

# ANTIOXIDANT ACTIVITY AND CYTOTOXICITY OF THE LEAVES OF *Melastoma decemfidum* Roxb. ex. Jack

Noraslamiah Sarju<sup>1</sup>, Maaruf Abd Ghani<sup>2</sup>, Salehuddin Hamdan<sup>1</sup> & Azman Abd Samad<sup>1</sup>

<sup>1</sup>Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor Darul Takzim, Malaysia.

<sup>2</sup>School of Chemical Science and Food Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Darul Ehsan, Malaysia.

(azman@fbb.utm.my)

## ABSTRACT

The aim of this investigation was to determine the content of kaempferol and naringenin, antioxidant activity and cytotoxicity of methanol extracts from the leaves of *Melastoma decemfidum* plants. The concentration of naringenin and kaempferol was determined and quantified using Gas Chromatography. The crude leaf extracts, single and combination of the two pure flavonoids were then screened for their antioxidant and cytotoxic activities. The antioxidant assay was carried out by the DPPH radical-scavenging and the cytotoxicity was measured by the MTT assay against the human cervical cancer cell lines. The combination of pure compound of naringenin and kaempferol had strongest antioxidant activity and cytotoxicity followed by the crude extracts, pure kaempferol and pure naringenin.

**Keywords:** Flavonoids, antioxidant, cytotoxicity, *Melastoma decemfidum*

## INTRODUCTION

*Melastoma decemfidum*, locally known as 'senduduk putih', is a member of Melastomataceae family. The species distribution covers Madagascar, India, and Australia [1]. The plants have also been used in traditional Malay medicine for the treatment of diarrhea as an astringent, post-partum treatment, hemorrhoids, hepatitis, leucorrhoea, swelling, piles, mouth ulcer, toothache, sinusities and as a diuretic [2, 3]. Recently, phytochemical analysis from *M. decemfidum* showed the presence of three compounds (naringenin, kaempferol and kaempferol-3-O-D-glucoside) in ethyl acetate extract and two compounds [(kaempferol-3-O-(2", 6"-di-O-p-trans-coumaroyl) glucoside and kaempferol-3-O-d-glucoside)] in methanol extracts. Cytotoxic activity tests indicated that naringenin and kaempferol-3-O-(2",6"-di-O-p-trans-coumaroyl)glucoside were found to be active in inhibiting cell proliferation of human breast cancer cell line (MCF7) with IC<sub>50</sub> values of 0.28 µM and 1.3 µM, respectively [4]. We did not find published data concerning isolation of high naringenin and kaempferol plant lines and potential of *M. decemfidum* methanolic extracts to act as antioxidant and anticancer against HeLa cell lines.

## METHODOLOGY

### Extraction of plant materials

Three to four month-old *M. decemfidum* plants were purchased from local nursery. Mature leaves were extracted from twenty-six plant lines using methanol solvent.

### Flavonoids detection of methanolic extracts

Detection and quantification were performed using gas chromatography (GC). Quantification of kaempferol and naringenin was carried out using response factor method [5].

### Cytotoxicity assay

Human cervical cancer cell lines (HeLa) was cultured RPMI 1640 with L-glutamine supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin. The MTT 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide)-based colorimetric assay was carried out to determine the cytotoxic effect [1]. The absorbance was measured at 575nm in a microplate reader. The 50% reduction in cell number relative to the control or IC<sub>50</sub> was determined.

### Scavenging activity of DPPH radical

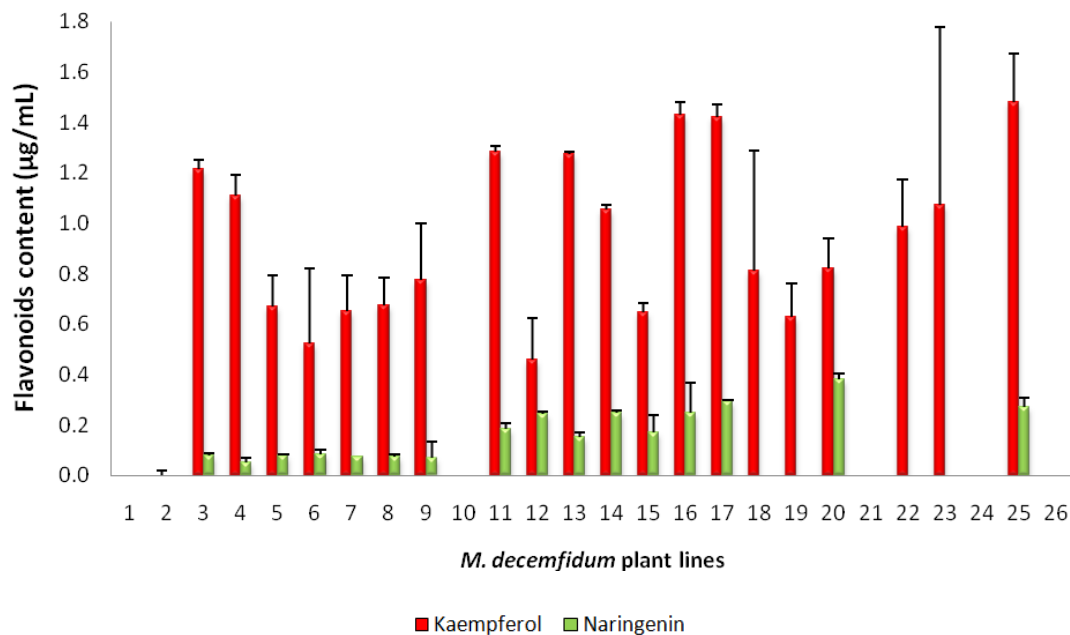
2, 2-diphenyl-1-picrylhydrazine (DPPH) was carried out [1]. The changes of DPPH colour from deep violet to light yellow were measured at 515nm. The kaempferol and naringenin were purchased from Sigma (Sigma-Aldrich, Germany) and used as the commercial standard for gas chromatography, antioxidant and cytotoxic assay.

## RESULTS AND DISCUSSION

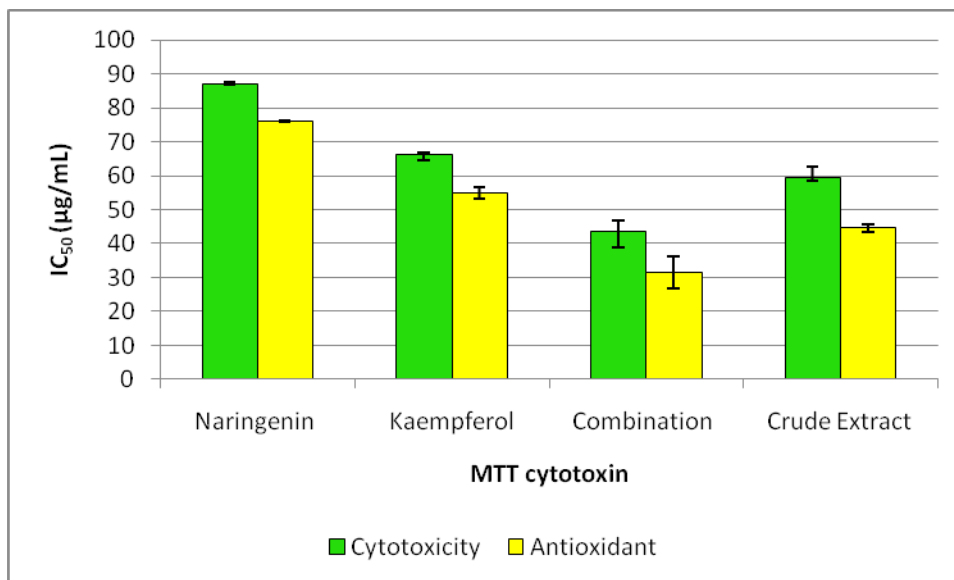
The mature leaves extracts from 26 plant lines of *M. decemfidum* were investigated for the presence of naringenin and kaempferol (Figure 1). Twenty leaf samples (76.9%) displayed the detection of kaempferol. Maximum kaempferol compound was detected in sample 25 ( $1.4728 \pm 0.009 \mu\text{g/mL}$ ) and significantly different at  $p < 0.05$  to all samples except sample 16 and 17 (7.69%). The average kaempferol intensity in all samples was  $0.7246 \pm 0.490 \mu\text{g/mL}$ . Naringenin was detected in 16 leaf samples (61.5%) of *M. decemfidum*. Maximum naringenin compound was detected in sample 20 ( $0.3840 \pm 0.004 \mu\text{g/mL}$ ) and significantly different at  $p < 0.05$  only to 4 samples (15.38%) The average naringenin intensity in all samples was  $0.1107 \pm 0.133 \mu\text{g/mL}$ .

Figure 2 shows the antioxidant activity and cytotoxicity of crude methanolic extracts, combination of both flavonoids and naringenin or kaempferol alone against HeLa cell lines. The combination of flavonoids compound had the strongest antioxidant activity and cytotoxicity with IC<sub>50</sub> value of  $31.394 \mu\text{g/mL}$  and  $43.729 \mu\text{g/mL}$ , respectively. The means were significantly different at  $p < 0.05$ . The amount of kaempferol and naringenin in combination or alone were proportional to concentration of naringenin and kaempferol in crude extract sample 25. The

concentration of naringenin and kaempferol in plant number 25 were  $0.367 \pm 0.004 \mu\text{g/mL}$  and  $1.4728 \pm 0.009 \mu\text{g/mL}$ , respectively.

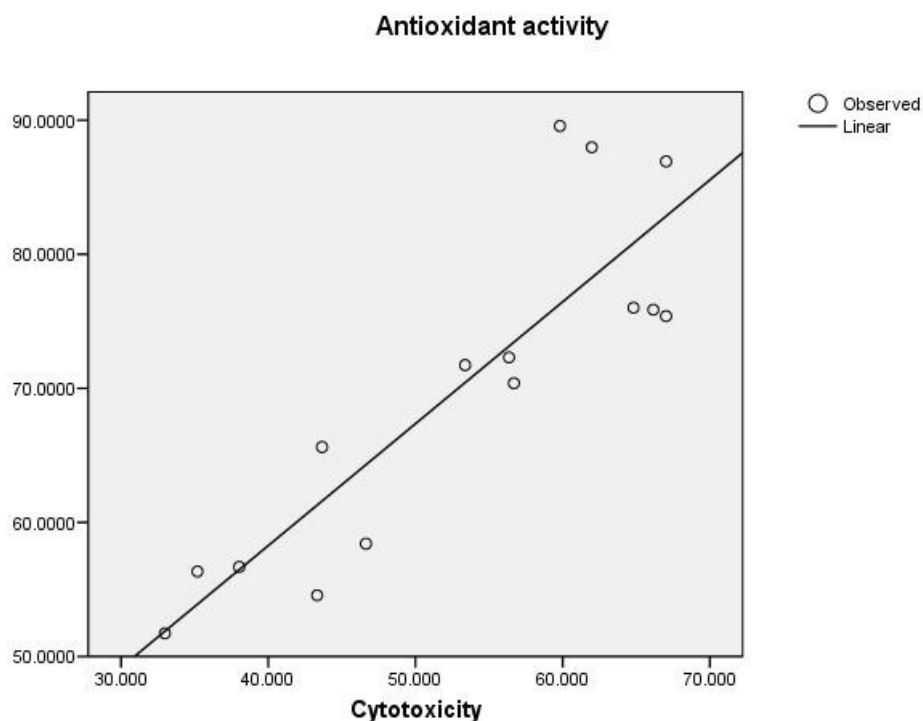


**Figure 1.** The content of naringenin and kaempferol in 26 plant lines of *M. decemfidum*.



**Figure 2.** The antioxidant activity and cytotoxicity of single pure compound (naringenin & kaempferol), combination of both flavonoids and crude extracts of *M. decemfidum* against HeLa cell lines.

The correlation and regression between cytotoxicity and antioxidant activity of methanolic leaf extracts of *M. decemfidum* were also determined. The scatter plot as in Figure 3 depicts a positive relationship. High scores on the antioxidant activity are associated with high scores on cytotoxicity of *M. decemfidum*. The plot revealed a strong positive relationship. The value of Pearson's correlation was 0.874.



**Figure 3.** Linear regression between antioxidant activity and cytotoxicity of methanolic leaf extracts of *M. decemfidum*.

The relationship between cytotoxic properties and the antioxidant capacity of *M. decemfidum* leaf extracts were determined. In general, the strongest antioxidant extracts were also the most cytotoxic. The antioxidant dose are smaller than the cytotoxic dose for every sample, hence the compounds are antioxidants at non-cytotoxic concentrations. A strong correlation was found between antioxidant activity and cytotoxicity in the human cervical cancer cell lines. This observation showed that the more cytotoxic the extracts, the better the antioxidant effect. Cytotoxicity and antioxidant activity was proportional to the high content of kaempferol and naringenin in the methanolic leaf extracts of *M. decemfidum*.

## CONCLUSION

Kaempferol and naringenin were successfully detected and quantified from *M. decemfidum* by gas chromatography. In this study, a combination of naringenin and kaempferol showed the strongest antioxidant activity and cytotoxicity among other treatments. It is suggested that combination of kaempferol and naringenin may serve as a promising anticancer agent. This study also proved the potential of *M. decemfidum* leaves as an anticancer agent against cervical cancer cell lines.

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