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*Full Length Research Paper*

# Antiproliferative effect of *Moringa oleifera* Lam. and *Pseuderanthemum palatiferum* (Nees) Radlk extracts on the colon cancer cells

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**Consumption of *Moringa oleifera* and *Pseuderanthemum palatiferum* for cancer therapy is becoming popular in Thailand. The aim of this study was therefore to investigate the inhibitory effect of the aqueous and ethanol extracts from *M. oleifera* and *P. palatiferum* leaves on colon cancer cell proliferation. Prior to the antiproliferative test, primarily safety test of the extracts was conducted by Ames test and the two extracts were clarified for their non-mutagenic activity. Test of antiproliferative effect on 3 types of colon cancer cell lines; HCT15, SW48 and SW480, using the extracts at the concentrations of 100, 250, 500 and 1,000 µg/ml for 24, 48 and 72 h, respectively by 3′-(4, 5 dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay showed that both extracts were toxic to all types of tested cell lines with increasing concentrations and time. It was also found that *M. oleifera* extract was more effective than that of *P. palatiferum*. Moreover, the ethanol extract of *M. oleifera* tended to give better antiproliferative results on all tested cell lines than the aqueous extract and SW48 was found to be the most sensitive cell line type. It has been shown from our results that these two plants might be new choices of naturally therapeutic products against colon cancer.**

**Key words:** *Moringa oleifera* Lam., *Pseuderanthemum palatiferum* (Nees) Radlk, colon cancer cells.

## INTRODUCTION

Reports from all over the world on the increase incident of cancer especially in Asia indicate that colon cancer rate is top on the list (Wargovich, 2001; Peng et al., 2002; Zhao et al., 2004). Although problems of cancer control have long been waiting for the best solution, challenges of drug discovery still remain. Due to the high incidence of colon cancer together with the increase trend of natural product utilization, researches into medicinal plants as a potential source of new remedies for colon cancer have been promoted. The antiproliferative effect of aqueous extract from the bark of *Millingtonia hortensis* against human colon cancer cell lines, DLD-1, HCT15, SW48 and SW480 has been proved by the study of Tansuwanwong et al. (2007). Similarly, cell cycle of human colon adenocarcinoma-derived, Caco-2 cells, were arrested when treated with the *Centella asiatica* extract (Bunpo et al., 2005). In recent years, *Moringa oleifera* Lam. and *Pseuderathemum palatiferum* (Nees) Radlk. have become the popular medicinal plants in Thailand. They have been used to cure many diseases and their efficiency in treating cancer was strongly believed. *M. oleifera* Lam. is a perennial softwood tree in the family Moringaceae. Its compound leaf contains 3 to 9 thin leaflets dispersed on a compound stalk. It has white flowers, long pods with 3 angled winged seeds (Foidl and Paull, 2008). Phytochemical studies showed that *M. oleifera* contained 4-(4'-*O*-acetyl-a-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(a-L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate and 4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate (Faizi et al., 1998; Fahey et al., 2001). *M. oleifera* is known in every Thai kitchen as almost all parts

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of it are commonly used in many dishes.

In addition to its nutritional values, *M. oleifera* offers many important health benefits, for instances, hypolipidaemic (Mehta et al., 2003), hypotensive (Faizi et al., 1998) and hepato-protective activities (Pari and Kumar, 2002). The inhibitory activity of *M. oleifera* on CEM, HL-60 (leukemia), B-16 (murine melanoma), HCT-8 (human colon carcinoma) and KB tumor cell lines was also claimed (Costa et al., 2005; Sreelatha et al., 2011). The ability of root extract of this plant to inhibit the antiproliferation of HCT-8 colon cell lines (Costa et al., 2005) gained our interest in testing this activity of the leave extract on colon cell. While the information of *M. oleifera* have been well documented, those of *P. palatiferum* (Nees) Radlk., however, were reported with very little information disclosed. This plant species belongs to the Acanthacea family, native to Vietnam. It is a bush with height of 1 to 2 m. The leaves are in whorls, green and glossy. The flowers are pink or purple (Dieu et al., 2005). Chemically, the leaves of *P. palatiferum* contain β-sitosterol, salicylic acid and phytol (Khanh, 1997; Padee et al., 2009).

It has been used by Vietnamese people for healing wound and gastric ulcer, treating diarrhea, sore throat and cancer (Grever, 2001; Dieu et al., 2006). Although, the latest property has gained tremendous interest among Thai people, there is no published scientific evidence supporting its anticancer potential. Only its abilities to treat diarrhea in pigs (Huynh and Tran, 2003; Phan et al., 2005), to inhibit fungal and bacterial growth (Kankamon, 2008) and to exhibit no toxicity in Vero cells of African green monkey kidney (Padee et al., 2009) have been scientifically reported. The leaves of *M. oleifera* and *P. palatiferum* recently become a widespread form of products and are promoted commercially in Thailand as having anticancer and antioxidant properties. Since laboratory evidence indicated the relationship of antioxidant and anticancer activities of some plants such as *Clausena lansium* and *Zingiber officinale* (Prasad et al., 2010, Rahman et al., 2011), the proof of capacity of *M. oleifera* and *P. palatiferum* in treating colon cancer is of interest. The evaluation on antiproliferative effect of *M. oleifera* and *P. palatiferum* in 3 colon cancer cell lines; H zCT15, SW48 and SW480 was therefore conducted in this study.

## MATERIALS AND METHODS

### Preparation of plant extracts

*M. oleifera* and *P. palatiferum* were obtained from a market in Muang District, Chiang Mai Province. The plants were identified by a botanist and the herbarium specimens were deposited at the Queen Sirikit Botanical Garden, Chiang Mai**,** ID: WP2614 for *M. oleifera* and ID: WP2615 for *P. palatiferum*. Fresh leaves of both plants were washed with clean tap water and ground in a ceramic mortar. 100 g of the ground leaves were then soaked in 1,000 ml of solvent (water or 80% ethanol) for 4 h and filtered through a piece of satin cloth. The filtrates were centrifuged at 5,000 rpm for 15 min and filtered again through Whatman filter paper No. 1. The filtrates were evaporated with rotating evaporator and freeze dried. The powdered extracts were kept at 5°C.

### Ames test

The safety of each extract was primarily evaluated using the mutagenicity test in *Salmonella typhimurium* (TA98), according to the method of Maron and Ames (1983). Different concentrations of the extracts were prepared and 50 µl of each concentration were mixed with 500 µl of the enzyme (S9 mix) or phosphate buffer in a test tube. Overnight culture (0.1 ml) of *S. typhimurium* was then added to the mixture and shaken at 30°C for 30 min. Two milliliters of molten agar was later added and mixed for 1 to 2 min. The mixtures were poured onto minimal glucose agar plate and incubated at 37°C for 48 h. Mutant colonies were counted under a stereomicroscope. Only the plates without the killing effect which may arise from the substances used in the experiment were taken into account. The number of mutant colonies obtained from each concentration was subtracted from the background colonies of control groups (dimethyl sulfoxide (DMSO) or distilled water) and mean values of three independent tests were then calculated. Only the non-mutagenic extracts will be selected for MTT assay.

### Cell lines and culture medium

The cancer cells used in the experiment were colon cancer cell line types HCT15, SW48 and SW480. Primary investigation had been done on the proper amount of cells for cultivation in 96 wells culture plate. The initial number of cell was 20,000 cultured in dulbecco's modified eagle's medium (DMEM) mixed with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin, incubated at 37°C with 5% $CO_2$ .

### MTT assay

Cells were cultured overnight in a 96 well plate and treated with the extracts that were declared safe by Ames test at the concentrations of 100,250,500 and 1,000 µg/ml for 24, 48 and 72 h. The culture mediums with the antibiotics were then changed to a new medium with MTT at the concentration of 0.5 mg/ml in each well. Culturing was carried on for 4 h and the medium with MTT was taken out and 150 µl of DMSO was added to all the wells. The colorimetric measurement was then conducted at 540 nm. The effectiveness of the extracts in causing cell death was deduced through the calculation of percent of cell viability and median inhibitory concentration ($IC_{50}$). The reported value is the mean of three independent assays.

### Statistical analysis

Analysis of variance between groups of mean ± SD was determined with Mann-Whitney U test at 95% confident level (*P<0.05*). The values of $IC_{50}$ were calculated by probit analysis.

## RESULTS

Both ethanol and aqueous extracts from the leaves of *M. oleifera* and *P. palatiferum* had no mutagenic effect. The number of mutant colonies of the tested bacterium at all

**Table 1.** Number of mutant colonies of *S. typhimurium* TA98 induced by *M. oleifera* and *P. palatiferum* extracts with or without activated enzyme.

| Treatment | Mutant colony | |
|---|---|---|
| | ±S9 mix | -S9 mix |
| 0 (DMSO) | 28 ± 6.63 | 18 ± 10.57 |
| 2-AA (0.5 µg/pl) | 103.00 ± 7.21 | - |
| AF-2 (0.025 µg/pl) | - | 248.33 ± 31.68 |
| Aqueous extracts of *M. oleifera* (25 µg/pl) | 27.50 ± 5.36 | 23.20 ± 4.09 |
| Aqueous extracts of *M. oleifera* (50 µg/pl) | 30.17 ± 2.86 | 22.00 ± 2.45 |
| Aqueous extracts of *M. oleifera* (100 µg/pl) | 33.83 ± 8.41 | 22.80 ± 5.36 |
| Ethanol extracts of *M. oleifera* (25 µg/pl) | 28.50 ± 7.74 | 20.33 ± 1.51 |
| Ethanol extracts of *M. oleifera* (50 µg/pl) | 33.50 ± 3.73 | 22.33 ± 2.16 |
| Ethanol extracts of *M. oleifera* (100 µg/pl) | 36.67 ± 6.80 | 32.80 ± 5.36 |
| 0 (DMSO) | 29.67 ± 7.00 | 23.17 ± 3.37 |
| 2-AA (0.5 µg/pl) | 372.00 ± 22.44 | - |
| AF-2 (0.025 µg/pl) | - | 313.17 ± 19.00 |
| Aqueous extracts of *P. palatiferum* (25 µg/pl) | 21.67 ± 2.88 | 23.17 ± 3.54 |
| Aqueous extracts of *P. palatiferum* (50 µg/pl) | 27.83 ± 5.19 | 24.83 ± 9.39 |
| Aqueous extracts of *P. palatiferum* (100 µg/pl) | 41.40 ± 5.03 | 25.40 ± 6.69 |
| Ethanol extracts of *P. palatiferum* (25 µg/pl) | 27.76 ± 7.55 | 22.00 ± 3.41 |
| Ethanol extracts of *P. palatiferum* (50 µg/pl) | 29.00 ± 5.29 | 22.50 ± 6.60 |
| Ethanol extracts of *P. palatiferum* (100 µg/pl) | 35.83 ± 2.71 | 28.83 ± 9.26 |

The data shown are the average ± standard deviation from 3 independent tests which the natural occurring colonies were already subtracted. 2-AA and AF-2 are the standard substances giving positive result with and without metabolic activation, respectively.

concentrations of the extracts was significantly lower than the positive control (*P<0.001*) but was not different from the negative control either in the presence or absence of the activating enzyme (Table 1).

### Inhibitory effect of *M. oleifera* and *P. palatiferum* extracts on colon cancer cell proliferation

Ethanol and aqueous extracts of *M. oleifera* and *P. palatiferum* were found to be toxic against the three types of colon cancer cells as the concentration and time increased. The *M. oleifera* extract was more effective in inhibiting cell proliferation than the *P. palatiferum* extract (Figures 1 and 2). The $IC_{50}$ at 72 h indicated that the ethanol extract of *M. oleifera* tended to inhibit cell proliferation of all the tested cell lines better than that of the aqueous extract (Table 2). SW48 was found to be most sensitive to the *M. oleifera* extract (Table 2).

## DISCUSSION

The bacterial mutation test is primarily used to confirm the gene safety of the substances before other tests, for example, anticancer were performed (Ghazali et al., 2011). From our results, *M. oleifera* and *P. palatiferum* extracts had no mutagenic effect on *S. typhimurium* TA98 whether the activating enzyme was used or not. There had been a report that aqueous extract of *M. oleifera* at 0.2 to 5.0 mg/pl had no mutagenic effect on *S. typhimurium* TA98 and TA100 (Charoensin and Wongpoomchai, 2010). In this study, although higher concentrations of both aqueous and ethanol extracts (25 to 100 mg/ml) were used, mutation did not occur. Moreover, *P. palatiferum* extract at the same range of concentration was not able to induce bacterial cell mutation. It is, therefore, confirmed by our results that both ethanol and aqueous extracts from *M. oleifera* and *P. palatiferum* leaves were not the mutagenic substances. MTT assay was used for toxicity test on the cancer cells. MTT will be changed to purple color compound, formazan by mitochondrial dehydrogenase which is active in the living cells (Mosmann, 1983). This purple compound is dissolved in DMSO and the intensity of color is directly proportional to the amount of living cells. The concentration of the extract giving 50% cancer cell survival ($IC_{50}$) is the comparative value of the extract in inhibiting the cancer cells.

In this study, each extract inhibited colon cancer cell proliferation differently. This might be due to the difference in cell properties and the phenotypic and genotypic characters of the three types of cells as well as

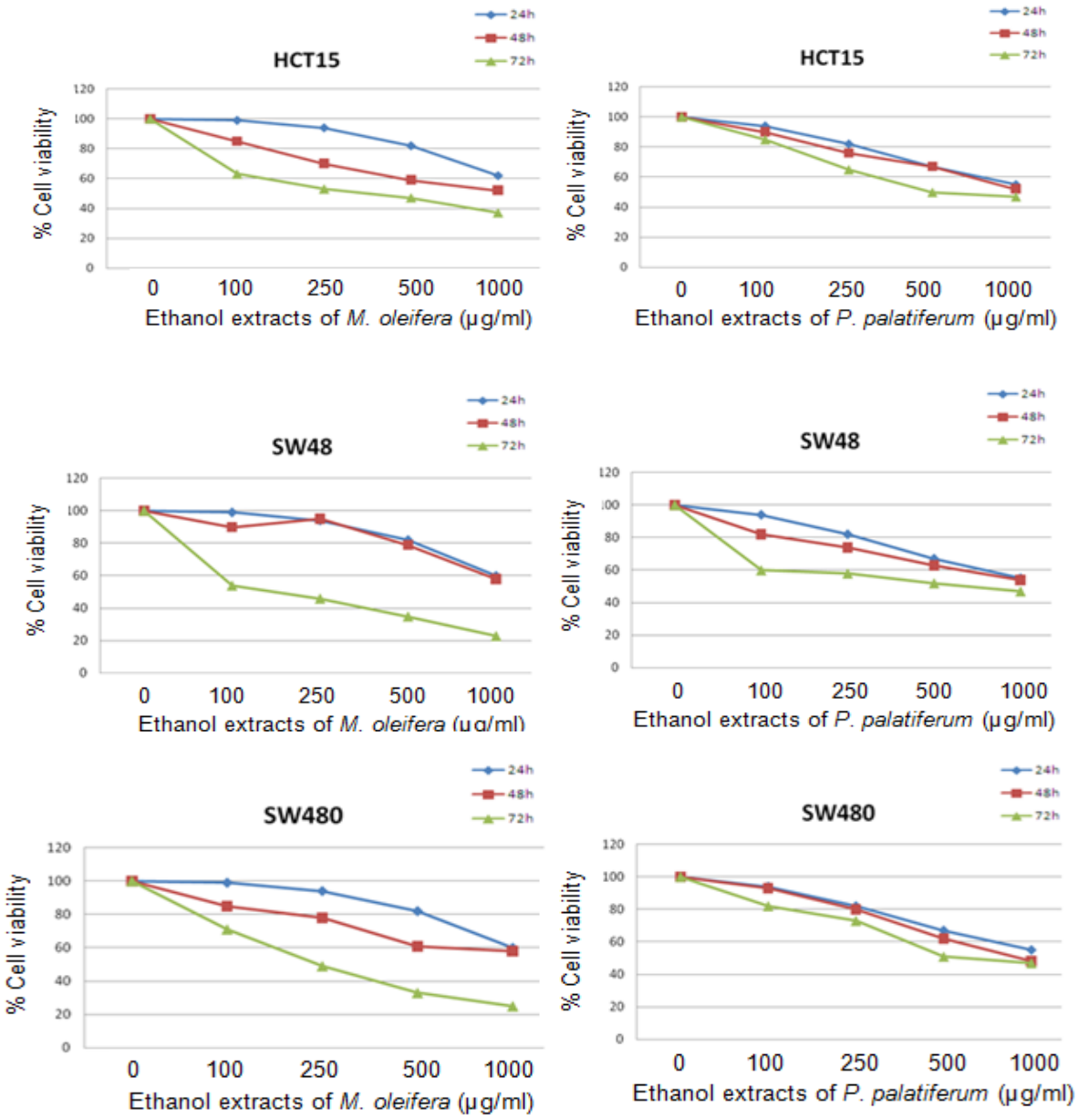


**Figure 1.** The antiproliferative effect of ethanol extracts from the leaves of *M. oleifera* and *P. palatiferum* on 3 types of human colon cancer cell line; HCT15, SW48 and SW480. Data are representative of three independent experiments (mean ± SD).

the active constituents in the crude extract from each part of the plant or from different plants as suggested by Tima et al. (2010). Moreover, the use of different solvents might give different proportions of bioactive substances which in turn, give different antiproliferative activity. Aqueous extract was used in the present study because the two plants are usually consumed with water for example, hot soup or tea infusion. As for ethanol extract, there are many reports on its effective inhibition of cancer cell proliferation (Padee et al., 2009: Tima et al., 2010). There is a report that the free radicals in the cell play an important role in carcinogenesis (Witz, 1991). Therefore, the antiproliferative efficiency of the extracts on colon cancer cells may arise from the antioxidative action of these two plants. There are several reports that *M. oleifera* leaves contain many antioxidants for example, vitamin C, α-tocopherol, flavonoids, phenolics and carotenoids (Makkar, 1996) while *P. palatiferum* contains flavanoids, apigenin, triterpenoids, saponin, sitosterol, stigmasterol, kaempferol and salicylic acid (Hung et al., 2004). These antioxidants are well known for their ability to delay cell degeneration and may prevent carcinogenesis.

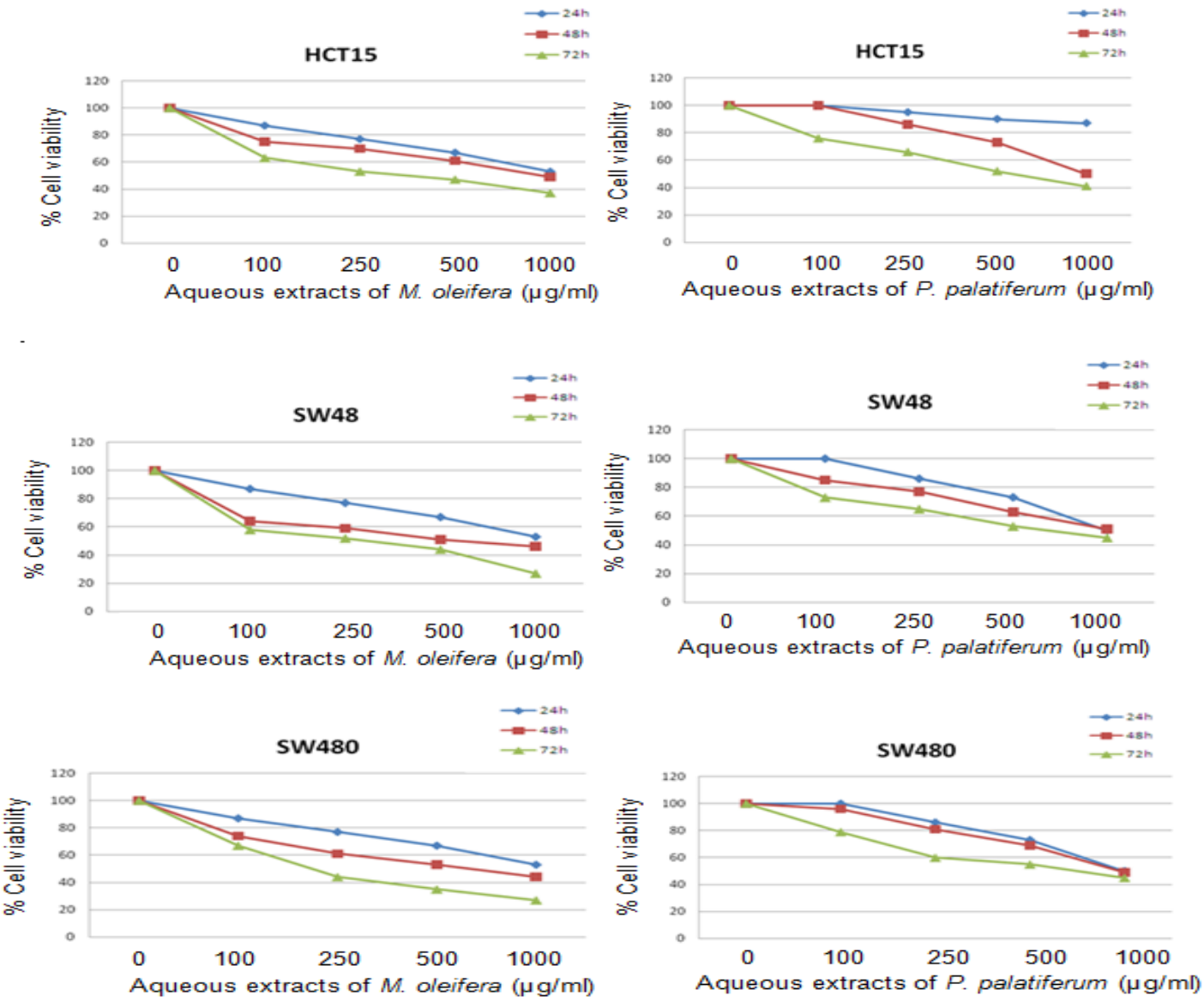


**Figure 2.** The antiproliferative effect of aqueous extracts from the leaves of *M. oleifera* and *P.palatiferum* on 3 types of human colon cancer cell line; HCT15, SW48 and SW480. Data are representative of three independent experiments (mean ± SD).

From the result of cell viability (Figures 1 and 2), it was shown that the *M. oleifera* extracts had better antiproferative activity on all types of cells than the *P. palatiferum* extracts. Moreover, the aqueous and ethanol extracts of *M. oleifera* had better antiproliferative effect on all types of tested colon cancer cell lines than other plant extracted with the same solvent for example, *M. hortensis* which has higher $IC_{50}$ than that of *M. oleifera* (Tansuwanwong et al., 2007).

Although the *P. palatiferum* extract did not show high activity on antiproliferation of cells, it had a tendency to inhibit colon cancer cell proliferation of all types. Therefore, it is possible to purify the extracts and analyze the active ingredients in order to develop into colon cancer drugs or dietary supplement for combined use with other chemotherapeutic agents in the future. The induction of cell cycle arrest, cell apoptosis and deoxyribonucleic acid (DNA) fragmentation have been suggested for mechanism of action of plant extracts in cancer cell inhibition (Ma et al., 2006; Tian et al., 2007) Besides, the research has been explored in the molecular level in some plant extracts. Ze et al. (2005) reported the cytotoxicity of three cycloartane triterpenoids isolated from the *Cimicifuga dahurica* on solid tumor (HepG2), blood tumor (HL-60) and drug resistant tumor (R-HepG2) cell lines with the suggestion that those three compounds

**Table 2.** Toxicity ($IC_{50}$ µg/ml) of *M. oleifera* and *P. palatiferum* extracts on 3 types of colon cancer cell lines. Data are representative of three independent experiments (mean ± SD).

| Treatment | Colon cell lines | $IC_{50}$ (µg/ml) |
|---|---|---|
| Aqueous extracts of *M. oleifera* | HCT15 | 266.67 ± 18.53 |
| | SW48 | 105.47 ± 23.50 |
| | SW480 | 200.26 ± 27.64 |
| Ethanol extracts of *M. oleifera* | HCT15 | 264.83 ± 23.33 |
| | SW48 | 102.40 ± 16.08 |
| | SW480 | 197.20 ± 32.52 |
| Aqueous extracts of *P. palatiferum* | HCT15 | 520.33 ± 24.74 |
| | SW48 | 503.75 ± 30.21 |
| | SW480 | 511.49 ± 21.22 |
| Ethanol extracts of *P. palatiferum* | HCT15 | 508.98 ± 26.87 |
| | SW48 | 500.39 ± 28.47 |
| | SW480 | 511.74 ± 31.21 |

possessed potentially cytotoxic activitiy on cancer cell lines via apoptosis and G2/M arrest which was resulted from the inhibition of cdc2 protein expression. Some medicinal plants such as *Elaeagnus glabra* and *Pluchea indica,* showed the inhibitory effects on cancer cell invasion, primarily via suppression of activity of matrix metalloproteinase (MMP, type 2 and 9), the enzymes involved in cancer invasion and metastasis activity (Ohtsuki et al., 2008; Li et al., 2009). Although, this research is only a preliminary study and only antiproliferative activity with MTT assay was studied, its results could support the folk use of *M. oleifera* and *P. palatiferum* as anticancer plants. Furthermore, this study is a promising first step towards further investigation concerning the anticancer efficiency of these 2 plant species. The combination of MTT and SRB assay to confirm their antiproliferative activity, the comparison of their ability to synthetic anticancer drug and the determination of mechanisms of action as conducted in other plant species (Prasad et al., 2005; Manosroi et al., 2006; Prasad et al., 2010; Sowemimo et al., 2011) will be investigated in our extensive researches.

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