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Research Article
Essence Supports Ethnomedicinal Uses of *Paederia foetida*, *Paederia linearis* and *Rotheca serrata*

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Abstract

Background and Objective: The plant species, *Paederia linearis*, *Paederia foetida* and *Rotheca serrata* have long been used as a traditional medicine for male vitality. So, they were investigated for data that supports their traditional safe uses including phytochemicals, investigating possible mechanisms and toxicity testing of their range of medicinal properties. **Materials and Methods:** Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) was were used for phytochemical analysis. Toxicity testing including cytotoxicity and genotoxicity as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and comet assays were investigated. **Results:** The results of this research were shown the following: Major phytochemicals were phytol and squalene in *P. linearis*, phytol in *P. foetida*, while other compounds were found in small amounts and most of them were unknown substances (whereas, *Rotheca serrata* was previously reported to contain phytol and oleamide). The L-dopa was found in the hexane extracts of all three plants. The rice bran oil extracts of *P. linearis* and *P. foetida* which can be edible showed higher L-dopa amounts and concentrations than the hexane extracts. The MTT assay indicated no toxicity at the cell level, but the comet assay showed significant ($p<0.01$) toxicity at the DNA level in all three plant extracts. However, when evaluated for human dosing, they can be safely consumed orally. **Conclusion:** These investigated compounds support the three studied plants having long been used traditionally in Thailand in a fresh form with several active phytochemicals including L-dopa.

Key words: L-dopa, *Paederia foetida*, *Paederia linearis*, *Rotheca serrata*, phytochemicals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Herbs are an alternative treatment for human disease that has been used for millennia as an essential part of human existence. Many species of the genus *Paederia* have long been studied along for their uses in human health. Many chemical constituents and activities have been found in these species, as follows. The *P. foetida* extract had a dose-dependent influence on serum testosterone levels, supporting its use of it as an aphrodisiac in traditionalmedicine[1]. *Paederia foetida* contains several phytochemicals such as peruloside, phenolic compounds, alkaloid, volatile oil, phytosterols, etc. and its properties against ulcer, diarrhoea, hyperglycemic and liver protection[2], the local people have popularly consumed the raw or steamed *P. foetida* rather than the traditional medicine, the leaves contained a high amount of vitamin C[3], *P. foetida* and *P. scandens* displayed iridoids, flavonoids, volatile oil and other metabolites and occupy several bioactivities against nociceptive, inflammatory, diarrheal, tussive and tumour; an injection that has been clinically used as an analgesic drug was created from *P. scandens*[4], *P. foetida* is a potent gastroprotective agent[5], *P. foetida* inhibits systemic and topical inflammation in a dose-dependent manner[6], *P. foetida* has antioxidant properties due to phenolics, particularly flavonoids[7]. One more species, *Rotheca serrata* has a long history of use, formerly named *Clerodendrum serratum,* with the following research review: Its properties are anti-rheumatic, anti-asthmatic, febrifuge, in cephalalgia and ophthalmia, composes many phytochemicals for example hispidulin, verbascoside, oleanolic acid, cleroflavone, apigenin, scutellarein, serratagenic acid and phytosterols[8], roots and leaves of *C. serratum* has therapeutic potential for many disease treatments such as allergy, inflammation and liver disorders characterized to various flavonoids, phenolics and saponins containing[9], a review reported that its activities including anti-activities for inflammatory, cancer, nociceptive, analgesic, allergic, cholinesterase and hepatoprotective effect, addition with potentiality for many disease treatments[10], antibacterial activity of pathogenic bacterial strains named *Staphylococcus hominis* ATCC27844, *Pseudomonas putida* ATCC2021, *Proteus vulgaris* ATCC13315, *Bacillus subtilis* ATCC2063 and *Escherichia coli* ATCC2065[11], *R. serrata* has antitussive, antioxidant, anticancer and vasorelaxant properties dealing with quantity of bioactive compounds like total phenolic and total flavonoids[12].

Many other species such as *P. linearis*, *P. scandens* and *P. pilifera* have been mentioned for ages with uses in ethnomedicine, but there is no scientific data to support their specific activities. Additionally, *P. linearis*, *P. foetida* and *R. serrata* have long been used traditionally for men's vitality or as an aphrodisiac by raw leaf consumption. So, the research aimed at studying these three species for their phytochemicals, elucidating and showing possible mechanisms of their range of medicinal properties. Toxicity testing has been also performed for consideration of safe use.

MATERIALS AND METHODS

Plant species: *Paederia linearis*, *P. foetida* and *Rotheca serrata* were collected, 1 kg of each, around Khon Kaen University Campus and identified by a proficient botanist, Prof. Arunrat Chaveerach. The sample leaves were rinsed and air-dried for at least 3 days or until dried. The research was studied in 2020-2021 at the Department of Biology, Faculty of Science, Khon Kaen University, Thailand.

Methods

Phytochemical analysis via GC-MS: The powder sample and hexane or ethanol solvents were mixed at a rate of 1 g: 5 mL and kept for 72 hrs, the mixture was filtered. The filtrates were phytochemical analyzed using Agilent Technologies GC 6890 N/5973. The constituents were expressed and determined by the relative percentage.

For toxicity testing, the solvent was evaporated from the extract by rotary evaporation, after that, an equal volume of dimethyl sulfoxide (DMSO) was added to the dried filtrate for re-dissolving, kept at -20°C as stock extracts.

PBMCs isolation: The buffy coats were derived from a blood bank and peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque Plus (GE Healthcare). The PBMCs were suspended at a concentration of 10^6 cells mL^{-1} in a modified RPMI-1640 medium, with 2 mM L-glutamine and 25 mM HEPES, supplemented with 10% FBS at a concentration of 2-10% providing attachment factors, nutrients and hormones for mammalian cells, as well as to be a buffer against disruptions like pH changes and endotoxins), 5 μg mL^{-1} phytohemagglutinin (PHA, caused blood cells to clump together), 100 μg mL^{-1} streptomycin and 100 U mL^{-1} penicillin (both are antibiotics solution for cell culture to prevent bacterial contamination of cell cultures due to their effective combined action against gram-positive and gram-negative bacteria).

MTT assay for cytotoxicity assessment of the three plants studied species: The five levels of working concentrations were prepared from serial 10-fold dilution from the stock extracts. The 125 µL of each prepared cell was seeded in 96-well plates, then 12.5 µL of the extract working concentrations, the untreated cells were negative control and the cells with UV light treated for 20 min to break DNA were used as positive controls. The plates were nurtured at 37°C with 5% CO_2 for 4 hrs. The 10 µL of 0.5 mg mL^{-1} MTT (Sigma, USA) was added to each well after the medium was displaced and the plates were kept under the same mentioned conditions. The DMSO was added to each well to solubilize formazan crystals, the plates were kept in the dark for 4 hrs and the absorbance was read at 570 nm. The IC_{50} values have been determined and applied to evaluate LD_{50} values following the Pesticides: Classifications, exposure and risks to human health[13].

Comet assay for genotoxicity assessment of the three plants studied species: The extract concentration at IC_{50} value or a maximum-treated concentration in cases that were found to have no IC_{50} value was applied in the cells. After that, the cells were used on a gel-coated slide and subjected to electrophoresis, the gel images were then the gel images were documented and the comets were examined by at least 150 cells (50 cells for each triplicate slide) in each experiment. The olive tail moment (OTM), which is the relative amount of DNA in the tail of the comet multiplied by the median migration distance using the CASP software (Wroclaw, Poland) was investigated for the level of DNA damage defining. The nonparametric Mann-Whitney test was used for statistical analysis of the comet assay results, at $p<0.05$.

HPLC analysis for L-dopa quantifying in hexane and rice bran oil extracts: The protocol was performed following Sawasdee *et al.*[14] as briefly described: A gram of powder sample was mixed with 5 mL hexane (1:5) and incubated for 72 hrs. The filtrates were solvent eliminated via a rotary evaporator and then 100% dimethyl sulfoxide (DMSO) was added equal to evaporated solvent volume. Then the filtrates were processed and analyzed by HPLC, using the mobile phase consisting of 0.1 mM formic acid and 0.2 mM EDTA, pH 3.1. The elution was carried out at a flow rate of 1 mL min^{-1}. The detection UV wavelength was 280 nm.

The rice bran oil extract preparation: A gram of powder sample was mixed with 3 mL (1:3) rice bran oil and incubated for 72 hrs. After filtering, the 1 mL filtrate was mixed with 1 mL hexane (1:1), gently shaken and incubated for 24 hrs. Then the hexane solution at the bottom was sucked out and evaluated, 10% DMSO was added instead and used for HPLC analysis in the identical condition as above.

Standard L-dopa was prepared at concentrations of 0.0005-0.05 mg mL^{-1}, 20 µL each for injection.

Statistical analysis: The calibration curve was constructed by plotting the standard concentrations (x) versus linear regression of the peak areas resulting from HPLC analysis (y) using Microsoft Excel released linear equation and correlation factor (R^2). The calibration equation was used for L-dopa evaluation from the studied samples. The correlation factor was applied for the reliability of the calibration equation. The results showed the calibration equation:

$$y = 5E+07x-460.47$$

and correlation coefficient:

$$R^2 = 0.9991$$

RESULTS

The phytochemical components of the studied hexane and ethanol leaf extracts, *P. liners* in Fig. 1a, b and *P. foetida* in Fig. 1c, d by GC-MS were detected in chromatograms according to the specific retention time (RT) of a compound. The compounds revealed the major groups with higher percentages than the others including 14.90 and 41.24% phytol in the hexane extracts of *P. linearis* and *P. foetida* and 15.56% squalene in the hexane extract of *P. linearis*. All phytochemical quantities were shown in Table 1.

When HPLC analysis was performed to determine L-dopa in the fresh (which have just been collected and dried) or dried leaves (which have been collected, dried and kept for more than 1 month) of the three studied species, following linearity equation derived from a linearity graph plotted from the L-Dopa standard concentrations and its peak area sizes producing the calibration equation. The studied samples were calculated for L-Dopa content following the calibration equation:

$$5E+07x-460.47$$

The calibration equation is:

$$y = mx+c$$

where, y is the peak area, m is the slope, c is the intercept of the linear curve and x is the derived area of the targeted substance) using the data and the correlation coefficient (R^2) was 0.9996, which indicated the reliability of the standard

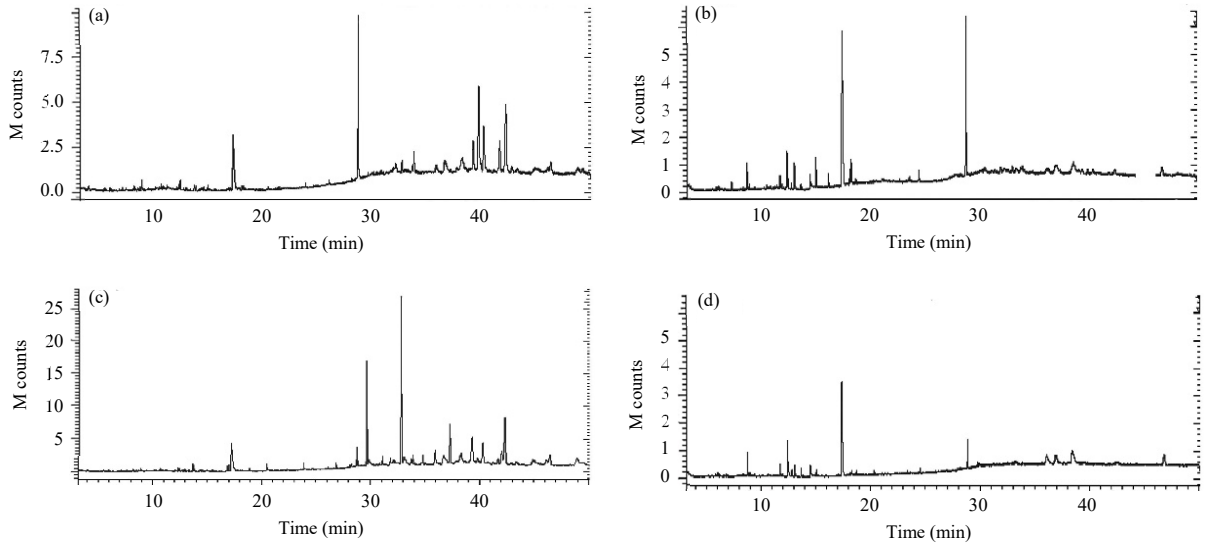

Fig. 1(a-d): GC-MS-chromatograms showing the mass spectrum of hexane and ethanol plant leaf extracts, (a) Hexane *P. linearis* leaf extract, (b) Ethanol *P. linearis* leaf extract, (c) Hexane *P. foetida* leaf extract and (d) Ethanol *P. foetida* leaf extract
Y-axis is relative abundance, the x-axis is the retention time (RT)

Table 1: Chemical constituents of hexane and ethanol *Paederia foetida*, *Paederia linearis* (and *Rotheca serrata*) leaf extracts[15]

		Relative content (%)					
		Paederia linearis		*Paederia foetida*		*Rotheca serrata*	
Compounds	Formula	Hexane	Ethanol	Hexane	Ethanol	Hexane	Ethanol
Benzoic acid, 4-ethoxy-, ethyl ester	$C_{11}H_{14}O_3$	-	2.02	-	3.58	-	-
Phytol acetate	$C_{22}H_{42}O_2$	-	-	-	8.48	-	-
N-hexadecanoic acid	$C_{16}H_{32}O_2$	-	1.90		2.41		
Hexadecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	-	3.27	-	1.46	-	-
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	-	-	0.77	-	-	-
1-[2-(1-azetidinyl)-1,2-diphenylethyl]azetidine	$C_{20}H_{24}N_2$	-	1.44	-	-	-	-
Phytol	$C_{20}H_{40}O$	14.90	-	8.65	41.24	3.98	30.41
Oleamide	$C_{18}H_{35}NO$	-	-	-		32.78	-
9,12,15-octadecatrien-1-ol	$C_{18}H_{32}O$	-	-	-	-	-	12.20
Palmitic acid	$C_{16}H_{32}O_2$	-	-	-		8.90	6.44
Stigmasterol	$C_{29}H_{48}O$	-	-	-	-	8.73	11.54
Squalene	$C_{30}H_{50}$	15.56	-	-	-	8.20	8.15
Cholest-4-en-3-ol	$C_{27}H_{46}O$	10.40					
24-Methylenecycloartan-3-one	$C_{31}H_{50}O$	7.22					
Stigmast-4-en-3-one	$C_{29}H_{48}$	16.85		13.47			
Neophytadiene	$C_{20}H_{38}$	-	-	-	-	-	7.86
Vitamin E	$C_{29}H_{50}O_2$	-	-	-	-	3.85	7.14
Eicosane	$C_{20}H_{42}$	-	-	-	-	3.86	-
Stearic acid	$C_{18}H_{36}O_2$	-	-	-	-	4.25	3.44
Tetracosane	$C_{24}H_{50}$	-	-	-	-	3.98	-
Palmitamide	$C_{16}H_{33}NO$	-	-	-	-	3.81	-
Nonanoic acid	$C_{21}H_{36}O_4$	-	-	-	-	-	2.85
Unknown	-	35.20	48.55	77.09	42.82	17.66	9.97

curve. Consequently, L-dopa was found in the fresh leaves, but not found in the dried leaves. Chromatogram characteristics of all substances included the L-dopa standard and the sample extracts in 10% DMSO were shown in Fig. 2a-c. The peak areas are shown in the HPLC chromatograms in Fig. 2d-f were substituted in the calibration equation to calculate the L-dopa content in the studied samples, *P. linearis*, *P. foetida* and *R. serrata*. They contained L-dopa quantities in 1 g leaves of 1.15×1^{-3}, 1.43×10^{-3} and 0.23×10^{-3} mg and concentrations at 3.40×10^{-3}, 2.80×10^{-3} and 0.80×10^{-3} mg mL^{-1},

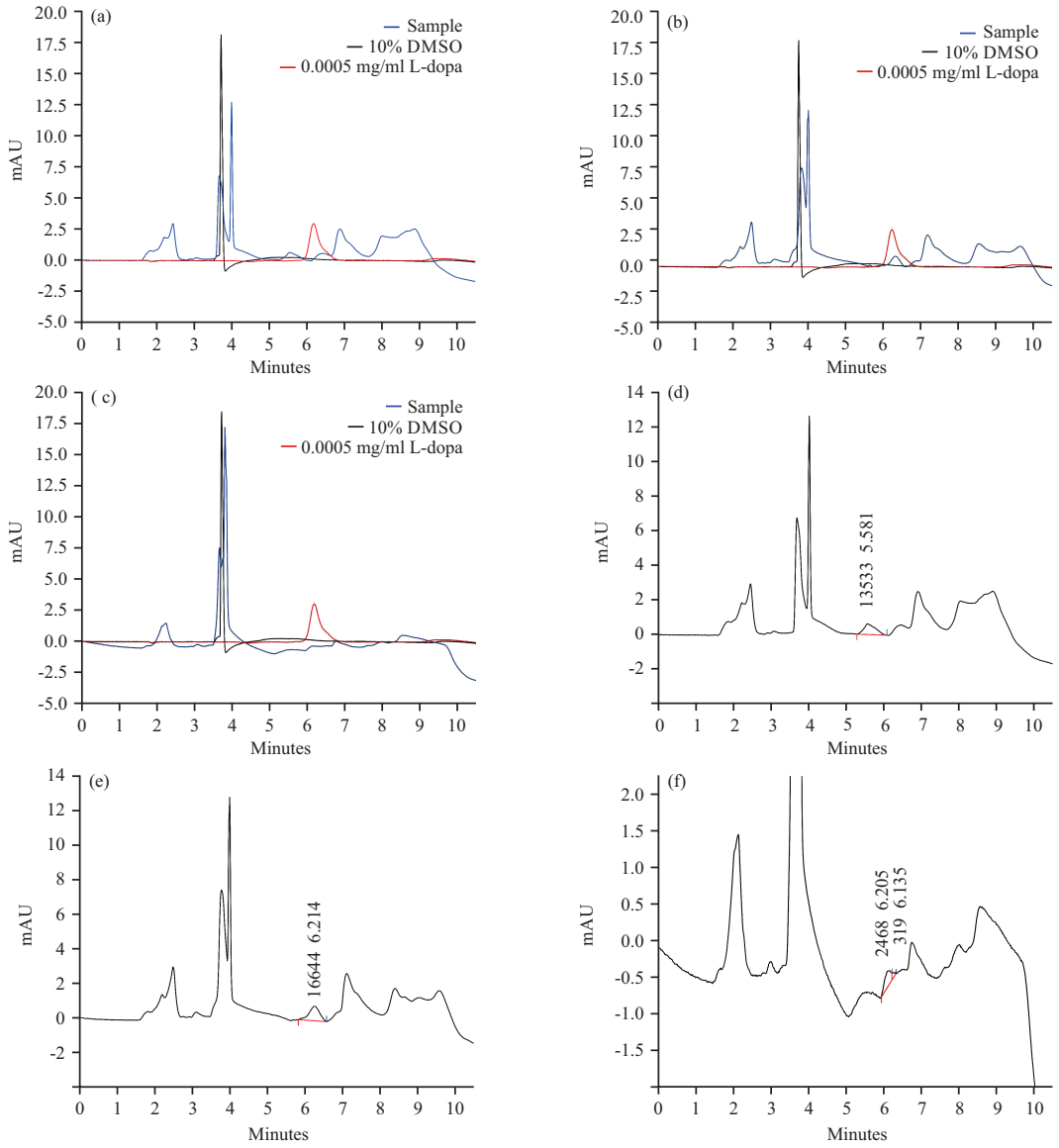

Fig. 2(a-f): HPLC chromatograms showing the mass spectrum of the three studies species, (a) *Paederia foetida* with L-dopa peak area, (b) *P. linearis* with L-dopa peak area, (c) *Rotheca serrata* with L-dopa peak area, (d) *P. foetida* standard calibration curve, (e) *P. linearis* standard calibration curve and (f) *R. serrata* standard calibration curve

Rotheca serrata (blue line)[15], standard L-dopa (red line) and control (black line), the peak areas of L-dopa: 16644 at an RT of 6.214, 13533 at RT of 5.581 and 2468 at RT of 6.205 and 319 at RT of 6.135 in the studied species. The y-axis is relative abundance, the x-axis is the retention time (RT), the linearity test result of the standard calibration curve is $r^2 = 0.9991$

respectively in Table 2. Repeated HPLC, which were shown chromatogram characteristics of the L-dopa standard and the sample extracts in 10% DMSO in Fig. 3a, b and the peak areas shown in Fig. 3c, d, to quantify L-dopa in rice bran oil leaf extracts of *P. linearis* and *P. foetida* showed higher amounts and concentrations at 3.8×10^{-3} and 6.60×10^{-3} mg in 1 g leaves and 11.00×10^{-3} and 22.00×10^{-3} mg mL^{-1} of these two sample species, respectively following chromatograms in Table 3.

The maximum mass concentrations of hexane and ethanol *P. linearis*, *P. foetida* and *R. serrata* extracts were 9.6 and 7.5, 19.6 and 9.2, 10.0 and 10.0 mg mL^{-1} in 100% DMSO, respectively. For working concentrations, 10% DMSO was used, thus the maximum working concentrations were 0.96 and 0.75, 1.96 and 0.92, 1.00 and 1.00 mg mL^{-1} in Table 4. These working solutions were 10-fold diluted across five levels using the MTT assay. The results showed no toxicity, without IC_{50} values in all three studied species, with high percentages

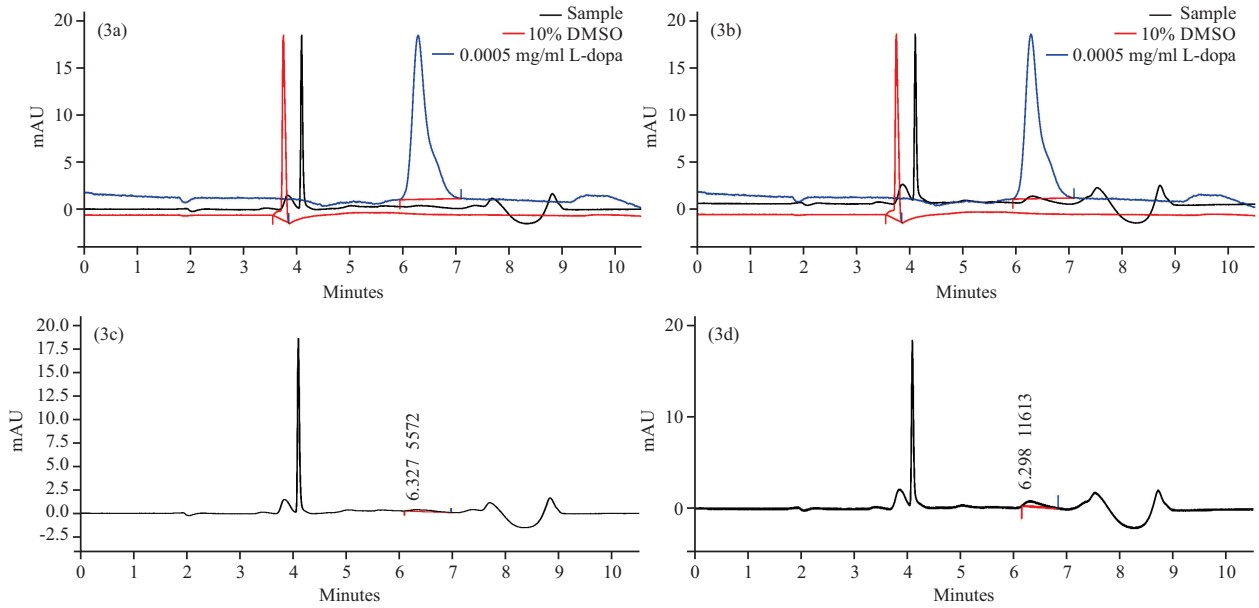

Fig. 3(a-d): HPLC chromatogram shows the mass spectrum indicating the number sizes of peak areas, (a) Rice bran oil *P. linearis*, (b) *P. foetida* leaf extracts, (c) *P. linearis*: 5572 at an RT of 6.327 and (d) *P. foetida* 11613 at RT of 6.298

Paederia foetida leaf extracts showing samples (black line), 10% DMSO (red line) and standard (blue line) and peak areas. The y-axis is relative abundance and the x-axis is the retention time (RT), the linearity test result of the standard calibration curve is $r^2 = 0.9991$

Table 2: Quantity of L-dopa detected in the leaf hexane extracts of *Paederia foetida*, *Paederia linearis* and *Rotheca serrata* by HPLC analysis

			L-dopa			
Plant extracts	Weight of flesh leaf (g)	Extracted volume (mL)	RT	Peak area	Concentration (mg mL^{-1})	Quantity (mg) in 1 mg leaf
P. linearis	71.27	24	6.214	16644	3.40×10^{-3}	1.15×10^{-3}
P. foetida	109.97	56	5.581	13533	2.80×10^{-3}	1.43×10^{-3}
R. serrata	67.69	24	6.205	2468	0.60×10^{-3}	0.23×10^{-3}
			6.135	319	0.20×10^{-3}	

Table 3: Data of *Paederia* species extracted by rice bran oil including weights and filtrate extract volume, peak areas derived by HPLC for calculation of L-dopa concentrations and amount

Plant leaves sample	Weight (g)	Filtrate (ml)	Peak area	L-Dopa conc. (mg mL^{-1})	L-Dopa (mg) in 1 g sample
Paederia linearis	29	90	5572	11.00×10^{-3}	3.8×10^{-3}
P. foetida	33	90	11613	22.00×10^{-3}	6.60×10^{-3}

Table 4: Cytotoxicity testing using the MTT assay of five hexane and ethanol *Paederia foetida*, *Paederia linearis* and *Rotheca serrata* leaf extract concentrations in human PBMCs

Plant	Solvent	Maximum working extract conc. (mg mL^{-1})	IC_{50} (mg mL^{-1})	Cell viability (%)
Paederia linearis	Hexane	0.96	-	63.93±0.09-71.51±0.16
	Ethanol	0.75	-	64.74±0.17-78.32±0.19
P. foetida	Hexane	1.96	-	68.01±0.09-74.54±0.13
	Ethanol	0.92	-	70.34±0.11-78.97±0.15
Rotheca serrata	Hexane	1.00	-	67.85±4.19-73.33±2.32
	Ethanol	1.00	-	61.42±3.00-82.51±1.39

of cell viability provided by the plotted graph of leaf extract concentrations in Fig. 4, for hexane *Paederia linearis* in Fig. 4a, ethanol *P. linearis* in Fig. 4b, hexane *P. foetida* in Fig. 4c, ethanol *P. foetida* in Fig. 4d, hexane *Rotheca serrata* in Fig. 4e and ethanol *R. serrata* in Fig. 4f. Without IC_{50} values, the highest working concentrations were used for in-depth toxicity testing using the comet assay. The comet assay images of PBMCs treated with the plant extracts were shown in Fig. 5 included negative and positive controls for *P. linearis*, *P. foetida* in Fig. 5a and b, hexane *P. linearis* extract in Fig. 5c,

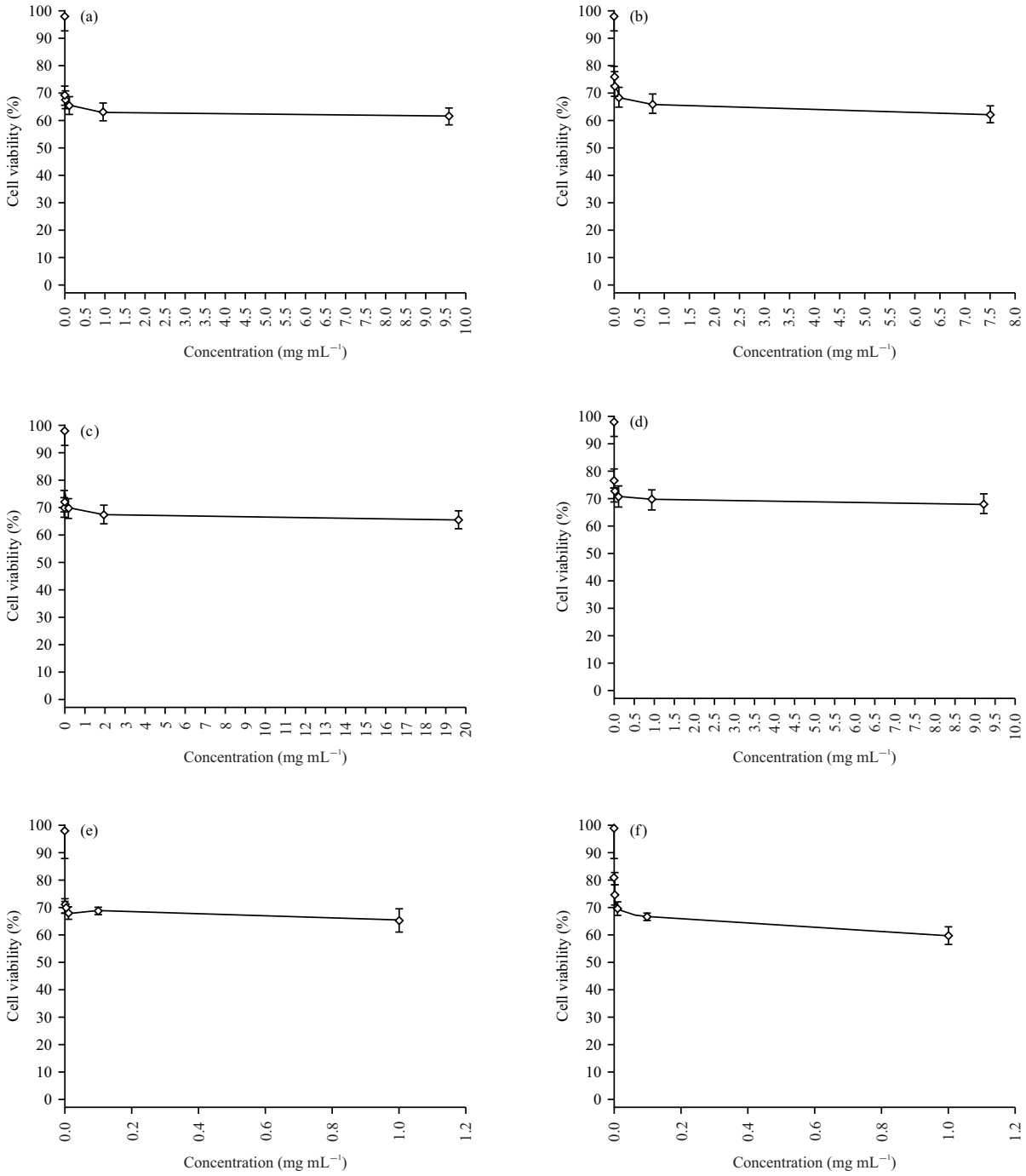

Fig. 4(a-f): Cytotoxicity testing using the MTT assay, (a) Hexane *P. linearis*, (b) Ethanol *P. linearis*, (c) Hexane *P. foetida*, (d) Ethanol *P. foetida*, (e) Hexane *Rotheca serrata* and (f) Ethanol *R. serrata*

Leaf extract concentrations used to treat PBMCs, the y-axis is cell viability (%) and the x-axis is the concentration (mg mL^{-1})

ethanol *P. linearis* in Fig. 5d, hexane *P. foetida* in Fig. 5e, ethanol *P. foetida* in Fig. 5f, negative and positive controls for *R. serrata* in Fig. 5g and h, hexane *R. serrata* extract in Fig. 5i, ethanol *R. serrata* extract in Fig. 5j. The results showed that the extracts induced significant ($p<0.01$) DNA damage compared with the negative controls, indicating broken nuclei with long tails as OTM values shown in Table 5.

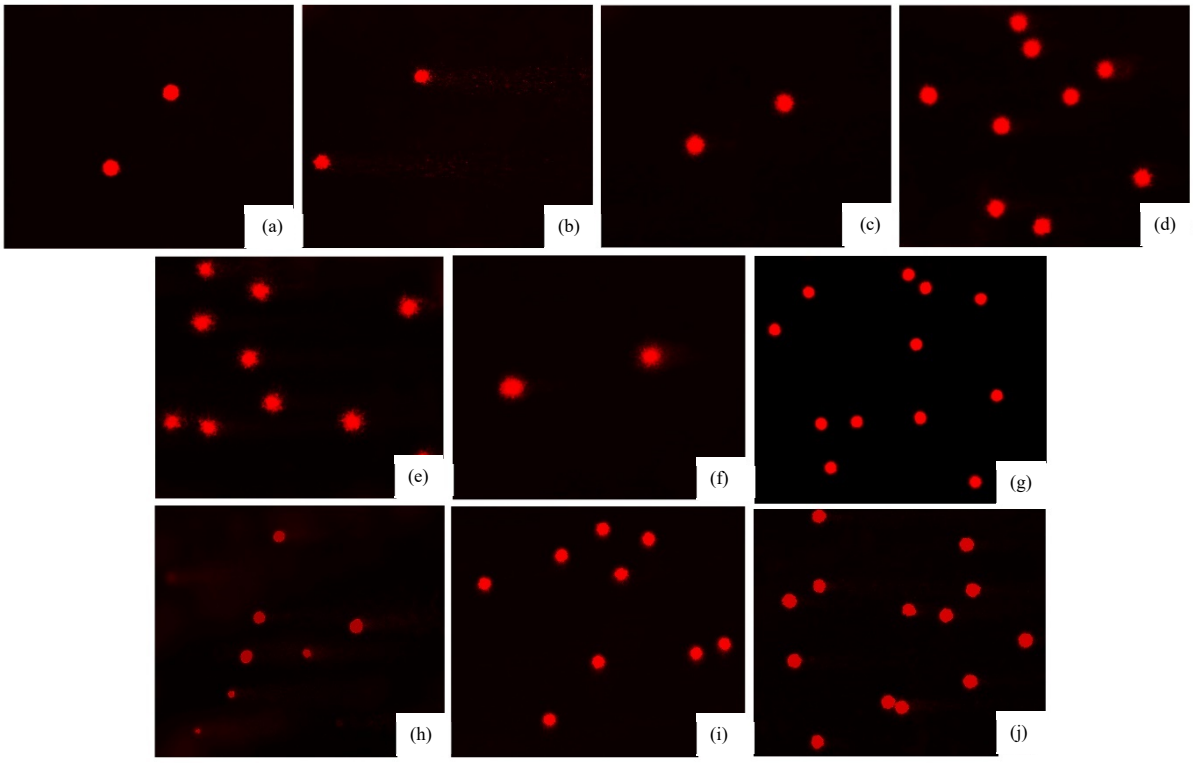

Fig. 5(a-j): Comet assay images of PBMCs (200×) treated with the hexane and ethanol leaf extracts compared with the negative and positive controls, (a) Negative and positive controls for *P. linearis*, (b) Negative and positive controls for *P. foetida*, (c) Hexane *P. linearis* extract, (d) Ethanol *P. linearis* (e) Hexane *P. foetida,* (f) Ethanol *P. foetida*, (g) Negative control for *R. serrata*, (h) Positive control for *R. serrata*, (i) Hexane *R. serrata* extract and (j) Ethanol *R. serrata* extract

Table 5: Level of DNA damage expressed as the olive tail moment (OTM) in PBMCs after treatment with hexane and ethanol *Paederia foetida*, *Paederia linearis* and *Rotheca serrata* leaf extracts

Plant	Solvent	Concentration (mg mL^{-1})	Olive tail moment	p-value
Paederia linearis	Hexane	0.96	0.14±0.13	<0.0001
	Ethanol	0.75	0.16±0.15	<0.0001
	Negative control	-	0.04±0.03	-
P. foetida	Hexane	1.96	0.18±0.15	<0.0001
	Ethanol	0.92	0.13±0.12	<0.0001
	Negative control	-	0.04±0.03	-
Rotheca serrata	Hexane	1.00	1.43±0.29	<0.0001
	Ethanol	1.00	0.33±0.22	<0.0001
	Negative control	-	0.04±0.02	-

DISCUSSION

The GC-MS analysis reported that the major phytochemicals found in *P. linearis* were phytol and squalene, *P. foetida* was phytol accorded to *Rotheca serrata* which was previously published by Boonthai *et al.*[15] showed 30.41% phytol and 32.78% oleamide in the hexane extracts. When HPLC analysis was performed, L-dopa was found in the hexane extracts of all the three plants. The rice bran oil extracts of *P. linearis* and *P. foetida* which can be edible showed higher L-dopa amounts and concentrations than the hexane extracts (Table 2, 3). The action of medicinal plants is a synergistic effect of the various substances contained in that plant. Accordingly, if any plant contains a high amount of any substance, it should be treated as having the main activity of that substance following Mohamed *et al.*[16] reported that a herb contains some pharmacologically active compounds but should be treated as a single activity. However, it does not mean that the plant will not be utilized to treat other diseases. The plant may be used according to the small number of other substances which depend on the deficiency and mechanism of the body. Therefore, a plant may be a treatment for many

diseases, as we see with our three studied plants which are used in various treatments according to the guidelines mentioned here and in the introduction section. One more substance found by HPLC analysis is L-dopa which acts as a male aphrodisiac[17] showing why these three studied plants also have identical activity along with traditional treatments that have been documented as local wisdom even though the L-dopa was found in small amounts, but it was assumed to be activated by the other substances found in the plants. Their discovery in small quantities of L-dopa seems to have positive effects, in addition to increasing sexual activity; there are no dangerous side effects. Moreover, direct administration of pure L-dopa is accompanied by side effects as well[17,18].

The research results lead to the best way of consuming fresh leaves instead of preserved forms such as dried leaves. There is a reason for one crucial activity of L-dopa it is rapidly oxidized by air. So, fresh leaves are best suited for consumption. For *R. serrata*, it can be grown under simple, low-cost garden conditions. However, the two other two studied species, *P. linearis* and *P. foetida* are difficult to be consumed in fresh leaves because they are hairy and have an objectionable smell. So, rice bran oil, the one effective solvent which can be edible as a non-polar property was selected for *P. linearis* and *P. foetida* leaf extraction because the rice bran oil has health advantages such as cholesterol decreasing, platelet aggregation inhibition and lowering earlytherosclerosis[19]. These two species were subjected to repeat L-dopa screening by HPLC due to having higher L-dopa quantities than *R. serrata*. This resulted in the first HPLC analysis and quantification of L-dopa in rice bran oil extraction of *Paederia* leaf. This extract showed a larger amount of L-dopa than the first extract with hexane (Table 2, 3). As rice bran oil is a beneficial food for human health, if these two plants were used to make natural product dietary supplements, people could take advantage of *Paederia* phytochemicals including L-dopa in rice bran oil. Another important role of L-dopa is as an orally active metabolic precursor of the neurotransmitter dopamine. The substance can cross the blood-brain barrier and is converted into dopamine in the brain and has anti-allodynia effects and the potential for treating Parkinson's disease[17]. Additionally, the three studied plant species that contain L-dopa would possibly benefit humans for vitiligo treatment and the delaying of white or grey hair formation, because the substance is a precursor of melanin synthesis[17,20,21].

Once there is information on the use and chemicals contained in these plants, toxicity tests of these three plants both on cells and DNA should be performed to promote safe human use. MTT and comet assays were chosen for this. The methods are the standard used worldwide, one of the several preclinical methods and alternative methods to animal testing[22,23]. Toxicity testing has shown that fresh leaves are safe for consumption. No toxicity was detected at the cell level (Table 4), but a toxic level was demonstrated in DNA with broken nuclei and a long tail at the maximum working concentrations (by hexane and ethanol solvents) of 0.96 and 0.75, 1.96 and 0.92, 1.00 and 1.00 mg mL^{-1} of *P. linearis*, *P. foetida* and *R. serrata* extracts, respectively. This is genotoxicity testing shown by the OTM value which is the level of significant DNA damage shown in the statistical value, $p<0.001$ (Table 5). It means that these examined concentrations exhibit a destructive effect on the cells' genetic materials namely DNA and RNA. When consumed in corresponding concentrations, the mutation in various cells may occur in people[24]. So, for rough consumption doses to identify the hazardous levels of the extracts, the highest concentration used (by hexane and ethanol solvents) of 0.96 and 0.75, 1.96 and 0.92, 1.00 and 1.00 mg mL^{-1}) in the comet assay instead of IC_{50} value would be supposedly evaluated for the LD_{50} calculation in common practice. The LD_{50} values for this test were 1,359.58 and 1,240.29, 1,773.04 and 1,338.22 and 1,380.38 and 1,380.38 mg of plant kg^{-1} body weight of rat. A 50 kg human who has consumed these dried plants at the doses of 67,979 and 62,014.50 mg, 88,652.00 and 66,911.00 mg and 69,019.00 and 69,019.00 mg of *P. linearis*, *P. foetida* and *R. serrata*, respectively, would develop toxicity by cell mutation at Pesticides: Classifications, exposure and risks to human health[13], Class III which would be slightly hazardous with oral consumption of over 500 mg of dried plant/body weight. Above these doses should not be used for humans.

CONCLUSION

In summary, these results of phytochemicals exactly L-dopa, without toxicity both in cell and DNA levels support ethnomedicinal uses of *P. foetida*, *P. linearis* and *Rotheca serrata*. They can be safely consumed orally as fresh leaves in various treatments as mentioned. Additionally, rice bran oil solvent can be a potential solvent for L-dopa preservation in modified form.

SIGNIFICANCE STATEMENT

The significant findings are the one major substance found as L-dopa contained in the three studied species, non-toxicity in the specific solvent which can maintain the condition of the substance from degradation.

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