

The anticancer effect of *Ocimum tenuiflorum* leaves

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Abstract

Breast cancer is the leading cause of cancer deaths among females in Malaysia. *Ocimum tenuiflorum* L., (*O. tenuiflorum*) commonly known as *ruku* in Malaysia, is usually cultivated as a garden ornamental plant because of its small purplish and some yellowish flower. The specific objective of this research is to investigate the anticancer of *O. tenuiflorum* against human breast cancer cell lines (MCF-7 and MDA-MB-231) and human fibroblast cell line (HS-27). In addition, another objective is to determine the mineral and heavy metal determination of *O. tenuiflorum*. *O. tenuiflorum* exhibited anticancer activity against MCF-7 (a hormone-dependent breast cancer cell line). The viability of MCF-7 cells decreased significantly after treatment with various concentrations of methanolic plant extracts (25 and 100 µg/mL), as shown via 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay. The crude extracts show the lower IC₅₀ (less than 100 µg/mL) value against the cancer cell lines and show no effect on HS-27. The high content of calcium in the leaves of *O. tenuiflorum* may play a role in decreasing the risk of certain cancer. The concentrations of heavy metals (Pb and As) detected in *O. tenuiflorum* are safe for consumption.

1. Introduction

In 2007, a report from the National Cancer Registry of Malaysia stated that 18 219 new cases of cancer had been diagnosed, 8123 (44.6%) of which were in males and 10 096 (55.4%) of which were in females (Zainal Ariffin and Nor Saleha, 2011). At that time, the ten leading cancers in the Malaysian population were breast, colorectal, lung, nasopharyngeal, and cervical cancers, lymphoma, leukemia, and ovarian, stomach and liver cancers (Zainal Ariffin and Nor Saleha, 2011).

In Malaysia, *Ocimum tenuiflorum* is commonly known as *ruku*, and it is usually cultivated as an ornamental garden plant because of its yellowish flower. Additionally, the young leaves of *Ocimum tenuiflorum* L. are used to make Nasi Ulam. *Ocimum tenuiflorum* L. is an herb with great medicinal and health potential (Kothari *et al.*, 2004); the leaves have an essential oil that contains various compounds with medicinal value (Rai *et al.*, 2004). Our previous study showed a possible use of *O. tenuiflorum* leaves in managing hyperglycemia and preventing the complications associated with it in

Type 2 Diabetes Mellitus (Leila *et al.*, 2016). *In vitro* and *in vivo* studies have shown that zinc and magnesium inhibit the development of several types of cancer (Singh *et al.*, 2012). The specific objective of this research was to investigate the anticancer activity of *O. tenuiflorum* against human breast cancer cell lines (MCF-7 and MDA-MB-231) and a human fibroblast cell line (HS-27). An additional objective was to determine the mineral and heavy metal content of *O. tenuiflorum*.

2. Materials and methods

2.1 Plant materials

The leaves of *O. tenuiflorum* were collected from Kuala Kurau in Peninsular Malaysia and were identified by Dr. Rahmad Zakaria from the School of Biological Sciences, Universiti Sains Malaysia. Leaves harvesting, and sample extraction were performed on the same day. Voucher specimen number 11400 was deposited in the herbarium laboratory of the School of Biological Sciences, Universiti Sains Malaysia.

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2.2 Sample preparation

The *O. tenuiflorum* leaves were separated from the stalks and the flowers. The leaves were washed thoroughly. Then, they were dried for 72 hours using a freeze dryer (ALPHA Freeze Dryer Model 1-2 LD plus, Vacuubrand, Germany). The dried leaves were then ground into a powder using a blender (Model BL 335, Kenwood, Selangor, Malaysia), stored in amber-colored glass bottles that were covered with aluminum foil, and kept airtight with Parafilm. The ground leaves were stored at -20°C until further use.

2.3 Water extraction

Approximately 50 g of ground leaves were extracted for 4 hours in a bath of hot distilled water (EYELA Oil Bath model OSB-2000, Tokyo Rikakika Co., Ltd., Japan). The plant-to-water ratio was 1:20 and was based on the procedure of Huang *et al.* (2003). Whatman No. 1 filter paper was used to filter the resulting crude extract. The same volume of hot (85°C) distilled water was added to repeat the extraction.

The crude extract was filtered using Whatman No. 1 filter paper. The filtrate was dried into a powder using a freeze dryer (ALPHA Freeze Dryer model 1-2 LD plus, Vacuubrand, Germany). The powdered extract was stored at -20°C until further use.

2.4 Methanol extraction

Methanol extraction of the plant samples was conducted according to the method of Wicaksono *et al.* (2009). Approximately 100 g of ground *O. tenuiflorum* leaves were weighed using an analytical balance (METTLER TOLEDO Balance Model PL6001-S, Mettler-Toledo Inc., Columbus) and soaked in 300 mL of absolute methanol for one day. After soaking, the crude extract was filtered through Whatman No. 1 filter paper. This procedure was repeated twice. After repeated soaking and filtration, the residual methanol in the filtrate was evaporated under reduced pressure at 40°C using a rotary evaporator (EYELA Rotary Evaporator model N-1000, Tokyo Rikakika Co., Ltd., Japan) connected to a water bath (EYELA Oil Bath model OSB-2000, Tokyo Rikakika Co., Ltd., Japan). The removal of residual methanol was followed by the evaporation of the methanolic extract at approximately 27°C (room temperature) for 2 days.

2.5 MTT assay

To produce a $1000\ \mu\text{g/mL}$ of stock solution, about 5 mg of plant extract by the mean of water extraction and

methanol extraction individually were mixed with 5 mL of PBS. The stock solution was diluted into concentration of $0.78\ \mu\text{g/mL}$, $1.56\ \mu\text{g/mL}$, $3.13\ \mu\text{g/mL}$, $6.25\ \mu\text{g/mL}$, $12.5\ \mu\text{g/mL}$, $25\ \mu\text{g/mL}$, $50\ \mu\text{g/mL}$, $100\ \mu\text{g/mL}$. Each increasing concentration of the sample was added for treating the cells from the column 3 ($0\ \mu\text{g/mL}$) to column 11 ($100\ \mu\text{g/mL}$). After treating, the cells were incubated at 37°C with 5% CO_2 for 48 and 72 hrs. The test was triplicated. When incubation period achieved, each well was added with $12\ \mu\text{L}$ of MTT labelling reagent. The cells were incubated again at 37°C with 5% CO_2 for 4hrs. Next, $100\ \mu\text{L}$ of acidified isopropanol and $100\ \mu\text{L}$ of distilled water were added into every well. Using ELISA reader (Thermo Scientific Spectrophotometer Model 1500-900, Fisher Scientific, Malaysia) at 550 nm, the cell viability was detected.

2.6 Measurement of the inhibition of cell proliferation

After a 48-72 hours incubation period, approximately $24\ \mu\text{L}$ of 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) solution (Sigma Chemical Company, St. Louis, MO, USA) was added to each well of a 96-well plate, and the assay results were obtained based on the method described by Mossman (1983). The plate was tapped and gently shaken to mix the solution into the media. Then, the cells were incubated at 37°C in a humidified CO_2 incubator for 4 hours. The media was removed, and $100\ \mu\text{L}$ of acidified isopropanol was added to each well. After 5 minutes, $100\ \mu\text{L}$ of autoclaved deionized water was added to each well and thoroughly mixed to permit color development. The absorbance at 570 nm was read using a spectrophotometric plate reader (Multiskan Spectrum; Thermo Electron Co., Waltham, MA, USA), and the proportion of surviving cells was calculated as:

$$\text{Viability (\%)} = \frac{\text{OD of drug-tested sample} - \text{OD of blank}}{\text{OD of control} - \text{OD of blank}} \times 100$$

Dose-response curves were constructed using probit analysis to obtain the LC_{50} and LC_{80} values. All experimental data were derived from at least three independent experiments.

A fibroblast cell line was used to analyze the extract's ability to kill normal cells. MCF-10A or HMEpC cells could have been used, but the former requires treatment with other factors that might affect the 'normal' character of the cells, whereas the latter are primary cells whose characteristics differ, making them unsuitable for the purposes of this study.

2.7 Sample digestion

Borgese *et al.* (2009) have reported a method for

sample digestion that uses a microwave digester. First, 10 mL of nitric acid (HNO₃) was diluted to 50 mL with deionized water. The diluted HNO₃ was used to rinse the apparatus and the centrifuge tubes to avoid contamination.

For both raw and powdered plant samples, between 500 mg and 800 mg was added to a digestion vessel using a clean spatula. The vessel was acidified with 7 mL HNO₃ and 1 mL HCl in a fume hood. A digestion vessel containing 7 mL HNO₃ and 1 mL HCl was used as a blank. The digestion vessel was placed into a rotor, and the rotor was then placed into the microwave digester (Multiwave 3000, Anton Paar, Austria). The process required 15 min to completely liquefy the sample. Once the digestion had finished, each vessel was carefully opened in the fume hood. The liquefied sample was filtered using ashless filter paper.

2.8 Standard preparation for mineral and heavy metal analysis

To determine the concentration of 21 standard elements [antimony (Sb), arsenic (As), beryllium (Be), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), strontium (Sr), thallium (Tl), titanium (Ti), vanadium (V) and zinc (Zn)], standards of 1-, 2-, and 3-ppm solutions were prepared for each element from 10-ppm stock solutions. To analyze additional elements, 1-, 2-, and 3-ppm potassium standards were needed. A 0.1 mL volume of a 10-ppm potassium standard was added to 1 mL of the standards of each of the 21 elements. Distilled water was added to the mixture to a final volume of 50 mL. Micropipettes were used to obtain accurate results during standard preparation. Standard solutions of 0, 1, 3 and 5 ppm were prepared for the analysis.

2.9 Sample preparation for mineral and heavy metal analysis

Firstly, eight centrifuge tubes were rinsed with nitric acid to prevent contamination. After the tubes dried, the raw plant sample solution obtained from the microwave digester was added to the tubes. One centrifuge tube was used as a blank. The mineral content (Ca, Pb, Na, K, Mg, Zn, Co, Fe and Cu) of the samples were analyzed via ICP-OES (Perkin Elmer, Optima 7000 DV). Five sample dilutions (1:10, 1:50, 1:100, 1:500 and 1:1000) and a blank were prepared for the analysis. The wavelength of each element was then set. The concentrations of the elements were calculated based on their obtained

intensity readings.

2.10 Statistical analysis

The cell viability results were calculated as means \pm standard deviations. To identify significant differences among multiple comparisons, one-way ANOVA analysis followed by Duncan's test with $p < 0.05$ was performed using SPSS version 20.0. The results of the mineral and heavy metal analysis were calculated as the means \pm standard deviations and were compared using t-tests.

3. Results and Discussion

3.1 Cell viability

The percentage of viable cells in the human cancer cell lines (MCF-7 and MDA-MB-231) and the non-cancerous cell line (HS-27) after treatment with aqueous *O. tenuiflorum* extracts for 48 and 72 hours are shown in Figures 1 and 2, respectively, and the results of similar experiments using methanol extracts are shown in Figures 3 and 4. In both cases, the extract concentrations were between 0 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$. The active compounds should be resistant to heat treatment, as has been cited in empirical tests showing that heat-treated extracts are effective in traditional treatments. In addition, the hot water extraction method was chosen to reduce the number of compounds in the active fraction.

Figure 1 shows the effects of a 48-h treatment with the aqueous extract. Treatment with 6.25 $\mu\text{g/mL}$ extract 127.6% reduced the viability of MCF-7 cells, and treatment with 3.13 and 12.5 $\mu\text{g/mL}$ significantly reduced their viability to an even greater extent (100.7% and 105.1%, respectively) ($p < 0.05$). In MD-MB-231 cells, treatment with 0.78 and 6.25 $\mu\text{g/mL}$ extract increased viability (121.7% and 126.4%, respectively).

Figure 2 shows that the viability of MCF-7 cells for 72-h increased after treatment with 3.13 and 100 $\mu\text{g/mL}$ extract (113.9% and 99.2%, respectively). The viability of MD-MB-231 cells increased significantly ($p < 0.05$) with 3.13 $\mu\text{g/mL}$ extract (113.9%). Therefore, the plant extract was more effective at decreasing MCF-7 cell viability at concentrations of between 6.25 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$.

Figure 3 shows the effect of 48-h treatments with methanolic plant extracts. After treatment with 25 and 100 $\mu\text{g/mL}$ extract, the viability of MCF-7 cells decreased significantly ($p < 0.05$) (94.1% and 95.3%, respectively). Thus, the plant extract inhibited the growth of these hormone-dependent breast cancer cells. The viability of MDA-MB-231 cells decreased significantly

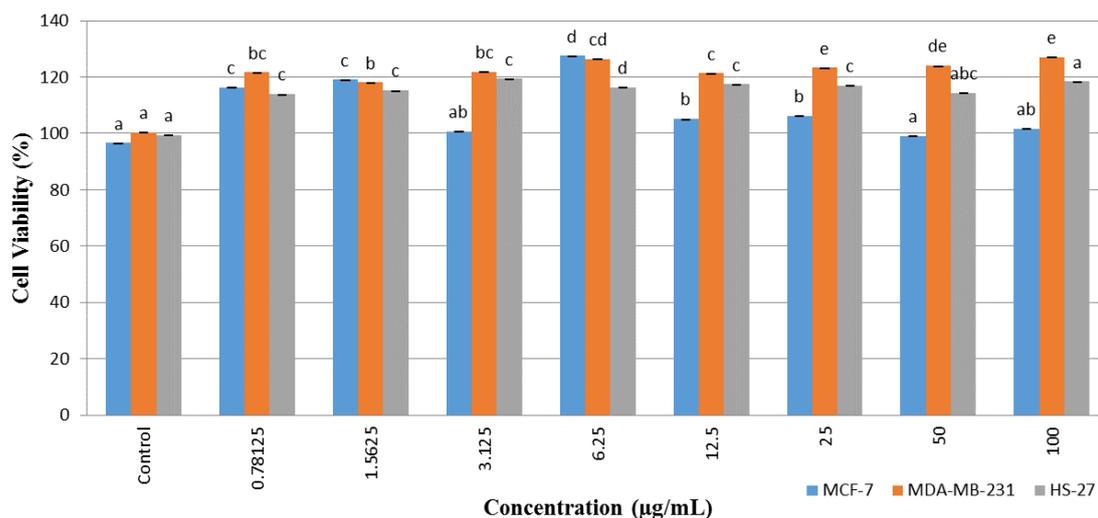


Figure 1. Cell Viability of *O. tenuiflorum* with water extraction at different concentration for 48 hours. Values are presented as mean \pm standard deviation of triplicate measurements.

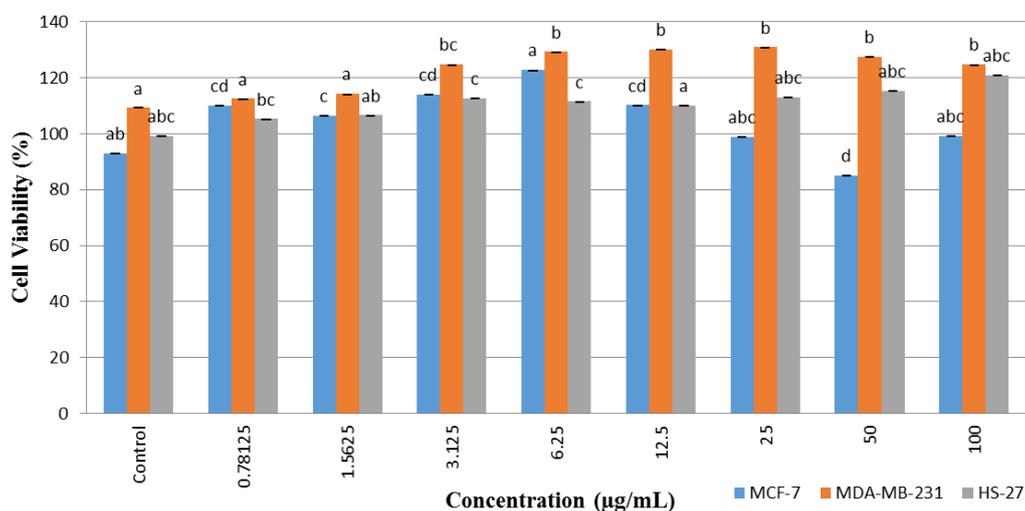


Figure 2. Cell Viability of *O. tenuiflorum* with water extraction at different concentration for 72 hours. Values are presented as mean \pm standard deviation of triplicate measurements.

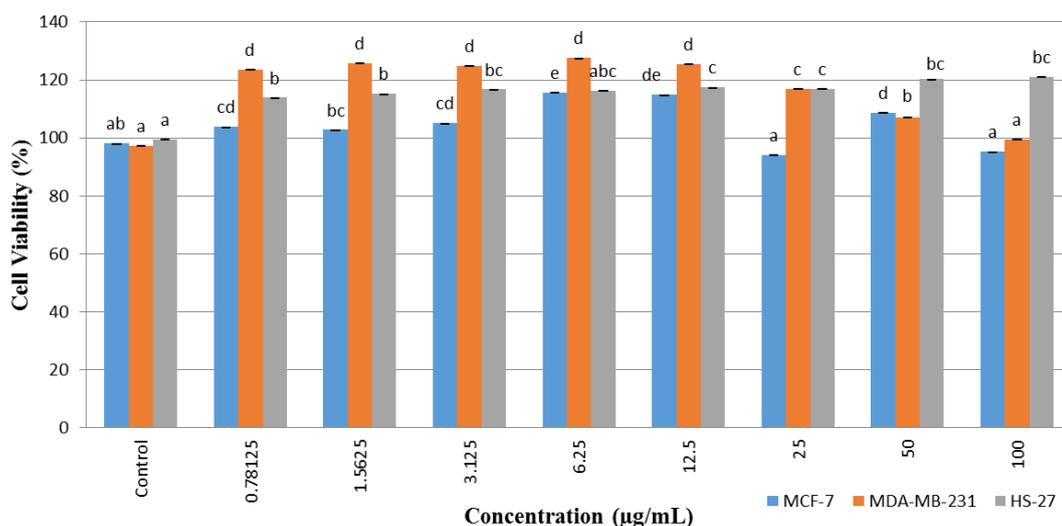


Figure 3. Cell Viability of *O. tenuiflorum* with methanol extraction at different concentration for 48 hours. Values are presented as mean \pm standard deviation of triplicate measurements.

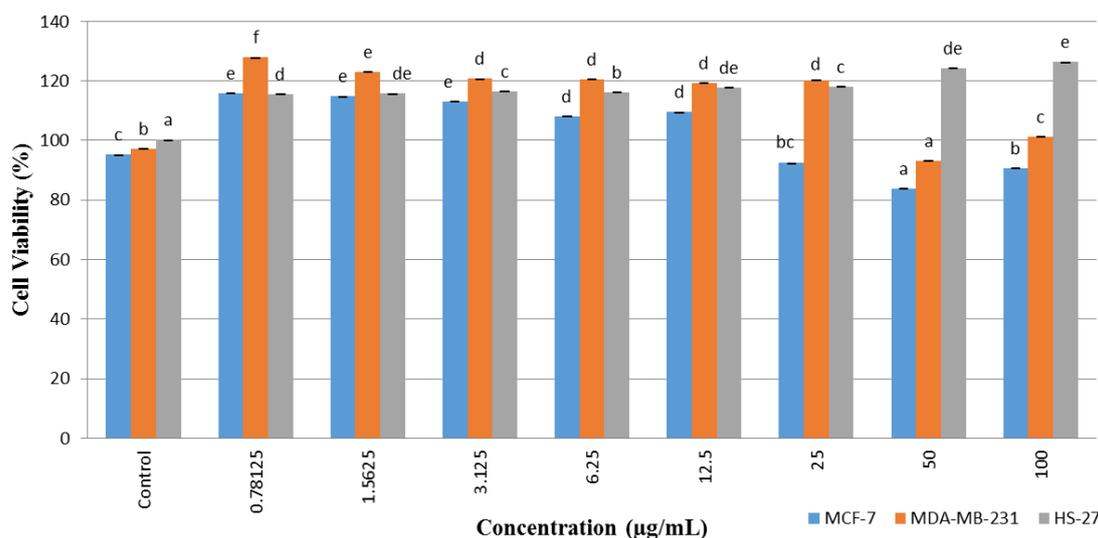


Figure 4. Cell Viability of *O. tenuiflorum* with methanol extraction at different concentration for 72 hours. Values are presented as mean \pm standard deviation of triplicate measurements.

with treatments of between 25 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ ($p < 0.05$) (116.9% and 99.6%).

Figure 4 shows that the viability of MCF-7 cells decreased significantly ($p < 0.05$) after 72 h of treatment with 25 or 50 $\mu\text{g/mL}$ methanolic plant extracts (92.4% and 83.8%, respectively). The difference in viability after treatment with 1.56 or 3.13 $\mu\text{g/mL}$ extract was not significant ($p > 0.05$) (114.8% and 113.2%, respectively). Thus, cancer cell growth was not inhibited. The HS-27 viability did not decrease significantly across the different concentrations ($p > 0.05$). Therefore, the 50 $\mu\text{g/mL}$ plant extract was the most effective against MCF-7 cells.

Samresh *et al.* (2003) have reported on the anticancer activity of *O. tenuiflorum* leaves, which inhibit chromosomal abnormalities in the bone marrow and suppress tumors in the forestomach of mice. Natural plant polyphenolic substances have been reported to inhibit cancer (Cognault *et al.*, 2000). One of the most important polyphenolic classes is that of the flavonoids (Rahmatullah *et al.*, 2010), which have been found in *O. tenuiflorum* (Samresh *et al.*, 2003). According to Umadevi (2001), orientin and vicenin, which are radioprotective flavonoids found in *O. tenuiflorum*, exhibit activity against hydroxyl radicals. Free radicals that damage tissues and cause diseases are produced regularly (Halliwell and Gutteridge, 1998).

The increased use of *O. tenuiflorum* in horticulture will be advantageous for cancer chemoprevention in the future. Moreover, *O. tenuiflorum* can be incorporated into food (Trevisan *et al.*, 2006).

Studies on the volatile constituents of oil isolated from *O. tenuiflorum* via hydrodistillation have been

described by the Association of Official Analytical Chemists (Tharmolwan *et al.*, 2010). Eugenol is a phenolic compound found in the volatile oils of *Ocimum* species (Prakash and Gupta 2005). A similar report by Kothari *et al.* (2004) showed that the oil isolated from *O. tenuiflorum* contains eugenol. *O. tenuiflorum* oil suppresses the genotoxicity of the MCF-7 cell line to regulate enzymes responsible for detoxification such as quinone oxidoreductase (Han *et al.*, 2007).

MCF-7, a hormone-dependent breast cancer cell line, is classified as estrogen receptor (ER)-positive, whereas MDA-MB-231, a non-hormone-dependent breast cancer cell line, is classified as ER-negative (Magdalena *et al.*, 2013). The treatments for these two types of breast cancer differ. ER-positive cancer requires radiation, chemotherapy, surgery and estrogen analogs. Estrogen analogs are not effective against ER-negative cancer because of its estrogen-independent property. Estrogen activity may be affected by the presence of polyphenols that suppress hormone-producing enzymes (Hakimuddin *et al.*, 2008).

Hakimuddin *et al.* (2008) reported that the polyphenols in *O. tenuiflorum* may inhibit MCF-7 cells. The estrogen-dependent proliferation of MCF-7 cells can be influenced by polyphenols (Hakimuddin *et al.*, 2008). This inhibitory effect has not been shown in MDA-MB-231 or HS-27 cells. The methanolic plant extracts had greater effects than the aqueous plant extracts, which may be due to the greater solubility of plant compounds in organic solvents. This result correlated with the finding of Gulluce *et al.* (2007), who stated that methanol extracts are particularly strong free radical scavengers because of their total phenol and flavonoid content (Rabeta and Lai, 2013).

3.2 Mineral and heavy metal analysis

Minerals might affect active compound activity and cell viability. Hence, because some minerals might be lost during sample preparation, it would be appropriate to determine the mineral content of the different extracts. The mineral and heavy metal contents of *O. tenuiflorum* are given in Tables 1 and 2. *O. tenuiflorum* had more K and Na (2534.38 ± 12.74 and 319.77 ± 6.45 mg/kg, respectively) than did *O. tenuiflorum* powder (2458.37 ± 25.38 and 240.05 ± 4.02 mg/kg, respectively). The K and Na content of the *O. tenuiflorum* powder was significantly less than that of *O. tenuiflorum* ($p < 0.05$). In a study by Mehmet *et al.* (2008), the K content of *O. basillum* ($11\ 160.4 \pm 1145.31$ mg/kg) was shown to be greater than that of *O. tenuiflorum*, whereas the Na content of *O. basillum* (65.24 ± 33.14 ppm) was less than that of *O. tenuiflorum*. Results from Pachkore and Dhale (2012) showed that the K content of *O. basillum*, *O. gratissimum* and *O. sanctum* was 3669.4 ± 4.53 , 1498.5 ± 8.34 and 3969.4 ± 4.98 mg/kg, respectively. The results obtained in the present study are in line with those of Pachkore and Dhale (2012). High potassium intake in the diet can reduce the risk of hypertension and possibly stroke (Devesh *et al.*, 2012).

Table 1. Concentration of mineral contents in *Ocimum tenuiflorum* and *Ocimum tenuiflorum* powder

Element	Concentration (mg/kg sample)	
	<i>Ocimum tenuiflorum</i>	<i>Ocimum tenuiflorum</i> powder
K	534.38 ± 12.74^a	458.37 ± 25.38^b
Na	319.77 ± 6.45^a	240.05 ± 4.02^b
Ca	200 ± 4.21^a	171.68 ± 3.90^b
Mg	34.29 ± 3.02^a	41.07 ± 2.41^b
Fe	2.57 ± 0.022^a	3.11 ± 0.27^b
Zn	1.49 ± 0.025^a	2.75 ± 0.35^b
Co	0.25 ± 0.049^a	0.24 ± 0.01^a
Cu	0.60 ± 0.01^b	0.67 ± 0.024^b

Values are mean \pm standard deviation (n=3). Values with different letters within rows are significantly different ($p < 0.05$).

Table 2. Concentration of heavy metals in *Ocimum tenuiflorum* and *Ocimum tenuiflorum* powder

Element	Concentration (mg/kg sample)	
	<i>Ocimum tenuiflorum</i>	<i>Ocimum tenuiflorum</i> powder
Pb	1.88 ± 0.47^a	1.15 ± 0.052^a
As	0.013 ± 0.001^a	0.014 ± 0.001^a

Values are mean \pm standard deviation (n=3). Values with different letters within rows are significantly different ($p < 0.05$).

Table 1 shows that the Ca content of *O. tenuiflorum* (200 ± 4.21 mg/kg) is higher than that of *O. tenuiflorum* powder (171.68 ± 3.90 mg/kg). Calcium is important for conducting nerve impulses and for building strong bones (Devesh *et al.*, 2012). *O. basillum* has a greater Ca content (574.7 ± 57 mg/kg) than does *O. tenuiflorum* (Mehmet *et al.*, 2008). Results from Ozcan (2004) show the Ca content of *O. basillum* to be even greater ($12\ 363$ mg/kg).

Some studies have suggested that high levels of calcium in the diet can decrease the risk of cancer (Wargovich *et al.*, 1991). A significant interaction between calcium and tumor formation has been discovered (Pence *et al.*, 1996); a study by Pence *et al.* (1996) found that calcium could inhibit carcinogen-induced colon tumorigenesis in rats. Calcium can be obtained from foods such as soybeans, tofu, sweet potatoes and low oxalate vegetables (e.g., kale and broccoli) (Connie *et al.*, 1999).

O. tenuiflorum had significantly less Mg than it did in its powder form (34.29 ± 3.02 mg/kg and 41.07 ± 2.41 mg/kg, respectively). These levels are low relative to those measured by Mehmet *et al.* (2008) in *Ocimum* species (513.2 ± 280.93 mg/kg). Magnesium is responsible for calcium metabolism in bones (Ishida *et al.*, 2000). Foods such as dark bread, cold cereal and bananas are sources of magnesium (Tucker *et al.*, 1999).

In addition to the macroelements, the concentrations of microelements such as Fe, Pb, Zn, Co, and Cu were determined. The Fe (2.57 ± 0.02 mg/kg) and Zn (1.49 ± 0.025 mg/kg) levels measured in *O. tenuiflorum* were significantly higher than the corresponding Fe and Zn levels in its powdered form. These results were not within the ranges of values reported by Ozcan (2004), who showed the Fe and Zn content of *O. basillum* to be 503 and 13.7 mg/kg, respectively. Aluko *et al.* (2012) also reported high Fe and Zn content in *O. canum* (185 ± 0.02 mg/kg and 130 ± 0.01 mg/kg, respectively). The WHO recommends a minimum Fe concentration of 15 mg/kg in food (Anthony *et al.*, 2013). The Cu content determined by Ozcan (2004) was between 0.6 mg/kg and 0.9 mg/kg, which was comparable with the Cu content of *O. tenuiflorum*. Copper deficiency can cause hypertension, hyperglycemia and antibiotic sensitivity (Devesh *et al.*, 2012).

The mineral content of plants can be influenced by differences in geography, growth conditions, species, genetics and analytical procedures (Ozcan, 2004). Geographic variations can include climatic variables such as temperature and the length of the growing season

(Reich and Oleksyn, 2004). Growth conditions such as climate, soil, and plant functional type are other factors that influence the mineral and heavy metal contents of plants (Reich and Oleksyn, 2004; Kara, 2009). The increased Cu, Zn and Fe content of *O. tenuiflorum* powder could be caused by the techniques and substances used during processing, such as are used in the cutting, grinding or blending of the plant. The Zn concentration in *Lycopersicon esculentum* increases by using iron pots during cooking (Anthony et al., 2013). Conversely, K and Na levels can be reduced by heating and washing. The passive diffusion of water-soluble Na and K in legume flour has been reported by Christine and Rosalind (2007).

From Table 2, the Pb content (2.10mg/kg) displayed by was more than that of *Ocimum tenuiflorum*. The Pb content determined in Aluko et al. (2012) was 20 ± 0.01 mg/kg that considered relatively higher than that of *Ocimum tenuiflorum*. Due to the accumulation potential of As, it was selected to be analyzed. The As content in both *Ocimum tenuiflorum* samples was lower than that of value collected by Mehmet et al. (2008) (5.3 ± 5.19 mg/kg). The result of Mehmet et al. (2008) showed that the As intake exceed 0.015 mg/kg set by Food and Agricultural Organization World Health Organization Joint Expert Committee on Food Additives (Harriet et al., 2012). Besides, the Scientific Committee of the European Food Safety Authority (EFSA) stated that it is important to ensure the botanical ingredients used in food supplements are safe to consume and *O. tenuiflorum* that is possible to cause reproduction toxicity was evaluated. The result of the evaluation showed that there was no information regarding toxic compounds for that effect (Gerrit et al., 2010).

Conclusion

O. tenuiflorum exhibited anticancer activity against some cancerous cell lines, and especially against MCF-7, a hormone-dependent breast cancer cell line. The viability of MCF-7 cells decreased significantly ($p < 0.05$) and the best concentration of *O. tenuiflorum* was 25 µg/ml of methanolic extracts after 48 and 72 h (94% and 92.4%, respectively). The concentrations of heavy metals (Pb and As) measured in *O. tenuiflorum* are low enough to be safe for consumption.

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