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Phytochemicals, cytotoxicity, and genotoxicity of three *Artocarpus* species reveal arbutin in *A. lacucha*

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ABSTRACT: Leaves of three *Artocarpus* species were hexane and ethanol extracted and chemically analysed. Cytotoxicity and genotoxicity tests were performed on peripheral blood mononuclear cells. The main phytochemical in the hexane extracts was (Z)-9-octadecenamide (oleamide). The ethanol extracts showed 21% 9,19-cyclolanost-24-en-3-ol in *A. altilis*, 19% *n*-hexadecanoic acid in *A. heterophyllus*, and 21% arbutin in *A. lacucha*. The hexane extract of *A. altilis* gave an IC_{50} of 0.00035 mg/ml but no IC_{50} values for *A. heterophyllus* and *A. lacucha* extracts. Ethanol extracts of *A. altilis* and *A. lacucha* had IC_{50} values of 0.868 and 0.218 mg/ml, respectively, but none for *A. heterophyllus*. All the hexane extracts showed non-significant DNA damage, while all the ethanol extracts significantly induced DNA damage ($p < 0.05$). *A. lacucha* leaf is newly reported as an alternative source of arbutin.

KEYWORDS: *Artocarpus altilis*, *Artocarpus heterophyllus*

INTRODUCTION

Asian countries possessing high biodiversity have consumed plant leaves in terms of cooking, i.e., vegetables, spices and seasoning, as well as fragrances. In addition, active compounds from extracts are applied in industries for the household, medicine, cosmetics and natural products. Leaves are sources of secondary metabolites which have long offered specific benefits to humans. Even though synthetic forms are comfortable amid daily use, they can be the cause of accumulated toxicity in the body amid long term use, for example, statin[1,2] and finasteride, a 5-α reductase inhibitor[3]. There are many well-known phytochemical substances which have long been used as treatments for diseases[4–6]. Statin can be replaced with β-sitosterol in the treatment of high blood cholesterol[7], which is a phytosterol synthesized only in plants for instance, *Polygonum multiflorum*[8], *Pygeum africanum*[4], *Serenoa repens*[4], *Gingko biloba*[4], *Viola odorata*[9] and *Cissus repens*[10]. Good health is supported by complete foods, essential phytochemicals (especially from plants as foods), vegetables, fruits and others for body mechanisms. As well as exercise, they form the basis of public health. With the mentioned reasons, leaves are factories for phytochemical productions including 5-α reductase inhibitors[5]; eugenol, isoeugenol, chavicol, chavibetol, caryophyllene, sabinene, phellandrene, germacrene A and germacrene D, and sesquiterpenes chavicol[11]; monoterpene, diterpene, triterpene and flavone[12]; squalene, α-tocopherol, a form of vitamin E[13,14]. With the aforementioned phytochemicals, gas chromatography-mass spectrometry (GC-MS), an important tool for chemical identification of plant extracts has successfully been applied for screening and identification of phytochemicals in many plant groups[14–17].

Plants found in genus *Artocarpus* have long been applied as foods and medicines. *A. lacucha* ('lakoocha') is one species of the genus shown to have resveratrol and oxyresveratrol in heartwood extracts and are employed as treatments of melanogenesis and microbial infections[18–20]. It is also a

valuable tropical tree species employed for its fruit, furniture production, timber, and animal feeding. The lakoocha fruits are generally eaten fresh, but can be utilized in pickles and sauces. Two more species, *A. altilis* and *A. heterophyllus* are edible fruit plants available worldwide, i.e., the renowned breadfruit and jackfruit. The other parts of plants have been shown to exhibit identical benefits to the *A. lacucha*. However, research has yet to take place regarding their leaves as a factory for phytochemicals. Leaves can provide worthwhile usage and are sustainable. Hence the aim of this study focuses on phytochemicals and the toxicity testing of the three species, *A. altilis*, *A. heterophyllus*, and *A. lacucha* leaves.

MATERIALS AND METHODS

Plant materials

Three *Artocarpus* species, *A. altilis* (voucher no. Chaveerach 957), *A. heterophyllus* (voucher no. Chaveerach 958), and *A. lacucha* (voucher no. Chaveerach 956) were collected from Khon Kaen province, northeastern Thailand and were identified by Prof. Dr Arunrat Chaveerach. The voucher specimens were kept at Department of Biology, Faculty of Science, Khon Kaen University. Mature leaves were subsequently used for preparation of crude extracts via hexane and ethanol. Then, phytochemical analysis via GC-MS, cytotoxicity via haemacytometer counting, and genotoxicity by comet assay were performed.

Phytochemical extracts

Phytochemical extraction was performed as explained in the previous research[17]. The samples were rinsed with water and air-dried until water evaporated from the leaves. The dried leaves were subsequently finely ground with an electronic blender. Twenty gram of leaf powder was soaked in 120 ml hexane or ethanol (analytical grade) for 72 h in the dark at room temperature. The mixture was then filtered through filter paper. The filtrates from this step were aliquoted, 1 ml each, for GC-MS analysis. For the remaining filtrates, the solvents were evaporated from the compounds using a vacuum concentrator (ScanVac LaboGene, Denmark) at −20 °C, 200 rpm for 2 h until completely evaporated and solids were obtained. Dimethyl sulphoxide (DMSO) was gradually added to the solids until completely dissolved and concentration was calculated and set as stock extract maintained at −20 °C until cytotoxicity and genotoxicity experiments were conducted. Prior to toxicity experiments, the stock extracts were 10-fold serial diluted for five levels in the culture medium as working concentrations.

Analysis of plant extract component by GC-MS

GC-MS analysis was performed as previously explained[17] using an Agilent Technologies GC 6890 N/5973 inert mass spectrometer fused with a capillary column (30.0 m × 250 μm × 0.25 μm). Helium gas was employed as the carrier at a constant flow rate of 1 ml/min. The injection and mass-transferred line temperature was set at 280 °C. The oven temperature was programmed for 70–120 °C at 3 °C/min, held isothermally for 2 min, then raised to 270 °C at 5 °C/min. A 1 μl aliquot of the crude extract was injected in split-mode. The relative percentage of the crude constituents was expressed as a percentage using peak area normalization. Component identification was determined by comparing the obtained mass spectra with the reference compounds in the Wiley 7N.1 library.

Isolation of human peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from sodium heparin anticoagulated venous blood from a blood bank using Ficoll-Paque Plus (GE Healthcare) following the manufacturer's protocol. After isolation, the cells, with a viability of at least 98%, were suspended at a concentration of $4–6 \times 10^5$ cells/ml in modified RPMI-1640 medium supplemented with 2.05 mM L-glutamine, 10% FBS, 100 μg/ml streptomycin, and 100 U/ml penicillin.

Extract treatment for cytotoxicity assay

The prepared cells in the RPMI-1640 medium were seeded in 96-well plates, 125 μl per well. Another 12.5 μl of the proper extract working concentrations were added to the corresponding wells in triplicate. The plates were subsequently incubated in a humidified incubator at 37 °C with supplied 5% CO_2 for 4 h. The untreated cells, without addition of any extracts were applied as a negative control.

The cytotoxicity of the plant extracts was estimated by haemacytometer cell counting method. Post-treatment, the numbers of viable cells were counted under a microscope following erythrosine staining. To reveal the cytotoxicity of the plant extracts, cell viability was calculated as: (treated cells)/(negative control cells) and expressed as a percentage. All values were expressed as mean ± SE. Doses inducing 50% inhibition of

Table 1 Phytochemical constituents in the six extracts of three *Artocarpus* species.

Chemical	Formula	Relative content (%)					
		A. altilis		*A. heterophyllus*		*A. lacucha*	
		hexane	ethanol	hexane	ethanol	hexane	ethanol
α-Amyrin	$C_{30}H_{50}O$	0.69	1.55	0.32	3.38	0.54	2.52
β-Amyrin	$C_{30}H_{50}O$	–	1.33	–	2.99	–	3.22
Arbutin	$C_{12}H_{16}O_7$	–	–	–	–	–	20.61
Benzyl β-D-glucoside	$C_{12}H_{18}O_6$	–	–	–	–	–	0.49
Campesterol	$C_{28}H_{48}O$	0.42	0.94		1.22	–	1.69
Cholesta-4,6-dien-3-ol	$C_{27}H_{44}$	–	–	–	0.11	–	0.08
(3β)-9,19-Cyclolanost-24-en-3-ol, acetate	$C_{32}H_{52}O_2$	–	21.42	–	2.30	–	–
Cyclopenta[d]anthracene-8,11-dione, 1,2,3,3a,4,5,6,6a,7,12-decahydro-3-isopropyl-6-methylene-	$C_{21}H_{26}O_2$	–	0.27	–	–	–	–
Docosane	$C_{22}H_{46}$	0.93		0.98		0.79	–
1,10-Epoxy-4-methoxy-7-methyl-8-(3,4-methylenedioxyphenyl)-spiro(5,5)undeca-1,4-dien-3-one	$C_{20}H_{20}O_5$	–	–	1.11	–	–	–
Friedelan-3-one	$C_{30}H_{50}O$	–	0.47		–	–	0.38
Germanicol	$C_{30}H_{50}O$	–	–	–	–	–	0.45
Glycerin 1-monostearate	$C_{21}H_{42}O_4$	1.63	–	1.61		1.12	0.28

cell viability (the IC_{50} value) were determined by plotting a graph between extract concentration and cell viability (%). Then the concentrations showing IC_{50} were used for LD_{50} calculation[21] to release hazardous levels[22].

Genotoxicity assay by comet assay

The prepared cells in the 96-well plates were treated with the proper extracts at concentrations showing IC_{50} values. In the case of no IC_{50} values, the highest concentrations were applied. Positive control cells were exposed to UV light for 15 min. The negative and vehicle controls as well as the incubation were performed as in the cytotoxicity assay. Subsequent to incubation, alkaline comet assay was performed as per the previously described method[23]. To quantify the level of DNA damage, the extent of DNA migration was defined using the 'Olive Tail Moment' (OTM), which is the relative amount of DNA in the tail of the comet multiplied by the median migration distance. Comets were observed at 360 magnifications with images obtained via an image analysis system (Isis) attached to a fluorescence microscope (Nikon, Japan), equipped with 560 nm excitation filter, 590 nm barrier filter and a CCD video camera PCO (Germany). At least 150 cells (50 cells for each triplicate slide) were examined for each experiment. Image analysis IMAGEJ was employed to analyse the OTM. All experiments were repeated at least three times. The nonparametric Mann-Whitney test was utilized for statistical analysis of the comet assay results and $p < 0.05$ was considered as the statistically significant value.

RESULTS AND DISCUSSION

To study the majority of phytochemical constituents in plants, non-polar and polar solvents, hexane and ethanol were employed for extraction. The three studied species showed higher extract concentrations as a result of applying ethanol rather than hexane. This indicates that phytochemicals exist for the most part, in a polar group. Extract concentrations with hexane and ethanol were 3.5 and 18 mg/ml; 2 and 9.4 mg/ml; and 2.45 and 29 mg/ml in *A. altilis*, *A. heterophyllus*, and *A. lacucha*, respectively.

Phytochemical constituents identified in all six extracts are shown in Table 1. Total ionic chromatograms showing compounds of the studied extracts are displayed in Fig. 1. Phytochemicals constituting higher than 10% in the hexane extracts were 20% (Z)-9-octadecenamide in *A. altilis*; 26% (Z)-9-octadecenamide, 12% hexacosane, and 10% pentacosane in *A. heterophyllus*; and 23% (Z)-9-octadecenamide in *A. lacucha*. For the ethanol extracts, phytochemicals were 21% 9,19-cyclolanost-24-en-3-ol and 15% *n*-hexanedecanoic acid in *A. altilis*; 19% *n*-hexadecanoic acid and 15% β-sitosterol in *A. heterophyllus*; and 21% arbutin in *A. lacucha*.

For extract cytotoxicity, haemacytometer counting revealed that hexane *A. altilis* extract exhibited

Table 1 (Cont.)

Chemical	Formula	Relative content (%)					
		A. altilis		*A. heterophyllus*		*A. lacucha*	
		hexane	ethanol	hexane	ethanol	hexane	ethanol
Glycerol β-palmitate	$C_{19}H_{38}O_4$	2.50	1.07	2.19	0.67	1.62	0.64
Heneicosane	$C_{21}H_{44}$	3.39	–	3.37	–	2.82	–
Hentriacontane	$C_{31}H_{64}$	2.56	–	2.20	–	6.29	–
Heptacosane	$C_{27}H_{56}$	7.46	–	9.26	–	9.10	1.50
Heptadecanoic acid	$C_{17}H_{34}O_2$	–	0.19	–	0.14	–	0.27
Heptadecanoic acid, ethyl ester	$C_{19}H_{40}O_2$	–	–	–	–	–	0.12
1-Heptatriacotanol	$C_{37}H_{76}O$	–	–	–	0.77	–	–
Hexacosane	$C_{26}H_{54}$	9.62	–	11.50	–	10.97	–
Hexadecanamide	$C_{16}H_{33}NO$	1.97	0.19	2.25	0.29	1.58	0.30
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	–	15.14	–	19.36	–	7.90
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	–	2.64	–	9.22	–	3.50
Hexatriacontane	$C_{36}H_{74}$	1.36	–	1.61		1.74	
2-Hydroxy-5-methylisophthalaldehyde	$C_9H_8O_3$	–	–	–	0.31	–	0.61
3-Isopropyl-6-methylene-1,2,3,3a,4,5,6,6a,7,12-decahydrocyclopenta[d]anthracene-8,11-dione	$C_{21}H_{26}O_2$	–	–	0.76	–	–	–
Lidocaine	$C_{14}H_{22}N_2O$	0.35	–	0.22	–	0.25	–
Linoleic acid	$C_{18}H_{32}O_2$	–	1.43	–	1.98	–	0.69
Linolenic acid	$C_{18}H_{30}O_2$	–	4.58	–	4.56	–	2.72
Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)-	$C_{21}H_{36}O_4$	–	0.18	–	0.53	–	–
Linolenic acid, ethyl ester	$C_{20}H_{36}O_2$	–	2.35	–	5.46	–	3.97
Lupeol acetate	$C_{32}H_{52}O_2$	6.20	1.10	–		0.78	8.10
24-Methylenecycloartan-3-one	$C_{31}H_{50}O$	–	–	–	0.67	–	–
3-(1-Methylhept-1-enyl)-5-methyl-2,5-dihydrofuran-2-one	$C_{13}H_{20}O_2$	–	0.58	–	–	–	–
Nonacosane	$C_{29}H_{60}$	4.09	–	5.27	–	8.18	–
Octacosane	$C_{28}H_{58}$	5.56	–	7.19	–	7.11	–
Octadecanamide	$C_{18}H_{37}NO$	1.29	0.16	1.70	0.20	1.21	0.16
Octadecanoic acid	$C_{18}H_{36}O_2$	–	1.96	–	3.32	–	3.06
Octadecanoic acid, ethyl ester	$C_{20}H_{42}O_2$	–	0.57	–	0.53	–	1.43
(Z)-9-Octadecenamide	$C_{18}H_{35}NO$	20.40	2.14	25.50	2.77	23.36	2.57
Olean-12-en-3-one	$C_{30}H_{48}O$	–	–	–	–	–	0.64
Pentacosane	$C_{25}H_{52}$	9.32	–	10.44	–	9.82	–
Pentadecanoic acid	$C_{15}H_{30}O_2$	–	0.11	–	0.17	–	–
Phenol, 2-propyl-	$C_9H_{12}O$	–	–	–	–	–	7.24
Phytol	$C_{20}H_{40}O$	0.94	5.31	0.28	6.58	0.05	2.99
Phytol, acetate	$C_{22}H_{42}O_2$	–	5.34	–	5.95	–	1.44
Simiarenol	$C_{30}H_{50}O$	–	–	–	–	–	0.46
β-Sitosterol	$C_{29}H_{50}O$	4.31	8.13	2.65	14.55	1.30	4.84
Squalene	$C_{30}H_{50}$	0.63	6.75	–	2.02	0.14	4.38
Stigmasterol	$C_{29}H_{48}O$	0.30	0.42	0.52	2.29		0.35
Tetracosane	$C_{24}H_{50}$	7.21	–	7.28	–	7.04	–
Tetradecanoic acid	$C_{14}H_{28}O_2$	–	–	–	–	–	0.22
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	–	2.61	–	2.43	–	0.62
dl-α-Tocopherol	$C_{31}H_{52}O_3$	2.31	5.26	–	0.57	0.45	3.86
γ-Tocopherol	$C_{28}H_{48}O_2$	2.44	4.78	–	0.60		0.79
δ-Tocopherol	$C_{28}H_{48}O_2$	–	0.26	0.53	2.07	0.34	1.02
Triacontane	$C_{30}H_{62}$	2.47	–	3.33	–	3.66	–
2,4,6-Trihydroxybenzoic acid	$C_7H_6O_5$	–	–	–	–	–	3.02
Vitamin E		–	0.35	–	0.43	–	0.16

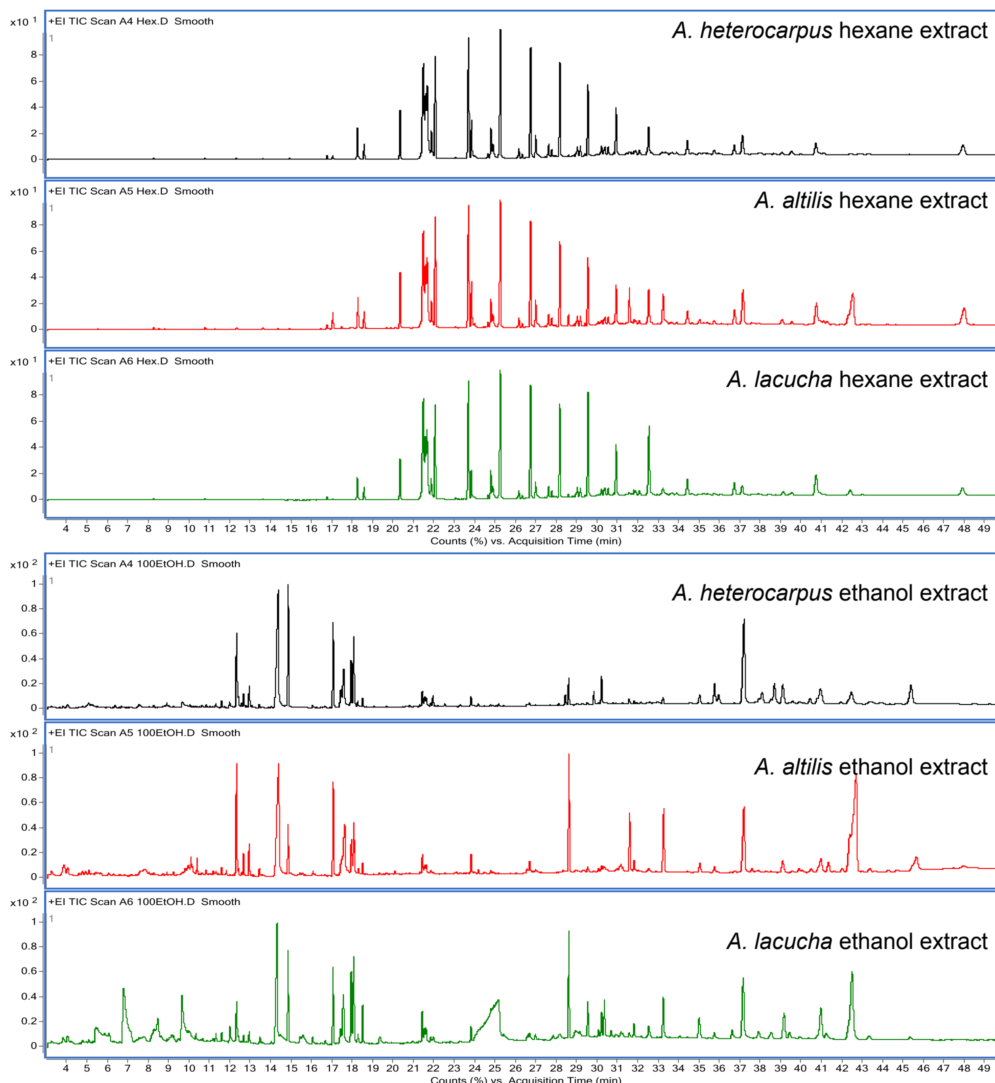

Fig. 1 Chromatograms of hexane and ethanol extracts from the leaves of *A. altilis*, *A. heterophyllus*, and *A. lacucha*.

IC_{50} value of 0.00035 mg/ml with calculated LD_{50} of 71.51 mg/kg. The predicted LD_{50} dose demonstrated that the *A. altilis* extracts belong to Class II (50–2000 mg/kg body weight, oral), moderately hazardous toxic chemicals according to WHO[22]. All extracts of *A. heterophyllus* and *A. lacucha* displayed cell viability percentages at 77 ± 15 and 62 ± 27 indicating cell viabilities of higher than 50%. Hence no IC_{50} values were calculated.

In addition IC_{50} values of the ethanol *A. altilis* and *A. lacucha* extracts were 0.868 and 0.218 mg/ml with calculated LD_{50} of 1309.57 and 783.26 mg/kg. Predicted LD_{50} dose demonstrated that all tested compounds of *A. altilis* and *A. lacucha* which also belong to the Class II of toxic chemicals. None exhibited IC_{50} value of *A. heterophyllus*, indicating cell viability percentage of 64 ± 13 (Fig. 2).

The extracts at IC_{50} values, 0.00035 mg/ml of hexane *A. altilis*, 0.868 mg/ml of ethanol *A. altilis* and 0.218 mg/ml of ethanol *A. lacucha*, as well as the highest concentrations of the species absent IC_{50} values including 2 mg/ml of hexane *A. heterophyllus*, 2.45 mg/ml of hexane *A. lacucha* and 9.4 mg/ml of ethanol *A. heterophyllus* were selected for further genotoxicity testing via comet assay. The results of comet assay showed that the hexane extracts of the three sample species exhibited no induced DNA damage in PBMCs, as indicated in Fig. 3 when compared to negative control, which was considered as the statistically non-significant DNA damage ($p > 0.05$). Meanwhile, the ethanol extracts inducing DNA damage in the cells displayed significant DNA

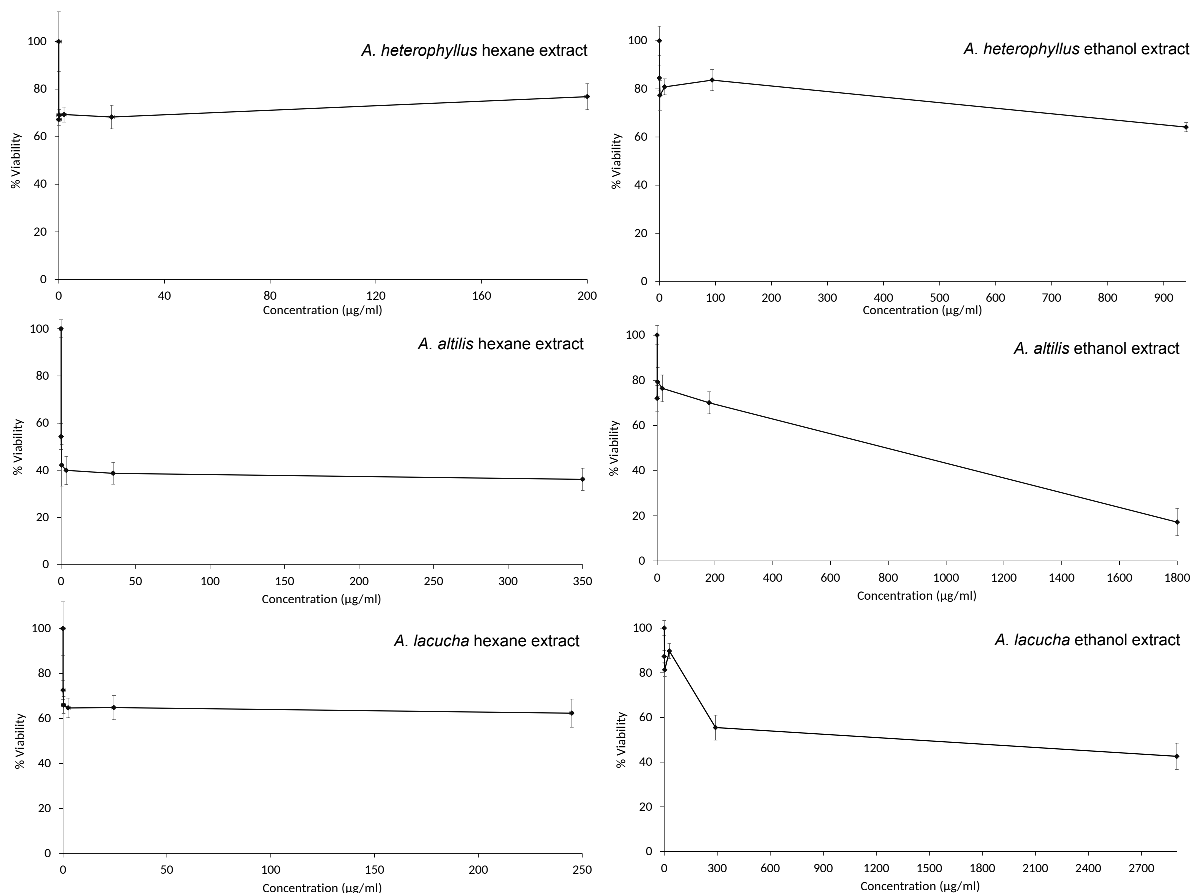

Fig. 2 Cytotoxicity tests of *A. altilis*, *A. heterophyllus*, and *A. lacucha* leaf hexane and ethanol extracts on human PBMCs. Percentages of cell viability are averaged from 3 replicate treatments.

Table 2 Comet assay results showing median of OTM of the PBMCs treated with the 6 extracts of three *Artocarpus* species. The numbers are averages of 150 cells from each of the three replicate treatments.

Plant	OTM			
	Control	Hexane	p val.	Ethanol
A. altilis	0.92 ± 0.73	0.99 ± 0.77	0.940	2.6 ± 2.2*
A. heterophyllus	1.01 ± 0.95	0.76 ± 0.62	0.103	2.6 ± 2.9*
A. lacucha	0.92 ± 0.73	0.91 ± 0.84	0.286	1.20 ± 0.70*

* $p < 0.0001$.

damage ($p < 0.05$) (Table 2). Presumably, these results are due to the fact that there are more polar than non-polar phytochemicals receiving high concentrations from ethanol extracts than hexane extracts.

In actuality, the phytochemicals contained within, are important factors for applying the appropriate plants to the appropriate treatments. Phytochemical functions ought to be concentrated on the substances at a high amount enough to be used despite hexane or ethanol solvent extractions. Besides that there are many significant phytochemicals identified in *Artocarpus* extracts. (Z)-9-Octadecenamide or oleamide, for example, was discovered at 20–26%, which is a substance important in human mechanisms. Furthermore it has been shown to be a protective agent against scopolamine-induced memory loss and is suggested to be a useful chemopreventive agent against Alzheimer's disease tested in vitro as well as mice at sufficient quantity[24, 25]. Here, a lower percentage may be applied. 9,19-Cyclolanost-24-en-3-ol found at 21% also known as cycloartenol, is an important triterpenoid in the sterol class which is found solely in plants. This is the starting subject for the synthesis of almost all plant steroids[26]. *n*-Hexadecanoic acid or palmitic acid, found at 15–19%, is a type of fatty acid. Also, β-sitosterol, one of the important phytosterol is beneficial for an array of human symptomatic treat-

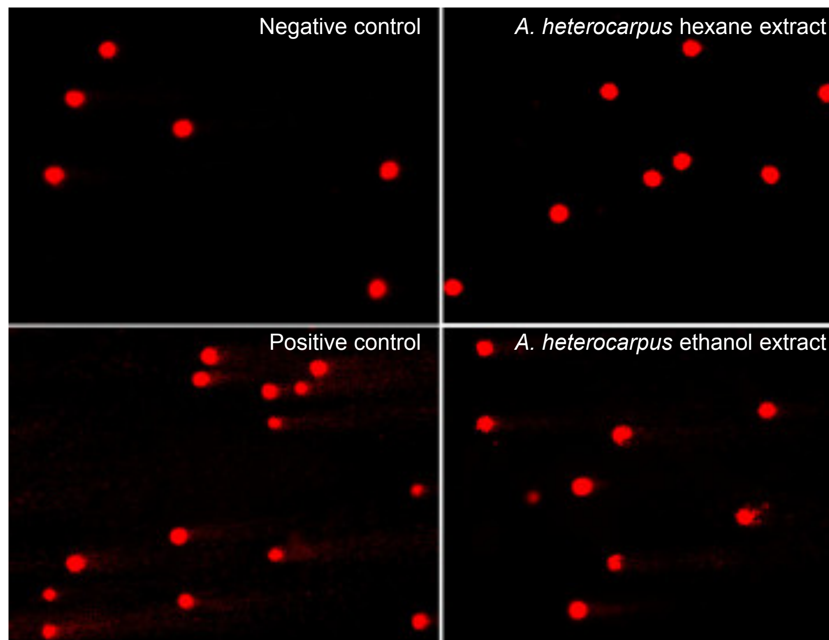

Fig. 3 Comet assay images of PBMCs showing no DNA damages in negative control cells and cells treated with hexane extract and significant DNA damages in positive control cells and cells treated with ethanol extract. Similar figures are not shown.

ments when utilized at suitable concentrations[27], i.e., at a rather low amount of 15%.

The most important phytochemical finding is arbutin in a high quantity at 21%, which is enough for actual use, as lower than 4% in health products has been tested to be safe for human consumption. Arbutin is a hydroquinone derivative that has been found in species of several plant genera, such as *Arctostaphylos*, *Bergenia*, *Lathyrus*, *Origanum*, *Pyrus*, and *Vaccinium*[28–32]. It is functioned as a tyrosinase inhibitor, which inhibits mammalian melanogenesis and is responsible for enzymatic browning reactions in damaged fruits during post-harvest handling and processing[28]. Thus it is used in a variety of cosmetics, particularly those aimed at lightening the skin, spot treatments, creams, lotions, soaps, serums and cleaners. The percentages found are rather similar as in the previous reported plants. Due to patent specifications, most skin care products contain plant extracts containing arbutin, for instance, bearberry (*Arctostaphylos uva-ursi*), pear (*Pyrus pyrifolia*) and lingonberry (*Vaccinium vitis-idaea*) rather than pure arbutin. Additional application of arbutin reasoned from tyrosinase function is that it may be used in fruit preservation[31–33].

Essentially, each phytochemical has specific functions, though not all are known. Hence for safe human usage, the testing of total substances contained for their cytotoxicity and genotoxicity was performed. Additionally, vehicle control (DMSO) was proved not to induce cell death at the highest (10%) tested concentration in PBMCs[17]. Hence those effects mentioned above are attributed only to the bioactive compounds of the plant extracts. Testing the effects of compounds on the viability of cells grown in culture is widely used as a predictor of potential toxic effect in whole animals[34]. Cytotoxicity assay revealed that all the extracts belong to Class II. However, there are no chemicals of interest in a high enough amount for treatments. For the *A. lacucha* extract with arbutin at a high quantity, it can certainly be utilized because low dose of arbutin is needed. Nonetheless, the comet assay result should be combined for consideration of the daily dose consumption.

All chemicals amid the three species studied induced DNA damage, though they were present in rather high concentrations.

Amid daily life, it is impossible to consume high doses per kilogram of body weight, as they present toxicity at over 500 mg/kg body weight when consumed orally. The authors suggest that applications of arbutin at concentration lower than 4%[33] may be used in a limited amount, during a limited time frame. The research revealed that the species of most interest, *A. lacucha*, contained high amounts

of arbutin; an alternative choice of arbutin in the context of Thailand. *Artocarpus* species are mainly distributed in Southeast Asia[35] and highly related to culture and life of the residences. These three studied species were listed as important species[36] among 50 species of the genus[37]. There have been reports on traditional uses as folk medicines[35–37]. Leaves of all the three species have been consumed as tea or foods. In Thailand, people have eaten young leaves of *A. heterophyllus* in wrapping tidbits. Furthermore, the *A. heterophyllus* leaves have been used as antisyphilitic and vermifuge agents, and for ulcer and wound healing, and its leaves and stem bark used to treat anaemia, asthma, dermatitis, diarrhoea, and coughs[37]. Formerly, resveratrol and oxyresveratrol were found in *A. lacucha* heartwood which has been used for inhibiting melanogenesis[20,38,39]. It can be used, though heartwood is cut from the stem, destroying the plants. Promisingly, leaves can be grown at any time, so they are worthwhile and sustainable amid application.

Arbutin or hydroquinone-beta-D-glucoside is one of the most important phytochemicals that have been used for health products, especially cosmetics. It was found in various plant species such as bearberry, blueberry, cranberry and mulberry. Arbutin is functioning as tyrosinase inhibitor which inhibits mammalian melanogenesis. Thus it is used in a variety of cosmetics such as spot treatments, creams, lotions, soaps, serums and cleaners, particularly those aimed at lightening the skin.

Even though its ethanol extract can induce DNA damage, this study suggested that, based on applications of arbutin lower than 4% in health products, usage in low amount can be safe for human consumption.

This study screens for phytochemical constituents in the three herbal edible plants including *A. altilis*, *A. heterophyllus*, and *A. lacucha* which are popularly grown and used as foods and medicines among the others in the genus. Arbutin was firstly reported in *A. lacucha* leaf with high quantity at 21%. It has never been reported in any Thai plants. Additionally, its existence in the leaves leads to convenient and sustainable use, the plant leaves can be high efficient sources of arbutin for cosmetic factories. However, its ethanol extract can induce DNA damage on human peripheral blood mononuclear cells. Hence the safety uses should be considered and further studies for its appropriate dose are required.

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