Asian Journal of Applied Chemistry Research

Volume 12, Issue 2, Page 53-60, 2022; Article no.AJACR.94324
ISSN: 2582-0273

Characterization of Chemical Constituent and Biological Activity of Roots from *Cleistanthus oblonggifolius* (Roxb.) Mull. Arg

Saranya Wattananon [a], Wilart Pompimon [b*], Phansuang Udomputtimekakul [b], Puttinan Maepowpan [c], Thanatcha Taratong [b], Amornrat Khamkaew [b], Atchariyaporn Mookaewkrue [b], Raksina Boonthadang [b], Nopawit Khamto [c,d], Puracheth Rithchumpon [c] and Narong Nuntasaen [e]

[a] Department of Food Science, Faculty of Agricultural Technology, Lampang Rajabhat University, Lampang, Thailand.
[b] Department of Chemistry, Faculty of Science, Lampang Rajabhat University, Lampang, Thailand.
[c] Department of Chemistry, Faculty of Science, Chiang Mai University, 239 Huay Kaew Road, Chiang Mai, Thailand.
[d] Graduate School, Chiang Mai University, 239 Huay Kaew Road, Chiang Mai, Thailand.
[e] Center of Innovation in Chemistry, Mahidol University, Rama 6 Road, Bangkok, Thailand.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJACR/2022/v12i2219

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/94324

Original Research Article

Received: 20/09/2022
Accepted: 30/11/2022
Published: 02/12/2022

**Corresponding author: Email: Pompimon.wilart@gmail.com, Wilart@lpru.ac.th;*

ABSTRACT

Background: *C. oblongifolius* is a species of Asian trees, originally described by William Roxburgh and later placed by Johannes Müller Argoviensis; it is now included in the family Phyllanthaceae. Distributed throughout Indochina and Malesia, its name in Vietnam is *cọc rào*; it has been recorded from the Andaman & Nicobar Islands, Australia (Queensland), Bangladesh, Borneo, Cambodia, Java, Lesser Sunda Islands, peninsular Malaysia, Maluku, Myanmar, New Guinea, Philippines, Solomon Islands, Sulawesi, Sumatera, Vietnam and Thailand. Several species of this genus are highly poisonous (*C. collinus*), some are brewed for treating dysentery (*C. decurrens*), and used for treatment of asthma (*C. myrianthus*).

Objective: This study was conducted to investigate the effect of crude extract and purified substance on inhibition of AIDS and cancer.

Methods: Phytochemical studies were extracts from these solvents used for the study: hexane, ethyl acetate and methanol. The dried herbs were ground and extracted by chromatography. Extracts and compound have been tested for HIV-1 RT, anti-syncytium and cytotoxicity. The obtained purification compounds were used to determine the structure by spectroscopic techniques such as UV. IR, NMR and MS.

Results: The study found that compound 3-*O*-methylellagic acid 4'-*O*-alpha-L-rhamnopyranoside from the roots of this plant, it was also found that ethyl acetate and methanol class extracts inhibited anti-HIV-1 RT up to 100% and 65% pure substance. For all extracts and purified compounds, syncytium inhibition assay showed inhibition HIV with EC_{50} <7.8 µM, TI>2.31. SH-SY5Y anticancer was showed with ED_{50} = 6.7 µM.

Conclusion: *C. oblongifolius* extract, most of these fractions were potent in inhibiting HIV-1 RT and syncytium (MC99+1A2) inhibition. In addition, hexane extract inhibited the growth of SH-SY5Y cancer cells more than other cancer cells. However, the isolated purified compound only had an inhibitory effect on HT-29 cells with ED_{50} 10.11 µg/mL.

Keywords: Cleistanthus oblonggifolius; PHYLLANTHACEAE; biological activity.

1. INTRODUCTION

The cleistanthus genus belongs to the Phyllanthaceae family and is distributed in the tropical regions of Africa, Asia, and Australia [1]. A total of 15 species are distributed in various regions of Thailand including decurrens, denudatus, glandulosus, gracilis, helferi, hirsutopetalus, hirsutulus, macrophyllus, oblongifolius, papyraceus, polyphyllus, praetermissus, refus, sumatranus and tomentosus [2]. As for the benefits of *C. collinus*, the bark, roots, leaves and berries are highly toxic, especially the extracts of this plant that cause severe gastrointestinal disorders, The Muser hill tribes of Thailand used *C. hirsutulus* to treat infected wounds in both humans and animals, in addition, the leaves and fruits of this plant are irritating to fish due to their intoxicating effect [3]. Further, parts of *C. decurrens* have been brewed for dysentery and *C. myrianthus* for asthma [4]. Phytochemical studies have found many groups of natural product chemicals, as well as new lignans, (70 R,80 S)-3,30 ,50 -trimethoxy-4,40 -dihydroxy-7-en-70 ,9- epoxy-8,80 - lignan and (70 R,80 S)-3,30 -dimethoxy-4,40 -dihydroxy-7-en-70 , 9- epoxy-8, 80 -lignan [5], gracicleistanthoside [6], cleistanthoside B [7], and Cleistanthin A [8]. In this study, compound extraction and structure determination were performed by NMR-based spectroscopy and AIDS and cancer bioactivity tests will be discussed below.

1.1 Plant Information

C. oblongifolius is a species of Asian trees, originally described by William Roxburgh and later placed by Johannes Müller Argoviensis; it is now included in the family Phyllanthaceae. They have a self-supporting growth form. They have simple, broad leaves. Individual tree can grow to 26 m. Distributed throughout Indochina and Malesia, its name in Vietnam is *cọc rào*; it has been recorded from the Andaman & Nicobar Islands, Australia (Queensland), Bangladesh, Borneo, Cambodia, Java, Lesser Sunda Islands, Peninsular Malaysia, Maluku, Myanmar, New Guinea, Philippines, Solomon Islands, Sulawesi, Sumatera, Thailand and Vietnam [9].

Fig. 1. Morphology

Source: *Dr***.** *Narong Nantasean, a botanist at the forest herbarium, ministry of natural resources and environment, Bangkok*

1.2 Chemical Constituent

Chemical composition of *C. oblongifolius,* there are several groups including lignan, furofuranoid lignans, dibenzylbutane lignans, triterpenoid, and ellagic derivatives. For this research, ellagic acid glycoside derivatives were found. Their structure was elucidated as *3-O*-methylellagic acid 4'-*O*-alpha-L-rhamnopyranoside [10] by IR, EI-MS and NMR spectroscopic techniques.

2. MATERIALS AND METHODS

2.1 Experimental

2.1.1 General experimental procedures

^{1}H (500 MHz), ^{13}C (125 MHz), and 2D NMR spectra were noted on a BrÜker AV-500 spectrometer in deuterated methanol (CD_3OD) solution. Melting points were explained by a Büchi 322 micro melting point apparatus and have to be uncorrected. UV-visible absorption spectra were carried out using a UV-2550 (SHIMADZU) UV-Vis spectrometer (Shimadzu). Infrared spectra (IR) were written down as KBr pellets using a Shimadzu 8900 FT-IR spectrophotometer and major bands were taken down in wavenumber (cm^{-1}). The mass spectra were recorded on a Thermo Finnigan Polaris Q mass spectrometer at 70 eV (probe) and EIMS were estimated by a BrÜker Esquire apparatus Column chromatography (CC) were demanded using silica gel 60 H from E. Merck. 70-230 mesh ASTM, cat. No. 7734 and No.7736. The thin-layer chromatography (TLC) technique was preceded on silica gel 60 PF_{254} at aluminum sheets ultraviolet light.

2.1.2 Plant material

The roots of *C. oblongifolius* (BKF.190459) were collected at, Amphoe Soi Dao, Chanthaburi province, Thailand, in October, 2020. The plant materials were recognized by Dr**.** Narong Nantasean, a botanist at the forest herbarium, ministry of natural resources and environment**.** Bangkok

2.1.3 Preparation of extraction and isolation

The air-dried powdered roots from *C. oblongifolius* (1.25 kg) were extracted with n-hexane (6 L× 3 days× 5 times), EtOAc (6 L× 3 days× 4 times), and MeOH (6.5 L× 3 days× 4 times) to give crude n-hexane extract (7.75 g), crude EtOAc extract (8.83 g) and crude MeOH extract (123.76 g), respectively.

The n-hexane extract (7.75 g) was separated by column chromatography on silica gel Merck No.7734 mesh 70-230 ASTM. The n-hexane extract was found stigmasterol 0.17. g

The EtOAc extract (8.83 g) was separated by column chromatography on silica gel on silica gel Merck No.7734 mesh 70-230 ASTM, eluted with a gradient system between n-hexane, EtOAc, and MeOH to give four fractions (A_1-A_{10}). Unfortunately, the results of the experiments did not reveal any interesting compounds. It's not suitable to be taken separately.

The MeOH extract (123.76 g) was further divided by column chromatography on silica gel eluted with a gradient system between n-hexane, EtOAc, and MeOH to give ten fractions (B_1-B_{10}). Fraction E_7 (2.57 g) was separated by flash CC with n-hexane: EtOAc (100:0-0:100) then with EtOAc: MeOH (100:0-0:100) to yield three subfractions (C_1-C_3). Subfraction C_2 was further separated by flash CC with n-hexane: EtOAc (100:0-0:100) to afford D_1-D_3. The subfraction D_3 was as well crystallized by ethanol to a white needle of *3-O*-methylellagic acid 4'-*O*-alpha-L-rhamnopyranoside 0.89 mg (**1**).

2.2 Test for Phytochemical Analysis

2.2.1 Anti-HIV1-RT activity and anti-syncytium assay

2.2.1.1 Anti-HIV1-RT (Reverse transcriptase) assay [*10*]

Anti-HIV1-RT and cytotoxicity assay of the extracts of *C. oblongifolius* were conducted at the Service Centre of Department of Physiology and Microbiology, Mahidol University, Thailand. The anti-HIV1-RT activities were decided by testing RT inhibition [11,12]. The extracts were diluted to give 20 mg/mL of 100% dimethyl sulfoxide (DMSO) after the removal of tannin by polyvinylpyrrolidone (PVP). The final volume was 200 µg/mL in 10% DMSO, and Nevirapine, 2 µg/mL was worked of as a positive control. The HIV1-RT (Amersham Pharmacia Biotech Asia Pacific Ltd., Hong Kong) kit was used. The 96-well plate (100 U/µL, 4 µL/well) was filled with samples (2 µL/well), and then 2.5 µg/µL of poly-A and 0.125 µg/mL of oligo dT16 primer were added to 4 µL/well and incubated at 37 °C for 20 mins. The reaction was affixed by 0.2 M EDTA (2 µL/well) and incubated at 4 °C for 15 mins. The signal of fluorescence was measured at an emission wavelength of 535 nm and excitation wavelength of 480 nm after Pico green dissolved in TE buffer (1:2000) was put in (volume 200 µL/well). The results were evaluated as a percentage of inhibition.

2.2.1.2 Cell-based assay for anti-HIV-1

The syncytium assay was performed in triplicate using $^{\Delta Tat/rev}$MC99 virus and 1A2 cell system [13, 14], starting at the final concentrations of 3.9–125 µg/mL or higher. Virus control and cell control wells contained neither the extracts nor the virus; cytotoxicity control wells containing cells with the extracts and positive control, i.e., azidothymidine, AZT, were included. The result was expressed as 50% effective concentration (EC_{50}). Cytotoxicity of the extracts was also carried out, in parallel and in duplicate, using a colorimetric XTT assay. The result was indicated as the concentration that inhibited 50% formazan formation in uninfected cells (IC_{50}). The therapeutic index (TI) was calculated using the equation: $TI=IC_{50}/EC_{50}$.

2.2.2 Cytotoxicity

Cytotoxicity activity of the extracts of *C. oblongifolius* was also investigated using the standard Sulforhodamine B (SRB) assay. Ellipticine was operated as a positive control [15, 16]. The concentrations of the samples were 20 - 0.16 µg/mL in 0.5% DMSO. The cancer cell lines were employed, including human intrahepatic cholangiocarcinoma (KKU-M213), human pharyngeal squamous carcinoma (FaDu), human colorectal adenocarcinoma (HT-29), human mammary gland/breast adenocarcinoma (MDA-MB-231), human neuroblastoma (SH-SY5Y), human lung carcinoma (A 549), and highly differentiated immortalized human cholangiocyte cell line (MMNK-1). MEM (minimum essential medium with Earles salt and L-glutamine) in 10% FBS were spending for culturing the cell lines. The cell lines were kept at temperature 37 °C for 72 hours 5% CO_2 in the air, and 100% relative humidity, followed by stabilizing with 20% trichloroacetic acid at 4 °C for 60 minutes and then stained for 30 minutes by 0.4% SRB in 1% acetic acid at room temperature. The unbound dye was cleaned with 1% acetic acid, while the already dried stain was mixed with 10 mM Tris base with pH = 10. The absorbance was gauged at 510 nm on a microplate reader, and the 50% effective dose (ED_{50}) was calculated.

3. RESULTS AND DISCUSSION

Phytochemical research on *C. oblongifolius*, this is the first time to reveal the bioactivity and spectroscopic potential of a chemical composition. This will be clear about the correct structure that can be referenced further.

Compound 1 was isolated as white needles**.** The molecular formula was determined to be $C_{21}H_{18}O_{12}$ on the basis of the pseudo molecular ion $[M+H]^+$ peak in EIMS at *m/z* 462. The UV spectrum displayed the absorption maxima at 258 (4.61), 300 (1**.**32) and 358 (1.06) nm with melting point (358**-**359.6) ◦C [17]. The IR spectrum showed characteristic absorptions for hydroxyl groups of sugar and phenolic at 3489 cm^{-1}, 2964, 2947 cm^{-1} for CH_2, CH_3, two carbonyl esters for 1697 cm^{-1} and 1464, 1373 cm^{-1} for C**-**O**-**C. The mass spectrum showed a characteristic fragment ion at *m/z* 163 (9.29 %), which was produced losing sugar, rhamnopyranosyl unit in the structure. The fragment ion at *m/z* 313 (77.81 %) in the mass spectrum due to ellagic acid fragmentation. Additionally, the fragment ions at *m/z* 299 (14.33 %) in the mass spectrum indicated the presence of the methyl group in the structure of ellagic moiety [18]. The ^{1}H signal of **1** at 7.59 (s, 1H) and 7.91 (s, 1H) were assigned at C-5 and C-5′,

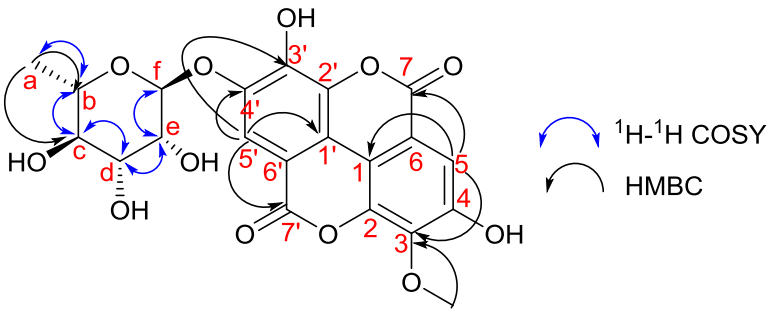

Fig. 2. Chemical structure, COSY and HMBC correlations

Table 1. ^{1}H (500 MHz, MeOD-d_4) and ^{13}C (125 MHz, MeOD-d_4) NMR spectra data of compound 1

Position	δ_H (Int., Mult., *J* in Hz)[c]	δ_C
1	-	111.9 (C)
2	-	141.7 (C)
3	-	140.5 (C)
4	-	152.6 (C)
5	7.59 (s, 1H, 5-ArCH)	111.6 (CH)
6	-	113.3 (C)
7	-	159.5 (C=O)
1’	-	114.9 (C)
2’	-	136.5 (C)
3’	-	140.5 (C)
4’	-	146.8 (C)
5’	7.91 (s, 1H, 5’-ArCH)	112.5 (C)
6’	-	113.3 (C)
7’	-	159.6 (C=O)
O-CH_3	4.19 (s, 3H, 3-ArC)	60.7 (CH3)
a	1.28 (d, J = 6.2 H_Z, 3H, a-CH_3)	16.7 (CH_3)
b	3.74 (dd, J = 9.3, 6.2H_Z, 1H, b-CH)	69.8 (CH)
c	3.52 (t, J = 9.3H_Z, 1H, c-CH)	72.3 (CH)
d	4.00 (dd, J = 9.3, 3.4H_Z, 1H, d-CH)	70.7 (CH)
e	4.19 (m, 1H, e-CH)	70.3 (CH)
f	5.58 (d, J = 1.6H_Z, 1H, f-CH)	100.2 (CH)

respectively. The dominant signal at position 3 (4.19, s, 3H) belongs to the only methoxy group on the ellagic acid structure.

The HMBC technique, showing the relationship between H-f (5.58 ppm) and C-4/ (146.8 ppm), confirmed that the sugar fraction was attached to the allergic acid at 4/ position. The COSY experimental results showed the connection of protons on the carbon structure of sugar as follows: H/C; H-a/C-a; H-b/C-b; H-c/C-c; H-d/C-d; H-e/C-e; H-f/C-f. From the data, it can be confirmed that it is a rhamnopyranose type. When analyzed with ^{13}C-NMR detects carbon resonance signal of ellagic acid at δ_C 4° 13 signals, 3° 1 signal. Similarly, the experiment also found the following sugar carbon signal groups: 3° CH_3, 1 signal, methine CH, 5 signals. The positions of the 7/ and 7 ester groups were confirmed by the HMBC relationship between H-5/ withC-7/ and H-5 with C-7, respectively. In addition, the HMBC relationship of protons and other carbons in the structure can be seen in Fig. 1. These data are in agreement with structure **1** for this first new ellagic acid glycoside derivatives compound of *C. oblonggifolius*, for which the trivial name *3-O*-methylellagic acid 4'-*O*-alpha-L-rhamnopyranoside [10,19,20].

Table 2. Anti-HIV-1 RT and Anti-syncytium (MC99+1A2 study of crude extracts and isolated compound

Crude extracts/ compound	Anti-HIV-1 RT[a] (% inhibition)		Anti-syncytium (MC99+1A2) [b]			
			IC_{50}	EC_{50}	TI[C]	Activity
Hexane	60.69	MA	19.85	<7.8	>2.54	Active
Ethyl acetate	100	VA	47.27	9.65	4.9	Active
Methanol	100	VA	50.22	12.6	3.99	Active
Compound **1**	65	MA	18.64	<7.8	>2.31	Active
AZT	-	-	$>10^{-8}$	$4.31x10^{-9}$	>2.32	Active

[a]Anti-HIV-1 RT activity express as % inhibition at 200 µg/mL: very active (VA) = >70% inhibition, moderately active (MA) = 50% to 69% inhibition, weakly active (WA)= 30% to 50% inhibition and inactive (I)= <30% inhibition; For determination of IC_{50} in the HIV-1 RT assay, the coefficients of determination, R2, were 0.98−0.99 in all assays for 50% end point. [b]Anti-syncytium (MC99+1A2) EC_{50} = dose of compound that reduced 50% syncytium formation by ΔTat/RevMC99 virus in 1A2 cells. AZT, averaged from three experiments, EC_{50} 3.95 × 10^{-3} µM; [c]TI, TherapeuticIndex: IC_{50}/EC_{50}

Table 3. Cytotoxicity study of crude extracts and isolated compound

Crude extracts/ compound	Cytotoxicity ED_{50} (µg/mL) [d]							
	KKu-M213	FaDu	HT-29	MDA-MB-231	A 549	SH-SY5Y	MNN-K1	Hep G2
Hexane	14.56	11.64	13.29	13.48	11.06	6.70	11.50	11.94
Ethyl acetate	-	-	-	-	-	-	15.33	-
Methanol	-	-	-	-	-	-	-	-
Compound **1**	-	-	10.11	-	-	-	-	-
Ellipticine	0.60	0.58	0.59	0.59	0.47	0.41	0.44	0.61

[d]Cytotoxic assay: ED_{50} less than 20 µg/mL were considered active for extracts and ED_{50} less than 4 µg/mL were considered active for pure compounds. Cancer cell lines: KKU-M213 (Human cholangiocarcinoma) FaDu (Human squamous cell carcinoma) HT-29 (Human colon adenocarcinoma) MDA-MB-231 (Human mammary gland/breast adenocarcinoma) A 549 (Human lung adenocarcinoma) SH-SY5Y (Human neuroblastoma) MNN-K1 (highly differentiated immortalized human cholangiocyte cell line) Hep G2 (Human hepatocellular carcinoma)

Biological Activities of *C. oblonggifolius* two mechanisms of resistance to HIVs were tested and the cell lines group was tested. The results of the anti-HIVs of crude extracts were evaluated for their anti-HIV-1 activity employing reverse transcriptase (RT) and syncytium reduction assays using the $^{\Delta Tat/Rev}$MC99 virus in 1A2 cell lines systems as shown in Table 2. In the reverse transcriptase assay, ethyl acetate and methanol extracts exhibited very actively with IC_{50} values of 100 % inhibition. All extracts and compound **1** displayed potent activity in syncytium inhibition assay with an effective concentration at 50% (EC_{50}) value of <7.8 µM (TI>2.31). Further, the hexane extract showed marked cytotoxicity (ED_{50} = 6.70 µg/ml against the SH-SY5Y cancer cell line. Furthermore, compound **1** was also exhibited moderately active with IC_{50} 65% inhibition (Table 3).

4. CONCLUSION

In this research, the researchers brought plants that are not well studied, extracted, isolated and purified to obtain 3 classes of extracts and 1 pure substance. Then, they were tested for anti-AIDS and cancer. In order to prove the structure of compounds, spectroscopic techniques are used, which can make the structure clearer and more chemically informative. In addition, the potential of extracts and purified substances were also found to be used as a guideline for the development of anti-AIDS and cancer drugs in the future.

DISCLAIMER

This paper is an extended version of a preprint document of the same author.

The preprint document is available in this link: https://assets.researchsquare.com/files/rs-1461253/v1_covered.pdf?c=1649349092.

ETHICAL APPROVAL

This study had been ethically approved by the institutional animal ethical committee of Mahidol University, Thailand.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pinho PM, Kijjoa A. Chemical constituents of the plants of the genus Cleistanthus and their biological activity. Phytochem. Rev. 2007;6:175–182.
2. Samitinan T. Thai plant names (botanical names-vernacular names) 2514;141.
3. Rodríguez PA. The occurrence of piscicides and stupefactants in the plant kingdom. Advances in economic botany. 1990;1-23.
4. Perry LM, Metzger J. Medicinal plants of East and Southeast Asia:attributed properties and uses. MIT Press, Cambridge, London.1980;620.
5. Shi RF, Guo JJ, Wang YT, Yang BJ, Chen DZ, Hao XJ. Two new bioactive lignans from leaves and twigs of *Cleistanthus concinnus* Croizat. Natural Product Research. 2019;1-7.
6. Pinho PM, Naengchomnong W, Kijjoa A, Nazareth N, Artur MSS, Eaton G, Herz W. An unusual glucoside from *Cleistanthus gracilis*. Phytochemistry. 2006;67:1789–1792.
7. Sastry KV, RAO EV, Buchanan JG, Stureons RJ. Cleitanthoside B, A diphyllin glycoside Patulus heartwood. Phytochemistry. 1987;26:1153-1154.
8. Meenakshi J, S Govindaswamy. Cleistanthin A, a diphyllin glycoside from *Cleistanthus collinus,* is cytotoxic to PHA-stimulated (proliferating) human lymphocytes. Drug Dev. Res. 2000; 51:187–190.
9. Hillis WE, Yazaki Y. Properties of some methylellagic acids and their glycosides, Phytochemistry. 1973;12: 2963-2968.
10. Pompimon W, Sombutsiri P, Baison W, Udomputtimekakul P, Chuajedton A, Suksen K,Limthongkul J, Naparswad C. Cancer cytotoxic and anti-HIV potential of triphala herb mixture on from Chae Son, Lampang, Thailand. Journal of Pharmaceutical Research International. 2019;1-9.
11. Sandhya T, Lathika KM, Pandey BN, Mishra KP. Potential of traditional ayurvedic formulation, Triphala, as a novel anticancer drug. Cancer Letters. 2006; 231:206–14.
12. Shi Y, Sahu RP, Srivastava SK. Triphala inhibits both in vitro and in vivo xenograft growth of pancreatic tumor cells by inducing apoptosis. BMC Cancer. 2008;8:294.
13. Kiser R, Makovsky S, Terpening SJ, Laing N, Clanton DJ. Assessment of a cytoprotection assay for the discovery and evaluation of anti-human immunodeficiency virus compounds utilizing a genetically- impaired virus. J Virol Meth. 1996;58:99–109.
14. Nara PL, Hatch WC, Dunlop NM, Robey WG, Arthur LO, Gonda MA, et al. HIV, Perinatal infections, and therapy: The role of the placenta. AIDS Res Hum Retrovirus. Phytochemistry.1973;12:2963-2968.
15. Sharma A, Sharma KK. Chemoprotective role of triphala against 1,2-dimethyl hydrazine dihydrochloride induced carcinogenic damage to mouse liver. Indian. Journal of Clinical Biochemistry. 2011;26:290–5.
16. Silprasit K, Thammaporn R, Tecchasakul S, Hannongbua S, Choowongkomon K Simple and rapid determination of the enzyme kinetics of HIV-1 reverse transcriptase and anti-HIV-1 agents by a fluorescence-based method. J Virol Methods. 2011;171(2):381–7.
17. Singha A, Bajpaia V, Kumara S, Sharmac KR, Kumara B. Profiling of gallic and ellagicacid derivatives in different plant parts of *Terminalia arjuna* by HPLC-ESI-QTOF-MS/MS. Natural Product Communications. 2016;11(2):239-244.
18. Jang DS, Yoo NH, Kim JM, Lee YM, Yoo JL, Kim YS and Kim JS. An ellagic acid rhamnoside from the roots of *Potentilla discolor* with protein glycation and rat lens aldose reductase inhibitory activity. Natural Product Sciences. 2007;13(2):160-163.
19. Lee SY, Kim YK, Park NIl, Kim CS, Lee CY, and Park SU. Chemical constituents and biological activities of the berry of

Panax ginseng. Journal of Medicinal PlantsResearch. 2010;4(5):349-353.

20. Sun Y. Structure and biological activities of the polysaccharides from the leaves, roots and fruits of *Panax ginseng* C.A. Meyer:An overview. Carbohydrate Polymers. 2011; 85:490-499.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/94324