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Interdisciplinary researches for potential developments of drugs and natural products

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ABSTRACT

Developments of drugs or natural products from plants are possibly made, simple to use and lower cost than modern drugs. The development processes can be started with studying local wisdom and literature reviews to choose the plants which have long been used in diverse areas, such as foods, traditional medicine, fragrances and seasonings. Then those data will be associated with scientific researches, namely plant collection and identification, phytochemical screening by gas chromatography-mass spectrometry, pharmacological study/review for their functions, and finally safety and efficiency tests in human. For safety testing, *in vitro* cell toxicity by cell viability assessment and *in vitro* testing of DNA breaks by the comet assay in human peripheral blood mononuclear cells can be performed. When active chemicals and functions containing plants were chosen with safety and efficacy for human uses, then, the potential medicinal natural products will be produced. Based on these procedures, the producing cost will be cheaper and the products can be evaluated for their clinical properties. Thus, the best and lowest-priced medicines and natural products can be distributed worldwide.

1. Introduction

Countries located on tropical and subtropical regions have rather high biodiversity. Therefore, there are sources of natural materials used for foods, household products, cosmetics, medicines, and perfumes, both in industry and in local products. Plants are beneficial sources of various products and have been used from ancient times to the present. Knowledge of plants' uses and their specific properties in human beings, cosmetics and disease treatments have been passed from generation to generation. In their earliest uses, plants were rather directly consumed without technological processing, such as extraction, modification, mixing or purification. So, plants were consumed fresh or simply ground, boiled, or dried before consumption. Additionally, the chronic toxicities of plants have been rarely studied, but their advantages and acute toxicities have been studied, as in the following examples: *Allamanda* [1], *Cinnamomum* [2], *Cissus* [3], *Guibourtia* [4], parasitic plants [5], *Piper* [6], and *Terminalia* [7]. As current ethnomedicines, plant extracts have been studied as sources of chemicals to be purified for further use in perfumes, cosmetics, medicines, household products, *etc.* Further experiments on the use of plant species for disease treatment have been conducted. Cytotoxicity and genotoxicity have more recently been studied to evaluate the safety of concentrated use of plant-derived products on human health. Such a series of tests allows prepared products to be packaged for easy use and wide distribution. For example, *Curcuma longa* is a well-known, worldwide rhizome containing important substances, such as curcumin, demethoxycurcumin and bisdemethoxycurcumin, as well as volatile oils, which have long been continuously used in traditional medicine and as food seasonings in Southern Thailand. This rhizome has anti-inflammatory, antiviral, antibacterial, antioxidant effects and for the treatment of osteoarthritis. The species has been studied under several

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conditions and most recently in the form of capsules, powders and mixtures [8,9]. Some chemicals, such as those derived from *Diospyros* species, induce toxicity. Many members of the genus are noted to have a benefit for humans but have also been shown to be toxic. The medicinal uses and chemical constituents of various *Diospyros* species were reviewed and about 300 organic chemicals have been isolated and identified [10]. *Diospyros* species are valuable of Chinese herbal medicine, Tibetan medicine, and Ayurvedic medicine. Fruits of 29 *Diospyros* species, methanol extract yields were shown the presence of bioactive constituents of alkaloids (82%), flavonoids (68.97%), tannin (55.17%), terpenoids (100%), and essential oils (100%) [11]. Chemical and functional analyses of the fruits of a number of *Diospyros* species will enable their safe and sustainable use.

The study and analysis tropical, subtropical plants for use in foods, cosmetics and disease treatments needs to be simple, with a limited preparation time and low cost. Information on plant-derived products can be extracted from widely distributed local knowledge in the countries and from chemical functions found in published papers. The simple steps to the interdisciplinary study of drug and natural product developments are as follows: local people and traditional healers are surveyed and or a literature review is conducted to acquire information on the ethnobotanical properties of native Thai plants; plants are collected and identified, chemical constituents are determined by gas chromatography-mass spectrometry (GC–MS); pharmacological literature review is conducted to acquire information on the activities of these constituents; cytotoxicity, viability, and genotoxicity tests for DNA breaks are conducted using the comet assay on human peripheral blood mononuclear cells (PBMCs); guidelines of safe plant use are established; and products are produced with the objectives of low cost, and shorten, and consistent clinical properties. Finally, the best and lowest-priced medicinal and natural products can reach the worldwide population by commercial processes as mentioned shown in Figure 1.

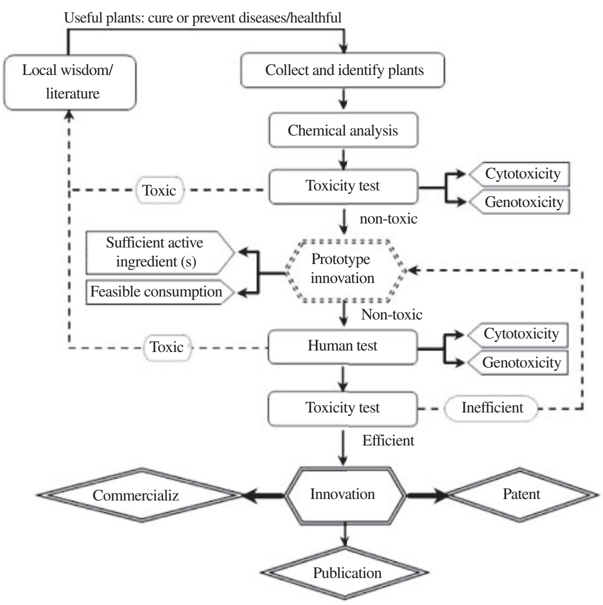

Figure 1. Diagram showing a simple step for the study, analysis and production of medicinal and natural products beneficial to humans for use in foods, cosmetics and disease treatments.

2. Literature reviews for ethnobotanical properties

The areas of high biodiversity are valuable for fruits, vegetables, lumber and several byproducts of plants, such as fragrances, resins and chemicals. Thus, the area's high biodiversity makes it golden location for foods of the world. Plants have long been used in the areas for foods, traditional medicines, cosmetics, seasonings, *etc*. without any technological management of their uses. Information passed from generation to generation, called local wisdom, is the best way to shorten the path of research on and draw attention to these useful plant-derived products. Therefore, studies on the use of these products in health care, health maintenance and disease treatment must be started from these points.

The popular *Piper* species, such as *Piper nigrum*, *Piper sarmentosum* and *Piper chaba*, have been widely used as vegetables and spices and in traditional medicines. Additionally, *Piper pendulispicum* is often used in kaeng khae, which is a type of Thai local food. *Piper betle* has been used in native ceremonies and has important volatile oils that are favored in household products, perfumes, medications and cosmetics. *Cissus* species have had important traditional uses in Thailand from ancient times to the present. A species, *Cissus repens* (*C. repens*) contains Khao-Yen Nuer and Khao-Yen Tai, which are also found in *Smilax china* and *Smilax glabra*, and has been used as an ingredient in several preparations, including those used in the treatment of lymphadenopathy, dermopathy, venereal diseases, leprosy, and cancers. Interestingly, despite substantial similarity between the drug preparations and the rhizomes, the drugs are available in traditional drug stores throughout the country. One more important, well-known species used worldwide, *Cissus quadrangularis* (*C. quadrangularis*), is used as a medication in both traditional and modified forms, including as tablets, capsules, and pure extracted substances, for weight loss and improved cardiovascular health [3]. Hemorrhoids and bone fractures have long been treated with *C. quadrangularis* in both its native and modernized forms. For hemorrhoids, it has analgesic properties and anti-inflammatory activity, and has been shown to reduce the size of the hemorrhoids, to hasten bone healing by initiating healing in fractures [12,13].

The medicinal recipes have included small amounts of the leaves, bark and milky sap of *Allamanda cathatica* (*A. cathatica*) in traditional medicines for use as laxatives and for inducing vomiting. When used in excess, however, these preparations become strong laxatives and cause excess vomiting and sometimes death. The active chemical of this species to be allamandin, which is a toxic iridoid lactone and cathartic agent, and thus a laxative [1,14,15].

The plants with potential interesting uses mentioned above should be studied at both the genus and species level. The discovery of these economically beneficial plants is important for industry and for efforts to provide a growing population with supplements from natural sources. The plant uses noted in the literature were brought to the attention of researches by local persons using them in communities, which can shorten the investigation process.

3. Plant collection and identification

For the reasons noted above, the species of an interesting genus have been investigated by exploration, collection and identification. For exploration, investigators should provide essential data, such as plant habits, area distribution, altitude, and approximate species numbers, as much as possible. Then, researchers should explore the areas similar to those initially explored. At least three complete specimens should then be collected, including mature leaves, flowers and fruit. Additionally, natural characteristics, such as for leaf shape, should be noted and described. Moreover, the local names of the plants and their uses exhibited by the forest guides, local people and traditional healers should be recorded. Investigations should then be repeated, concentrating on complete specimens and plant characteristic data. Plant morphological data should then be compared within specimens of a given plant species from a different growing area to determine character variations. These data are generally required for a formal description and consequent taxonomic identification. The collected plants should then be prepared as plant specimens and kept in a public national herbarium with collector numbers. The natural data need to be recorded on a plant sheet. Finally, approximately two years later, the collectors will have herbarium specimen numbers.

Investigating the uses of the several *Piper* species and following the steps for collection and identification, the species diversity of the *Piper* genus in Thailand was reported to extend or develop alternative uses for the species. The investigation has covered almost the entire area of the country, both in fields and herbariums. There were 43 species including new species in the genus [16,17]. The samples and type specimens are kept at the BK Herbarium, Bangkok, Thailand.

The species diversity of the *Cissus* genus in Thailand was explored, and specimens were collected to further study of chemicals to be used in parallel with *C. quadrangularis*. There are eight species of this genus: *Cissus assamica* (*C. assamica*), *Cissus carnosa* (*C. carnosa*), *Cissus elongata, Cissus hastata* (*C. hastata*), *Cissus javana, Cissus pteroclada, C. quadrangularis*, and *C. repens*. The sample specimens were kept at Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand [3].

Finally, the *Allamanda* genus was found to have the following species in Thailand using the steps outlines above: *Allamanda blanchetii* (*A. blanchetii*), *A. cathatica, Allamanda neriifolia* (*A. neriifolia*), *Allamanda schottii* (*A. schottii*) and *Allamanda violacea* (*A. violacea*). The sample specimens were kept at Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand [1].

4. Chemical constituents by gas chromatography-mass spectrometry based on chemical and genetic relationships

The chemical constituents of *Piper betloides*, *Piper crocatum*, *Piper maculaphyllum*, *Piper rubroglandulosum*, *Piper semiimmersum*, *Piper submultinerve*, *Piper tricolor*, and *Piper yinkiangense* were investigated using hexane as the solvent. *Piper betle* or betel leaf has been used in the aromatic oil industry, with the oils being used in several household products, including perfumes, seasonings and cosmetics. The authors found that there were some Piper plants other than *Piper betle* that possess a betel-like scent, viz. the eight species mentioned above. Thus, it was expected that these plants would contain similar useful chemicals. The extracts were screened for chemical constituents by GC–MS. The extracts contained some important chemicals that are similar to the betel extract, including eugenol, isoeugenol, chavicol, caryophyllene, sabinene, phellandrene, germacrene A and D, and sesquiterpenes. The results indicate that the eight plant species should be as useful as the betel plant for industrial purposes. The eight species are wild, so they have been documented to have greater vigor, i.e., better growing and producing more branches and leaves, than betel. These are important factors for sustainable use and can enable conservation management for posterity [6].

It is clear that *C. quadrangularis* has served human beings as a natural source of traditional treatments and chemicals from ancient times to the present, in both Thailand and worldwide. There are eight *Cissus* species in Thailand, so the question is whether other species in the group may be used in the same ways as *C. quadrangularis*. Therefore, the research aimed to analyze the genetics and chemical constituents of some *Cissus* species by comparing them to *C. quadrangularis*. This enables shortened scope and timing as well as specific destination species based on the genetic relationships established by the inter simple sequence repeat (ISSR). From this investigation, four species were selected based on having higher S values (the S values of the four species, *C. assamica, C. carnosa, C. hastata*, and *C. repens* are 0.64–0.72) than those of the remaining four species (*C. assamica, C. carnosa, C. hastata*, and *C. repens*, whose values are 0.59, 0.57, 0.59, and 0.60, respectively), compared to *C. quadrangularis* and selected to be undergone chemical study by GC–MS. Phytochemical screening of the methanol crude extract of *C. assamica, C. carnosa, C. hastata*, and *C. repens* species showed the presence of both varied and identical chemical compounds as represented by a total ion chromatographs (TIC) in Figure 2. The substances found are as follows. Four compounds, β-sitosterol, phytol/phytol isomer, hexadecanoic acid, and n-hexadecanoic acid found in the studied species [3], are identical compounds reported in *C. quadrangularis* [18,19] with different percentages. Vitamin E was found in all four studied species, but not reported in *C. quadrangularis*. Three compounds, namely, hexadecanoic acid, methyl ester; 2,6,10,14,18,22-tetracosahexaene; *n*-hexadecanoic acid were found in three of the species studied. Four chemicals such as 4-vinylphenol, 9,12,15-octadecatrien-1-ol, neophytadiene, stigmasta-5,22-dien-3-β-ol/stigmasta-5,22-dien-3-ol were found in two of the species studied. Three compounds were found in only one species including (1R, 3R, 4R, 5R)-(−)-quinic acid in *C. assamica*; 4-methoxy-3,5-dihydroxybenzoic acid; 23-R-methylcholesterol in *C. hastata* (Table 1). The four selected species were found to have S values, 0.64–0.72, higher than those of *C. quadrangularis*, in accordance with the types of compounds found [3].

The various chemicals found in the plants are in agreement with their various medicinal uses. *Cissus quadrangularis* has properties that aid in weight loss, improve cardiovascular health, hemorrhoids and bone fracture healing, as well as having anti-inflammatory properties [12,13]. The other species that were studied, such as *C. repens*, are used as medical treatments in Thailand by superseding or being used concomitantly with *Smilax china* and *Smilax glabra* for many disease treatments. All three species are used in the traditional medicine setting under the local names ‘khao-yen nuer’ and ‘khao-yen tai’. In addition, *C. carnosa*, locally called ‘hun’ in Thai, is used to treat inflammation of the hands and feet [3].

The above discussion outlines the shortened, efficient pathway to use indigenous Thai species, such as *C. quadrangularis*, that have long been used for disease

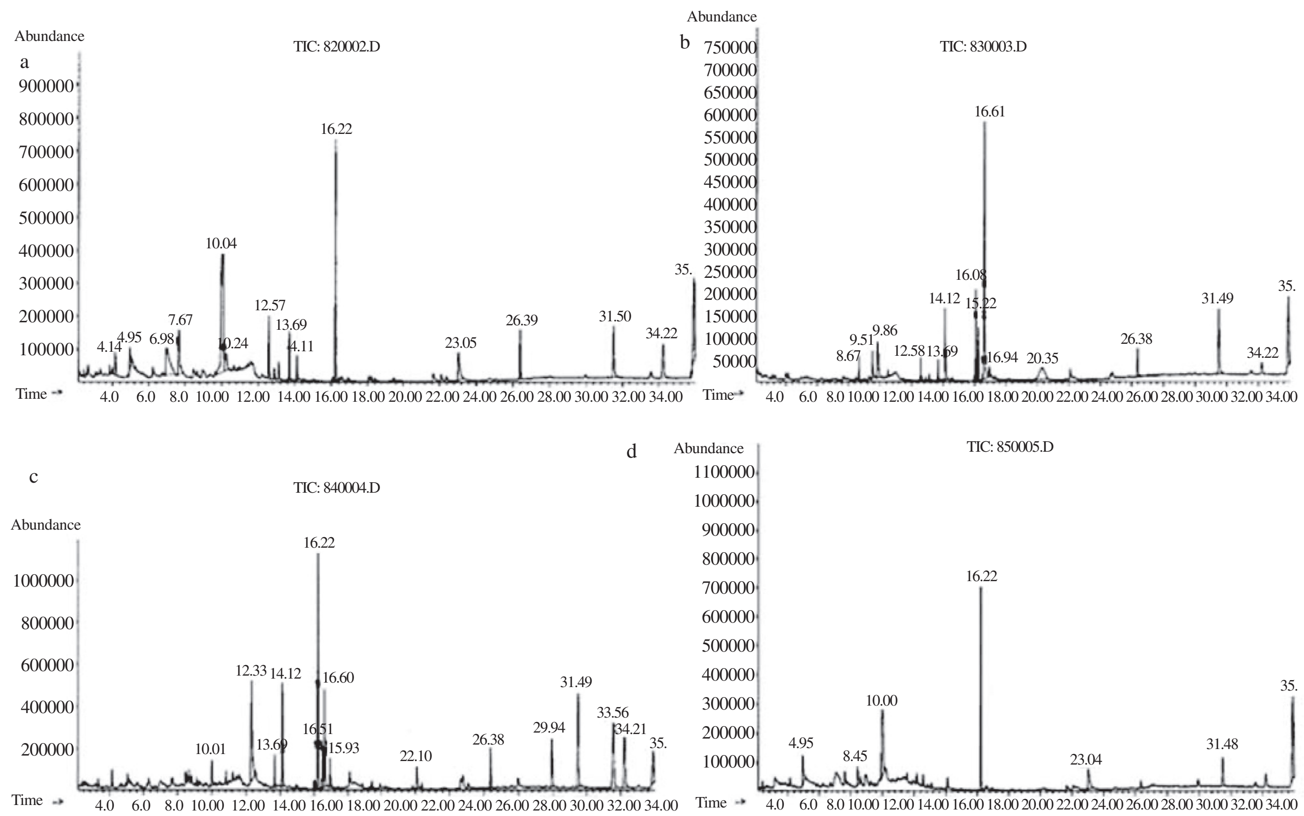

Figure 2. GC–MS chromatogram of methanol crude extracts on the leaves of the four *Cissus* species, *C. assamica* (a), *C. carnosa* (b), *C. hastata* (c) and *C. repens* (d) [3].

Table 1

Chemical constituents in each studied *Cissus* species [3] compared to *C. quadrangularis* [18,19].

Chemical	Relative content (%)				
	C. assamica	*C. carnosa*	*C. hastata*	*C. repens*	*C. quadrangularis*
(1R, 3R, 4R, 5R)-(−)-Quinic acid	27.24	nd	nd	nd	nd
4-Methoxy-3,5-dihydroxybenzoic acid	nd	nd	14.66	nd	nd
Phytol, phytol isomer	12.51	4.86	14.38	22.46	15.98
23-R-Methylcholesterol	nd	nd	11.60	nd	nd
Vitamin E or D-α-Tocopherol	6.05	11.64	16.58	7.01	nd
2,6,10,14,18,22-Tetracosahexaene	3.52	2.97	3.04	nd	nd
Hexadecanoic acid, methyl ester	2.33	1.36	9.1	nd	7.57
β-Sitosterol	10.1	13.54	5.41	25.17	na.
4-Vinylphenol	1.77	nd	nd	7.17	nd
9,12,15-Octadecatrien-1-ol	nd	33.5	5.4	nd	nd
Neophytadiene	3.58	1.61	nd	nd	nd
n-Hexadecanoic acid	1.53	6.12	6.67	nd	25.63
Stigmasta-5,22-dien-3-β-ol/Stigmasta-5,22-dien-3-ol	4.94	nd	8.66	nd	nd

nd: Not detected.

treatments to produce safe products with low cost and high medical effectiveness.

Currently, there are medicinal products produced by Chao Phraya Abhaibhubejhr Hospital, Prachinburi Province, Thailand that are directly dispensed to patients by doctors. These include *Cissus* species products.

There was another research result that demonstrated the matching of disease treatment and chemical content. There are five *Allamanda* species in Thailand: *A. blanchetii, A. cathatica, A. neriifolia, A. schottii* and *A. violacea*. Some publications have reported the chemical constituents of *A. cathartica* [14,15]; so, the remaining four *Allamanda* species were later studied, and their hexane crude extracts revealed some beneficial chemicals as follows. A large amount of squalene was found in *A. blanchetii* and *A. violacea*, at 55.81% and 51.09%, respectively, and a minor amount of squalene was found in *A. neriifolia*, at 6.08% [1]. All plants, animals, and humans produce squalene, which is a triterpene necessary for life. In the human body, squalene is a natural and essential component used for a precursor of cholesterol biosynthesis. It is extensively used as an excipient in pharmaceutical formulations for disease management and therapy. In addition, squalene acts as a protective agent and has been shown to decrease chemotherapy induced side effects, exhibit chemopreventive activity [20]. It shows some advantages for the skin as an emollient and antioxidant, and for hydration and its antitumor activities. It is also used as a material in topically applied vehicles such as lipid emulsions and nanostructured lipid carriers [21]. α-Tocopherol, a form of vitamin E that can be absorbed and accumulated in humans, is one of the predominant components of three of the four species, including *A. violacea*, *A. schottii*, and *A. neriifolia* at 26.33%, 15.41%, and 9.16%, respectively. A final substance, 9,12,15-octadecatrien-1-ol, was found at relatively high levels in *A. neriifolia* (15.51%) and *A. schottii* (17.31%); however, its activity has never been reported [1]. The discovery of such chemicals in these species and other plant species can provide an alternative and supplemental method for improving human well-being that can be used by pharmaceutical industries using natural resources. Such use is particularly important given to the increasing world population. However, please concentrate that there are phytochemicals beneficial to human health, but actually *A. cathartica*, *A. violacea* which are in the genus *Allamanda* have toxicity to PBMCs and DNA, so human be careful to use (data not shown). Additionally, LD_{50} values will inform consumers that how much to take for health or may be toxic both acute and chronic poisoning [22].

5. Pharmacological study/review

Pharmacology is a scientific branch related to medicine and concerned with the study of drug activities beneficial for living things, such as humans. The study of animal bodies is a vital part of this research process. Alternatively, basic cell processes can be investigated for the same purposes. The results of interest are the activities of the drug and its short-term effects and the selection of studied animals. The animals need to be similar to humans in their physiological functions, such as breathing, digestion, movement, sight, hearing and reproduction. Humans share common illnesses with animals; therefore, animals can act as models for the study of human illness. For example, rabbits suffer from atherosclerosis (hardening of the arteries); as well as other diseases, such as emphysema; and birth defects, such as spina bifida. Dogs suffer from cancer, diabetes, cataracts, ulcers and bleeding disorders, such as hemophilia, which make them natural candidates for research on these disorders. Cats suffer from some of the same visual impairments as humans. From such models, we learn how disease affects the body, how the immune system responds, who will be affected, and more (http://www.animalresearch.info/en/designing-research/why-animals-are-used).

Additionally, humans may be directly experimented on in some cases. The following investigations were carried out on humans, mice, rats and dogs. A comparison of the effects of proprietary extracts of *C. quadrangularis* (CQR-300) to those of a proprietary formulation containing CQR-300 (CORE) on weight, bold lipids, and oxidative stress was performed in overweight and obese people [3,23]. The effects associated with hemorrhoids, *i.e.*, the analgesic and anti-inflammatory activities, and the venotonic effect of the methanol extract of *C. quadrangularis* provoked a significant reduction in the number of writhes in the acetic acid-induced writhing response in mice. In an experiment in dogs, an effect of *C. quadrangularis* in accelerating the healing process in an experimentally fractured dog radius-ulna was observed [12,13]. Experiments on the effects of oleamide from *Zizyphus jujuba* on choline acetyltransferase and the associated cognitive activities were performed in mice. The oleamide enhanced the activity of the enzyme choline acetyltransferase, which is crucial to the production of the hormone acetylcholine, which contributes to Alzheimer's disease [24].

Currently, the functions of plant chemicals can be determined by searching or reviewing publications as mentioned above. More information on medicinal plants, chemicals and their activities are revealed in books, for example, Herbal Bioactives and Food Fortification, Extraction and Formulation [25].

Therefore, one step of pharmacological study is to review the literature in this way, shortening one time-consuming research step.

Similarly, natural products are derived in a same way as mentioned above, differently in herbal register or supplements.

6. *In vitro* cell toxicity by cell viability assessment

Cytotoxicity tests are a popular method for determining the cell death, cell division and cell growth induced by various chemicals, pesticides, herbicides and heavy metals in studies of phytochemical toxicity, drug development, and environmental toxicity [26]. The one kind popular cell for viability assessment is human white blood cells or PBMCs.

After the previous steps, cytotoxicity needs to be evaluated to support the safe use of the plants. PBMCs are commonly available and effective for this purpose. The concentration of viable PBMCs for further cytotoxic tests was calculated as 4–6 × 10^5 cells/mL using a hemocytometer and a trypan blue staining. The cells were incubated with the plant extracts, for examples, *Allamanda* and *Plumeria* species and *Tiliacora triandra* [27], in various concentrations diluted from mass extracts with dimethyl sulfoxide (DMSO) starting from harmless percentage (less than 10%) at 37 °C for 4 h. The untreated cells (negative control) were incubated in culture medium only. Positive control cells were incubated with 100 μmol/L H_2O_2 for 15 min. Each experiment was performed in triplicate. The viability percentage was calculated using the following equation: cell viability (%) = (average of treated cells/average of untreated cells) × 100. Additionally, the concentration of the extract that produced 50% cell death (IC_{50}) was reported by plotting graph of extract concentrations against cell viabilities as an example in Figure 3. The cytotoxic effect of the plant extracts was defined by the loss of membrane activity when treated with trypan blue dye. The experiments indicated a clear dose-dependent cytotoxic effect of the plants on PBMCs with a final concentration range for the plant extracts. Alternatively, one more method for cytotoxicity test is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay performing in 96-well microplates. Cellular reduction of MTT, formed a violet crystal formazan through mitochondrial succinate dehydrogenase activity of the viable cells, and the violet crystal formazan was quantified by a

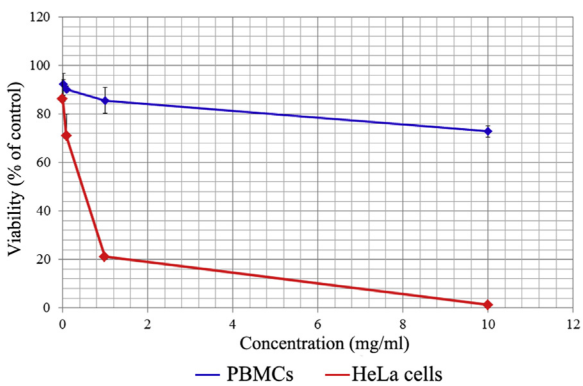

Figure 3. Cytotoxicity of the *Tiliacora triandra* extract toward human peripheral blood mononuclear cells (PBMCs) and HeLa cells [27].

microplate spectrophotometer (Fluorescence microplate reader) at the absorbance of 570 nm. The wells containing medium and MTT without cells were used as blanks. When the percentage of cell viability and IC_{50} were reported, LD_{50} will be calculated following Walum [28] to release hazardous levels [22].

7. *In vitro* testing of DNA breaks by the comet assay

Genotoxicity is a deeper level of toxicity than cytotoxicity. The comet assay is a popular technique used to measure genotoxicity. For this assay, PBMCs are treated with the plant and product extracts. Comet assay is a well-established, sensitive method for detecting single and double-strand DNA breaks, alkali labile sites, DNA cross-links, base, base-pair damages and apoptotic nuclei. It is a simple and cost-effective procedure with numerous variations and applications to provide answers to important questions concerning the background levels of DNA damage in normal and abnormal cells, variation in repair capacity within the human population and regulation of DNA repair at the molecular level within the nucleus [29]. Thus, the authors used the comet assay to detect DNA damage in PBMCs after treatment with the plant and product extracts. The comet assay was performed using the IC_{50} of each species according to the methods described previously [27,30,31] with slight modifications. In case plants have no IC_{50}, the highest plant extract concentrations have to be used instead. The alkaline comet assay was used to assess the genotoxicity of plant extracts. Briefly, after incubation of freshly isolated PBMCs with the extracts, the comet assay was performed. 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. The comets were observed at 200 magnifications, images were obtained using Isis (http://www.metasystems-international.com/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 of triplicate slides) were examined for each experimental point. Image analysis ImageJ software (http://imagej.nih.gov/ij) was used to analyze the OTM. The nonparametric Mann–Whitney test was used for statistical analysis, $P < 0.05$ was considered as the statistically significant value. DNA comets of PBMCs when treated with the plant extract indicating significant DNA damage ($P < 0.05$) compared to untreated negative control are shown in Figure 4 and examples of the median of OTM values are shown in Figure 5.

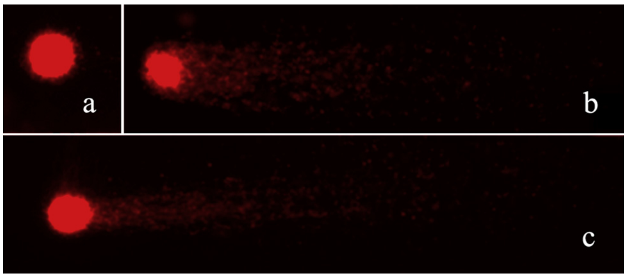

Figure 4. DNA comets of PBMCs (a–b) untreated (negative control) (a) and treated with 10 mg/mL of the plant extract (b), indicating significant DNA damage ($P < 0.05$), and HeLa cells treated with 0.41 mg/mL (the IC_{50}) of the plant extract (c), indicating significant DNA damage ($P < 0.05$) (200×) [27].

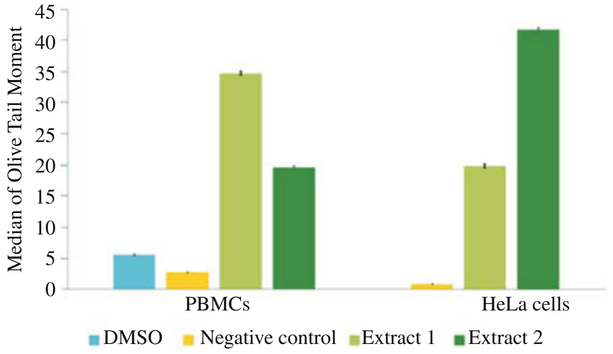

Figure 5. The median of Olive Tail Moment (OTM) values of DNA from PBMCs and HeLa cells treated with the plant extracts compared to negative control cells not treated with any chemical.
PBMCs treated with DMSO alone, a dissolving agent for plant extract, was included for reference [27].

8. Conclusion

Then, medicinal, natural products were potentially produced at low cost and checked for clinical properties. The processes were started with edible or medicinal plants considered with the determination of plant cytotoxicity and genotoxicity coupled with the high concentration of important chemicals met the objectives, and other substances beneficial to human health warranted the developments of the drug, natural products produced from the plants prepared as a capsule, powder, tablet or another forms depending on the plant parts used and the convenience of the use without costly purification or extraction. The advantage of this method of preparation is its low price, with benefits from other nutrients equivalent to those gained from consuming the vegetables.

The medicinal, natural products were tested to confirm its human safety, and doses. Finally, the patent should be reviewed and finally, the best natural medicines can reach the world's population with the following product advantages:

1) Low cost and a short research time.
2) Low cost for medicinal production as there is no extraction or purification but only plant collection, a manufacturing process for the medication, natural production and packaging.
3) No side effects, equivalent to consumption of the vegetables.
4) Low prices for instantly consumable forms.
5) Convenient for the use.

Conflict of interest statement

We declare that we have no conflict of interest.

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