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Anti-Proliferative Activity and Preliminary Phytochemical Screening of *Ipomoea quamoclit* Leaf Extracts

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ABSTRACT

Ipomoea quamoclit is a plant traditionally used to treat hemorrhoids, ulcers, diabetes and cancer. However, the anticancer property of this plant have not yet been scientifically tested. Hence, the present study aims to examine the anti-proliferative effect of the methanol, dichloromethane, ethyl acetate and hexane extracts of *Ipomoea quamoclit* leaves ($15-1000 \mu\text{g mL}^{-1}$) on MCF-7 (breast adenocarcinoma), HeLa (cervix adenocarcinoma), CNE-1 (nasopharyngeal carcinoma), HT-29 (colorectal adenocarcinoma) and 3T3 (normal mouse fibroblast) cell lines. Besides, preliminary phytochemical screening of each extract was also conducted. The methanol leaf extract of *Ipomoea quamoclit* was shown to possess the highest anti-proliferative activity against the tested cell lines. The greatest activity was observed on CNE-1 ($\text{IC}_{50} = 18 \pm 1.00 \mu\text{g mL}^{-1}$) and HT-29 ($\text{IC}_{50} = 18 \pm 1.00 \mu\text{g mL}^{-1}$). Dichloromethane, ethyl acetate and hexane extracts showed weak anti-proliferative activity. Phytochemical screening detected the presence of steroids, triterpenes, phenol, flavonoids and diterpenes in all four extracts but carbohydrates were only found in the methanol and ethyl acetate extracts. Whereas, hexane was the only extract that contains saponins.

Key words: Anti-proliferative activity, *Ipomoea quamoclit*, breast cancer, nasopharyngeal carcinoma, cervical cancer, colon cancer, mouse fibroblast

INTRODUCTION

Cancer remains one of the leading causes of death around the globe, with 7.6 million deaths recorded in 2008 alone (American cancer society, cancer prevention and early detection facts and figures 2012. Atlanta: American cancer society) (ACS., 2012). Cancer is not a simple molecular event but a multifunctional and dynamic one, requiring changes that affect the neoplastic cell, its interaction with surrounding stroma, as well as the immune system. Despite the availability of a wide range of chemotherapeutic drugs as well as the advancement in medical surgery and radiotherapy, the current management for cancer have failed to successfully lower the high morbidity and mortality rates. Besides, the current anti-cancer commonly used in chemotherapy have a lot of side effects including immunosuppression, myelosuppression, gastrointestinal distress, anemia, hair loss, secondary neoplasm and tumor lysis syndrome (Chitwood *et al.*, 2013; Rodgers *et al.*, 2012). Hence, there is a need in searching for new anti-cancer agents that are free of side effects. Plants are potential sources for the discovery of new anticancer agents. An estimated 80% of the world population make use of plant-sourced medicine as their primary health care.

Ipomoea quamoclit is commonly known as cypressvine or Morning-glory, an ornamental plant found in many countries with temperate climates. It is used as folk medicine around the world for various illnesses, such as hemorrhoids, ulcers, breast pain, snake bites, diabetes, fever, piles and as an antibiotic (Rajendran *et al.*, 2007; Sajem *et al.*, 2008). Furthermore, it found a total of 11 different resin glycosides in *Ipomoea quamoclit*, namely Quamoclins I-IV and Quamoclinic acids B-H, which are the bioactive components of the plant. Various species of the *Ipomoea* genus have been found to exhibit anticancer properties. One of them is *Ipomoea leari*, which was found to be active against Walker carcinoma-256 in rats (Sarin *et al.*, 1973). *Ipomoea bahiensis* was also found to be active against sarcoma 180 in mice in concentration as low as 7.5 mg kg⁻¹ (Bieber *et al.*, 1996). Besides, *Ipomoea squamosa* was found to be cytotoxic against human ovarian cancer (A2780) and human leukemic monocyte lymphoma (U937) cell lines (Cao *et al.*, 2005). *Ipomoea aquatica* leaf was also previously found to be cytotoxic on normal Vero, Hep-2 (human larynx epithelial carcinoma) and A-549 (human small cell lung carcinoma) cell lines (Prasad *et al.*, 2005).

Even though there have been some evidences of the anti-proliferative properties among plants of the *Ipomoea* genus, the anticancer activity of *Ipomoea quamoclit* is not yet evaluated. Hence, the present study aims to investigate the anti-proliferative activity of different extracts of *Ipomoea quamoclit* leaf on MCF-7 (human breast adenocarcinoma) cells, CNE-1 (nasopharyngeal carcinoma) cells, HeLa (human cervical cancer) cells, HT-29 (human colonic carcinoma cells) cells and 3T3 (mouse fibroblast) cells. A preliminary phytochemical screen was also carried out to determine the types of phytochemicals present.

MATERIALS AND METHODS

Plant material: *Ipomoea quamoclit* leaves were collected from Johor, Malaysia and were authenticated by a specialist in plant taxonomy and coordinator of biodiversity unit in Institute of Bioscience, University Putra Malaysia. The leaves were then air-dried at room temperature.

Preparation of extract: Leaf extracts were prepared with four solvents (methanol, dichloromethane, ethyl acetate and hexane) (Ayinde *et al.*, 2012). The dried leaves were first grounded into coarse form using an electronic blender. A total of 45 g of leaves were then soaked in 300 mL of solvent for 4 days. After the filtrate was decanted, a second maceration was carried out with 200 mL of solvent. The extracts were filtered after 4 days. The solvent was then evaporated using a rotary evaporator at controlled temperature (methanol: 64.7°C, dichloromethane: 34.6°C, ethyl acetate: 77.1°C, hexane: 68.7°C). The crude extracts was dissolved in Dimethyl Sulphoxide (DMSO) solution to produce a 100,000 µg mL⁻¹ stock solution. Both the stock solutions and the remaining crude extracts were stored at 4°C (Kamalraj and Devdass, 2011).

Cell lines: The selected cell lines (MCF-7, HeLa, CNE-1, HT-29 and 3T3) were obtained from International Medical University Research Lab. They were then cultured in Dulbecco's Modified Eagle Medium (DMEM) (MediaTech, USA) supplemented with 10% foetal bovine serum (Cell Culture Company, Australia) and 1% penicillin-streptomycin (i-DNA Biotechnology, Singapore) at 37°C in a humidified air atmosphere with 5% CO₂.

Anti-proliferative assay: After 100 µL of cultured cells were seeded into the 96-well plate (Falcon, USA) at the density of 10,000 cells per well, they were allowed to adhere overnight at 37°C in a humidified air atmosphere with 5% CO₂. Then, the cells were treated with 100 µL of the

extract, which had been diluted from the stock solution to a series of different concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625 $\mu\text{g mL}^{-1}$). The first row of the wells served as negative controls, where no extract were seeded. The cells were further incubated for 48 h. After that, 20 μL of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) solution (Sigma-Aldrich, USA) was added into each well and incubated at 37°C for 4 h. The supernatant layer was then removed and the formazan crystal was dissolved by adding 100 μL of DMSO. The absorbance was then measured at 570 nm using an ELISA plate reader (Opsys, USA). The percentage of cell viability was calculated by using the following equation:

$$\text{Percentage of cell viability (\%)} = \frac{\text{Absorbance (treated)}}{\text{Absorbance (negative control)}} \times 100\%$$

The assay was repeated for a total of 3 times, then the graphs of concentration-response curve were plotted and IC_{50} values were determined (RW.ERROR-Unable to find reference: 552; Denizot and Lang, 1986). Selectivity Index (SI) was calculated with the following equation:

$$\text{Selectivity Index (SI)} = \frac{\text{IC}_{50} \text{ of 3T3}}{\text{IC}_{50} \text{ of cancer cell line}}$$

Preliminary phytochemical screening: Chemical tests were carried out according to the methods reported by Tiwari *et al.* (2011):

- **Alkaloids-dragendorff's test:** Each extract was dissolved in dilute hydrochloric acid. Dragendorff's reagent was added and observed for the presence of red precipitate
- **Carbohydrates-benedict's test:** Each extract was dissolved in distilled water and filtered. The filtrate was then heated gently with Benedict's reagent and observed for the presence of red-orange precipitate
- **Saponins-foam test:** The extract was shaken with water. The presence of foam, which persists for about 10 min was observed
- **Steroids and triterpenes-salkowski's test:** The extract was treated with chloroform and filtered. The filtrate was then treated with a few drops of concentrated sulphuric acid, shaken and allowed to stand. It was observed for the presence of golden yellow colour
- **Phenols-ferric chloride test:** The extract was dissolved in distilled water. A few drops of ferric chloride solution were added and it was observed for the presence of bluish black colour
- **Flavonoids-alkaline reagent test:** The extract was treated with a few drops of sodium hydroxide solution and observed for the formation of intense yellow colour. Diluted acid was added and the solution was observed for the color changes from yellow to colorless
- **Proteins and amino acids-ninhydrin test:** The extract was dissolved in distilled water. It was then boiled at 100°C with ninhydrin reagent and observed for the presence of blue colour
- **Diterpenes-copper acetate test:** The extract was dissolved in distilled water and treated with a few drops of copper acetate solution. It was then observed for the presence of emerald green colour

Statistical analysis: All the data were subjected to analysis of variance (ANOVA) using the Statistical Package for the Social Science (SPSS) 18.0. As p-value lower than 0.05 was considered to be significant.

RESULTS

Anti-proliferative assay: The four different leaf extracts of *Ipomoea quamoclit* were studied for their anti-proliferative activity on five different cell lines (CNE-1, HT-29, MCF-7, HeLa and normal 3T3). All extracts generally showed a concentration-dependent effect against all cell lines tested (Fig. 1). The IC_{50} value, a parameter to compare the cytotoxic activity in the present study

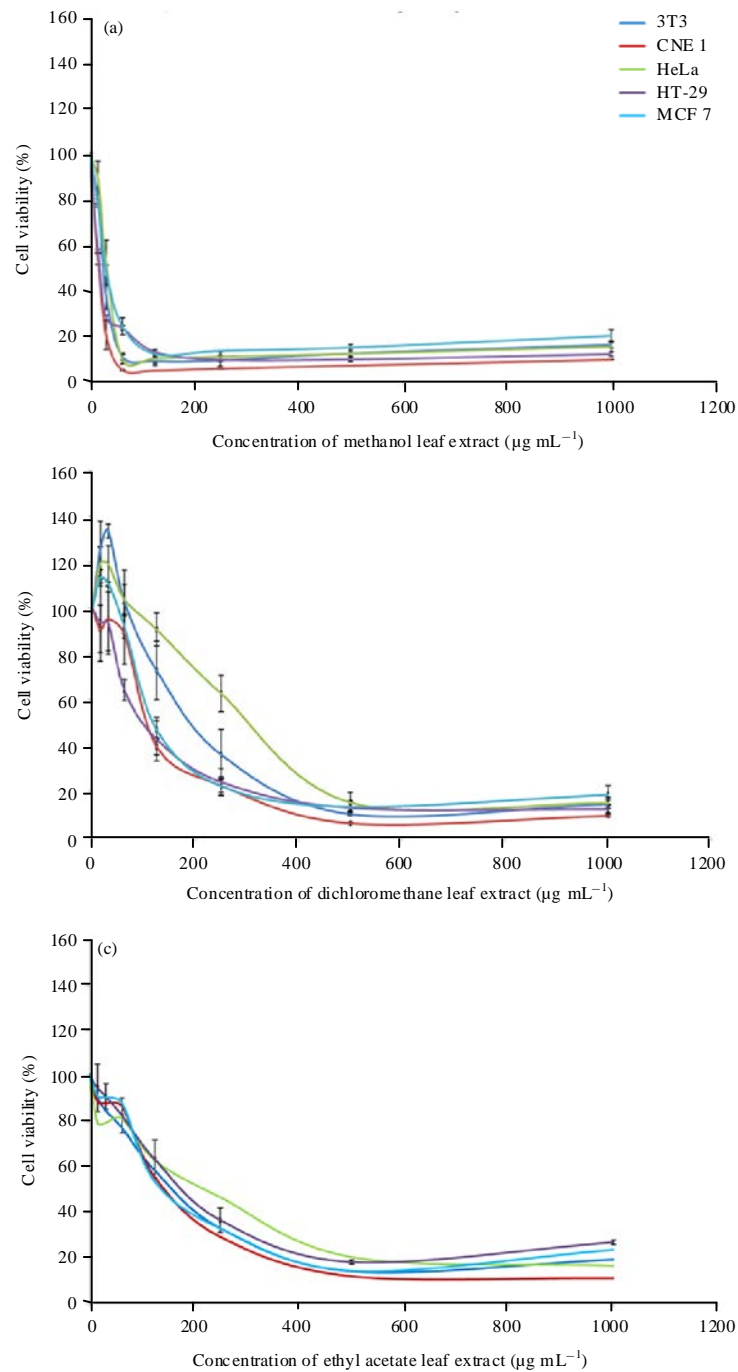


Fig. 1(a-d): Continue

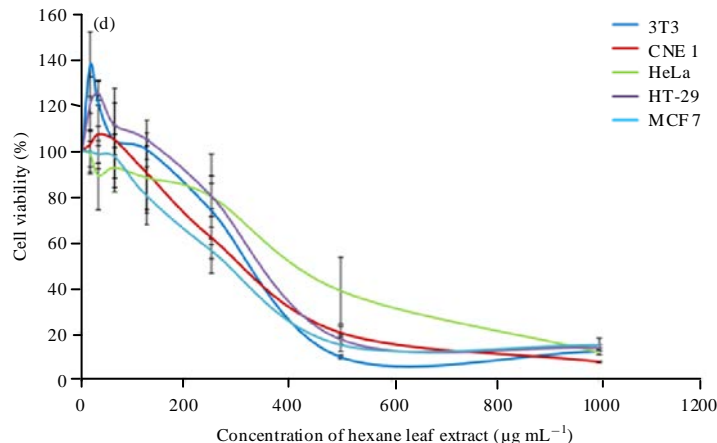


Fig. 1(a-d): Anti-proliferative activity of (a) Methanol, (b) Dichloromethane, (c) Ethyl acetate and (d) Hexane extract of *Ipomoea quamoclit* leaves on different cell lines *in vitro*

Table 1: IC₅₀ values of methanol, dichloromethane, ethyl acetate and hexane leaf extracts of *Ipomoea quamoclit* on CNE-1, HT-29, MCF-7, HeLa and normal 3T3 cell lines after 48 h of incubation

Cell lines	IC ₅₀ values (µg mL ⁻¹)			
	Methanol	Dichloromethane	Ethyl acetate	Hexane
CNE-1	18±1.00	129±18.72	142±23.31	273±35.27
HT-29	18±1.00	118±30.33	175±29.88	350±10.00
MCF-7	31±1.20	143±23.79	137±14.42	270±47.26
HeLa	33±2.73	311±34.86	222±58.54	447±76.89
3T3	26±1.20	214±39.63	159±37.95	300±41.63

were summarized in Table 1. Among the four extracts, the methanol leaf extract of *Ipomoea quamoclit* demonstrated the highest anti-proliferative activity against the five tested cell lines with lowest IC₅₀ values (Table 1). The methanol extract was found to exert the greatest effect and better sensitivity on CNE-1 and HT-29 cell lines (IC₅₀ < 20 µg mL⁻¹), while its sensitivity against MCF-7 and HeLa cell lines were lower than the normal 3T3 cell line.

Comparatively, the IC₅₀ values of the dichloromethane, ethyl acetate and hexane leaf extract of *Ipomoea quamoclit* are much greater than the methanol leaf extracts of *Ipomoea quamoclit* (Table 1). For the dichloromethane extract, the anti-proliferative effect was most significant (p-value < 0.05) towards HT-29 cell lines. While for ethyl acetate and hexane extracts, the anti-proliferative effect is most significant (p-value < 0.05) towards MCF-7 cell lines. However, all IC₅₀ values of dichloromethane, ethyl acetate and hexane leaf extract of *Ipomoea quamoclit* are greater than 20 µg mL⁻¹.

Phytochemical screening test: Phytochemical screening using various chemical tests was conducted on each *Ipomoea quamoclit* leaf extract to test for the presence of alkaloids, carbohydrates, saponins, steroids and triterpenes, phenols, flavonoids, diterpenes and protein. The results of phytochemical screening are shown in Table 2.

Steroids, triterpenes, phenol, flavonoids and diterpenes were detected in all four extracts, while carbohydrates were only detected in the methanol and ethyl acetate extract. Furthermore, hexane was the only extract that did not contain saponins.

Table 2: Phytochemical screening of methanol, dichloromethane, ethyl acetate and hexane leaf extracts of *Ipomoea quamoclit*

Phytochemicals	Crude extract of <i>Ipomoea quamoclit</i>			
	Methanol	Dichloromethane	Ethyl acetate	Hexane
Alkaloids	-	-	-	-
Carbohydrates	+	-	+	-
Saponins	+	+	+	-
Steroids and triterpenes	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Diterpenes	+	+	+	+
Amino acids and proteins	-	-	+	-

+: Present, -: Absent

Table 3: Selectivity Index (SI) values

Extracts	Cell lines and selectivity index			
	HT-29	CNE-1	MCF-7	HeLa
Methanol	1.40	1.40	0.80	0.80
Dichloromethane	1.81	1.65	1.49	0.68
Ethyl acetate	0.91	1.12	1.16	0.77
Hexane	0.86	1.10	1.11	0.67

DISCUSSION

This study investigates the anti-proliferative activity of methanol, dichloromethane, ethyl acetate and hexane leaf extracts of *Ipomoea quamoclit* against four cancer cell lines (CNE-1, HT-29, MCF-7 and HeLa) and a normal 3T3 cell line. Besides, preliminary phytochemical screening was also carried out.

Generally, the anti-proliferation assay showed that each *Ipomoea quamoclit* leaf extract possesses concentration-dependent anti-proliferative activity on the tested cell lines as shown in Fig. 1. According to American National Cancer Institute, active cytotoxicity of crude extract is indicated by an $IC_{50} \leq 20 \mu\text{g mL}^{-1}$ (Hazalin *et al.*, 2009). Therefore, methanol leaf extract of the plant has shown promising anti-proliferative effect against the tested cell lines, especially against CNE-1 ($IC_{50} = 18 \pm 1.00 \mu\text{g mL}^{-1}$) and HT-29 cell lines ($IC_{50} = 18 \pm 1.00 \mu\text{g mL}^{-1}$). On the other hand, dichloromethane, ethyl acetate and hexane extracts of *Ipomoea quamoclit* have shown weak cytotoxicity since they have IC_{50} values $\geq 20 \mu\text{g mL}^{-1}$ (Table 1).

In this study, normal 3T3 cell line played an important role in assessing the basal cytotoxicity as recommended by US National Institute of Environmental Health Sciences (NIEHS) (NIEHS., 2001). New chemical entity suitable as anti-cancer medication should exhibit cytotoxicity specific for cancer cells only, with minimal effect to normal human cells.

Selectivity Index (SI) was commonly used as a parameter to determine the selectivity of an extract towards cancer cell lines as compared to the normal 3T3 cell line (Badisa *et al.*, 2009). The selectivity of an extract increases proportionally with increasing SI. The SI value of less than 2 indicates general toxicity of the pure compound. From the study, it has been observed that the SI values of all four extracts are less than 2 (Table 3). Hence, all the extracts lack selectivity towards the cancer cell lines studied. However, it should be noted that the low selectivity may be caused by the usage of crude extracts in this study instead of pure compound.

The phytochemical components present in all four extracts are steroids and triterpenes, phenol, flavonoids and diterpenes. Phenolic compounds and flavonoids are well known for their antioxidant and anticancer properties and have been widely used in cancer treatments (Boudet, 2007; Dai and Mumper, 2010; Havsteen, 2002). According to Haddad *et al.* (2006) flavonoids inhibit the growth

of human prostate cancer cells through alteration in cell cycle (Haddad *et al.*, 2006). Moreover, phenolic compounds like anthocyanins have also been found to contribute to the cytotoxic activity of *Ipomoea batatas* (Kaneshiro *et al.*, 2005). Prasad *et al.* (2005) also found that the presence of polyphenolic compounds in the crude methanol leaf extract of *Ipomoea aquatica* is related to its anti-proliferative activity on Hep-2 cells and A549 human small cell lung carcinoma (Prasad *et al.*, 2005).

However, the quantitative amount of the phytochemicals remains unknown. The difference in the anti-proliferative activity may be due to the different amount of phytochemicals in each extract. Theoretically, methanol extract should contain the highest amount of the phytochemicals with desired activity as methanol has the greatest anti-proliferative activity towards the four different cell lines. Therefore, further quantitative analysis should be conducted to determine the amount of phytochemicals presence in each extract.

CONCLUSION

In conclusion, methanol leaf extract of *Ipomoea quamoclit* shows good anti-proliferative activity especially against CNE-1 and HT-29 cell lines. As preliminary phytochemical screening has been conducted, future study could focus on the identification of the bioactive molecule.

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