Tropical Journal of Pharmaceutical Research November 2019; 18 (11): 2341-2348
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v18i11.16

Original Research Article

Evaluation of *in vitro* cytotoxicity effect of *Clinacanthus nutans* (Brum. f.) Lindau standardized leaf extracts

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Sent for review: 5 May 2016 *Revised accepted: 18 October 2019*

Abstract

Purpose: *To standardize Clinacanthus nutans (CN) leaf extracts, evaluate their contents of orientin, vitexin and isovitexin using a reversed-phase high-performance liquid chromatography (RP-HPLC) method, and also to investigate in vitro cytotoxicity of CN.*
Methods: *CN leaf powder was macerated in distilled water, methanol, methanol (50 %), ethanol, and ethanol (50 %) over a hot water bath at 50 - 55 °C for 24 h. The extracts were standardized for total phenolic, flavonoid, proteins and polysaccharides content by ultra-violet (UV) spectrophotometry. Moreover, RP-HPLC was used to determine the contents of orientin, vitexin and isovitexin in the extracts. The anti-proliferative effect of the extracts against human colorectal carcinoma cell line (HCT-116) and human colon normal cell line (CCD-18Co) was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The most active extract was fractionated using silica gel flash column chromatography to produce 20 fractions. All the fractions were subjected to the MTT test.*
Results: *The extracts showed modest cytotoxicity against HCT-116 and non-cytotoxicity against CCD-18Co cell lines. Of all the extracts tested, the methanol extract (CN-M) showed the highest activity of all the extracts and had the highest content of flavonoid and phenolic compounds. Twenty fractions were obtained from this extract. Fraction nos. F3, F4, F14 and F16 showed significant ($p < 0.05$) cytotoxicity against HCT-116, with F14 having the highest activity.*
Conclusion: *Fraction F14 has the potential to be developed to anti-colon cancer agent. However, further studies including chemical profiling, mechanism of action and safety profile of this fraction are required.*

Keywords: *Clinacanthus nutans, Cytotoxicity, Standardization, Orientin, Vitexin, Isovitexin*

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INTRODUCTION

Plants are widely valued for their aromas and tastes and many of these plants are medicinal and used to treat various human illnesses. Malaysian plants are widely valued for their medicinal properties and has been traditionally used to treat various human illnesses. One of the plants of interest is *Clinacanthus nutans* (CN) Lindau (Acanthaceae). CN is commonly known as Sabah Snake Grass or Belalai Gajah in Malaysia [1]. This plant is an important traditional herbal medicine in Malaysia, Indonesia, and Thailand. CN is also widely used for treating skin

rashes, insects and snake bites, lesions caused by herpes simplex virus, diabetes mellitus, fever and diuretics [2]. The anti-inflammatory activity of the n-butanol (n-BuOH) soluble fraction from the CN leaves has long been reported [3]. Despite its long history of use as traditional medicine, the ethnopharmacological reports of CN are only partly established. Therefore, extensive scientific investigations are required to justify the therapeutic potential of CN. Previous chemical studies on CN had shown that the presence of lupeol, β-sitosterol, stigmasterol and myricyl alcohol [4]. Several compounds have been isolated from methanolic extract of the CN leaves collected in Thailand such as six known C-glycosyl flavones, vitexin, isovitexin, shaftoside, isomollupentin 7-O-b-glucopyranoside, orientin, and isoorientin from the n-BuOH and water-soluble portion of the extract and five sulfur containing glucosides from the n-BuOH soluble portion of the extract [5,6]. C-glycosyl flavones' presence in the CN leaves possesses potential therapeutic properties such as antioxidant and anti-cancer [7]. Standardization of herbal medicines is an assurance of quality, efficacy, safety, and reproducibility through the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values. Therefore, the present investigation was performed to standardize the CN extracts including aqueous (CN-W), ethanolic (CN-E), methanolic (CN-M), 50 % ethanolic (CN-EW) and 50 % methanolic (CN-MW) extracts that led to the process of prescribing a set of characteristics exhibited by a particular plant. Another main objective was to investigate their anti-colon cancer activities on humans' colon carcinoma cells (HCT-116) and humans' normal colon fibroblasts (CCD-18Co) cells by MTT assay. Moreover, anti-colon cancer effect of the fractions of the most active extract would also be investigated.

EXPERIMENTAL

Chemicals and reagents

Human colorectal carcinoma (HCT-116) and human normal colon fibroblasts (CCD-18Co) cells were purchased from ATCC (Manassas, Virginia). RPMI 1640 medium, minimum essential medium (MEM), trypsin, penicillin-streptomycin and fetal bovine serum (FBS) were obtained from Bio-Diagnostics (Petaling Jaya, Selangor, Malaysia). Dimethylsulfoxide (DMSO), phosphate buffered saline (PBS), 3-(4,5-dimethylthiazol-2-yl-2,5-diphenyl) tetrazolium bromide (MTT), vincristine sulphate, quercetin, gallic acid, Folin-Ciocalteau reagent, sodium carbonate, aluminium chloride, potassium acetate, anthrone and glucose were purchased from Sigma-Aldrich (Subang Jaya, Selangor, Malaysia). Orientin, vitexin, and isovitexin standard compounds were purchased from Indofine (New Jersey, USA). Analytical grade of methanol, ethanol, chloroform, n-hexane, ethyl acetate, hydrochloric acid, sulfuric acid and HPLC grade methanol, and formic acid were purchased from Merck (Petaling Jaya, Selangor, Malaysia). De-ionized water for HPLC was prepared using Ultra-pure water purifier system (Elgastat, Bucks, UK).

Plant materials

The leaves of CN were purchased from Herbagus Sdn. Bhd. Penang, Malaysia and identified through plant morphology Mr. Shunmugam from the school of Biological Sciences, University Sains Malaysia. A voucher sample of the plant, reference number 11338 was deposited at the herbarium of School of Biological Sciences, Universiti Sains Malaysia.

Plant leaves extraction

The dried leaves of CN were pulverised into fine powders using a milling machine (Retsch GmbH, Germany). The fine powder (500 g) was macerated in distilled water, methanol, ethanol, ethanol (50 %) and methanol (50 %) at 50 - 55 °C degree water bath for 24 h. After cooling, the extracts were filtered using Whatman filter paper no. 1, concentrated at 50 ºC under vacuum using a rotary evaporator (RE121 Buchi, Switzerland), and dried using a freeze-dryer (Labconco, USA). The extracts were stored in the refrigerator at -20 °C until further use. The extraction solvents play an important role in extraction of total solid and phytochemical composition as well as antioxidant capacity [8]. Polar solvents are frequently used for recovering polyphenols from plant matrices. The most suitable solvents are ethanol and methanol or aqueous mixtures containing these solvents. Methanol, methanol (50 %), ethanol and ethanol (50 %) have been known to be good solvents for bioactive components extraction [9]. The present study was undertaken to compare the effect of different extraction solvents in extracting the active components.

Preliminary phytochemical screening of cn extracts

Total polyphenolic content

Total phenolic content was determined according to the method described previously [10]. Furthermore, 20 µL extract (4 mg/mL) or gallic

acid (0.0625 - 4 mg/mL) was added to 1.58 mL deionized water and 100 µL Folin-Ciocalteu reagent, mixed thoroughly and incubated at room temperature for 8 min. Subsequently, 300 µL of 20 % sodium carbonate was added and incubated for 2 h. The absorbance was measured at 765 nm by a UV-Visible spectrophotometer (PerkinElmer, USA). The results are expressed as average mg gallic acid equivalents per gram of the extract.

Total flavonoid content

The total flavonoid content was determined using a previously described method [11]. Aluminium chloride (0.1 mL, 10 % w/v), 0.1 mL of 1 M potassium acetate solution, 1.5 mL methanol, and 2.8 mL of distilled deionized water were added to 500 µL of extract (4 mg/mL) or quercetin (0.0078 - 4 mg/mL), mixed, and incubated at room temperature for 30 min. Absorbance was measured at 415 nm by a UV-Visible spectrophotometer (PerkinElmer, USA). Aluminium chloride was substituted by water for the blank. The total flavonoid content was determined as average mg quercetin equivalents per gram of the extract.

Total proteins

The total protein estimation was determined as per the method described [12]. Furthermore, sodium carbonate (2 % w/v) and sodium hydroxide (0.1 M) were mixed together to make solution A. Solution B was prepared by adding copper sulphate (1.56 % w/v) and potassium sodium tartarate (2.37 % w/v). Solution A (100 mL) was mixed with 2 mL of solution B to produce the analytical reagent. Distilled deionized water (900 µL) and 3 mL of the analytical reagent were added to 100 µL extract (5 mg/mL) or bovine serum albumin (BSA) (50 – 250 µg/mL) and incubated for 10 min at room temperature. Following the incubation, 200 µL of the Folin-Ciocalteu reagent was added and the mixture was further incubated for another 30 min. The absorbance of the test extracts was determined at 600 nm against a blank using a UV-Visible spectrophotometer (PerkinElmer, USA). Total protein content was determined as mg BSA equivalents per gram of the extract.

Determination of polysaccharides

The total polysaccharides of the test extracts were determined using the anthrone method with a minor modification [13]. The anthrone reagent was prepared in cold sulphuric acid (0.2 mg/mL). Glucose as standard in this study was prepared in distilled deionised water (20 - 100 µg/mL). Hot ethanol 80 % (7 mL) was added to 0.2 g of extract in labelled centrifuge tubes. The mixture was then centrifuged at 2700 rpm for 10 min in which the alcoholic solution was discarded. This step was repeated two more times. The remaining residue was then dried in the oven at 90 °C. Distilled water (5 mL) was added to the residue before the addition of 5 mL 25 % hydrochloric acid (HCl) with constant stirring; the mixture was allowed to stand in ice water for 20 min before the addition of 20 mL distilled water.

The reaction mixture was then centrifuged; the aqueous solution was poured into a 100 mL volumetric flask. Another set of 5 mL distilled water and 5 mL of 25 % HCl was added and the previous step was repeated. The aqueous solution in the volumetric flask was filled up to 100 mL and filtered. Then, 0.1 mL of the diluted solution or glucose solution (20 - 100 µg/mL) was withdrawn into a new separate test tube. Anthrone reagent (4 mL) was then added into each tube of samples, standard solution and blank. The tubes were then heated for 3 min in a boiling water bath. The tubes were then cooled rapidly in the ice for 1 min before proceeding to measure their absorbance at 630 nm using a UV-Visible spectrophotometer (PerkinElmer, USA). The total polysaccharides content was determined, as mg glucose equivalents per gram of the extract.

High performance liquid chromatography (HPLC)

Standard compounds

To 5 mg of each standard (orientin, vitexin, and isovitexin) 5 mL methanol was added to make stock solutions of 1000 µg/mL. The stock solutions were then filtered through a 0.45 µm filter (Whatman). A series of working standard solutions (0.01 - 1000 µg/mL) were prepared by diluting the stock solution with methanol.

Samples

CN-W, CN-M, CN-E, CN-MW and, CN-EW (100 mg) were dissolved in 50 mL of deionized water and sonicated for 15 min. Working sample solution of a concentration of 2 mg/mL was prepared by diluting the stock solution with deionized water. The sample was filtered through a 0.45 µm filter (Whatman).

HPLC was performed using an Agilent Technologies series 1260 infinity (Waldbronn, Germany) system equipped with quaternary pump (G 1311 C), auto sampler (G 1329 B), column oven (G 1316A), and ultraviolet (UV)

detector (G 1314 F). The HPLC analysis was performed according to a previously described method [14]. The chromatographic conditions included a reversed phase Eclipse C_{18} column (250 × 4.6 mm) packed with 5 µm diameter particle size (Agilent), with a flow rate of 1 mL/min, injection volume of 10 µL and temperature of 30 ºC. The detection of orientin, vitexin, and isovitexin was carried out at 330 nm. The isocratic elution of methanol: formic acid (1 %) in volume ratio 33 : 67 was used in the analysis. The identification of marker compounds was evaluated by comparing the retention time and spiking technique. The content of the marker compounds in the extracts was calculated using the regression parameters obtained from the calibration curve.

Cytotoxicity assay

HCT-116 and CCD-18Co cell lines were cultured in RPMI 1640 growth medium and minimum essential medium (MEM), respectively supplemented with 10 % fetal bovine serum and 1 % penicillin-streptomycin at 37 °C in a humidified atmosphere of 5 % CO_2. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay was used to study the cytotoxic properties of CN extracts. Furthermore, the cell line under study in 100 µL of medium containing about 5000 cells was seeded into each well of the microtiter plate. After overnight incubation, another 100 µL of culture medium containing the test sample was added into each well to produce the desired final concentration with 0.1 % DMSO in each well. After overnight incubation, a 100 µL aliquot of culture medium containing the test sample in 0.1 % DMSO was added into each well to make the desired final concentration.

The extracts that showed significant differences of inhibition after being compared to the vehicle control were further tested at 0, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/mL to generate the dose-response curve. For positive control, the cells were treated with 600 ng/mL of vincristine sulphate. The untreated cells that received only the medium in 0.1% DMSO were used as vehicle control. All the cells were treated for 72 h. Each experiment was performed three times, with four replicates for each concentration. At the end of the treatment period (72 h), 20 µL of MTT solution (5 mg/mL) was added to each well. After incubation for 4 h at 37°C, the medium was removed, and 200 µL of DMSO was added to the well to solubilize the formazan crystals. After 5 min of shaking, the optical density was recorded by a Multiskan Ascent plate reader (Thermo Scientific) at 570 nm for absorbance and 650 nm as reference. The IC_{50} value of each sample was derived from the linear regression curve of drug concentration against response.

Preparation of fraction

The crude CN-M extract (4 g) was dissolved in methanol (10 mL) and mixed properly with 8 g of silica gel. The mixture was completely dried by using rotary evaporator at 40 °C. Subsequently, the fine powdered material was subjected to flash column chromatography under vacuum using column (30 × 5 cm) packed with silica gel with pore size 60 Å, 0.063 - 0.200 mm particle size, and 230 - 400 mesh particle size. Elution was started with n-hexane, and continued with a mixture of n-hexane : ethyl acetate (95 : 5 %). The elution was continued by decreasing the percentage of n-hexane and increasing the percentage of ethyl acetate to 75 : 25; 50 : 50; 25 : 75, and 0 : 100 %, respectively. The further separation was performed with a mixture of ethyl acetate : methanol (95 : 5 %) with decreasing the percentage of ethyl acetate and increasing the percentage of methanol to 95 : 5; 75 : 25; 50 : 50; 25 : 75, and 0 : 100 %, respectively. The volume of the eluent in each cycle was 200 mL, and a total of 20 fractions were obtained. Fraction 1 (F1) to fraction 10 (F10) were obtained from elution of n-hexane : ethyl acetate from 100 : 0 % to 0 : 100 %, respectively, while fraction 11 (F11) to fraction 20 (F20) were obtained from elution of ethyl acetate : methanol from 100 : 0 % to 0 : 100 %, respectively. The solvent was evaporated at room temperature in a fume hood for 3 - 4 days.

Cytotoxicity assay

All fractions were tested on HCT-116 and CCD-18Co cell lines by MTT assay in order to evaluate their anti-colon cancer properties.

Statistical analysis

The samples were analysed in triplicates and results are presented as mean ± SD, (n = 3). Comparisons were made by one-way ANOVA and differences were considered significant at $p < 0.05$. Statistical analysis was carried out using SPSS version 16.0 (SPSS, Chicago, Illinois).

RESULTS

Phytochemical profile of CN Extracts

The total polyphenolic, flavonoid, protein and carbohydrate contents of CN-W, CN-M, CN-E, CN-MW and CN-MW are shown in Table 1. The results reveal that CN-M contains the highest

content of phenolics and flavonoids compared to other extracts. However, CN-W had the highest amount of proteins and carbohydrate among all other extracts.

HPLC chromatograms and data

As shown in Figure 1, selected standard compounds are well separated by the used of applied HPLC method. Identification of marker compounds was evaluated by comparing the retention time and spiking technique. The retention time for orientin, vitexin and isovitexin are 10.23, 13.84 and 18.41min, respectively. Good linearity (R^2 = 0.9999) is obtained in the concentration range of 7.81 - 1000 µg/mL). The linear regression equations are Y = 24.43X + 66.13, Y = 28.73X + 193.9, and Y = 33.56X + 517.3 for orientin, vitexin and isovitexin, respectively. In Table 2, the concentration of standard compounds determined by HPLC in CN-W, CN-M, CN-E, CN-MW, and CN-EW extracts is presented. The results are derived from the mean of peak area from three replicates injections. The results demonstrated orientin as a major component in all CN extracts, while vitexin as the minor component. However, all CN extracts contain significant (p < 0.05) amount of marker compounds but CN-M has the highest concentration of orientin, vitexin, and isovitexin among other extracts.

Cytotoxicity

Figure 2 demonstrate the dose-dependent activity of extracts and vincristine sulphate (positive control) on HCT-116 and CCD-18Co cells proliferation after 48 h. The IC_{50} values of the extracts and vincristine sulphate on HCT-116 and CCD-18Co cell lines are presented in Table 3. It indicates that all the extracts have modest cytotoxic activity against HCT-116 cancer cell compared to the positive controls, but CN-M shows more cytotoxicity compared to the other extracts. Moreover, all the extracts are found to be non-cytotoxic against CCD-18Co normal cell compared to vincristine sulphate.

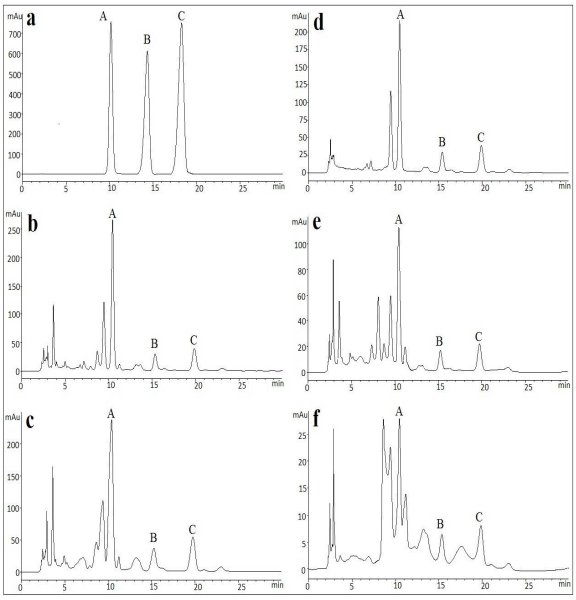

Figure 1: High performance liquid chromatography chromatogram of orientin, vitexin and isovitexin. (a) standard of (A) orientin, (B) vitexin and (C) isovitexin, (b) CN-M, (c) CN-E, (d) CN-MW, (e) CN-EW and (f) CN-W

Table 1: Phytochemical profile of *Clinacanthus nutans* leaf extracts

Extract	Total Proteins (mg/g)	Total polysaccharides (mg/g)	Total Flavonoid (mg/g)	Total polyphenolic (mg/g)
CN-W	544.33 ± 5.65	323.31 ± 20.63	13.14 ± 0.63	20.5 ± 0.42
CN-M	376 ± 13.32	106.5 ± 6.25	108.14 ± 4.35	63.76 ± 0.17
CN-E	269.73 ± 9.16	85.83 ± 0.25	102.53 ± 2.31	51.3 ± 0.24
CN-MW	522.5 ± 20.55	268.92 ± 14.34	21.18 ± 1.49	47.8 ± 0.67
CN-EW	383.03 ± 18.69	134.15 ± 10.39	20.1 ± 2.21	30.68 ± 0.23

Results are shown as mean ± SD (n = 3)

Table 2: Content of orientin, vitexin and isovitexin in *Clinacanthus nutans* leaf extracts

Sample	Orientin (mg/g)	Vitexin (mg/g)	Isovitexin (mg/g)
CN-M	27.52 ± 0.20	13.86 ± 0.25	20.87 ± 1.39
CN-E	23.41 ± 0.25	12.83 ± 0.29	14.11 ± 0.56
CN-W	8.95 ± 0.34	0.71 ± 0.00	6.12 ± 0.21
CN-MW	17.83 ± 0.63	6.55 ± 0.32	11.17 ± 0.23
CN-EW	12.75 ± 0.34	3.09 ± 0.24	9.41 ± 1.49

Results are shown as mean ± SD (n = 3)

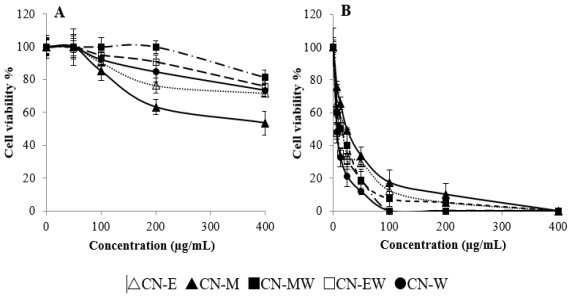

Figure 2: Anti-proliferative effect of *Clinacanthus nutans* leaves extracts against (A) CCD-18Co cell lines and (B) HCT-116 cell lines. Values are shown as mean ± SD (n = 3)

Table 3: Median inhibitory concentrations (IC_{50}) of *Clinacanthus nutans* leaf extracts on human colon cancer and normal cell lines

Sample	HCT-116 (µg/mL)	CCD-18Co (µg/mL)
CN-M	52.90 ± 1.79	394.25 ± 3.09
CN-E	108.83 ± 2.71	1197.72 ± 13.06
CN-W	276.70 ± 8.37	1946.33 ± 16.84
CN-MW	108.68 ± 3.75	1420.05 ± 11.34
CN-EW	245.12 ± 9.13	1840.56 ± 16.70
Vincristine sulphate	4.57 ± 0.03	10.06 ± 0.43

Results are shown as mean ± SD (n = 3)

Among the twenty fractions of CN-M extract, fractions number 3, 4, 14 and 16 (F3, F4, F14 and F16, respectively) significantly ($p < 0.05$) show cytotoxicity on HCT-116 human colon cancer cell line with IC_{50} 21.74 ± 1.75, 43.97 ± 6.63, 10.78 ± 1.46, and 12.20 ± 2.07 µg/mL, respectively. However, fraction F14 is more cytotoxic compared to other fractions. Moreover, all the fractions are non-cytotoxic against CCD-18Co normal colon cell line, while fractions F3 and F4 with IC_{50} 779.77 ± 14.76, and 1554.2 ± 20.89 µg/mL, respectively are more non-cytotoxic compared to fractions F14 and F16 with IC_{50} 165.90 ± 1.68 and 184.57 ± 5.35 µg/mL, respectively. Figure 3 demonstrated the dose-dependent activity of the most active fractions on HCT-116 and CCD-118Co cells proliferation after 48 h. The IC_{50} values of the fractions on HCT-116 and CCD-118Co cell lines are presented in Table 4.

Table 4: Median inhibitory concentrations (IC_{50}) of fractions of methanol extract of *Clinacanthus nutans* leaves on human colon cancer and normal cell lines

Fraction	HCT-116 (µg/mL)	CCD-18Co (µg/mL)
F3	21.74 ± 1.75	779.77 ± 14.76
F4	43.97 ± 6.63	1554.2 ± 20.89
F14	10.78 ± 1.46	165.90 ± 1.68
F16	12.20 ± 2.07	184.57 ± 5.35

Results are shown as mean ± SD (n = 3)

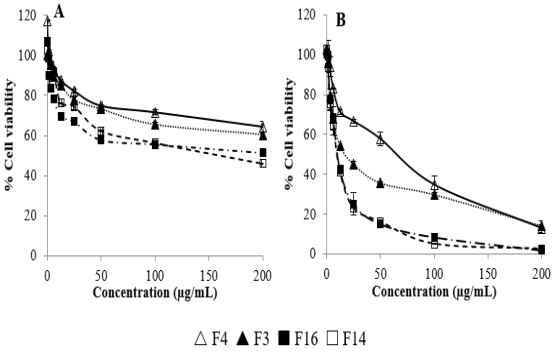

Figure 3: Anti-proliferative effect of fractions of methanolic extract of *Clinacanthus nutans* against (A) CCD-18Co and (B) HCT-116 cell lines. Values are shown as mean ± SD (n = 3)

DISCUSSION

The maceration method is applied to prepare crude extracts of water (CN-W), methanol (CN-M), ethanol (CN-E), 50 % methanol (CN-MW) and 50 % ethanol (CN-EW) from the dried green leaves of CN. All the CN extracts are estimated for their phytochemical composition by different colorimetric and gravimetric techniques. The results of this study reveal that all the extracts contain primary and secondary metabolites such as proteins, polysaccharide, flavonoids and phenolic compounds.

The variation in the percentage of these primary and secondary metabolites in each CN extracts is also detected. CN-M has the highest content of phenolics and flavonoids with 63.76 ± 0.17 and 108.14 ± 4.35 mg/g, respectively while CN-W contains the highest amount of proteins (544.33 ± 5.65 mg/g) and polysaccharides (323.31 ± 20.63 mg/g) among all the other extracts. HPLC analysis also confirms the presence of higher phenolic compounds particularly the C-glycosylflavones, such as orientin, vitexin, and isovitexin CN-M extract contained the highest level of phenolic/flavonoid components.

The antioxidant activity of phenolic and flavonoid compounds has received much attention, as it is well documented that antioxidant activities of these compounds can help in preventing some serious pathological and chronic conditions such as osteoarthritis, cancer, diabetes and cardiovascular diseases [14-18]. The antioxidant property of phenolics is derived from their ability to directly scavenge the reactive oxygen species. Phenolics are able to chelate free radicals immediately by donating a hydrogen atom or by single-electron transfer [17]. Phenolics can also act as an intracellular antioxidant through inhibition of free radical generating enzymes

such as xanthine oxidase, lipoxygenase, protein kinase C, cyclooxygenase, microsomal monooxygenase, mitochondrial succinoxidase, and NADPH oxidase [17,18].

Previous research on CN showed the presence of relatively high content of polyphenols and flavonoids, which were also known as antioxidant compounds [19]. The results from this study were in line with previous reports suggesting the presence of a high level of C-glycosyl flavones in CN extracts [5]. C-glycosyl flavones presence in plant extracts were known to possess various pharmacological activities including antiangiogenic and cytotoxic effects on a number of human cancer cell lines [1-3].

MTT cytotoxicity assay provided a simple method for the determination of live cell number in order to assess the rate of cell proliferation and to screen cytotoxic agents. MTT assay measures cell viability based on the activity of mitochondria enzymes in living cells that reduce MTT to water-insoluble formazan crystals to be easily solubilized by DMSO. The preliminary cytotoxicity study demonstrated a modest cytotoxic activity of CN-W, CN-M, CN-E, CN-MW and CN-EW when screened against human colorectal carcinoma cell line (HCT-116) but no toxicity against human colon normal cell line (CCD-18Co). CN-M with higher phenolic and flavonoid contents showed higher cytotoxicity activity against the HCT-116 cell line. Therefore, these results therefore supported the role of flavonoids and phenolics compounds as antioxidants in cytotoxicity activity and the findings were in agreement with the results obtained in other studies [16,20].

Thus, the extract (CN-M) was subjected to silica gel column chromatography. Twenty fractions were obtained and tested on HCT-116 cell line. Among the twenty fractions of CN-M, fractions number F3, F4, F14, and F16 significantly showed cytotoxicity on HCT-116 human tumor cell line and non-cytotoxicity against human colon normal cell line (CCD-18Co). F14 fraction with the lowest IC_{50} value against HCT-116 cell compared to other fractions may be considered as anti-colon cancer candidate.

CONCLUSION

The findings suggest that the high cytotoxicity of CN-M extract is related to the presence of relatively high contents of phenolics and flavonoids such as orientin, vitexin and isovitexin. The selective cytotoxicity towards colon cancer cell lines indicates a potential anti-cancer effect of CN-M extract. F14 fraction showed stronger cytotoxicity than the crude CN-M extract. The present observations provided preliminary data exposing F14 fraction obtained from CN-M extract had potent cytotoxic activity against HCT-116 cells. Further studies on isolation and characterisation of the active compounds from F14 fraction for proper assessment of their anti-proliferation activity are suggested as well as their possible development as promising anticancer drugs.

DECLARATIONS

Acknowledgement

The authors would like to acknowledge Universiti Sains Malaysia for providing facilities for this research.

Conflict of interest

The authors declare that they have no competing interests with regard to this work.

Authors' contributions

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. AFAA and ZI designed the study and assisted KE and FSRA in conducting the study. AS and KE interpreted the data and drafted the manuscript. All authors reviewed the data and approved the final version of the manuscript.

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