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Original Research Article

Cytotoxic Effect and Antioxidant Activity of Bioassay-guided Fractions from *Solanum nigrum* Extracts

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Abstract

Purpose: *To evaluate the cytotoxic effect and antioxidant activity of bioassay-guided fractions from Malaysian species of Solanum nigrum.*
Methods: *Methanol leaf and ethanol fruit extracts of Solanum nigrum were subjected to bioassay-guided fractionation using column chromatography. The semi-purified fractions were investigated for their in vitro cytotoxic effect against various cancer cell lines using 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay, and for antioxidant activity using 2, 2-diphenyl-1-picryhydrazyl (DPPH) assay.*
Results: *From bioassay-guided fractionation, 13 and 17 fractions were obtained from the methanol leaf and ethanol fruit extracts, respectively. In MTT assay, fractions 1, 2 and 4 from methanol extract showed the highest cytotoxic effect against the cancer cells with IC_{50} of 13.0 µg/mL at 48 h incubation. For the ethanol extract, fractions 14 and 15 showed the highest cytotoxic effect with IC_{50} of 12.0 µg/mL against K-562 cells, while fractions 13, 14 and 17 showed IC_{50} of 13.0 µg/mL against HeLa cells. Doxorubicin hydrochloride and vinblastine sulfate inhibited the cancer cells with IC_{50} range of 1.3 to 17.0 µg/mL. The highest radical scavenging activity was exhibited by fraction 2 from methanol extract with ED_{50} value of 0.10 mg/ml, while fraction 15 from ethanol extract showed ED_{50} of 0.79 mg/mL. Ascorbic acid and α-tocopherol exhibited radical scavenging activity of 95.0 ± 0.01 % (ED_{50} = 0.05 mg/mL) and 93.0 ± 0.01 % (ED_{50} = 0.10 mg/mL), respectively.*
Conclusion: *Solanum nigrum leaves and fruits are potential sources of cytotoxic and antioxidant agents.*

Keywords: *Solanum nigrum, Cytotoxic activity, Anti-oxidant activity, Bioassay-guided fractionation, Doxorubicin, Vinblastine*

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INTRODUCTION

Solanum nigrum is a medicinal plant from Solanaceae family and is usually cultivated in tropical and subtropical agro climatic regions. This genus herb has been extensively used in traditional folk medicine to treat various diseases at different parts of the world such as South America, Europe and North America [1]. The plant is used as antitumourigenic, antioxidant, anti-inflammatory, hepatoprotective and antipyretic agents the plant is a small green leaves spread around and grows in a diffused manner to an approximate height less than a

metre [1]. It exhibits soft arching branches and stems. The flowers are in a cluster formation. The berries are green when young and turn to purple, brown or black when they become ripe [1].

Solanum nigrum usually grows as a weed in moist habitats in different kinds of soils including dry, stony, shallow, or deep soils. These species are only semi-cultivated in a few countries such as Africa and Indonesia and are largely utilized as vegetables, fruit sources or even some community semi-cultivates the vegetable in home gardens [2]. It is also commonly grows wild and abundantly in open fields. Traditionally, *Solanum nigrum* has been used in Oriental medicine and is believed to have various biological activities [3].

The whole plant of *Solanum nigrum* is used as an antiseptic, anti-inflammatory, expectorant, cardiotonic, digestive, diuretic, laxative, diaphoretic, sedative, as well as for the treatment of swelling, cough and asthma [1]. The plant is also effective in curing cardiopathy, leprosy, haemorrhoids, nephropathy, ophthalmopathy, dropsy and general debility [1]. There is lack of scientific evidence on the plant's cytotoxic activity against cancer cells in Malaysian species of *Solanum nigrum*. Thus, the aim of this study was to investigate the cytotoxic and antioxidant activities of the semi-purified fractions from the plant extract.

EXPERIMENTAL

Plant collection and identification

The whole plant of *Solanum nigrum* was collected in January 2012 from Batu Gajah, Perak, Malaysia. The plant was authenticated by Prof Ong, Institute of Biological Sciences, Faculty of Science, University of Malaya. A voucher specimen (20120117) was kept in Biomedical Science Laboratory, Universiti Tunku Abdul Rahman, Malaysia. The leaves and fruits were separated and dried in an oven (40 ºC) (Binder, Germany). The powdered samples were stored at -20 ºC for further analysis.

Cold sequential extraction

The powdered leaves (184.2 g) were soaked in approximately 500 mL of hexane, ethyl acetate and methanol sequentially, while dried fruits (129.4 g) were soaked in 300 mL of ethanol at room temperature for one week with occasional shaking. The extracts were filtered and evaporated using a rotary evaporator at temperature ranging from 40 to 60 °C. The extraction process was repeated three times and was dried in oven at less than 40 °C. The weight of each crude extracts were shown in Fig 1. Methanol leaves and ethanol fruits extracts were proceeded to fractionation due to its active properties in cytotoxic assay.

Bioassay-guided fractionation

The methanol leaves extract (3 g) and ethanol extract (3 g) were subjected to gravity column chromatography separately using silica gel 60 (Merck, US) which was packed in a glass column. The respective extract layered onto the top of the column. The column was first eluted with n-hexane (100 %) and the polarity was increased by a stepwise gradient solvent system using ethyl acetate, followed by methanol and finally ethanol. The semi-purified fractions obtained were analyzed using thin layer chromatography (TLC). The final weights of the fractions were measured and kept at -20 °C until further analysis.

Thin layer chromatography (TLC)

Pre-coated TLC plates were prepared by drawing the baseline and solvent front on the plate. A thin capillary tube was dipped into the sample solution and was spotted onto the baseline. The plate was then put into the developing chamber saturated with non-polar and polar solvents at different ratios. The spots developed were visualized under ultra-violet lamp with both short and long wavelengths 254 and 365 nm, respectively. TLC was performed to select suitable solvent system for gravity chromatography and to combine similar fractions after isolation.

Preparation of fractions

The column fractions were dissolved in 100 % dimethyl sulphoxide (DMSO) at concentration of 10 mg/mL. In MTT assay, the fractions were further diluted using DMEM basic medium at concentration ranging 20 to 100 μg/mL (DMSO < 1 %). While, in DPPH assay, the fractions were further diluted using either DMSO or methanol concentrations ranging from 2 to 10 mg/mL.

Cell lines culture

Human chronic myelogenous leukemia cells (K-562), human promyelocytic leukemia cells (HL-60), liver cells (HepG2) and cervical cells (HeLa) were cultured in Dulbecco's Modified Eagle Medium (DMEM)(Nacalai tesque, Japan), supplemented with 10 % fetal bovine serum

(FBS), 100 units/ml penicillin and 100 µg/ml streptomycin. The cells were maintained at 37 °C in a 5 % carbon dioxide (CO_2) incubator. The cell growth was observed daily to check for confluency. Subculture was performed every 2 to 3 days upon 80 to 90 % confluence.

MTT assay

The cancer cells were seeded into 96-well plates (Nunc, Denmark) at concentration ranging from 1 x 10^4 to 1 x 10^5 cells per well. The fractions at various concentration, DMSO (negative control), doxorubicin hydrochloride and vinblastine sulfate (positive controls) (Nacalai Tesque, Japan) were added into respective wells. The treated plates were incubated for 48 h at 37 ºC in CO_2 incubator. After incubation, 50 µL of MTT reagent (5 mg/mL) (Sigma, UK) was added to each treated well and incubated further for 4 h. The plates were centrifuged for 10 min at 1000 rpm and 100 µL of DMSO was added into the wells to solubilize the purple crystal formazan [4]. The absorbance was measured using a microplate reader at wavelength of 550 nm. The results were used to construct a graph of percentage cell viability against concentration of fractions. The percentage of cell viability was calculated using formula as in Eq 1 [5].

Percentage cell viability = 100 - {(At-Ab)/(Ac-Ab)} x 100 ....(1)

Where At is the absorbance of test sample or positive controls, Ab is the absorbance of blank and Ac is the absorbance of negative control. The 50 % growth inhibition concentrations (IC_{50}) of the semi-purified fractions were estimated from the graphical interpolation.

DPPH assay

DPPH was performed in 96-well U-shape plates (Nunc, Denmark). Approximately 100 µl of fractions, ascorbic acid and α-tocopherol at various concentrations were added into wells containing 50 µL of DPPH. The plates were then incubated at room temperature in dark for 30 min. After incubation, the absorbance was measured at 517 nm and the results were recorded. A graph of percentage of radical scavenging activity (R) versus concentration was plotted and effective dose at 50 % (ED_{50}) values were determined for each test sample. The DPPH radical scavenging activity was calculated as in Eq 2 [6]. Where A0 is the absorbance of the control and A1 is the absorbance of test or standard samples.

R (%) = {(A0 – A1/ A0)}100 .... (2)

Data analysis

Cytotoxic and DPPH assays were repeated thrice and the mean value and standard deviation (mean ± SD) of the data were obtained using Microsoft excel 2007. $P < 0.05$ was considered statistically significant.

RESULTS

Bioassay-guided fractionation

Methanol leaves and ethanol fruits extracts were subjected to fractionation due to their active preliminary biological activities. Its isolated yield is as shown in Fig 1.

Cytotoxic activity

K-562, HepG2 (Fig 2) and HL-60 cells were inhibited by fractions from methanol extract after 48 h treatment. These cell lines exhibited IC_{50} values less than 50 µg/mL and are potential as a cytotoxic agent. Meanwhile, fractions isolated from ethanol fruits extract inhibited K-562 and Hela cells at IC_{50} value of 12.0 µg/mL and 13.0 µg/mL, respectively. Vinblastine sulfate reduced cell viability of K-562 and Hela cell lines at IC_{50} value less than 4.0 µg/mL (Table 1).

Antioxidant activity

All the fractions from both methanol and ethanol extracts were screened for antioxidant activity using DPPH assay. The effective doses at 50 % (ED_{50}) of each fraction from both extracts are presented in Table 2. Fractions 2 and 5 from methanol leaves and fractions 13 to 17 of ethanol fruits exhibited the highest radical scavenging as shown in Fig 3(a) and (b). The ED_{50} value of ascorbic acid and α-tocopherol were 0.017 and 0.04 mg/mL, respectively. Ascorbic acid and α-tocopherol are well-known antioxidant agents and exhibited lower ED_{50} values as compared to isolated fractions as shown in Fig 3 (c).

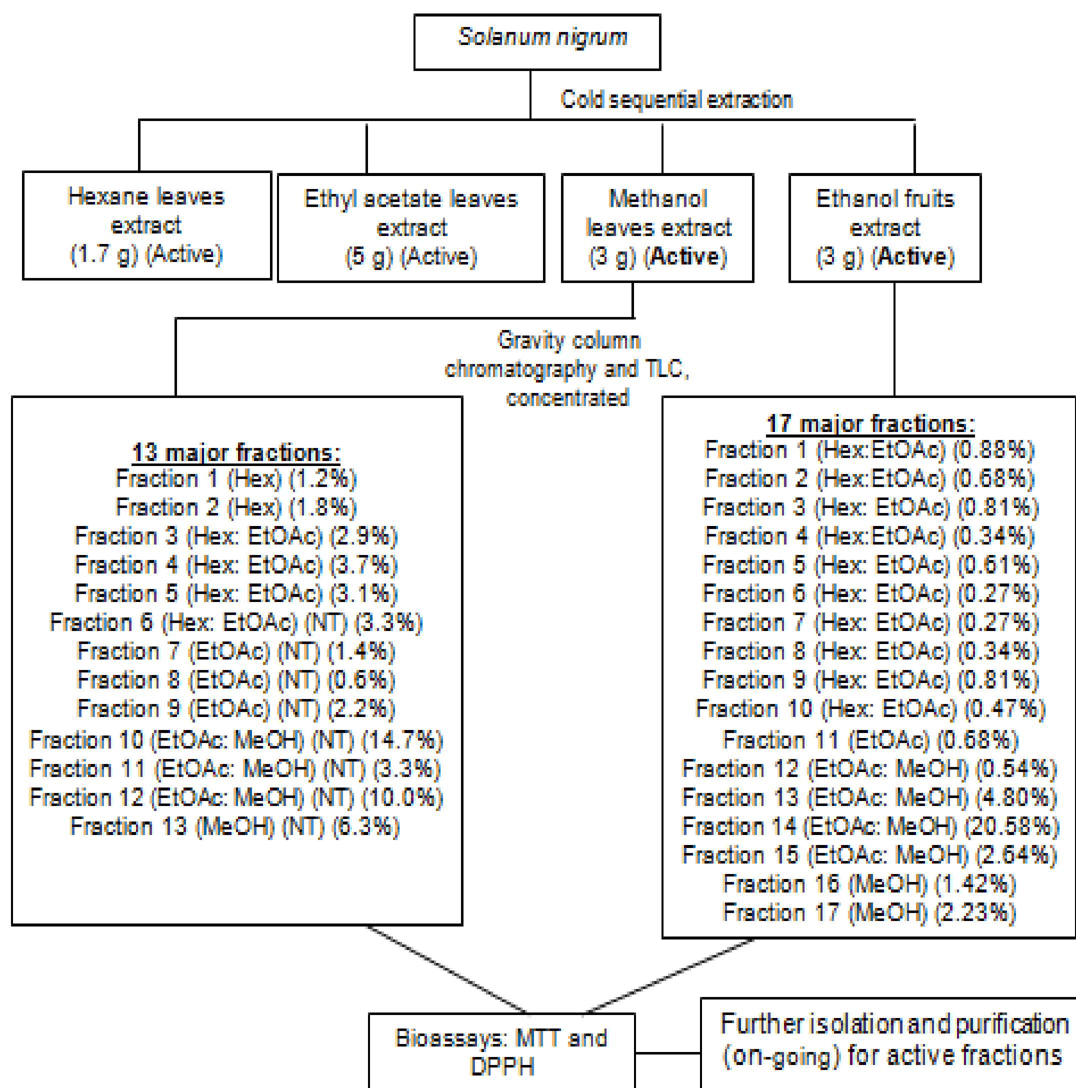


Fig 1: Overall flowchart in the extraction of fractions from methanol leaves and ethanol fruits extracts of *Solanum nigrum*

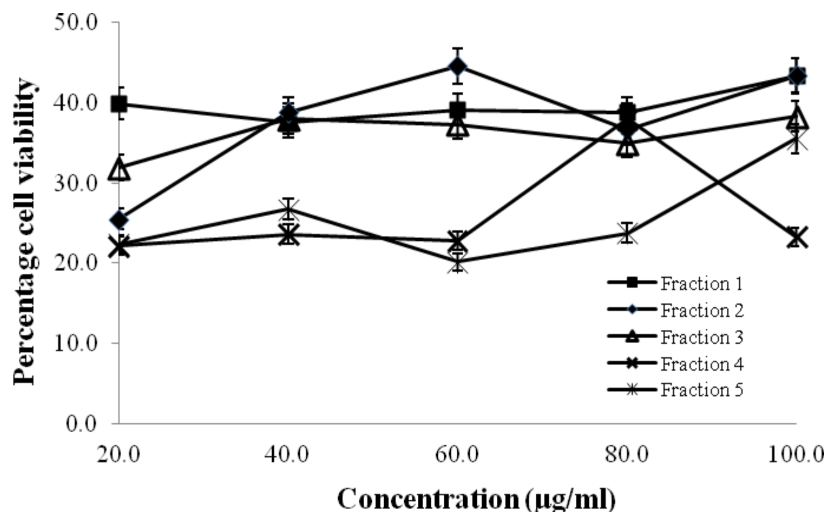


Fig 2: Cell viability of HepG2 against fractions isolated from *Solanum nigrum* extract after 48 h treatment

Table 1: The IC_{50} values of test samples against K-562, HL-60, HeLa and HepG2 cancer cells after 48h

Sample	Fraction	IC_{50} (µg/mL)			
		K-562	**HL-60**	**HeLa**	**HepG2**
Methanol extract	1	**13.0**	18.0	-	14.0
	2	-	20.0	-	**13.0**
	3	60.0	17.0	-	19.0
	4	-	**14.0**	-	**13.0**
	5	-	16.0	-	16.5
Ethanol extract	13	16.0	NT	**13.0**	NT
	14	**12.0**	NT	**13.0**	NT
	15	**12.0**	NT	16.0	NT
	16	14.0	NT	14.0	NT
	17	16.0	NT	**13.0**	NT
Positive controls	Doxorubicin hydrochloride	-	17.0	15.0	12.0
	Vinblastine sulfate	1.5	NT	1.3	NT

Footnote: - IC_{50} value cannot be determined from the graph as the percentage cell viability was above 50µg/ml, NT: Not tested

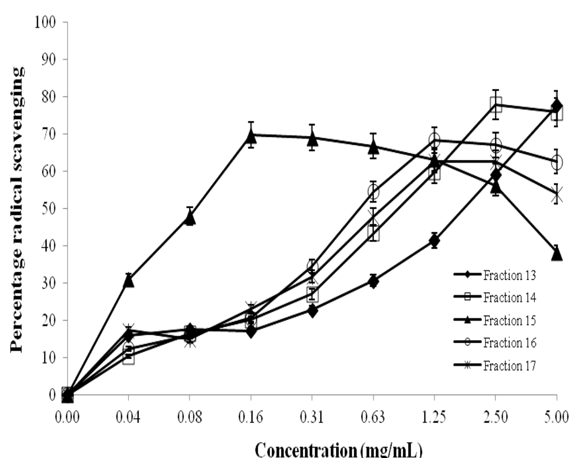
Table 2: The ED_{50} values of samples and positive controls in DPPH assay after 30 min incubation

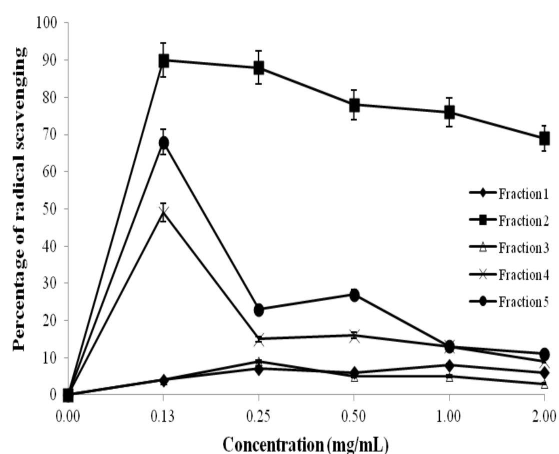
Extract	Sample	ED_{50} (mg/mL)	Extract	Sample	ED_{50} (mg/mL)
Methanol leaves	1	>5	Ethanol fruits	1	>10
	2	**0.10**		2	>10
	3	>5		3	>10
	4	>5		4	>10
	5	0.12		5	>10
	6	>5		6	>10
	7	>5		7	1.25
	8	>5		8	1.56
	9	>5		9	>10
	10	>5		10	>10
	11	>5		11	2.18
	12	2.00		12	2.18
	13	2.00		13	1.87
				14	0.90
				15	**0.08**
				16	0.55
				17	0.79
Positive controls	ascorbic acid		ED_{50} = 0.05 mg/mL		
	α-tocopherol		ED_{50} = 0.10 mg/mL		

DISCUSSION

Based on the previous investigations on *Solanum nigrum*, it was reported that the secondary metabolites present are responsible for its antiproliferative activity. These bioactive components are glycoalkaloids (solamargine, solasonine, solanine), glycoproteins, polysaccharides and polyphenolic compounds (gallic acid, catechin, PCA, caffeic acid, epicatechin, rutin, naringenin) [2]. The bioactive compounds are mainly polar in nature which can be extracted by polar solvents such as methanol and ethanol. Semi-purified fractions obtained from *Solanum nigrum* exhibited the highest antioxidant activity as well as promising cytotoxic activity against the selected cancer cell lines. Thus, the presence of respective secondary metabolites in this plant may play a role in the observed biological activity. Considering that the sample used was semi-purified fractions, it is important to note that the pure active compound(s) would possibly show stronger cytotoxic effect. Further isolation and purification is essential and on-going to identify these bioactive compounds.

Previous reports suggested that enrichment of phenolic compounds within plant extracts is correlated with their enhanced antioxidant activity and capability to scavenge free radicals [7]. According to Lee et al, *Solanum nigrum* extracts have higher amount of phenolic compounds as compared to some Korean herbal plants [8]. Phenolic compounds are large and diverse group of molecules with an aromatic ring bearing one or





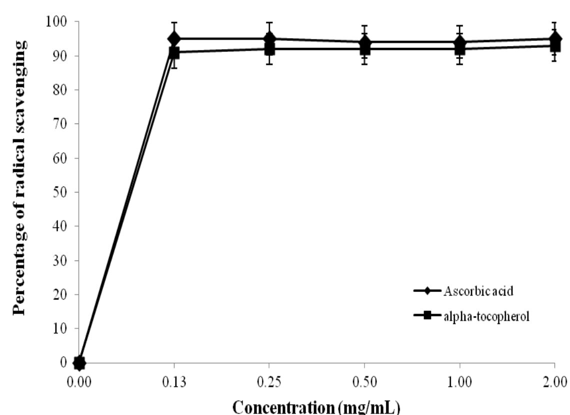


Fig 3: The percentage of radical scavenging activity of few fractions isolated from methanol leaves extract **(a)** ethanol fruits extract **(b)** and positive controls: Ascorbic acid and α-tocopherol **(c)** in DPPH assay

more hydroxyl groups that determines its antioxidant activity. The properties of phenolic compounds are as free radical scavengers, hydrogen donators, metal chelators and singlet oxygen quenchers [9]. Gallic acid is one of the major polyphenolic compounds present in *Solanum nigrum* [2]. Plant extracts containing high levels of gallic acid may be able to scavenge excessive free radicals such as superoxide anion radicals and peroxy radicals in the human body and protect human cells or tissues against oxidative stress [10].

CONCLUSION

The semi-purified fractions obtained from Solanum nigrum through fractionation possess promising antioxidant and cytotoxic activities. They are thus a potential source of agents that may be developed as anti-cancer medicines.

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