB-231 | A 549 | SH-SY5Y | MMN-K1 |
| hexane extract                 | -       | -    | -     | -           | -     | -       | -      |
| ethyl acetate extract          | -       | -    | -     | -           | -     | -       | -      |
| methanol extract               | -       | -    | -     | -           | -     | -       | -      |
| Compound 1                     | -       | -    | -     | -           | -     | -       | -      |
| Compound 2                     | -       | -    | -     | -           | -     | -       | -      |
| Compound 3                     | -       | -    | -     | -           | -     | -       | -      |
| *Ellipticine*                  | 0.50    | 0.41 | 0.52  | 0.54        | 0.41  | 0.45    | 0.51   |

*Ellipticine is reference standard

Cytotoxic assay: ED<sub>50</sub> less than 20 mg/ml were considered active for extracts; KKU-M213: Human intrahepatic cholangiocarcinoma, FaDu: Human pharyngeal squamous cell carcinoma, HT-29: Human colorectal adenocarcinoma, MDA-MB-231: Human mammary gland/breast adenocarcinoma, SH-SY5Y: Human neuroblastoma, A 549: Human lung adenocarcinoma, MMNK-1: Highly differentiated immortalized human cholangiocyte cell line.
Table 4. $^{13}$C- and $^1$H-NMR data of epifriedelanol (1), compound (2), and bergapten (3)

| Position | $\delta^{13}$C (1) | $\delta^1$H (1) | $\delta^{13}$C (2) | $\delta^1$H (2) | $\delta^{13}$C (3) | $\delta^1$H (3) |
|----------|-------------------|-----------------|-------------------|-----------------|-------------------|-----------------|
| 1        | 15.78 (CH$_2$)    | 37.28 (CH$_2$)  | -                 | -               | 161.29 (C)        | -               |
| 2        | 35.18 (CH$_2$)    | 31.87 (CH$_2$)  | -                 | 112.62 (CH)     | 6.24 (d,9.7)      | -               |
| 3        | 72.74 (CH)        | 71.81 (CH)      | 3.52 (m)          | 149.70 (C)      | -                 | -               |
| 4        | 49.16 (CH)        | 42.34 (CH$_2$)  | -                 | 149.70 (C)      | -                 | -               |
| 5        | 37.09 (C)         | -               | -                 | -               | -                 | -               |
| 6        | 41.72 (CH$_2$)    | 121.68 (CH)     | 5.35 (m)          | 112.62 (C)      | -                 | -               |
| 7        | 17.53 (CH$_2$)    | 31.87 (CH$_2$)  | -                 | 158.49 (C)      | -                 | -               |
| 8        | 53.18 (CH)        | 31.92 (CH)      | -                 | 93.90 (C)       | 7.09 (s)          | -               |
| 9        | 38.36 (C)         | 50.18 (CH)      | -                 | 152.82 (C)      | -                 | -               |
| 10       | 61.35 (CH)        | 36.52 (C)       | -                 | 106.52 (C)      | -                 | -               |
| 11       | 35.32 (CH$_2$)    | 21.10 (CH$_2$)  | -                 | -               | -                 | -               |
| 12       | 30.62 (CH$_2$)    | 39.81 (CH$_2$)  | -                 | -               | -                 | -               |
| 13       | 37.82 (C)         | 42.34 (C)       | -                 | -               | -                 | -               |
| 14       | 39.66 (C)         | 56.79 (CH)      | -                 | -               | -                 | -               |
| 15       | 29.67 (CH$_2$)    | 28.23 (CH$_2$)  | -                 | -               | -                 | -               |
| 16       | 36.07 (CH$_2$)    | 29.71 (CH$_2$)  | -                 | -               | -                 | -               |
| 17       | 30.01 (C)         | 56.11 (CH)      | -                 | -               | -                 | -               |
| 18       | 42.82 (CH)        | 36.14 (CH)      | -                 | -               | -                 | -               |
| 19       | 35.55 (CH$_2$)    | 18.78 (CH$_3$)  | 0.92 (d, 3H, J = 6.6 Hz) | - | - | - |
| 20       | 28.15 (C)         | 33.99 (CH$_3$) and 138.27 (CH) | - | - | - | - |
| 21       | 32.81 (CH$_2$)    | 24.30 (CH$_3$) and 129.31 (CH) | - | - | - | - |
| 22       | 39.26 (CH$_2$)    | 45.89 (CH)      | -                 | -               | -                 | -               |
| 23       | 11.58 (CH$_3$)    | 23.11 (CH$_3$)  | -                 | -               | -                 | -               |
| 24       | 16.37 (CH$_3$)    | 11.98 (CH$_3$)  | 0.84 (m)          | -               | -                 | -               |
| 25       | 18.22 (CH$_3$)    | 29.69 (CH)      | -                 | -               | -                 | -               |
| 26       | 18.61 (CH$_3$)    | 19.79 (CH$_3$)  | 0.82 (m)          | -               | -                 | -               |
| 27       | 20.09 (CH$_3$)    | 19.38 (CH$_3$)  | 0.81 (m)          | -               | -                 | -               |
| 28       | 31.77 (CH$_3$)    | 18.71 (CH$_3$)  | 0.67 (s)          | -               | -                 | -               |
| 29       | 34.99 (CH$_3$)    | 11.86 (CH$_3$)  | 1.00 (s)          | -               | -                 | -               |
| 30       | 32.07 (CH$_3$)    | 1.17 (s)        | -                 | -               | -                 | -               |
| 2'       |                   |                 | 144.90 (CH)       | 7.57 (d,2.5)    | -                 | -               |
| 3'       | 105.14 (CH)       | 7.01 (d,2.4)    | -                 | -               | -                 | -               |
| OCH$_3$  |                   |                 | 60.20 (CH$_3$)    | 4.25 (s)        | -                 | -               |
Compound (2): white solid, m.p. = 137–139 °C (lit. 134–136 °C) [20]; IR (KBr). \( \nu_{\text{max}} \) = 3406, 2939, 1649, 1458 cm\(^{-1}\), \(^{13}\)C and \(^{1}\)H NMR data, see Table 4.

Bergapten (3): white solid, m.p. = 187–188 °C (lit. 188–190 °C) [21]; IR (KBr). \( \nu_{\text{max}} \) = 3146, 3078, 2956, 1728, 1470, 1360, 1213, 1123, 1155, 835 cm\(^{-1}\), \(^{13}\)C and \(^{1}\)H NMR data, see Table 4.

4. CONCLUSION

Anti-HIV and antibacterial activities of crude extract of *Ficus dubia* were found that the methanol extract showed moderated active toward HIV-1RT and MC99+1A2, and also inhibited *E. Coli* (EPEC). Cytotoxicity investigation of crude extract and isolated pure compounds of *Ficus dubia* were inactive toward all strains of the cancer cell line. Phytochemical investigation of twigs and stems of *Ficus dubia* has founded four known compounds including, epifriedelanol (1), compound (2) (\( \beta \)-sitosterol and stigmasteryl), and bergapten (3) respectively. This research revealed the important role of compounds in plants showed good anti-syncytium (MC99+1A2) and antibacterial activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All protocols involving cell experiments were approved by the cell Ethics Committee of Lampang Rajabhat University, Lampang, Thailand.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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