Journal of Pharmaceutical Research International

32(41): 84-91, 2020; Article no.JPRI.64104
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

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

Wilart Pompimon[1], Phansuang Udomputtimekakul[1*], Aphiruk Chaisena[1], Saranya Wattananon[2], Arnuphap Arsa[1], Kitipong Konsantha[1] and Narong Nuntasaen[3]

[1]Department of Chemistry, Faculty of Science, Lampang Rajabhat University, 52100 Lampang, Thailand.
[2]Department of Food Science and Technology, Faculty of Agricultural Technology, Lampang Rajabhat University, 52100, Lampang, Thailand.
[3]The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand.

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.

Article Information

DOI: 10.9734/JPRI/2020/v32i4131046
Editor(s):
(1) Dr. Jongwha Chang, University of Texas, College of Pharmacy, USA.
Reviewers:
(1) Alison Honório de Oliveira, Federal University of Para (UFPA), Brazil.
(2) Asna Khan, Gulbarga Istitute of Medical Sciences, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64104

Original Research Article

Received 29 October 2020
Accepted 01 January 2021
Published 18 January 2021

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

**Corresponding author: E-mail: phansuang@yahoo.com;*

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 fubia* 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, anti-bacterial, 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_{254}/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. ^{1}H- and ^{13}C-NMR spectra were recorded on NMR Bruker AV-500 (500 MHz for ^{1}H-NMR, 125 MHz for ^{13}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 give night fractions (A1-A9). Fraction A3 (5.83 g, eluted with hexane-ethyl acetate, v/v 95:5) was purified by silica gel CC (12 g, Merck, 7631, 86-9 mesh) to afford three subtractions (B1-B3). Subfraction B2 (3.97 g, was recrystallized with ethanol: ethyl acetate (1:1) to give compound 1 (0.47 g) as a white solid. Subfraction B3 (2.38 g) was recrystallized with ethanol to yield compound 2 (0.39 g). The ethyl acetate extract (24.53 g) was chromatographed with 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 produce night fractions (C1-C9). Fraction C5 (1.46 g, eluted with hexane-ethyl acetate, v/v 90:10) was purified by silica gel CC (25 g, Merck, 7631, 86-9 mesh) to afford three subtractions (D1-D3). Subfraction D2 (0.62 g, eluted with hexane: ethyl acetate; v/v, 94:6-90:10) was recrystallized with ethanol: ethyl acetate (3:1) to give compound 3 (0.44 g) as a white solid. The methanol extract (94.00 g) was chromatographed with CC (940 g, Merck 7734, 70-230 mesh) using a stepped gradier 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 subtractions (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_{50} at 42.32-132.45, EC_{50} 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.

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	IC_{50}	EC_{50}	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	-	-	$>10^8$	$2.65x10^9$	>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; M = 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 : EC_{50} = dose of compound that reduced 50% syncytium formation by $^{\Delta Tat/Rev}$MC99 virus in 1A2 cells. AZT (positive control), averaged from three independent experiments, EC_{50} 3.18 x 10^{-3} µM, IC_{50} > 10^{-2} µM; A = active (SI >1),I = inactive, SI =Selectivity Index (SI = IC_{50}/EC_{50})

From Table 2, the methanol extract was inhibited *E. coli* (EPEC) with MIC/MBC values at 100/>200 mg/mL, while the three pure compounds were inhibited all of the bacterial strains with MIC/MBC values at >0.1/>0.1 mg/mL. The results of this experiment showed that the methanol extract and compounds (1-3) from the twigs and stems of *ficus dubia* had antibacterial activity, which is consistent with previous research [11,12,13].

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

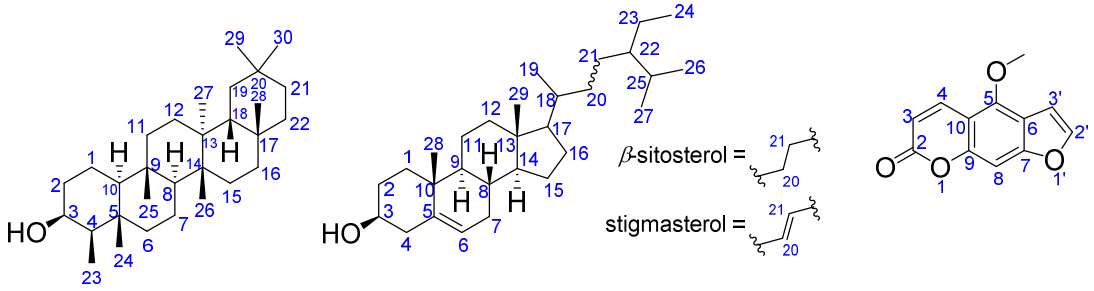
The result of the cytotoxicity study of three crude hexane, ethyl acetate, methanol extracts, and pure compounds of stems and twigs from *Ficus dubia* were shown in Table 3.

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 pseudopalma* 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}; ^{13}C and ^{1}H NMR data, see Table 4.



epifriedelanol (**1**) compound (**2**) bergapten (**3**)

Fig. 2. Chemical structure of epifriedelanol (1), compound (2), and bergapten (3)

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	-	-	-	-	100/ >200	-	-	-	-
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/ 0.125	<0.03/ 0.125	<0.03/ 0.125	<0.03/ 0.125	<0.03/ 0.125	<0.03/ 0.125	<0.03/ 0.125	<0.03/ 0.125	<0.03/ 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_{50} 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_{50} less than 20 mg/ml were considered active for extracts; KKU-M213: Human intrahepatic cholangiocarcinama, 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	δ^{13}C (1)	δ^{1}H (1)	δ^{13}C (2)	δ^{1}H (2)	δ^{13}C (3)	δ^{1}H (3)
1	15.78 (CH_2)		37.28 (CH_2)	-		
2	35.18 (CH_2)	1.90 (H-2a, d, J=10.1, 2.7 Hz) 1.54 (H-2b, m)	31.87 (CH_2)	-	161.29 (C)	-
3	72.74 (CH)	3.73(d)	71.81 (CH)	3.52(m)	112.62 (CH)	6.24 (d,9.7)
4	49.16 (CH)	1.23-1.31(m)	42.34 (CH_2)	-	139.33 (CH)	8.12 (d,9.8)
5	37.09 (C)	-	140.77 (C)	-	149.70 (C)	-
6	41.72 (CH_2)	1.73 (H-6a, dt,J = 12.8, 3.0 Hz) 1.02-1.14 (H-6b, m)	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)		26.18 (CH_2)	-		
16	36.07 (CH_2)		28.23 (CH_2) and 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_2) and 138.27 (CH)	-		
21	32.81 (CH_2)		24.30 (CH_2) and 129.31 (CH)	-		
22	39.26 (CH_2)		45.89 (CH)	-		
23	11.58 (CH_3)	0.94 (d, J=7.3 Hz)	23.11 (CH_2)	-		
24	16.37 (CH_3)	0.96 (s)	11.98 (CH_3)	0.84 (m)		
25	18.22 (CH_3)	0.85 (s)	29.69 (CH)	-		
26	18.61 (CH_3)	1.01 (s)	19.79 (CH_3)	0.82 (m)		
27	20.09 (CH_3)	0.99 (s)	19.38 (CH_3)	0.81 (m)		
28	31.77 (CH_3)	0.99 (s)	18.71 (CH_3)	0.67 (s)		
29	34.99 (CH_3)	0.94 (s)	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), ν_{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), ν_{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) (*β*-sitosterol and stigmasterol), 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.

ACKNOWLEDGEMENT

This research project was supported by the Thailand Research Fund (TRF). Partial supports from Mahidol University, the Center for Innovation in Chemistry (PERCH-CIC) are gratefully acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chantarasuwan B, Thongaree, S. Five species of *Ficus* (Moraceae) new for Thailand. Thai. For. Bull. 2006; 34:25-37.
2. Mava S, Husain K, Jantan I. *Ficus carica*. (Moraceae):Phytochemistry, traditional uses and biological activities. Evidence−Based Complementary and Alternative Medicine. 2013;1-8.
3. Hossain MA. A review on *Ficus sycomorus*: A potential indigenous medicinal plant in Oman. Journal of King Suad University−Science. 2019; 31:961-965.
4. Pompimon W**,** Sombutsiri P, Baison W, Udomputtimekakul P, Chuajedton A, Wingwon B, Suksen K, Limthongkul J, Naparswad C. Cancer Cytotoxic and anti-HIV potential of triphala herb mixture on from Chae Son, Lampang, Thailand. J. Pharm. Res. Int. 2019; 30(19):1-9.
5. Udomthawee K, Chainok K, Jiajaroen S, Nuntasaen N, Pompimon W. New ent-kaurane-type diterpenoid with cytotoxic activity from *Croton mekongensis*. Asian Nat. Prod. Res. 2020;14:1-8.
6. Limjiasahapong S, Tuchinda P, Reutrakul V, Pohmakotr M, Akkarawongsapat R, Limthongkul J, Napaswad C, Nuntasaen N. Anti-HIV-1 Activities and Chemical Constituents from Leaves and Twigs of *Santisukia pagetii* (Bignoniaceae). Nat Prod Commun. 2018;13(11):1449-1452.
7. Pompimon W, Baison W, Chuajedton A, Udomputtimekakul P, Sombutsiri P, Wingwon B, Udomtanakunchai C. A comparative study on antioxidation and antibacterial activities Triphala herb extracts from Chae Son, Lampang, Thailand. J. Pharm. Res. Int. 2019; 31(6): 1-14.
8. Aiyelaagbe OO, Hamid AA, Fattorusso E. Taglialatela-Scafati O, SchrÖder HC, MÜller WEG. Cytotoxic activity of crude extracts as well as of pure components from *Jatropha* species, plants used extensively in African traditional medicine. *Evid Based Complement Alternat Med*. 2011;1-7.
9. Caesar LK, Cech NB. Synergy and antagonism in natural product extracts:

when 1 + 1 does not equal 2. Nat. Prod. Rep. 2019;36:869-888.
10. Carmona F, Pereira, AMS. Herbal medicines: old and new concepts, truths and misunderstandings. Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy. 2013; 23(2): 379-385.
11. Salem MZM, Salem AZM, Camacho LM, Ali HM. Antimicrobial activities and phytochemical composition of extracts of *Ficus* species: An over view. Afr. J. Microbiol. Res. 2013;7(33):4207-4219.
12. Tkachenko H, Buyun H, Terech-Majewska E, Osadowski Z. *In vitro* antimicrobial activity of ethanolic extracts obtained from *Ficus* spp. leaves against the fish pathogen *Aeromonas hydrophila*. Arch. Pol. Fish. 2016;24:219-230.
13. Bhawana, Robin, Kaur J, Vig AP, Arora S, Kaur R. Evaluation of antibacterial potential of *Ficus* species. J. Pharm. Sci. & Res. 2018;10(5):1251-1255.
14. Llagas MCDL, Santiago L, Ramos JD. Cytotoxicity and apoptotic activity of Ficus pseudopalma Blanco Leaf Extracts Against Human Prostate Cancer Cell Lines. Trop J Pharm Res. 2014;13(1):93-100.
15. Mazumder K, Biswas B, Raja IM, Fukase K. A review of cytotoxic plants of the indian subcontinent and a broad-spectrum analysis of their bioactive compounds. Molecules. 2020;25:1-40.
16. Sathiyamoorthy J, Sudhakar N. *In vitro* Cytotoxicity and Apoptotic Assay in HT-29 Cell Line Using Ficus hispida Linn: Leaves Extract. Pharmacogn Mag. 2020;13(52): S756-S761.
17. Monkodkaew S, Loetchutinat C, nuntasean N, Pompimon W. Identification and antiproliferative activity evaluation of a series of triterpenoids isolated from Flueggea virosa (Roxb.ex. Willd.). Am J Appl Sci. 2009;6(10):1800-1806.
18. Kiem PV, Minh CV, Huong HT, Nam NH, Lee JJ. Pentacyclic triterpenoids from Mallotus apelta. Arch Pharm Res. 2004; 27(11):1109-1113.
19. Utami R, Khalid N, Sukari MA, Rahmani M, Abdul AB, Darchriyanus. Phenolic contents, antioxidant and cytotoxic activities of *Elaeocarpus floribundus* Blume. Pak J. Pharm. Sci. 2013;26(2): 245-250.
20. Chaturvedula VSP, Prakash I. Isolation of stigmasterol and *β*-sitosterol from the dichloromethane extract of *Rubus suavissimus*. Int. Curr. Pharm. J. 2012; 1(9):239-242.
21. Chunyan C, Bo S, Ping L. Isolation and punrification of psoralen and bergapten from Ficus carica L leaves by high-speed countercurrent chromatography. HHS public access Author manuscript peer-reviewed and accepted for publication, 2010; 32(1):136-143.
22. Tan GT, Pezzuto JM, Kinghorn AD, Hughes SH. Evaluation of natural products as inhibitor of human immunodeficiency virus type 1(HIV-1) reverse transcriptase. J. Nat. Prod. 1991; 54(1):143-154.
23. Reutrakul V, Anantachoke N, Pohmakotr M, Jaipetch T, Sophasan S, Yoosook C, Kasisit J, Napaswat C, Santisuk T, Tuchinda P. Cytotoxic and anti-HIV-1 caged xanthones from the resin and fruits of *Garcinia hanburyi*. Planta Medica. 2007; 73:33-40.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/64104