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## Nutrient composition, antioxidant and antiproliferative activities of *Clausena excavata* and *Murraya koenigii*

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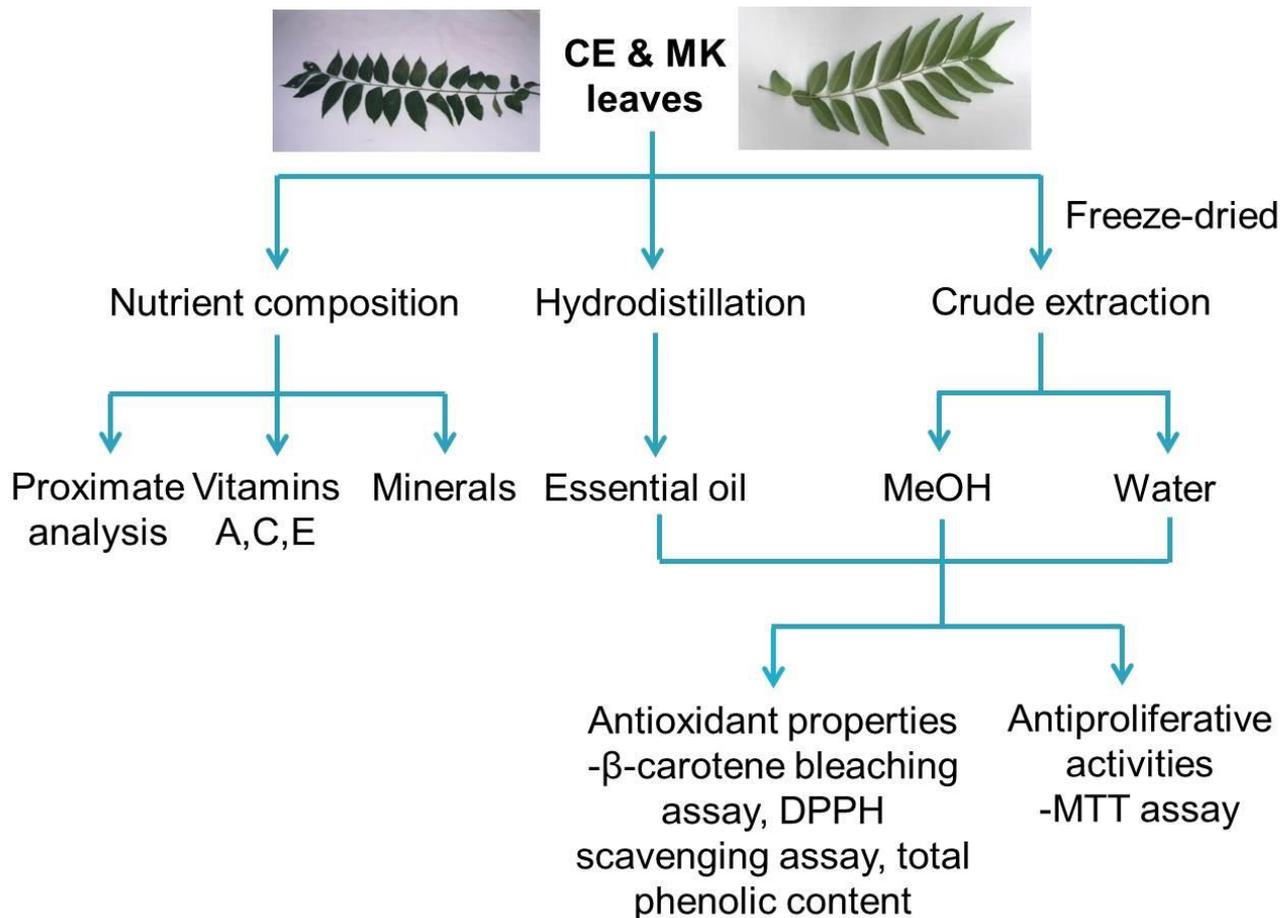


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# Nutrient composition, antioxidant and antiproliferative activities of *Clausena excavata* and *Murraya koenigii*



## Abstract:

*Clausena excavata* (CE) and *Murraya koenigii* (MK) have shown potential medicinal values of herbal plants, thus they were investigated for their nutrient composition. MK possessed higher carbohydrate but lower fiber than CE. Vitamin A is higher in MK but lower vitamin C and E than CE. Antioxidant and antiproliferative activities of CE and MK crude extracts and essential oils and the composition of essential oil were also examined. The hydrodistilled essential oil was analysed by GC/MS. CE and MK leaf oils were made up of safrole and  $\beta$ -farnesene. Phenolic contents of the methanolic extracts of both plants were higher than the water extracts with CE exhibited higher phenolic content. Antioxidant activities were measured via inhibition of linoleic acid oxidation and scavenging of DPPH radicals. The methanolic extracts exhibited significant activities in both assays. MK methanolic extract and oil significantly inhibited linoleic acid oxidation but weakly scavenged DPPH radical than CE. Antiproliferative activities against HepG2, MCF-7, MDA-MB-231, HeLa and CAOV3 were determined using MTT assay. MK methanolic extract and oil possessed the most potent antiproliferative effects. In conclusion, the methanolic extracts especially MK have the great potential in antioxidant and antiproliferative activities. Further investigations are required to explain the underlying mechanisms.

**Keywords:** Antioxidant; Antiproliferative; *Clausena excavata*; *Murraya koenigii*; Nutrient composition



# Introduction

## ***Clausena excavata* (CE)**

- Rutaceae family
- known as Chemama, Kemantu hitam, Pokok cherek in Malaysia; Sicherek in Sumatra; Fia fan & San Soak in Thailand (Soepadmo et al 1991)
- a shrub, with a strong smell; the leaves have a characteristic curry-like smell when crushed
- in traditional medicine, various parts of the plants have been used for abdominal pain, as a poultice for sores, headaches, cold, ulceration of the nose (Ridley 1925), for malaria & dysentery (Wu et al 1992)

## ***Murraya koenigii* (MK)**

- Rutaceae family
- a curry leaf plant; the leaves being used as a flavoring in curries
- a small tree & found widely in East Asia
- in traditional medicine, various parts of the plant have been used for the treatment of headache, toothache, stomachache, influenza, rheumatism, traumatic injury, insect & snake bites, dysentery & astringent (Burkill 1966, Kong et al 1986).



# Introduction

## Biological activities of CE

antiproliferative activities against cancer cell lines (Arbab et al 2012, Al-Abboodi et al 2017, Waziri et al 2016, Andas et al 2015), antioxidant (Albaayit et al 2014), antilarval (Cheng et al 2009), antibacterial (Sunthitikawinsakul et al 2003), antifungal (Sunthitikawinsakul et al 2003), anti-HIV1 (Kongkathip et al 2005), gastroprotective (Albaayit et al 2016), wound healing (Albaayit et al 2015), anti-obesity (Cho et al 2018)

## Biological activities of MK

antioxidant (Ramkissoon et al 2013, Husna et al 2018), antidiabetic (Yadav et al 2002, Ponnusamy et al 2010, Bhat et al 2011, Husna et al 2018), antibacterial, antihypertensive, antiproliferative (Bhattacharya et al 2010, Syam et al 2011, Ahmadipour et al 2015, Arun et al 2017, Hobani et al 2017, Utaipan et al 2017, Nooron et al 2017), antimicrobial (Panghal et al 2011), nephroprotective (Mahipal and Pawar 2017), antilarval (Patil et al 2010)



# Introduction

## Importance of study

- Although there are countless studies being carried out, scientific interests on both plants continue to develop
- There are limited literatures reporting on the nutrient composition of CE. Moreover, little is known about the antioxidative and antiproliferative properties of CE's essential oil
- Provides additional information on the usefulness of CE and MK



# Introduction

## Specific objectives

1. To examine the nutrient composition of CE & MK
2. To determine the antioxidant properties of CE & MK
3. To elucidate the antiproliferative activities of CE & MK on several human tumour cell lines



# Experiment 1

## Nutrient composition

- Proximate analysis (moisture, ash, crude fiber, protein, carbohydrate and fat) (Association of Official Analytical Chemists, 1984 & Tee et al 1996)
- Vitamins A, C & E → HPLC analysis (Tee et al 1996, Abushita et al 1997, Shin & Godber 1993)
- Minerals content (calcium, magnesium, sodium, potassium, iron, copper & zinc content) → AAS



# Results: Nutrient composition

Composition	<i>Clausena excavata</i>	<i>Murraya koenigii</i>
<b>Proximate composition (%)</b>		
Moisture	64.50 ± 2.20 <sup>a</sup>	62.30 ± 1.89 <sup>b</sup>
Ash	4.22 ± 0.03	3.46 ± 0.35
Protein	2.03 ± 0.33	2.33 ± 0.03
Carbohydrate	7.77 ± 0.13 <sup>c</sup>	11.66 ± 0.16 <sup>d</sup>
Crude fiber	3.33 ± 1.80 <sup>e</sup>	1.60 ± 0.80 <sup>f</sup>
Fat (ether extract)	0.03 ± 0.01	0.05 ± 0.01
<b>Vitamins and Minerals (mg/100g)</b>		
Vitamin A	47.78 ± 0.31 <sup>g</sup>	1406.32 ± 13.95 <sup>h</sup>
Vitamin C	586.30 ± 7.96 <sup>i</sup>	374.38 ± 3.71 <sup>j</sup>
Vitamin E	267.67 ± 0.58 <sup>k</sup>	18.52 ± 0.15 <sup>l</sup>
Zinc	0.01 ± 0.01	0.09 ± 0.02
Copper	0.01 ± 0.02	0.1 ± 0.01
Iron	0.32 ± 0.11	0.14 ± 0.24
Sodium	0.37 ± 0.03	0.40 ± 0.05
Potassium	0.73 ± 0.28	0.91 ± 0.16
Magnesium	0.96 ± 0.08	0.76 ± 0.37
Calcium	5.46 ± 0.09	5.28 ± 0.14

**Table 1.** Data shown as mean ± S.D (n=6). Different letters indicate significant difference at the level of  $p < 0.05$ . Results for each constituent were compared between *Clausena excavata* and *Murraya koenigii*.



# Experiment 2

## Hydrodistillation of essential oil

- Fresh leaves (300 g) -> Hydrodistillation (6 hours) using Clavenger apparatus
- Essential oil obtained -> GC-MS-analysis



# Results: Hydrodistillation of CE oil

- Oil yield : 0.7%
- The oil was mainly made up of safrole (89.85%)
- Minor components > 1% :  $\alpha$ - $\alpha$ , 4-trimethyl benzenemethanol (3.13%), 3-cyclohexene-1-carboxaldehyde (1.34%) & terpinolene (1.16%)
- The others < 1%



# Results: Hydrodistillation of MK oil

- Oil yield : 0.2%
- MK oil was mainly made up of  $\beta$ -farnesene (42.85%)
- Other components : naphthalene (12.17%),  $\beta$ -caryophyllene (8.09%), caryophyllene (5.47%) and eudesmol (4.34%)
- Minor components > 1% : caryophyllene oxide (1.93%), nerolidyl acetate (1.83%), globulol (1.69%), cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane (1.65%), pseudocumene (1.49%),  $\beta$ -farnesene (1.16%) and spathulenol (1.00%)
- The others < 1%



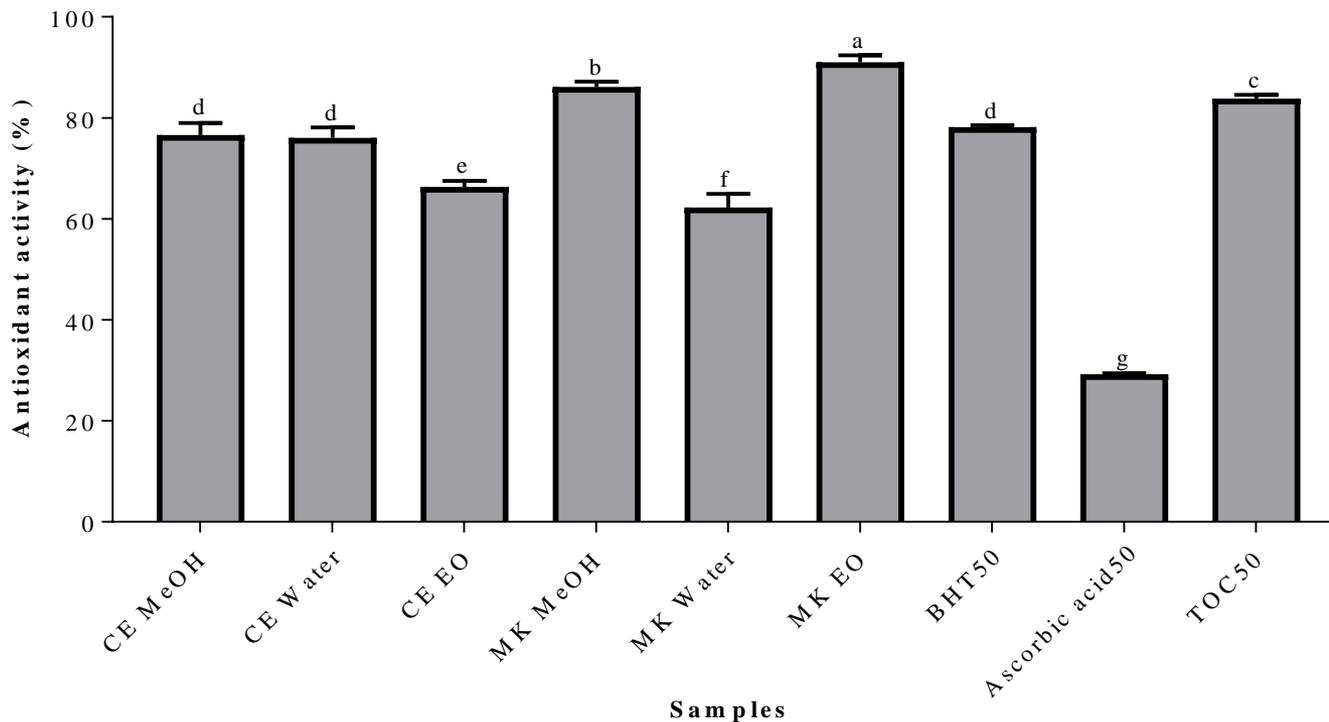
# Experiment 3

## Antioxidant properties (Antioxidant activity)

- $\beta$ -carotene bleaching assay
- The rate of  $\beta$ -carotene bleaching can be slowed down in the presence of antioxidants (Velioglu et al 1998)
- The antioxidant activity was measured



# Results: Antioxidant properties ( $\beta$ -carotene bleaching assay)



**Fig. 1:** *Murraya koenigii* essential oil (MK EO) showed the highest AA ( $91.01 \pm 1.40$  %), followed by *Murraya koenigii* methanol extract (MK MeOH) ( $86.33 \pm 0.07$  %) which were significantly higher than the standards: BHT (BHT50),  $\alpha$ -tocopherol (TOC50) and ascorbic acid. Data shown as mean  $\pm$  S.D; n=6. Different letters indicate significant difference at the level of  $p < 0.05$ . Comparison was made between all samples and standards.



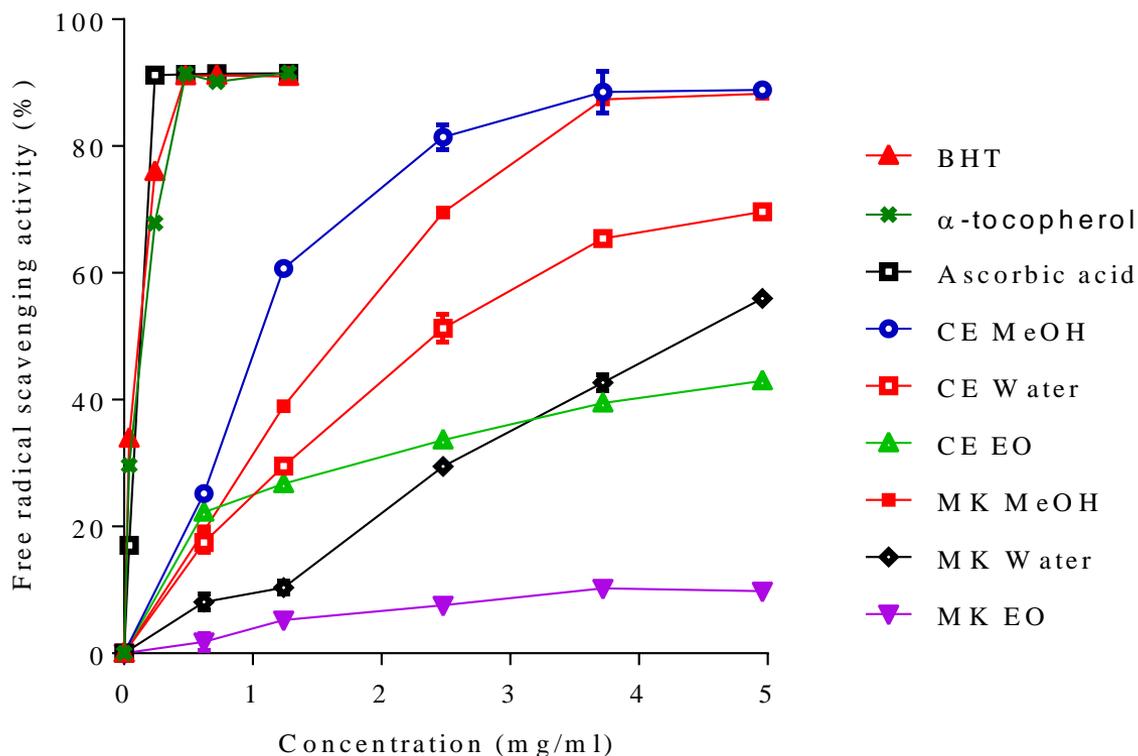
# Experiment 4

## Antioxidant properties (Free radical scavenging activity)

- DPPH free radical scavenging assay (Blois, 1958, Lai et al 2001)
- The capability of sample to scavenge the DPPH radical was measured
- Dose-response curve was plotted to obtain EC50
- BHT, ascorbic acid and  $\alpha$ -tocopherol were used as standards



# Results: Antioxidant properties (DPPH free radical scavenging assay)



**Fig. 2:** All standards inhibit 50% DPPH radical at 0.11-0.14 mg/ml; which were stronger than other studied samples. The methanol extracts from both samples inhibit 50% DPPH radical at lower concentrations (CE MeOH:  $0.89 \pm 0.25$  mg/ml) (MK MeOH:  $1.69 \pm 0.01$  mg/ml) compared to water extracts.  $EC_{50}$  values of both CE and MK essential oils were not detected at the concentration tested. Data shown as mean  $\pm$  S.D; n=6.



# Experiment 5

## Antioxidant properties (Total phenolic content)

- Folin-Ciocalteu assay (Singleton and Rossi 1965)
- Absorbance was measured spectrophotometrically at 725 nm
- The total phenolic content was expressed as gallic acid equivalents (GAEs) in milligrammes per g sample extract



# Results: Antioxidant properties (Total phenolic content)

Samples	Gallic acid equivalents (GAEs) (mg of GAEs/g of sample extract)
<b><i>Clausena excavata</i></b>	
Methanol extract	103.33 ± 0.95 <sub>c</sub>
Water extract	53.46 ± 0.75 <sub>a</sub>
<b><i>Murraya koenigii</i></b>	
Methanol	63.92 ± 0.81 <sub>b</sub>
Water	53.62 ± 0.96 <sub>a</sub>

**Table 2:** Methanol extracts from both samples showed higher total phenolic content than the water extracts. Data shown as mean ± S.D; n=6. Different letters indicate significant difference at the level of p<0.05. Comparison was made between methanol and water extracts of the respective samples.



# Experiment 6

## Antiproliferative activities

- HepG2 (hepatic cancer), MCF-7 (hormone-dependent breast cancer), MDA-MB-231 (non-hormone-dependent breast cancer), HeLa (cervical cancer) & CAOV3 (ovarian cancer) were obtained from American Type Culture Collection (ATCC)
- HepG2, MCF-7, HeLa; cultured in RPMI-1640, MDA-MB-231, CAOV3; in DMEM + 10% foetal bovine serum + 1% penicillin-streptomycin with fungizone, at 37°C with 5% CO<sub>2</sub>
- CE & MK methanol extracts, water extracts & essential oils; dissolved with 100% DMSO to 10 mg/ml, diluted with medium to 100 µg/ml for MTT assay



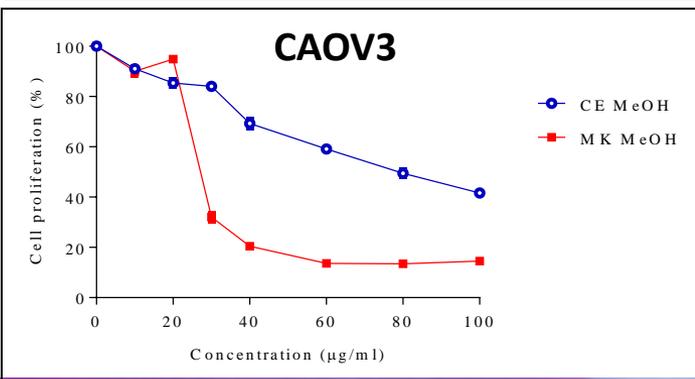
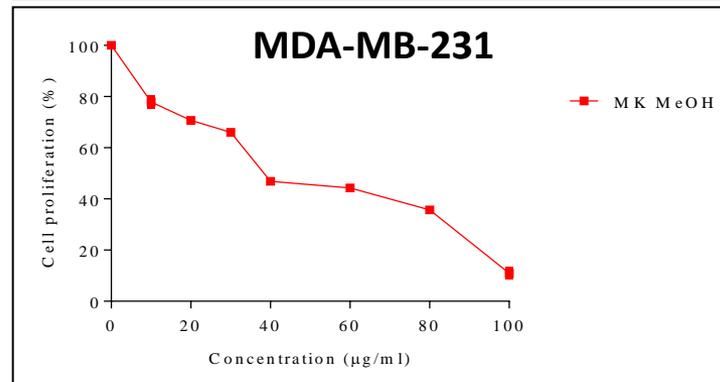
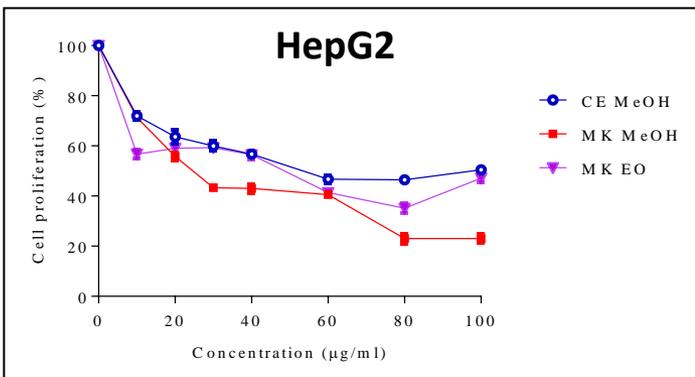
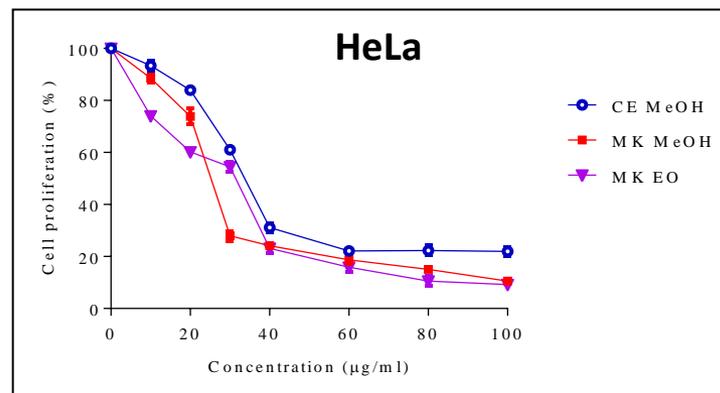
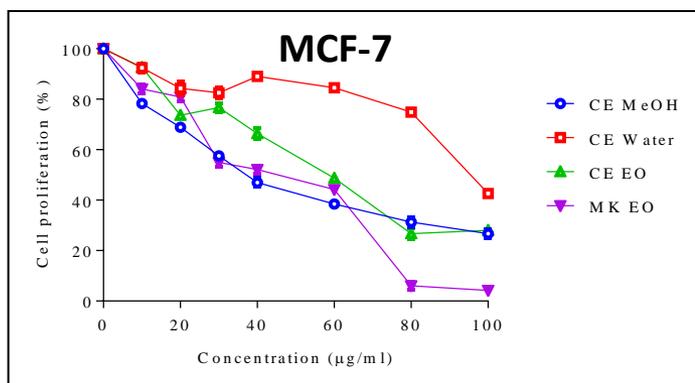
# Experiment 6

## Antiproliferative activities (Cell proliferation assay)

- MTT assay (Roche, Germany)
- Tumour cells were treated with CE & MK methanol extracts, water extracts & essential oils (10-100  $\mu\text{g}/\text{ml}$ ) & incubated for 96 h
- An equivalent serial dilution of DMSO was used as control treatment
- ELISA measurement at 550 nm
- Dose-response curve was plotted to obtain  $\text{IC}_{50}$



# Results: MTT assay



**Fig. 3.** The effect of CE & MK methanol extracts, water extracts & essential oils on proliferation of MCF-7, HeLa, HepG2, MDA-MB-231 & CAOV3 assessed using the MTT assay. DMSO (vehicle) does not inhibit 50% cell growth (data not shown). Data shown as mean  $\pm$  S.D; n=6.



## Results: MTT assay (cont.)

Sample	IC <sub>50</sub> (µg/ml)				
	HeLa	MCF-7	HepG-2	MDA-MB-231	CAOV3
<b><i>Clausena excavata</i></b>					
Methanol extract	34.51 ± 0.82 <sup>b</sup>	36.50 ± 0.91 <sup>a</sup>	53.03 ± 0.53 <sup>c</sup>	ND	79.00 ± 0.97 <sup>b</sup>
Water extract	ND	95.00 ± 1.40 <sup>d</sup>	ND	ND	ND
Essential oil	ND	59.00 ± 0.95 <sup>c</sup>	ND	ND	ND
<b><i>Murraya koenigii</i></b>					
Methanol extract	25.00 ± 1.25 <sup>a</sup>	ND	23.90 ± 0.41 <sup>a</sup>	37.98 ± 0.97	27.90 ± 0.78 <sup>a</sup>
Water extract	ND	ND	ND	ND	ND
Essential oil	31.10 ± 0.90 <sup>b</sup>	46.01 ± 2.14 <sup>b</sup>	48.00 ± 0.92 <sup>b</sup>	ND	ND

**Table 3.** IC<sub>50</sub> values of CE & MK methanol extracts, water extracts & essential oils on HeLa, MCF-7, HepG2, MDA-MB-231 & CAOV3. Different letters indicate significant difference at the level of p<0.05. Results were compared between samples treated on the same cell line (same row). The IC<sub>50</sub> value is defined as the concentration of sample required to inhibit 50% of the cancer cells proliferation. ND indicates IC<sub>50</sub> value was not detected at the concentration tested. Data shown as mean ± S.D; n=6.



## Discussion

- CE & MK leaves contained a moderate amount of proximate composition as well as minerals and vitamins which may be useful for the evaluation of dietary information. The nutrient composition might also contribute to the total antioxidant activity (de Souza et al 2014).
- The MeOH extracts of both plants performed better in the lipid peroxidation and free radical scavenging activities suggesting the antioxidant active and probably phenolic compound in this study has a high activity.
- MK oil exhibited higher antioxidant & antiproliferative activities compared to CE oil which could be due to the compounds in their essential oils.



## Discussion

- The MTT assay revealed that the methanol extracts of both CE and MK also contain antiproliferative compound, which exhibited growth inhibitory effect on almost all human cancer cell lines tested.
- Both CE & MK methanol extract contained high amount of phenolic than the water extracts suggesting the growth inhibitory effects of the MeOH extract. Therefore, it is believed that inhibition of cell growth is closely associated with antioxidant properties of the extract (Rodriguez 2016).



## Conclusions

- CE & MK proved to possess interesting properties, emerging from their nutrients composition & the evaluation of their in vitro biological activities.
- The MeOH extracts especially MK MeOH extract has the great potential in antioxidant & antiproliferative activities.
- However, further chemical work and pharmacological evidences are required to establish the possible correlation among the mentioned activities of the extract.



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